Testing Methods for Fertilizers
(2021)

Incorporated Administrative Agency
Food and Agricultural Materials Inspection Center

Contents (2021)

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Explanation of Testing Methods for Fertilizers (2021) Explanation - 1

1. General Rule

1.1 Common Items

(1) Applicable range

The Testing Methods for Fertilizers stipulate the official method of analysis of fertilizers and fertilizer materials. The type of samples in the tests is shown in the summary of respective test items.

Reference: The Ministry of Agriculture, Forestry and Fisheries (Hereinafter referred to as MAFF) Public Notices which adopt the "Testing Methods for Fertilizers" are shown below.

1) MAFF Public Notice: Prescribing for the quality labeling standards of special fertilizers, MAFF Public Notice No. 1163 of August 31, 2000, MAFF Public Notice No. 2087 (2020) of October 27, 2020, as amended.
2) MAFF Public Notice: Prescribing for the particulars detailed in the assurance label regarding the type of raw material as well as material, name and an amount of usage of normal fertilizers, pursuant to Article 11-2 Paragraph 1 and Paragraph 2 of Regulation for Enforcement of Fertilizer Regulation Act, MAFF Public Notice No. 700 of March 16, 1984, MAFF Public Notice No. 2085 (2020) of October 27, 2020, as amended.
3) MAFF Public Notice: Prescribing for the official specification of normal fertilizers pursuant to Fertilizer Regulation Act, MAFF Public Notice No. 284 of February 22, 1986, MAFF Public Notice No. 2126 (2020) of October 30, 2020, as amended.
4) MAFF Public Notice: Prescribing for the major components whose contents are detailed in the assurance label of normal fertilizers listed in Fertilizer Regulation Act Article 4 Paragraph 1 Clause 3, pursuant to Fertilizer Regulation Act Article 17 Paragraph 1 Clause 3, MAFF Public Notice No. 96 of January 27, 2000, MAFF Public Notice No. 2086 (2020) of October 27, 2020, as amended.

(2) General matters in common, procedures and terms

(2.1) Terms related to laws and ordinances

a) Main components or major components: The main components or major components in fertilizers in Table 1 are stipulated as components to be calculated by a public notice of the Ministry of Agriculture, Forestry and Fisheries.

Table 1 Components to be calculated as main component

Main component or major component	Component to be calculated
Phosphoric acid	Phosphorus pentaoxide (P_2O_5)
Potassium	Potassium oxide (K_2O)
Silicate	Silicon dioxide (SiO_2)
Magnesia	Magnesium oxide (MgO)
Manganese	Manganese oxide (MnO)
Boron	Diboron trioxide (B_2O_3)
Sulfur content	Sulfur trioxide (SO_3)
Lime	Calcium oxide (CaO)

(2.2) General matters and terms that cite Japanese Industrial Standards (JIS)

- **a)** **General notices**: General matters common to analysis are according to JIS K 0050.
- **b)** **Definition**: The definitions of major terms used in the Testing Methods for Fertilizers are according to JIS K 0211, JIS K 0214, JIS K 0215, JIS Z 8101-1, JIS Z 8101-2 or JIS Z 8101-3.
- **c)** **Laboratory sample**: A sample transferred to a laboratory. Laboratory sample as specified in JIS K 0211.
- **d)** **Test sample**: A sample obtained from a laboratory sample after pretreatment such as grinding. Test sample as specified in JIS K 0211.
- **e)** **Analytical sample**: A sample measured from a laboratory sample or a test sample and to be used in one test. Test sample or analytical sample as specified in JIS K 0211.
- **f)** **Sample**: A sample in the testing methods indicates **c**) a laboratory sample, **d**) a test sample or **e**) an analytical sample.
- **g)** **Rounding numbers**: Methods of rounding the numbers are according to JIS Z 8401.
- **h)** **Absorptiometric analysis**: General rules for absorptiometric analysis are according to JIS K 0115.
- **i)** **Atomic absorption spectrometry**: Atomic absorption spectrometry includes flame atomic absorption spectrometry, electrically heated atomic absorption spectrometry (hereinafter referred to as "electrically heated atomic absorption spectrometry") and other atomic absorption spectrometry. General matters common to these are according to JIS K 0121.
- **j)** **Gas chromatography**: General matters common to gas chromatography are according to JIS K 0114.
- **k)** **Gas Chromatography/Mass Spectrometry**: General matters common to Gas Chromatography/Mass Spectrometry are according to JIS K 0123.
- **l)** **Electrical conductivity measuring method**: General matters common to electrical conductivity measuring methods are according to JIS K 0130.
- **m)** **Test sieving**: General matters common to test sieving are according to JIS Z 8815.
- **n)** **High-Performance Liquid Chromatography**: General matters common to High-Performance Liquid Chromatography are according to JIS K 0124.
- **o)** **High-Performance Liquid Chromatography/ Mass Spectrometry**: General matters common to High-Performance Liquid Chromatography/ Mass spectrometry are according to JIS K 0136.
- **p)** **ICP Optical Emission Spectrometry**: General matters common to ICP Optical Emission Spectrometry are according to JIS K 0116.
- **q)** **ICP Mass Spectrometry**: General matters common to ICP Mass Spectrometry are according to JIS K 0133.
- **r)** **Ion Chromatography**: General matters common to Ion Chromatography are according to JIS K 0127.

(2.3) Description methods, procedures and terms in testing methods for fertilizers.

- **a)** **Reagent name**: Unless otherwise specified, conform to the names by the chemical nomenclature established by the Chemical Society of Japan [in accordance with the International Union of Pure and Applicable Chemistry (IUPAC) nomenclature of inorganic chemistry and nomenclature of organic chemistry] and the names of JIS reagents.
- **b)** **Organic matters**: Fertilizers such as Organic fertilizers, sludge fertilizers and compost and fertilizer materials. However, organic compounds such as urea and urea compounds are excluded.
- **c)** **Actual article**: A laboratory sample in original state.
- **d)** **Drying matter**: The matter which remains after drying the actual article.
- **e)** **Notes, comments, figures, tables and formulae**: Serial numbers for each test item should be recorded in notes, comments, figures, tables and formulae.

f) **Dilution of solution**: "Transfer accurately a predetermined amount (to a vessel)" means the procedure to measure any volume of solution with a measuring instrument specified in JIS K 0050 (into a vessel).

Also, "dilute accurately a predetermined amount (with solvent or solution)" means the procedure to measure any volume of solution with a measuring instrument specified in JIS K 0050 into a volumetric flask of arbitrary volume and fill up to the marked line (with solvent or solution) (1).

g) **Description of mixture solution**: Mixture solutions are described as shown in **1)** - **4)**.

1) **Reagent + reagent**: Describe as reagent name 1−reagent name 2 (V_1 + V_2). In this case, V_1 volume of reagent name 1 is mixed with V_2 volume of reagent name 2.
Example: acetonitrile−water (1+1), hexane−ethyl acetate (2+1), methanol−buffer solution (3+1)

2) **Reagent + water**: Describe as reagent name 1 (V_1+V_2). In the case of reagents described in Table 1 in JIS K 0050, it means V_1 volume of reagent name 1 is diluted by mixing with V_2 volume of water.
Example: hydrochloric acid (1+1), sulfuric acid (1+2), ammonia solution (1+3)

3) **Solution + reagent**: Describe as solution name a (concentration) - reagent name b [V_1+V_2]. In this case, it means V_1 volume of solution name a of a certain concentration is mixed with V_2 volume of reagent name b.
Example: sodium hydroxide solution (4 g/L) − methanol [1+4]

4) **Diluted reagent + reagent**: Describe as reagents name a (V_1+V_2) - reagent name b [V_3+V_4]. In this case, V3 volume of the solution in which V_1 volume of reagent name a described in Table 1 in JIS K 0050 diluted by mixing with V_2 volume of water, is mixed with V4 volume of reagent name b.
Example: hydrochloric acid (1+100) − methanol [2+3]

h) **Preparation of a calibration curve**: "Put A mL - B mL of the standard solution in volumetric flasks step-by-step." means the procedure to put a volume of 4 - 6 steps (2) in the range from A mL to B mL of the standard solution in respective volumetric flasks step-by-step.
Prepare a calibration curve every time a test is conducted. Also, when the same test item is measured under the same conditions for multiple samples continuously, measure the standard solution at regular intervals to check the indicated value.

i) **Washing of apparatus**: Wash containers with a detergent and tap water before usage and wash sufficiently with water of A2 specified in JIS K 0577 or water that is confirmed not to affect a quantification value. In case of sampling a sample to test a metallic element and organic materials, after previous washing, dip with nitric acid (1+9) or hydrochloric acid (1+9) as appropriate and further wash sufficiently with water of A2, A3 or A4.

j) **Handling of reagents and liquid waste, etc.**: Handle with care and in compliance with relevant laws and regulations. When treatment methods are specified in respective test items, comply with the methods.

k) **Referential matters related to the validity of testing methods**: Information related to the validity of respective testing methods such as quantification range (minimum limits of quantification, etc.), mean recovery, repeatability, intermediate precision and reproducibility is described in a Comment, etc. Note that the numerical values such as Minimum Limit of Quantification, etc. are not standards to be targeted but examples.

Note (**1**) When the dilution factor is large, accuracy should be secured by procedures such as repeating the dilution procedure.

(**2**) Set according to the specification and operation method of the measurement instrument used. There is no need to include the minimum and the maximum values of the calibration curb range described in the Testing Methods for Fertilizers.

(3) Water

a) Water: Water used in the Testing Methods for Fertilizers herein is water of A2 specified in JIS K 0557 or water that is confirmed not to affect a quantitation value. However, when otherwise specified in respective test items, use the specified water.

(4) Reagents

a) Reagents: When the reagent is JIS-specified, use one of highest quality among those marked with the JIS symbol; when none of the reagent is marked with the JIS symbol, use one of quality that will not cause a problem in the test. Use reference materials for volumetric analysis specified in JIS K 8005 for the standardization of titration solutions. Note that ethanol (95) specified in JIS K 8102 to prepare indicators, etc. can be replaced with ethanol (99.5) specified in JIS K 8101 and ethanol-water (19+1) prepared with water.

b) Reference materials: The preparation of standard solutions or standardization of titration solutions using reference materials **1**) - **2**) below other than materials specified in respective testing items is possible.

1) Reference materials provided by National Metrology Institute: Reference materials traceable to International System of Unit (SI) provided by National Metrology Institute (NMI: National Institute of Advanced Industrial Science and Technology NMIJ, NIST, BAM, etc.) which signed CIPM MRA (Global Mutual Recognition Arrangement based on the Meter Convention)

2) Reference materials for volumetric analysis: Reference materials for volumetric analysis specified in JIS K 8005.

c) Standard Solutions: In the cases of specifying in the comment in respective testing items, the preparation of standard solutions for a calibration curve using the solution which is traceable to the National Metrology of **1**) - **3**) below other than specified in respective testing items is possible. However, use standard solutions which do not cause a problem in the test with the kinds and concentration of chemical compounds or added acid used. In addition, in (**2.1**) **a**) Main components or major components, calculate main components or major components using conversion factors specified in the comment of respective items.

1) Standard Solutions provided by National Metrology Institute: Standard solutions traceable to International System of Unit provided by National Metrology Institute (NMI: National Institute of Advanced Industrial Science and Technology NMIJ, NIST, BAM, etc.) which signed CIPM MRA.

2) JCSS (Japan Calibration Service System) Standard Solutions: Standard solutions prepared by JCSS (Japan Calibration Service System) the registered provider for Chemical Analysis, Atomic Absorption Spectrometry, ICP or Ion Chromatography traceable to specific reference materials based on Measurement Act Article 134. In addition, it is recommended to use standard solutions which indicate the uncertainty of concentration and factors.

3) Standard Solutions traceable to National Metrology: Standard Solutions traceable to National Metrology (National Institute of Advanced Industrial Science and Technology NMIJ, NIST, BAM reference materials, etc. traceable to International System of Unit) provided by National Metrology Institutes which signed CIPM MRA, but at the same time they are standard solutions for Chemical Analysis, Atomic Absorption Spectrometry, ICP or Ion Chromatography prepared by the providers who obtained the certification of ISO Guide 34(JIS Q 0034 : General requirements for the competence of reference material producers). In addition, it is recommended to use standard solutions which indicate the uncertainty of concentration and factors.

d) Titrant: A Titrant described in **1**) is usable if it is specified in the comment of a testing item. In addition, the titrant may be diluted to a predetermined concentration as necessary. In this case, however, dilution treatment should be conducted when it is used and factors of the titrant before dilution should be applied.

1) **Titrant conforming to ISO/IEC 17025**: A Titrant which is prepared and standardized and whose factors are calculated by a laboratory which obtained an accreditation (accredited range: JIS K 8001 JA.5 solutions for titration) based on ISO/IEC 17205. In addition, it is recommended to use titrant which indicates the uncertainty of concentration and factors.

e) **Concentration of reagent solution**: Unless otherwise specified, the mass concentration is expressed as g/L or mg/L, while the molar concentration is expressed as mol/L or mmol/L. The concentration of the standard solution is expressed as the mass in 1 mL (mg/mL, µg/mL or ng/mL) except for the ion-selective electrode method.

f) **Concentration in parenthesis shown after the name of reagent solution**: It indicates that the solution is about that concentration except the standard solution. For example, sodium hydroxide solution (0.1 mol/L) means that it is about 0.1 mol/L sodium hydroxide solution. Also, the concentration shown in front of the name of solution means that it is the accurate concentration. However, the concentration is generally expressed as a round figure; calculate the factor separately.

(5) Apparatus

a) **Glass apparatus**: Unless otherwise specified, use glass apparatus specified in JIS R 3503 and JIS R 3505. Also, when a heating procedure is involved, use borosilicate glass-1 specified in JIS R 3503.

b) **Non-glass apparatus**: Unless otherwise specified, use plastic apparatus.

c) **Desiccants for desiccators**: Unless otherwise specified, use silica gel.

d) **Porcelain crucibles and porcelain evaporating dishes**: Use ones specified in JIS R 1301 and JIS R 1302.

e) **Platinum crucibles and platinum evaporating dishes**: Use ones specified in JIS H 6201 and JIS H 6202.

f) **Filter paper**: Use that specified in JIS P 3801. However, the type of filter paper is specified in respective test items.

g) **Absorbance measurement (absorptiometric analysis) absorbance cells**: Unless otherwise specified, use ones of 10 mm in optical path length.

1.2 Validity check of testing methods

The Testing Methods for Fertilizers are methods which have been discussed and approved by the Technical Committee for Fertilizers etc. or include some methods in the Official Methods of Analysis of Fertilizers (1992) whose performance has been evaluated and style was rewritten according to the Testing Methods for Fertilizers. The Testing Methods for Fertilizers will be revised by adding, modifying, or deleting testing methods with the approval of the Technical Committee for Fertilizers etc. due to the needs such as progress in analytical techniques and changing social situation.

The procedure for the testing methods validity check is shown in Annex A of this Testing Methods for Fertilizers. This procedure was made based on 7.2.2 Validation of methods in JIS Q 17025 “General requirements for the competence of testing and calibration laboratories” or 2.4 Tests for validation requirements in “Guidelines for the design and implementation of surveillance and monitoring and for the evaluation and publication of the results” which was issued by the Ministry of Agriculture, Forestry and Fisheries and with reference to the guideline of Codex Alimentarius Commission (CAC), IUPAC protocol and the guideline of AOAC INTERNATIONAL, etc. Validated testing methods are methods which are conducted according to this procedure and confirmed to conform to the standards such as required accuracy (trueness and precision), quantification range (maximum and minimum limits of quantification) and so on.

In addition, according to the validation levels in Table 1, an individual testing method is classified from Type A to Type E in Table 2.

Table 1 Testing method validation level

Symbol of Validation, etc.	Validation method, etc.
Def-M (Defining method)	Procedures of a testing method define measurement items. No relation to a validation level.
Def-C (Defining calculation)	Only calculation methods in a testing method define measurement items. The part of the definition has no relation to a validation level.
Def-E (Defining extraction)	Only extraction procedures in a testing method define measurement items. The part of the definition has no relation to a validation level.
HCV (Harmonized collaborative validation)	Evaluation by a collaboration test using samples of no less than 5 concentrations in no less than 8 laboratories with a testing method validation method regarded as an international standard (Guideline of AOAC-International, IUPAC protocol, etc.).
MLV(Multi laboratory validation)	Evaluation of validation using multiple laboratories, though it does not satisfy MLV (H) criteria.
SLV (Single laboratory validation)	Evaluation in a single laboratory using a testing method validation method regarded as an international standard (IUPAC/ISO/AOAC-International harmonized guideline, etc.).
RNV (Research non validated)	A testing method that does not carry out the validation by SLV or higher level.

Table 2 Classification of an individual testing method

Symbol of Classification	Validation level
Type A	A defined testing method
Type B	A testing method whose evaluation results by HCV and SLV satisfies the requirements of "Appendix: The procedure to validate characteristics of testing methods".
Type C	A testing method whose evaluation results by MLV and SLV satisfies the requirements of "Appendix: The procedure to validate characteristics of testing methods".
Type D	A testing method whose evaluation results by SLV satisfies the requirements of "Appendix: The procedure to validate characteristics of testing methods".
Type E	A testing method that does not carry out the validation by SLV or higher level.

1.3 Procedure of testing methods

1.3.1 Competence evaluation of laboratory

When an individual testing method is used, it is recommended that the following competence evaluation of a laboratory is conducted.

As for the testing methods of Type A, Type B and Type C, conduct a replicate testing with five samples, whose measured component concentration is known and to which certified reference materials or standard solutions are added, to confirm trueness and precision in advance. As for the testing methods of Type D and Type E, conduct a validity check in a single laboratory anew.

In order to ensure the reliability of a series of testing, conduct internal quality control (internal quality assurance control, internal precision control) for each testing by a duplicate testing with samples, whose measured component concentration is known, to confirm trueness and precision.

If it is possible, participate in an external quality assessment (external precision control, competence test) in order to evaluate the consistency with testing results of other laboratories and confirm the evaluation by z score.

1.3.2 Evaluation of test result

The results of a testing method which substitutes for the Testing Methods for Fertilizers can be used if it conforms to criteria required in validation of a testing method. However, in case where the result of the testing method does not agree with the result of the Testing Methods for Fertilizers (1), the testing result of the latter is used to make a final judgement. In addition, if multiple methods are described for a testing component, it is recommended that the result of a testing method for final judgement is adopted in the following order: Type A, Type B, Type C, Type D and Type E.

Note **(1)** Refer to the separate sheet: "Target of trueness and criteria of precision in respective concentration levels" or "the reproducibility of respective testing methods" in order to determine if there is mutual agreement.

2. Handling of samples

2.1 Sampling

Refer to the "Sampling Method of Fertilizers (2020)".

References

1) Incorporated Administrative Agency Food and Agricultural Materials Inspection Center: Sampling Method of Fertilizers (2020)

2) JIS M 8100: Particulate materials - General rules for methods of sampling (1992)

3) JIS K 0060: Sampling method of industrial wastes (1992)

4) JIS Z 8816: Particulate materials − General requirements for methods of sampling (2001)

5) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p. 12 - 17, Yokendo, Tokyo (1988)

2.2 Storage of samples

(1) Summary

Put a sample in a container suitable for its characteristics and form and seal it tightly, and then store the sample at room temperature (10 ºC - 30 ºC) or cold temperature. Care should be taken not to freeze it when it is stored at cold temperature.

(2) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Refrigerator**: A refrigerator that can be adjusted to 1 ºC - 8 ºC.

b) **Storage container for a sample**: A storage container for a sample should be clean, strong and completely sealed airtight. In particular, in case it contains sludge for raw materials, the container should be made of non-degradable, non-absorbable material. Additionally, it should be airtight, water-proof, vapor-proof and non-corrosive.

(3) **Procedure**: Conduct storage as shown below.

a) Store a relatively stable sample in a tightly sealed container to avoid direct sunlight.

b) Store a sample in a desiccator, etc. by tightly sealing it if test results are affected by moisture absorption.

c) Store a sample in a tightly sealed container in a dark place at 1 ºC - 8 ºC if it is easily deteriorated by moisture.

2.3 Preparation of test samples

(1) Summary

a) Prepare a test sample by pre-drying, reducing, and grinding laboratory samples as necessary.

b) Conduct pre-drying if a laboratory sample is moist and hard to grind.

c) A laboratory sample made from such fertilizers as a fluid fertilizer and a particle-fertilizer that is sufficiently homogeneous can be used as a test sample.

d) If contamination by apparatus affects a test result, procedures such as pre-drying, reduction and grinding are prohibited

e) Note that part of a test sample should not scatter, nor should surrounding fine particles or other alien substances be mixed with the test sample being prepared

2.3.1 Pre-drying

(1) Summary

This procedure is applicable to fertilizers whose laboratory sample is moist and hard to grind. The symbol of the procedure is 2.3.1-2017 or PD.-1.

Conduct pre-drying using a drying apparatus, and measure the loss on drying in this procedure. In addition, calculate a conversion factor (actual article), if necessary, to convert the component content obtained in respective tests to the component content in a laboratory sample (actual article).

(2) Apparatus and instruments: Apparatus and instruments are shown below.

a) Drying apparatus: A drying apparatus that can be adjusted to the pre-drying temperature at ± 2 ºC.

b) Sample drying dish: Measure the mass to the order of 0.1g in advance. Additionally, use materials of a quality that do not affect the measurement of test components.

(3) Procedure: Conduct pre-drying as shown below.

a) Put 250 g - 1 kg of a laboratory sample in a sample drying dish, spread uniformly and measure the mass to the order of 0.1g.

b) Place a sample drying dish containing a laboratory sample in a drying apparatus and dry [(1)].

c) Remove a sample drying dish from a drying apparatus and leave at rest at room temperature until it is balanced with atmospheric temperature [(2)].

d) After leaving at rest, measure the mass of c) to the order of 0.1 g.

e) Calculate loss on drying in the pre-drying by the following formula (1). If necessary, calculate a conversion factor (actual article) by the following formula (2).

$$\text{Loss on drying (\% (mass fraction))} = ((W_1 - A)/W_1) \times 100 \quad \cdots\cdots\cdots (1)$$

$$\text{Conversion factor (actual article)} = A/W_1 \quad \cdots\cdots\cdots\cdots (2)$$

W_1 : Mass (g) of the sampled laboratory sample

A : Mass (g) of the laboratory sample after drying

Note (**1**) Examples of drying temperature and drying time: About 70 hours at 40 ºC, no less than 5 hours at 65 ºC.

(**2**) An example of time to leave at rest: About 20 minutes

Comment 1 When calculating major components in a laboratory sample (actual article) such as compost and sludge fertilizers, etc. where the test sample is prepared conducting pre-drying, convert component contents in the analytical sample obtained in respective tests by the following formula.

$$\text{Component content in a laboratory sample (actual article)} = B \times C$$

B : Component content in an analytical sample obtained in each test

C : Conversion factor (actual article)

References

1) Mariko AIZAWA, Yuji SHIRAI, Yasushi SUGIMURA, Yuichi TAKAHASHI, Jun OKI, Yukio FUKUCHI and Norio HIKICHI: Evaluation of Pre-drying Procedure to Prepare Test Samples from Sludge Fertilizer, Research Report of Fertilizer **Vol. 1**, p. 122 - 128 (2008)

(**4**) **Flow sheet for pre-drying** The flow sheet for the pre-drying of a moist laboratory sample is shown below.

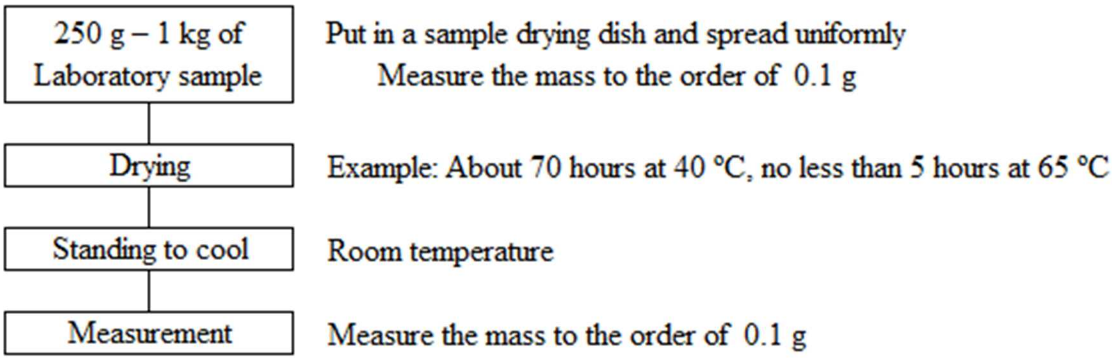


Figure Flow sheet for pre-drying

2.3.2 Reduction (Separation)

(1) Summary

This procedure is applicable to fertilizers. The symbol of the procedure is 2.3.2-2017 or Red.-1.

In order to distinguish a test sample from a sample for granularity test and physical characteristics test, etc., reduce (separate) a laboratory sample with an increment reduction method, a riffle sampler method or a conical quartering method.

(2) Apparatus

a) **Scoop for increment reduction**: A scoop for increment reduction specified in the "Sampling Method of Fertilizers (2020)".

b) **Riffle sampler**: A riffle sampler specified in the "Sampling Method of Fertilizers (2020)".

(3) Procedure: Conduct reduction (separation) as shown below.

a) **Increment reduction method**: Refer to the "Sampling Method of Fertilizers (2020)" 4.2 (2) Increment reduction method.

b) **Riffle sampler method**: Refer to the "Sampling Method of Fertilizers (2020)" 4.2 (3) Riffle sampler method.

c) **Conical quartering method**: Refer to the "Sampling Method of Fertilizers (2020)" 4.2 (4) Conical quartering method

References

1) Incorporated Administrative Agency Food and Agricultural Materials Inspection Center: Sampling Method of Fertilizers (2020)
2) JIS M 8100: Particulate materials - General rules for methods of sampling (1992)
3) JIS K 0060: Sampling method of industrial wastes (1992)
4) JIS Z 8816: Particulate materials − General requirements for methods of sampling (2001)

2.3.3 Grinding

(1) Summary

This procedure is applicable to fertilizers. The symbol of the procedure is 2.3.3-2017 or GRD.-1.
In order to prepare a homogeneous test sample, grind a laboratory sample with an adequate grinder until it completely passes through the designated granularity.

(2) Apparatus and instruments: Apparatus and instruments are shown below.

a) **Grinder**: Use a grinder (3) of a type and suitability for the granularity and the physical characteristics (1) of a laboratory sample. In addition, the grinder apparatus which come into contact with a laboratory sample should be made of materials (2) which do not affect the analytical value.

b) **Primary grinder**: A grinder (4) that can primarily grind a large lump.

c) **Cutter machine**: A cutter that can cut long stems, etc.

d) **Sieve**: A sieve for the test specified in JIS Z 8801-1 or JIS Z 8801-2 or equivalents.

Note **(1)** The physical characteristics of a laboratory sample are defined by their solidity, toughness, specific gravity and adhesiveness.

(2) (Ex.) Do not use stainless steel apparatus when preparing a test sample for chromium or nickel.

(3) A centrifuging type grinder, a cutting mill, a vibration mill type grinder, etc.

(4) A blender with an attachable blade, etc.

(3) Procedure: Conduct grinding as shown below.

(3.1) Fertilizers except ones specified in (3.2): Conduct as specified in 6.4 of JIS M 8100 and as shown below.

a) Break or cut a laboratory sample with a primary grinder or a cutting machine as necessary.

b) Grind with a grinding machine until it completely passes through a sieve of 500 µm - 1 mm aperture.

c) Mix ground samples to make the test sample.

Comment 1 If the sampling amount of an analytical sample is less than 1 g, use a test sample which will completely pass through 500 µm aperture sieve. In addition, in case a test sample which suits the aforementioned condition cannot be obtained due to a deliquescent laboratory sample, etc., make one by crushing a test sample with a mortar and pestle, which completely passes through 1 mm aperture sieve.

(3.2) Fused phosphate fertilizer, silicate slag fertilizer, etc.: Conduct as specified in 6.4 of JIS M 8100 and as shown below.

a) Grind a laboratory sample with a vibration mill type grinder.

b) Transfer the ground laboratory sample to a sieve of 212 µm apertures.

c) Incline the sieve about 20 degrees, supporting it with one hand or a bent arm, and tap the sieve frame with the other hand at the rate of about 120 times per minute. During the procedure, place the sieve in a horizontal position at the rate of 4 times per minute, rotate it 90 degrees and tap the sieve frame firmly one or two times.

d) When fine powder attaches to the back side of a sieve screen, remove it gently from the back side to make minus sieve.

e) Regarding the plus sieve of a sample, make them pass through by repeating the procedure in **a**) - **d**).

f) Combine and mix the sample passed to make the test sample.

Comment 2 Conduct the procedures in (**3.2**) to obtain observed value of citric acid-soluble main components in a stable manner. For examples of applicable laboratory samples, fused matters such as fused phosphate and silicate slag fertilizer, fertilizers made from fused materials and calcined phosphate fertilizer, etc. are listed.

Comment 3 The procedures in **b**) - **d**) are the procedures in 6.1.3 (1.4) of JIS Z 8815.

References

1) JIS Z 8801-1: Test sieves - Part 1: Test sieves of metal wire cloth (2019)
2) JIS Z 8801-2: Test sieves - Part 2: Test sieves of metal wire cloth (2020)
3) JIS M 8100: Particulate materials - General rules for methods of sampling (1992)

3. General tests
3.1 Moisture or moisture content
3.1.a Loss on drying method with drying apparatus
(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type A (Def-M) and its symbol is 3.1.a-2017 or Mois.a-1.

Use drying apparatus under conditions suitable for the kind of fertilizers to be measured to heat analytical samples to measure loss on drying and obtain moisture in an analytical sample or the moisture content of a quality labeling standard of a special fertilizer (herein after referred to as "moisture"). Additionally, calculate a conversion factor (drying matter) to convert component content obtained by respective tests to component content in a drying matter as necessary.

This testing method corresponds to loss on heating in the Official Methods of Analysis of Fertilizers (1992).

(2) Apparatus and instruments: Apparatus and instruments are shown below.

a) Drying apparatus: Drying apparatus that can be adjusted to the test temperature ± 2 ºC.

b) Ground-in stoppered weighing bottles (1): Low-form weighing bottles, 50 mm × 30 mm, specified in JIS R 3503. Dry by heating in advance in a drying apparatus at 75 ºC - 130 ºC, stand to cool in a desiccator, and measure the mass to the order of 1 mg.

Note (1) Aluminum weighing dishes described in the Handbook of the Feed Analysis Standards -2009- can also be used.

(3) Measurement: Conduct measurement as shown below.

a) Put 2 g-5 g of an analytical sample in a ground-in stoppered weighing bottle, spread so that the thickness is no more than 10 mm, and measure the mass to the order of 1 mg.

b) Place the ground-in stoppered weighing bottle containing the analytical sample in a drying apparatus at 100 ºC ± 2 ºC, and heat for 5 hours (2).

c) After heating, fit the stopper into the ground-in stoppered weighing bottle, and immediately move to a desiccator to let it stand to cool.

d) After standing to cool, remove the ground-in stoppered weighing bottle from the desiccator, and measure the mass to the order of 1 mg.

e) Calculate loss on drying in the analytical sample by the following formula (1) as moisture. If necessary, calculate a conversion factor (actual article) by the following formula (2).

$$\text{Loss on drying (\% (mass fraction))} = ((W_1 - A)/W_1) \times 100 \quad \cdots\cdots (1)$$

$$\text{Conversion factor (drying matter)} = W_1/A \quad \cdots\cdots (2)$$

W_1 : Mass (g) of the sampled analytical sample

A : Mass (g) of the analytical sample after drying

Note (2) Heat simultaneously the slightly moved or removed stopper of the ground-in stoppered weighing bottle.

Comment 1 When pre-drying a laboratory sample such as compost and sludge fertilizers to prepare a test sample, calculate the moisture of the laboratory sample (actual article) by the following formula:

Moisture (% (mass fraction)) in the laboratory sample (actual article) = $B + C \times ((100 - B)/100)$

B : Loss on drying (% (mass fraction)) of the laboratory sample (actual article) by the pre-drying procedure
C : Loss on drying (% (mass fraction)) in the analytical sample by moisture measurement

Comment 2 When calculating harmful content in a drying matter of sludge fertilizers, etc., convert component content in a test sample obtained from respective tests by the following formula.

Component content in a drying matter = $D \times E$

D : Component content in an analytical sample obtained in each test
E : Conversion factor (drying matter)

Comment 3 Use drying conditions in Table 1 for fertilizers of the types shown below:

Table 1 Drying conditions

Type of fertilizers	Sampling amount of analytical samples	Drying temperature	Drying time
Superphosphate of lime, triple superphosphate of lime, or fertilizer containing these	About 5 g	100 ºC ± 2 ºC	3 hours
Ammonium sulfate, sodium nitrate, and potassium salts	2 g - 5 g	130 ºC ± 2 ºC	Until a constant weight is achieved.
Urea and urea-containing fertilizer	About 5 g	75 ºC ± 2 ºC	4 hours
Silica gel fertilizer and fertilizer containing silica gel, and silica hydrogel fertilizer	About 5 g	180 °C±5 °C	3 hours

Comment 4 For samples containing volatile matters, subtract the volatile matter content by the following **a**) and **b**) from loss on drying to obtain moisture.

a) Fertilizers containing guano or diammonium hydrogen phosphate, etc.: Determine total nitrogen in the test sample, and in the analytical sample after the drying procedure; convert the difference between the quantitation values into ammonia (NH_3) to make the volatile matter content.

b) Potassium hydrogen carbonate: Determine carbon dioxide in the test sample, and in the analytical sample after the drying procedure; the difference between the quantitation values is the volatile matter content.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p. 20 - 23, Yokendo, Tokyo (1988)
2) Society for the Study of Feed Analysis Standards: Methods of Analysis of Feeds and

Feed Additives-2009-I, p. 37 - 39, Incorporated Administrative Agency Food and Agricultural Materials Inspection Center, Saitama (2009)
3) JIS Z 0701 : Silica gel desiccants for packaging (1997)

(**4**) **Flow sheet for moisture**: The flow sheet for moisture in fertilizers is shown below:

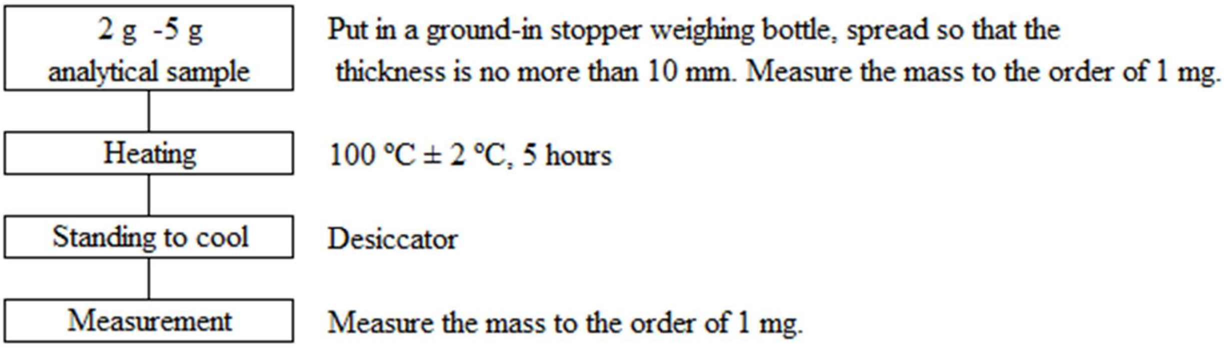


Figure Flow sheet for loss on drying in fertilizers
by loss on drying method with a drying apparatus (Example)

3.1.b Loss on drying method by moisture analyzers

(1) Summary

The testing method is applicable to sludge fertilizers, compost, and organic fertilizers, etc. This testing method is classified as Type B and its symbol is 3.1.b-2017 or Mois.b-1.

Use a moisture analyzer in the heat drying method to measure loss on drying and obtain moisture in an analytical sample or the moisture content of a quality labeling standard of a special fertilizer (herein after referred to as "moisture"). Additionally, calculate a conversion factor (drying matter) to convert component content obtained by respective tests to component content in a drying matter as necessary.

In addition, the performance of this testing method is shown in **Comment 3**.

(2) Instruments: Instruments are as shown below:

a) Moisture analyzer: A moisture analyzer consisted of a heat source to heat an analytical sample (halogen lamp, infrared heater, ceramic heater, etc.) and a balance (1) with calibration function.

Note (1) There is a method to calibrate with calibration weights or a method to calibrate automatically with built-in weights.

(3) Measurement: Conduct measurement as shown below. However, conduct in advance a comparative test with 3.1.a Loss on drying with drying apparatus using sludge fertilizers, compost, and organic fertilizers, etc., to confirm that there is no difference in the quantitation value of moisture.

a) Put about 5 g of an analytical sample in a ground-in stoppered weighing bottle, spread so that the thickness is no more than 10 mm, and measure the mass to the order of 1 mg.

b) Heat at 100 ºC (2), until a constant weight is achieved.

c) After the end of heating (2), measure the mass to the order of 1 mg.

d) Calculate loss on drying in the analytical sample by the formula (1) as moisture. If necessary, calculate a conversion factor (drying matter) by the formula (2).

$$\text{Loss on drying (\% (mass fraction))} = ((W_1 - A)/W_1) \times 100 \quad \text{.... (1)}$$

$$\text{Conversion factor (drying matter)} = W_1/A \quad \text{.... (2)}$$

W_1 : Mass (g) of the sampled analytical sample
A : Mass (g) of the analytical sample after drying

Note (2) The setup of the drying program and the determination parameter for the end of heating (constant weight) is according to the specification and the operation method of the moisture analyzer used.

Comment 1 When pre-drying is conducted, calculate the moisture of the laboratory sample (actual article) by the following formula:

$$\text{Moisture (\% (mass fraction)) in the laboratory sample (actual article)} = B + C \times ((100 - B)/100)$$

B : Loss on drying (% (mass fraction)) of the laboratory sample (actual article) by the pre-drying procedure
C : Loss on drying (% (mass fraction)) in the analytical sample by moisture measurement

Comment 2 When calculating harmful content in a drying matter of sludge fertilizers, etc., convert component content in a test sample obtained from respective tests by the following formula.

Component content in a drying matter = $D \times E$

D : Component content in an analytical sample obtained in each test
E : Conversion factor (drying matter)

Comment 3 Table 1 shows the results of the comparison of the measurement values by loss on drying method with drying apparatus and the measurement values by loss on drying method with a moisture analyzer using organic fertilizers, compost and sludge fertilizers in order to evaluate trueness.
Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 2.

Table 1 Analysis results of the comparison test results of respective methods

Symbol of measurement value		Sample		Range of	Regression coefficient		Correlation
Drying apparatus method 1)	Moisture method 2)	Kind	Number of samples	yi～yj (%)3)	($y = a + bx$) a	b	coefficient r
x_i	y_i	Sludge fertilizer4)	26	5.50～90.61	0.188	0.998	0.999
x_j	y_j	Organic fertilizer5)	25	2.96～12.33	0.185	0.986	0.994

1) 3.1.a Loss on drying method with drying apparatus
2) 3.1.b Loss on drying method with a moisture analizer
3) Mass fraction
4) Sewage sludge fertilizer, Human waste sludge fertilizer, Industrial sludge fertilizer, Composted sludge fertilizer
5) Fish meal, Byproduct organic fertilizer of vegetable origin, Compost, Steamed leather meal, Rape seed meal and powdered rape seed meal

Table 2 Analysis results of the collaborative study for the validation of the moisture test method.

Sample name	Number of laboratories1)	Mean2) (%)3)	s_r 4) (%)3)	RSD_r 5) (%)	s_R 6) (%)3)	RSD_R 7) (%)
Sewage sludge fertilizer	9	21.93	0.32	1.4	0.47	2.1
Human waste sludge fertilizer	8	13.36	0.14	1.1	0.37	2.8
Industrial sludge fertilizer	9	34.28	0.21	0.6	0.50	1.5
Calcined sludge fertilizer	9	38.75	0.59	1.5	0.59	1.5
Composted sludge fertilizer	9	27.1	0.26	0.9	0.60	2.2

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Takeshi UCHIYAMA and Chiyo SAKASEGAWA: Validation of a Heating Method Using a Moisture Analyzer for Moisture Content in Sludge Fertilizer. Research Report of Fertilizer **Vol. 1**, p. 1- 5 (2008)

2) Takeshi UCHIYAMA and Yuji SHIRAI: Determination of Moisture Content in Sludge Fertilizer by a Heating Method Using Moisture Analyzer: A Collaborative Study. Research Report of Fertilizer **Vol. 1**, p. 6 - 11 (2008)

3) Satono AKIMOTO and Sakiko TAKAHASHI: Validation of a Heating Method Using a Moisture Analyzer for Moisture Content in Sludge Fertilizer. Research Report of Fertilizer **Vol. 2**, p. 1 - 5 (2009)

(**4**) **Flow sheet for moisture**: The flow sheet for moisture in sludge fertilizers, compost, and organic fertilizers, etc. is shown below:

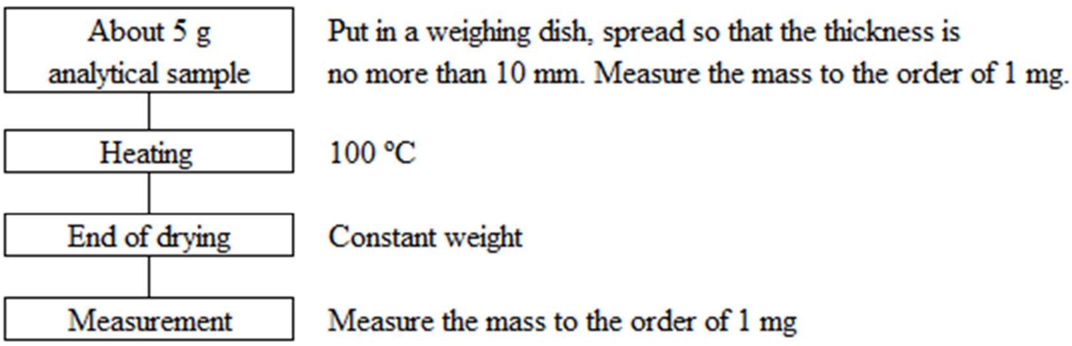


Figure Flow sheet for moisture in sludge fertilizers, compost, and organic fertilizers, etc. by the loss on drying method with a moisture analyzer.

3.2 Ash content

3.2.a Ignition residue method

(1) Summary

The method is applicable to organic fertilizers and fertilizers containing organic matters. This testing method is classified as Type A (Def-M) and its symbol is 3.2.a-2017 or Ash.a-1.
The method ignites an analytical sample with an electric furnace and measures residue on ignition to obtain ash content in an analytical sample.

(2) Apparatus and instruments: Apparatus and instruments are shown below.

- **a) Electric furnace**: Electric furnace that can be adjusted to 550ºC ± 5 ºC.
- **b) Crucible**: After heating a porcelain crucible for chemical analysis specified in JIS R 1301 with an electric furnace at 550 ºC ± 5 ºC, stand to cool in a desiccator in advance and measure the mass to the order of 1 mg.

(3) Measurement: Conduct measurement as shown below.

- **a)** Put about 2 g of an analytical sample into a crucible, and measure the mass to the order of 1 mg.
- **b)** Place it into an electric furnace, heat gently until carbonized (1).
- **c)** Heat at 550 ºC ± 5 ºC for no less than 4 hours (1).
- **d)** After heating, move the crucible into a desiccator and let it stand to cool.
- **e)** After standing to cool, remove the crucible from the desiccator and measure the mass to the order of 1 mg.
- **f)** Calculate the residue on ignition in the analytical sample by the following formula to make ash content.

$$\text{Residue on ignition (\% (mass fraction))} = (A/W) \times 100$$

W : Mass (g) of the sampled analytical sample
A : Mass (g) of the ignited analytical sample

Note (1) Example of carbonizing and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 550 °C in 1 to 2 hours.

(4) Flow sheet for ash content: The flow sheet for ash content in fertilizers is shown below:

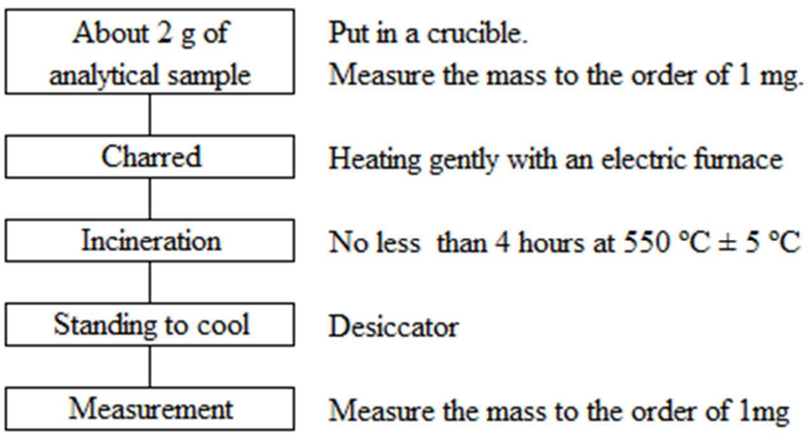


Figure Flow sheet for ash content in fertilizers.

3.3 pH
3.3.a Glass electrode method
(1) Summary
This testing method is applicable to fertilizers. This testing method is classified as Type A (Def-M) and its symbol is 3.3.a-2017 or pH.a-1.
Measure the pH of fertilizers with a pH meter using a glass electrode.

(2) Reagent: Reagents are as shown below.
- **a) Oxalate pH standard solution**: Oxalate pH standard solution class 2 traceable to National Metrology.
- **b) Phthalate pH standard solution**: Phthalate pH standard solution class 2 traceable to National Metrology.
- **c) Neutral phosphate pH standard solution**: Neutral phosphate pH standard solution class 2 traceable to National Metrology.
- **d) Borate pH standard solution**: Borate pH standard solution class 2 traceable to National Metrology.
- **e) Carbonate pH standard solution**: Carbonate pH standard solution class 2 traceable to National Metrology.

Comment 1 Respective pH standard solutions stored for long time should not be used since the pH value may change during storage period. In particular, note that borate pH standard solution and carbonate pH standard solution easily absorb carbon dioxide in the air, so that the pH values deteriorate.
The pH standard solution that was used once or left exposed to the air should not be used.

(3) Instruments: Instruments are as shown below:
- **a) pH meter**: Use type II specified in JIS Z 8802.

Comment 2 Conduct the calibration of a pH meter as indicated in JIS Z 8802. Actual calibration operation is according to the operation procedure of the pH meter used for measurement.
When the pH of a sample solution is no more than 7, use neutral phosphate pH standard solution and oxalate pH standard solution, or phthalate pH standard solution. When it exceeds 7, use neutral phosphate pH standard solution and borate pH standard solution, or carbonate pH standard solution.

(4) Test procedures
(4.1) Preparation of sample solution: Conduct preparation of a sample solution as shown below.
(4.1.1) Fertilizers except inorganic fertilizers
- **a)** Put a predetermined amount of an analytical sample (1) into a ground-in stopper flask and add water 5 - 10 times the volume (2).
- **b)** Mix with a magnetic stirrer, filter with Type 3 filter paper to make a sample solution.

Note (1) In the case of a moist laboratory sample, it is recommended to use a sample that is not pre-dried.
(2) If the sample solution becomes hard to measure because of gelling due to the influence of flocculants in sludge fertilizer, etc., increase the volume of water to be added.

(4.1.2) Inorganic fertilizers

a) Transfer a predetermined amount of an analytical sample [(1)] into a ground-in stopper flask and add water 100 times the volume.

b) Mix with a magnetic stirrer, filter with Type 3 filter paper to make a sample solution.

Comment 3 The procedure in (**4.1.1**) is the same as **3.4.a** (**4.1**). Additionally, the sample solution prepared in **4.2.4.a** (**4.1**) can be used instead of the sample solution prepared by (**4.1.2**).

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS Z 8802 and as shown below. Actual calibration operation is according to the operation procedure of the pH meter used for measurement.

a) Wash the read station of a calibrated pH meter repeatedly no less than 3 times with water and wipe out with clean and soft paper, etc.

b) Put a sample solution into a beaker [(3)], dip the read station in the solution and measure the pH value.

Note (**3**) It is necessary to put sufficient volume of sample solution to keep a measurement value stable.

Comment 4 If a pH meter has a temperature correction dial or a digital switching, measure the pH value after adjusting the graduation of the pH meter with the temperature of a sample.

References

1) JIS Z 8802 : Methods for determination of pH of aqueous solutions (2011)

(**5**) **Flow sheet for pH value**: The flow sheet for pH value in fertilizers is shown below.

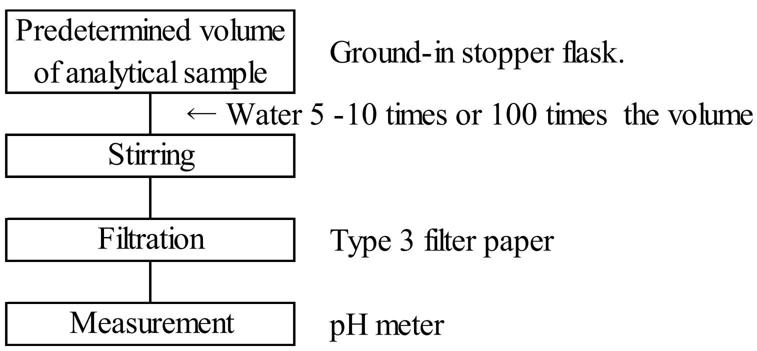


Figure Flow sheet for pH in fertilizers.

3.4 Electrical conductivity

3.4.a Measurement method with an electrical conductivity meter

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type A (Def-M) and its symbol is 3.4.a-2017 or EC.a-1.

Measure the electrical conductivity of organic fertilizers such as compost or sludge fertilizers with an electrical conductivity meter.

(2) Reagent: Reagents are as shown below.

a) Potassium chloride: Grind potassium chloride used for measurement of electrical conductivity specified in JIS K 8121 with an agate mortar to powder and heat for 4 hours at 500 ºC ± 5 ºC, and then stand to cool in a desiccator.

b) Potassium chloride standard solution (1)**:** Measure predetermined volume (2) of potassium chloride of **a**) on a weighing dish, dissolve in a small amount of water, transfer it into a 1000-mL volumetric flask, and add up to the marked line with water.

Note (**1**) Store potassium chloride standard solution in a polyethylene or borosilicate glass bottle and seal the bottle.

(**2**) The volume that is recommended for an instrument or a cell used.

Comment 1 Potassium chloride standard solution used once or left in the air should not be used.

(3) Instruments: Instruments are as shown below:

a) Electrical conductivity meter: An electrical conductivity meter specified in JIS K 0130

Comment 2 Check the indicated value as shown in 6.2 in JIS K 0130 as necessary. Actual procedure to check is according to the operation procedure of the electrical conductivity meter used for measurement.

(4) Test procedures

(4.1) Preparation of sample solution: Conduct preparation of a sample solution as shown below.

a) Put the predetermined volume of an analytical sample (3) into a ground-in stopper flask and add water 10 times the equivalent volume of dry matter (4).

b) Mix with a magnetic stirrer, filter with Type 3 filter paper to make a sample solution.

Note (**3**) In the case of a moist laboratory sample, it is recommended to use a sample that is not pre-dried.

(**4**) If the sample solution becomes hard to measure because it is gelled by the influence of flocculants in sludge fertilizer, etc., increase the volume of water added. However, this fact should be expressed in the test result.

Comment 3 The procedure in (**4.1**) is the same as **3.3.a** (**4.1.1**).

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0130 and as shown below. Actual measurement operation is according to the operation procedure of the electrical conductivity meter used for measurement.

a) Wash the read station of an electrical conductivity meter repeatedly no less than 3 times with water.

b) Put a sample solution into a beaker (5), dip the read station and measure electrical conductivity.

Note (**5**) It is necessary to put sufficient volume of sample solution to keep a measurement value stable.

References

1) JIS K 0130 : General rules for electrical conductivity measuring method (2008)

(**5**) **Flow sheet for electrical conductivity**: The flow sheet for electrical conductivity is showed below.

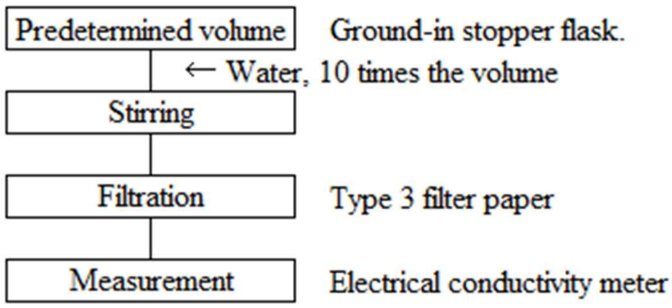


Figure Flow sheet for electrical conductivity in fertilizers.

3.5 Granularity

3.5.a Dry-type sieving testing method

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type A (Def-M) and its symbol is 3.5.a-2017 or P-size.a-1.

Measure the particle diameter distribution of solid fertilizers with a dry-type sieving analysis.

(**2**) **Apparatus**: Apparatus are shown below.

a) **Sieve**: A sieve for testing specified in JIS Z 8815

b) **Clogging removal brush**: A brush which is adequately hard for the apertures and does not damage the sieving screen.

c) **Weighing dish**: A container that can contain about 250 g of a sample. Measure the mass to the order of 0.1g in advance.

(**3**) **Dry-type sieving analysis procedure**: Conduct sieving analysis corresponding to the aperture size of a sieve used as indicated in JIS Z 8815 and as shown below.

(**3.1**) **More than 1mm and no more than 4mm**

a) Stack a large aperture sieve on an acceptor so that the large sieve is on top.

b) Measure a laboratory sample to the order of 0.1g and put it in the sieve at the top section.

c) After putting a stopper on it, hold the stacked sieves with both hands, and vibrate (1) them back and forth along a unidirectional and horizontal plane at about 60 times per minute with about 70 mm amplitude.

d) Put respective plus and minus sieves in a weighing dish (2).

Note (**1**) Conduct more circular motion at the rate of about 3 revolutions per minute as necessary.

(**2**) Turning over the back side of a sieve, remove the clogged particles from the sieve screen with a clogging removal brush and combine them with the plus sieve.

(**3.2**) **Less than or equal to 1mm**

a) Stack a large aperture sieve on an acceptor so that the large sieve is on top.

b) Measure the laboratory sample or minus sieve of (**3.1**) **c**) to the order of 0.1g and put it in the sieve at the top section.

c) After putting a stopper on it, incline the stacked sieves about 20 degrees, supporting with one hand or a bent arm, and tap the sieve frame with the other hand at the rate of about 120 times per a minute.

d) During the procedure in **c**), place the sieve in a horizontal position at the rate of 4 times per minute, rotate it 90 degrees and tap the sieve frame firmly one or two times.

e) Put respective plus and minus sieves (3) in a weighing dish (2).

Note (**3**) When fine powder attaches to the back side of a sieve screen, remove them gently from the back side with a clogging removal brush and combine them with the minus sieve

(**4**) **Measurement of granularity distribution**: Calculate the granularity distribution in an analytical sample as shown below.

a) Measure respective mass of plus and minus sieves to the order of 0.1g.

b) Calculate “plus sieve percentage” and “minus sieve percentage” with the following formula and round the results to the first decimal place.

c) Confirm that the sum of the mass of the plus sieve and the mass of the minus sieve with the smallest aperture is in the range of ± 2 % of the mass of sample measured in (**3.1**) **b**) or (**3.2**) **b**).

Mass percentage of plus sieve or minus sieve (%) (R) = (A/T) × 100

A : Mass of plus sieve or minus sieve (g)
T : Sum of the mass of plus and minus sieve (g)

References

1) JIS Z 8815 : Test sieving - General requirements (1994).
2) JIS K 0069 : testing methods for sieving of chemical products (1992).

(**5**) **Flow sheet for granularity**: The flow sheet of granularity of solid fertilizers is shown below.

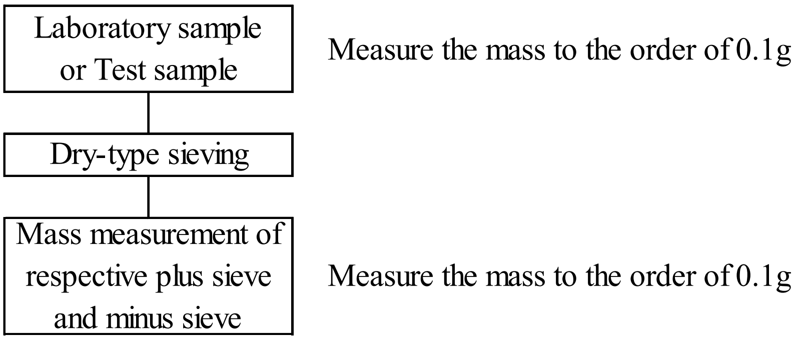


Figure Flow sheet for granularity of solid fertilizers

3.6 Oil content

3.6.a Diethyl ether extraction method

(1) Summary

This testing method is applicable to organic fertilizers. This testing method is classified as Type A (Def-M) and its symbol is 3.6.a-2017 or Oil.a-1.

Extract an analytical sample with diethyl ether using a Soxhlet extractor and measure the extract to obtain oil content in an analytical sample. The oil content contains not only fat but also fat-soluble pigments (carotenoid, chlorophyll, etc.), wax, and free fatty acids, etc.

(2) Reagent: Reagents are as shown below.

a) Diethyl ether: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Drying apparatus: Drying apparatus that can be adjusted to the test temperature ± 2 ºC.

b) Soxhlet extractor: An inter-changeable Soxhlet extractor, cooling apparatus and weighing bottles (Example: JIS R 3503, attached figure 71)

c) Water bath: A water bath that can be adjusted to about 60 ºC

d) Weighing bottle: A flat bottle flask connectable to a Soxhlet extractor. After heating with a drying apparatus at 100 ºC - 105 ºC in advance, stand to cool in a desiccator and measure the mass to the order of 1mg.

e) Cylindrical filter paper: A cylindrical filter paper made of cellulose. Example: 22 mm external diameter, 20 mm internal diameter, 90 mm total length (1).

(4) Measurement: Conduct measurement as shown below.

a) Weigh 2 g - 5 g of an analytical sample to the order of 1mg, and put it in a cylindrical filter paper.

b) Place absorbent cotton on the upper end of an analytical sample (2), as if gently pushing it, and heat it at 100 ºC - 105 ºC for 2 hours.

c) As soon as heating is complete, move the cylindrical filter paper to a desiccator and stand to cool.

d) After standing to cool, put it in a Soxhlet extractor and connect it to a cooling apparatus.

e) Put adequate volume of diethyl ether (3) in a weighing bottle, connect it to the Soxhlet extractor and heat (4) it for 8 hours to extract.

f) Recover the diethyl ether (5).

g) Disconnect the weighing bottle from the Soxhlet extractor and vaporize the diethyl ether (6).

h) Heat the weighing bottle (7) at 100 ºC - 105 ºC for 3 hours.

i) As soon as heating is complete, move the weighing bottle to the desiccator and stand to cool.

j) After standing to cool, remove the weighing bottle from the desiccator and measure the mass to the order of 1mg.

k) Calculate oil content with the following formula.

$$\text{Oil content (\% (mass fraction))} = (B/A) \times 100$$

A : Mass (g) of the sampled analytical sample

B : Mass of extract of diethyl ether (g)

Note **(1)** Select a scale according to the volume of a Soxhlet extractor.

(2) The purpose is to prevent overflow at the upper end of an analytical sample.

(**3**) The amount of diethyl ether depends on the volume of a weighing bottle.
(**4**) Adjust the temperature for diethyl ether to circulate 16 - 20 times per hour. (Target temperature is about 60 ºC)
(**5**) Remove the cylindrical filter paper from the Soxhlet extractor. In the case of a cock attached Soxhlet extractor, open the cock and recover it.
(**6**) It is dangerous if diethyl ether resides in a weighing bottle when the bottle is put in a drying apparatus.
(**7**) Wipe the outside of a weighing bottle since there is a risk of garbage or stain sticking to it.

References

1) Japan Oil Chemist ‘Society: Standard Method for the Analysis of Fats, Oils and Related Materials (2003), 1.5 Oil content p. 1 - 2, Incorporated Foundation Japan Oil Chemist ‘Society, Tokyo (2009)
2) Feed Analysis Standard Task Force: Feed Analysis Method/Handbook -2009 - I, p. 37 - 39, Incorporated Administrative Agency, Food and Agriculture Materials Inspection Center, Saitama (2009)

(**5**) **Flow sheet for oil content**: The flow sheet for oil content in organic fertilizers is shown below:

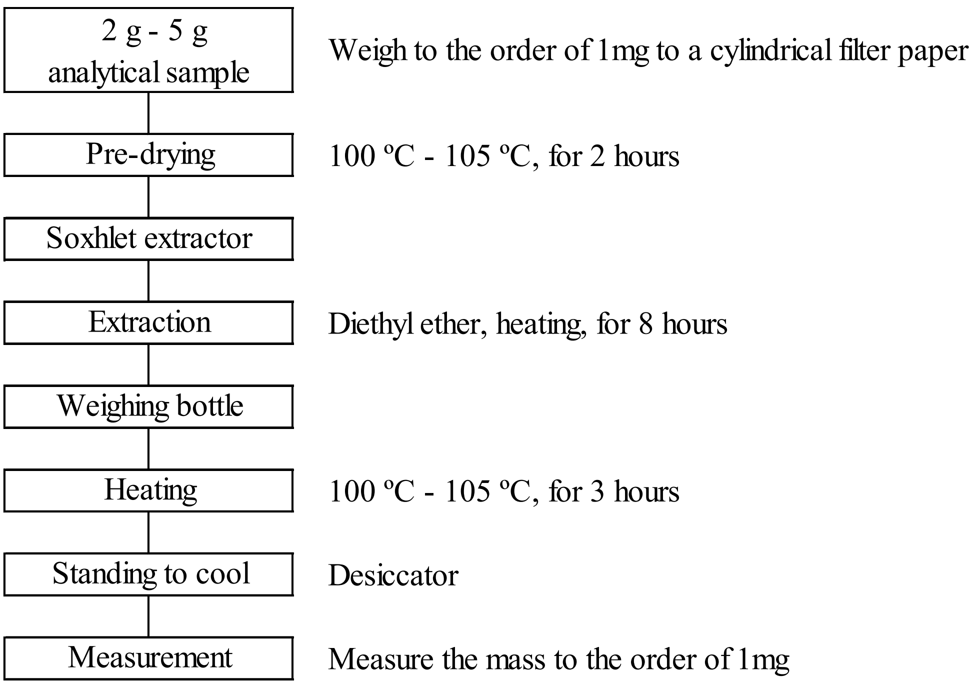


Figure Flow sheet for oil content in organic fertilizers.

4. Main components, guaranteed components, etc.

4.1 Nitrogen

4.1.1 Total nitrogen

4.1.1.a Kjeldahl method

(1) Summary

This testing method is applicable to fertilizers containing no nitrate nitrogen. This testing method is classified as Type C and its symbol is 4.1.1.a-2017 or T-N.a-1.

Add sulfuric acid, potassium sulfate and copper (II) sulfate pentahydrate to an analytical sample, pretreat by Kjeldahl method to change total nitrogen (T-N) to ammonium ion, and add a sodium hydroxide solution to subject to steam distillation. Collect isolated ammonia with 0.25 mol/L sulfuric acid and measure surplus sulfuric acid by (neutralization) titration using a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain the total nitrogen (T-N) in an analytical sample. Or collect isolated ammonia with a boric acid solution and measure ammonium ion by (neutralization) titration using 0.25 mol/L sulfuric acid to obtain the total nitrogen (T-N) in an analytical sample. This testing method corresponds to the sulfuric acid method in the Official Methods of Analysis of Fertilizers (1992). In addition, the performance of this testing method is shown in **Comment 8**.

(2) Reagent: Reagents are as shown below.

a) 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

- W_1 : Mass (g) of sulfamic acid sampled
- A : Purity (% (mass fraction)) of sulfamic acid
- V_1 : Aliquot volume (mL) of sulfamic acid solution
- V_2 : Constant volume (250 mL) of sulfamic acid solution
- V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
- C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.25 mol/L sulfuric acid** (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Put a predetermined amount (3) of 0.25 mol/L sulfuric acid in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L -0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green [4]. Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula [2]:

Volume (B) of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$= V_4/V_5$ ……… (1)

Factor of 0.25 mol/L sulfuric acid (f_2)

$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ ……… (2)

- V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
- V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
- C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
- C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.

e) **Catalyst** [5]: Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate [6] specified in JIS K 8983 in the ratio of 9 to 1.

f) **Sodium hydroxide solution (200 g/L - 500 g/L)** [1]: Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.

g) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.

h) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

i) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.

j) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).

k) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.

l) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).

(**3**) 5 mL -10 mL

(**4**) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

(**5**) A tablet is commercially available.

(**6**) Crush into powder as appropriate.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

Comment 2 0.25 mol/L sulfuric acid in (**2**) **c**) can be replaced with 0.25 mol/L sulfuric acid conforming to ISO/IEC 17025.

(3) **Apparatus and instruments**: Apparatus and instruments are shown below.
a) **Steam distillation apparatus**
b) **Digestion flask**: Kjeldahl flask
c) **Distillation flask**: A Kjeldahl flask or round bottom flask that can be connected to a steam distillation apparatus.

(4) **Test procedures**
(4.1) **Kjeldahl digestion**: Conduct digestion as shown below.
a) Weigh 0.5 g - 5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL digestion flask.
b) Add 5 g - 10 g of catalyst, and further add 20 mL - 40 mL of sulfuric acid, shake to mix (7) and heat gently.
c) After bubbles cease to form, heat until white smoke of sulfuric acid evolves.
d) Ignite until organic matters are completely digested (8).
e) After standing to cool, add a small amount of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water (9), and further mix by shaking.
f) After cooling is complete, add water up to the marked line to make a digestion solution.

Note (7) Leaving at rest overnight is preferable.
(8) When the solution has finished changing color, heat further for no less than 2 hours.
(9) When the entire sample solution volume is used in measurement, it is not necessary to transfer it to a volumetric flask.

Comment 3 A digestion solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.
Comment 4 In the case of fish meal containing amino acids that are not easily digested, use 0.5 g - 1 g analytical sample, 10 g catalyst and 30 mL - 40 mL sulfuric acid.
Comment 5 In the case of nitrolime, moisten by adding a small amount of water before the procedure in (**4.1**) **b**). Care should be taken because bubbles are produced by the addition of sulfuric acid.

(4.2) **Distillation**: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.
a) Put a predetermined amount (10) of 0.25 mol/L sulfuric acid in an acceptor (11), add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (10) of boric acid solution (40 g/L) in an acceptor (11), add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.
b) Put a predetermined amount of the digestion solution in a 300-mL distillation flask, add a proper amount of sodium hydroxide solution (200 g/L - 500 g/L) (12), and immediately connect this distillation flask to the steam distillation apparatus.
c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.
d) Stop distilling when the distillate has reached 120 mL - 160 mL.
e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (10) 5 mL - 20 mL

(**11**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).
(**12**) An amount sufficient to make the solution strong alkalinity. A blue color will appear.

(**4.3**) **Measurement**: Conduct measurement as shown below.
(**4.3.1**) When 0.25 mol/L sulfuric acid is used in (**4.2**):
a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).
b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (14.007/W_3) \times (100/1000)$
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (1.4007/W_3)$

B : Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
V_6 : Volume (mL) of 0.25 mol/L sulfuric acid put in the acceptor in (**4.2**) **a**)
V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_8 : Constant volume (mL) of the digestion solution in (**4.1**) **f**)
V_9 : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.2**) **b**)
W_3 : Mass (g) of the analytical sample

(**4.3.2**) When a boric acid solution (40 g/L) is used in (**4.2**):
a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (13).
b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_2) \times (100/1000)$
$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_2)$

V_{10} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid
f_2 : Factor of 0.25 mol/L sulfuric acid
V_{11} : Constant volume (mL) of the digestion solution in (**4.1**) **f**)
V_{12} : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.2**) **b**)
W_2 : Mass (g) of the analytical sample

Note (**13**) The endpoint is reached when the color changes from green to light red.

Comment 6 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **c**) **Standardization** and (**4.3**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 7 The nitrogen content in the analytical sample can be measured by using an automatic nitrogen analyzer (Kjeldahl digestion method) instead of the test procedure in (**4**). The setup of the program and the parameter of the analyzer as well as vessels etc. are according to the specification and the operation method of the automatic nitrogen analyzer used. However, conduct in advance a comparative test with the test procedure in (**4**) using fertilizers containing no nitrate nitrogen, to confirm that there is no difference in the quantitation value of total nitrogen.

Comment 8 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 10 % (mass fraction) - 20 % (mass fraction) and 1 % (mass fraction) - 5 % (mass fraction) were 98.5 % - 100.6 % and 97.1%-99.2 % as total nitrogen (T-N) respectively.

The results of the collaborative study (limited to reported values with the Kjeldahl method) to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 1 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.2 % (mass fraction) for solid fertilizers and about 0.02 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the collaborative study to determine the total nitrogen of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1]	Average[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)	s_R[8] (%)[3]	RSD_R[9] (%)
FAMIC-A-10	11	14.68	0.07	0.5	0.07	0.5	0.13	0.9

1) The number of laboaratories used for analysis conducting Kjeldahl method
2) Mean (the number of laboratory(p) × test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p. 27 - 31, Yokendo, Tokyo (1988)

2) Feed Analysis Standard Task Force: Feed Analysis Method/Handbook -2009 - I, p. 28 - 33, Incorporated Administrative Agency, Food and Agriculture Materials Inspection Center, Saitama (2009)

3) Takashi KUBOTA, Tomoko OSHIDA, Kozue YANAI, Yuzuru INOUE, Seiji MATSUI, Takaharu MATSUMOTO, Eiichi ISHIKURO and Akemi YASUI: Improvement of the Conditions for the Determination of Total Nitrogen in Fish Meal in Kjeldahl Method and Its Comparison with Dumas Method, Bunsekikagaku, **60**, p. 67 - 74 (2011)

4) Kimie KATO, Masaki CHIDA and Erina WATANABE: Verification of Performance Characteristics of Testing Method for Total Nitrogen Content in Fertilizer by Kjeldahl method, Research Report of Fertilizer, **Vol. 5**, p. 156 - 166 (2012)

(5) **Flow sheet for total nitrogen**: The flow sheet for total nitrogen in fertilizers is shown below:

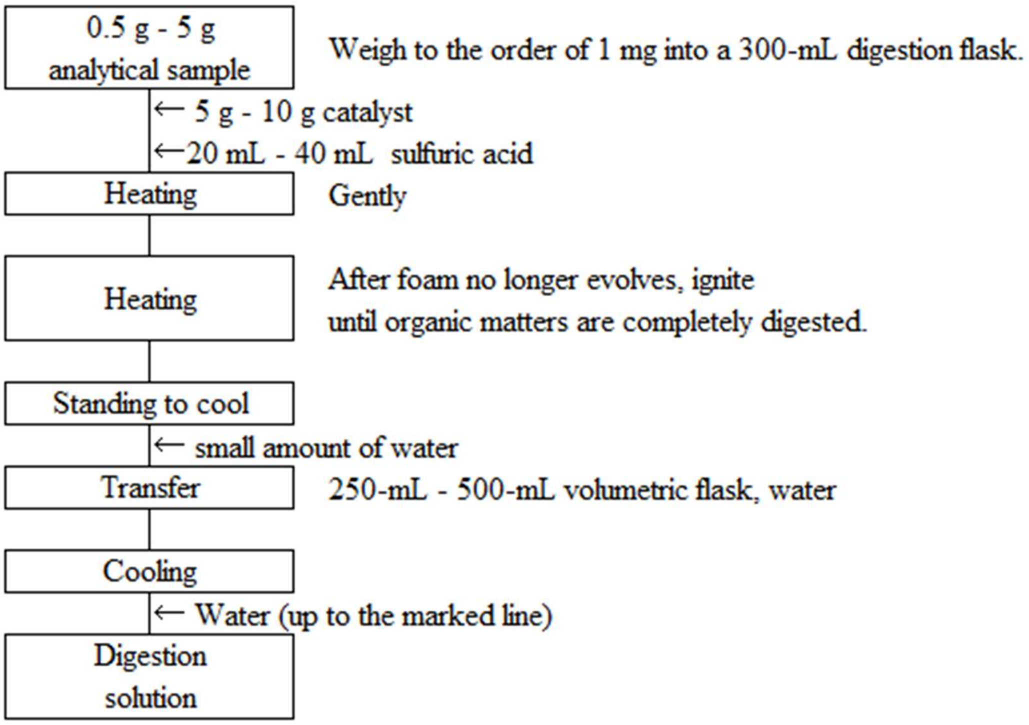


Figure 1 The flow sheet for total nitrogen in fertilizers (Kjeldahl digestion procedure)

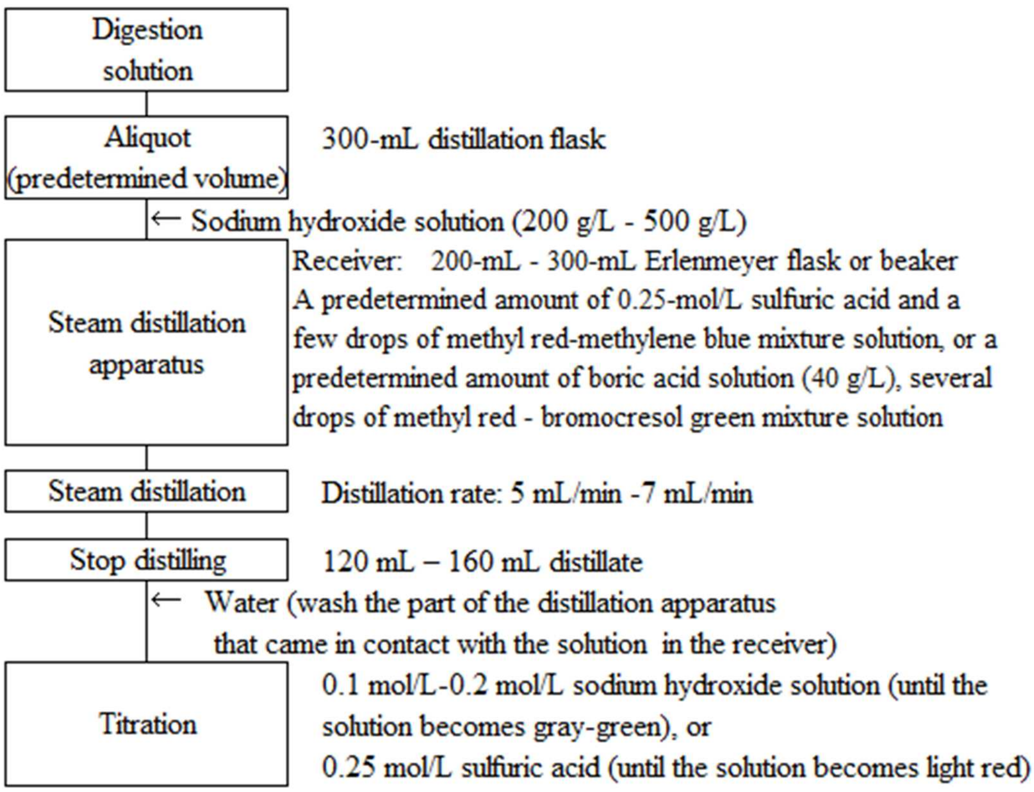


Figure 2 Flow sheet for total nitrogen in fertilizers (Distillation and mesurement procedure)

4.1.1.b Combustion method

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 4.1.1.b-2017 or T-N.b-1.

Thermally decompose nitrogen compounds in an analytical sample using a total nitrogen analyzer by the combustion method to produce nitrogen gas and nitroxide gas. Reduce the nitroxide gas to nitrogen, and measure the nitrogen gas content with a thermal conductivity detector to obtain the total nitrogen (T-N) in an analytical sample. This testing method is also referred to as a modified Dumas' method. In addition, the performance of this testing method is shown in **Comment 4**.

(**2**) **Instruments**: Instruments are as shown below:

a) **Total nitrogen analyzer by the combustion method**: A total nitrogen analyzer configured on the basis of the principle of the combustion method (modified Dumas' method).

1) Turn on the total nitrogen analyzer by the combustion method (1), and adjust so that stable indicated values can be obtained.

(i) Combustion gas: Oxygen having purity no less than 99.99 % (volume percentage)

(ii) Carrier gas: Helium having purity no less than 99.99 % (volume percentage)

(**3**) **Measurement:** Conduct measurement as shown below. However, confirm in advance using an analytical sample that there is no difference from the measured value of total nitrogen obtained according to **4.1.1.a**, **4.1.1.c**, **4.1.1.d** or **4.1.1.e**.

a) **Measurement conditions for the total nitrogen analyzer by the combustion method**: Set up the measurement conditions for the total nitrogen analyzer considering the following:
Combustion temperature: No less than 870 ºC

b) **Calibration curve preparation**

1) Turn on the total nitrogen analyzer by the combustion method (1), and adjust so that stable indicated values can be obtained.

2) Weigh a predetermined amount of the standard for calibration curves (2) to the order of 0.1 mg into a combustion vessel.

3) Insert the combustion vessel into the total nitrogen analyzer by the combustion method, and read the indicated value.

4) Conduct the procedure in **3**) for another combustion vessel for a blank test, and read the indicated value.

5) Prepare a curve for the relationship between the nitrogen content and the indicated value of the standard for calibration curves and the blank test for calibration curves.

c) **Sample measurement**

1) Weigh a predetermined amount of an analytical sample (3) to the order of 0.1 mg into a combustion vessel.

2) Insert the combustion vessel containing the analytical sample to the total nitrogen analyzer by the combustion method, and read the indicated value.

3) Obtain the nitrogen content from the calibration curve, and calculate total nitrogen in the analytical sample.

Note (**1**) The setup of the program and the parameter of the analyzer are according to the specification and the operation method of the total nitrogen analyzer by the combustion method used.

(**2**) Standard for calibration curves: DL-Aspartic acid (purity no less than 99 % (mass fraction)), EDTA (purity no less than 99 % (mass fraction)), hippuric acid (purity no less than 98 % (mass fraction)) or other reagents having equivalent purity recommended by the total nitrogen analyzer by the combustion method used.

(**3**) The sampling amount of an analytical sample is as shown in Table 1. In addition, set the sampling amount of an analytical sample upon consideration of the estimated content of total nitrogen in the test sample and the measurement range of the total nitrogen analyzer by the combustion method.

Comment 1 Sample an analytical sample from a test sample prepared in **2.3.3 Grinding** (**3.1**) by grinding with a mill until it completely passes through a sieve of 500 µm aperture or from a test sample prepared in **2.3.3 Grinding Comment 1**.

Table 1 Sampling amount of analytical samples

Type of fertilizers	Sampling amount (g)
Compound fertilizers and designated blended fertilizer	0.02 - 0.5
Organic fertilizers and compost	0.05 - 0.5
Sludge fertilizer	0.05 - 0.5

Comment 2 Compound fertilizers, designated blended fertilizers and nitrolime may have high contents of phosphoric acid (P_2O_5), alkali metals (Na, K), alkaline earth metals (Ca, Mg), etc., causing contamination of packing or damage in quartz parts, etc. To avoid their influences, it is recommended to add tungsten oxide (elemental analysis reagent or heat-treated reagent) to completely cover the analytical sample.

Comment 3 When a sample with a low content of organic compounds, such as compound fertilizers and designated blended fertilizers etc., and thus with low combustion efficiency is measured, it is recommended to add sucrose to the analytical sample so that the carbon content will be comparable to the standard for calibration curves. Additionally, confirm in advance that sucrose to be used has a nitrogen content that does not affect the measured value of total nitrogen of the analytical sample.

Comment 4 Table 2, in order to evaluate trueness, shows the results of the comparison of measurement values by the Combustion method and the Kjeldahl method with sludge fertilizers, organic fertilizers, and inorganic fertilizers, etc.

Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 3.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 % (mass fraction) for fluid fertilizers for home gardening and about 0.05 % (mass fraction) for the other fertilizers.

Table 2 Analysis results of comparison test results between methods

Symbol of measurement value		Sample		Range of	Regression coefficient		Correlation
Kjaeldahl method[1)]	Combustion method[2)]	Kind	Number of samples	y_i - y_k (%)[3)]	($y=a+bx$) a	b	coefficient r
x_i	y_i	Sludge fertilizers[4)]	81	0.31～8.35	-0.006	1.018	0.999
x_j	y_j	Organic fertilizers, etc.[5)]	31	1.10～12.90	0.009	1.012	1.000
x_k	y_k	Inorganic fertilizers, etc.[6)]	36	0.60～46.35	0.000	1.004	1.000

1) 4.1.1.a Kjeldahl method
2) 4.1.1.b Combustion method
3) Mass fraction
4) Sewage sludge fertilizers, Human waste sludge fertilizers, Industrial sludge fertilizers, Calcined sludge fertilizers, Composted sludge fertilizers
5) Fish meal, Byproduct organic fertilizer of vegetable origin, Compost, Crustose fertilizer meal, Rape seed meal and powdered rape seed meal, etc.
6) Nitrogenous fertilizers, Compound fertilizers, Blended fertilizers, Fluid fertilizers

Table 3 Aanalysis results of the collaborative study for the total nitrogen testing method validation

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Compound fertilizer (containing nitrate nitrogen)	11	9.32	0.07	0.8	0.25	2.7
Compound fertilizer (containing urea)	11	18.34	0.06	0.3	0.45	2.5
Designated blended fertilizer (containing organic fertilizer)	12	14.06	0.12	0.9	0.42	3.0
Nitrolime	8	19.96	0.07	0.4	0.17	0.8
Fish meal	10	8.34	0.04	0.4	0.10	1.3
Steamed wool waste	11	13.42	0.10	0.7	0.26	2.0
Rape seed meal and powdered rape seed meal	11	6.21	0.07	1.1	0.25	4.0
Composted sludge fertilizer A	13	6.20	0.02	0.3	0.09	1.4
Composted sludge fertilizer B	12	2.36	0.01	0.6	0.04	1.8
Human waste sludge fertilizer	11	4.44	0.02	0.4	0.06	1.3
Industrial sludge fertilizer	11	8.06	0.03	0.4	0.07	0.9
Calcined sludge fertilizer	13	0.80	0.02	2.8	0.03	4.3

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Mariko AIZAWA, Yasushi SUGIMURA, Yuichi TAKAHASHI, Jun OKI, Yukio FUKUCHI, Yuji SHIRAI and Norio HIKICHI: Validation of a Combustion Method for Determination of Total Nitrogen Content in Sludge Fertilizer. Research Report of Fertilizer **Vol. 1**, p. 12 - 17, (2008)

2) Mariko AIZAWA and Yuji SHIRAI: Determination of Total Nitrogen content in Sludge

Fertilizer by a Combustion Method: A Collaborative Study. Research Report of Fertilizer **Vol. 1**, p. 18 - 24, (2008)

3) Mariko AIZAWA and Yuji SHIRAI: Validation of a Combustion Method for Determination of Total Nitrogen Content in Organic Fertilizer. Research Report of Fertilizer **Vol. 2**, p. 6 - 11, (2009)

4) Mariko AIZAWA and Yuji SHIRAI: Validation of a Combustion Method for Determination of Total Nitrogen Content in Inorganic Fertilizer. Research Report of Fertilizer **Vol. 3**, p. 1 - 10, (2010)

5) Mariko AIZAWA, Yuko SEKINE and Yuji SHIRAI: Determination of Total Nitrogen Content in Fertilizer by a Combustion Method: A Collaborative Study. Research Report of Fertilizer **Vol. 3**, p. 11 - 18, (2010)

6) Kazumi UCHIYAMA and Yoshio MAEBASHI: Effective Analysis of Organic Trace Element, p. 99, Mimizuku-sha, Tokyo (2008)

(**4**) **Flow sheet for total nitrogen**: The flow sheet for total nitrogen in fertilizers is shown below:

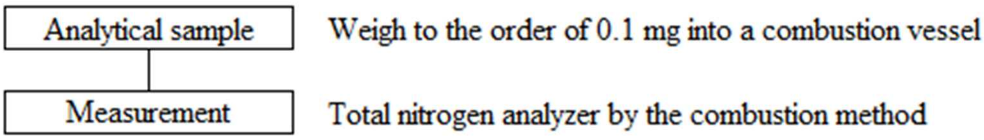
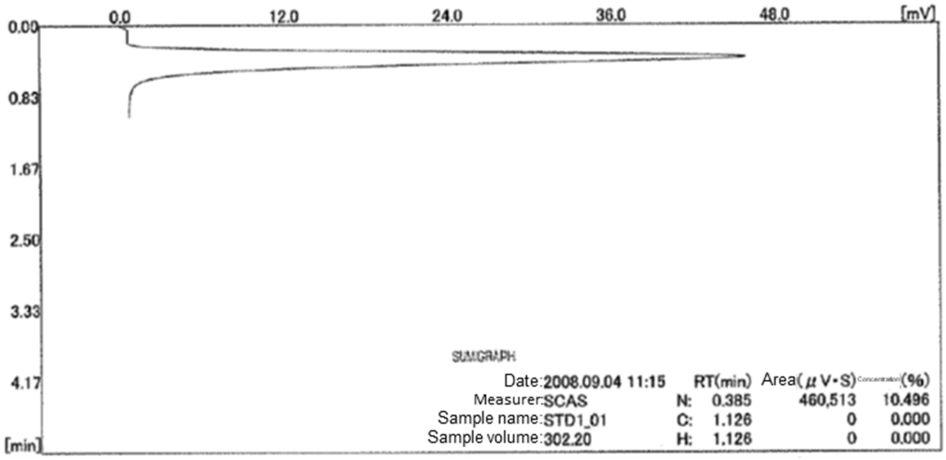
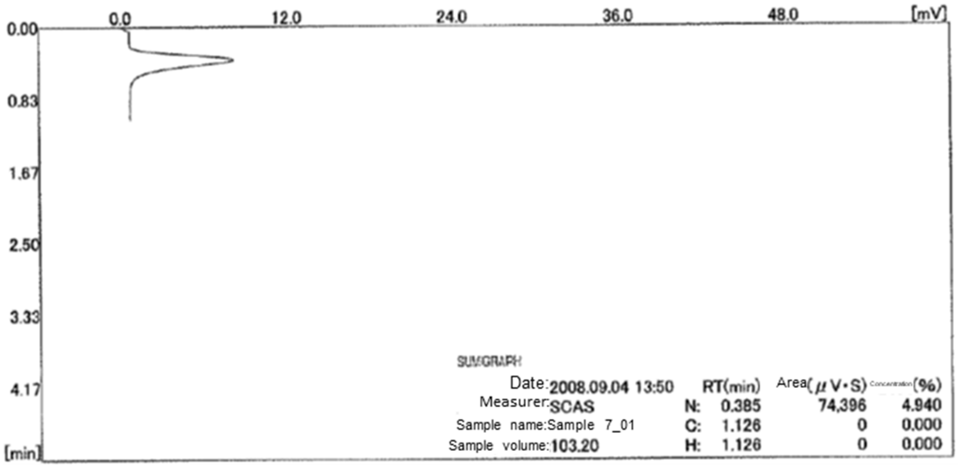


Figure Flow sheet for total nitrogen in fertilizers by the combustion method.

Reference: Chromatograms of the standard for calibration curves and an analytical sample are shown below:



1) Standard for calibration curves (DL-aspartic acid)



2) Analytical sample (sludge fertilizer)

Reference figures: Chromatograms of total nitrogen.

Measurement conditions for total nitrogen analyzer by the combustion method

Combustion gas: Highly pure oxygen, purity no less than 99.9999 % (volume fraction), flow rate 200 mL/min

Carrier gas: Highly pure helium, purity no less than 99.9999 % (volume fraction), flow rate 80 mL/min

Separation column: A silica gel stainless column (1m)

Detector: Thermal conductivity detector (TCD)

Measurement cycle: Purge time = 60 seconds, circulation combustion time = 200 seconds, measurement time = 100 seconds

Current value of Detector: 160 mA

Temperature conditions: Reaction furnace temperature: 870 ºC
Reaction furnace temperature: 600 °C
Column oven temperature: 70 °C
Detector temperature: 100 °C

4.1.1.c Devarda's alloy – Kjeldahl method

(1) Summary

The method is applicable to the fertilizers that contain nitrate nitrogen (N-N) and guarantee total nitrogen. This testing method is classified as Type E and its symbol is 4.1.1.c-2017 or T-N.c-1.

Add hydrochloric acid (1+1) and tin (II) chloride dihydrate to an analytical sample and further add devarda's alloy to reduce nitrate nitrogen (N-N), and then add sulfuric acid (1+1), pretreat by Kjeldahl method to change total nitrogen (T-N) to ammonium ion and add sodium hydroxide to subject to steam distillation. Collect isolated ammonia with 0.25 mol/L sulfuric acid and measure surplus sulfuric acid by (neutralization) titration using a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain the total nitrogen (T-N) in an analytical sample. Or collect isolated ammonia with a boric acid solution and measure ammonium ion by (neutralization) titration using 0.25 mol/L sulfuric acid to obtain the total nitrogen (T-N) in an analytical sample. This testing method corresponds to the devarda's alloy - sulfuric acid method in the Official Methods of Analysis of Fertilizers (1992).

(2) Reagent: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

W_1 : Mass (g) of sulfamic acid sampled
A : Purity (% (mass fraction)) of sulfamic acid
V_1 : Aliquot volume (mL) of sulfamic acid solution
V_2 : Constant volume (250 mL) of sulfamic acid solution
V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.25 mol/L sulfuric acid** (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Put a predetermined amount (3) of 0.25 mol/L sulfuric acid in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4). Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$= V_4/V_5$ ……… (1)

Factor of 0.25 mol/L sulfuric acid (f_2)

$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ ……… (2)

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.
e) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
f) **Tin (II) chloride dihydrate**: A JIS Guaranteed Reagent specified in JIS K 8136 or a reagent of mercury analysis grade or equivalent quality.
g) **Devarda's alloy**: A reagent of nitrogen analysis grade specified in JIS K 8653 or a reagent of equivalent quality.
h) **Sodium hydroxide solution (200 g/L - 500 g/L)** (1): Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
i) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.
j) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
k) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
l) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
m) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
n) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(**3**) 5 mL -10 mL
(**4**) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

(3) **Apparatus and instruments**: Apparatus and instruments are shown below.
a) **Steam distillation apparatus**
b) **Digestion flask**: Kjeldahl flask
c) **Distillation flask**: A Kjeldahl flask or round bottom flask that can be connected to a steam distillation apparatus.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

Comment 2 0.25 mol/L sulfuric acid in (**2**) **c**) can be replaced with 0.25 mol/L sulfuric acid conforming to ISO/IEC 17025.

(**4**) **Test procedures**

(**4.1**) **Reduction and Kjeldahl digestion**: Conduct reduction and digestion as shown below:

a) Weigh 0.5 g - 1 g (no more than the equivalents of N-N 50 mg) of an analytical sample to the order of 1 mg, and put it in a 300-mL- 500-mL digestion flask (5)

b) Add 60 mL of hydrochloric acid (1+1) and 2 g of tin (II) chloride dihydrate, and shake to mix and leave at rest for about 20 minutes.

c) Add 3.5 g of devarda's alloy and leave at rest for about 40 minutes while sometimes shaking to mix.

d) Add 70 mL of sulfuric acid (1+1) and one boiling stone as necessary, and heat at low temperature (6).

e) As soon as white smoke start evolving, strengthen heating gradually and further continue heating for about 90 minutes.

f) After standing to cool, add 100 mL - 200 mL of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water, and further mix by shaking (7).

g) After cooling is complete, add water up to the marked line to make a digestion solution.

Note (**5**) In the case of direct distillation, a 500-mL Kjeldahl flask connectable to a steam distillation apparatus is preferable.

(**6**) If the bubbles foam strongly and excessively, suspend heating for a little while.

(**7**) When the entire sample solution volume is used in measurement, it is not necessary to precisely adjust.

(**4.2**) **Distillation**: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Put a predetermined amount (8) of 0.25 mol/L sulfuric acid in an acceptor, (9) add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (8) of boric acid solution (40 g/L) in an acceptor (9), add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Put a predetermined amount of the digestion solution in a 300-mL distillation flask, add a proper amount of sodium hydroxide solution (200 g/L - 500 g/L) (10), and connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (**8**) 5 mL - 20 mL

(**9**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).

(**10**) An amount sufficient to make the solution strong alkalinity. A blue color will appear.

(**4.3**) **Measurement**: Conduct measurement as shown below.
(**4.3.1**) When 0.25 mol/L sulfuric acid is used in (**4.2**):
a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).
b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (14.007/W_2) \times (100/1000)$
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (1.4007/W_2)$

B : Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
V_6 : Volume (mL) of 0.25 mol/L sulfuric acid put in the acceptor in (**4.2**) **a**)
V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_8 : Constant volume (mL) of the digestion solution in (**4.1**) **g**)
V_9 : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.2**) **b**)
W_2 : Mass (g) of the analytical sample

(**4.3.2**) When a boric acid solution (40 g/L) is used in (**4.2**):
a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (11).
b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample
$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_3) \times (100/1000)$
$= V_{10} \times C_2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_3)$

V_{10} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid
f_2 : Factor of 0.25 mol/L sulfuric acid
V_{11} : Constant volume (mL) of the digestion solution in (**4.1**) **g**)
V_{12} : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.2**) **b**)
W_3 : Mass (g) of the analytical sample

Note (**11**) The endpoint is reached when the color changes from green to light red.

Comment 3 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **c**) **Standardization** and (**4.3**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.31- 33, Yokendo, Tokyo (1988)

(**5**) **Flow sheet for total nitrogen**: The flow sheet for total nitrogen in fertilizers is shown below:

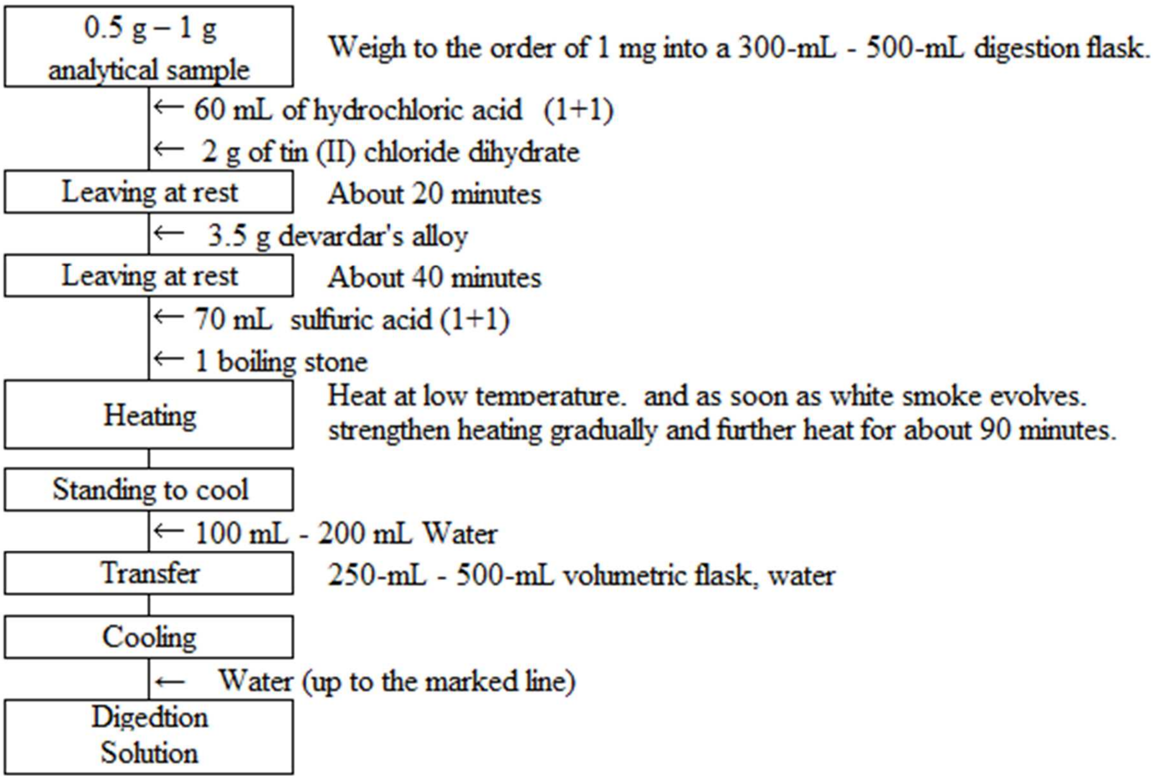


Figure 1 The flow sheet for total nitrogen in fertilizers (Reduction and Kjeldahl digestion procedure)

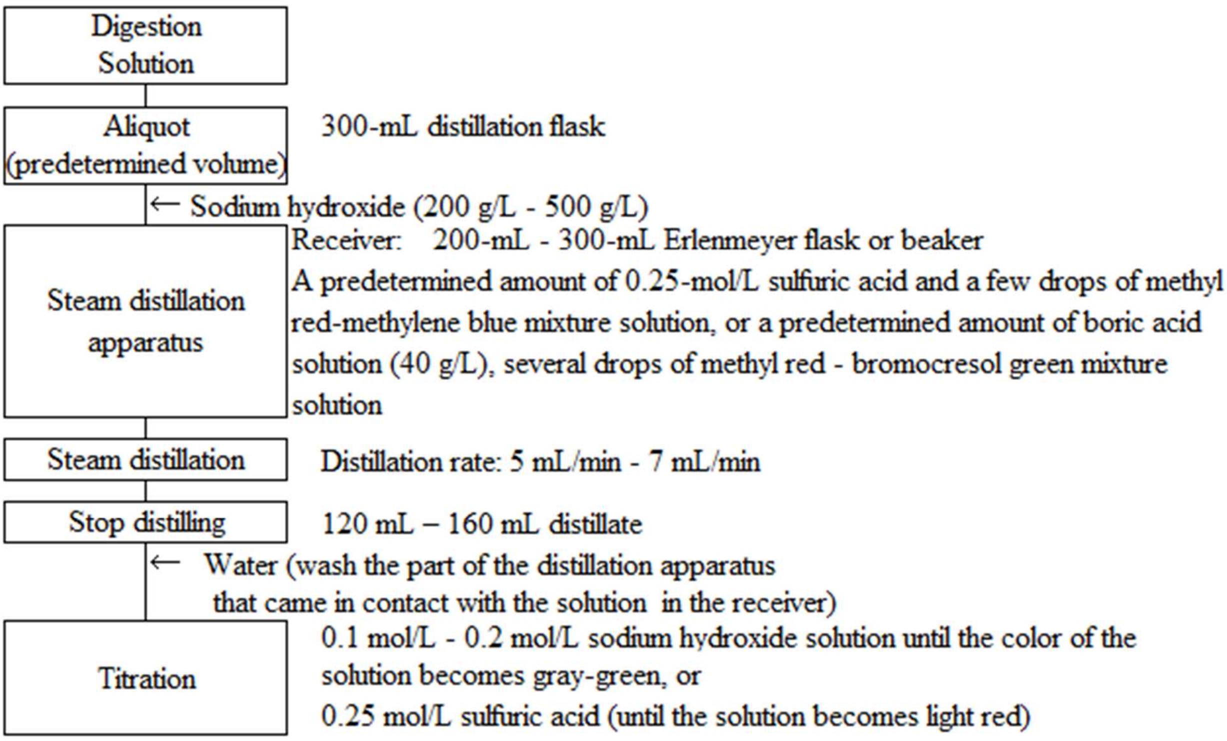


Figure 2 Flow sheet for total nitrogen in fertilizers (Distillation and measurement procedure).

4.1.1.d Reduced iron - Kjeldahl method

(1) Summary

The method is applicable to the fertilizers that contain nitrate nitrogen (N-N) and guarantee total nitrogen. This testing method is classified as Type E and its symbol is 4.1.1.d-2017 or T-N.d-1.

Add water, reduced iron and sulfuric acid (1+1) to an analytical sample to reduce nitrate nitrogen (N-N) and heat at low temperature, and then add sulfuric acid and pretreat by Kjeldahl method to change total nitrogen (T-N) to ammonium ion, and add a sodium hydroxide solution to subject to steam distillation. Collect isolated ammonia with 0.25 mol/L sulfuric acid and measure surplus sulfuric acid by (neutralization) titration using a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain the total nitrogen (T-N) in an analytical sample. Or collect isolated ammonia with a boric acid solution and measure ammonium ion by (neutralization) titration using 0.25 mol/L sulfuric acid to obtain the total nitrogen (T-N) in an analytical sample. This testing method corresponds to the reduced iron - sulfuric acid method in the Official Methods of Analysis of Fertilizers (1992).

(2) Reagent: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Transfer 5.5 mL -11 mL of the supernatant to a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

W_1 : Mass (g) of sulfamic acid sampled
A : Purity (% (mass fraction)) of sulfamic acid
V_1 : Aliquot volume (mL) of sulfamic acid solution
V_2 : Constant volume (250 mL) of sulfamic acid solution
V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.25 mol/L sulfuric acid** (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Put a predetermined amount (3) of 0.25 mol/L sulfuric acid in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L -0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4). Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$= V_4/V_5$ ………. (1)

Factor of 0.25 mol/L sulfuric acid (f_2)

$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ ………. (2)

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.

e) **Reduced iron**: Nitrogen content is no more than 0.005 % (mass fraction)

f) **Catalyst** (5): Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate (6) specified in JIS K 8983 in the ratio of 9 to 1.

g) **Sodium hydroxide solution (200 g/L - 500 g/L)** (1): Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.

h) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.

i) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

j) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.

k) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).

l) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.

m) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).

(**3**) 5 mL -10 mL

(**4**) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

(**5**) A tablet is commercially available.

(**6**) Crush into powder as appropriate.

(3) **Instruments**: Instruments are as shown below:

a) **Steam distillation apparatus**

b) **Digestion flask**: Kjeldahl flask

c) **Distillation flask**: A Kjeldahl flask or round bottom flask that can be connected to a steam distillation apparatus.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

Comment 2 0.25 mol/L sulfuric acid in (**2**) **c**) can be replaced with 0.25 mol/L sulfuric acid conforming to ISO/IEC 17025.

(4) Test procedures

(**4.1**) **Reduction and Kjeldahl digestion**: Conduct reduction and digestion as shown below:

a) Weigh 0.5 g - 1 g of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL digestion flask.

b) Add 30 mL of water and mix well.

c) As soon as 5 g of reduced iron and 30mL of sulfuric acid (1+1) are added, insert a long stem funnel to a digestion flask and shake to mix gently while cooling the outside of the container under flowing water (7).

d) Leave at rest for about 5 minutes (8), boil in a low flame for about 15 minutes.

e) After standing to cool, add 5 g - 10 g of catalyst, 30 mL of sulfuric acid and, if necessary, one boiling stone, heat gradually until water evaporates and white smoke of sulfuric acid evolves (9).

f) Ignite until it is completely digested (10).

g) After standing to cool, add a small amount of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water, and further mix by shaking.

h) After cooling is complete, add water up to the marked line to make a digestion solution.

Note (**7**) A sudden reaction generates heat, and unreacted nitric acid vaporizes or digests to make nitrogen oxide etc. through which process losses occur easily. Careful and efficient operation should be taken

(**8**) Until a sudden reaction is settled.

(**9**) If the bubbles foam strongly and excessively, suspend heating for a little while.

(**10**) When the solution has finished changing color, heat further for no less than 2 hours.

(**4.2**) **Distillation:** Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Put a predetermined amount (11) of 0.25 mol/L sulfuric acid in an acceptor (12), add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (11) of boric acid solution (40 g/L) in an acceptor (12), add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Put a predetermined amount of the digestion solution in a 300-mL distillation flask, add a proper amount of sodium hydroxide solution (200 g/L - 500 g/L) (13), and connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (**11**) 5 mL - 20 mL

(**12**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).

(**13**) An amount sufficient to make the solution strong alkalinity. A blue or reddish-brown color will appear.

(**4.3**) **Measurement**: Conduct measurement as shown below.

(**4.3.1**) When 0.25 mol/L sulfuric acid is used in (**4.2**):

a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample

$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (14.007/W_2) \times (100/1000)$$

$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (1.4007/W_2)$$

B : Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

V_6 : Volume (mL) of 0.25 mol/L sulfuric acid put in the acceptor in (**4.2**) **a**)

V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

V_8 : Constant volume (mL) of the digestion solution in (**4.1**) **h**)

V_9 : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.2**) **b**)

W_2 : Mass (g) of the analytical sample

(**4.3.2**) When a boric acid solution (40 g/L) is used in (**4.2**):

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (14).

b) Calculate the total nitrogen (T-N) in the analytical sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the analytical sample

$$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_3) \times (100/1000)$$

$$= V_{10} \times C_2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_3)$$

V_{10} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration

C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

f_2 : Factor of 0.25 mol/L sulfuric acid

V_{11} : Constant volume (mL) of the digestion solution in (**4.1**) **h**)

V_{12} : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.2**) **b**)

W_3 : Mass (g) of the analytical sample

Note (**14**) The endpoint is reached when the color changes from green to light red.

Comment 3 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **c**) S**tandardization** and (**4.3**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.33- 34, Yokendo, Tokyo (1988)

(5) **Flow sheet for total nitrogen**: The flow sheet for total nitrogen in fertilizers is shown below:

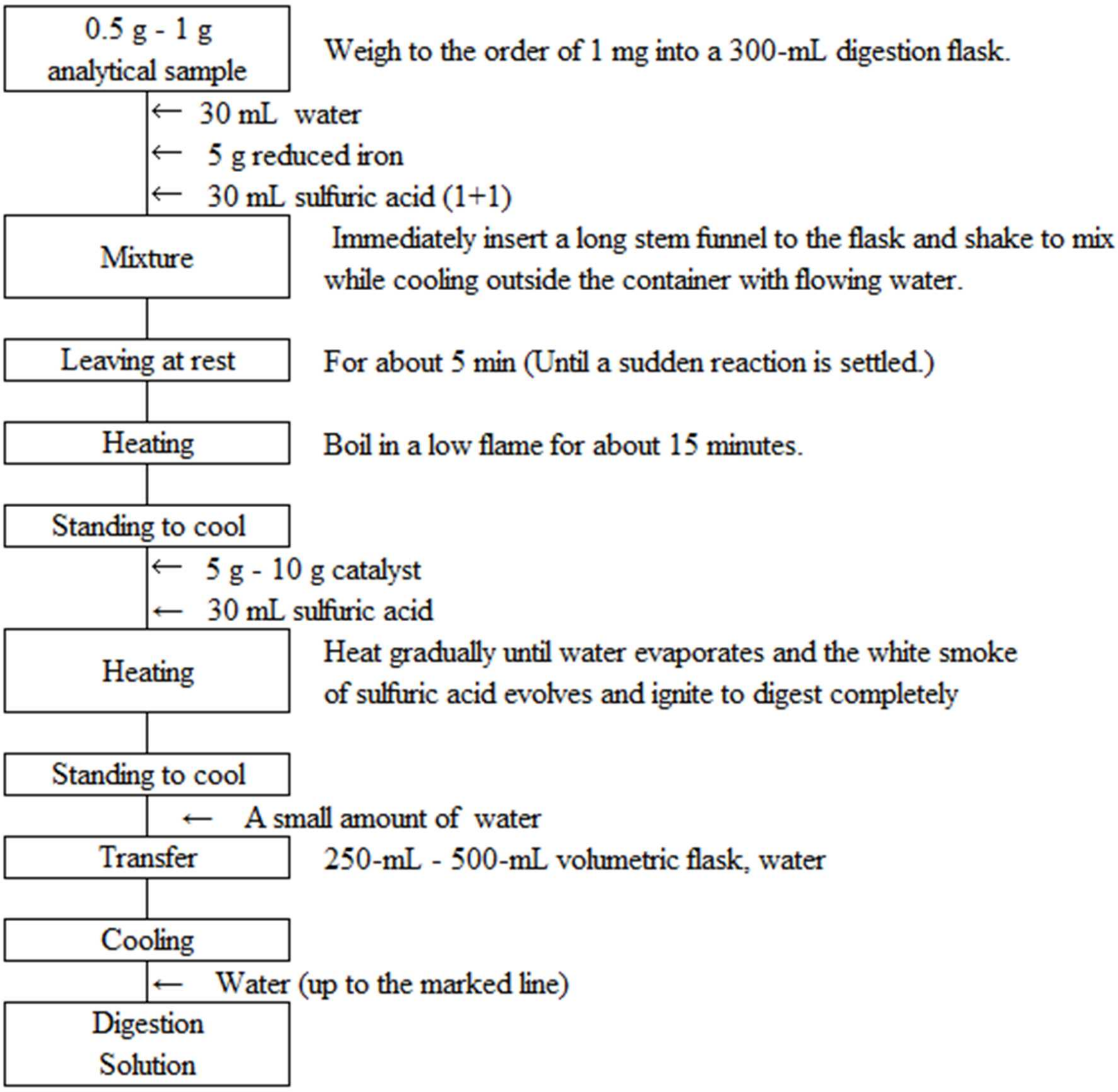


Figure 1 The flow sheet for total nitrogen in fertilizers
(Reduction and Kjeldahl digestion procedure)

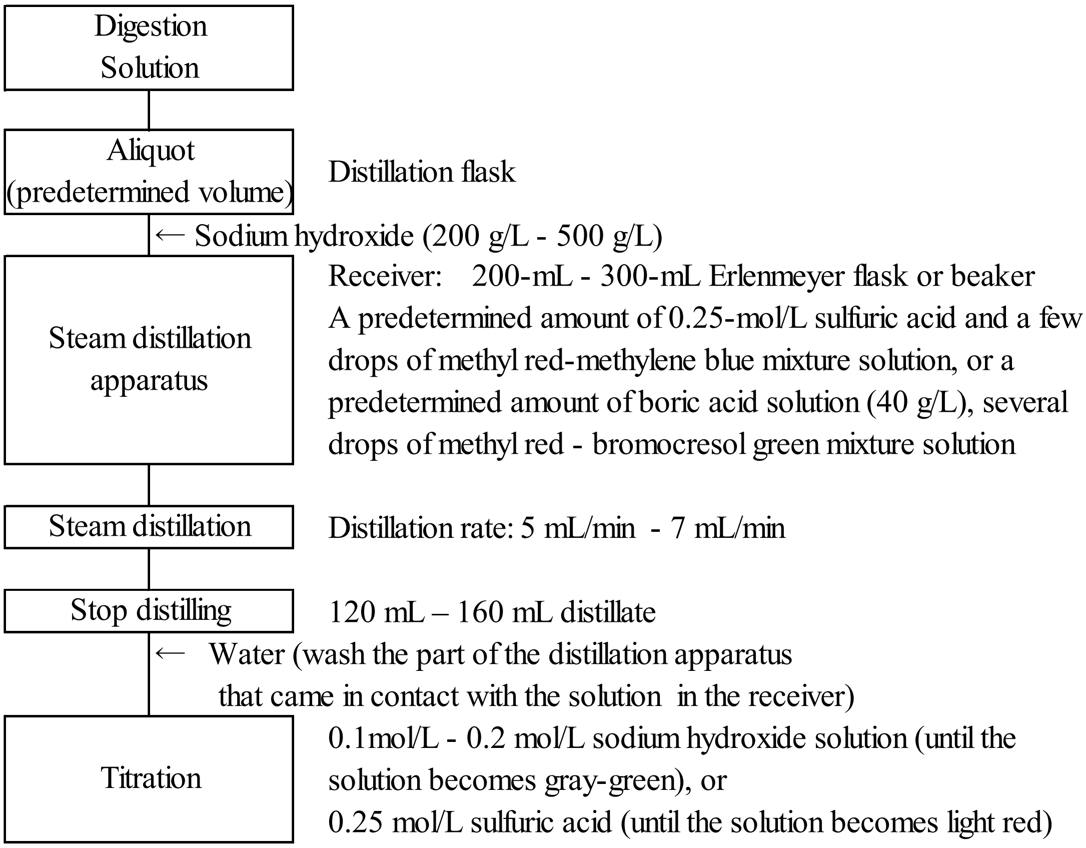


Figure 2 Flow sheet for total nitrogen in fertilizers (Distillation and measurement procedure).

4.1.1.e Calculation with ammoniacal nitrogen and nitrate nitrogen

(1) Summary

This testing method is applicable to the fertilizers that contain ammoniacal nitrogen (A-N) and nitrate nitrogen (N-N), and that does not contain the fertilizers guaranteeing total nitrogen (T-N). This testing method is classified as Type A (Def-C) and its symbol is 4.1.1.e-2017 or T-N.e-1.
Calculate total nitrogen (T-N) by adding the ammoniacal nitrogen (A-N) obtained in **4.1.2** to the nitrate nitrogen (N-N) obtained in **4.1.3**.

(2) The calculation of total nitrogen

a) Calculate the total nitrogen (T-N) in the test sample by the following formula:

Total nitrogen (T-N) (% (mass fraction)) in the test sample
= (A-N) ＋ (N-N)

A-N : Ammoniacal nitrogen (% (mass fraction)) in the analytical sample (1) obtained by **4.1.2**

N-N : Nitrate nitrogen (% (mass fraction)) in the analytical sample (1) obtained by **4.1.3**

Note (**1**) A-N and N-N use raw data without rounding numerical value

4.1.2 Ammoniacal nitrogen
4.1.2.a Distillation method
(1) Summary

The testing method is applicable to the fertilizers that contain ammonium salt. However, in some cases, it is not applicable to the fertilizers that contain such compounds as nitrolime that digests by heating. This testing method is classified as Type B and its symbol is 4.1.2.a-2021 or A-N.a-2.

Add water to an analytical sample or extract an analytical sample with hydrochloric acid (1+23), further add magnesium oxide or a sodium hydroxide solution to make the solution alkalinity and subject it to steam distillation. Collect isolated ammonia with 0.25 mol/L sulfuric acid and measure surplus sulfuric acid by (neutralization) titration using a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain ammoniacal nitrogen (A-N) in an analytical sample. Or collect isolated ammonia with a boric acid solution and measure ammonium ion by (neutralization) titration using 0.25 mol/L sulfuric acid to obtain ammoniacal nitrogen (A-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7** to **Comment 10**.

(2) Reagent: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

W_1 : Mass (g) of sulfamic acid sampled
A : Purity (% (mass fraction)) of sulfamic acid
V_1 : Aliquot volume (mL) of sulfamic acid solution
V_2 : Constant volume (250 mL) of sulfamic acid solution
V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Magnesium oxide**: A JIS Guaranteed Reagent specified in JIS K 8432 or a reagent of equivalent quality.

c) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

d) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

e) **0.25 mol/L sulfuric acid** (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Put a predetermined amount [3] of 0.25 mol/L sulfuric acid in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L -0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green [4]. Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$= V_4/V_5$ ………. (1)

Factor of 0.25 mol/L sulfuric acid (f_2)

$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ ………. (2)

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

f) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.
g) **Sodium hydroxide solution (200 g/L - 500 g/L)** [1]: Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
h) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.
i) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
j) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
k) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
l) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
m) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(**3**) 5 mL -10 mL
(**4**) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.
Comment 2 0.25 mol/L sulfuric acid in (**2**) **e**) can be replaced with 0.25 mol/L sulfuric acid

conforming to ISO/IEC 17025.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Extractor: A vertical rotating mixer or vertical reciprocating shaker as described below.**

aa) **Vertical rotating mixer:** A vertical rotating mixer that can vertically rotate a 250-mL volumetric flask at a rate of 30 - 40 revolutions/min.

ab) **Vertical reciprocating shaker:** A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

b) **Steam distillation apparatus**

c) **Distillation flask**: A Kjeldahl flask or round bottom flask that can be connected to a steam distillation apparatus.

(**4**) **Test procedures**

(**4.1**) **Sample solution preparation**: Prepare a sample solution as shown below:

(**4.1.1**) **Addition of water**

a) Weigh 0.25 g - 2g (5) (the equivalents of 20 mg - 100 mg as N) of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL distillation flask

b) Add about 25 mL of water to make a sample solution.

(**4.1.2**) **Extraction using a vertical rotating mixer**

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumaetric flask.

b) Add about 150 mL of hydrochloric acid (1+23) and mix at a rate of 30 - 40 revolutions/min for about 30 minutes.

c) Add water to the marked line to make a sample solution.

(**4.1.3**) **Extraction using a vertical reciprocating shaker**

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add about 150 mL of hydrochloric acid (1+23) and mix at a rate of 300 reciprocations/min (amplitude of 40 mm) for about 30 minutes.

c) Add water to the marked line to make a sample solution.

Note (**5**) The sampling amount of the analytical sample is 5 g when there is less nitrogen content in the fertilizers such as a home garden-use fertilizer.

Comment 3 When it is a fertilizer which contains ammonium urate, humus acid ammonium or nitrate nitrogen, etc., or when it is not a fertilizer in which phosphate, ammonium and magnesium coexist, conduct the procedure in (**4.1.1.1**) **a**) - **c**) in 4.**2.4.a**, the procedure in (**4.1.1.2**) **a**) - **c**) in **4.2.4.a** or the procedure in (**4.1.2**) **a**) - **c**) in **4.2.4.a** and put a predetermined amount of suspension (the equivalents of 20 mg - 100 mg as N) in a 300-mL - 500-mL distillation flask to make a sample solution.

(**4.2**) **Distillation**: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Put a predetermined amount (6) of 0.25 mol/L sulfuric acid in an acceptor (7), add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (6) of boric acid solution (40 g/L) in an acceptor (7), add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Add (9) no less than 2 g of magnesium oxide (8) to the distillation flask containing the sample solution prepared in (**4.1.1**), and connect this distillation flask to a steam distillation apparatus. Or, put a predetermined amount (the equivalents of 20 mg - 100 mg as N) of the sample solution prepared in (**4.1.2**) or (**4.1.3**) in a 300-mL distillation flask, add (9) no less than 5 g of magnesium oxide (8), and connect this distillation flask to a steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (**6**) 5 mL - 20 mL

(**7**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).

(**8**) An amount sufficient to make the solution strong alkalinity.

(**9**) Add a small amount of silicone oil as necessary.

Comment 4 When the sample does not contain organic matters or urea, a proper amount of sodium hydroxide solution (200 g/L - 500 g/L) (8) can be added instead of magnesium oxide.

(**4.3**) **Measurement**: Conduct measurement as shown below.

(**4.3.1**) When 0.25 mol/L sulfuric acid is used in (**4.2**):

(**4.3.1.1**) In the case the sample solution was prepared by the procedure in (**4.1.1**)

a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Calculate the ammoniacal nitrogen (A-N) in the analytical sample by the following formula:

Ammoniacal nitrogen (A-N) (% (mass fraction)) in the analytical sample

$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (14.007/W_2) \times (100/1000)$$
$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (1.4007/W_2)$$

B : Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

V_6 : Volume (mL) of 0.25 mol/L sulfuric acid put in the acceptor in (**4.2**) **a**)

V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

W_2 : Mass (g) of the analytical sample

(**4.3.1.2**) In the case the sample solution was prepared by the procedure in (**4.1.2**) or (**4.1.3**)

a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Calculate the ammoniacal nitrogen (A-N) in the analytical sample by the following formula:

Ammoniacal nitrogen (A-N) (% (mass fraction)) in the analytical sample

$= (B \times V_8 - V_9) \times C_1 \times f_1 \times (250/V_{10}) \times (14.007/W_3) \times (100/1000)$

$= (B \times V_8 - V_9) \times C_1 \times f_1 \times (250/V_{10}) \times (1.4007/W_3)$

B : Volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
V_8 : Volume of 0.25 mol/L sulfuric acid put in the acceptor in (4.2) a)
V_9 : Volume (mL) of sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_{10} : Aliquot volume (mL) of the sample solution subjected to distillation in (**4.2**) **b**)
W_3 : Mass (g) of the analytical sample

(**4.3.2**) When a boric acid solution (40 g/L) is used in (**4.2**):
(**4.3.2.1**) In the case the sample solution was prepared by the procedure in (**4.1.1**)
a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (10).
b) Calculate the ammoniacal nitrogen (A-N) in the analytical sample by the following formula:

Ammoniacal nitrogen (A-N) (% (mass fraction)) in the analytical sample

$= V_{11} \times C_2 \times 2 \times f_2 \times (14.007/W_4) \times (100/1000)$

$= V_{11} \times C_2 \times f_2 \times (2.8014/W_4)$

V_{11} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid
f_2 : Factor of 0.25 mol/L sulfuric acid
W_4 : Mass (g) of the analytical sample

(**4.3.2.2**) In the case the sample solution was prepared by the procedure in (**4.1.2**) or (**4.1.3**)
a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (10).
b) Calculate the ammoniacal nitrogen (A-N) in the analytical sample by the following formula:

Ammoniacal nitrogen (A-N) (% (mass fraction)) in the analytical sample

$= V_{12} \times C_2 \times 2 \times f_2 \times (250/V_{13}) \times (14.007/W_5) \times (100/1000)$

$= V_{12} \times C_2 \times f_2 \times (250/V_{13}) \times (2.8014/W_5)$

V_{12} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid
f_2 : Factor of 0.25 mol/L sulfuric acid
V_{13} : Aliquot volume (mL) of the sample solution subjected to distillation in (**4.2**) **b**)
W_5 : Mass (g) of the analytical sample

Note (**10**) The endpoint is reached when the color changes from green to light red.

Comment 5 If it is hard to confirm the endpoint due to the carbon dioxide resulting from carbonate in the extract when magnesium oxide is used, it is recommended to boil the extract for 1-2 minute(s) after distilling and cool, and then titrate.

Comment 6 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **e**) **Standardization** and (**4.3**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 7 Recovery testing was conducted to evaluate trueness of the extraction procedure (**4.1.1**) using a preparation sample. As a result, the mean recovery rates at the content level of 10 % (mass fraction) - 21 % (mass fraction) and 1 % (mass fraction) were 100.2 % - 100.8 % and 102.5 % as ammoniacal nitrogen (A-N) respectively.

Comment 8 The comparison of the measurement value (y_i: 0.27 % (mass fraction) - 21.34 % (mass fraction)) of the extraction procedure using a vertical rotating mixer (**4.1.2**) and the measurement value (x_i) of the extraction procedure (**4.1.1**) was conducted to evaluate trueness of the extraction procedure (**4.1.2**) and the extraction procedure (**4.1.3**) using fertilizers (22 samples). As a result, a regression equation was $y = 0.188 + 0.990x$, and its correlation coefficient (r) was 0.998. In addition, the comparison of the measurement value (y_i) and the measurement value (x_i) of **4.1.2.b** formaldehyde method was conducted using fertilizers (13 samples). As a result, a regression equation was $y = -0.255 + 1.041x$, and its correlation coefficient (r) was 0.999. Similarly, the comparison of the measurement value (y_i: 0.25 % (mass fraction) - 21.44 % (mass fraction)) of the extraction procedure using a vertical reciprocating shaker (**4.1.3**) and the measurement value (x_i) of the extraction procedure (**4.1.1**) was conducted using fertilizers (22 samples). As a result, a regression equation was $y = 0.193 + 0.990x$, and its correlation coefficient (r) was 0.999. Further, the comparison of the measurement value (y_i) and the measurement value (x_i) of **4.1.2.b** formaldehyde method was conducted. As a result, a regression equation was $y = -0.220 + 1.039x$, and its correlation coefficient (r) was 0.998.

Comment 9 In order to evaluate precision of the extraction procedure (**4.1.1**), results from a collaborative study for test method validation and its analysis are shown in Table 1. Further, the results of the collaborative study (limited to reported values with Distillation method) to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 2 shows the calculation results of reproducibility, intermediate precision and repeatability. In addition, in order to evaluate precision of the extraction procedure (**4.1.2**) and the extraction procedure (**4.1.3**), the results of the repeatability tests on different days using ammonium sulfate fertilizer, by-product organic fertilizer, compound fertilizer, magnesium ammonium phosphate, mixed compost compound fertilizer, by-product compound fertilizer, and composted sludge fertilizer were analyzed by the one-way analysis of variance. Table 3 shows the calculation results of intermediate precision and repeatability.

Comment 10 The minimum limit of quantification of the extraction procedure (**4.1.1**) was estimated to be about 0.1 % (mass fraction) for solid fertilizers and about 0.01 % (mass fraction) for fluid fertilizers, that of the extraction procedure (**4.1.2**) was estimated to be about 0.07 % (mass fraction) for solid fertilizers and about 0.003 % (mass fraction) for fluid fertilizers, and that of the extraction procedure (**4.1.3**) was estimated to be about 0.1 % (mass fraction) for solid fertilizers and about 0.005 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the collaborative study
for the test method validation of ammoniac nitrogen

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Ammonium chloride	12	25.20	0.18	0.7	0.33	1.3
Ammonium sulfate	10	21.03	0.04	0.2	0.16	0.7
Compund fertilizer 1	11	5.55	0.05	1.0	0.09	1.7
Compund fertilizer 2	12	4.14	0.10	2.4	0.13	3.2
Compund fertilizer 3	11	1.94	0.04	2.2	0.05	2.3

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2 Analysis results of the collaborative study to determine a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1)]	Average[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)	s_R[8)] (%)[3)]	RSD_R[9)] (%)
FAMIC-B-10	11	8.38	0.09	1.0	0.11	1.3	0.15	1.8
FAMIC-B-14	11	8.06	0.03	0.4	0.05	0.6	0.07	0.9

1) The number of laboaratories used for analysis conducting Distillation method
2) Mean (the number of laboratory(p)× test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

Table 3Analysis results of the repeatability tests on different days of ammoniacal nitrogen

Extraction method	Sample name	Test days of repeatability (T)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)
Vertical rotating mixer	Ammonium sulfate	5	20.52	0.39	1.9	0.39	1.9
	Compound fertilizer	5	8.85	0.05	0.5	0.06	0.7
	Byproduct organic fertilizer	5	0.53	0.01	1.4	0.02	4.3
Vertical reciprocating shaker	Ammonium sulfate	5	20.79	0.15	0.7	0.15	0.7
	Compound fertilizer	5	8.70	0.29	3.3	0.29	3.3
	Byproduct organic fertilizer	5	0.53	0.02	3.4	0.04	8.4

1) The number of test days conducting a duplicate test
2) Mean ([Number of test days (T)] × [Number of duplicate testing (2)]
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers

(Details), p.36- 37, Yokendo, Tokyo (1988)

2) Kimie KATO, Masaki CHIDA and Erina WATANABE: Verification of Performance Characteristics of Testing Method for Ammonia Nitrogen Content in Fertilizer by Distillation Method, Research Report of Fertilizer, **Vol. 6**, p. 130 - 138 (2013)

3) Erika HIRATA, Hideo SOETA, Hidemi YOSHIMURA and Keiji YAGI: Performance Evaluation of Determination Method for Nitrogen in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 12**, p. 84 – 93 (2019)

(**5**) **Flow sheet for ammoniacal nitrogen**: The flow sheet for ammoniacal nitrogen in fertilizers is shown below.

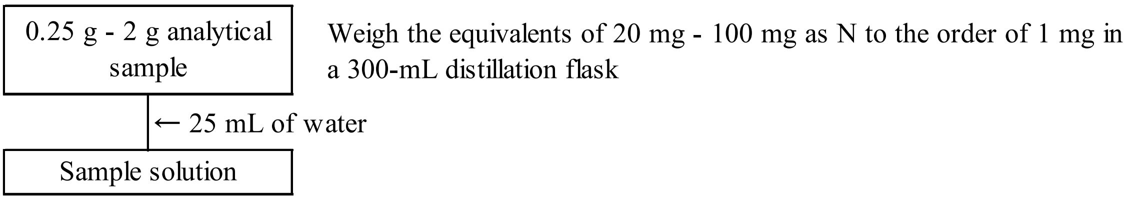


Figure 1-1 Flow sheet for ammoniacal nitrogen in fertilizers (Extraction procedure (4.1.1))

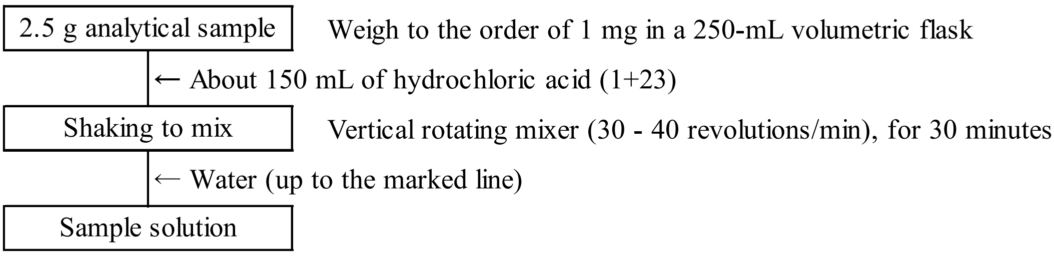


Figure 1-2 Flow sheet for ammoniacal nitrogen in fertilizers (Extraction procedure (4.1.2))

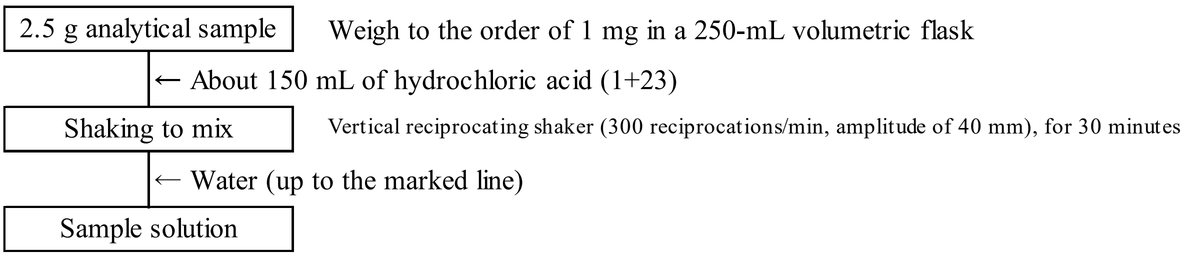


Figure 1-3 Flow sheet for ammoniacal nitrogen in fertilizers (Extraction procedure (4.1.3))

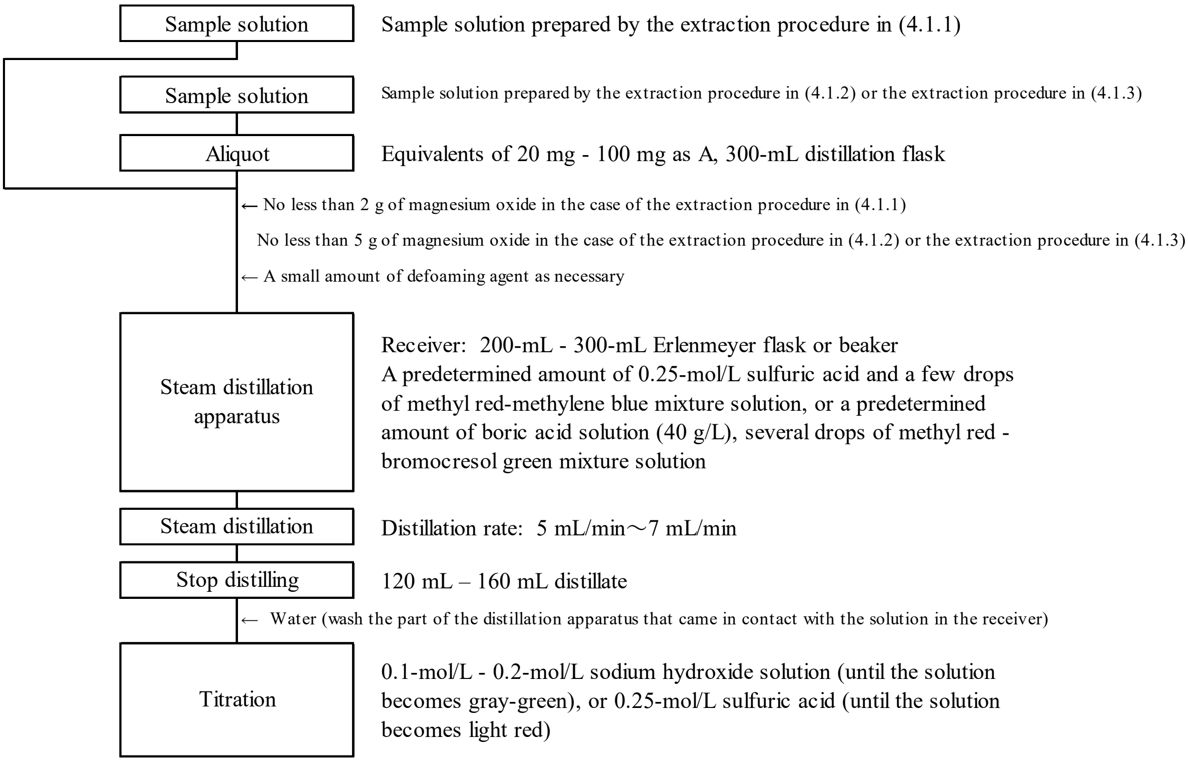


Figure 2 Flow sheet for ammoniacal nitrogen in fertilizers (Distillation and measurement procedure)

4.1.2.b Formaldehyde method

(1) Summary

The testing method is applicable to the fertilizers which do not contain a large amount of flora and fauna sample. This testing method is classified as Type C and its symbol is 4.1.2.b-2017 or A-N.b-1. After adding water or hydrochloric acid (1+20) to an analytical sample to extract ammonium ion, add an aluminum chloride solution, add a potassium hydroxide solution drop by drop and precipitate phosphoric acid and excessive aluminum to make a sample solution. Adjust the sample solution to slight acidity, add a formaldehyde solution and measure ammonium ion by complexometric titration with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain the ammoniacal nitrogen (A-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(2) Reagent: Reagents are as shown below.

a) 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C)$$

- W_1 : Mass (g) of sulfamic acid sampled
- A : Purity (% (mass fraction)) of sulfamic acid
- V_1 : Aliquot volume (mL) of sulfamic acid solution
- V_2 : Constant volume (250 mL) of sulfamic acid solution
- V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
- C : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) Potassium chloride solution (1 mol/L) (1): Dissolve 75 g of potassium chloride specified in JIS K 8121 in water to make 1000 mL.

c) Aluminum chloride solution (1 mol/L) (1): Dissolve 240 g of aluminum chloride (III) hexahydrate specified in JIS K 8114 in water to make 1000 mL.

d) Potassium hydroxide solution (170 g/L) (1): Dissolve 170 g of potassium hydroxide in water to make 1000 mL.

e) Formaldehyde solution: Add one volume of water to one volume of the 36 % (mass fraction) - 38 % (mass fraction) formaldehyde specified in JIS K 8872.

f) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

g) Bromothymol blue solution (0.1 g/100 mL): Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, and add water to make 100 mL.

h) Methyl red solution (0.1 g/100 mL): Dissolve 0.1 g of methyl red specified in JIS K 8896 in

100 mL of ethanol (95) specified in JIS K 8102.

i) **Bromothymol blue solution (1 g/100 mL)**: Dissolve 1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, and add water to make 100 mL.

Note (1) This is an example of preparation; prepare an amount as appropriate.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

Comment 2 Thymol blue can be dissolved if it is sodium salt. The thymol blue specified in JIS K 8643 is slightly hard to dissolve in ethanol and hard to dissolve in water. Therefore, add about 2.15 mL of sodium hydroxide solution (0.1 mol/L) per 0.1 g of thymol blue to neutralize, and then prepare the thymol blue solution (1 g/100 mL) through the same procedure as (**2**) **i**).

(**3**) **Instruments**: Instruments are as shown below:

a) **Vertical rotating mixer:** A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Ammonium salts**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Compound fertilizers**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 300 mL of hydrochloric acid (1 + 20) and shake to mix at the rate of 30 - 40 revolutions/min for about 30 minutes.
c) Add an aluminum chloride solution (1 mol/L) (2) to the solution and add a few drops of methyl red solution (1mol/L) as an indicator. After that, add immediately potassium hydroxide (170 g/L) while shaking the flask until the color of the solution changes to light yellow (3).
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Add 3 mL of aluminum chloride per 0.04 g of P or 0.1 g of P_2O_5 in the sample solution.
(**3**) Form precipitate of aluminum hydroxide and aluminum phosphate to separate the phosphate.

Comment 4 In the procedure of (**4.1.1**) **a**) and (**4.1.2**) **a**), it is also allowed to weigh 2.5g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask

Comment 5 When it is not a fertilizer in which phosphate, ammonium and magnesium coexist, about 400 mL of potassium chloride solution (1 mol/L) can be used instead of about 300 mL of hydrochloric acid (1+20) in the procedure of (**4.1.2**) **b**).

Comment 6 In the case of the compound fertilizers containing bentonite, after shaking to mix using about 300 mL of hydrochloric acid (1+20) or about 400 mL of potassium chloride solution (1 mol/L) according to **Comment 5** in (**4.1.2**) **b**), add water to the marked line, filter with Type 3 filter paper and transfer 50 ml - 100 mL to a 250-mL volumetric flask, and then conduct the procedure in (**4.1.2**) **c**) - **e**).

(**4.2**) **Measurement**: Conduct measurement as shown below.

a) Put a predetermined amount of sample solution (the equivalents of 50 mg as A-N) in a 300-mL volumetric flask (4).

b) Add water to the solution to make about 100 mL.

c) Add one or two drop(s) of methyl red solution (0.1 g/100 mL) and add hydrochloric acid (1+200) until the color of the solution changes to light pink.

d) Add 10 mL of formaldehyde solution.

e) Add one or two drop(s) of thymol blue solution (1 g/100 mL) and titrate with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution changes to blue (5).

f) As a blank test, put 100 mL of water in another 300-mL Erlenmeyer flask and conduct the procedure in **c**) - **e**).

g) Calculate the ammoniacal nitrogen (A-N) in the analytical sample by the following formula:

Ammoniacal nitrogen (A-N) (% (mass fraction)) in the analytical sample

$$= (V_S - V_B) \times C \times f \times (V_4/V_5) \times (14.007/W_2) \times (100/1000)$$
$$= (V_S - V_B) \times C \times f \times (V_4/V_5) \times (1.4007/W_2)$$

V_S : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration in (**4.2**) **e**)

V_B : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration of the blank test in (**4.2**) **f**)

C : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

V_4 : Constant volume (mL) of the sample solution in (**4.1.1**) **c**) or (**4.1.2**) **d**)

V_5 : Aliquot volume (mL) of the sample solution in (**4.2**) **a**)

W_2 : Mass (g) of the analytical sample

Note (**4**) The volume to be transferred should be up to 100 mL.

(**5**) The endpoint is reached when the color changes from green to light red. It is easy to observe the change of color under fluorescent light.

Comment 7 The titration procedures in (**2**) **a**) **Standardization** and (**4.2**) **e**) - **f**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 8 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 10 % (mass fraction) - 21 % (mass fraction) and 1 % (mass fraction) were 100.4 % - 101.0 % and 100.1 % as ammoniacal nitrogen (A-N) respectively.

The results of the collaborative study (limited to reported values by Formaldehyde method) to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 1 shows the calculation results of

reproducibility, intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.03 % (mass fraction) for solid fertilizers and about 0.02 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the collaborative study
to determine ammmoniac nitrogenof a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)	s_R[8] (%)[3]	RSD_R[9] (%)
FAMIC-A-10	10	10.66	0.07	0.7	0.09	0.8	0.16	1.5
FAMIC-A-13	9	10.36	0.06	0.5	0.08	0.8	0.21	2.0

1) The number of laboaratories used for analysis conducting Formaldehyde method
2) Mean (the number of laboratory(p) × test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.39- 42, Yokendo, Tokyo (1988)
2) Kimie KATO, Masaki CHIDA and Erina WATANABE: Verification of Performance Characteristics of Testing Method for Ammonia Nitrogen Content in Fertilizer by Formaldehyde Method, Research Report of Fertilizer **Vol. 6**, p. 139 - 147 (2013)

(**5**) **Flow sheet for ammoniacal nitrogen**: The flow sheet for ammoniacal nitrogen in fertilizers is shown below.

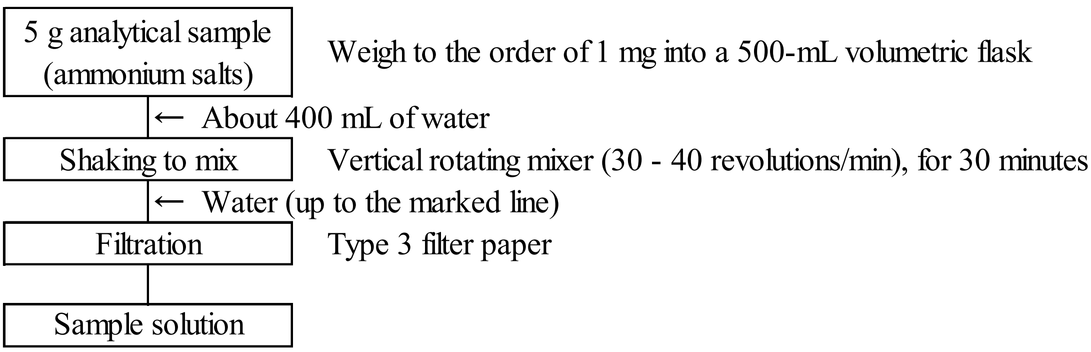


Figure 1-1 Flow sheet for ammoniacal nitrogen in fertilizers (Extraction procedure (4.1.1))

5 g analytical sample (compound fertilizer)	Weigh to the order of 1 mg into a 500-mL volumetric flask

← About 300 mL of hydrochloric acid (1 + 20)

Shaking to mix	Vertical rotating mixer (30 - 40 revolutions/min), for 30 minutes

← Aluminum chloride solution (1 mol/L)
← One or two drop(s) of methyl red
← Potassium hydroxide solution (170 g/L)
(Until the solution changes to light yellow)
← Water (up to the marked line)

Filtration	Type 3 filter paper

Sample solution

Figure 1-2 Flow sheet for ammoniacal nitrogen in fertilizers (Extraction procedure (4.1.2))

Sample solution

Aliquot	Up to the equivalents of 50 mg as A-N ,300-mL Erlenmeyer flask

← Add water to make about 100 mL of solution
← One or two drop(s) of methyl red (0.1 g/100 mL)
← Hydrochloric acid (1+200) [light pink]
← 10 mL of formaldehyde solution
← One or two drop (s) of thymol blue solution (1 g/100 mL)

Titration	0.1 mol/L - 0.2 mol/L sodium hydroxide solution (until the solution becomes blue)

Figure 2 Flow sheet for ammoniacal nitrogen in fertilizers (Measurement procedure)

4.1.3 Nitrate nitrogen

4.1.3.a Devarda's alloy - distillation method

(1) Summary

The testing method is applicable to the fertilizer containing nitrate. However, it is not applicable to fertilizers containing urea, nitrolime and organic matters that digest by heating and isolate ammonia. This testing method is classified as Type E and its symbol is 4.1.3.a-2017 or N-N.a-1.

Add water to an analytical sample to dissolve ammoniacal nitrogen (A-N) and nitrate nitrogen (N-N), and further add devarda's alloy and sodium hydroxide to subject it to steam distillation. In this process, nitrate nitrogen (N-N) is reduced to ammonia. Collect isolated ammonia with 0.25 mol/L sulfuric acid and measure surplus sulfuric acid by (neutralization) titration using 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain nitrogen content (N-N+A-N) in an analytical sample. Or collect isolated ammonia with a boric acid solution and measure ammonium ion by (neutralization) titration using 0.25 mol/L sulfuric acid to obtain nitrogen content (N-N+A-N) in an analytical sample. Subtract separately obtained the ammoniacal nitrogen (A-N) by **4.1.2** to calculate nitrate nitrogen (N-N). This testing method corresponds to the devarda's alloy method in the Official Methods of Analysis of Fertilizers (1992).

(2) Reagent: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

W_1 : Mass (g) of sulfamic acid sampled
A : Purity (% (mass fraction)) of sulfamic acid
V_1 : Aliquot volume (mL) of sulfamic acid solution
V_2 : Constant volume (250 mL) of sulfamic acid solution
V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.25 mol/L sulfuric acid** (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Put a predetermined amount (3) of 0.25 mol/L sulfuric acid in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L -0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4). Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$= V_4/V_5$ ……… (1)

Factor of 0.25 mol/L sulfuric acid (f_2)

$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ ……… (2)

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.
e) **Sodium hydroxide solution (200 g/L - 500 g/L)** (1): Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
f) **Devarda's alloy**: A reagent of nitrogen analysis grade specified in JIS K 8653 or a reagent of equivalent quality.
g) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, and add water to make 100 mL.
h) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
i) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
j) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
k) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
l) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(**3**) 5 mL -10 mL
(**4**) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.
Comment 2 0.25 mol/L sulfuric acid in (**2**) **c**) can be replaced with 0.25 mol/L sulfuric acid conforming to ISO/IEC 17025.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Steam distillation apparatus**

b) **Distillation flask**: A Kjeldahl flask or round bottom flask that can be connected to a steam distillation apparatus.

(**4**) **Test procedures**

(**4.1**) **Sample solution preparation**: Prepare a sample solution as shown below:

a) Weigh 0.25 g - 1 g (5) (the equivalents of 20 mg - 100 mg as N) of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL distillation flask

b) Add about 25 mL of water to make a sample solution.

Note (**5**) Conduct the procedure in **Comment 3** when there is much nitrogen content in the fertilizers such as simple salt fertilizers.

Comment 3 In the case of nitrate fertilizer, etc. containing much nitrogen content, weigh 2 g - 5g of an analytical sample to the order of 1 mg, put it into a 250- mL volumetric flask, dissolve it in water, and further add water up to the marked line. Put predetermined volume of suspension (the equivalents of 20 mg -100 mg as N) into a 300- mL - 500-mL distillation flask.

(**4.2**) **Distillation**: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Put a predetermined amount (6) of 0.25 mol/L sulfuric acid in an acceptor (7), add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (6) of boric acid solution (40 g/L) in an acceptor (7), add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Add (10) no less than 3 mg of devarda’s alloy and adequate volume of sodium hydroxide (200 g/L - 500 g/L) (8) (9) and connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (**6**) 5 mL - 20 mL

(**7**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).

(**8**) Sudden reaction makes bubbles foam drastically and the bubbles overflow from a distillation flask. Therefore, it is required to add an alkali solution gradually and mix quietly.

(**9**) An amount sufficient to make the solution strong alkalinity.

(**10**) Add a small amount of silicone oil as necessary.

(**4.3**) **Measurement**: Conduct measurement as shown below.

(**4.3.1**) When 0.25 mol/L sulfuric acid is used in (**4.2**):

a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Calculate nitrogen content (N-N+A-N) in the analytical sample by the following formula:

c) Subtract the ammoniacal nitrogen (A-N) separately obtained in **4.1.2** from the obtained nitrogen content (N-N+A-N) to calculate nitrate nitrogen (N-N) (11) (12).

Nitrogen content (N-N+A-N) (% (mass fraction)) in the analytical sample

$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (14.007/W_2) \times (100/1000)$

$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (1.4007/W_2)$

- B : Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
- V_6 : Volume (mL) of 0.25 mol/L sulfuric acid put in the acceptor in (**4.2**) **a**)
- V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration in (**4.3**) **a**)
- C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
- f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
- W_2 : Mass (g) of the analytical sample

Note (**11**) The nitrogen content (N-N+A-N) and ammoniacal nitrogen (A-N) use raw data without rounding the numerical value.

(**12**) When no ammoniacal nitrogen (A-N) is contained, the nitrogen content (N-N+A-N) calculated in (**4.3**) **b**) is regarded as nitrate nitrogen (N-N).

(**4.3.2**) When a boric acid solution (40 g/L) is used in (**4.2**):

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (13).

b) Calculate nitrogen content (N-N+A-N) in the analytical sample by the following formula:

c) Subtract the ammoniacal nitrogen (A-N) separately obtained in **4.1.2** from the obtained nitrogen content (N-N+A-N) to calculate nitrate nitrogen (N-N) (11) (12).

Nitrogen content (N-N+A-N) (% (mass fraction)) in the analytical sample

$= V_{10} \times C_2 \times 2 \times f_2 \times (14.007/W_3) \times (100/1000)$

$= V_{10} \times C_2 \times f_2 \times (2.8014/W_3)$

- V_{10} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
- C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid
- f_2 : Factor of 0.25 mol/L sulfuric acid
- W_3 : Mass (g) of the analytical sample

Note (**13**) The endpoint is reached when the color changes from green to light red.

Comment 4 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **c**) **Standardization** and (**4.3**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.49- 50, Yokendo, Tokyo (1988)

(5) **Flow sheet for nitrate nitrogen**: The flow sheet for nitrate nitrogen in fertilizers is shown below.

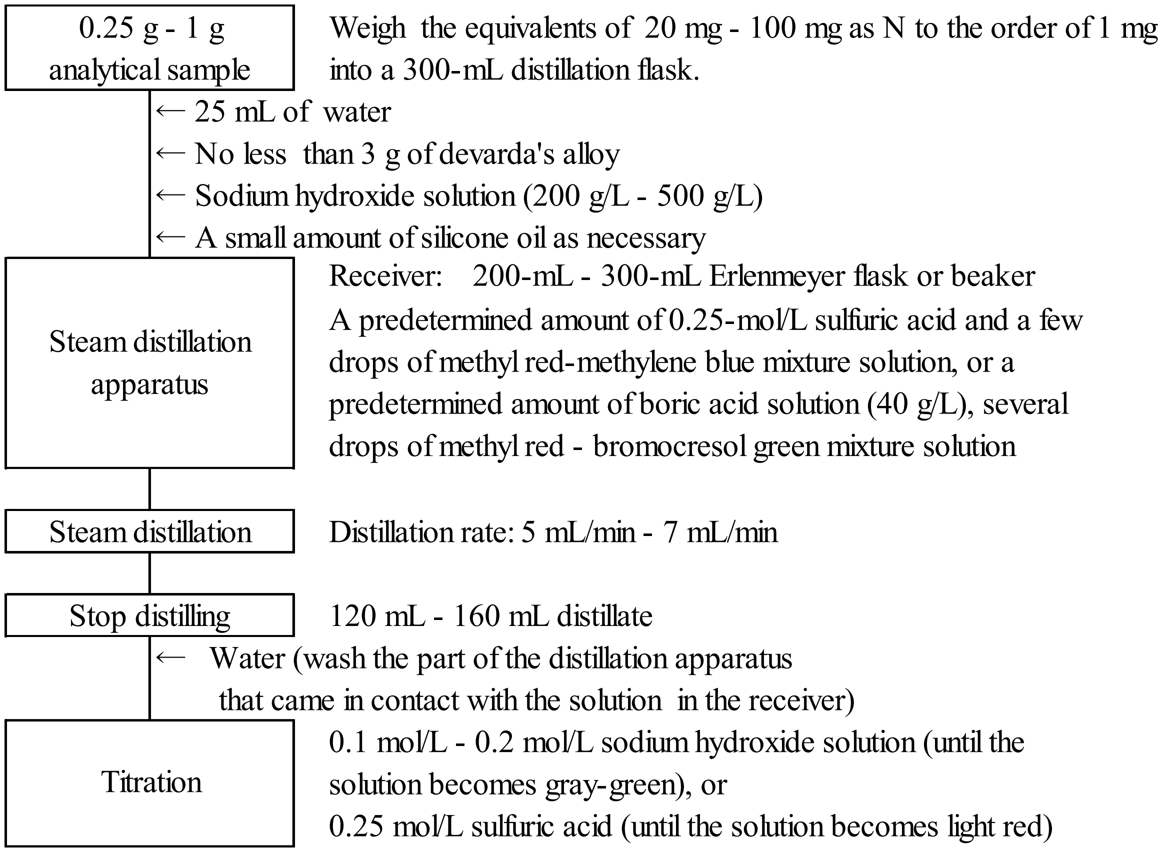


Figure Flow sheet for nitrate nitrogen in fertilizers

4.1.3.b Reduced iron- distillation method

(1) Summary

The testing method is applicable to the fertilizer containing nitrate. However, it is not applicable to fertilizers containing urea, nitrolime and organic matters that digest by heating and isolate ammonia. This testing method is classified as Type E and its symbol is 4.1.3.b-2017 or N-N.b-1.

Add water to an analytical sample to dissolve ammoniacal nitrogen (A-N) and nitrate nitrogen (N-N), and add reduced iron and a sulfuric acid solution to boil lightly. In this process, nitrate nitrogen (N-N) is reduced to ammonia. And further add a sodium hydroxide solution to distillate. Collect isolated ammonia with 0.25 mol/L sulfuric acid and measure surplus sulfuric acid by (neutralization) titration using 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain nitrogen content (N-N+A-N) in an analytical sample. Or collect isolated ammonia with a boric acid solution and measure ammonium ion by (neutralization) titration using 0.25 mol/L sulfuric acid to obtain nitrogen content (N-N+A-N) in an analytical sample. Subtract separately obtained the ammoniacal nitrogen (A-N) by **4.1.2** to calculate nitrate nitrogen (N-N). This testing method corresponds to the reduced iron method in the Official Methods of Analysis of Fertilizers (1992).

(2) Reagent: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

W_1 : Mass (g) of sulfamic acid sampled
A : Purity (% (mass fraction)) of sulfamic acid
V_1 : Aliquot volume (mL) of sulfamic acid solution
V_2 : Constant volume (250 mL) of sulfamic acid solution
V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sulfuric acid:** A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.25 mol/L sulfuric acid** (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Put a predetermined amount (3) of 0.25 mol/L sulfuric acid in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L -0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4). Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$=V_4/V_5$ ………. (1)

Factor of 0.25 mol/L sulfuric acid (f_2)

$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ ………. (2)

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.
e) **Sodium hydroxide solution (200 g/L - 500 g/L)** (1): Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
f) **Reduced iron**: Nitrogen content is no more than 0.005 % (mass fraction)
g) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, and add water to make 100 mL.
h) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
i) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
j) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
k) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
l) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(**3**) 5 mL -10 mL
(**4**) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.
Comment 2 0.25 mol/L sulfuric acid in (**2**) **c**) can be replaced with 0.25 mol/L sulfuric acid conforming to ISO/IEC 17025.

(**3**) **Instruments**: Instruments are as shown below:
a) **Steam distillation apparatus**
b) **Distillation flask**: A Kjeldahl flask or round bottom flask that can be connected to a steam

distillation apparatus.

(4) Test procedures

(4.1) Preparation of sample solution: Conduct preparation of a sample solution as shown below.

a) Weigh 0.5 g - 1 g (5) (the equivalents of 20 mg - 100 mg as N) of an analytical sample to the order of 1 mg, and put it in a 300-mL - 500-mL distillation flask

b) Add about 30 mL of water and mix well.

c) As soon as adding 5 g of reduced iron and 10 mL of sulfuric acid (1+1), insert a long stem funnel to a distillation flask and shake to mix gently while cooling the outside of the container under flowing water (6).

d) After leaving at rest for about 5 minutes (7), heat gradually by low temperature and boil in a low flame for about 15 minutes, and then stand to cool to make a sample solution.

Note (**5**) Conduct the procedure in **Comment 3** when there is much nitrogen content in the fertilizers such as simple salt fertilizers.

(**6**) Sudden reaction generates heat, and unreacted nitric acid vaporizes or digests to make nitrogen oxide etc. through which process losses occur easily. Careful and efficient operation should be taken

(**7**) Until a sudden reaction is settled.

Comment 3 In the case of nitrate fertilizer, etc. containing much nitrogen content, weigh 2 g - 5g of an analytical sample to the order of 1 mg, put it into a 250- mL volumetric flask, dissolve it in water, and further add water up to the marked line. Put predetermined volume of suspension (the equivalents of 20 mg - 100 mg as N) into a 300-mL - 500-mL distillation flask.

(4.2) Distillation: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Put a predetermined amount (8) of 0.25 mol/L sulfuric acid in an acceptor (9), add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (8) of boric acid solution (40 g/L) in an acceptor (9), add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Add adequate volume of sodium hydroxide (200 g/L - 500 g/L) (10) and connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (**8**) 5 mL - 20 mL

(**9**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).

(**10**) An amount sufficient to make the solution strong alkalinity.

(4.3) Measurement: Conduct measurement as shown below.

(4.3.1) When 0.25 mol/L sulfuric acid is used in (**4.2**) **a**),

a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green [4].

b) Calculate nitrogen content (N-N+A-N) in the analytical sample by the following formula:

c) Subtract the ammoniacal nitrogen (A-N) separately obtained in 4.1.2 from the obtained nitrogen content (N-N+A-N) to calculate nitrate nitrogen (N-N) [11] [12].

Nitrogen content (N-N+A-N) (% (mass fraction)) in the analytical sample

$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (14.007/W_2) \times (100/1000)$$
$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (1.4007/W_2)$$

B : Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

V_6 : Volume (mL) of 0.25 mol/L sulfuric acid put in the acceptor in (**4.2**) **a**)

V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

W_2 : Mass (g) of the analytical sample

Note (**11**) The nitrogen content (N-N+A-N) and ammoniacal nitrogen (A-N) use raw data without rounding the numerical value.

(**12**) When no ammoniacal nitrogen (A-N) is contained, the nitrogen content (N-N+A-N) calculated in (**4.3**) **b**) is regarded as nitrate nitrogen (N-N).

(**4.3.2**) When a boric acid solution (40 g/L) is used in (**4.2**) **a**):

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red [13].

b) Calculate nitrogen content (N-N+A-N) in the analytical sample by the following formula:

c) Subtract the ammoniacal nitrogen (A-N) separately obtained in **4.1.2** from the obtained nitrogen content (N-N+A-N) to calculate nitrate nitrogen (N-N) [11] [12].

Nitrogen content (N-N+A-N) (% (mass fraction)) in the analytical sample

$$= V_{10} \times C_2 \times 2 \times f_2 \times (14.007/W_3) \times (100/1000)$$
$$= V_{10} \times C_2 \times f_2 \times (2.8014/W_3)$$

V_{10} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration

C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

f_2 : Factor of 0.25 mol/L sulfuric acid

W_3 : Mass (g) of the analytical sample

Note (**13**) The endpoint is reached when the color changes from green to light red.

Comment 4 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **c**) **Standardization** and (**4.3**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.48- 49, Yokendo, Tokyo (1988)

(5) Flow sheet for nitrate nitrogen: The flow sheet for nitrate nitrogen in fertilizers is shown below.

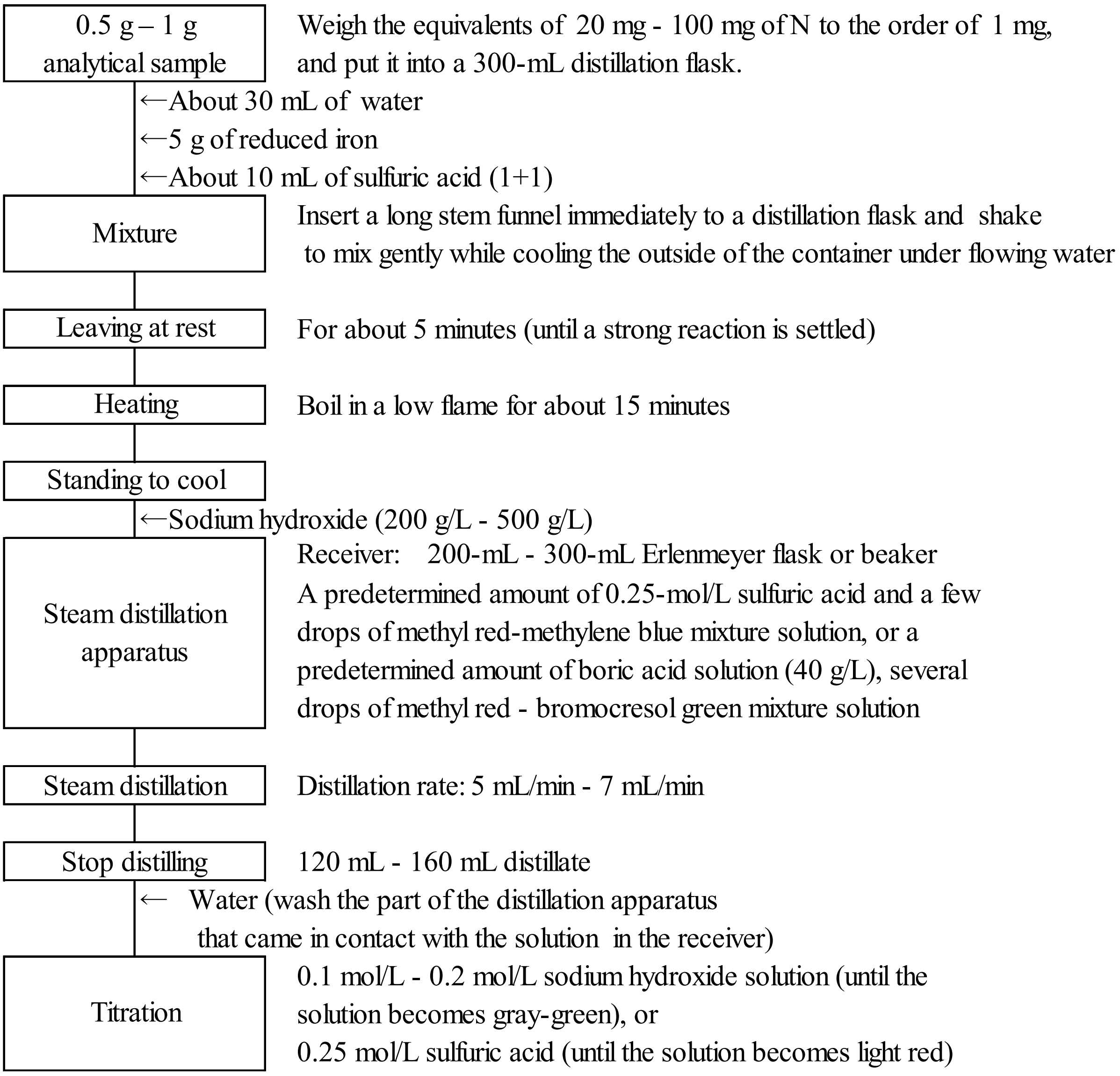


Figure Flow sheet for nitrate nitrogen in fertilizers

4.1.3.c Phenol sulfuric acid method

(1) Summary

The testing method is applicable to the fertilizer containing nitrate. It is also applicable to the fertilizers containing chemical compounds such as urea, nitrolime and organic matters that digest by heating and isolate ammonia. This testing method is classified as Type B and its symbol is 4.1.3.c-2021 or N-N.c-2.

Add a copper sulfate - silver sulfate solution, calcium hydroxide and basic magnesium carbonate to an analytical sample, extract nitrate nitrogen (N-N) as well as removing chloride and organic matters, and measure the absorbance of nitro phenol ammonium sulfate formed by the reaction with phenol sulfuric acid and an ammonia solution to calculate nitrate nitrogen (N-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(2) Reagent: Reagents are as shown below.

- **a) Nitrate standard solution (N-N 5 mg/mL)** (1): Heat potassium nitrate (no less than 99.9 (%) (mass fraction) in purity) at 110 ºC for no less than 1 hour, and after standing to cool in a desiccator, put 36.09 g in a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.
- **b) Nitrate standard solution (N-N 0.01 mg/mL)**: Dilute predetermined volume of nitrate standard solution (N-N 5 mg/mL) with water to prepare a nitrate standard solution (N-N 0.01 mg/mL).
- **c) Copper sulfuric - silver sulfuric solution** (1): Dissolve 5 g of copper (II) sulfate pentahydrate specified JIS K 8983 in 900 mL of water, and dissolve while adding 4 g of silver sulfate specified in JIS K 8965 to make 1000 mL (2).
- **d) Phenol sulfuric acid**: Dissolve 15 g of phenol specified in JIS K 8798 in 100 mL of sulfuric acid specified in JIS K 8965, heat in a water bath at 80 ºC - 100 ºC for two hours and then let it stand to cool (2).
- **e) Ammonia solution**: A JIS Guaranteed (NH_3 28 % (mass fraction)) reagent specified in JIS K 8085 or a reagent of equivalent quality.
- **f) Calcium hydroxide**: A JIS Guaranteed Reagent specified in JIS K 8575 or a reagent of equivalent quality.
- **g) Basic magnesium carbonate**: Basic magnesium carbonate that contains no nitrate nitrogen.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store in an amber bottle.

Comment 1 Instead of the nitrate standard solution in (**2**), a nitrate standard solution for a calibration curve may be prepared using nitrate nitrogen (NO_3-N 0.1 mg/mL or 1 mg/mL) traceable to National Metrology.

(3) Instruments: Instruments are as shown below:

- **a) Extractor: A vertical rotating mixer or vertical reciprocating shaker as described below.**
- **aa) Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 250-mL volumetric flask at a rate of 30 - 40 revolutions/min.
- **ab) Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).
- **b) Spectrophotometer**: A spectrophotometer specified in JIS K 0115
- **c) Water bath**: Water bath that can be adjusted to no less than 80 ºC

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

(4.1.1.1) Vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.
b) Add about 200 mL of copper sulfuric - silver sulfuric solution and shake to mix at 30 - 40 revolutions /min for about 20 minutes.
c) Add about 1 g of calcium hydroxide and about 1 g of basic magnesium carbonate and shake to mix at 30 - 40 revolutions /min for about 10 minutes.
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make a sample solution (3).

(4.1.1.2) Vertical reciprocating shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.
b) Add about 200 mL of copper sulfuric - silver sulfuric solution, and shake to mix at 300 reciprocations/min (amplitude of 40 mm) for about 20 minutes.
c) Add about 1 g of calcium hydroxide and about 1 g of basic magnesium carbonate and shake to mix at 300 reciprocations/min (amplitude of 40 mm) for about 10 minutes.
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make a sample solution (3).

(4.1.2) Fluid test sample

a) Weigh 0.4 g of an analytical sample to the order of 1 mg, and put it in a 100-mL volumetric flask.
b) Add about 80 mL of copper sulfuric - silver sulfuric solution, and shake to mix.
c) Add about 0.4 g of calcium hydroxide and about 0.4 g of basic magnesium carbonate, and shake to mix (4).
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make a sample solution (3).

Note **(3)** As soon as the sample solution is prepared, conduct the procedure in (**4.2**) **a**).
(4) Shake horizontally for at least five times, and turn upside down once. Repeat that about 10 times. Keep shaking until precipitation originating from the analytical sample, copper sulfuric - silver sulfuric solution, calcium hydroxide or basic magnesium carbonate is not present and fully dispersed.

Comment 2 If the filtrate of (**4.1.1.1**), (**4.1.1.2**), (**4.1.2**) **e**) is colored, add no more than 0.5 g of active carbon and filter with Type 3 filter paper to make a sample solution.

(4.2) Coloring: Conduct coloring as shown below.

a) Put a predetermined amount of sample solution (the equivalents of 0.01 mg - 0.1 mg as N-N) into a small evaporating dish (4).
b) Evaporate water on a water bath at no less than 80 ºC to exsiccate.
c) After standing to cool, swiftly add 2 mL of phenol sulfuric acid (5) and then rotate the evaporating dish so that the whole residue comes in contact with the acid.
d) After leaving at rest for about 10 minutes, add 20 mL of water (6).
e) After standing to cool, transfer it with water to a 100- mL volumetric flask.

f) Add an ammonia solution (1+2), until the color of a solution changes to light yellow, to allow it to be weak alkalinity, and further add 3 mL of ammonia solution (1+3) (7).
g) After cooling is complete, add water up to the marked line and leave at rest for 30 minutes.

Note (**4**) A round-bottom glass or porcelain evaporating dish is preferable.
(**5**) Add at the center of a small evaporating dish with Komagome pipet.
(**6**) If residue does not dissolve easily, grind it with a glass rod.
(**7**) As no color appears from a blank test solution for a calibration curve preparation, add almost the same volume of ammonia solution (1+2) as the nitrate standard solution.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.
a) **Measurement conditions of spectrophotometer**: Set up the measurement conditions of spectrophotometer considering the following.
Detection wavelength: 410 nm
b) **Calibration curve preparation**
1) Put 1 mL - 10 mL of nitrate standard solution (N-N 0.01 mg/mL) in small evaporating dishes (4) step-by-step.
2) Conduct the same procedure as (**4.2**) **b**) - **g**) to make the nitrate standard solution for the calibration curve preparation.
3) Put 40 mL of water in a 100-mL volumetric flask, and shake to mix while gently adding 2 mL of phenol sulfuric acid. Let it stand to cool and conduct the same procedure as (**4.2**) **f**) - **g**) to make the blank test solution for calibration curve.
4) Measure absorbance at a wavelength 410 nm of the nitrate standard solution for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control.
5) Prepare the calibration curve of concentration of the nitrate nitrogen and absorbance of the nitrate standard solutions for the calibration curve preparation.
c) **Sample measurement**
1) Regarding the solution in (**4.2**) **g**), measure absorbance by the same procedure as **b**) **4**).
2) Obtain the nitrate nitrogen (N-N) content from the calibration curve and calculate nitrate nitrogen (N-N) in the analytical sample.

Comment 3 Tripricates recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 16 % (mass fraction) and 1 % (mass fraction) - 3% (mass fraction) were 103.4 % and 101.1 % - 100.9 % as nitrate nitrogen (N-N) respectively.
In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1.
The comparison of the measurement value (y_i: 0.04 % - 16.48 % (mass fraction)) of extraction by a vertical reciprocating shaker and the measurement value (x_i) of extraction by a vertical rotating mixer was conducted using sodium nitrate (2 samples), lime nitrate (1 sample), fluid nitrogen fertilizer (2 samples), coating compound fertilizer (1 sample), compound fertilizer (5 samples), fluid compound fertilizer (4 samples), blended fertilizer (3 samples), home garden-use compound fertilizer (2 samples), and compost (1 sample). As a result, a regression equation was $y = 0.0059 + 0.9995x$, and its correlation coefficient (r) was 0.9999.
The results of repeated analyses on different days using a compound fertilizer and home garden-use compound fertilizer were analyzed by the one-way analysis of variance. Table 2 shows the estimation results of intermediate precision and

repeatability.

The comparison of the measurement value (y_i: 1.15% - 8.48 % (mass fraction)) of simple extraction and the measurement value (x_i) of extraction by a vertical rotating mixer was conducted using fluid nitrogen fertilizer (3 samples), fluid compound fertilizer (7 samples), and home garden-use compound fertilizer (4 samples). As a result, a regression equation was $y = -0.0098 + 0.9967x$, and its correlation coefficient (r) was 0.9999.

The results of repeated analyses on different days using a fluid nitrogen fertilizer and fluid compound fertilizer were analyzed by the one-way analysis of variance. Table 2 shows the estimation results of intermediate precision and repeatability.

Comment 4 The minimum limit of quantification of the extraction procedure (4.1.1) was estimated to be about 0.01 % (mass fraction) for solid fertilizers and about 0.002 % (mass fraction) for fluid fertilizers, and that of the extraction procedure (4.1.2) was estimated to be about 0.003 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the collaborative study for the test method validation of nitrate nitrogen

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Ammonium nitrate	12	18.04	0.13	0.7	0.64	3.6
Compund fertilizer 1	12	14.03	0.17	1.2	0.40	2.8
Compund fertilizer 2	10	5.04	0.07	1.4	0.24	4.8
Compund fertilizer 3	12	3.87	0.05	1.2	0.10	2.7
Compund fertilizer 4	12	1.18	0.03	2.4	0.04	3.4

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2Estimation results of repeatability and intermediate precision

Sample name	Days of repeatability T[1)]	Mean[2)] (%)[3)]	Repeatability s_r[4)] (%)[3)]	Repeatability RSD_r[5)] (%)	Intermediate precision $s_{I(T)}$[6)] (%)[3)]	Intermediate precision $RSD_{I(T)}$[7)] (%)
Compound fertilizer	5	15.89	0.07	0.5	0.1	0.8
Home garden-use fertilizer	5	4.23	0.01	0.3	0.01	0.3
Compost	5	0.03	0.001	3.5	0.01	0.03
Fluid nitrogen fertilizer	5	1.62	0.01	0.4	0.01	1.0
Fluid compound fertilizer	5	8.43	0.05	0.6	0.06	0.7

1) The number of days conducting duplicate analysis
2) Mean ([Number of days of repeatability (T)] × [Number of duplicates (2)])
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.52- 55, Yokendo, Tokyo (1988)
2) Kimie KATO, Sakiko TAKAHASHI and Yuji SHIRAI: Validation of a Color metric Method for Determination of Nitrogen, Phosphorus and Boron: Evaluation of Calibration curve, Research Report of Fertilizer **Vol. 2**, p. 137 - 144 (2009)
3) Kimie KATO, Masaki CHIDA and Erina WATANABE: Verification of Performance Characteristics of Testing Method for Nitrate Nitrogen Content in Fertilizer by Phenol Sulfuric Acid Method, Research Report of Fertilizer **Vol. 6**, p. 148 - 155 (2013)
4) Erika HIRATA, Hideo SOETA, Hidemi YOSHIMURA and Keiji YAGI: Performance Evaluation of Determination Method for Nitrogen in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 12**, p. 84 – 93 (2019)

(5) **Flow sheet for nitrate nitrogen:** The flow sheet for nitrate nitrogen in fertilizers is shown below.

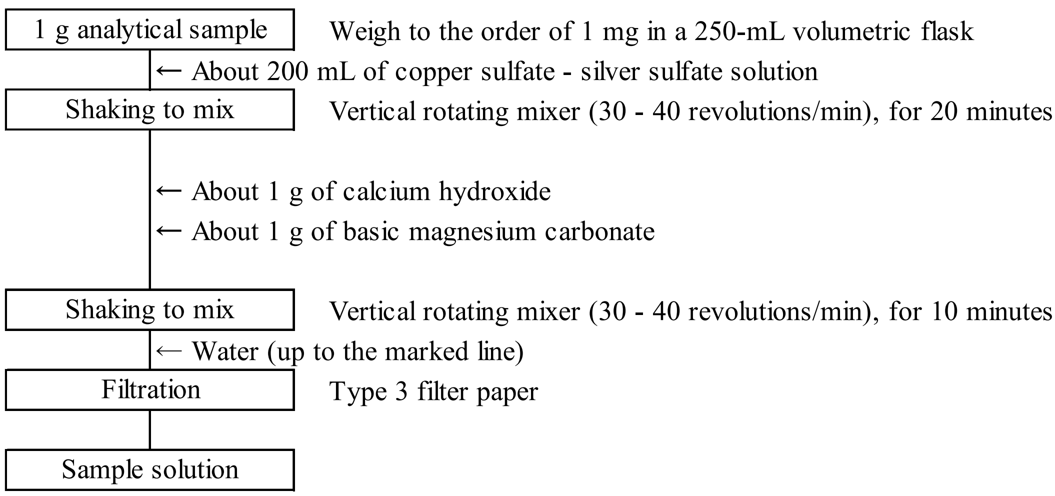


Figure 1-1 Flow sheet for nitrate nitrogen in fertilizers (Extraction procedure (4.1.1.1))

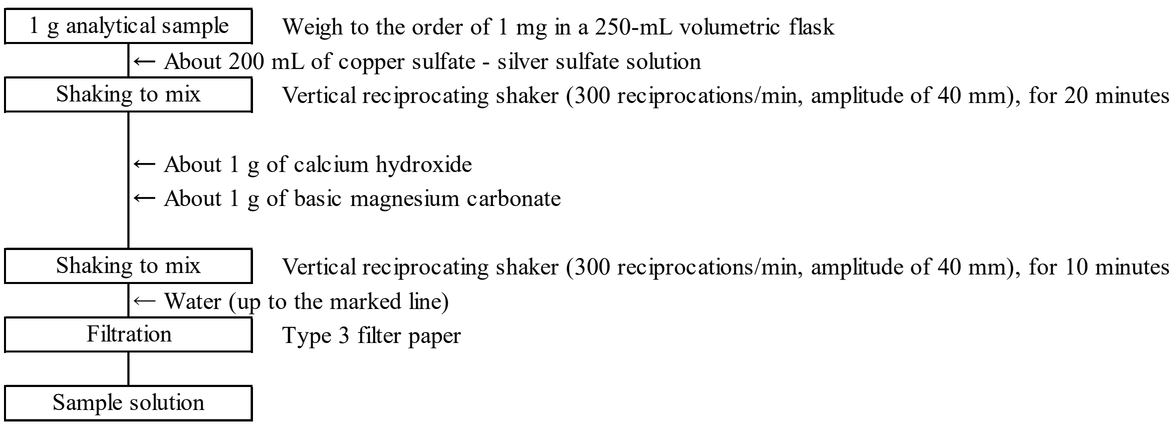


Figure 1-2 Flow sheet for nitrate nitrogen in fertilizers (Extraction procedure (4.1.1.2))

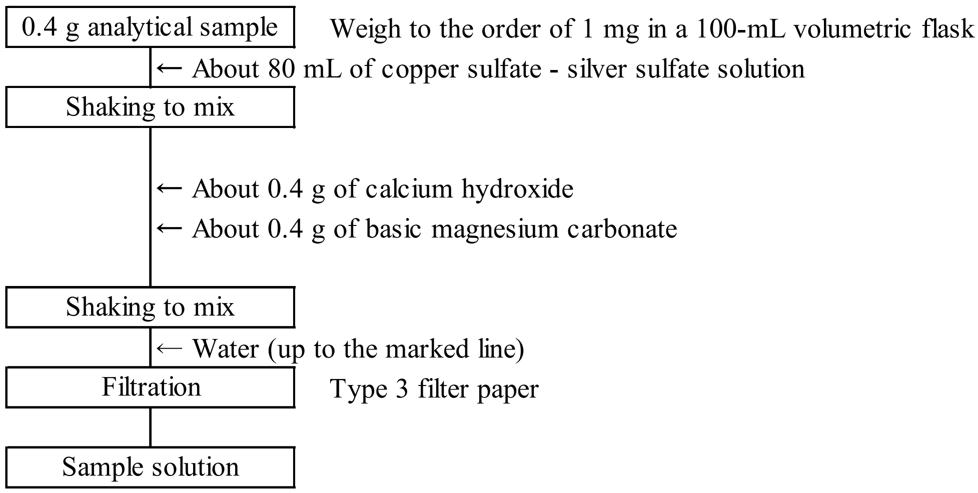


Figure 1-3 Flow sheet for nitrate nitrogen in fertilizers (Extraction procedure (4.1.2))

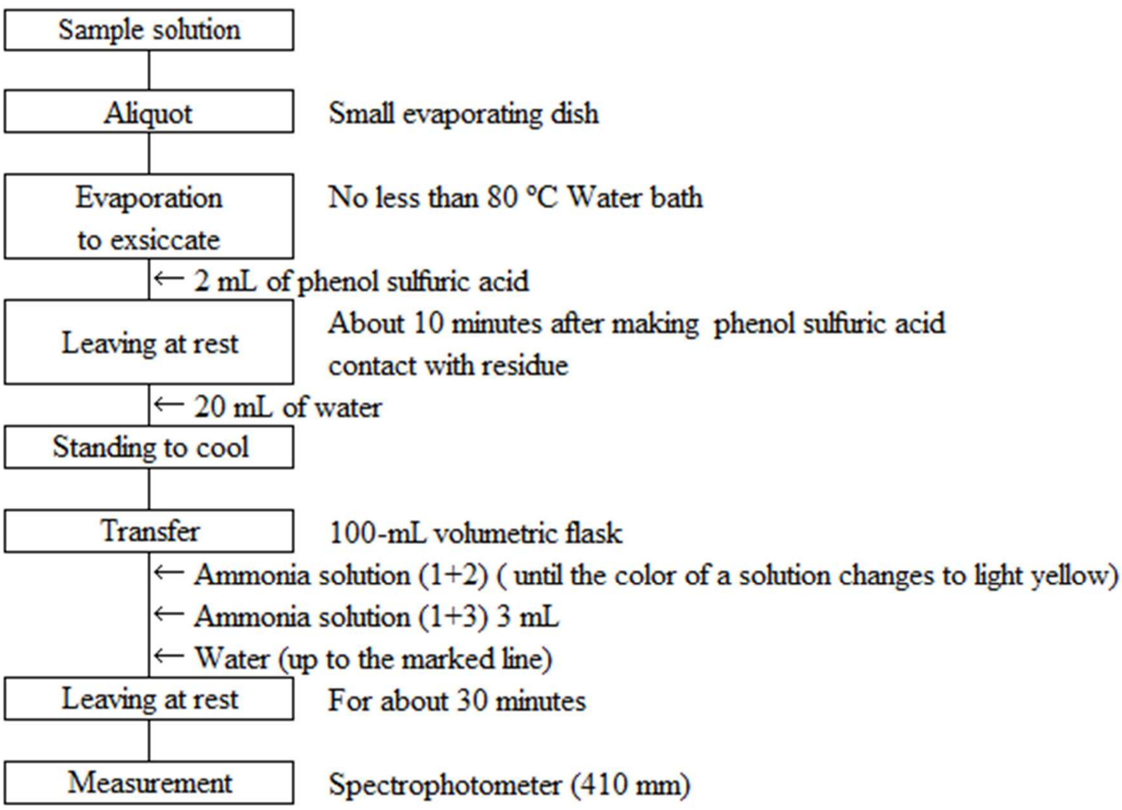


Figure 2 Flow sheet for nitrate nitrogen in fertilizers (Coloring and measurement procedure)

4.2 Phosphoric acid
4.2.1 Total phosphoric acid
4.2.1.a Ammonium vanadomolybdate absorptiometric analysis
(1) Summary

This testing method is applicable to fertilizers containing organic matters. This testing method is classified as Type B and its symbol is 4.2.1.a-2017 or T-P.a-1.

Add sulfuric acid, potassium sulfate and copper (II) sulfate pentahydrate to an analytical sample. Pretreat by Kjeldahl digestion, incineration-hydrochloric acid boiling or aqua regia digestion to convert total phosphorus to phosphate ion, and measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid, to obtain total phosphoric acid (T-P_2O_5) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 9**.

(2) Reagent: Reagents are as shown below.

a) Sulfuric acid: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

b) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

c) Nitric acid: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

d) Ammonia solution: A JIS Guaranteed (NH_3 28 % (mass fraction)) reagent specified in JIS K 8085 or a reagent of equivalent quality.

e) Catalyst (1): Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate (2) specified in JIS K 8983 in the ratio of 9 to 1.

f) Coloring reagent solution (3) (4): Dissolve 1.12 g of ammonium vanadate (V)(5) specified in JIS K 8747 in water, add 250 mL of nitric acid, then add 27 g of hexaammonium heptamolybdate tetrahydrate (6) specified in JIS K 8905 dissolved in water, and further add water to make 1000 mL (7).

g) Phenolphthalein solution (1 g/100 mL): Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

h) Phosphoric acid standard solution (P_2O_5 10 mg/mL) (3): Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, add 2 mL-3 mL of nitric acid, and add water up to the marked line.

i) Phosphoric acid standard solution (P_2O_5 0.5 mg/mL) (3): Put 50 mL of phosphoric acid standard solution (P_2O_5 10 mg/mL) in a 1000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note (**1**) A tablet is commercially available.

(**2**) Crush into powder as appropriate.

(**3**) This is an example of preparation; prepare an amount as appropriate.

(**4**) This corresponds to reagent “a” reagent solution in the Official Methods of Analysis of Fertilizers (1992).

(**5**) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).

(**6**) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).

(**7**) Store in an amber bottle.

Comment 1 Instead of the phosphoric acid standard solution in (**2**), a phosphoric acid standard solution for a calibration curve can be prepared using a phosphoric acid standard solution (P 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate the total phosphoric acid (T-P_2O_5) in an analytical sample by multiplying the concentration (P) of a phosphoric acid standard solution for a calibration curve or a measured value obtained in (**4.3**) by a conversion factor (2.2914).

Comment 2 When using a sample solution obtained in the procedure in (**4.1.3**) **h**) for the measurement of cadmium, nickel, chromium or lead, sulfuric acid and hydrochloric acid in (**2**) should be a reagent of harmful metal analysis grade, microanalysis grade or equivalents.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115

b) **Electric furnace**: An electric furnace that can be adjusted to 550 ºC ± 5 ºC.

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

d) **Digestion flask**: Kjeldahl flask

(**4**) **Test procedures**

(**4.1**) **Sample solution preparation**: Prepare a sample solution as shown below:

(**4.1.1**) **Kjeldahl digestion**

a) Weigh 0.5 g - 5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL digestion flask.

b) Add 5 g - 10 g of catalyst, and further add 20 mL - 40 mL of sulfuric acid, shake to mix and heat gently.

c) After bubbles cease to form, heat until white smoke of sulfuric acid evolves.

d) Ignite until organic matters are completely digested (8).

e) After standing to cool, add a small amount of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water, and further mix by shaking.

f) After cooling is complete, add water up to the marked line

g) Filter with Type 3 filter paper to make a sample solution.

Note (**8**) When the solution has finished changing color, heat further for no less than 2 hours.

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Incineration-hydrochloric acid boiling**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (9).

c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate (9).

d) After standing to cool, moisten the residue with a small amount of water, gradually add about 10 mL of hydrochloric acid, and further add water to make 20 mL.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.

f) After cooling is complete, transfer to a 250-mL - 500-mL volumetric flask with water.

g) Add water up to the marked line.

h) Filter with Type 3 filter paper to make a sample solution.

Note (**9**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 550 °C in 1 to 2 hours.

Comment 4 Do not conduct the procedures in (**4.1.2**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 5 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.1.3**) **Incineration-aqua regia digestion**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char (10).
c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (10).
d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
f) Slightly move the watch glass (11), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated (12).
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (13) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

Note (**10**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.
(**11**) The watch glass can be removed.
(**12**) When exsiccating it, phosphoric acid may not dissolve completely and the concentration may become a low value in the procedure in **g**).
(**13**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 6 Do not conduct the procedures in (**4.1.3**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 7 A sample solution obtained in the procedure in (**4.1.3**) is also applicable to the components shown in Annex B.

(**4.2**) **Coloring**: Conduct coloring as shown below.

a) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 6 mg as P_2O_5) in a 100-mL volumetric flask.
b) Add 1 - 2 drop(s) of phenolphthalein solution (1 g/100 mL), and neutralize by adding ammonia solution (1+1) until the color of the solution becomes light red-purple (14).
c) Add nitric acid (1+10) until the light red-purple color of the solution disappears to make it slightly acidic, and add a proper amount of water (15).
d) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes.

Note (**14**) It is not necessary to add a phenolphthalein solution (1 g/100 mL) when determination can be done by the color change of copper ion (light blue → blue-purple).

(**15**) Without the addition of water, precipitate may be produced when a coloring reagent solution is added.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of spectrophotometer**: Set up the measurement conditions of spectrophotometer considering the following.
Detection wavelength: 420 nm

b) **Calibration curve preparation**

1) Put 1 mL - 12 mL of phosphoric acid standard solution (P_2O_5 0.5 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Add a proper amount of water (15), and conduct the same procedure as (**4.2**) **d**) to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphoric acid standard solutions for the calibration curve preparation.

3) Conduct the same procedures as **2**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 420 nm of the phosphoric acid standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (16).

5) Prepare the calibration curve of the phosphoric acid concentration and absorbance of the phosphoric acid standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the solution in (**4.2**) **d**), measure absorbance by the same procedure as **b**) **4**) (16).

2) Obtain the phosphoric acid (P_2O_5) content from the calibration curve, and calculate total phosphoric acid (T-P_2O_5) in the analytical sample.

Note (**16**) Measure within 6 hours after adding the coloring reagent solution in the procedure in (**4.2**) **d**).

Comment 8 After the procedure in (**4.2**) **a**), it is possible to measure another measurement solution of citrate-soluble phosphoric acid at the same time by adding 4 mL of nitrate acid (1+1) and 2 mL of Petermans citrate solution and by conducting the procedures from (**4.2**) **d**) to (**4.3**) in 4.2.2.a (using b reagent solution in the Official Methods of Analysis of Fertilizers (1992)).
After the procedure in (**4.2**) **a**), it is possible to measure another measurement solution of citrate-soluble phosphoric acid at the same time by adding 4 mL of nitrate acid (1+1) and 17 mL of citric acid solution and by conducting the procedures from (**4.2**) **d**) to (**4.3**) in **4.2.3.a** (using b reagent solution in the Official Methods of Analysis of Fertilizers (1992)).

Comment 9 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 10 % - 20 % (mass fraction) and 1 % (mass fraction) - 5 % (mass fraction) were 99.4 % - 100.2 % and 101.0 % - 105.7 % as total phosphoric acid (T-P_2O_5) respectively.
In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1. In addition, the results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 2 shows the calculation results of reproducibility, intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to

be about 0.04 % (mass fraction) for solid fertilizers and about 0.01 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the collaborative study for the test method validation of total phosphoric acid

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Designated blended fertilizer	11	25.36	0.12	0.5	0.20	0.8
Compund fertilizer	10	15.07	0.05	0.4	0.18	1.2
Fish cakes powder	11	8.57	0.08	0.9	0.16	1.9
Castor oill cakes and powder	9	4.17	0.01	0.1	0.03	0.6
Crustacea grade fertilizer powder	10	3.26	0.01	0.5	0.03	0.8

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2 Analysis results of the collaborative study to determine total phosphoric acid of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)	s_R 8) (%) 3)	RSD_R 9) (%)
FAMIC-C-12	9	8.62	0.03	0.4	0.04	0.4	0.08	0.9

1) The number of laboaratories used for analysis conducting Ammonium vanadomolybdate absorptiometric analysis
2) Mean (the number of laboratory(p) × test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.108- 114, Yokendo, Tokyo (1988)

2) Kimie KATO, Sakiko TAKAHASHI and Yuji SHIRAI: Validation of a Color metric Method for Determination of Nitrogen, Phosphorus and Boron: Evaluation of Calibration curve, Research Report of Fertilizer **Vol. 2**, p. 137 - 144 (2009)

3) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, **Vol. 3** p. 107 - 116 (2010)

4) Yoshiyuki SUNAGA, Yasushi SUGIMURA, Ichiro YOSHIDA and Hidenori KONISHI: Verification of Performance Characteristics of Testing Methods for Phosphorus Content in Fertilizer by Ammonium Vanadomolybdate Absorption Photometry, Research Report of Fertilizer **Vol. 5**, p. 167 - 179 (2012)

5) Toshio HIRABARA, Shin ABE, and Masahiro ECHI: Performance Evaluation of Determination Method for Phosphoric Acid in fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 12**, p. 94 – 108 (2019)

(5) Flow sheet for total phosphoric acid: The flow sheet for total phosphoric acid in fertilizers is shown below:

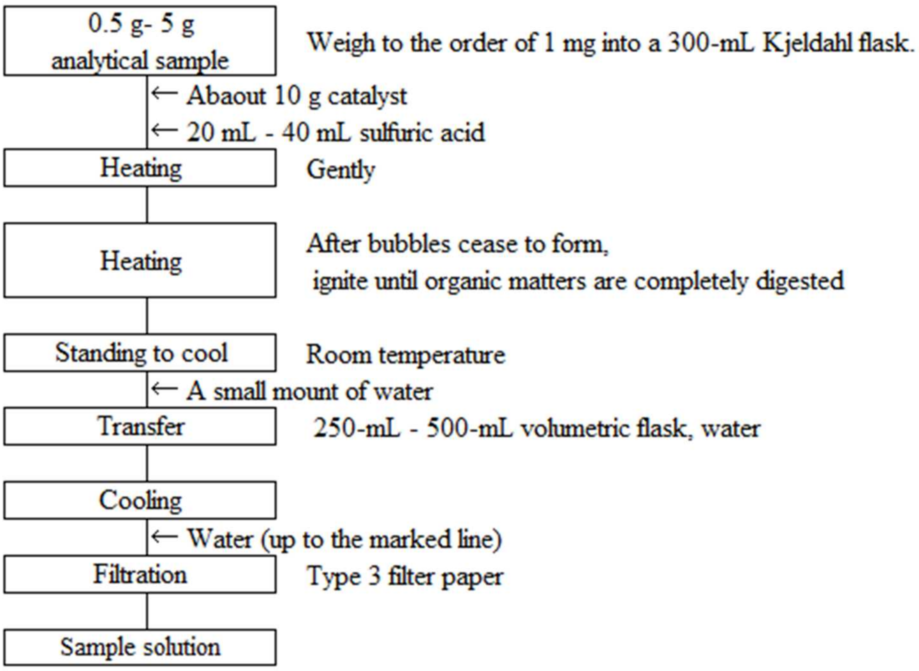


Figure 1-1 Flow sheet for total phosphoric acid in fertilizers
(Kjeldahl digestion procedure (4.1.1))

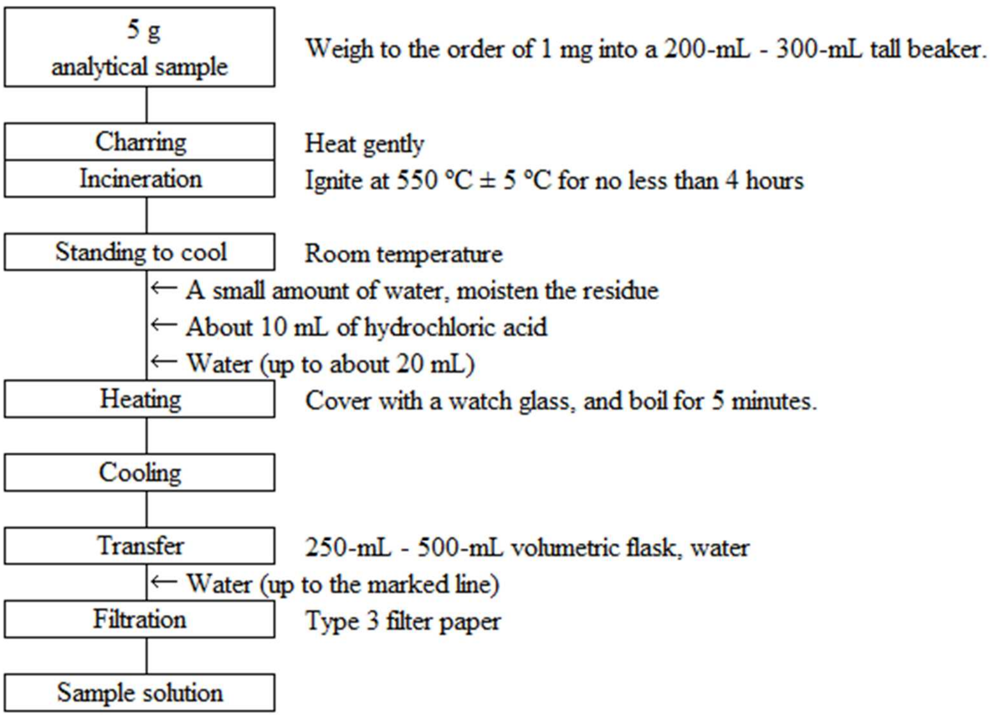


Figure 1-2 Flow sheet for total phosphoric acid in fertilizers
(Incineration-hydrochloric acid boiling procedure (4.1.2))

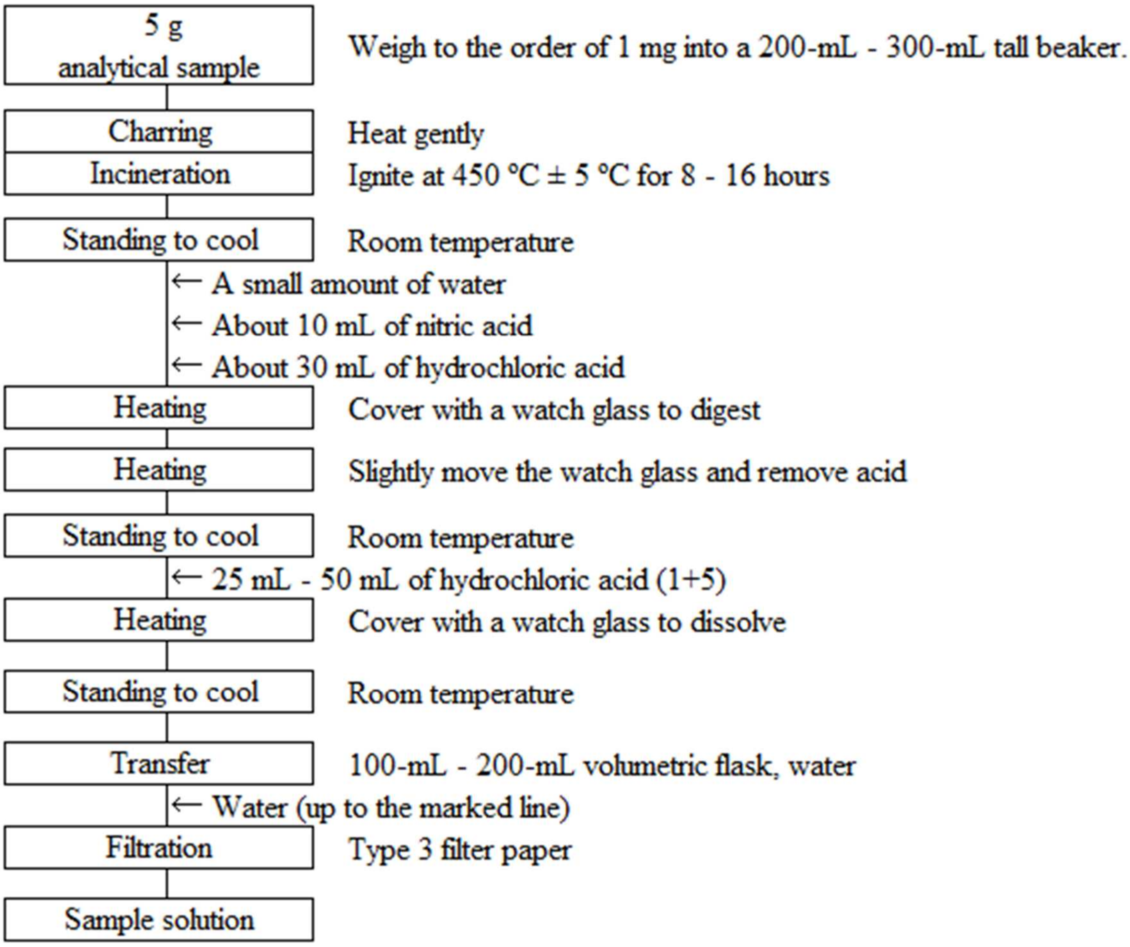


Figure 1-3 Flow sheet for total phosphoric acid in fertilizers
(Incineration-aqua regia digestion orocedure (4.1.3))

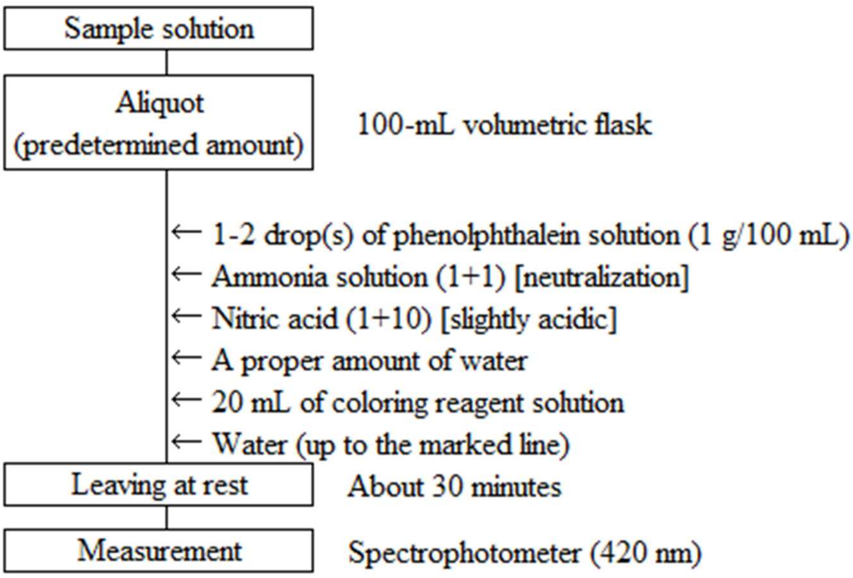


Figure 2 Flow sheet for total phosphoric acid in fertilizers
(Coloring and measurement procedure)

4.2.1.b Quinoline gravimetric analysis

(1) Summary

This testing method is applicable to fertilizers containing organic matters. It is suitable for the fertilizers with relatively a high content of phosphoric acid. This testing method is classified as Type E and its symbol is 4.2.1.b-2017 or T-P.b-1.

Add sulfuric acid, potassium sulfate and copper (II) sulfate pentahydrate to an analytical sample. Pretreat by the Kjeldahl digestion method to convert the total phosphoric acid (T-P_2O_5) to phosphate ion, and measure the mass of quinolinium phosphomolybdate formed by the reaction with quinoline, molybdic acid and nitric acid to obtain the total phosphoric acid (T-P_2O_5) in an analytical sample.

(2) Reagent: Reagents are as shown below.

a) Sulfuric acid: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

b) Nitric acid: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

c) Sodium molybdate solution (1): Dissolve 70 g of sodium molybdate dihydrate in 150 mL of water.

d) Quinoline solution (1): Add 5 mL of quinolone specified in JIS K 8279 to the mixture solution of 35 mL of nitric acid and 100 mL of water.

e) Quimosiac solution (1): Add 60 g of citric acid monohydrate specified in JIS K 8283 to the mixture solution of 85 mL nitric acid and 150 mL of water to dissolve. Add gradually total volume of the sodium molybdate solution to mix. Add gradually the total volume of the quinoline solution while mixing the solution. After leaving at rest overnight, filter the total volume with Type 3 filter paper. Add 280 mL of acetone specified in JIS K 8034, and further add water to make 1000 mL.

f) Catalyst (2): Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate (3) specified in JIS K 8983 in the ratio of 9 to 1.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) A tablet is commercially available.

(**3**) Crush into powder as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Water bath: Water bath that can be adjusted to 60 ºC - 65 ºC.

b) Drying apparatus: A drying apparatus that can be adjusted to 220 ºC ± 5 ºC.

c) Crucible type glass filter: A crucible type glass filter 1G4 specified in JIS R 3503. Let it stand to cool in a desiccator after heating at 220 ºC ± 5 ºC in advance and measure the mass to the order of 1 mg.

d) Digestion flask: Kjeldahl flask

(4) Test procedures

(4.1) Kjeldahl digestion: Conduct digestion as shown below.

a) Weigh 0.5 g - 5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL digestion flask.

b) Add 5 g - 10 g of catalyst, and further add 20 mL - 40 mL of sulfuric acid, shake to mix and heat gently.

c) After bubbles cease to form, heat until white smoke of sulfuric acid evolves.

d) Ignite until organic matters are completely digested (4).

e) After standing to cool, add a small amount of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water.

f) After cooling is complete, add water up to the marked line
g) Filter with Type 3 filter paper to make a sample solution.

Note (**4**) When the solution has finished changing color, heat further for no less than 2 hours.

Comment 1 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measuremen**t: Conduct measurement as shown below.

a) Put a predetermined volume (the equivalents of 10 mg - 30 mg as P_2O_5 and no more than 5 mL as sulfuric acid) of sample solution in a 300-mL tall beaker.
b) Add 5 mL of nitric acid and add water to make about 80 mL.
c) Cover with a watch glass. After boiling for about 3 minutes, wash the watch glass and the inside the tall beaker with water and add water to make about 100 mL.
d) Immediately, add 50mL of quimosiac solution, heat for about 15 minutes while sometimes mixing in a water bath at 60 ºC - 65 ºC to produce the precipitate of quinonium phosphomolybdate.
e) After standing to cool down to room temperature while sometimes mixing, filter under reduced pressure with a crucible type glass filter, wash the tall beaker 3 times with water and transfer the whole precipitate into a crucible type glass filter, and further wash 7 - 8 times with water.
f) Put the precipitate together with the crucible type glass filter in a drying apparatus and heat at 220 ºC ± 5 ºC for about 30 minutes.
g) As soon as heating is complete, move it into a desiccator and let it stand to cool.
h) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.
i) Calculate total phosphoric acid (T-P_2O_5) in the analytical sample by the following formula.

Total phosphoric acid (T-P_2O_5) (% (mass fraction)) in an analytical sample
$=A \times 0.03207 \times (V_1/V_2) \times (1/W) \times 100$

A : Mass (g) of the precipitate in **h**)
W : Mass (g) of the analytical sample
V_1 : Constant volume (mL) of sample solution
V_2 : Aliquot volume (mL) of the sample solution in **a**)

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.98- 106, Yokendo, Tokyo (1988)

(5) Flow sheet for total phosphoric acid: The flow sheet for total phosphoric acid in fertilizers is shown below:

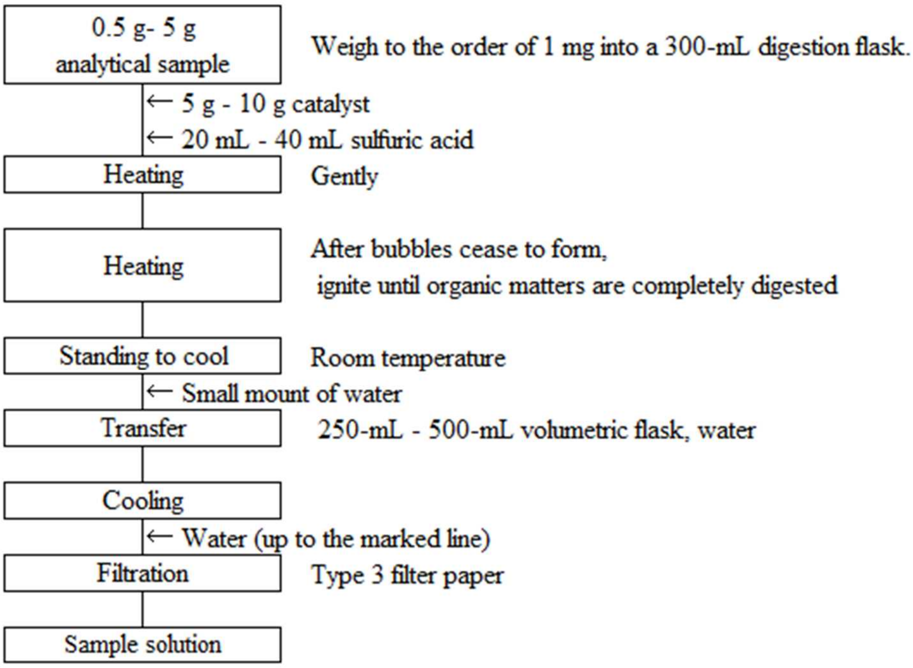


Figure 1 Flow sheet for total phosphoric acid in fertilizers (Kjeldahl digestion procedure)

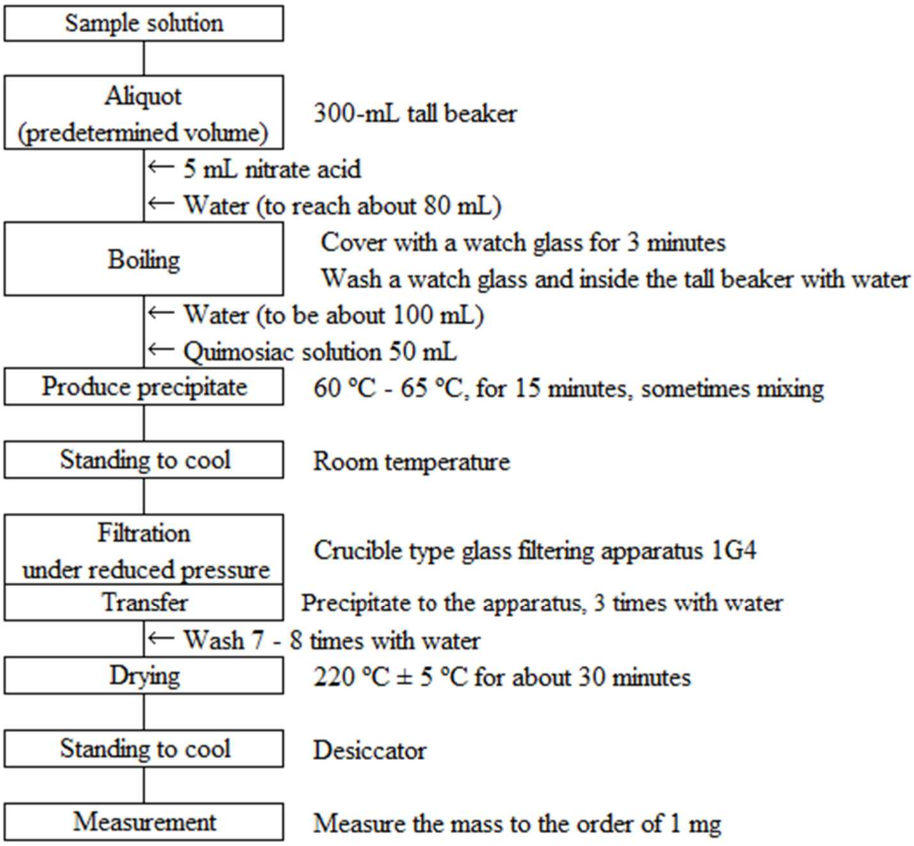


Figure 2 Flow sheet for total phosphoric acid in fertilizers (Measurement procedure)

4.2.2 Citrate-soluble phosphoric acid
4.2.2.a Ammonium vanadomolybdate absorptiometric analysis
(1) Summary
This testing method is applicable to fertilizers that do not contain matter not colored by hydrolysis with nitrate acids such as phosphonic acid. This testing method is classified as Type B and its symbol is 4.2.2.a-2017 or S-P.a-1.
Extract by adding water to an analytical sample, then extract by adding an ammonium citric acid solution, and combine respective pre-determined amounts of extract (equivalent volume). Heat after adding nitric acid (1+1), hydrolyze nonorthophosphoric acid to orthophosphate ion and measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid to obtain ammonia alkaline ammonium citrate-soluble phosphoric acid (citrate-soluble phosphoric acid (S-P_2O_5)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(2) **Reagent**: Reagents are as shown below.
a) **Nitric acid**: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.
b) **Ammonia solution**: A JIS Guaranteed (NH_3 28 % (mass fraction)) reagent specified in JIS K 8085 or a reagent of equivalent quality.
c) **Petermans citrate solution**: Add 173 g of citric acid monohydrate specified in JIS K 8283 in water to dissolve and add gradually an ammonia solution equivalent to 42 g of nitrogen while cooling. After cooling is complete, add water to make 1000 mL. Additionally, check that the specific gravity of the solution is 1.082 - 1.083 (15 ºC) and the nitrogen content per 1 mL is 42 mg.
d) **Coloring reagent solution** (1) (2): Dissolve 1.12 g of ammonium vanadate (V)(3) specified in JIS K 8747 in water, add 150 mL of nitric acid, then add 50 g of hexaammonium heptamolybdate tetrahydrate (4) specified in JIS K 8905 dissolved in water, and further add water to make 1000 mL (5).
e) **Phosphoric acid standard solution (P_2O_5 10 mg/mL)** (1): Heat potassium dihydrogenphosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, add 2 mL-3 mL of nitric acid, and add water up to the marked line.
f) **Phosphoric acid standard solution (P_2O_5 0.5 mg/mL)** (1): Put 50 mL of phosphoric acid standard solution (P_2O_5 10 mg/mL) in a 1000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.
(2) This corresponds to reagent “b” reagent solution in the Official Methods of Analysis of Fertilizers (1992).
(3) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).
(4) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).
(5) Store in an amber bottle. However. the reagent solution cannot tolerate long term preservation.

Comment 1 The coloring reagent solution in **d**) can also be prepared by the following method. Dissolve 2.24 g of ammonium vanadate (V) (3) specified in JIS K 8747 in water, add 300 mL of nitric acid, and add water to make 1000 mL. Separately, add 100 g of hexaammonium heptamolybdate tetrahydrate (4) specified in JIS K 8905 while dissolving in water, and further add water to make 1000 mL. In the case of usage, mix equal volumes of the two solutions.

Comment 2 Instead of the phosphoric acid standard solution in (**2**), a phosphoric acid standard solution for a calibration curve can be prepared using a phosphoric acid standard solution (P 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citrate-soluble phosphoric acid (S-P_2O_5) in an analytical sample by multiplying the concentration (P) of a phosphoric acid standard solution for a calibration curve or a measured value obtained in (**4.3**) by a conversion factor (2.2914).

(**3**) **Instruments**: Instruments are as shown below:

a) **Water bath**: Water bath that can be adjusted to 65 ºC ± 2 ºC.

b) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

c) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a small mortar.

b) Add about 20 mL - 25 mL of water, grind and filter (6) the supernatant with Type 6 filter paper into a 250-mL volumetric flask.

c) Further, after repeating procedure in **b**) 3 times, transfer non-dissolved matter in the small mortar onto a filter paper with water and wash with water until the filtrate becomes about 200 mL.

d) Add a small amount of nitric acid to the filtrate, and further add water up to the marked line to make a sample solution (1).

e) Transfer the non-dissolved matter on the filter paper together with the filter paper to another 250- mL (7) volumetric flask, and add 100 mL of Petermans citrate solution and stopple. Then shake to mix until the filter paper breaks down.

f) Heat the volumetric flask in **e**) in water bath at 65 ºC ± 2 ºC for 1 hour while shaking to mix every 15 minutes.

g) After immediate cooling is complete, add water up to the marked line

h) Filter with Type 3 filter paper to make a sample solution (2).

Note (**6**) It is recommended to use a long stem funnel.

(**7**) It is recommended to use a 250-mL short-neck volumetric flask.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

Comment 4 When the determination is affected by the coloring of the sample solution of **d**) and **h**), put the predetermined volume (8) of the sample solution (1) and the sample solution (2) in a 100-mL volumetric flask, add a few drops of hydrochloric acid (1+1) to acidify, then add no more than 0.1 g of active carbon. After leaving at rest for a little while, add water up to the marked line and filter with Type 3 filter paper. The filtrate is to be the mixture solution for the sample solution of (**4.2**) **a**). Additionally, as phosphorus contained in active carbon has the possibility to elute and affects the determination value, a blank test is required.

(**4.2**) **Coloring**: Conduct coloring as shown below.

a) Put a predetermined amount (the equivalents of 0.5 mg - 6 mg as P_2O_5 and no more than the equivalents of 2 mL of Petermans citrate solution) (8) of the sample solution (1) and the sample solution (2) in a 100-mL volumetric flask.

b) Add the solution to make Petermans citrate solution equivalent to 2 mL.

c) Add 4 mL of nitric acid (1+1) (9), and heat to boil (10).

d) After cooling is complete, add a proper amount of water (11).

e) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes (9).

Comment 5 The volumetric flask used in the procedure in **a**) should be distinguished as a flask to be used for phosphate coloring operation and should not be used for other purposes.

Note (**8**) The transferred volume of the sample solution (1) and the sample solution (2) should be equivalent.

(**9**) When the solution is muddled by adding nitric acid (1+1), conduct centrifugation with about 1700 × *g* centrifugal force (12) for about five minutes after the procedure in **e**).

(**10**) When it does not contain nonorthophosphoric acid, the boiling operation is not necessary.

(**11**) Without the addition of water, precipitate may be produced when a coloring reagent solution is added.

(**12**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of spectrophotometer**: Set up the measurement conditions of spectrophotometer considering the following.
Detection wavelength: 420 nm

b) **Calibration curve preparation**

1) Put 1 mL - 12 mL of phosphoric acid standard solution (P_2O_5 0.5 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Add 2 mL of Petermans citrate solution, 4 mL of nitric acid (1+1) and a proper amount of water (11), and conduct the same procedure as (**4.2**) **e**) to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphoric acid standard solutions for the calibration curve preparation.

3) Conduct the same procedures as **2**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 420 nm of the phosphoric acid standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (13).

5) Prepare the calibration curve of the phosphoric acid concentration and absorbance of the phosphoric acid standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the solution in (**4.2**) **e**), measure absorbance by the same procedure as **b**) **4**) (13).

2) Obtain the phosphoric acid (P_2O_5) content from the calibration curve, and calculate citrate soluble phosphoric acid (S-P_2O_5) in the analytical sample.

Note (**13**) Measure within 2 hours after adding the coloring reagent solution.

Comment 6 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 10 % (mass fraction) - 20 % (mass fraction) and 1 % (mass fraction) - 5 % (mass fraction) were 99.4 % - 100.6 % and 98.6 % - 103.0 % as citrate-soluble phosphoric acid (S-P_2O_5) respectively.

In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1. In addition, the results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 2 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.08 % (mass fraction).

Table 1 Analysis results of the collaborative study for the test method validation of citrate-soluble phosphoric acid

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Compund fertilizer 1	11	1.55	0.02	1.5	0.06	3.6
Compund fertilizer 2	10	5.57	0.04	0.8	0.17	3.1
Compund fertilizer 3	11	9.43	0.13	1.3	0.30	3.2
Double superphosphate of lime	10	44.90	0.32	0.7	0.26	0.6
Compund fertilizer 4	10	51.80	0.21	0.4	0.48	0.9

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2 Analysis results of the collaborative study to determine citrate-soluble phosphoric acid of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)	s_R 8) (%) 3)	RSD_R 9) (%)
FAMIC-B-10	10	8.62	0.04	0.4	0.05	0.6	0.06	0.7
FAMIC-B-14	10	9.18	0.03	0.4	0.04	0.5	0.09	1.0

1) The number of laboaratories used for analysis conducting Ammonium vanadomolybdate absorptiometric analysis
2) Mean (the number of laboratory(p)×test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.108- 114, Yokendo, Tokyo (1988)

2) Kimie KATO, Sakiko TAKAHASHI and Yuji SHIRAI: Validation of a Color metric

Method for Determination of Nitrogen, Phosphorus and Boron: Evaluation of Calibration curve, Research Report of Fertilizer **Vol. 2**, p. 137 - 144 (2009)

3) Akira SHIMIZU and Shin ABE: Verification of Performance Characteristics of Testing Method for Citrate-Soluble Phosphorus Content by Ammonium Vanadomolybdate Absorption Photometry, Research Report of Fertilizer **Vol. 5**, p. 180 - 189 (2012)

4) Toshio HIRABARA, Shin ABE, and Masahiro ECHI: Performance Evaluation of Determination Method for Phosphoric Acid in fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 12**, p. 94 – 108 (2019)

(5) Flow sheet for citrate soluble phosphoric acid: The flow sheet for citrate soluble phosphoric acid in fertilizers is shown below:

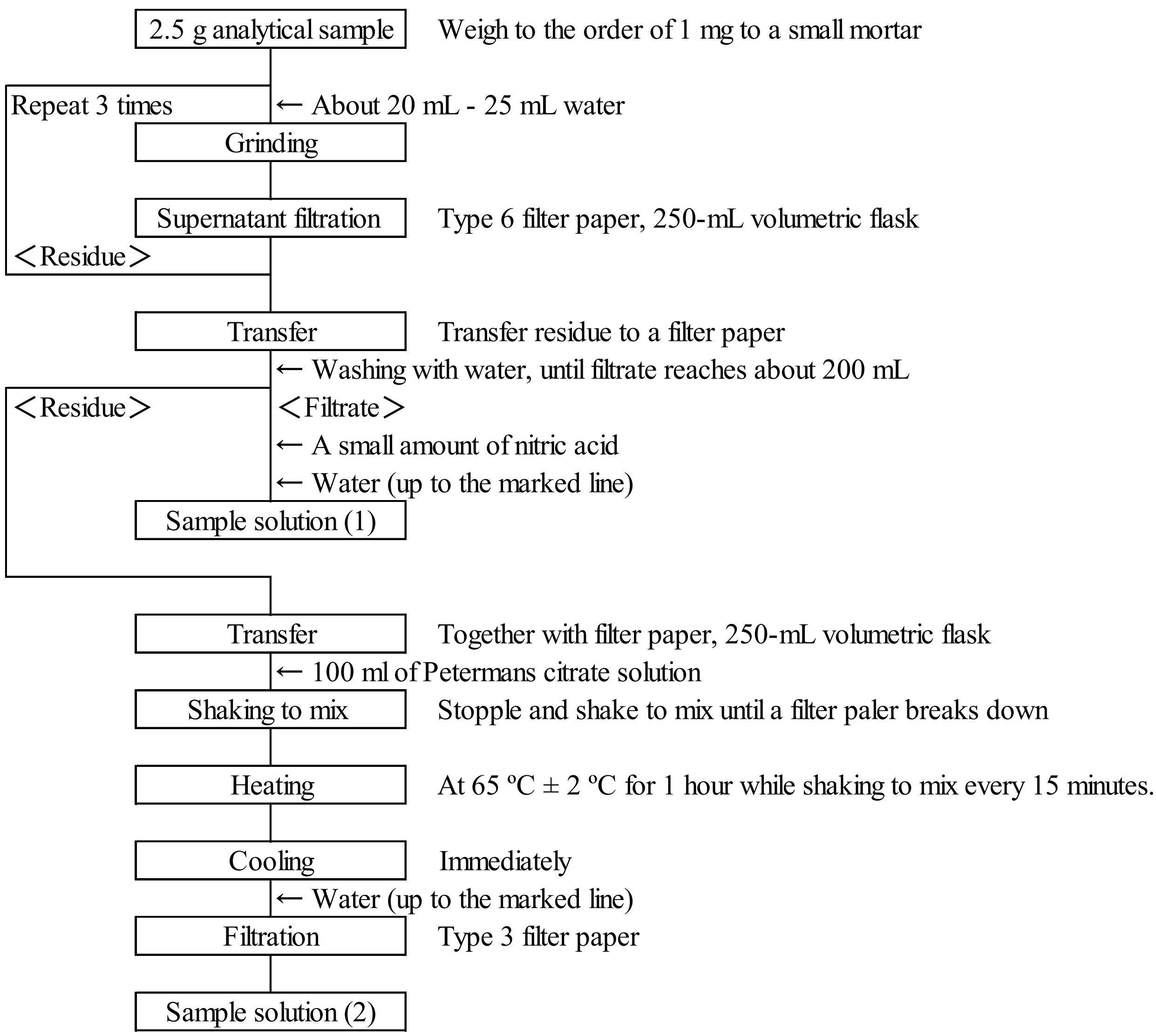


Figure 1 Flow sheet for citrate-soluble phosphoric acid in fertilizers (Extraction procedure)

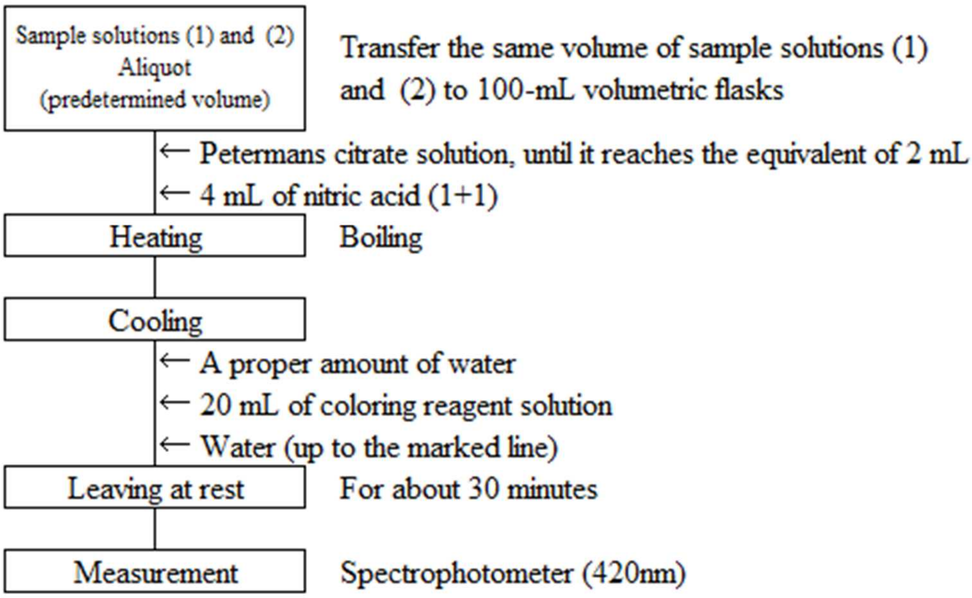


Figure 2 Flow sheet for citrate-soluble phosphoric acid in fertilizers
(Coloring and measurement procedure)

4.2.2.b Quinoline gravimetric analysis

(1) Summary

This testing method is applicable to fertilizers containing no Phosphorous acid, etc. It is suitable for the fertilizers with relatively a high content of phosphoric acid. This testing method is classified as Type E and its symbol is 4.2.2.b-2017 or S-P.b-1.

Extract by adding water to an analytical sample, then extract by adding an ammonium citric acid solution, and combine respective pre-determined amounts of extract (equivalent volume). Heat after adding nitric acid and water, hydrolyze nonorthophosphoric acid to orthophosphate ion and measure the mass of quinolinium phosphomolybdate formed by the reaction with quinoline, molybdic acid and nitric acid to obtain ammonia alkaline ammonium citrate-soluble phosphoric acid (citrate-soluble phosphoric acid (S-P_2O_5)) in an analytical sample.

(2) Reagent: Reagents are as shown below.

a) Nitric acid: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

b) Ammonia solution: A JIS Guaranteed (NH_3 28 % (mass fraction)) reagent specified in JIS K 8085 or a reagent of equivalent quality.

c) Petermans citrate solution: Add 173 g of citric acid monohydrate specified in JIS K 8283 in water to dissolve and add gradually an ammonia solution equivalent to 42 g of nitrogen while cooling. After cooling is complete, add water to make 1000 mL. Additionally, check that the specific gravity of the solution is 1.082 - 1.083 (15 ºC) and the nitrogen content per 1 mL is 42 mg.

d) Sodium molybdate solution: Dissolve 70 g of sodium molybdate dihydrate in 150 mL of water.

e) Quinoline solution: Add 5 mL of quinolone specified in JIS K 8279 to the mixture solution of 35 mL of nitric acid and 100 mL of water.

f) Quimosiac solution: Add 60 g of citric acid monohydrate specified in JIS K 8283 to the mixture solution of 85 mL nitric acid and 150 mL of water to dissolve. Add gradually total volume of the sodium molybdate solution to mix. Add gradually the total volume of the quinoline solution while mixing the solution. After leaving at rest overnight, filter the total volume with Type 3 filter paper. Add 280 mL of acetone specified in JIS K 8034, and further add water to make 1000 mL.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Water bath: Water bath that can be adjusted to 65 °C ± 2 °C and 60 ºC - 65 ºC.

b) Drying apparatus: A drying apparatus that can be adjusted to 220 °C ± 5 ºC.

c) Crucible type glass filter: A crucible type glass filter 1G4 specified in JIS R 3503. Let it stand to cool in a desiccator after heating at 220 ºC ± 5 ºC in advance and measure the mass to the order of 1 mg.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a small mortar.

b) Add about 20 mL - 25 mL of water, grind well and filter [(1)] the supernatant with Type 6 filter paper into a 250-mL volumetric flask.

c) Further, after repeating procedure in **b)** 3 times, transfer non-dissolved matter in the small mortar onto a filter paper with water and wash with water until the filtrate becomes about 200 mL.

d) Add a small amount of nitric acid to the filtrate, and further add water up to the marked line to make a sample solution (1).

e) Transfer the non-dissolved matter on the filter paper together with the filter paper to another 250- mL volumetric flask (2), and add 100 mL of Petermans citrate solution and stopple. Then shake to mix until the filter paper breaks down completely.
f) Heat the volumetric flask in **e**) in water bath at 65 ºC ± 2 ºC for 1 hour while shaking to mix every 15 minutes.
g) After immediate cooling is complete, add water up to the marked line
h) Filter with Type 6 filter paper to make a sample solution (2).

Note (**1**) It is recommended to use a long stem funnel.
(**2**) It is recommended to use a 250-mL short-neck volumetric flask.

Comment 1 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement as shown below.
a) Put a predetermined amount (the equivalents of 10 mg - 30 mg as P_2O_5 and no more than the equivalents of 2 mL of Petermans citrate solution) (3) of the sample solution (1) and the sample solution (2) in a 300-mL tall beaker.
b) Add 5 mL of nitric acid and add water to make about 80 mL.
c) Cover with a watch glass. After boiling for about 3 minutes, wash the watch glass and the inside the tall beaker with water and add water to make about 100 mL.
d) Immediately, add 50mL of quimosiac solution, heat for about 15 minutes while sometimes mixing in a water bath at 60 ºC - 65 ºC to produce the precipitate of quinonium phosphomolybdate.
e) After standing to cool down to room temperature while sometimes mixing, filter under reduced pressure with a crucible type glass filter, wash the tall beaker 3 times with water and transfer the whole precipitate into a crucible type glass filter, and further wash 7 - 8 times with water.
f) Put the precipitate together with the crucible type glass filter in a drying apparatus and heat at 220 ºC ± 5 ºC for about 30 minutes.
g) As soon as heating is complete, move it into a desiccator and let it stand to cool.
h) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.
i) Calculate citrate-soluble phosphoric acid (S-P_2O_5) in the analytical sample by the following formula.

Citrate-soluble phosphoric acid (% (mass fraction)) in an analytical sample
$= A \times 0.03207 \times (V_1/V_2) \times (1/W) \times 100$

A : Mass (g) of the precipitate in **h**)
W : Mass of an analytical sample (2.5 g)
V_1 : Constant volume (250 mL) of the sample solution
V_2 : Aliquot volume (mL) of the sample solution in **a**)

Note (**3**) The transferred volume of the sample solution (1) and the sample solution (2) should be equivalent.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.98- 106, Yokendo, Tokyo (1988)

(5) Flow sheet for citrate soluble phosphoric acid: The flow sheet for citrate soluble phosphoric acid in fertilizers is shown below:

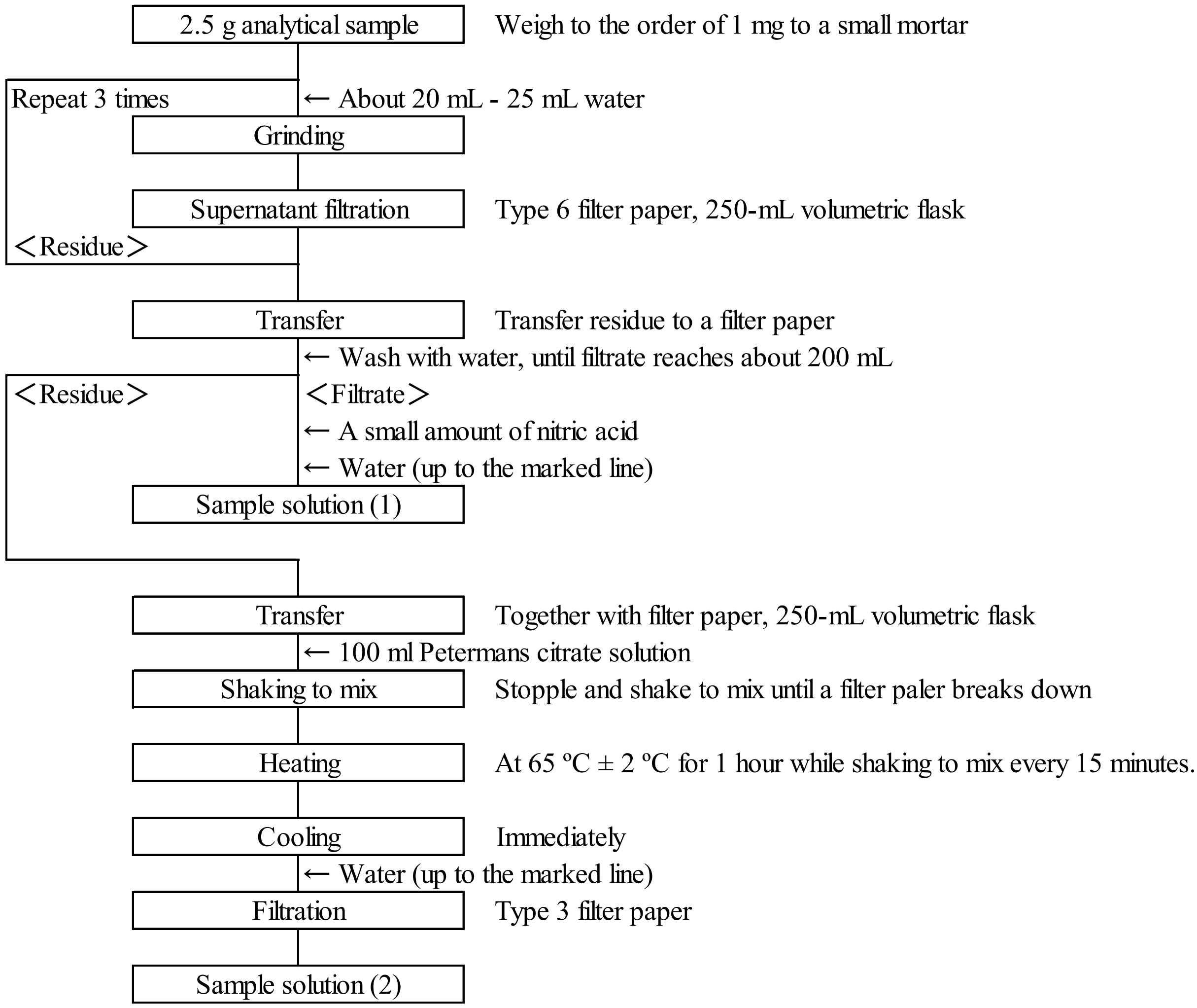


Figure 1 Flow sheet for citrate-soluble phosphoric acid in fertilizers
(Extraction procedure)

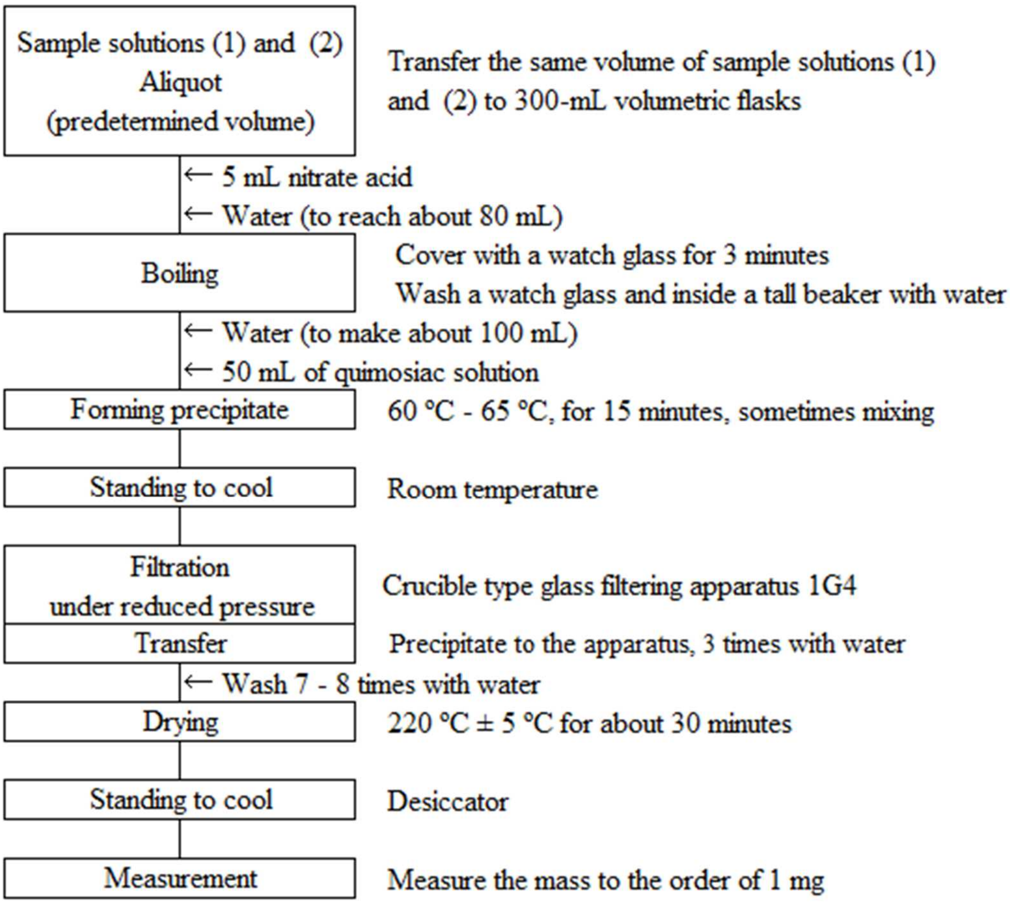


Figure 2 Flow sheet for citrate-soluble phosphoric acid in fertilizers
(Measurement procedure)

4.2.3 Citric acid-soluble phosphoric acid
4.2.3.a Ammonium vanadomolybdate absorptiometric analysis
(1) Summary

This testing method is applicable to fertilizers that do not contain matter not colored by hydrolysis with nitrate acids such as phosphonic acid. This testing method is classified as Type B and its symbol is 4.2.3.a-2018 or C-P.a-2.

Extract by adding a citric acid solution to an analytical sample. Heat after adding nitric acid (1+1), hydrolyze nonorthophosphoric acid to orthophosphate ion and measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid to obtain citric acid-soluble phosphoric acid (C-P_2O_5) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 9**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Nitric acid**: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

b) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

c) **Coloring reagent solution** (1) (2): Dissolve 1.12 g of ammonium vanadate (V)(3) specified in JIS K 8747 in water, add 150 mL of nitric acid, then add 50 g of hexaammonium heptamolybdate tetrahydrate (4) specified in JIS K 8905 dissolved in water, and further add water to make 1000 mL (5).

d) **Phosphoric acid standard solution (P_2O_5 10 mg/mL)** (1): Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, add 2 mL-3 mL of nitric acid, and add water up to the marked line.

e) **Phosphoric acid standard solution (P_2O_5 0.5 mg/mL)** (1): Put 50 mL of phosphoric acid standard solution (P_2O_5 10 mg/mL) in a 1000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This corresponds to reagent "b" reagent solution in the Official Methods of Analysis of Fertilizers (1992).

(**3**) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).

(**4**) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).

(**5**) Store in an amber bottle. However, the reagent solution cannot tolerate long term preservation.

Comment 1 The coloring reagent solution in **c**) can also be prepared by the following method. Dissolve 2.24 g of ammonium vanadate (V) (3) specified in JIS K 8747 in water, add 300 mL of nitric acid, and add water to make 1000 mL. Separately, add 100 g of hexaammonium heptamolybdate tetrahydrate (4) specified in JIS K 8905 while dissolving in water, and further add water to make 1000 mL. In the case of usage, mix equal volumes of the two solutions.

Comment 2 Instead of the phosphoric acid standard solution in (**2**), a phosphoric acid standard solution for a calibration curve can be prepared using a phosphoric acid standard solution (P 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble phosphoric acid (C-P_2O_5) in an analytical sample by multiplying the concentration (P) of a phosphoric acid standard solution for a calibration curve or a measured value obtained in (**4.3**) by a conversion factor (2.2914).

(**3**) **Instruments**: Instruments are as shown below:

a) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

aa) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

ab) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

b) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

c) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Constant-temperature vertical rotating mixer**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (6), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**6**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Horizontal reciprocating water bath shaker**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (7).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (6), and shake to mix by reciprocating horizontally at 160 times /min with amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**7**) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 5 For a by-product phosphate fertilizer, if the pH of the sample solution in (**4.1.1**) **d**) and (**4.1.2**) **d**) is neutral or basic, prepare a sample solution anew by replacing “1 g of an analytical sample” in the procedures in (**4.1.1**) **a**) and (**4.1.2**) **a**) with “0.5 g of an analytical sample”.

Comment 6 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

Comment 7 When the determination is affected by the coloring of the sample solution of (**4.1.1**) **d**) and (**4.1.2**) **d**), transfer the predetermined volume of the sample solution to a 100-mL volumetric flask, add a few drops of hydrochloric acid (1+1) to acidify, then add no more than 0.1 g of active carbon. After leaving at rest for a little while, add water up to the marked line and filter with Type 3 filter paper. The filtrate is prepared as the sample solution of (**4.2**) **a**). Additionally, as phosphorus contained in active carbon has the possibility to elute and affects the determination value, a blank test is required.

(**4.2**) **Coloring**: Conduct coloring as shown below.

a) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 6 mg as P_2O_5 and no more than the equivalents of 17 mL of citric acid solution) in a 100-mL volumetric flask.

b) Add the solution to make citric acid solution equivalent to 17 mL.

c) Add 4 mL of nitric acid (1+1) (8), and heat to boil (9).

d) After cooling is complete, add a proper amount of water (10).

e) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes.

Comment 8 The volumetric flask used in the procedure in **a**) should be distinguished as a flask to be used for phosphate coloring operation and should not be used for other purposes.

Note (**8**) When the solution is muddled by adding nitric acid (1+1), conduct centrifugation with about 1700 × *g* centrifugal force (11) for about five minutes after the procedure in **e**).

(**9**) When it does not contain nonorthophosphoric acid, the boiling operation is not necessary.

(**10**) Without the addition of water, precipitate may be produced when a coloring reagent solution is added.

(**11**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of the spectrophotometer**: Set up the measurement conditions of the spectrophotometer considering the following.
Detection wavelength: 420 nm

b) **Calibration curve preparation**

1) Put 1 mL - 12 mL of phosphoric acid standard solution (P_2O_5 0.5 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Add 17 mL of a citric acid solution, then add 4 mL of nitric acid (1+1), further add a proper amount of water (10) and conduct the same procedure as (**4.2**) **e**) to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphoric acid standard solution for the calibration curve preparation.

3) Conduct the same procedures as **2**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 420 nm of the phosphoric acid standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (12).

5) Prepare the calibration curve of the phosphoric acid concentration and absorbance of the phosphoric acid standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the solution in (**4.2**) **e**), measure absorbance by the same procedure as **b**) **4**) (12).

2) Obtain the phosphoric acid (P_2O_5) content from the calibration curve, and calculate citric acid-soluble phosphoric acid (C-P_2O_5) in the analytical sample.

Note (12) Measure within 2 hours after adding the coloring reagent solution.

Comment 9 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 10 % (mass fraction) - 20 % (mass fraction) and 1 % (mass fraction) - 5 % (mass fraction) were 96.6 % - 103.4 % and 102.0 % - 103.8 % as citric acid-soluble phosphoric acid (C-P_2O_5) respectively.

In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1. In addition, the results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 2 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.03 % (mass fraction) for solid fertilizers and about 0.01 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the collaborative study for the test method validation of citric acid-soluble phosphoric acid

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Processed phosphorus fertilizer	11	42.29	0.14	0.3	0.37	0.9
Fused phosphorus fertilizer	9	20.72	0.21	1.0	0.24	1.2
Compund fertilizer 1	11	10.77	0.12	1.1	0.18	1.7
Compund fertilizer 2	10	4.15	0.02	0.5	0.03	0.8
Compund fertilizer 3	11	1.58	0.02	1.2	0.03	1.9

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2 Analysis results of the collaborative study to determine citric acid-soluble phosphoric acid of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)	s_R[8)] (%)[3)]	RSD_R[9)] (%)
FAMIC-A-10	11	10.05	0.04	0.4	0.05	0.5	0.13	1.3
FAMIC-A-13	10	10.79	0.06	0.6	0.08	0.8	0.09	0.8

1) The number of laboaratories used for analysis conducting Ammonium vanadomolybdate absorptiometric analysis
2) Mean (the number of laboratory (p) × test days(2) × the number of replicate testing (3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.108- 114, Yokendo, Tokyo (1988)

2) Kimie KATO, Sakiko TAKAHASHI and Yuji SHIRAI: Validation of a Color metric Method for Determination of Nitrogen, Phosphorus and Boron: Evaluation of Calibration curve, Research Report of Fertilizer **Vol. 2**, p. 137 - 144 (2009)

3) Yoshiyuki SUNAGA, Yasushi SUGIMURA, Ichiro YOSHIDA and Hidenori KONISHI: Verification of Performance Characteristics of Testing Methods for Phosphorus Content in Fertilizer by Ammonium Vanadomolybdate Absorption Photometry, Research Report of Fertilizer **Vol. 5**, p. 167 - 179 (2012)

4) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)

5) Toshio HIRABARA, Shin ABE, and Masahiro ECHI: Performance Evaluation of Determination Method for Phosphoric Acid in fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 12**, p. 94 – 108 (2019)

(5) **Flow sheet for citric acid-soluble phosphoric acid**: The flow sheet for citric acid-soluble phosphoric acid in fertilizers is shown below:

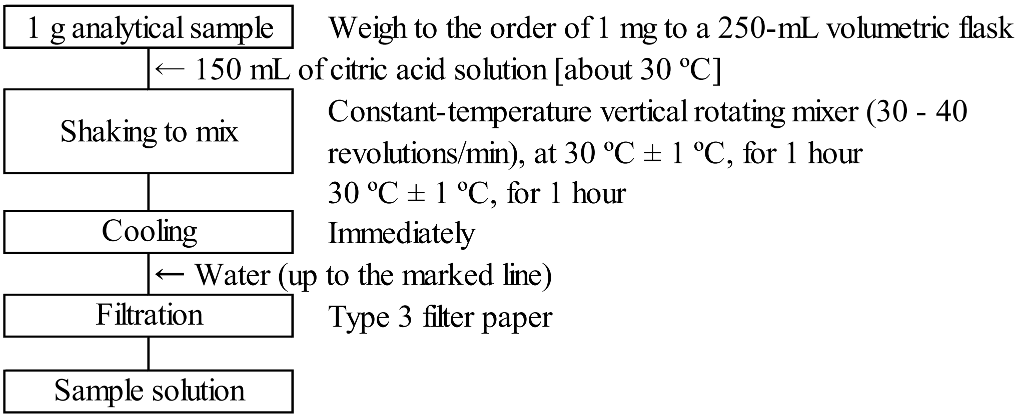


Figure 1-1 Flow sheet for citric acid-soluble phosphoric acid in fertilizers (Extraction procedure (4.1.1))

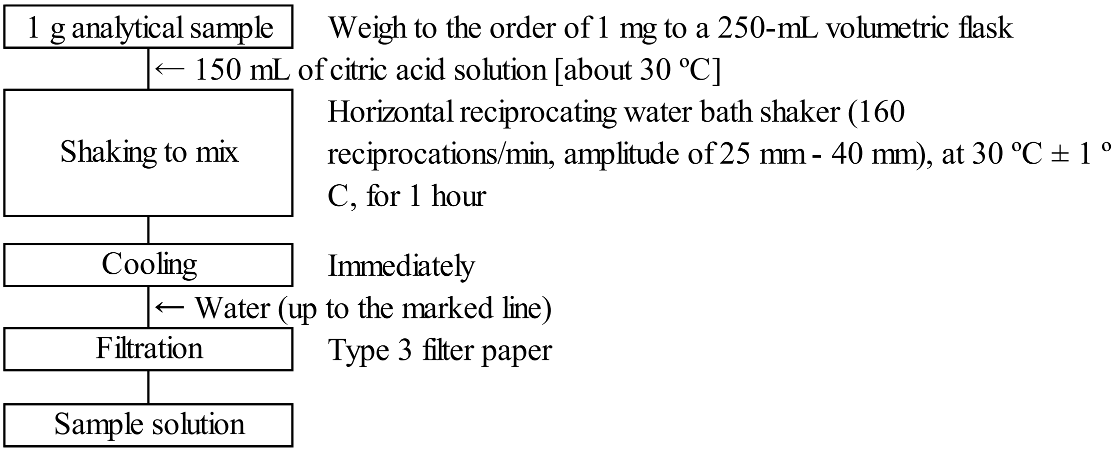


Figure 1-2 Flow sheet for citric acid-soluble phosphoric acid in fertilizers (Extraction procedure (4.1.2))

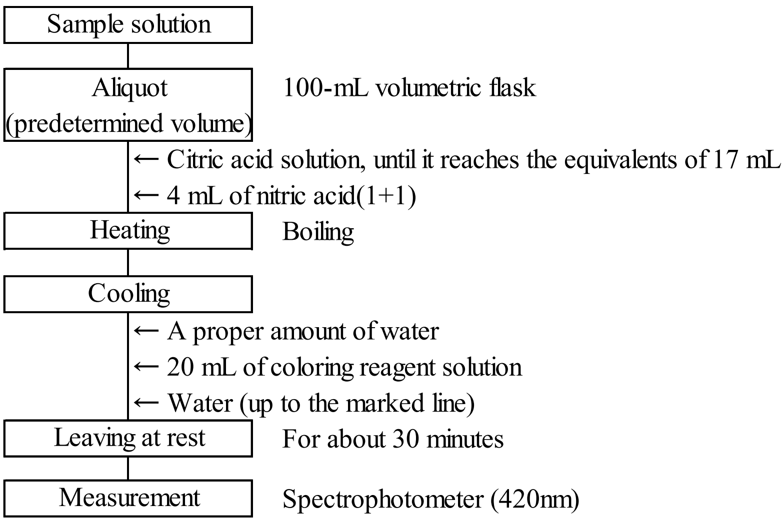


Figure 2 Flow sheet for citric acid-soluble phosphoric acid in fertilizers (Coloring and measurement procedure)

4.2.3.b Ammonium vanadomolybdate absorptiometric analysis (Fertilizers containing phosphorous acid or phosphite)

(1) Summary

This testing method is applicable to the fertilizers containing phosphorous acid or phosphite. This testing method is classified as Type B and its symbol is 4.2.3.b-2018 or C-P.b-2.

Extract by adding a citric acid solution to an analytical sample, add hydrochloric acid - sulfuric acid to heat, and oxygenate phosphorous acid ion to orthophosphate ion, and then measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid to obtain citric acid-soluble phosphoric acid (C-P_2O_5) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(2) Reagent: Reagents are as shown below.

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Nitric acid: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

c) Citric acid solution (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

d) Coloring reagent solution (1) (2): Dissolve 1.12 g of ammonium vanadate (V)(3) specified in JIS K 8747 in water, add 150 mL of nitric acid, then add 50 g of hexaammonium heptamolybdate tetrahydrate (4) specified in JIS K 8905 dissolved in water, and further add water to make 1000 mL (5).

e) Phosphoric acid standard solution (P_2O_5 10 mg/mL) (1): Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, add 2 mL-3 mL of nitric acid, and add water up to the marked line.

f) Phosphoric acid standard solution (P_2O_5 0.5 mg/mL) (1): Put 50 mL of phosphoric acid standard solution (P_2O_5 10 mg/mL) in a 1000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This corresponds to reagent "b" reagent solution in the Official Methods of Analysis of Fertilizers (1992).

(**3**) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).

(**4**) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).

(**5**) Store in an amber bottle. However, the reagent solution cannot tolerate long term preservation.

Comment 1 The coloring reagent solution in **d**) can also be prepared by the following method. Dissolve 2.24 g of ammonium vanadate (V) (3) specified in JIS K 8747 in water, add 300 mL of nitric acid, and add water to make 1000 mL. Separately, add 100 g of hexaammonium heptamolybdate tetrahydrate (4) specified in JIS K 8905 while dissolving in water, and further add water to make 1000 mL. In the case of usage, mix equal volumes of the two solutions.

Comment 2 Instead of the phosphoric acid standard solution in (**2**), a phosphoric acid standard solution for a calibration curve can be prepared using a phosphoric acid standard solution (P 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble phosphoric acid (C-P_2O_5) in an analytical sample by multiplying the concentration (P) of a phosphoric acid standard solution for a calibration curve or a measured value obtained in (**4.3**) by a conversion factor (2.2914).

(**3**) **Instruments**: Instruments are as shown below:

a) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

aa) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

ab) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

b) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

c) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Constant-temperature vertical rotating mixer**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (6), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**6**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Horizontal reciprocating water bath shaker**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (7).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (6), and shake to mix by reciprocating horizontally at 160 times/min with amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**7**) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 5 For a by-product phosphate fertilizer, if the pH of the sample solution in (**4.1.1**) **d**) and (**4.1.2**) **d**) is neutral or basic, prepare a sample solution anew by replacing “1 g of an analytical sample” in the procedures in (**4.1.1**) **a**) and (**4.1.2**) **a**) with “0.5 g of an analytical sample”.

Comment 6 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(**4.2**) **Coloring**: Conduct coloring as shown below.

a) Put a predetermined amount (up to 25 mL, the equivalents of 0.5 mg - 6 mg as P_2O_5) of the sample solution in a 100-mL - 200-mL tall beaker.

b) Add 3 mL of hydrochloric acid and 1 mL of nitric acid.

c) Cover the tall beaker with a watch glass (8), heat on a hot plate or sand bath at 200 ºC - 250 ºC and condense until the solution volume becomes about 2 mL (9).

d) After standing to cool, transfer it with water to a 100- mL volumetric flask (10).

e) Add the citric acid solution to make the equivalents of 17 mL of the citric acid and further add 2 mL of nitric acid (1+1).

f) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes.

Note (**8**) A watch glass should not be uncovered when bubbles form while heating because they may splash.

(**9**) It is recommended to transfer 2 mL of water to a 100-mL - 200-mL tall beaker in advance and confirm the volume.

(**10**) The volume of the solution after transferring should be up to about 50 mL.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of the spectrophotometer**: Set up the measurement conditions of the spectrophotometer considering the following.
Detection wavelength: 420 nm

b) **Calibration curve preparation**

1) Put 1 mL - 12 mL of phosphoric acid standard solution (P_2O_5 0.5 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Add 17 mL of a citric acid solution, then add 4 mL of nitric acid (1+1), further add a proper amount of water (11). Conduct the same procedure as (**4.2**) **f**) to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphoric acid standard solutions for the calibration curve preparation.

3) Conduct the same procedures as **2**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 420 nm of the phosphoric acid standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (12).

5) Prepare the calibration curve of the phosphoric acid concentration and absorbance of the phosphoric acid standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the solution in (**4.2**) **f**), measure absorbance by the same procedure as **b**) **4**) (12).

2) Obtain the phosphoric acid (P_2O_5) content from the calibration curve, and calculate citric acid-soluble phosphoric acid (C-P_2O_5) in the analytical sample.

Note (**11**) If water is not added, precipitate may form when adding the coloring reagent solution.

(**12**) Measure within 2 hours after adding the coloring reagent solution in the procedure in (**4.2**) **f**).

Comment 7 Additive recovery testing was conducted to evaluate trueness using solid fertilizers (10 samples) containing the equivalents of 1.03 % (mass fraction) - 51.04 % (mass fraction) as citric acid-soluble phosphoric acid. As a result, the mean recovery rate was 99 % - 100 %.

The results of the repeatability tests on different days using a solid preparation sample to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Additionally, results from a collaborative study for test method validation and their analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.05 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of citric acid-soluble phosphoric acid (Solid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Preparation sample 1	5	51.01	0.12	0.2	0.16	0.3
Preparation sample 2	5	2.57	0.01	0.6	0.03	1.1

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) × the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Results and analysis results from a collaborative study for the test method validation of citric acid-soluble phosphoric acid

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Processed phosphorus fertilizer	12	47.21	0.13	0.3	0.69	1.5
Compund fertilizer 1	11	17.71	0.07	0.4	0.19	1.1
Compund fertilizer 2	12	5.08	0.08	1.6	0.17	3.3
Absorptive compound fertiliser	11	14.32	0.06	0.4	0.18	1.2
Regent	11	50.89	0.14	0.3	0.57	1.1

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Toshiaki HIROI, Masayuki YAMANISHI Determination Method for Citric Acid-Soluble Phosphorus in Solid Fertilizer Containing Phosphonate (Phosphite) using Spectrophotometer, Research Report of Fertilizer, **Vol. 9**, p. 43 - 58 (2016)
2) Masayuki YAMANISHI, Toshiaki HIROI and Fumika TAKATSU Determination Method for Citric Acid-Soluble and Water-Soluble Phosphorus in Solid Fertilizer Containing Phosphonic Acid or Phosphonate (Phosphite) using Spectrophotometer: A Collaborative Study, **Vol. 9**, p. 59 - 68 (2016)
3) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)

(5) **Flow sheet for citric acid-soluble phosphoric acid containing phosphorus acid, etc.**: The flow sheet for citric acid-soluble phosphoric acid in fertilizers containing phosphorus acid, etc.is shown below:

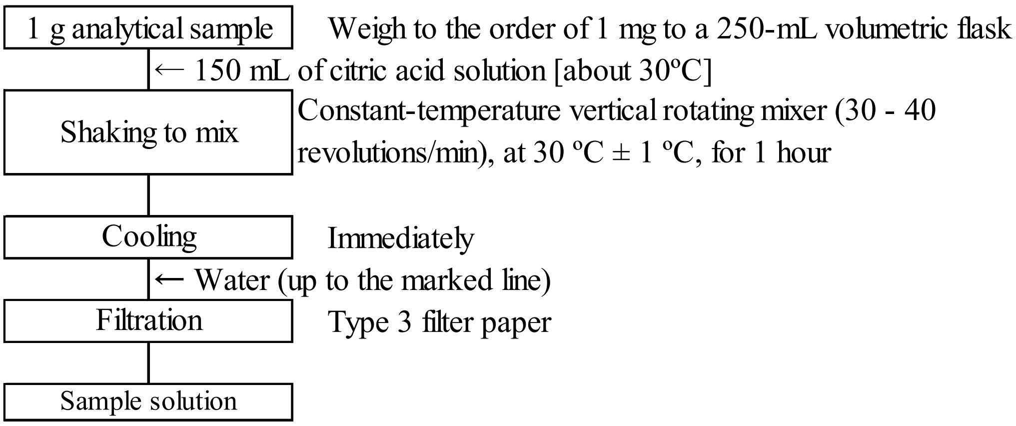


Figure 1-1 Flow sheet for citric acid-soluble phosphoric acid in fertilizers containing phosphorus acid, etc. (Extraction procedure (4.1.1))

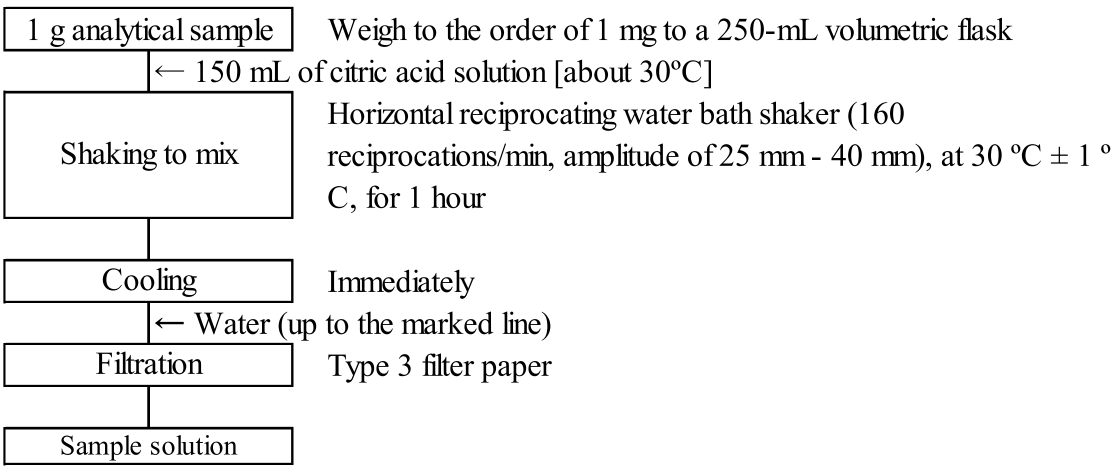


Figure 1-2 Flow sheet for citric acid-soluble phosphoric acid in fertilizers containing phosphorus acid, etc. (Extraction procedure (4.1.2))

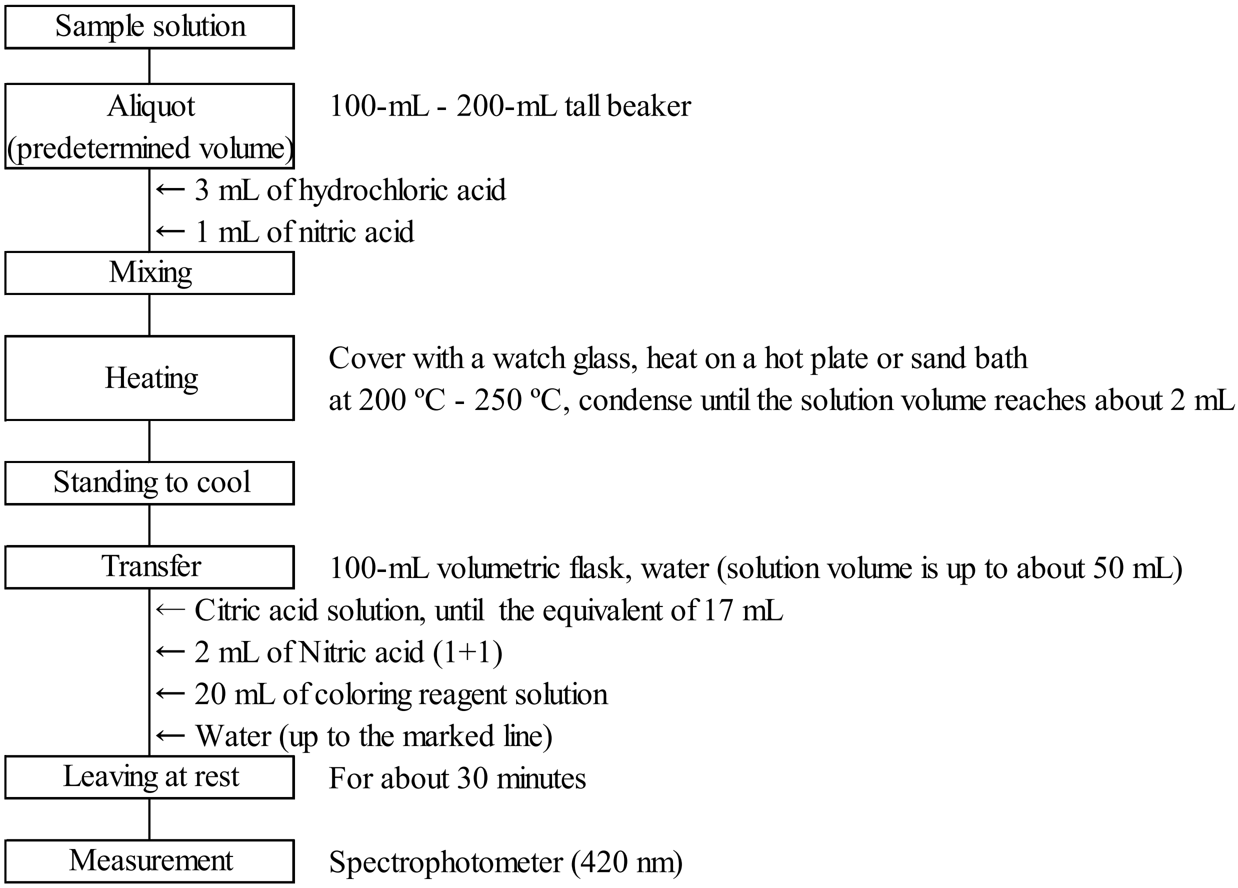


Figure 2 Flow sheet for citric acid-soluble phosphoric acid
in fertilizers containing phosphorus acid, etc. (Coloring and measurement procedure)

4.2.3.c Quinoline gravimetric analysis

(1) Summary

This testing method is applicable to fertilizers containing no phosphorous acid, etc. It is suitable for the fertilizers with relatively a high content of phosphoric acid. This testing method is classified as Type E and its symbol is 4.2.3.c-2017 or C-P.c-1.

Extract by adding a citric acid solution to an analytical sample. Heat after adding nitric acid and water, hydrolyze nonorthophosphoric acid to orthophosphate ion and measure the mass of quinolinium phosphomolybdate formed by the reaction with quinoline, molybdic acid and nitric acid to obtain citric acid-soluble phosphoric acid (C-P_2O_5) in an analytical sample.

(**2**) **Reagent**: Reagents are as shown below.

a) **Nitric acid**: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

b) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

c) **Sodium molybdate solution**: Dissolve 70 g of sodium molybdate dihydrate in 150 mL of water.

d) **Quinoline solution**: Add 5 mL of quinoline specified in JIS K 8279 to the mixture solution of 35 mL of nitric acid and 100 mL of water.

e) **Quimosiac solution**: Add 60 g of citric acid monohydrate specified in JIS K 8283 to the mixture solution of 85 mL nitric acid and 150 mL of water to dissolve. Add gradually total volume of the sodium molybdate solution to mix. Add gradually the total volume of the quinoline solution while mixing the solution. After leaving at rest overnight, filter the total volume with Type 3 filter paper. Add 280 mL of acetone specified in JIS K 8034, and further add water to make 1000 mL.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

b) **Water bath**: Water bath that can be adjusted to 60 ºC - 65 ºC.

c) **Drying apparatus**: A drying apparatus that can be adjusted to 220 ºC ± 5 ºC.

d) **Crucible type glass filter**: A crucible type glass filter 1G4 specified in JIS R 3503. Let it stand to cool in a desiccator after heating at 220 ºC ± 5 ºC in advance and measure the mass to the order of 1 mg.

(4) Test procedures

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 1 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

Comment 2 For a by-product phosphate fertilizer or a fertilizer containing a by-product phosphate, if the pH of the sample solution of **d**) is neutral or basic, prepare a sample solution anew by replacing “1 g of an analytical sample” in the procedure in **a**) with “0.5 g of an analytical sample”.

Comment 3 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedure of (**4.1**) **b**).

(**4.2**) **Measurement**: Conduct measurement as shown below.

a) Put a predetermined volume (the equivalents of 10 mg - 30 mg as P_2O_5) of sample solution in a 300-mL tall beaker.

b) Add 5 mL of nitric acid and add water to make about 80 mL.

c) Cover with a watch glass. After boiling for about 3 minutes, wash the watch glass and the inside the tall beaker with water and add water to make about 100 mL.

d) Immediately, add 50mL of quimosiac solution, heat for about 15 minutes while sometimes mixing in a water bath at 60 ºC - 65 ºC to produce the precipitate of quinolinium phosphomolybdate.

e) After standing to cool down to room temperature while sometimes mixing, filter under reduced pressure with a crucible type glass filter, wash the tall beaker 3 times with water and transfer the whole precipitate into a crucible type glass filter, and further wash 7 - 8 times with water.

f) Transfer the precipitate together with the crucible type glass filter into a drying apparatus and heat at 220 ºC ± 5 ºC for about 30 minutes.

g) As soon as heating is complete, move it into a desiccator and let it stand to cool.

h) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.

i) Calculate citric acid-soluble phosphoric acid (C-P_2O_5) in the analytical sample by the following formula.

Citric acid-soluble phosphoric acid (C-P_2O_5) (% (mass fraction)) in an analytical sample

$$= A \times 0.03207 \times (V_1/V_2) \times (1/W) \times 100$$

A : Mass (g) of the precipitate in **h**)

W : Mass of an analytical sample (1 g)

V_1 : Constant volume (250 mL) of the sample solution

V_2 : Aliquot volume (mL) of the sample solution in **a**)

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.98- 106, Yokendo, Tokyo (1988)

(5) **Flow sheet for citric acid-soluble phosphoric acid**: The flow sheet for citric acid-soluble phosphoric acid in fertilizers is shown below:

1 g analytical sample	Weigh to the order of 1 mg to a 250-mL volumetric flask
← 150 mL of citric acid solution [about 30 ºC]	
Shaking to mix	Constant-temperature vertical rotating mixer (30 - 40 revolutions/min), at 30 ºC ± 1 ºC, for 1 hour
Cooling	Immediately
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Sample solution	

Figure 1 Flow sheet for citric acid-soluble phosphoric acid in fertilizers (Extraction procedure)

Sample solution	
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Aliquot (predetermined volume)	300-mL tall beaker
← 5 mL nitric acid	
← Water (to reach about 80 mL)	
Boiling	Cover with a watch glass for 3 minutes Wash a watch glass and the inside the tall beaker with water
← Water (to be about 100 mL)	
← 50mL of quimosiac solution	
Forming precipitation	60 °C - 65 °C, for 15 minutes, sometimes mixing
Standing to cool	Room temperature
Filtration under reduced pressure	Crucible type glass filtering apparatus 1G4
Transfer	Precipitate to the apparatus, 3 times with water
←Wash 7 - 8 times with water	
Drying	220 °C ± 5 °C for about 30 minutes
Standing to cool	Desiccator
Measurement	Measure the mass to the order of 1 mg

Figure 2 Flow sheet for citric acid-soluble phosphoric acid in fertilizers (Measurement procedure)

4.2.3.d ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fertilizers. It is also suitable for the fertilizers containing phosphite. This testing method is classified as Type D and its symbol is 4.2.3.d-2018 or C-P.d-1.

Extract by adding a citric acid solution to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the phosphorus at a wavelength of 178.287 nm to obtain citric acid-soluble phosphoric acid (citric acid-soluble phosphoric acid (C-P_2O_5)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

d) **Phosphoric acid standard solution (P_2O_5 10 mg/mL)** (1): Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, add 2 mL-3 mL of nitric acid, and add water up to the marked line.

e) **Phosphoric acid standard solution (P_2O_5 1 mg/mL)** (1): Put 10 mL of phosphoric acid standard solution (P_2O_5 10 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.

f) **Phosphoric acid standard solution (P_2O_5 20 µg/mL - 0.4 mg/mL) for the calibration curve preparation** (1): Put 2 mL - 40 mL of phosphoric acid standard solution (P_2O_5 1 mg/mL) in a 100-mL volumetric flask step by step and add hydrochloric acid (1+23) up to the marked line.

g) **Phosphoric acid standard solution ((P_2O_5 5 µg/mL - 20 µg/mL) for the calibration curve preparation** (1): Put 5 mL - 20 mL of phosphoric acid standard solution (P_2O_5 0.1 mg/mL) in a 100-mL volumetric flask step by step and add hydrochloric acid (1+23) up to the marked line.

h) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**) - **g**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the phosphoric acid standard solution in (**2**), a phosphoric acid standard solution for a calibration curve can be prepared using a phosphoric acid standard solution (P 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble phosphoric acid (C-P_2O_5) in an analytical sample by multiplying the concentration (P) of a phosphoric acid standard solution for a calibration curve or a measured value obtained in (**4.2**) by a conversion factor (2.2914).

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Instruments**: Instruments are as shown below:

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0115

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

ba) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

bb) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Constant-temperature vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (2) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Horizontal reciprocating water bath shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (3).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix by reciprocating horizontally at 160 times/min with amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (3) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 5 For a by-product phosphate fertilizer, if the pH of the sample solution in (**4.1.1**) **d**) and (**4.1.2**) **d**) is neutral or basic, prepare a sample solution anew by replacing “1 g of an analytical sample” in the procedures in (**4.1.1**) **a**) and (**4.1.2**) **a**) with “0.5 g of an analytical sample”.

Comment 6 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometry used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following: Analytical line wavelength: 178.287 nm

b) Calibration curve preparation

1) Spray the phosphoric acid standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 178.287 nm.

2) Prepare a curve for the relationship between the phosphoric acid concentration and the indicated value of the phosphoric acid standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 40 mg as P_2O_5) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b) 1)** to read the indicated value.

4) Obtain the phosphoric acid (P_2O_5) content from the calibration curve, and calculate citric acid-soluble phosphoric acid (C-P_2O_5) in the analytical sample.

Comment 7 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 8 The comparison of the measurement value (y_i : 1.74 % (mass fraction) - 49.04 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Ammonium vanadomolybdate absorptiometric analysis was conducted to evaluate trueness using processed phosphorus fertilizers (2 samples), compound fertilizers (12 samples), home garden-use compound fertilizers (1 sample), mixed compost compound fertilizers (2 samples), mixed phosphate fertilizers (2 samples), designated blended fertilizers (4samples), blended fertilizers (5 samples), byproduct compound fertilizers (1 sample), byproduct phosphorus fertilizers (2 samples), organic compound fertilizers (1 sample) and fused phosphate fertilizers (1 sample). As a result, a regression equation was $y=-0.0027+1.001x$, and its correlation coefficient (r) was 1.000. In addition, additive recovery testing was conducted using a preparation sample. As a result, the mean recovery rate at the additive level of 0.260 % (mass fraction) - 49.99 % (mass fraction) was 96.3 % - 100.8 %.

The results of the repeatability tests on different days using compound fertilizers and blended fertilizers to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of citric acid-soluble phosphoric acid (Solid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Compound fertilizer	7	20.90	0.13	0.6	0.18	0.9
Blended fertilizer	7	6.44	0.06	0.9	0.06	1.0

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) × the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination of Water-Soluble Principal Ingredients (W-P2O5, W-K2O, W-MgO, W-MnO and W-B2O3) in Liquid Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 8**, p. 1 - 9 (2015)

2) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)

3) Shingo MATSUO: Simultaneous Determination of Citrate-Soluble Principal Ingredients (C-P2O5, C-K2O, C-MgO, C-MnO and C-B2O3) in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 11**, p. 14 – 28 (2018)

(5) **Flow sheet for citric acid-soluble phosphoric acid**: The flow sheet for citric acid-soluble phosphoric acid in fertilizers is shown below:

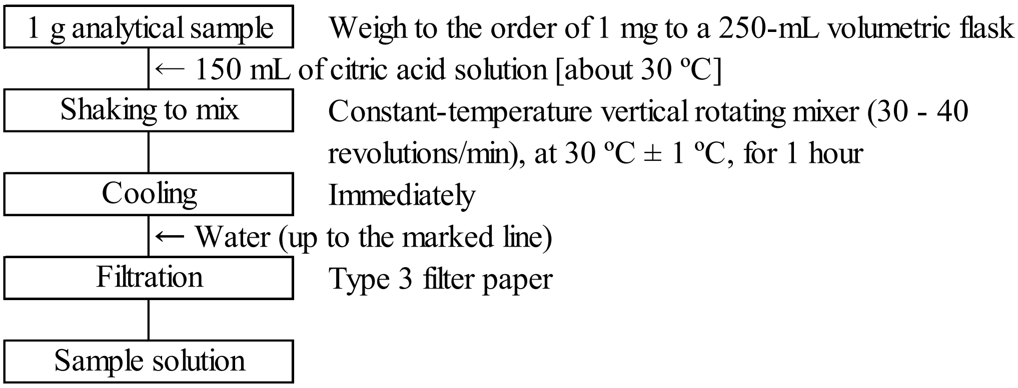


Figure 1-1 Flow sheet for citric acid-soluble phosphoric acid in fertilizers
(Extraction procedure (4.1.1))

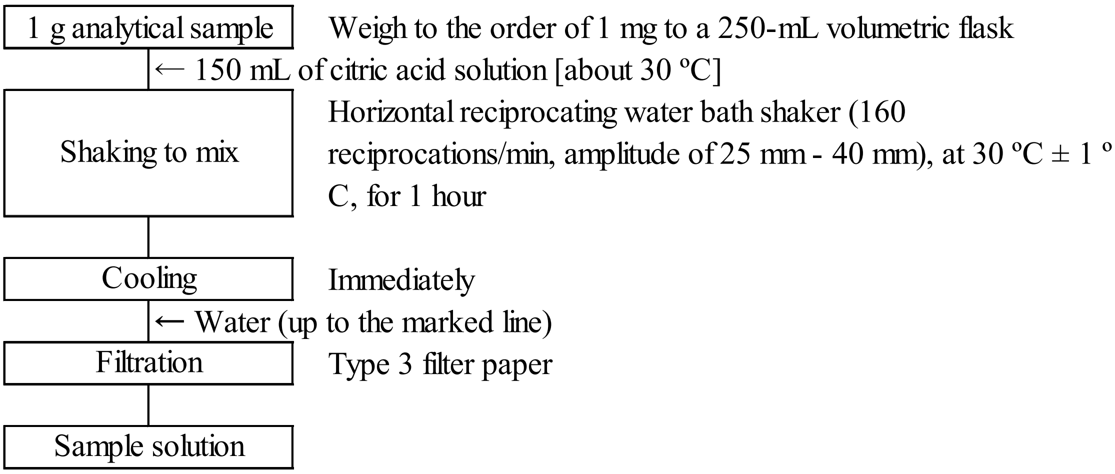


Figure 1-2 Flow sheet for citric acid-soluble phosphoric acid in fertilizers
(Extraction procedure (4.1.2))

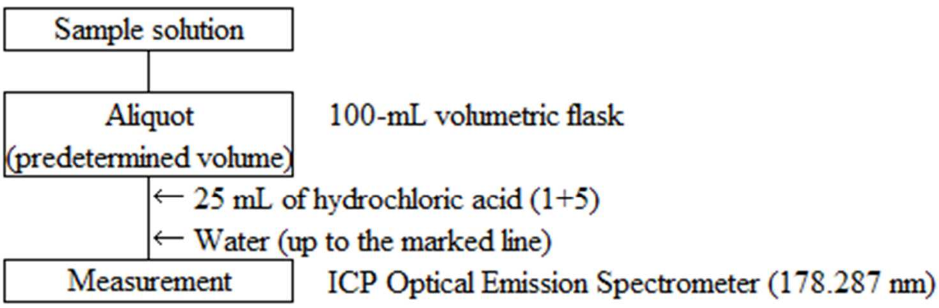


Figure 2 Flow sheet for citric acid-soluble phosphoric acid in fertilizers
(Mmeasurement procedure)

4.2.4 Water-soluble phosphoric acid
4.2.4.a Ammonium vanadomolybdate absorptiometric analysis
(1) Summary

This testing method is applicable to fertilizers that do not contain matter not colored by hydrolysis with nitrate acids such as phosphonic acid. This testing method is classified as Type B and its symbol is 4.2.4.a-2017 or W-P.a-1.

Extract by adding water to an analytical sample, add nitric acid (1+1) to heat, and hydrolyze nonorthophosphoric acid to orthophosphate ion, then measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid to obtain water-soluble phosphoric acid (W-P_2O_5) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 9**.

(2) Reagent: Reagents are as shown below.

a) **Nitric acid**: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

b) **Ammonia solution**: A JIS Guaranteed (NH_3 28 % (mass fraction)) reagent specified in JIS K 8085 or a reagent of equivalent quality.

c) **Coloring reagent solution** (1) (2): Dissolve 1.12 g of ammonium vanadate (V)(3) specified in JIS K 8747 in water, add 250 mL of nitric acid, then add 27 g of hexaammonium heptamolybdate tetrahydrate (4) specified in JIS K 8905 dissolved in water, and further add water to make 1000 mL (5).

d) **Phenolphthalein solution (1 g/100 mL)**: Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

e) **Phosphoric acid standard solution (P_2O_5 10 mg/mL)** (1): Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, add 2 mL-3 mL of nitric acid, and add water up to the marked line.

f) **Phosphoric acid standard solution (P_2O_5 0.5 mg/mL)** (1): Put 50 mL of phosphoric acid standard solution (P_2O_5 10 mg/mL) in a 1000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This corresponds to reagent “a” reagent solution in the Official Methods of Analysis of Fertilizers (1992).

(**3**) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).

(**4**) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).

(**5**) Store in an amber bottle.

Comment 1 Instead of the phosphoric acid standard solution in (**2**), a phosphoric acid standard solution for a calibration curve can be prepared using a phosphoric acid standard solution (P 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble phosphoric acid (W-P_2O_5) in an analytical sample by multiplying the concentration (P) of a phosphoric acid standard solution for a calibration curve or a measured value obtained in (**4.3**) by a conversion factor (2.2914).

(3) Instruments: Instruments are as shown below:

a) **Extractor**: A vertical rotating mixer or vertical reciprocating shaker as described below.

aa) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

ab) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

b) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

c) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

(4.1.1.1) Vertical rotating mixer

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 2 In the procedure of (**4.1.1.1**) **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 3 A sample solution obtained in the procedure in (**4.1.1.1**) is also applicable to the components shown in Annex B.

(4.1.1.2) Vertical reciprocating shaker:

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.
b) Add about 200 mL of water, and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 4 A sample solution obtained in the procedure in (**4.1.1.2**) is also applicable to the components shown in Annex B.

(4.1.2) Fluid test sample

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 5 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 6 When the determination is affected by the coloring of the sample solution of (**4.1.1.1**) **d)**, (**4.1.1.2**) **d**) and (**4.1.2**) **d**), put the predetermined volume of the sample solution in a 100-mL volumetric flask, add a few drops of hydrochloric acid (1+1) to acidify, then add no more than 0.1 g of active carbon. After leaving at rest for a little while, add water up to the marked line and filter with Type 3 filter paper. The filtrate is prepared as the sample solution of (**4.2**) **a**). Additionally, as phosphorus contained in active carbon has the possibility to elute and affects the determination value, a blank test is required.

(**4.2**) **Coloring**: Conduct coloring as shown below.

a) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 6 mg as P_2O_5) in a 100-mL volumetric flask.

b) Add 4 mL of nitric acid (1+1) (6), and heat to boil (7).

c) After cooling is complete, add 1 - 2 drop(s) of phenolphthalein solution (1 g/100 mL), and neutralize by adding ammonia solution (1+1) until the color of the solution becomes light red-purple (14).

d) Add nitric acid (1+10) until the light red-purple color of the solution disappears to make it slightly acidic, and add a proper amount of water (8).

e) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes (6).

Comment 7 The volumetric flask used in the procedure in **a**) should be distinguished as a flask to be used for phosphate coloring operation and should not be used for other purposes.

Note (**6**) When the solution is muddled by adding nitric acid (1+1), conduct centrifugation with about 1700 × *g* centrifugal force (9) for about five minutes after the procedure in **e**).

(**7**) When it does not contain nonorthophosphoric acid, the procedure in **b**) is not necessary.

(**8**) Without the addition of water, precipitate may be produced when a coloring reagent solution is added.

(**9**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of the spectrophotometer**: Set up the measurement conditions of the spectrophotometer considering the following.
Detection wavelength: 420 nm

b) **Calibration curve preparation**

1) Put 1 mL - 12 mL of phosphoric acid standard solution (P_2O_5 0.5 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Add a proper amount of water (8), and conduct the same procedure as (**4.2**) **e**) to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphoric acid standard solutions for the calibration curve preparation.

3) Conduct the same procedures as **2**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 420 nm of the phosphoric acid standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (10).

5) Prepare the calibration curve of the phosphoric acid concentration and absorbance of the phosphoric acid standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the solution in (**4.2**) **e**), measure absorbance by the same procedure as **b**) **4**) (10).

2) Obtain the phosphoric acid (P_2O_5) content from the calibration curve, and calculate water-soluble phosphoric acid (W-P_2O_5) in the analytical sample.

Note (**10**) Measure within 6 hours after adding the coloring reagent solution in the procedure in (**4.2**) **e**).

Comment 8 After the procedure in (**4.2**) **a**), it is possible to measure another measurement solution of citrate-soluble phosphoric acid at the same time by adding 2 mL of Petermans citrate solution and by conducting the procedures from (**4.2**) **c**) to (**4.3**) in **4.2.2.a** (using b reagent solution in the Official Methods of Analysis of Fertilizers (1992)).

After the procedure in (**4.2**) **a**), it is possible to measure another measurement solution of citrate-soluble phosphoric acid at the same time by adding 17 mL of citric acid solution and by conducting the procedures from (**4.2**) **c**) to (**4.3**) in **4.2.3.a** (using b reagent solution in the Official Methods of Analysis of Fertilizers (1992)).

Comment 9 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 10 % (mass fraction) - 20 % (mass fraction) and 1 % (mass fraction) - 5 % (mass fraction) were 100.5 % - 101.2 % and 99.0 % - 101.7 % as water-soluble phosphoric acid (W-P_2O_5) respectively. The comparison of the measurement value (y_i: 0.292 % (mass fraction) - 40.40 % (mass fraction)) of extraction by a vertical reciprocating shaker and the measurement value (x_i) of extraction by a vertical rotating mixer was conducted using fertilizers (12 samples). As a result, a regression equation was $y = -0.041 + 0.999x$, and its correlation coefficient (r) was 1.000. The comparison of the measurement value (y_i: 1.92 % (mass fraction) - 12.21 % (mass fraction)) of simple extraction and the measurement value (x_i) of extraction by a vertical rotating mixer was conducted using fluid fertilizers (12 samples). As a result, a regression equation was $y = 0.005 + 1.005x$, and its correlation coefficient (r) was 0.999.

The results of the repeatability tests on different days using compound fertilizers, designated blended fertilizer and fluid compound fertilizer (2 samples) to evaluate precision were analyzed by one-way analysis of variance. Tables 1-1 and 1-2 show the calculation results of intermediate precision and repeatability. Table 2 shows results and analysis results from a collaborative study for testing method validation. In addition, the results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 3 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.03 % (mass fraction) for solid fertilizers and about 0.004 % (mass fraction) for fluid fertilizers.

Table 1-1 Analysis results of the repeatability tests on different days of water-soluble phosphoric acid (Solid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Designated blended fertilizer	7	13.77	0.03	0.2	0.06	0.5
Compound fertilizer	7	1.19	0.01	0.5	0.01	0.5

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) × the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 1-2 Analysis results of the repeatability tests on different days of water-soluble phosphoric acid (Fluid fertilizer)

Name of sample	Test days of repeatability (T)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Fluid compound fertilizer 1	7	12.19	0.02	0.2	0.05	0.4
Fluid compound fertilizer 2	7	2.88	0.01	0.2	0.02	0.5

Footnote: Refer to Table 1-1

Table 2 Analysis resultsof the collaborative study for the test method validation of water-soluble phosphoric acid

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Compund fertilizer 1	11	48.43	0.26	0.5	0.34	0.7
Double superphosphate of lime	11	36.21	0.29	0.8	0.47	1.3
Compund fertilizer 2	11	12.67	0.14	1.1	0.25	2.0
Compund fertilizer 3	10	2.82	0.02	0.6	0.05	1.7
Compund fertilizer 4	11	0.91	0.01	1.4	0.07	7.2

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 3 Analysis results of the collaborative study to determine water-soluble phosphoroc acid of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)	s_R[8] (%)[3]	RSD_R[9] (%)
FAMIC-B-10	9	7.00	0.02	0.3	0.03	0.5	0.07	1.0
FAMIC-B-14	15	6.70	0.02	0.3	0.03	0.5	0.06	0.9

1) The number of laboaratories used for analysis conducting Ammonium vanadomolybdate absorptiometric analysis
2) Mean (the number of laboratory(p) × test days(2) × the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.108- 114, Yokendo, Tokyo (1988)

2) Kimie KATO, Sakiko TAKAHASHI and Yuji SHIRAI: Validation of a Color metric Method for Determination of Nitrogen, Phosphorus and Boron: Evaluation of Calibration curve, Research Report of Fertilizer **Vol. 2**, p. 137 - 144 (2009)

3) Yoshiyuki SUNAGA, Yasushi SUGIMURA, Ichiro YOSHIDA and Hidenori KONISHI:

Verification of Performance Characteristics of Testing Method for Citrate-Soluble Phosphorus Content by Ammonium Vanadomolybdate Absorption Photometry, Research Report of Fertilizer **Vol. 5**, p. 167 - 179 (2012)
4) Shinji KAWAGUCHI: Simple Extraction Method for Water-Soluble Components in Liquid Compound Fertilizers, Research Report of Fertilizer, **Vol. 9**, p. 10 - 20 (2016)
5) Shinji KAWAGUCHI: Extraction Method for the Water-soluble Principal Ingredients in the Solid Fertilizer using a General-purpose Equipment, Research Report of Fertilizer, **Vol. 10**, p. 1 - 8 (2017)
6) Toshio HIRABARA, Shin ABE, and Masahiro ECHI: Performance Evaluation of Determination Method for Phosphoric Acid in fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 12**, p. 94 – 108 (2019)

(**5**) **Flow sheet for water-soluble phosphoric acid**: The flow sheet for water-soluble phosphoric acid: in fertilizers is shown below:

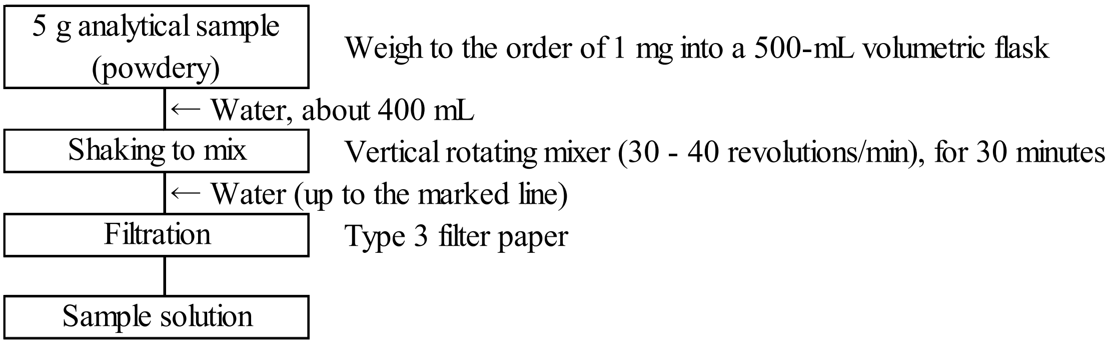


Figure 1-1 Flow sheet for water-soluble phosphoric acid in fertilizers
(Extraction procedure (4.1.1.1))

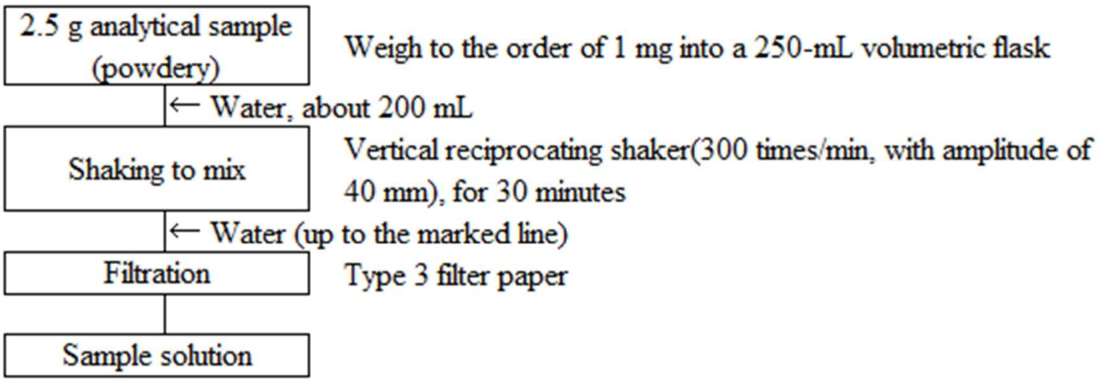


Figure 1-2 Flow sheet for water-soluble phosphoric acid in fertilizers
(Extraction procedure (4.1.1.2))

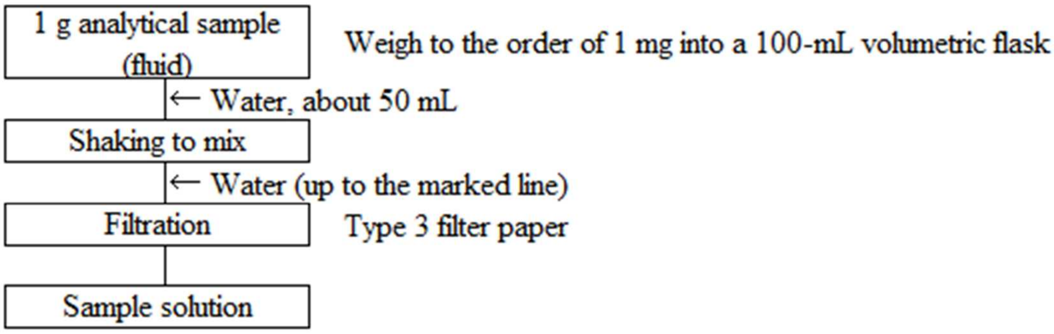


Figure 1-3 Flow sheet for water-soluble phosphoric acid in fertilizers (Extraction procedure (4.1.2))

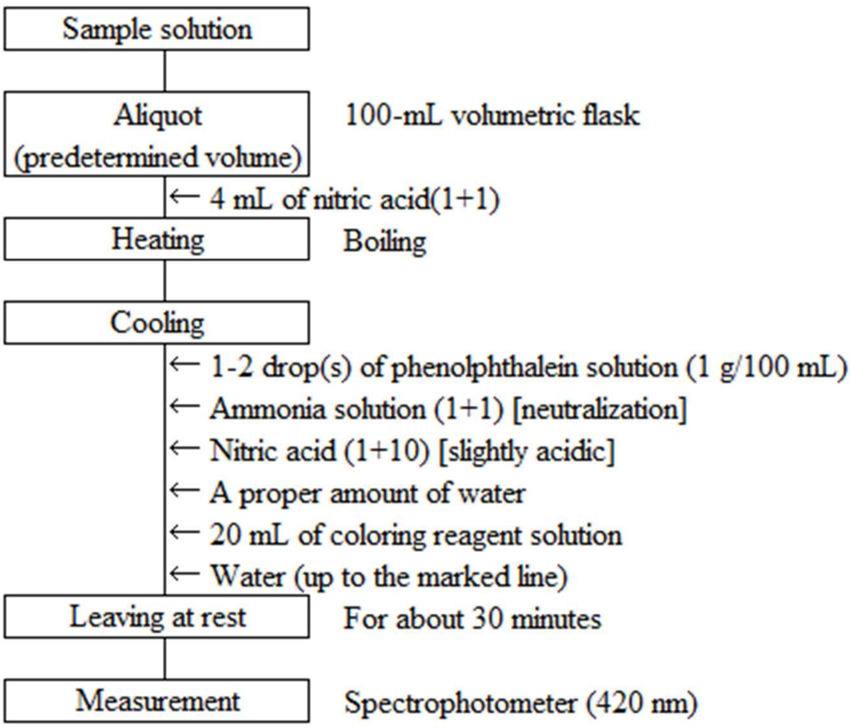


Figure 2 Flow sheet for water-soluble phosphoric acid in fertilizers (Coloring and measurement procedure)

4.2.4.b Ammonium vanadomolybdate absorptiometric analysis (Fertilizers containing phosphorous acid or phosphite)

(1) Summary

This testing method is applicable to the fertilizers containing phosphorous acid or phosphite. This testing method is classified as Type B and its symbol is 4.2.4.b-2017 or W-P.b-1.

Extract by adding water to an analytical sample, add hydrochloric acid - sulfuric acid to heat, and oxygenate phosphorous acid ion to orthophosphate ion, and then measure the absorbance of phosphovanadomolybdate salt formed by the reaction with ammonium vanadate (V), hexaammonium heptamolybdate and nitric acid to obtain water-soluble phosphoric acid (W-P_2O_5) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(**2**) **Reagen**t: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Nitric acid**: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

c) **Ammonia solution**: A JIS Guaranteed (NH_3 28 % (mass fraction)) reagent specified in JIS K 8085 or a reagent of equivalent quality.

d) **Coloring reagent solution** (1) (2): Dissolve 1.12 g of ammonium vanadate (V)(3) specified in JIS K 8747 in water, add 250 mL of nitric acid, then add 27 g of hexaammonium heptamolybdate tetrahydrate(4) specified in JIS K 8905 dissolved in water, and further add water to make 1000 mL (5).

e) **Phenolphthalein solution (1 g/100 mL)**: Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

f) **Phosphoric acid standard solution (P_2O_5 10 mg/mL)** (1): Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, add 2 mL-3 mL of nitric acid, and add water up to the marked line.

g) **Phosphoric acid standard solution (P_2O_5 0.5 mg/mL)** (1): Put 50 mL of phosphoric acid standard solution (P_2O_5 10 mg/mL) in a 1000-mL volumetric flask, add 2 mL - 3 mL of nitric acid, and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This corresponds to reagent "a" reagent solution in the Official Methods of Analysis of Fertilizers (1992).

(**3**) This corresponds to ammonium metavanadate in the Official Methods of Analysis of Fertilizers (1992).

(**4**) This corresponds to ammonium molybdate in the Official Methods of Analysis of Fertilizers (1992).

(**5**) Store in an amber bottle.

Comment 1 Instead of the phosphoric acid standard solution in (**2**), a phosphoric acid standard solution for a calibration curve can be prepared using a phosphoric acid standard solution (P 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble phosphoric acid (W-P_2O_5) in an analytical sample by multiplying the concentration (P) of a phosphoric acid standard solution for a calibration curve or a measured value obtained in (**4.3**) by a conversion factor (2.2914).

(3) Instruments: Instruments are as shown below:

a) **Extractor**: A vertical rotating mixer or vertical reciprocating shaker as described below.
aa) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 250-mL - 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.
ab) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).
b) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.
c) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115

(4) Test procedure
(4.1) Extraction: Conduct extraction as shown below.
(4.1.1) Powdery test sample
(4.1.1.1) Vertical rotating mixer
a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 2 In the procedure of (**4.1.1.1**) **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).
Comment 3 A sample solution obtained in the procedure in (**4.1.1.1**) is also applicable to the components shown in Annex B.

(4.1.1.2) Vertical reciprocating shaker:
a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.
b) Add about 200 mL of water, and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 4 A sample solution obtained in the procedure in (**4.1.1.2**) is also applicable to the components shown in Annex B.

(4.1.2) Fluid test sample
a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 5 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(4.2) Coloring: Conduct coloring as shown below.
a) Put a predetermined amount (the equivalents of 0.5 mg - 6 mg as P_2O_5) of the sample solution

in a 100-mL - 200-mL tall beaker.

b) Add 3 mL of hydrochloric acid and 1 mL of nitric acid.

c) Cover the tall beaker with a watch glass, heat on a hot plate or sand bath at 200 ºC - 250 ºC and condense (7) until the solution volume becomes about 2 mL (6).

d) After standing to cool, transfer it with water to a 100- mL volumetric flask (8).

e) Add 1 - 2 drop (s) of phenolphthalein solution (1 g/100 mL), and neutralize by adding ammonia solution (1+1) until the color of the solution becomes light red-purple.

f) Add nitric acid (1+10) until the light red-purple color of the solution disappears to make it slightly acidic.

g) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes.

Note (**6**) It is recommended to transfer about 2 mL of water to a 100-mL - 200-mL tall beaker in advance and confirm the volume.

(**7**) Care should be taken not to exsiccate it. When it exsiccates, the determined value becomes lower than usual in some cases.

(**8**) The volume of the solution after transferring should be up to about 50 mL.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of the spectrophotometer**: Set up the measurement conditions of the spectrophotometer considering the following.
Detection wavelength: 420 nm

b) **Calibration curve preparation**

1) Put 1 mL - 12 mL of phosphoric acid standard solution (P_2O_5 0.5 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Add a proper amount of water (9), and conduct the same procedure as (**4.2**) **g**) to make the P_2O_5 0.5 mg/100 mL - 6 mg/100 mL phosphoric acid standard solutions for the calibration curve preparation.

3) Conduct the same procedures as **2**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength of 420 nm of the phosphoric acid standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (10).

5) Prepare the calibration curve of the phosphoric acid concentration and absorbance of the phosphoric acid standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the solution in (**4.2**) **g**), measure absorbance by the same procedure as **b**) **4**) (10).

2) Obtain the phosphoric acid (P_2O_5) content from the calibration curve, and calculate water-soluble phosphoric acid (W-P_2O_5) in the analytical sample.

Note (**9**) If water is not added, precipitate may form when adding the coloring reagent solution.

(**10**) Measure within 6 hours after adding the coloring reagent solution in the procedure in (**4.2**) **g**).

Comment 6 Recovery testing was conducted to evaluate trueness using a fluid preparation sample. As a result, the mean recovery rates at the content level of 30 % (mass fraction) - 50 % (mass fraction), 10 % (mass fraction) - 20 % (mass fraction), 4 % (mass fraction) and 0.2 % (mass fraction) were 101.1 % - 101.8 %, 101.1 % - 101.5 %, 100.8 % and

102.5 % as water-soluble phosphoric acid (W-P_2O_5) respectively. In the case of using a solid preparation sample, the average rate of recovery at the content level of 30 % (mass fraction) - 59 % (mass fraction), 12 % (mass fraction) - 21 % (mass fraction) and 1 % (mass fraction) - 9 % (mass fraction) are 99.5 % - 100.4 %, 99.3 % - 100.3 %, and 96.9 % - 100.4% respectively.

The results of the repeatability tests on different days using a solid preparation sample to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Also, table 2-1 and 2-2 show results from a collaborative study for test method validation using solid and fluid fertilizers and its analysis.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.04 % (mass fraction) for solid fertilizers and about 0.01 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the repeatability tests on different days of water-soluble phosphoric acid (Solid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Preparation sample 1	7	59.36	0.09	0.2	0.13	0.2
Preparation sample 2	7	5.90	0.07	1.2	0.07	1.2

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) × the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2-1 Analysis results of the collaborative study for the test method validation of water-soluble phosphoric acid (Solid fertilizer)

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Compound fertilizer 1	12	58.47	0.13	0.2	0.42	0.7
Blended fertilizer	12	21.80	0.12	0.5	0.18	0.8
Compound fertilizer 2	12	13.37	0.10	0.7	0.20	1.5
Absorptive compound fertilizer	12	7.16	0.03	0.4	0.16	2.3
Compound fertilizer 3	12	3.92	0.04	1.0	0.08	2.1

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2-2 Analysis results of the collaborative study
for the test method validation of water-soluble phosphoric acid (Fluid mixed fertilizer)

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Fluid compound fertilizer 1	12	33.56	0.25	0.7	0.59	1.8
Fluid compound fertilizer 5	11	24.10	0.08	0.3	0.47	2.0
Fluid compound fertilizer 2	12	17.93	0.08	0.5	0.30	1.7
Fluid compound fertilizer 4	11	11.93	0.13	1.1	0.33	2.8
Fluid compound fertilizer 3	12	7.99	0.12	1.5	0.31	3.8

Footnote: Refer to Table 2-1

References

1) Toshiaki HIROI, Masakazu SAIKI and Kimie KATO: Development and Validation of Spectrophotometry for Determination of Water-Soluble Phosphoric acid in Liquid Mixed Fertilizer Containing Phosphonic acid, Research Report of Fertilizer, **Vol. 1** P. 25 - 33 (2008)

2) Toshiaki HIROI, Masakazu SAIKI and Kimie KATO: Determination of Water-Soluble Phosphoric Acid in Liquid Mixed Fertilizer Containing Phosphonic Acid by Spectrophotometry: A Collaborative Study, Research Report of Fertilizer, **Vol. 1** p. 34 - 40 (2008)

3) Fumihiro ABE, Noriyuki SASAKI and Toshio HIRABARA: Determination of Water-Soluble Phosphorus in Solid Fertilizer Containing Phosphonic Acid by Spectrophotometry, Research Report of Fertilizer, **Vol. 8**, p. 10 - 16 (2015)

4) Masayuki YAMANISHI, Toshiaki HIROI and Fumika TAKATSU Determination Method for Citric Acid-Soluble and Water-Soluble Phosphorus in Solid Fertilizer Containing Phosphonic Acid or Phosphonate (Phosphite) using Spectrophotometer: A Collaborative Study, **Vol. 9**, p. 59 - 68 (2016)

(5) **Flow sheet for water-soluble phosphoric acid containing phosphorus acid, etc.**: The flow sheet for water-soluble phosphoric acid in fertilizers containing phosphorus acid, etc.is shown below:

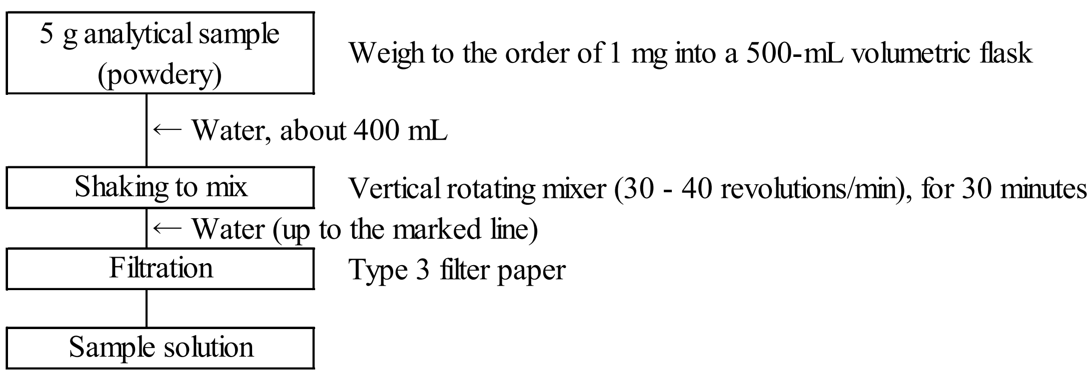


Figure 1-1 Flow sheet for water-soluble phosphoric acid in fertilizers containing phosphorus acid, etc. (Extraction procedure (4.1.1.1))

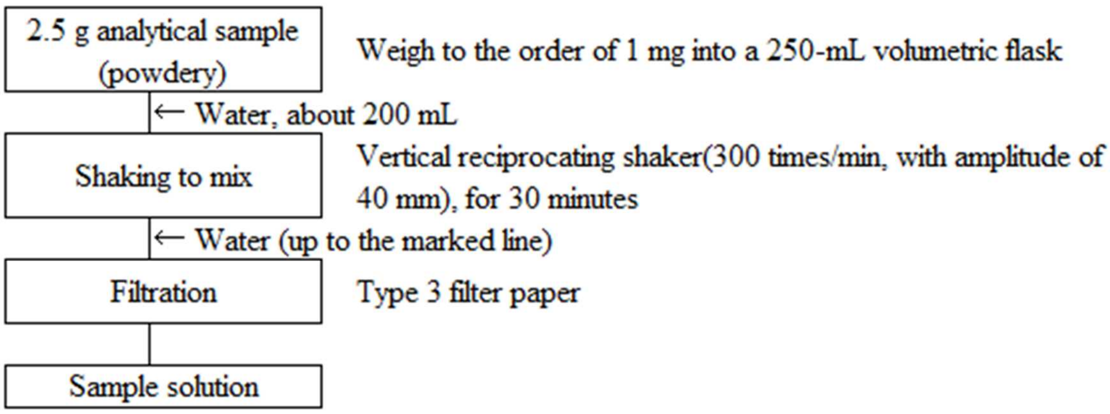


Figure 1-2 Flow sheet for water-soluble phosphoric acid in fertilizers containing phosphorus acid, etc. (Extraction procedure (4.1.1.2))

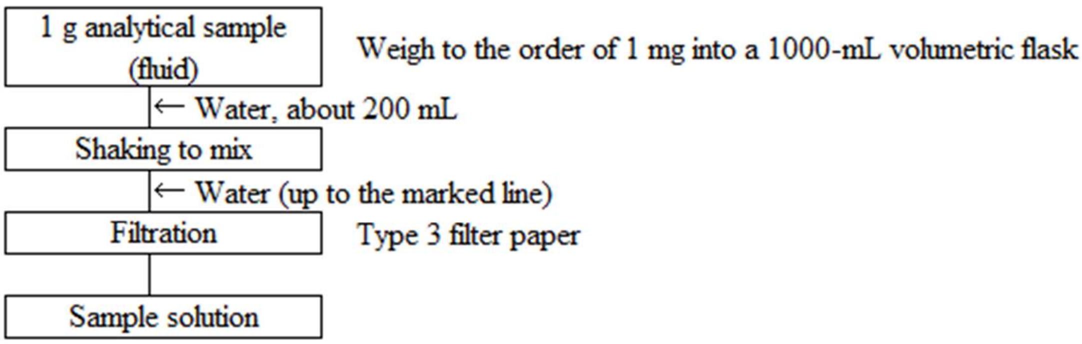


Figure 1-3 Flow sheet for water-soluble phosphoric acid in fertilizers containing phosphorus acid, etc. (Extraction procedure (4.1.2))

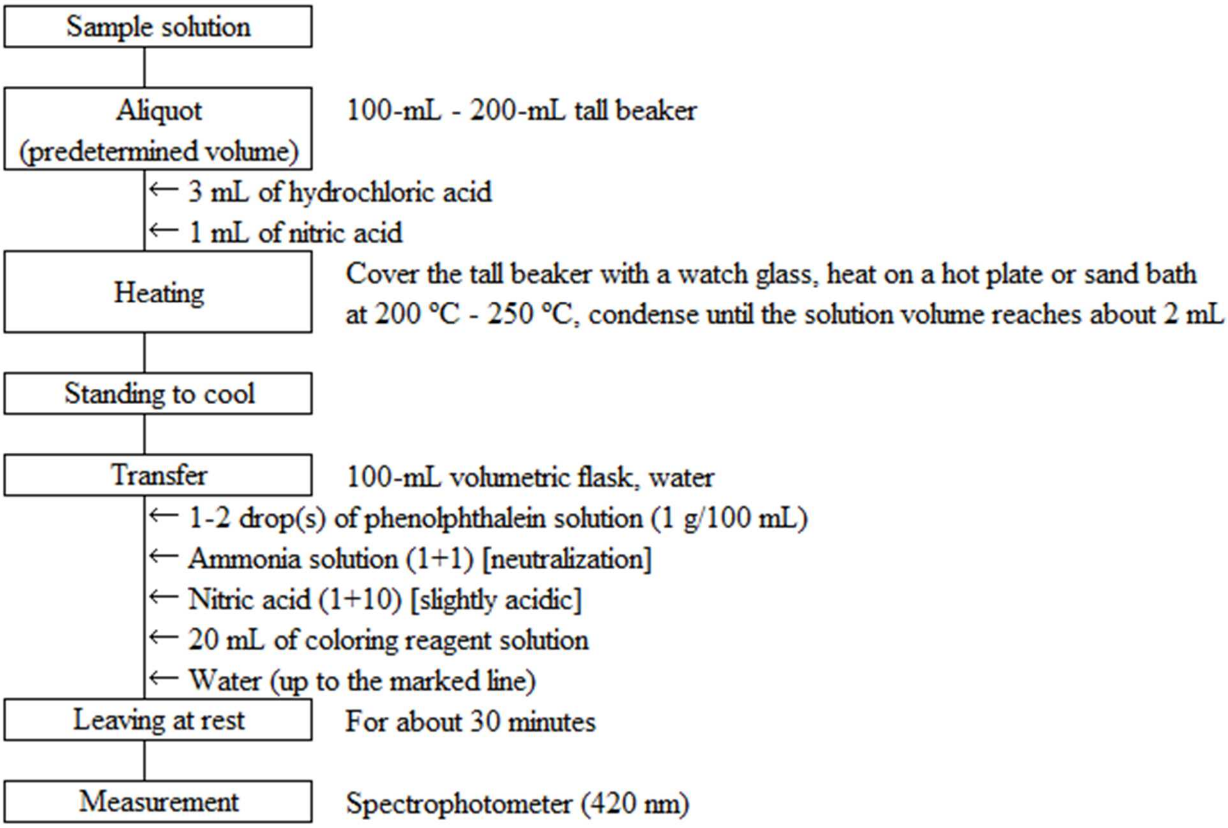


Figure 2 Flow sheet for water-soluble phosphoric acid in fertilizers
containing phosphorus acid, etc. (Coloring and measurement procedure)

4.2.4.c Quinoline gravimetric analysis

(1) Summary

This testing method is applicable to fertilizers containing no phosphorous acid, etc. It is suitable for the fertilizers with relatively a high content of phosphoric acid. This testing method is classified as Type E and its symbol is 4.2.4.c-2017 or W-P.c-1.

Extract by adding water to an analytical sample. Heat after adding nitric acid and water, hydrolyze nonorthophosphoric acid to orthophosphate ion and measure the mass of quinonium phosphomolybdate formed by the reaction with quinoline, molybdic acid and nitric acid to obtain water-soluble phosphoric acid (W-P_2O_5) in an analytical sample.

(**2**) **Reagent**: Reagents are as shown below.

a) **Nitric acid**: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

b) **Sodium molybdate solution**: Dissolve 70 g of sodium molybdate dihydrate in 150 mL of water.

c) **Quinoline solution**: Add 5 mL of quinoline specified in JIS K 8279 to the mixture solution of 35 mL of nitric acid and 100 mL of water.

d) **Quimosiac solution**: Add 60 g of citric acid monohydrate specified in JIS K 8283 to the mixture solution of 85 mL nitric acid and 150 mL of water to dissolve. Add gradually total volume of the sodium molybdate solution to mix. Add gradually the total volume of the quinoline solution while mixing the solution. After leaving at rest overnight, filter the total volume with Type 3 filter paper. Add 280 mL of acetone specified in JIS K 8034, and further add water to make 1000 mL.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

b) **Water bath**: Water bath that can be adjusted to 60 ºC - 65 ºC.

c) **Drying apparatus**: A drying apparatus that can be adjusted to 220 ºC ± 5 ºC.

d) **Crucible type glass filter**: A crucible type glass filter 1G4 specified in JIS R 3503. Let it stand to cool in a desiccator after heating at 220 ºC ± 5 ºC in advance and measure the mass to the order of 1 mg.

(4) Test procedures

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask**.**

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Comment 1 In the procedure in **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it into a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 2 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement as shown below.

a) Put a predetermined volume (the equivalents of 10 mg - 30 mg as P_2O_5 and no more than 20 mL as the total solution volume) of sample solution in a 300-mL tall beaker.

b) Add 5 mL of nitric acid and add water to make 80 mL.
c) Cover with a watch glass. After boiling for about 3 minutes, wash the watch glass and the inside the tall beaker with water and add water to make 100 mL.
d) Immediately, add 50mL of quimosiac solution, heat for about 15 minutes while sometimes mixing in a water bath at 60 ºC - 65 ºC to produce the precipitate of quinolinium phosphomolybdate.
e) After standing to cool down to room temperature while sometimes mixing, filter under reduced pressure with a crucible type glass filter, wash the tall beaker 3 times with water and transfer the whole precipitate into a crucible type glass filter, and further wash 7 - 8 times with water.
f) Transfer the precipitate together with the crucible type glass filter into a drying apparatus and heat at 220 ºC ± 5 ºC for about 30 minutes.
g) As soon as heating is complete, move it into a desiccator and let it stand to cool.
h) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.
i) Calculate water-soluble phosphoric acid (W-P_2O_5) by the following formula.

Water-soluble phosphoric acid (% (mass fraction)) in an analytical sample

$$= A \times 0.03207 \times (V_1/V_2) \times (1/W) \times 100$$

A : Mass (g) of the precipitate in **h**)
W : Mass of an analytical sample (5 g)
V_1 : Constant volume (500 mL) of the sample solution
V_2 : Aliquot volume (mL) of the sample solution in **a**)

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.98- 114, Yokendo, Tokyo (1988)

(5) **Flow sheet for water-soluble phosphoric acid** The flow sheet for water-soluble phosphoric acid: in fertilizers is shown below:

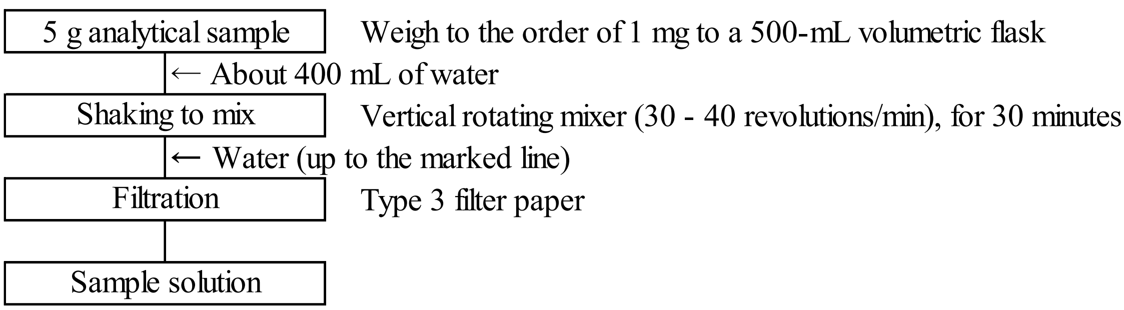


Figure 1 Flow sheet for water-soluble phosphoric acid in fertilizers (Extraction procedure)

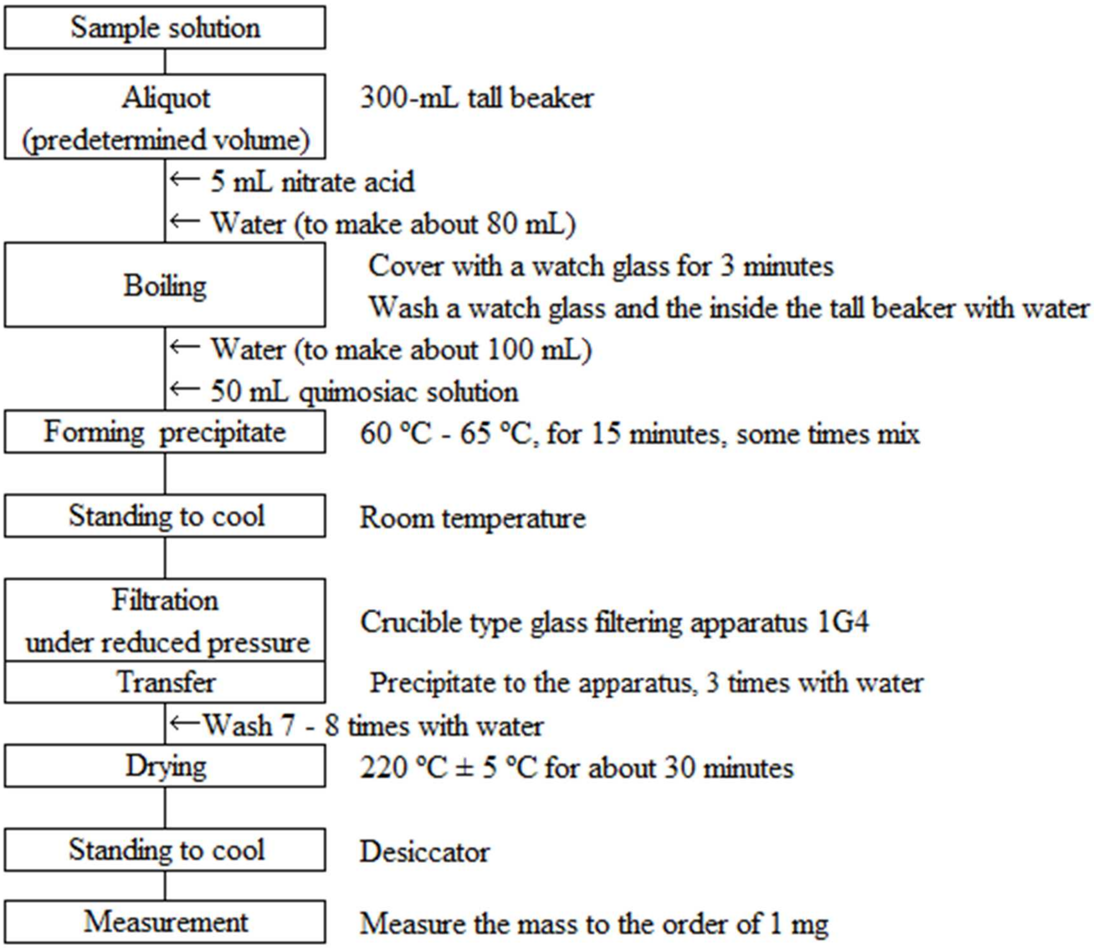


Figure 2 Flow sheet for water-soluble phosphoric acid in fertilizers
(Measurement procedure)

4.2.4.d ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fertilizers. It is also suitable for the fertilizers containing phosphorus acid (phosphite). This testing method is classified as Type D for solid fertilizers and Type B for fluid fertilizers. Its symbol is 4.2.4.d-2019 or W-P.d-2.

Extract by adding water to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the phosphorus at a wavelength of 178.287 nm to obtain water-soluble phosphoric acid W-P_2O_5) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Phosphoric acid standard solution (P_2O_5 10 mg/mL)** (1): Heat potassium dihydrogen phosphate specified in JIS K 9007 at 105 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 19.17 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, add 2 mL-3 mL of nitric acid, and add water up to the marked line.

d) **Phosphoric acid standard solution (P_2O_5 1 mg/mL)** (1): Put 10 mL of phosphoric acid standard solution (P_2O_5 10 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.

e) **Phosphoric acid standard solution (P_2O_5 20 µg/mL - 0.4 mg/mL) for the calibration curve preparation** (1): Put 2 mL - 40 mL of phosphoric acid standard solution (P_2O_5 1 mg/mL) in a 100-mL volumetric flask step by step and add hydrochloric acid (1+23) up to the marked line.

f) **Phosphoric acid standard solution ((P_2O_5 5 µg/mL - 20 µg/mL) for the calibration curve preparation** (1): Put 5 mL - 20 mL of phosphoric acid standard solution (P_2O_5 0.1 mg/mL) in a 100-mL volumetric flask step by step and add hydrochloric acid (1+23) up to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**), **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the phosphoric acid standard solution in (**2**), a phosphoric acid standard solution for a calibration curve can be prepared using a phosphoric acid standard solution (P 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble phosphoric acid (W-P_2O_5) in an analytical sample by multiplying the concentration (P) of a phosphoric acid standard solution for a calibration curve or a measured value obtained in (**4.2**) by a conversion factor (2.2914).

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Extractor**: A vertical rotating mixer or vertical reciprocating shaker as described below.

aa) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

ab) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

b) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

(4.1.1.1) Vertical rotating mixer

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 3 In the procedure of (**4.1.1.1**) **a**), it is also allowed to weigh 2.5g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask

Comment 4 A sample solution obtained in the procedure in (**4.1.1.1**) is also applicable to the components shown in Annex B.

(4.1.1.2) Vertical reciprocating shaker

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.
b) Add about 200 mL of water, and shake to mix at 300 times/min (amplitude of 40 mm) for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 5 A sample solution obtained in the procedure in (**4.1.1.2**) is also applicable to the components shown in Annex B.

(4.1.2) Fluid test sample

a) Weigh 1 g of an analytical sample (2) to the order of 1 mg, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, shake to mix and add water up to the marked line.
c) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) The sampling amount of the analytical sample is 10 g when there is less phosphoric acid content in the fertilizers such as a home garden-use fertilizer.

Comment 6 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(4.2) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 178.287 nm

b) Calibration curve preparation

1) Spray the phosphoric acid standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 178.287 nm.

2) Prepare a curve for the relationship between the phosphoric acid concentration and the indicated value of the phosphoric acid standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 40 mg as P_2O_5) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the phosphoric acid (P_2O_5) content from the calibration curve, and calculate water-soluble phosphoric acid (W-P_2O_5) in the analytical sample.

Comment 7 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 8 The comparison of the measurement value (y_i : 0.390 % (mass fraction) - 29.5 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Ammonium vanadomolybdate absorptiometric analysis was conducted to evaluate trueness using powdery test fertilizers (26 samples). As a result, a regression equation was $y=-0.115+0.977x$, and its correlation coefficient (r) was 0.998. Similarly the comparison of the measurement value (y_i : 0.179 % (mass fraction) - 10.88 % (mass fraction)) and the measurement value (x_i) was conducted using fluid fertilizers (12 samples). As a result, a regression equation was $y=-0.022+1.008x$, and its correlation coefficient (r) was 0.999. In addition, additive recovery testing was conducted using preparation fertilizers (5 samples). As a result, the mean recovery rate at the additive level of 1.64 % (mass fraction) - 52.15 % (mass fraction) was 99.8 % - 103.0 %. Additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 10 % (mass fraction) and 1 % (mass fraction) were 98.1 % and 101.9 % respectively.

The results of the repeatability tests on different days using a home garden-use compound fertilizer (solid), a blended fertilizer, a fluid compound fertilizer and a home garden-use compound fertilizer (fluid) to evaluate precision were analyzed by the one-way analysis of variance. Table 1-1 and 1-2 show the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.04 % (mass fraction) for solid fertilizers and about 0.02 % (mass fraction) for fluid fertilizers.

Table 1-1 Analysis results of the repeatability tests on different days of water-soluble phosphoric acid (Solid fertilizer)

Name of sample	Test days of repeatability (T)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Home garden-use compound fertilizer (solid)	5	28.91	0.54	1.9	0.54	1.9
Blended fertilizer	5	1.58	0.05	3.3	0.07	4.5

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) × the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 1-2 Analysis results of the repeatability tests on different days of water-soluble phosphoric acid (Fluid fertilizer)

Name of sample	Test days of repeatability (T)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Fluid compound fertilizer	7	10.83	0.10	0.9	0.14	1.3
Home garden-use compound fertilizer (fluid)	7	0.829	0.008	0.9	0.015	1.8

Footnote: Refer to Table 2-1

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble phosphoric acid

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Preparation sample (Fluid) 1	10	9.45	0.16	1.7	0.29	3.0
Preparation sample (Fluid) 2	10	2.02	0.02	1.2	0.04	2.0
Preparation sample (Fluid) 3	10	5.07	0.06	1.1	0.09	1.9
Preparation sample (Fluid) 4	9	1.04	0.01	0.8	0.04	3.5
Preparation sample (Fluid) 5	10	0.519	0.003	0.6	0.013	2.5

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination of Water-Soluble Principal Ingredients ($W\text{-}P_2O_5$, $W\text{-}K_2O$, W-MgO, W-MnO and $W\text{-}B_2O_3$) in Liquid Fertilizer using Inductively

Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 8**, p. 1 - 9 (2015)

2) Norio FUNAKI: Simultaneous Determination of Water-soluble Principal Ingredients in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 12**, p. 28 – 51 (2019)

3) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(**5**) **Flow sheet**: The flow sheet for water-soluble phosphoric acid of the fluid mixed fertilizers is shown below:

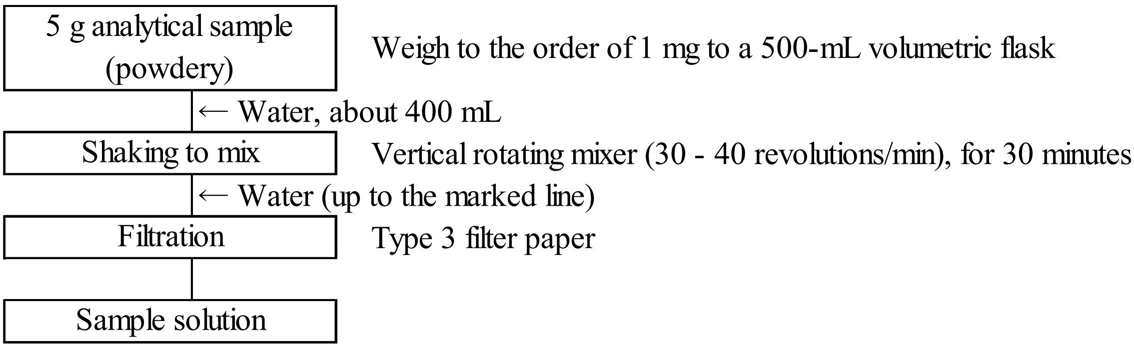


Figure 1-1 Flow sheet for water-soluble phosphoric acid in fluid fertilizers (Extraction procedure (4.1.1.1)

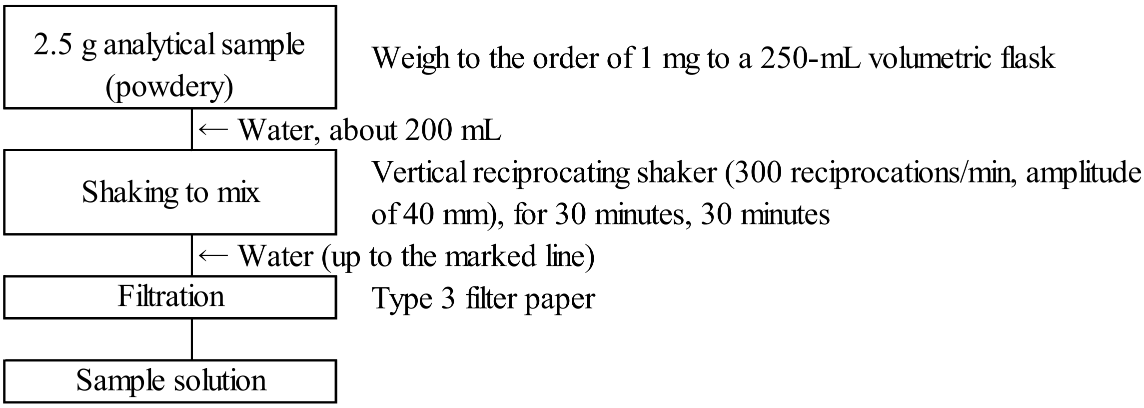


Figure 1-2 Flow sheet for water-soluble phosphoric acid in fluid fertilizers (Extraction procedure (4.1.1.2)

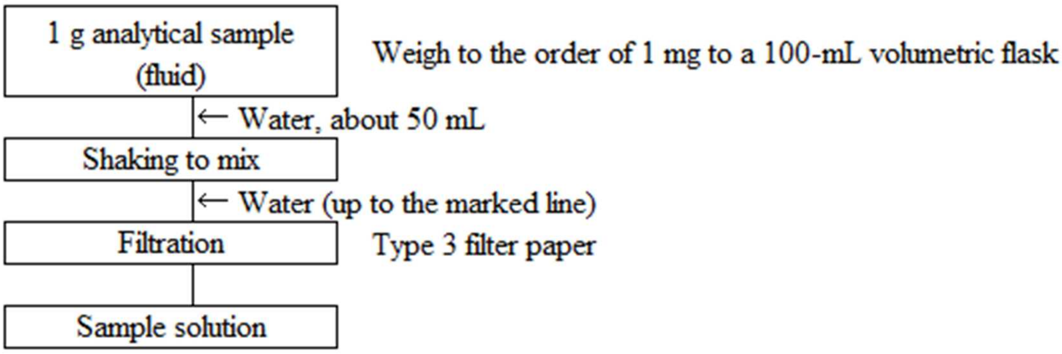


Figure 1-3 Flow sheet for water-soluble phosphoric acid in fluid fertilizers
(Extraction procedure (4.1.2)

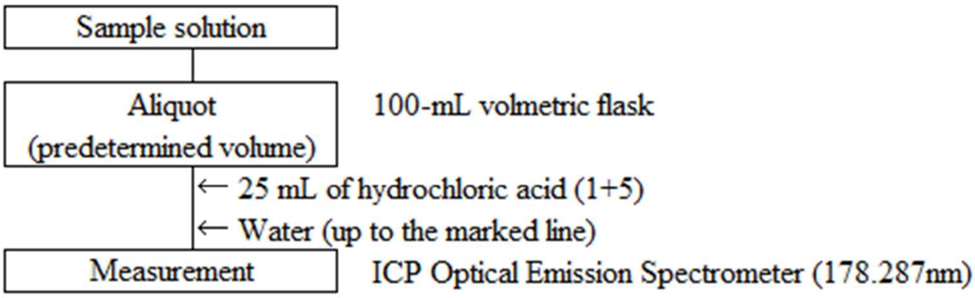


Figure 2 Flow sheet for water-soluble phosphoric acid in fluid fertilizers
(Measurement procedure)

4.3 Potassium
4.3.1 Total potassium
4.3.1.a Flame atomic absorption spectrometry or flame photometry
(1) Summary

This testing method is applicable to fertilizers containing organic matters. This testing method is classified as Type B and its symbol is 4.3.1.a-2021 or T-K.a-2.

Pretreat an analytical sample with incineration-hydrochloric acid boiling or aqua regia digestion to convert the total potassium into potassium ion, add an interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with potassium at a wavelength of 766.5 nm or 769.9 nm to quantify the total potassium. Or, determine the intensity of the emission line at a wavelength of 766.5 nm or 769.9 nm produced in flame to obtain the total potassium (T-K_2O) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Nitric acid**: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

c) **Interference suppressor solution**: Weigh 12.5 g of calcium carbonate specified in JIS K 8617 into a 2000-mL beaker, add a small amount of water, gradually add 105 mL of hydrochloric acid, and heat for a little while. After cooling is complete, add water to make 1000 mL.

d) **Potassium standard solution (K_2O 1 mg/mL)** (1): Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.583 g into a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

e) **Potassium standard solution (K_2O 5 µg/mL - 50 µg/mL) for the calibration curve preparation** (2): Put 2.5 mL - 25 mL of potassium standard solution (K_2O 1 mg/mL) in 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and add water up to the marked line.

f) **Blank test solution for the calibration curve preparation** (1): Put about 50 mL of interference suppressor solution in a 500-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This is an example of preparation in the case of a wavelength of 769.9 nm; prepare an amount appropriate for the actual condition.

(**3**) Add an interference suppressor solution that is 1/10 of the volume to be prepared.

Comment 1 Instead of the potassium standard solution in (**2**), a potassium standard solution for the calibration curve preparation can be prepared by using a potassium standard solution (K 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate total potassium (T-K_2O) in the analytical sample by multiplying the concentration (K) of a potassium standard solution for calibration curve preparation or a measurement value (K) obtained in (**4.2**) by a conversion factor (1.2046).

Comment 2 When using a sample solution obtained in the procedure in (**4.1.2**) **h**) for the measurement of cadmium, nickel, chromium or lead, sulfuric acid and hydrochloric acid in (**2**) should be a reagent of harmful metal analysis grade, microanalysis grade or equivalents.

(3) **Instruments**: Instruments are as shown below:

a) **Analytical instrument**: An atomic absorption spectrometer or a flame photometer as shown below:

aa) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A potassium hollow cathode lamp

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

ab) **Flame photometer:**

1) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Electric furnace**: An electric furnace that can be adjusted to 550 ºC ± 5 ºC.

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) **Test procedure**

(4.1) **Extraction**: Conduct extraction as shown below.

(4.1.1) **Incineration-hydrochloric acid boiling**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (4).

c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate (4).

d) After standing to cool, moisten the residue with a small amount of water, gradually add about 10 mL of hydrochloric acid, and further add water to make about 20 mL.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.

f) After standing to cool, transfer to a 250-mL - 500-mL volumetric flask with water.

g) Add water up to the marked line.

h) Filter with Type 3 filter paper to make a sample solution.

Note (4) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 550 °C in 1 to 2 hours.

Comment 3 Do not conduct the procedures in (**4.1.1**) **b**) **- c**) in the case of fertilizers not containing organic matters.

Comment 4 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) **Incineration-aqua regia digestion**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (5).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (5).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (6), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (7) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

Note (**5**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.

(**6**) The watch glass can be removed.

(**7**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 5 Do not conduct the procedures in (**4.1.2**) **b**) **- c**) in the case of fertilizers not containing organic matters.

Comment 6 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer or flame photometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer or the flame photometer:** Set up the measurement conditions for the atomic absorption spectrometer or the flame photometer considering the following:
Analytical line wavelength: 766.5 nm or 769.9 nm

b) **Calibration curve preparation**

1) Spray the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 766.5 nm or 769.9 nm.

2) Prepare a curve for the relationship between the potassium concentration and the indicated value of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as K_2O) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the potassium content from the calibration curve, and calculate total potassium (T-K_2O) in the analytical sample.

Comment 7 The analytical line wavelength may be set to 404.4 nm with lower sensitivity. At the wavelength of 404.4 nm, the slit width needs to be reduced to separate neighboring lines. If the slit width is specified for the instrument, set the slit width to the specified value. An example preparation of standard solutions for calibration curve preparation for a wavelength of 404.4 nm is 3 μg/mL - 90 μg/mL as K_2O, and the minimum limit of quantification was estimated to be about 3 μg/mL in a measurement solution. However, it is necessary to understand in advance the concentration range of calibration curve suitable for the instrument and prepare standard solutions for calibration curve preparation.

Comment 8 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 10 % (mass fraction) - 20 % (mass fraction) and 1 % (mass fraction) - 5 % (mass fraction) were 97.8 % - 100.1 % and 100.9 % - 103.1 % as total potassium acid (T-K_2O) respectively.

In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1. In addition, the results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 2 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.08 % (mass fraction) for solid fertilizers and about 0.03 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the collaborative study for the test method validation of total potassium

Sample name	Number of laboratories1)	Mean2) (%)3)	s_r 4) (%)3)	RSD_r 5) (%)	s_R 6) (%)3)	RSD_R 7) (%)
Compound fertilizer A	9	25.11	0.19	0.8	0.33	1.3
Compound fertilizer B	9	14.05	0.12	0.8	0.25	1.8
Compound fertilizer C	10	9.00	0.11	1.2	0.24	2.6
Dry fungus body fertilizer	10	2.66	0.03	1.3	0.04	1.5
Cator oil cales and powder	9	1.82	0.02	1.0	0.03	1.5

1) Number of laboratories used in analys
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2 Analysis results of the collaborative study to determine total pottasium of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)1)	Mean2) (%)3)	s_r 4) (%)3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%)3)	$RSD_{I(T)}$ 7) (%)	s_R 8) (%)3)	RSD_R 9) (%)
FAMIC-C-12	11	0.584	0.005	0.9	0.011	1.9	0.038	6.5

1) The number of laboaratories used for analysis conducting flame atomic absorbance spectrometry
2) Average (the number of laboratory(p) × test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.132- 138, Yokendo, Tokyo (1988)

2) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, **Vol. 3** p. 107 - 116 (2010)

3) Yasuharu KIMURA and Hisanori ARAYA Verification of Performance Characteristics of Testing Methods for Potassium Content in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer **Vol. 5**, p. 190 - 200 (2012)

4) Hisanori ARAYA and Kimie KATO: Performance Evaluation of Determination Methods for Potassium in Fertilizer - Harmonized Collaborative Validation - Research Report of Fertilizer, **Vol. 12**, p. 109 – 122 (2019)

(5) **Flow sheet for total potassium:** The flow sheet for total potassium in fertilizers is shown below:

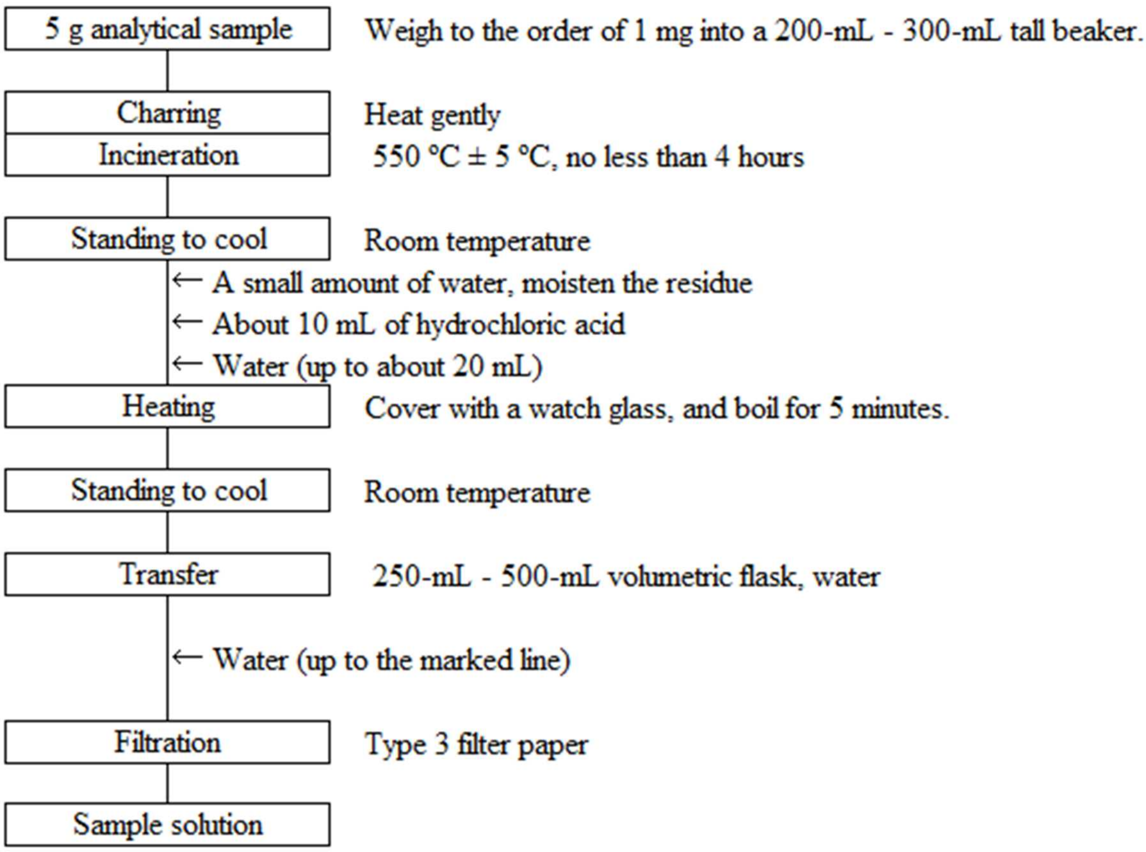


Figure 1-1 Flow sheet for total potassium in fertilizers
(Incineration-hydrochloric acid boiling procedure (4.1.1))

5 g analytical sample	Weigh to the order of 1 mg into a 200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 °C ± 5 °C for 8 - 16 hours
Standing to cool	Room temperature
← A small amount of water	
← About 10 mL of nitric acid	
← About 30 mL of hydrochloric acid	
Heating	Cover with a watch glass to digest
Heating	Slightly move the watch glass and remove acid
Standing to cool	Room temperature
← 25 mL - 50 mL of hydrochloric acid (1+5)	
Heating	Cover with a watch glass to dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Sample solution	

Figure 1-2 Flow sheet for total potassium in fertilizers
(Incineration-aqua regia digestion procedure (4.1.2))

Sample solution	
Aliquot (predetermined amount)	100-mL volumetric flask, water
← About 10 mL interference suppressor solution	
← Water (up to the marked line)	
Measurement	Atomic absorption spectrometer or flame photometer

Figure 2 Flow sheet for total potassium in fertilizers (Measurement procedure)

4.3.1.b Sodium tetraphenylborate gravimetric analysis

(1) Summary

This testing method is applicable to fertilizers containing organic matters. It is suitable for fertilizers containing relatively a high content of potassium. This testing method is classified as Type D and its symbol is 4.3.1.b-2017 or T-K.b-1.

Pretreat an analytical sample by incineration and hydrochloric acid to convert the total potassium into potassium ion, mask co-existing ammonium and other salts with formaldehyde and ethylenediamine tetraacetate, and measure the mass of potassium tetraphenylborate formed by the reaction with tetraphenylborate to obtain the total potassium (T-K_2O) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 2**.

(2) Reagent: Reagents are as shown below.

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Formaldehyde solution: A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.

c) Sodium hydroxide solution (200 g/L) (1): Dissolve 200 g of sodium hydroxide specified in JIS K 8576 in water to make 1000 mL.

d) Aluminum chloride solution (1 mol/L) (1): Dissolve 12 g of aluminum chloride (III) hexahydrate specified in JIS K 8114 in water to make 100 mL.

e) Tetraphenylborate solution (1): Put 6.1 g of sodium tetraphenylborate specified in JIS K 9521 in a 250-mL volumetric flask, dissolve by adding about 200 mL of water and add 10 mL of aluminum chloride solution. Add a methyl red solution (0.1 g/100 mL) as an indicator, and neutralize with a sodium hydroxide solution (200 g/L) until the color of the solution changes to yellow, and then add water up to the marked line. Filter with Type 3 filter paper and add 0.5 mL of sodium hydroxide solution (200 g/L) to the total filtrate. Filter with Type 3 filter paper in the case of usage.

f) Tetraphenylborate washing solution (1): Dilute 40 mL of tetraphenylborate solution with water to make 1000 mL.

g) Ethylenediaminetetraacetate - Sodium hydroxide solution (1): Dissolve 10 g of ethylenediaminetetraacetic acid disodium dihydrogen dihydrate specified in JIS K 8107 and 8 g of sodium hydroxide specified in JIS K 8576 in a proper amount of water. Add 6 mL - 10 mL of tetraphenylborate solution while mixing according to the potassium content coexisting as impurity after standing to cool, and then add water to make 100 mL. After leaving at rest for about 30 minutes while sometimes mixing, filter with Type 3 filter paper.

h) Methyl red solution (0.1 g/100 mL): Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Electric furnace: An electric furnace that can be adjusted to 550 ºC ± 5 ºC.

b) Drying apparatus: A drying apparatus that can be adjusted to 120 ºC ± 2 ºC.

c) Crucible type glass filter: A crucible type glass filter 1G4 specified in JIS R 3503. Let it stand to cool in a desiccator after heating at 120 ºC ± 2 ºC in advance and measure the mass to the order of 1 mg.

d) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh about 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char [(2)].

c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate [(2)].

d) After standing to cool, moisten the residue with a small amount of water, gradually add about 10 mL of hydrochloric acid, and further add water to make 20 mL.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.

f) After cooling, transfer to a 250-mL - 500-mL volumetric flask with water.

g) Add water up to the marked line.

h) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 550 °C in 1 to 2 hours.

Comment 1 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement as shown below.

a) Put a predetermined amount (the equivalents of 15 mg - 30 mg as K_2O) of the sample solution in a 100-mL tall beaker.

b) Add water to the solution to reach 50 mL when the procedure in **e**) is complete.

c) Add hydrochloric acid (1+9), so that the hydrochloric acid becomes equivalent to 0.2 mL.

d) Add 5 mL of formaldehyde solution, and then add 5 mL of ethylenediamine tetraacetate−sodium hydroxide solution.

e) Add necessary volume [(3)] of tetraphenylborate solution at the rate of one or two drop(s) per second while mixing, and further add 4 mL of the same solution in the same manner.

f) Leave at rest for about 30 minutes while sometimes mixing to form the precipitate of potassium tetraphenylborate.

g) Filter supernatant under reduced pressure with a crucible type glass filter, wash the tall beaker 5 times with 5 mL of tetraphenylborate washing solution and transfer the whole precipitate to the crucible type glass filter and further wash 2 times with 2 ml of water.

h) Transfer the precipitate together with the crucible type glass filter into a drying apparatus and heat at 120 ºC ± 2 ºC for about 1hour.

i) As soon as heating is complete, move it into a desiccator and let it stand to cool.

j) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.

k) Calculate total potassium (T-K_2O) in the analytical sample by the following formula.

Total potassium (T-K_2O) (% (mass fraction)) in an analytical sample

$$= A \times 0.1314 \times (V_1/V_2)/W \times 100$$

A : Mass (g) of the precipitate

V_1 : Constant volume (mL) of the sample solution in (**4.1**) **g**)

V_2 : Aliquot volume (mL) of the sample solution in (**4.2**) **a**)

W : Mass (g) of the analytical sample

Note (**3**) About 3 ml of tetraphenylborate solution per 10 mg of K_2O is required to form the precipitate of potassium tetraphenylborate

Comment 2 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 25 % (mass fraction) - 30 % (mass fraction) and 10 % (mass fraction) - 20 % (mass fraction) were 99.5 % - 100.8 % and 99.5 % - 100.6 % as total potassium acid (T-K_2O) respectively.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.3 % (mass fraction) for solid fertilizers.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.122- 128, Yokendo, Tokyo (1988)

2) Keiji YAGI, Aiko YANO and Hideo SOETA: Verification of Performance Characteristics of Testing Method for Nitrate Nitrogen Content in Fertilizer by Phenol Sulfuric Acid Method, Research Report of Fertilizer **Vol. 5**, p. 201 - 211 (2012)

(**5**) **Flow sheet for total potassium:** The flow sheet for total potassium in fertilizers is shown below:

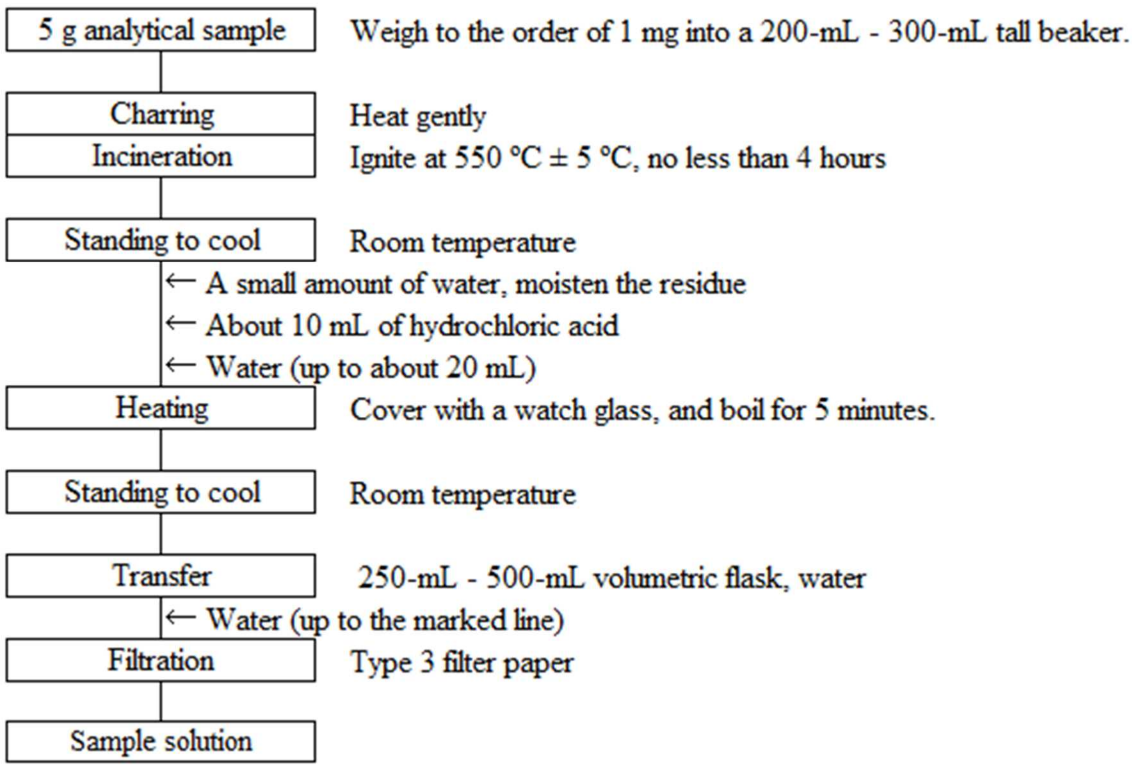


Figure 1 Flow sheet for total potassium in fertilizers (Extraction procedure)

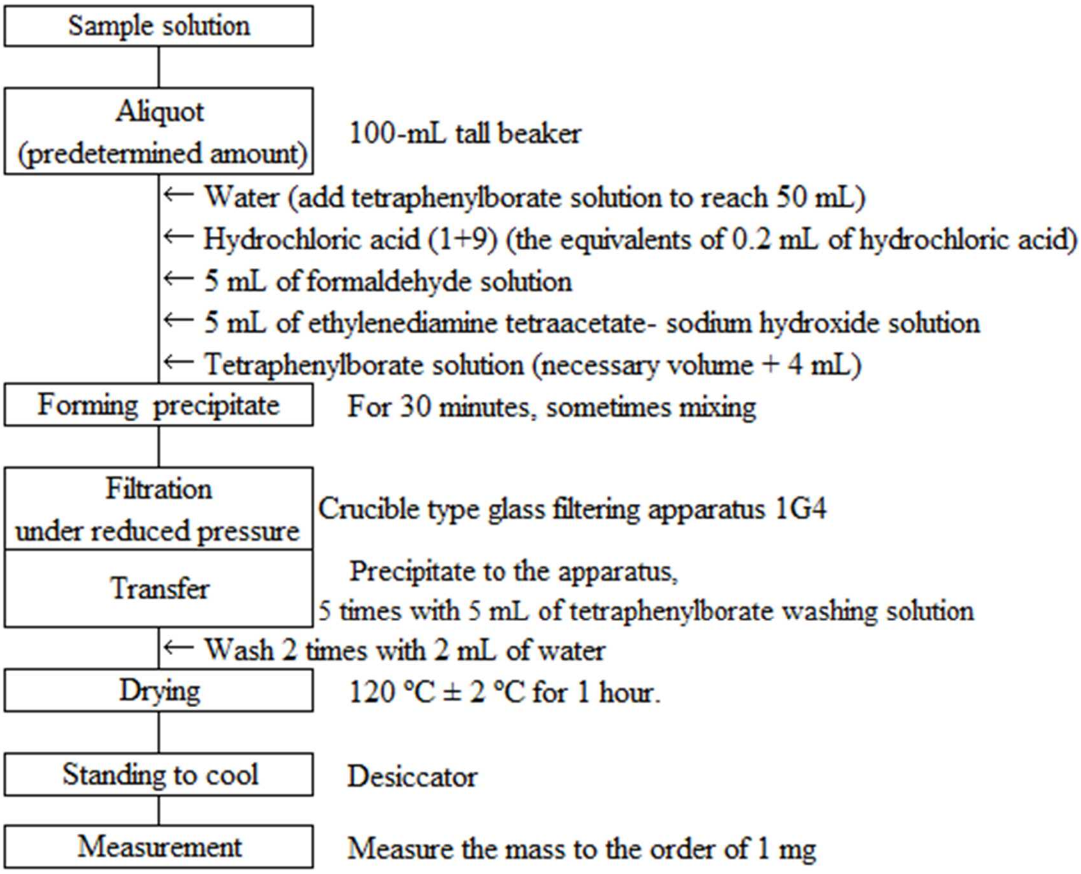


Figure 2 Flow sheet for total potassium in fertilizers (Measurement procedure)

4.3.2 Citric acid-soluble potassium

4.3.2.a Flame atomic absorption spectrometry or flame photometry

(1) Summary

This testing method is applicable to fertilizers containing potassium silicate fertilizers, etc. This testing method is classified as Type B and its symbol is 4.3.2.a-2021 or C-K.a-3.

Extract by adding a citric acid solution to an analytical sample, add an interference suppressor solution, and then spray in an acetylene−air flame, and measure the atomic absorption with potassium at a wavelength of 766.5 nm or 769.9 nm to quantify citric acid-soluble potassium (C-K_2O). Or, measure the intensity of the emission line at a wavelength of 766.5 nm or 769.9 nm produced in flame to quantify citric acid-soluble potassium (C-K_2O) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(2) Reagent: Reagents are as shown below.

- **a) Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **b) Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.
- **c) Interference suppressor solution** (1): Weigh 12.5 g of calcium carbonate specified in JIS K 8617 into a 2000-mL beaker, add a small amount of water, gradually add 105 mL of hydrochloric acid, and heat for a little while. After standing to cool, add water to make 1000 mL.
- **d) Potassium standard solution (K_2O 1 mg/mL)** (1): Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.583 g into a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.
- **e) Potassium standard solution (K_2O 5 µg/mL - 50 µg/mL) for the calibration curve preparation** (2): Put 2.5 mL - 25 mL of potassium standard solution (K_2O 1 mg/mL) in 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and add water up to the marked line.
- **f) Blank test solution for the calibration curve preparation** (1): Put about 50 mL of interference suppressor solution in a 500-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This is an example of preparation in the case of a wavelength of 769.9 nm; prepare an amount appropriate for the actual condition.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the potassium standard solution in (**2**), a potassium standard solution for the calibration curve preparation can be prepared by using a potassium standard solution (K 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble potassium (C-K_2O) in the analytical sample by multiplying the concentration (K) of a potassium standard solution for calibration curve preparation or a measurement value (K) obtained in (**4.2**) by a conversion factor (1.2046).

(3) Instruments: Instruments are as shown below:

- **a) Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.
- **aa) Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating

mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

ab) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

b) **Analytical instrument**: An atomic absorption spectrometer or a flame photometer as shown below:

ba) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A potassium hollow cathode lamp

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

bb) **Flame photometer:**

1) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Constant-temperature vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (4), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note **(4)** Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 2 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Horizontal reciprocating water bath shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (5).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (4), and shake to mix by reciprocating horizontally at 160 times/min with amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note **(5)** Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 3 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 4 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer or the flame photometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer or the flame photometer**: Set up the measurement conditions for the atomic absorption spectrometer or the flame photometer considering the following:
Analytical line wavelength: 766.5 nm or 769.9 nm

b) **Calibration curve preparation**

1) Spray the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 766.5 nm or 769.9 nm.

2) Prepare a curve for the relationship between the potassium concentration and the indicated value of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as K_2O) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the potassium content from the calibration curve, and calculate citric acid-soluble potassium (C-K_2O) in the analytical sample.

Comment 5 The analytical line wavelength may be set to 404.4 nm with lower sensitivity. At the wavelength of 404.4 nm, the slit width needs to be reduced more than for other wave lengths to separate neighboring lines. If the slit width is specified for the instrument, set the slit width to the specified value. An example preparation of standard solutions for calibration curve preparation for a wavelength of 404.4 nm is 3 μg/mL - 90 μg/mL as K_2O, and the minimum limit of quantification was estimated to be about 3 μg/mL in a measurement solution. However, it is necessary to understand in advance the concentration range of calibration curve suitable for the instrument and prepare standard solutions for calibration curve preparation.

Comment 6 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 10 % (mass fraction) - 20 % (mass fraction) and 1 % (mass fraction) - 5 % (mass fraction) were 100.2 % - 101.7 % and 100.4 % - 101.8 % as citric acid-soluble potassium (C-K_2O) respectively.

In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.05 % (mass fraction) for solid fertilizers and about 0.06 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the collaborative study
for the test method validation of citric acid-soluble potassium

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Byproduct compound fertilizer	10	37.98	0.77	2.0	1.00	2.6
Potassium silicate fertilizer	10	20.32	0.12	0.6	0.32	1.6
Compound fertilizer A	10	10.59	0.16	1.5	0.28	2.6
Compound fertilizer B	10	4.79	0.02	0.4	0.12	2.5
Home garden-use compound fertilizer	9	1.95	0.01	0.6	0.03	1.7

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.136- 138, Yokendo, Tokyo (1988)

2) Yasuharu KIMURA and Hisanori ARAYA Verification of Performance Characteristics of Testing Methods for Potassium Content in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer **Vol. 5**, p. 190 - 200 (2012)

3) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)

4) Hisanori ARAYA and Kimie KATO: Performance Evaluation of Determination Methods for Potassium in Fertilizer - Harmonized Collaborative Validation - Research Report of Fertilizer, **Vol. 12**, p. 109 – 122 (2019)

(5) Flow sheet for citric acid-soluble potassium: The flow sheet for citric acid-soluble potassium in fertilizers is shown below:

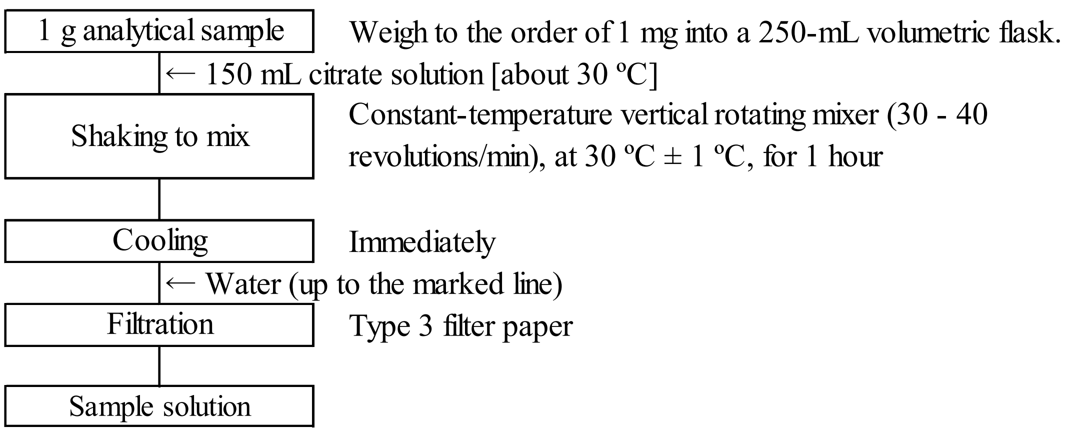


Figure 1-1 Flow sheet for citric acid-soluble potassium in fertilizers (Extract procedure 4.1.1)

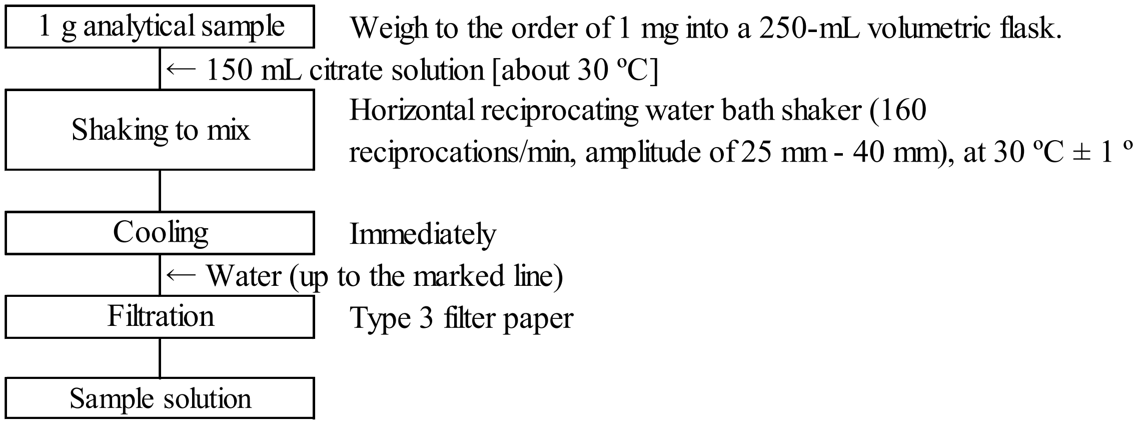


Figure 1-2 Flow sheet for citric acid-soluble potassium in fertilizers (Extract procedure 4.1.2)

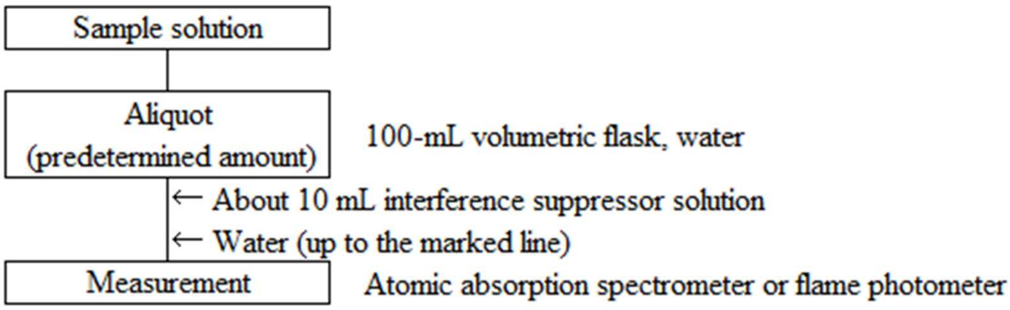


Figure 2 Flow sheet for total potassium in fertilizers (Measurement procedure)

4.3.2.b Sodium tetraphenylborate gravimetric analysis

(1) Summary

This testing method is applicable to fertilizers containing potassium silicate fertilizers, etc. This testing method is classified as Type D and its symbol is 4.3.2.b-2017 or C-K.b-1.

Extract by adding a citric acid solution to an analytical sample, mask co-existing ammonium and other salts with formaldehyde and ethylenediamine tetraacetate and measure the mass of citric acid-soluble potassium (citric acid-soluble potassium (C-K_2O)) and the mass of potassium tetraphenylborate formed by the reaction with tetraphenylborate to obtain citric acid-soluble potassium (C-K_2O) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(2) Reagent: Reagents are as shown below.

a) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

b) **Formaldehyde solution**: A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.

c) **Sodium hydroxide solution (200 g/L)** (1): Dissolve 200 g of sodium hydroxide specified in JIS K 8576 in water to make 1000 mL.

d) **Aluminum chloride solution (1 mol/L)** (1): Dissolve 12 g of aluminum chloride (III) hexahydrate specified in JIS K 8114 in water to make 100 mL.

e) **Tetraphenylborate solution** (1): Put 6.1 g of sodium tetraphenylborate specified in JIS K 9521 in a 250-mL volumetric flask, dissolve by adding about 200 mL of water and add 10 mL of aluminum chloride solution. Add a methyl red solution (0.1 g/100 mL) as an indicator, and neutralize with a sodium hydroxide solution (200 g/L) until the color of the solution changes to yellow, and then add water up to the marked line. Filter with Type 3 filter paper and add 0.5 mL of sodium hydroxide solution (200 g/L) to the total filtrate. Filter with Type 3 filter paper in the case of usage.

f) **Tetraphenylborate washing solution** (1): Dilute 40 mL of tetraphenylborate solution with water to make 1000 mL.

g) **Ethylenediaminetetraacetate - Sodium hydroxide solution** (1): Dissolve 10 g of ethylenediaminetetraacetic acid disodium dihydrogen dihydrate specified in JIS K 8107 and 8 g of sodium hydroxide specified in JIS K 8576 in a proper amount of water. Add 6 mL - 10 mL of tetraphenylborate solution while mixing according to the potassium content coexisting as impurity after standing to cool, and then add water to make 100 mL. After leaving at rest for about 30 minutes while sometimes mixing, filter with Type 3 filter paper.

h) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

b) **Drying apparatus**: A drying apparatus that can be adjusted to 120 ºC ± 2 ºC.

c) **Crucible type glass filter**: A crucible type glass filter 1G4 specified in JIS R 3503. Let it stand to cool in a desiccator after heating at 120 ºC ± 2 ºC in advance and measure the mass to the order of 1 mg.

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 1 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

Comment 2 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedure of (**4.1**) **b**).

(**4.2**) **Measurement**: Conduct measurement as shown below.

a) Put 20 mL of sample solution in a 100-mL tall beaker.

b) Add water to the solution to reach 50 mL when the procedure in **d**) is complete.

c) Add 5 mL of formaldehyde solution, and then add 5 mL of ethylenediamine tetraacetate - sodium hydroxide solution.

d) Add necessary volume (3) of tetraphenylborate solution at the rate of one or two drop(s) per second while mixing, and further add 4 mL of the same solution in the same manner.

e) Leave at rest for about 30 minutes while sometimes mixing to form the precipitate of potassium tetraphenylborate.

f) Filter supernatant under reduced pressure with a crucible type glass filter, wash the vessel 5 times with 5 mL of tetraphenylborate washing solution and transfer the whole precipitate to the crucible type glass filter and further wash 2 times with 2 ml of water.

g) Put the precipitate together with the crucible type glass filter into a drying apparatus and heat at 120 ºC ± 2 ºC for about 1hour.

h) As soon as heating is complete, move it into a desiccator and let it stand to cool.

i) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.

j) Calculate citric acid-soluble potassium (C-K_2O) by the following formula in the analytical sample.

Citric acid-soluble potassium (C-K_2O) (% (mass fraction)) in an analytical sample

$$=A\times 0.1314\times (V_1/V_2)/W\times 100$$

A : Mass (g) of the precipitate

V_1 : Constant volume (mL) of the sample solution in (**4.1**) **c**)

V_2 : Aliquot volume (mL) of the sample solution in (**4.2**) **a**)

W : Mass (g) of the analytical sample

Note (**3**) About 3 ml of tetraphenylborate solution per 10 mg of K_2O is required to form the precipitate of potassium tetraphenylborate

Comment 3 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 25 % (mass fraction) - 30 %

(mass fraction) and 10 % (mass fraction) - 20 % (mass fraction) were 98.6 % - 100.6 % and 100.6 % - 100.7 % as citric acid-soluble potassium (C-K_2O) respectively.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.6 % (mass fraction) for solid fertilizers.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.122- 128, Yokendo, Tokyo (1988)

2) Keiji YAGI, Aiko YANO and Hideo SOETA: Verification of Performance Characteristics of Testing Method for Nitrate Nitrogen Content in Fertilizer by Phenol Sulfuric Acid Method, Research Report of Fertilizer **Vol. 5**, p. 201 - 211 (2012)

(**5**) **Flow sheet for the testing method**: The flow sheet for citric acid-soluble potassium in fertilizers is shown below:

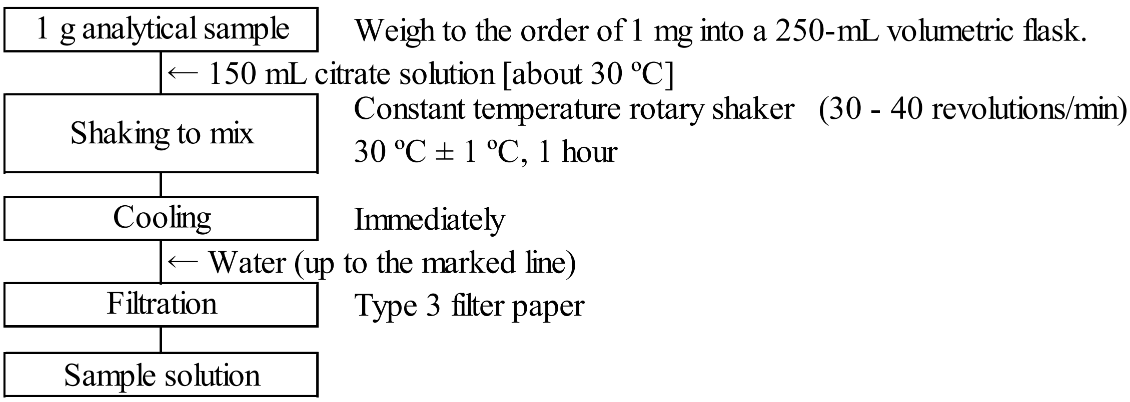


Figure 1 Flow sheet for citric acid-soluble potassium in fertilizers (Extraction procedure)

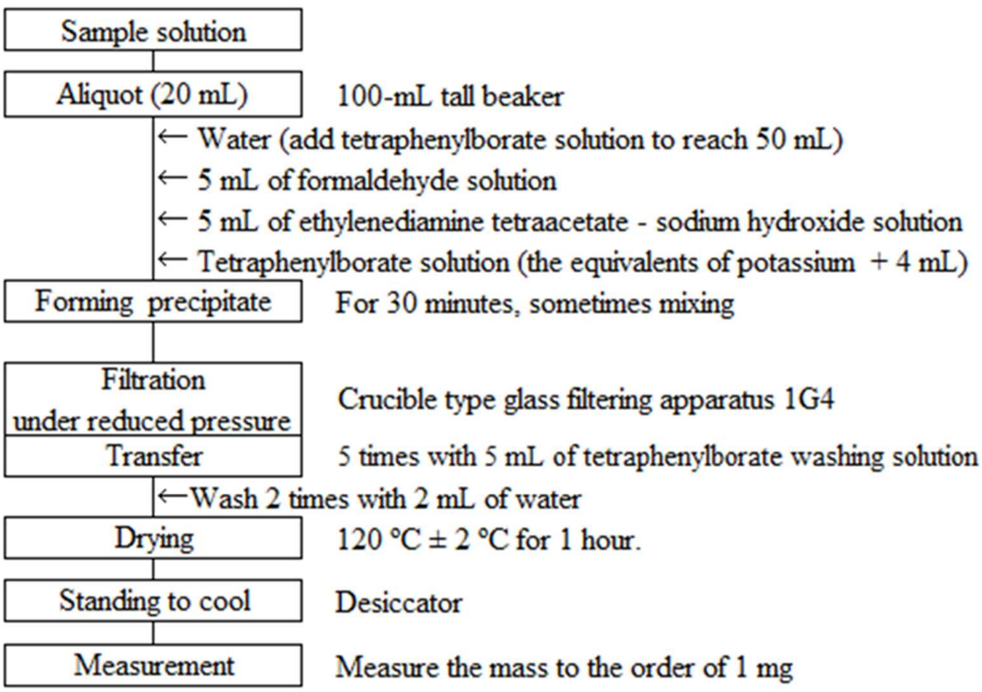


Figure 2 Flow sheet for citric acid-soluble potassium in fertilizers (Measurement procedure)

4.3.2.c Sodium tetraphenylborate volumetric analysis

(1) Summary

This testing method is applicable to fertilizers containing potassium silicate fertilizer, etc. but not organic matters. This testing method is classified as Type E and its symbol is 4.3.2.c-2017 or C-K.c-1.

Extract by adding a citric acid solution to an analytical sample, mask co-existing ammonium and other salts with formaldehyde, and make potassium ion and tetraphenylborate react with each other. Measure unconsumed tetraphenylborate by conducting a precipitate titration to obtain citric acid-soluble potassium (C-K_2O) in an analytical sample.

(2) Reagent: Reagents are as shown below.

a) Citric acid solution (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

b) Formaldehyde solution: A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.

c) Sodium hydroxide solution (120 g/L) (1): Dissolve 30 g of sodium hydroxide specified in JIS K 8576 in water to make 250 mL.

d) Tetraphenylborate solution (1): Put 12.2 g of sodium tetraphenylborate in a 1000-mL volumetric flask, dissolve by adding about 800 mL of water and add 3 mL of sodium hydroxide (120 g/L) to the total filtrate, and further add water up to the marked line. Filter with Type 3 filter paper in the case of usage.

e) Benzalkonium chloride solution (3.3 g/500 mL) (1): Dissolve 3.3 g of benzalkonium chloride in 500 mL of water.

f) Methyl red solution (0.1 g/100 mL): Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

g) Titan Yellow solution (0.04 g/100mL): Dissolve 0.04 g of Titan Yellow in 100 mL of water in the case of usage.

h) Potassium standard solution (K_2O 2 mg/mL) (1): Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 3.166 g into a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as shown below:

a) Constant-temperature vertical rotating mixer: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 1 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

Comment 2 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedure of (**4.1**) **b**).

(**4.2**) **Precipitate formation**: Form precipitate as shown below.

a) Put 5 mL - 15 mL (no more than the equivalents of 30 mg as K_2O) of the extract in a 100-mL volumetric flask.

b) Add water to the solution to make about 30 mL.

c) Add about 5 mL of formaldehyde solution and add 5 mL of sodium hydroxide solution (120 g/L).

d) Add 25 mL of tetraphenylborate solution at the rate of one or two drop(s) per second while shaking to mix.

e) After adding water up to the marked line, leave at rest for 10 minutes.

f) Filter with Type 3 filter paper to make a sample solution.

(**4.3**) **Measurement**: Conduct measurement as shown below.

a) **Calibration curve preparation**

1) Put 1 mL - 15 mL of potassium standard solution (K_2O 2 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Conduct the same procedures as (**4.2**) **b**) - **f**) to make K_2O 2 mg/100 mL - 30 mg/100 mL of the potassium standard solutions for the calibration curve preparation.

3) Conduct the same procedures as **2**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

4) Put 40 mL of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation in an Erlenmeyer flask respectively.

5) Add a few drops of Titan Yellow solution.

6) Titrate with a benzalkonium chloride solution (3.3 g/500 mL) until the color of the solution changes to light red (2).

7) Prepare a curve for the relationship between the potassium concentration and the volume of the benzalkonium chloride solution (3.3 g/500 mL) required for the titration of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

b) **Sample measurement**

1) Put 40 mL of the sample solution of (**4.2**) **f**) in a 100-mL Erlenmeyer flask.

2) Conduct similarly as in **a**) **5**) - **6**) to obtain the volume of the benzalkonium chloride solution (3.3 g/500 mL) required for the titration.

3) Obtain the potassium content from the calibration curve, and calculate citric acid-soluble potassium (C-K_2O) in the analytical sample.

Note (**2**) If the solution temperature is no more than 20 ºC, the reaction does not advance in some cases. Therefore, it is recommended to heat the solution up to about 30 ºC.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.128- 132, Yokendo, Tokyo (1988)

(5) **Flow sheet for citric acid-soluble potassium**: The flow sheet for citric acid-soluble potassium in fertilizers is shown below:

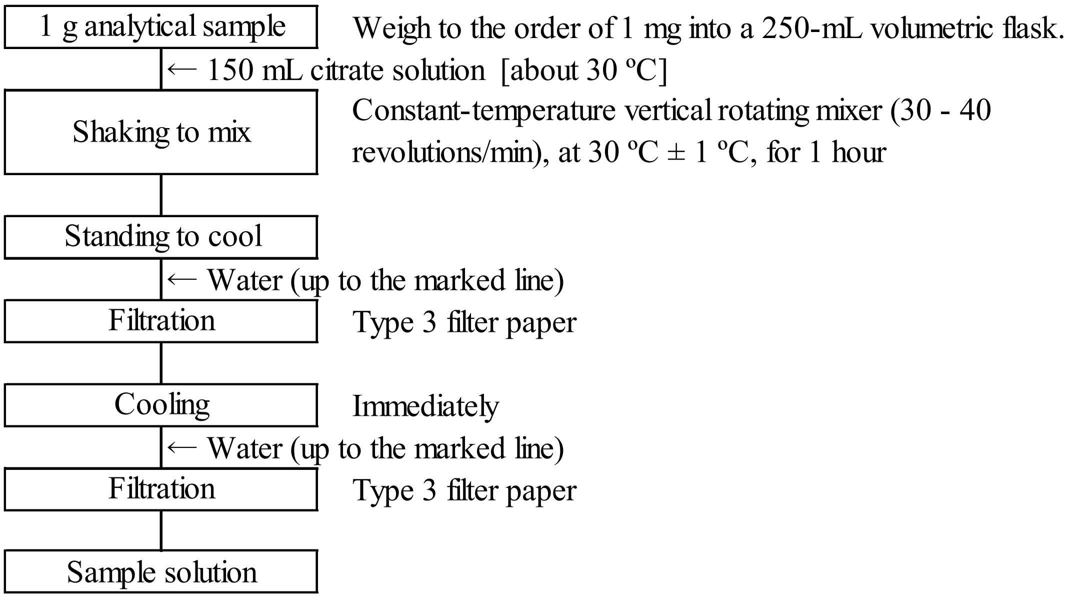


Figure 1 Flow sheet for citric acid-soluble potassium in fertilizers (Extraction procedure)

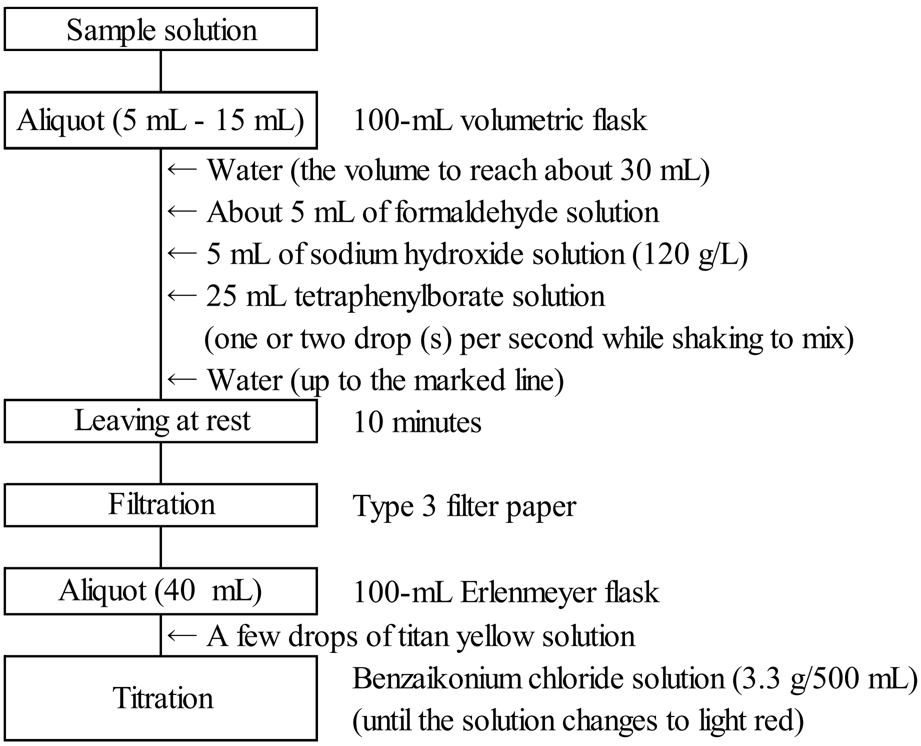


Figure 2 Flow sheet for citric acid-soluble potassium in fertilizers (Measurement procedure)

4.3.2.d ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 4.3.2.d-2018 or C-K.d-1.

Extract by adding a citric acid solution to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the potassium at a wavelength of 766.491 nm to obtain citric acid-soluble potassium (citric acid-soluble potassium (C-K_2O)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(2) Reagent: Reagents are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

d) **Potassium standard solution (K_2O 1 mg/mL)** (1): Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.583 g into a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

e) **Potassium standard solution (K_2O 20 µg/mL - 0.16 mg/mL) for the calibration curve preparation** (1): Put 2 mL - 16 mL of potassium standard solution (K_2O 1 mg/mL) in 100-mL volumetric flasks step-by-step, add about 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

f) **Potassium standard solution (K_2O 2 µg/mL - 20 µg/mL) for the calibration curve preparation** (1): Put 2 mL - 20 mL of potassium standard solution (K_2O 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, add about 25 mL of hydrochloric acid (1+23), and add water up to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the potassium standard solution in (**2**), a potassium standard solution for the calibration curve preparation can be prepared by using a potassium standard solution (K 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble potassium (C-K_2O) in the analytical sample by multiplying the concentration (K) of a potassium standard solution for calibration curve preparation or a measurement value (K) obtained in (**4.2**) by a conversion factor (1.2046).

Comment 2 There are two modes to observe emission from an ICP-OES, a horizontal observation mode and an axial observation mode. The axial observation mode does not apply to potassium since interference is serious.

Comment 3 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K

0116.

1) **Gas:** Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

ba) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

bb) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

(4) **Test procedure**

(4.1) **Extraction**: Conduct extraction as shown below.

(4.1.1) **Constant-temperature vertical rotating mixer**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 4 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) **Horizontal reciprocating water bath shaker**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (3).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix by reciprocating horizontally at 160 times/min with amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (3) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 5 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 6 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(4.2) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometry used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:

Analytical line wavelength: 766.491 nm

b) **Calibration curve preparation**

1) Spray the potassium standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 766.491 nm.

2) Prepare a curve for the relationship between the potassium concentration and the indicated value of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Transfer a predetermined amount of the sample solution (the equivalents of 0.2 mg - 16 mg as K_2O) to a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b)** **1)** to read the indicated value.

4) Obtain the potassium content from the calibration curve, and calculate citric acid-soluble potassium (C-K_2O) in the analytical sample.

Comment 7 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b)** - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 8 The comparison of the measurement value (y_i : 3.57 % (mass fraction) - 34.24 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Flame atomic absorption spectrometry analysis was conducted to evaluate trueness using compound fertilizers (9 samples), mixed compost compound fertilizers (2 samples), designated blended fertilizers (1sample), blended fertilizers (4 samples), and byproduct compound fertilizers (1 sample). As a result, a regression equation was $y = -0.0058+1.0027x$, and its correlation coefficient (r) was 0.999. In addition, additive recovery testing was conducted using a preparation sample. As a result, the mean recovery rate at the additive level of 0.329 % (mass fraction) - 63.18 % (mass fraction) was 98.0 % - 100.3 %.

The results of the repeatability tests on different days using compound fertilizers and blended fertilizers to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.09 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of citric acid-soluble potassium

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Compound fertilzier	7	16.17	0.13	0.8	0.15	1.0
Blended fertilizer	7	4.42	0.04	1.0	0.04	1.6

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)

2) Shingo MATSUO: Simultaneous Determination of Citrate-Soluble Principal Ingredients (C-P2O5, C-K2O, C-MgO, C-MnO and C-B2O3) in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 11**, p. 14 – 28 (2018)

(5) **Flow sheet for citric acid-soluble potassium**: The flow sheet for citric acid-soluble potassium in fertilizers is shown below:

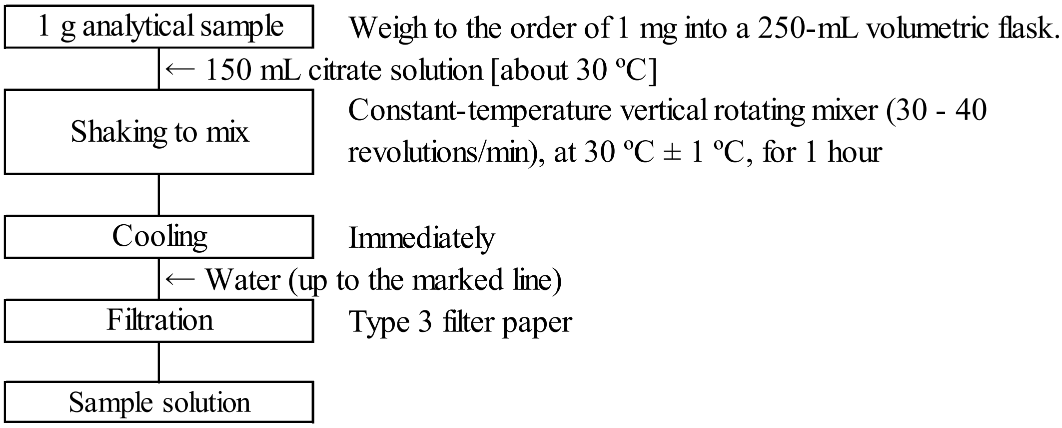


Figure 1-1 Flow sheet for citric acid-soluble potassium in fertilizers (Extract procedure 4.1.1)

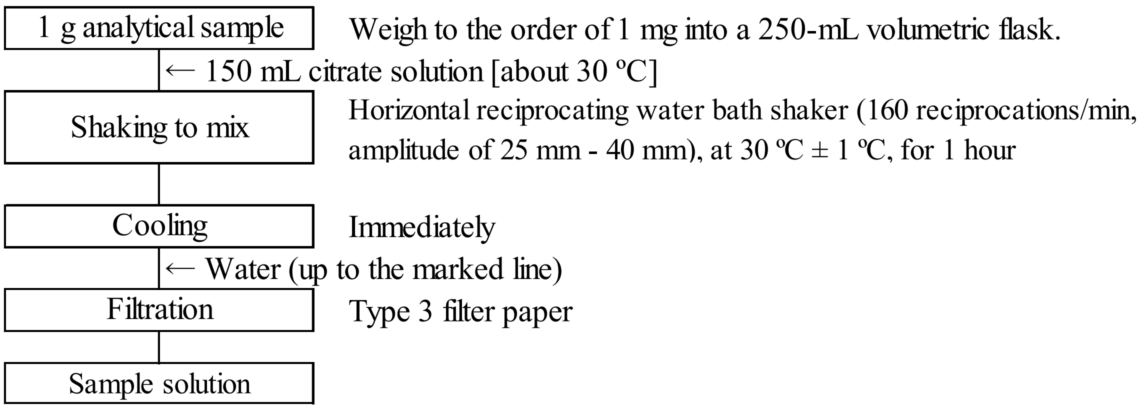


Figure 1-2 Flow sheet for citric acid-soluble potassium in fertilizers (Extract procedure 4.1.2)

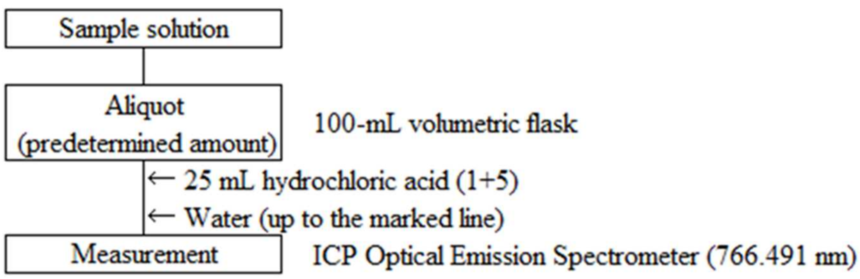


Figure 2 Flow sheet for citric acid-soluble potassium in fertilizers (Measurement procedure)

4.3.3 Water-soluble potassium

4.3.3.a Flame atomic absorption spectrometry or flame photometry

(1) Summary

This testing method is applicable to fertilizers containing potassium salts. This testing method is classified as Type B and its symbol is 4.3.3.a-2021 or W-K.a-2.

Extract by adding water to an analytical sample, add an interference suppressor solution, and then spray in an acetylene–air flame, and measure the atomic absorption with potassium at a wavelength of 766.5 nm or 769.9 nm to quantify water-soluble potassium (W-K_2O). Or, determine the intensity of the emission line at a wavelength of 766.5 nm or 769.9 nm produced in flame to quantify water-soluble potassium (W-K_2O) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 9**.

(2) Reagent: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Interference suppressor solution**: Weigh 12.5 g of calcium carbonate specified in JIS K 8617 into a 2000-mL beaker, add a small amount of water, gradually add 105 mL of hydrochloric acid, and heat for a little while. After cooling is complete, add water to make 1000 mL.

c) **Potassium standard solution (K_2O 1 mg/mL)** (1): Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.583 g into a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

d) **Potassium standard solution (K_2O 5 µg/mL - 50 µg/mL) for the calibration curve preparation** (2): Put 2.5 mL - 25 mL of potassium standard solution (K_2O 1 mg/mL) in 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and add water up to the marked line.

e) **Blank test solution for the calibration curve preparation** (1): Put about 50 mL of interference suppressor solution in a 500-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This is an example of preparation in the case of a wavelength of 769.9 nm; prepare an amount appropriate for the actual condition.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the potassium standard solution in (**2**), a potassium standard solution for the calibration curve preparation can be prepared by using a potassium standard solution (K 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble potassium (W-K_2O) in the analytical sample by multiplying the concentration (K) of a potassium standard solution for calibration curve preparation or a measurement value (K) obtained in (**4.2**) by a conversion factor (1.2046).

(3) Instruments: Instruments are as shown below:

a) **Extractor**: A vertical rotating mixer or vertical reciprocating shaker as described below.

aa) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

ab) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

b) **Analytical instrumen**t: An atomic absorption spectrometer or a flame photometer as shown below:

ba) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A potassium hollow cathode lamp

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

bb) **Flame photometer:**

1) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

c) **Hot plate** : A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Compound fertilizers containing potassium salts and magnesia potassium sulfate

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL tall beaker.

b) Add about 200 mL of water, and cover with a watch glass and heat on a hot plate to boil for about 15 minutes.

c) After immediate cooling is complete, transfer it to a 250- mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make a sample solution.

Comment 2 In the procedure in **a**), a 250-mL volumetric flask can be used instead of a 300-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, "cover with a watch glass" in **b**) is replaced by "place a funnel". Skip "transfer it to a 250-mL volumetric flask with water" in the procedure in **c**).

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Compound fertilizers containing no magnesia potassium sulfate

(4.1.2.1) Vertical rotating mixer

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Comment 4 In the procedure of (**4.1.2.1**) **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 5 A sample solution obtained in the procedure in (**4.1.2.1**) is also applicable to the components shown in Annex B.

(4.1.2.2) Vertical reciprocating shaker:

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add about 200 mL of water, and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 6 A sample solution obtained in the procedure in (**4.1.2.2**) is also applicable to the components shown in Annex B.

(**4.1.3**) Fluid test sample
a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 7 A sample solution obtained in the procedure in (**4.1.3**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer or flame photometer used in measurement.
a) **Measurement conditions for the atomic absorption spectrometer or flame photometer**: Set up the measurement conditions for the atomic absorption spectrometer or flame photometer considering the following:
Analytical line wavelength: 766.5 nm or 769.9 nm
b) **Calibration curve preparation**
1) Spray the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 766.5 nm or 769.9 nm.
2) Prepare a curve for the relationship between the potassium concentration and the indicated value of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.
c) **Sample measurement**
1) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as K_2O) in a 100-mL volumetric flask.
2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.
3) Subject to the same procedure as in **b) 1)** to read the indicated value.
4) Obtain the potassium content from the calibration curve, and calculate water soluble potassium (W-K_2O) in the analytical sample.

Comment 8 The analytical line wavelength may be set to 404.4 nm with lower sensitivity. At the wavelength of 404.4 nm, the slit width needs to be reduced to separate neighboring lines. If the slit width is specified for the instrument, set the slit width to the specified value. An example preparation of standard solutions for calibration curve preparation for a wavelength of 404.4 nm is 3 μg/mL - 90 μg/mL as K_2O, and the minimum limit of quantification was estimated to be about 3 μg/mL in a measurement solution. However, it is necessary to understand in advance the concentration range of calibration curve suitable for the instrument and prepare standard solutions for calibration curve preparation.
Comment 9 Recovery testing was conducted to evaluate trueness using a preparation sample. As a

result, the mean recovery rates at the content level of 10 % (mass fraction) - 20 % (mass fraction) and 1 % (mass fraction) - 5 % (mass fraction) were 97.9 % - 100.2 % and 97.3 % - 100.6 % as water soluble potassium (W–K_2O) respectively. The comparison of the measurement value (y_i: 2.69 % (mass fraction) - 26.64 % (mass fraction)) of extraction by a vertical reciprocating shaker and the measurement value (x_i) of extraction by a vertical rotating mixer was conducted to evaluate trueness of the extraction of solid fertilizers using fertilizers (12 samples). As a result, a regression equation was $y = 0.022 + 1.001x$, and its correlation coefficient (r) was 1.000. The comparison of the measurement value (y_i: 2.69 % (mass fraction) - 26.64 % (mass fraction)) of extraction by a vertical reciprocating shaker and the measurement value (x_i) of extraction by a vertical rotating mixer was conducted to evaluate trueness of the extraction of fluid fertilizers using fertilizers (12 samples). As a result, a regression equation was $y = 0.022 + 1.001x$, and its correlation coefficient (r) was 1.000.

The results of the repeatability tests on different days using compound fertilizers, designated blended fertilizer and fluid compound fertilizer (2 samples) to evaluate precision were analyzed by one-way analysis of variance. Table 1-1 and 1-2 show the calculation results of intermediate precision and repeatability. Results from a collaborative study for test method validation and its analysis are shown in Table 3.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.04 % (mass fraction) for solid fertilizers and about 0.007 % (mass fraction) for fluid fertilizers.

Table 1-1 Analysis results of the repeatability tests on different days of water-soluble potassium (Solid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Compound fertilzier	7	19.67	0.09	0.5	0.15	0.7
Designated blended fertilizer	7	6.50	0.07	1.1	0.07	1.1

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) × the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 1-2 Analysis results of the repeatability tests on different days of water-soluble potassium (Fluid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluid compound fertilizer 1	7	9.66	0.02	0.2	0.07	0.7
Fluid compound fertilizer 2	7	2.44	0.01	0.4	0.02	0.8

Footnote: Refer to Table 1-1

Table 2-1 Analysis results of the collaborative study
for the test method validation of water-soluble potassium
(Compound fertilizers containing potassium salts and magnesia potassium sulfate)

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Potassium sulfate	10	51.19	0.24	0.5	0.63	1.2
Byproduct compound fertilizer	10	36.22	0.20	0.6	0.57	1.6
Magnesia potassium sulfate	10	22.37	0.27	1.2	0.54	2.4
Compound fertilizer 1	10	3.47	0.01	0.4	0.05	1.4
Home garden-use compound fertilizer 1	10	1.73	0.02	1.1	0.03	1.8

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2-2 Analysis results of the collaborative study
for the test method validation of water-soluble potassium
(Compound fertilizers not containing magnesia potassium sulfate)

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Compound fertilizer 1	10	26.72	0.15	0.6	0.25	0.9
Compound fertilizer 2	10	20.79	0.14	0.7	0.27	1.3
Compound fertilizer 3	10	15.25	0.11	0.7	0.27	1.8
Compound fertilizer 4	10	4.47	0.04	0.8	0.09	2.1
Home garden-use compound fertilizer	10	1.71	0.01	0.7	0.03	1.9

Footnote: Refer to table 2-1

Table 3 Analysis results of the collaborative study to determine water-soluble pottasium
of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)	s_R[8)] (%)[3)]	RSD_R[9)] (%)
FAMIC-A-10	11	13.59	0.08	0.6	0.09	0.6	0.16	1.2
FAMIC-A-13	10	13.07	0.08	0.6	0.11	0.8	0.16	1.2
FAMIC-B-10	9	8.85	0.04	0.4	0.07	0.7	0.12	1.4
FAMIC-B-14	14	8.32	0.03	0.4	0.07	0.8	0.13	1.6

1) The number of laboaratories used for analysis conducting flame atomic absorbance spectrometry
2) Mean (the number of laboratory(p) × test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.136- 138, Yokendo, Tokyo (1988)

2) Yasuharu KIMURA and Hisanori ARAYA Verification of Performance Characteristics of Testing Methods for Potassium Content in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer **Vol. 5**, p. 190 - 200 (2012)

3) Shinji KAWAGUCHI: Simple Extraction Method for Water-Soluble Components in Liquid Compound Fertilizers, Research Report of Fertilizer, **Vol. 9**, p. 10 - 20 (2016)

4) Shinji KAWAGUCHI: Extraction Method for the Water-soluble Principal Ingredients in the Solid Fertilizer using a General-purpose Equipment , Research Report of Fertilizer, **Vol. 10**, p. 1 - 8 (2017)

5) Hisanori ARAYA and Kimie KATO: Performance Evaluation of Determination Methods for Potassium in Fertilizer - Harmonized Collaborative Validation - Research Report of Fertilizer, **Vol. 12**, p. 109 – 122 (2019)

(5) **Flow sheet for water-soluble potassium**: The flow sheet for water-soluble potassium in fertilizers is shown below:

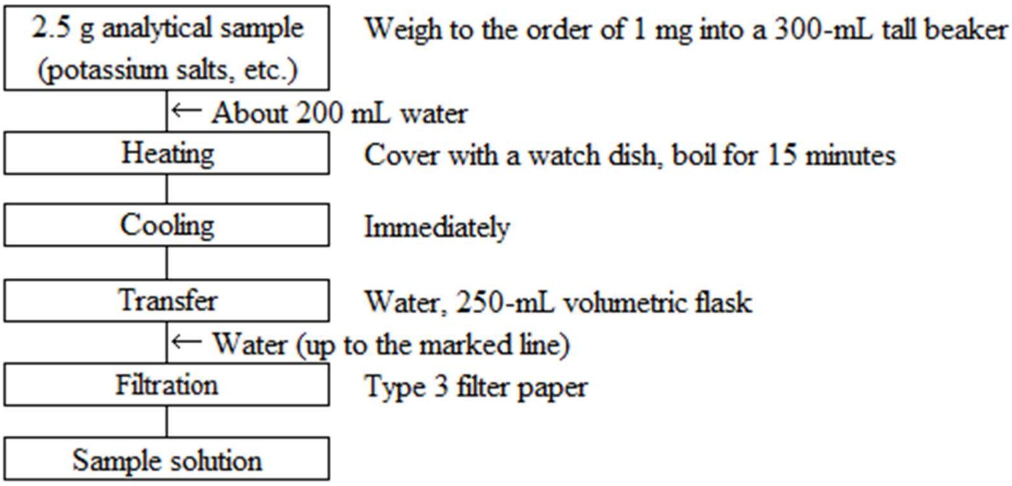


Figure 1-1 Flow sheet for water-soluble potassium in fertilizers (Extraction procedure (4.1.1))

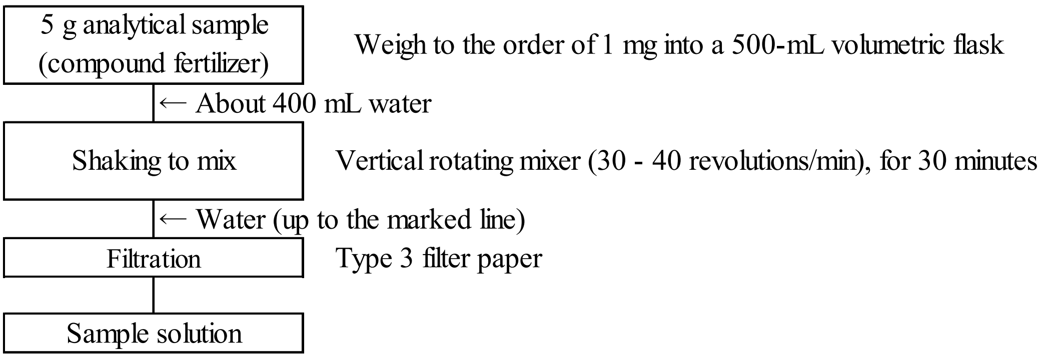


Figure 1-2 Flow sheet for water-soluble potassium in fertilizers (Extraction procedure (4.1.2.1))

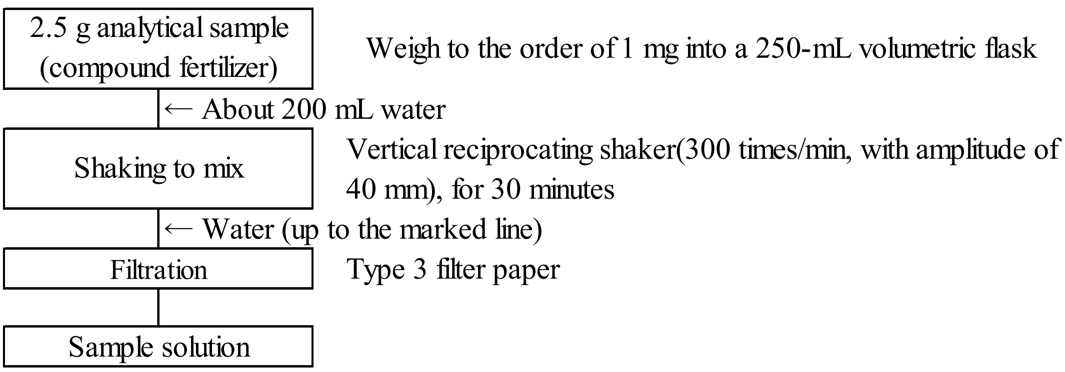


Figure 1-3 Flow sheet for water-soluble potassium in fertilizers (Extraction procedure (4.1.2.2))

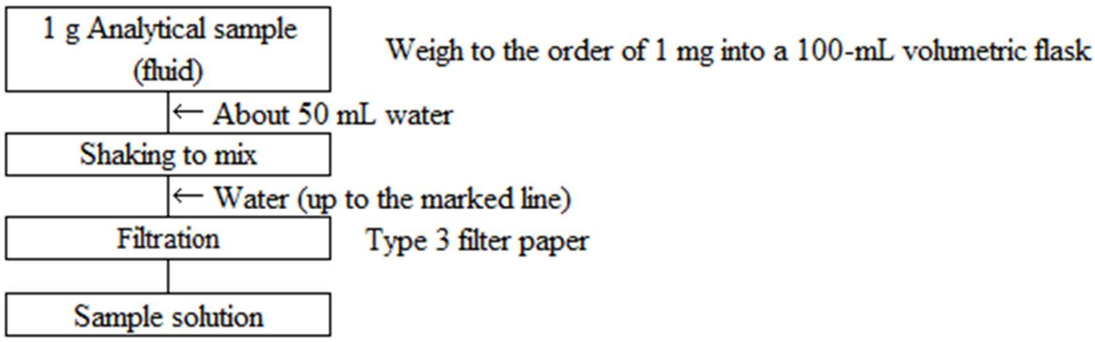


Figure 1-4 Flow sheet for water-soluble potassium in fertilizers (Extraction procedure (4.1.3))

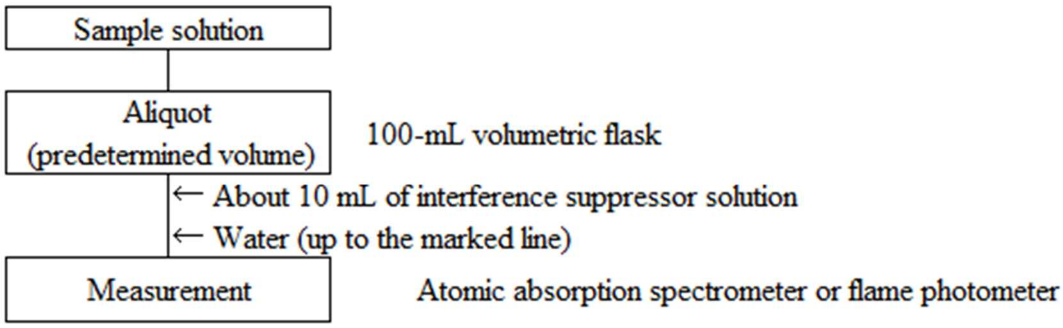


Figure 2 Flow sheet for water-soluble potassium in fertilizers (Measurement procedure)

4.3.3.b Sodium tetraphenylborate gravimetric analysis

(1) Summary

This testing method is applicable to fertilizers containing potassium salts. This testing method is classified as Type D and its symbol is 4.3.3.b-2017 or W-K.b-1.

Extract by adding water to an analytical sample, mask co-existing ammonium and other salts with formaldehyde and ethylenediamine tetraacetate and measure the mass of potassium tetraphenylborate formed by the reaction with tetraphenylborate to obtain water-soluble potassium (W-K_2O) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(2) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Formaldehyde solution**: A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.

c) **Sodium hydroxide solution (200 g/L)** (1): Dissolve 200 g of sodium hydroxide specified in JIS K 8576 in water to make 1000 mL.

d) **Aluminum chloride solution (1 mol/L)** (1): Dissolve 12 g of aluminum chloride (III) hexahydrate specified in JIS K 8114 in water to make 100 mL.

e) **Tetraphenylborate solution** (1): Put 6.1 g of sodium tetraphenylborate specified in JIS K 9521 in a 250-mL volumetric flask, dissolve by adding about 200 mL of water and add 10 mL of aluminum chloride solution. Add a methyl red solution (0.1 g/100 mL) as an indicator, and neutralize with a sodium hydroxide solution (200 g/L) until the color of the solution changes to yellow, and then add water up to the marked line. Filter with Type 3 filter paper and add 0.5 mL of sodium hydroxide solution (200 g/L) to the total filtrate. Filter with Type 3 filter paper in the case of usage.

f) **Tetraphenylborate washing solution** (1): Dilute 40 mL of tetraphenylborate solution with water to make 1000 mL.

g) **Ethylenediaminetetraacetate - Sodium hydroxide solution** (1): Dissolve 10 g of ethylenediaminetetraacetic acid disodium dihydrogen dihydrate specified in JIS K 8107 and 8 g of sodium hydroxide specified in JIS K 8576 in a proper amount of water. Add 6 mL - 10 mL of tetraphenylborate solution while mixing according to the potassium content coexisting as impurity after standing to cool, and then add water to make 100 mL. After leaving at rest for about 30 minutes while sometimes mixing, filter with Type 3 filter paper.

h) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

b) **Drying apparatus**: A drying apparatus that can be adjusted to 120 ºC ± 2 ºC.

c) **Crucible type glass filter**: A crucible type glass filter 1G4 specified in JIS R 3503. Let it stand to cool in a desiccator after heating at 120 ºC ± 2 ºC in advance and measure the mass to the order of 1 mg.

d) **Hot plate** : A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Compound fertilizers containing potassium salts and magnesia potassium sulfate

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL tall beaker.
b) Add about 200 mL of water, and cover with a watch glass and heat on a hot plate to boil for about 15 minutes.
c) After immediate cooling is complete, transfer it to a 250- mL volumetric flask with water.
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make a sample solution.

Comment 1 In the procedure in **a**), a 250-mL volumetric flask can be used instead of a 300-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, "cover with a watch glass" in **b**) is replaced by "place a funnel". Skip "transfer it to a 250-mL volumetric flask with water" in the procedure in **c**).
Comment 2 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Compound fertilizers containing no magnesia potassium sulfate**
a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 3 In the procedure in **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it into a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).
Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement as shown below.
a) Put a predetermined amount (the equivalents of 15 mg - 30 mg as K_2O) of the sample solution in a 100-mL tall beaker.
b) Add water to the solution to reach 50 mL when the procedure in **e**) is complete.
c) Add 2 mL of hydrochloric acid (1+9).
d) Add 5 mL of formaldehyde solution, and then add 5 mL of ethylenediamine tetraacetate - sodium hydroxide solution.
e) Add necessary volume (2) of tetraphenylborate solution at the rate of one or two drop(s) per second while mixing, and further add 4 mL of the same solution in the same manner.
f) Leave at rest for about 30 minutes while sometimes mixing to form the precipitate of potassium tetraphenylborate.
g) Filter supernatant under reduced pressure with a crucible type glass filter, wash the vessel 5 times with 5 mL of tetraphenylborate washing solution and transfer the whole precipitate to the crucible type glass filter and further wash 2 times with 2 ml of water.
h) Transfer the precipitate together with the crucible type glass filter into a drying apparatus adjusted to 120 ºC ± 2 ºC and heat for 1 hour.
i) As soon as heating is complete, move it into a desiccator and let it stand to cool.
j) After standing to cool, remove the ground-in stoppered weighing bottle from the desiccator, and measure the mass to the order of 1 mg.
k) Calculate water soluble potassium (W-K_2O) in the analytical sample by the following formula.

Water-soluble potassium (W-K_2O) (% (mass fraction)) in an analytical sample

$$=A \times 0.1314 \times (V_1/V_2)/W \times 100$$

A : Mass (g) of the precipitate
V_1 : Constant volume (mL) of the sample solution in (**4.1.1**) **d**) or (**4.1.2**) **c**)
V_2 : Aliquot volume (mL) of the sample solution in (**4.2**) **a**)
W : Mass (g) of the analytical sample

Note (**2**) About 3 ml of tetraphenylborate solution per 10 mg of K_2O is required to form the precipitate of potassium tetraphenylborate

Comment 5 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 30 % (mass fraction) - 50 % (mass fraction) and 10 % (mass fraction) - 20 % (mass fraction) were 100.2 % - 100.8 % and 99.3 % - 102.2 % as water soluble potassium (W-K_2O) respectively.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.7 % (mass fraction) for solid fertilizers.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.122- 128, Yokendo, Tokyo (1988)
2) Keiji YAGI, Aiko YANO and Hideo SOETA: Verification of Performance Characteristics of Testing Method for Nitrate Nitrogen Content in Fertilizer by Phenol Sulfuric Acid Method, Research Report of Fertilizer **Vol. 5**, p. 201 - 211 (2012)

(**5**) **Flow sheet for water-soluble potassium**: The flow sheet for water-soluble potassium in fertilizers is shown below:

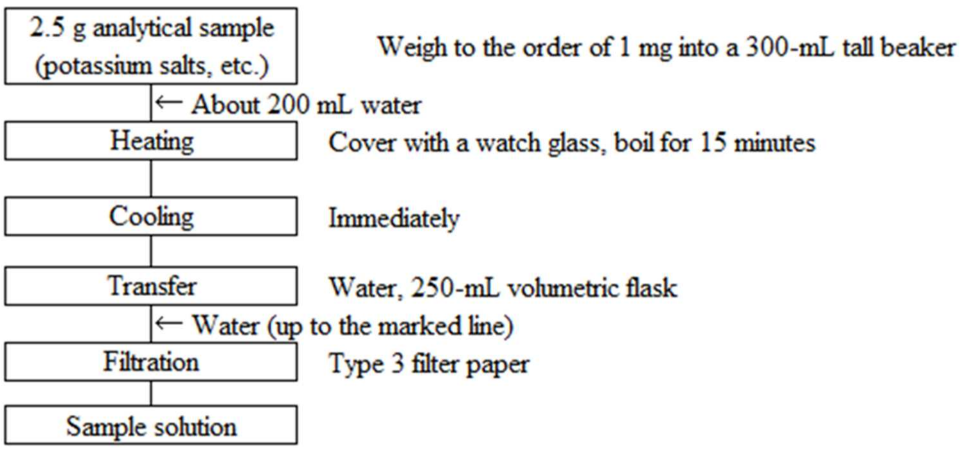


Figure 1-1 Flow sheet for water-soluble potassium in fertilizers
(Extraction procedure (4.1.1))

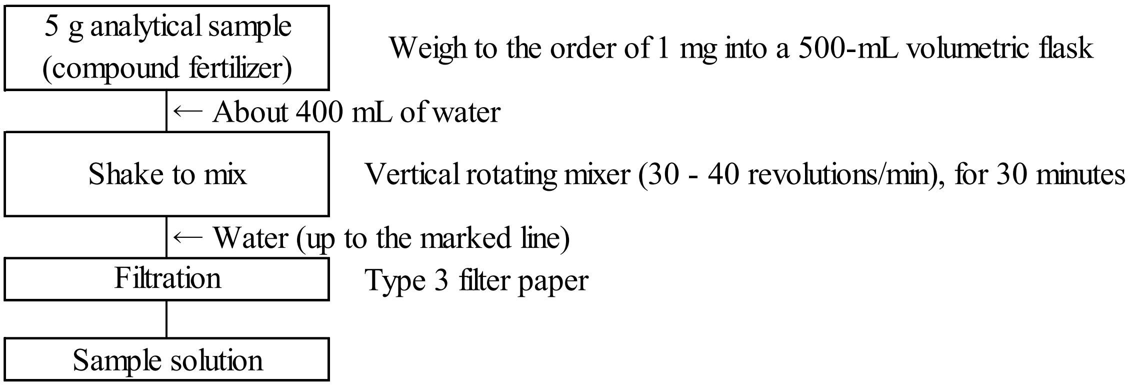


Figure 1-2 Flow sheet for water-soluble potassium in fertilizers
(Extraction procedure (4.1.2))

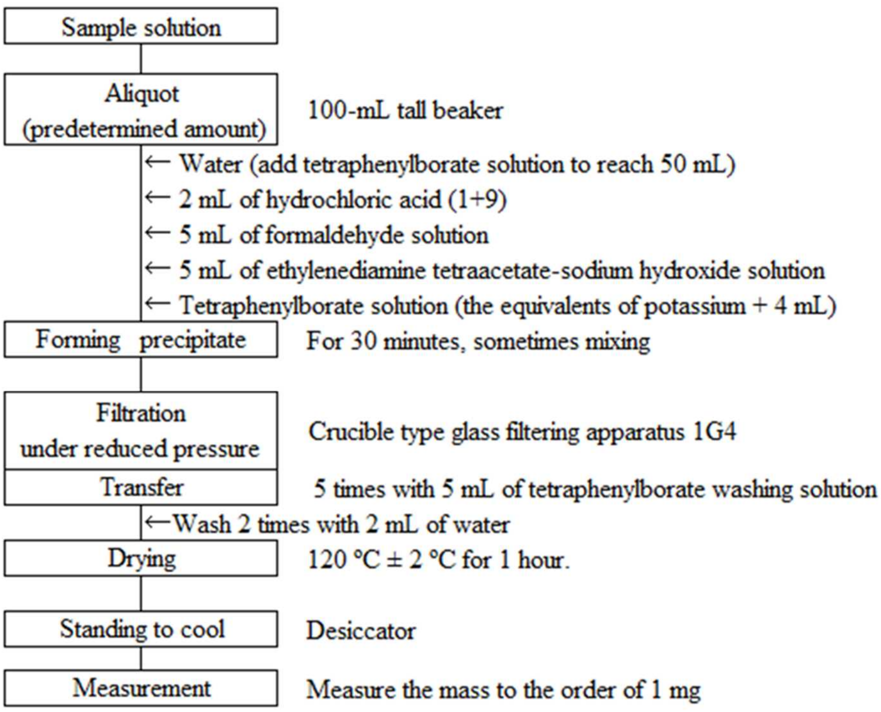


Figure 2 Flow sheet for water-soluble potassium in fertilizers
(Measurement procedure)

4.3.3.c Sodium tetraphenylborate volumetric analysis

(1) Summary

This testing method is applicable to fertilizers containing potassium salt but not organic matters. This testing method is classified as Type E and its symbol is 4.3.3.c-2017 or W-K.c-1.

Extract by adding water to an analytical sample, mask co-existing ammonium and other salts with formaldehyde, and make potassium ion and tetraphenylborate react with each other. Measure unconsumed tetraphenylborate by conducting a precipitate titration to obtain water-soluble potassium (W-K_2O) in an analytical sample.

(2) Reagent: Reagents are as shown below.

a) **Formaldehyde solution**: A JIS Guaranteed Reagent specified in JIS K 8872 or a reagent of equivalent quality.

b) **Sodium hydroxide solution (120 g/L)** (1): Dissolve 30 g of sodium hydroxide specified in JIS K 8576 in water to make 250 mL.

c) **Tetraphenylborate solution** (1): Put 12.2 g of sodium tetraphenylborate in a 1000-mL volumetric flask, dissolve by adding about 800 mL of water and add 3 mL of sodium hydroxide (120 g/L) to the total filtrate, and further add water up to the marked line. Filter with Type 3 filter paper in the case of usage.

d) **Benzalkonium chloride solution (3.3 g/500 mL)** (1): Dissolve 3.3 g of benzalkonium chloride in 500 mL of water.

e) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

f) **Titan Yellow solution (0.04 g/100mL**): Dissolve 0.04 g of Titan Yellow in 100 mL of water in the case of usage.

g) **Potassium standard solution (K_2O 2 mg/mL)** (1): Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 3.166 g into a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as shown below:

a) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

b) **Hot plate** : A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Compound fertilizers containing potassium salts and magnesia potassium sulfate

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL tall beaker.

b) Add about 200 mL of water, and cover with a watch glass and heat on a hot plate to boil for about 15 minutes.

c) After immediate cooling is complete, transfer it to a 250- mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make an extract.

Comment 1 In the procedure in **a**), a 250-mL volumetric flask can be used instead of a 300-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, “cover with a watch glass” in **b**) is replaced by “place a funnel”. Skip “transfer it to a 250-mL volumetric flask with water" in the procedure in **c**).

Comment 2 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Compound fertilizers containing no magnesia potassium sulfate**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make an extract.

Comment 3 In the procedure in **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Precipitate formation**: Form precipitate as shown below.

a) Put 5 mL - 15 mL (no more than the equivalents of 30 mg as K_2O) of the extract in a 100-mL volumetric flask.
b) Add water to the solution to make about 30 mL.
c) Add about 5 mL of formaldehyde solution and add 5 mL of sodium hydroxide solution (120 g/L).
d) Add 25 mL of tetraphenylborate solution at the rate of one or two drop (s) per second while shaking to mix.
e) After adding water up to the marked line, leave at rest for 10 minutes.
f) Filter with Type 3 filter paper to make a sample solution.

(**4.3**) **Measurement**: Conduct measurement as shown below.

a) **Calibration curve preparation**

1) Put 1 mL - 15 mL of potassium standard solution (K_2O 2 mg/mL) in 100-mL volumetric flasks step-by-step.
2) Conduct the same procedures as (**4.2**) **b**) - **f**) to make K_2O 2 mg/100 mL - 30 mg/100 mL of the potassium standard solutions for the calibration curve preparation.
3) Conduct the same procedures as **2**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.
4) Put 40 mL of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation in an Erlenmeyer flask respectively.
5) Add a few drops of Titan Yellow solution.
6) Titrate with a benzalkonium chloride solution (3.3 g/500 mL) until the color of the solution changes to light red (2).
7) Prepare a curve for the relationship between the potassium concentration and the volume of the benzalkonium chloride solution (3.3 g/500 mL) required for the titration of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

b) **Sample measurement**

1) Put 40 mL of the sample solution of (**4.2**) **f**) in a 100-mL Erlenmeyer flask.
2) Conduct similarly as in **a**) **5**) - **6**) to obtain the volume of the benzalkonium chloride solution (3.3 g/500 mL) required for the titration.
3) Obtain the potassium content from the calibration curve, and calculate water soluble potassium

($W\text{-}K_2O$) in the analytical sample.

Note (**2**) If the solution temperature is no more than 20 ºC, the reaction does not advance in some cases. Therefore, it is recommended to heat the solution up to about 30 ºC.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.128- 132, Yokendo, Tokyo (1988)

(**5**) **Flow sheet for water-soluble potassium**: The flow sheet for water-soluble potassium in fertilizers is shown below:

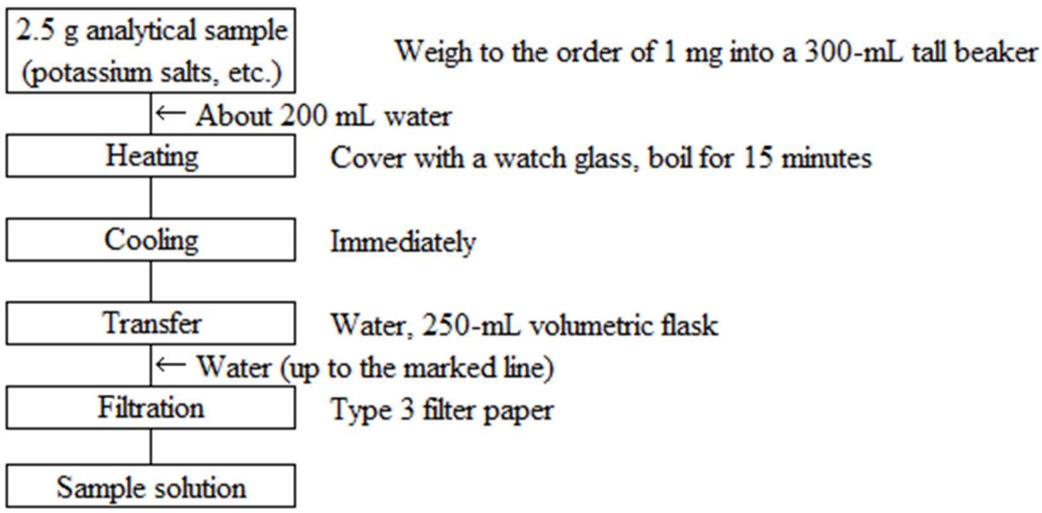


Figure 1-1 Flow sheet for water-soluble potassium in fertilizers (Extraction procedure (4.1.1))

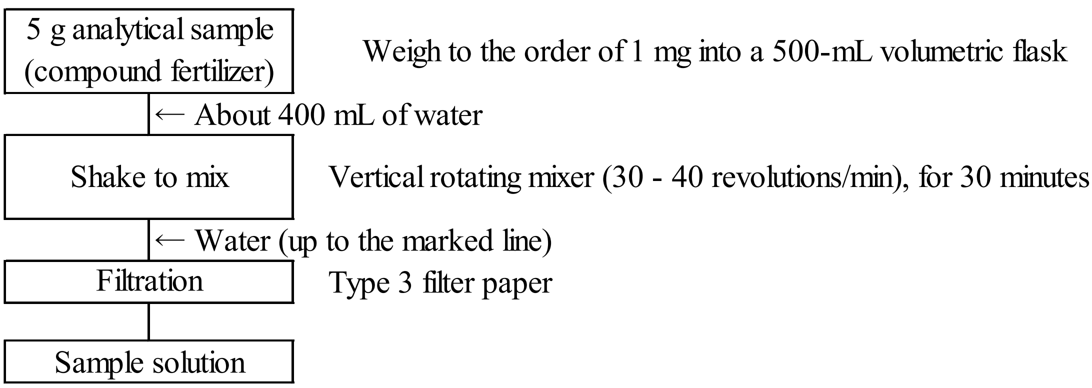


Figure 1-2 Flow sheet for water-soluble potassium in fertilizers (Extraction procedure (4.1.2))

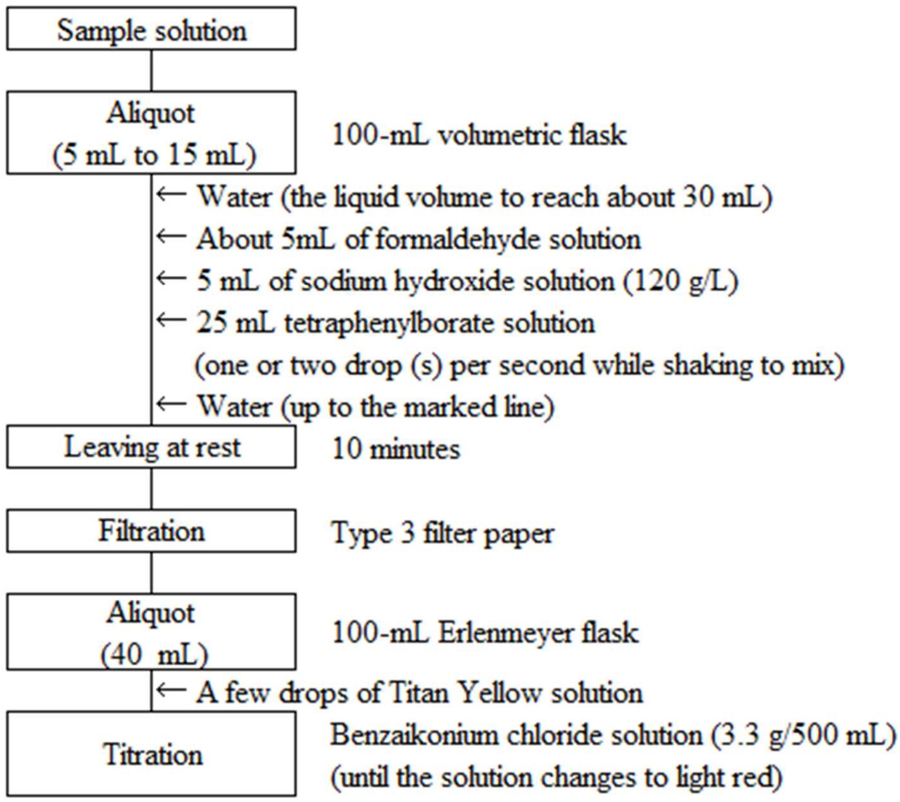


Figure 2 Flow sheet for water-soluble potassium in fertilizers
(Precipitate formation and measurement procedure)

4.3.3.d ICP Optical Emission Spectrometry

(1) Summary

This testing method is classified as Type D for solid fertilizers and Type B for fluid fertilizers.
Its symbol is 4.3.3.d-2019 or W-K.d-2.
Add water to an analytical sample to extract, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the potassium at a wavelength of 766.491 nm, etc. to obtain water-soluble potassium (W-K_2O) in the analytical sample. In addition, the performance of this testing method is shown in **Comment 11**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Potassium standard solution (K_2O 1 mg/mL)** (1): Heat potassium chloride specified in JIS K 8121 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.583 g into a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

d) **Potassium standard solution (K_2O 20 µg/mL - 0.16 mg/mL) for the calibration curve preparation** (1): Put 2 mL - 16 mL of potassium standard solution (K_2O 1 mg/mL) in 100-mL volumetric flasks step-by-step, add about 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

e) **Potassium standard solution (K_2O 2 µg/mL - 20 µg/mL) for the calibration curve preparation** (1): Put 2 mL - 20 mL of potassium standard solution (K_2O 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

f) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the potassium standard solution in (**2**), a potassium standard solution for the calibration curve preparation can be prepared by using a potassium standard solution (K 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble potassium (W-K_2O) in the analytical sample by multiplying the concentration (K) of a potassium standard solution for calibration curve preparation or a measurement value (K) obtained in (**4.2**) by a conversion factor (1.2046).

Comment 2 There are two modes to observe emission from an ICP-OES, a horizontal observation mode and an axial observation mode. The axial observation mode does not apply to potassium since interference is serious.

Comment 3 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Extractor**: A vertical rotating mixer or vertical reciprocating shaker as described below.

aa) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

ab) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

ac) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

b) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Powdery test sample** (Solid compound fertilizers containing potassium salts and magnesia potassium sulfate)

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 300-mL tall beaker.

b) Add about 200 mL of water, and cover with a watch glass and heat on a hot plate to boil for about 15 minutes.

c) After immediate cooling is complete, transfer it to a 250- mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make a sample solution.

Comment 4 In the procedure in **a**), a 250-mL volumetric flask can be used instead of a 300-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, “cover with a watch glass” in **b**) is replaced by “place a funnel”. Skip “transfer it to a 250-mL volumetric flask with water” in the procedure in **c**).

Comment 5 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Powdery test sample** (Solid compound fertilizers not containing potassium salts and magnesia potassium sulfate)

(**4.1.2.1**) **Vertical rotating mixer**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Comment 6 In the procedure of (**4.1.2.1**) **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 7 A sample solution obtained in the procedure in (**4.1.2.1**) is also applicable to the components shown in Annex B.

(**4.1.2.2**) **Vertical reciprocating shaker**

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add about 200 mL of water, and shake to mix at 300 times/min (amplitude of 40 mm) for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Comment 8 A sample solution obtained in the procedure in (**4.1.2.2**) is also applicable to the components shown in Annex B.

(4.1.3) Fluid test sample

a) Weigh 1 mg of an analytical sample (2) to the order of 1 g, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, shake to mix and add water up to the marked line.

c) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) The sampling amount of the analytical sample is 10 g when there is less potassium content in the fertilizers such as a home garden-use fertilizer.

Comment 9 A sample solution obtained in the procedure in (**4.1.3**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometry used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 766.491 nm or 769.896 nm (3)

b) **Calibration curve preparation**

1) Spray the potassium standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at an analytical line wavelength.

2) Prepare a curve for the relationship between the potassium concentration and the indicated value of the potassium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.2 mg - 16 mg as K2O) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the potassium content from the calibration curve, and calculate water soluble potassium (W-K_2O) in the analytical sample.

Note (**3**) 769.896 nm can also be used. However, since the intensity of emission obtained is different from the one of 766.491 nm, it is necessary to understand the suitable concentration range of the calibration curve and prepare a standard solution for the calibration curve in advance.

Comment 10 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 11 The comparison of the measurement value (y_i: 3.30 % (mass fraction) - 35.22 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Flame atomic absorption spectrometry was conducted to evaluate trueness using powdery test fertilizers (25 samples). As a result, a regression equation was $y = -0.233 + 1.018x$, and its correlation coefficient (r) was 0.997. Similarly the comparison of the measurement value (y_i: 0.641 % (mass fraction) - 7.23 % (mass fraction)) and the measurement value (x_i) was conducted using fluid fertilizers (12 samples). As a result, a regression equation was $y = -0.021 + 0.969x$, and its correlation coefficient (r) was 0.999. In addition, additive recovery testing was conducted using preparation fertilizers (7 samples). As a result, the mean recovery rate at the additive level of 1.09 % (mass fraction) - 63.18 % (mass fraction) was 98.4 % - 102.4 %. Additionally, additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 5 % (mass fraction) and 0.4 % (mass fraction) were 102.3 % and 104.0 % respectively.

The results of the repeatability tests on different days using potassium sulfate, potassium bicarbonate, a home garden-use compound fertilizer (solid fertilizer), a blended fertilizer, a fluid compound fertilizer and a home garden-use compound fertilizer (fluid fertilizer) to evaluate precision were analyzed by the one-way analysis of variance. Table 1-1 and 1-2 show the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for test method validation and their analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.08 % (mass fraction) for solid fertilizers and about 0.05 % (mass fraction) for fluid fertilizers.

Table 1-1 Analysis results of the repeatability tests on different days of water soluble potassium (Solid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Potassium sulfate	5	50.57	0.42	0.8	1.43	2.8
Potassium bicarbonate	5	45.03	0.18	0.4	0.69	1.5
Home garden-use compound fertilizer (solid)	5	20.52	0.43	2.1	0.43	2.1
Blended fertilizer	5	7.15	0.18	2.5	0.21	2.9

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 1-2 Analysis results of the repeatability tests on different days of water soluble potassium (Fluid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluid compound fertilizer	7	5.69	0.02	0.4	0.06	1.1
Home garden-use compound fertilizer(fluid)	7	2.29	0.02	0.8	0.04	1.6

Footnote: Refer to Table 1-1

Table 2 Analysis results of the collaborative study
for the test method validation of water-soluble potassium

Analytical line wavelength (nm)	Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
766.491	Preparation sample (Liquid) 1	11	2.20	0.03	1.3	0.08	3.8
	Preparation sample (Liquid) 2	9	10.24	0.11	1	0.31	3.0
	Preparation sample (Liquid) 3	10	5.03	0.07	1.4	0.35	7.0
	Preparation sample (Liquid) 4	10	1.04	0.01	1.2	0.04	3.7
	Preparation sample (Liquid) 5	11	0.50	0.006	1.2	0.04	8.1
769.896	Preparation sample (Liquid) 1	8	2.18	0.03	1.2	0.13	6.1
	Preparation sample (Liquid) 2	10	10.41	0.18	1.7	0.58	5.5
	Preparation sample (Liquid) 3	9	5.13	0.06	1.2	0.33	6.5
	Preparation sample (Liquid) 4	9	1.07	0.01	1.4	0.07	6.9
	Preparation sample (Liquid) 5	10	0.51	0.005	1.0	0.05	8.8

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination of Water-Soluble Principal Ingredients (W-P_2O_5, W-K_2O, W-MgO, W-MnO and W-B_2O_3) in Liquid Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 8**, p. 1 - 9 (2015)

2) Norio FUNAKI: Simultaneous Determination of Water-soluble Principal Ingredients in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 12**, p. 28 – 51 (2019)

3) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(5) **Flow sheet for water-soluble potassium**: The flow sheet for water-soluble potassium in the fluid fertilizers is shown below:

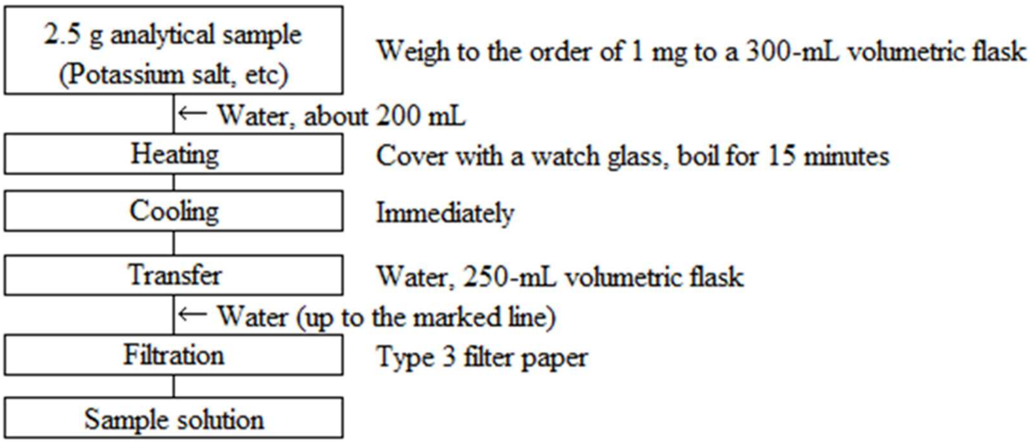


Figure 1-1 Flow sheet for water-soluble potassium in fertilizers
(Extraction procedure (4.1.1))

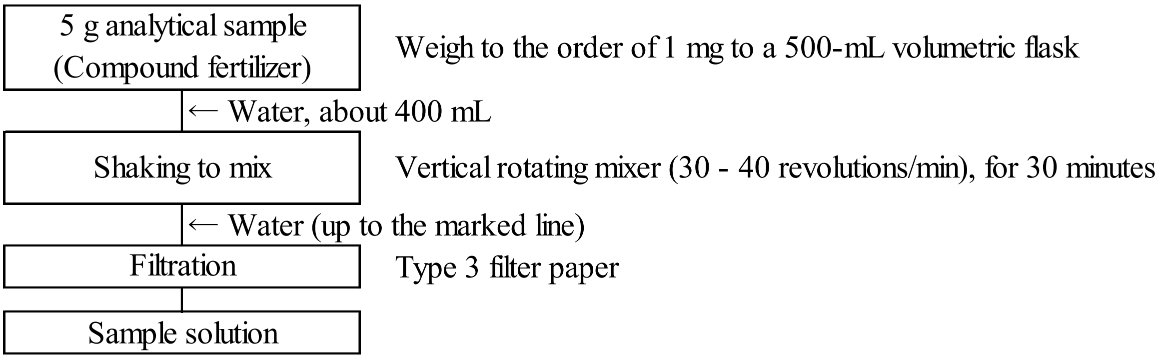


Figure 1-2 Flow sheet for water-soluble potassium in fluid fertilizers
(Extraction procedure (4.1.2.1))

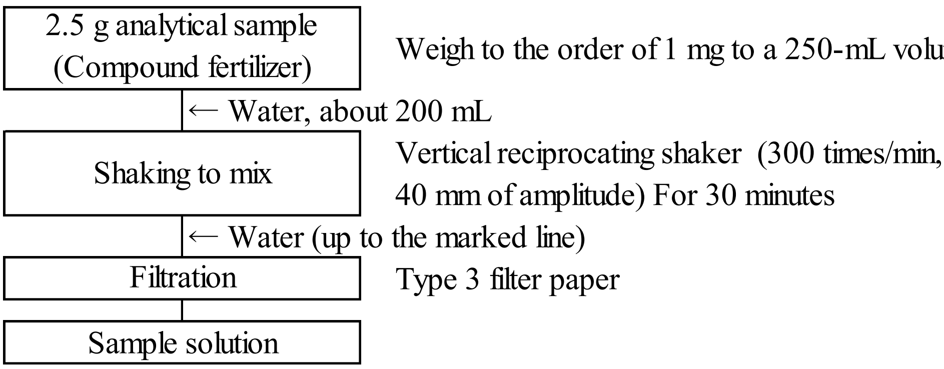


Figure 1-3 Flow sheet for water-soluble potassium in fluid fertilizers
(Extraction procedure (4.1.2.2))

1 g analytical sample (Fluid) — Weigh to the order of 1 mg to a 100-mL volumetric flask

← Water, about 50 mL

Shaking to mix

← Water (up to the marked line)

Filtration — Type 3 filter paper

Sample solution

Figure 1-4 Flow sheet for water-soluble potassium in fluid fertilizers
(Extraction procedure (4.1.3))

Sample solution

Aliquot (predetermined volume) — 100-mL volmetric flask

← 25 mL of hydrochloric acid (1+5)

← Water (up to the marked line)

Measurement — ICP Optical Emission Spectrometer (766.491nm)

Figure 2 Flow sheet for water-soluble potassium in fluid fertilizers
(Measurement procedure)

4.4 Silicic acid
4.4.1 Acid and base-soluble silicic acid
4.4.1.a Potassium fluoride method
(1) Summary

This testing method is applicable to fertilizers containing no silica gel fertilizers. This testing method is classified as Type B and its symbol is 4.4.1.a-2019 or S-Si.a-2.

Extract by adding hydrochloric acid (1+23) to an analytical sample, add hydrochloric acid, potassium fluoride solution and potassium chloride and cool in a refrigerator, and then filter after forming precipitate as potassium silicofluoride (K_2SiF_6). Put the precipitate in water and heat, and titrate dissolved potassium silicofluoride (K_2SiF_6) with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain the hydrochloric acid (1+23) soluble silicic acid (acid and base-soluble silicic acid (S-SiO2)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(2) Reagent: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** [(1)]: Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line [(1)]. Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C)$$

W_1 : Mass (g) of sulfamic acid sampled
A : Purity (% (mass fraction)) of sulfamic acid
V_1 : Aliquot volume (mL) of sulfamic acid solution
V_2 : Constant volume (250 mL) of sulfamic acid solution
V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Hydrochloric acid:** A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

c) **Potassium chloride**: A JIS Guaranteed Reagent specified in JIS K 8121 or a reagent of equivalent quality.

d) **Potassium chloride solution** [(1)]: Add 250 mL of ethanol specified in JIS K 8101 to 750 mL of water to mix, and add 150 mL of potassium chloride to dissolve. Add a few drops of methyl red solution (0.1 g/100 mL) as an indicator and add hydrochloric acid drop by drop until the color of the solution becomes red to make it acidic. After leaving at rest for 1 day, neutralize with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution.

e) **Potassium fluoride solution** [(1)]: Dissolve 58 g of potassium fluoride specified in JIS K 8815 in 1000 mL of water [(2)].

f) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.10 g of methyl red specified in JIS K 8896 in

100 mL of ethanol (95) specified in JIS K 8102.

g) **Phenolphthalein solution (1 g/100 mL)**: Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store in a container made of polymer that contains no silicon.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

aa) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

ab) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

b) **Hot plate**: A hot plate or a water bath, etc. which can raise the liquid temperature up to 80 ºC.

c) **Beaker made of polymer**: A beaker made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the measurement procedure in (**4.2**).

d) **Filter made of polymer**: A Gooch crucible made of polymer (compatible filter diameter: 25 mm) or a funnel for reduced pressure filtering made of polymer (compatible filter diameter: 21 mm) A Gooch crucible made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the measurement procedure in (**4.2**).

Comment 2 A funnel for reduced pressure filtering made of polymer (compatible filter diameter: 21 mm) is sold under the name Polyethylene Kiriyama Funnel PSB-21.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Constant-temperature vertical rotating mixer**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of hydrochloric acid (1+23) heated up to about 30 ºC (3), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) Since processed slag phosphoric acid fertilizers and mixed phosphoric acid fertilizers, etc. stick to the bottom of a volumetric flask easily, shake to mix the volumetric flask gently and disperse an analytical sample into the hydrochloric acid (1+23).

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Horizontal reciprocating water bath shaker**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (4).

b) Add about 150 mL of hydrochloric acid heated up to about 30 ºC (3), and shake to mix by reciprocating horizontally at 160 times/min with amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Note (**4**) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 4 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(**4.2**) **Measurement**: Conduct measurement as shown below.

a) Put a predetermined volume (the equivalents of 20 mg - 50 mg as SiO_2 and no more than 25 mL of liquid volume) in a 200-mL beaker made of polymer.

b) Add about 10 mL of hydrochloric acid and about 15 mL of potassium fluoride solution, and further add about 2 g of potassium chloride to dissolve, and then cool in a refrigerator for about 30 minutes or more (5) to form the precipitate of potassium fluoride.

c) Filter under reduced pressure with a filter made of polymer (6) topped with Type 6 filter paper, and wash the container 3 times with a potassium chloride solution, then transfer the whole precipitate into the filter, and further wash 6 - 7 times with a small amount of potassium chloride solution (7).

d) Transfer the precipitate on the filter together with the filter paper into a 300-mL tall beaker with water, and further add water to make about 200 mL and heat it and raise the liquid temperature up to 70 ºC - 80 ºC on a hot plate, etc.

e) Add a few drops of phenolphthalein solution (1 g/100 mL) to the sample solution as an indicator and titrate with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes light red.

f) Calculate acid and base-soluble silicic acid (S-SiO_2) by the following formula.

Acid and base-soluble silicic acid (S-SiO_2) (% (mass fraction)) in an analytical sample

$$= V_4 \times C \times f \times (V_5/V_6) \times (15.021/W_2) \times (100/1000)$$

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

V_5 : Constant volume (mL) of the extract in (**4.1**) **c**)

V_6 : Aliquot volume (mL) of the extract in (**4.2**) **a**)

W_2 : Mass (g) of the analytical sample

Note (**5**) To be no more than 10 ºC

(**6**) Filter paper pulp can be stuffed to restrain precipitate from outflowing.

(**7**) Until the filtrate becomes neutral.

Comment 5 (**2**) **a)** Standardization and (**4.2**) **e**) Titration procedure can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 6 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 25 % (mass fraction) - 40 % (mass fraction) and 10 % (mass fraction) were 98.4 % - 100.5 % and 101.0 % as acid-soluble silicic acid (S-SiO_2) respectively.

The comparison of the measurement value (y_i: 13.24 % (mass fraction) - 37.08 % (mass fraction)) of extraction by a horizontal reciprocating water bath shaker and the measurement value (x_i) of extraction by a constant-temperature vertical rotating mixer was conducted to evaluate trueness using fertilizers (25 samples). As a result, a regression equation was $y = -0.250 + 0.987x$, and its correlation coefficient (r) was 0.999. In addition, the results of the repeatability tests on different days using silicate slag fertilizers and mixed phosphorus fertilizer to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.3 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of acid and base-soluble silicic acid

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Silicate slag fertilizer	5	37.22	0.15	0.4	0.17	0.4
Mixed phosphorus fertilizer	5	11.82	0.09	0.8	0.16	1.4

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table2 Analysis results of the collaborative study for the test method validation of acid and base-soluble silicic acid

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Silicate slag fertilizer	11	34.66	0.19	0.6	0.53	1.5
Mixed phosphorus fertilizer	11	21.35	1.0	1.0	0.45	2.1
Processed slag phosphate fertilizer	10	28.92	1.0	1.0	0.65	2.2
Mixed potassium fertilizer	11	16.15	0.90	0.9	0.44	2.7
Autoclaved lightweight concrete powdery fertilizer	11	25.00	0.7	0.7	0.46	1.9

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.144- 146, Yokendo, Tokyo (1988)

2) Yasushi MIYASHITA Verification of Performance Characteristics of Testing Method for Soluble Silicic Acid in Fertilizer by Potassium Fluoride Method, Research Report of Fertilizer **Vol. 7**, p. 123 - 130 (2014)

3) Toshiharu YAGI and Kenta SAKUMA: Extraction Method for the Silicic Acid in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 12**, p. 1 – 9 (2019)

4) Kenta SAKUMA, Tarou MOTOKI and Toshiharu YAGI: Performance Evaluation of Determination Methods for Soluble Silicic Acid and Water-soluble Silicic Acid in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 62 – 75 (2020)

5) **Flow sheet for acid-soluble silicic acid**: The flow sheet for acid and base-soluble silicic acid in fertilizers is shown below:

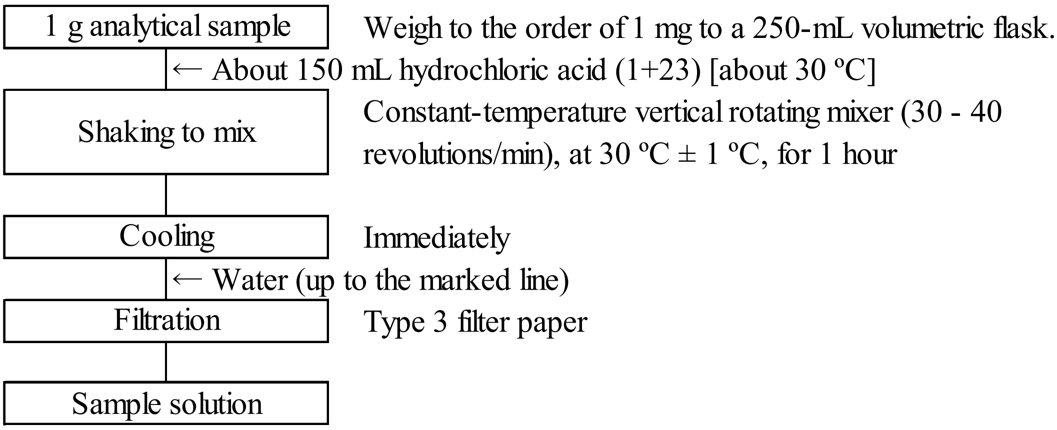


Figure 1-1 Flow sheet for acid and base-soluble silicic acid in fertilizers (Extraction procedure (4.1.1))

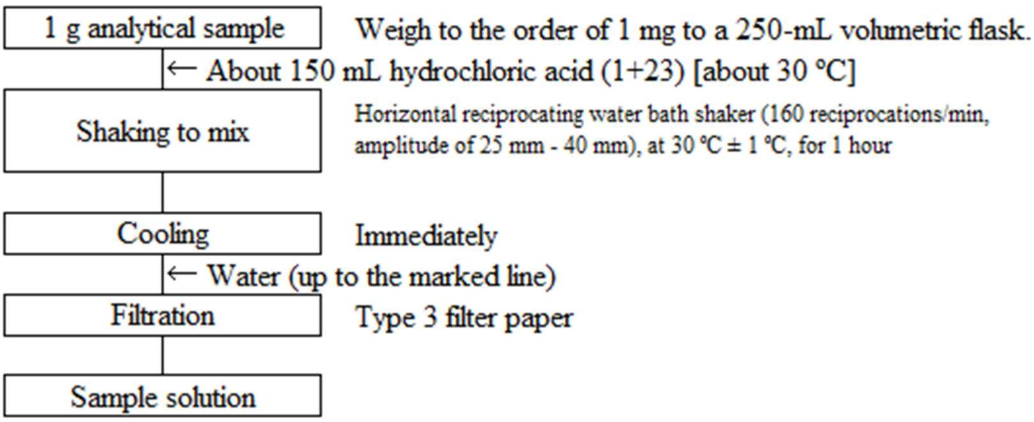


Figure 1-2 Flow sheet for acid and base-soluble silicic acid in fertilizers (Extraction procedure (4.1.2))

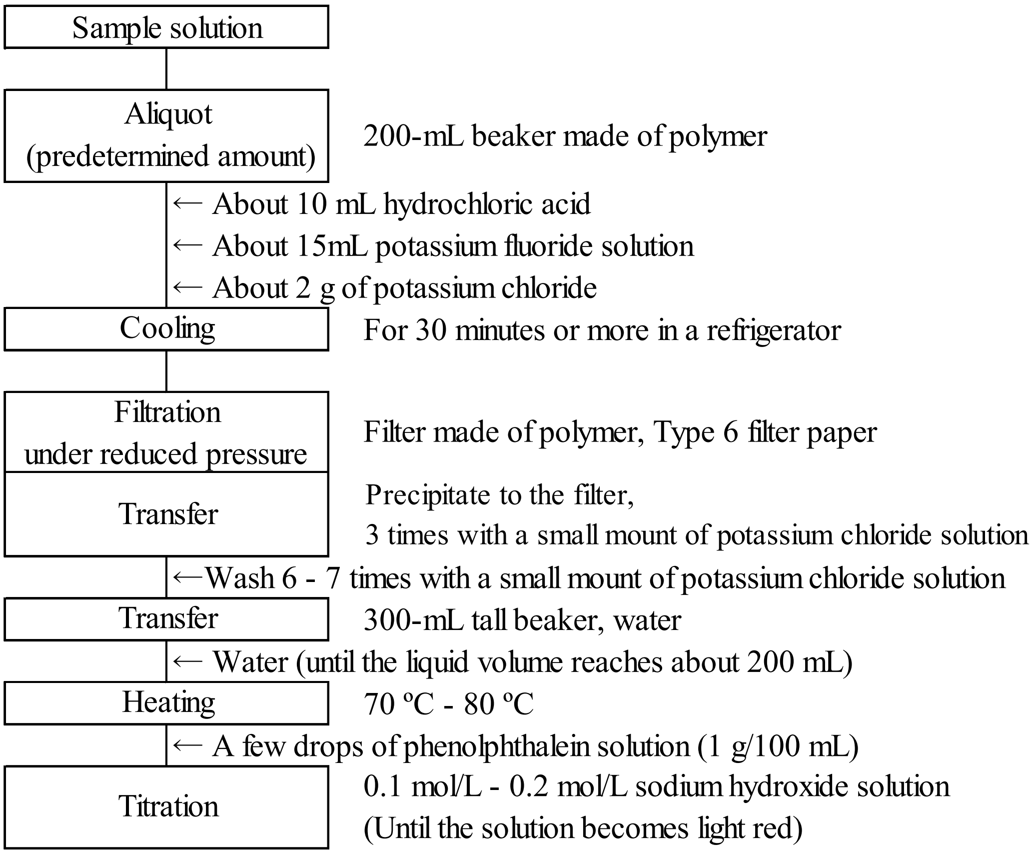


Figure 2 Flow sheet for acid and base-soluble silicic acid in fertilizers
(Measurement procedure)

4.4.1.b Potassium fluoride method (Silica gel fertilizers, etc.)

(1) Summary

This testing method is applicable to silica gel fertilizers and silica hydrogel fertilizers. This testing method is classified as Type B and its symbol is 4.4.1.b-2017 or S-Si.b-1.

Extract by adding sodium hydroxide (20 g/L) to an analytical sample, add hydrochloric acid, potassium fluoride solution and potassium chloride and cool in a refrigerator, and then filter after forming precipitate as potassium silicofluoride (K_2SiF_6). Put the precipitate in water and heat, and titrate dissolved potassium silicofluoride (K_2SiF_6) with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain the sodium hydroxide solution (20 g/L)) soluble silicic acid (acid and base-soluble silicic acid (S-SiO_2)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(2) **Reagent**: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C)$$

W_1 : Mass (g) of sulfamic acid sampled
A : Purity (% (mass fraction)) of sulfamic acid
V_1 : Aliquot volume (mL) of sulfamic acid solution
V_2 : Constant volume (250 mL) of sulfamic acid solution
V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sodium hydroxide**: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

c) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

d) **Potassium chloride**: A JIS Guaranteed Reagent specified in JIS K 8121 or a reagent of equivalent quality.

e) **Potassium chloride solution** (1): Add 250 mL of ethanol specified in JIS K 8101 to 750 mL of water to mix, and add 150 mL of potassium chloride to dissolve. Add a few drops of methyl red solution (0.1 g/100 mL) as an indicator and add hydrochloric acid drop by drop until the color of the solution becomes red to make it acidic. After leaving at rest for 1 day, neutralize with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution.

f) **Potassium fluoride solution** (1): Dissolve 58 g of potassium fluoride specified in JIS K 8815 in 1000 mL of water (2).

g) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
h) **Phenolphthalein solution (1 g/100 mL)**: Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store in a container made of polyethylene, etc. that contains no silicon.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.
a) **Water bath:** A water bath that can be adjusted to 65 ºC ± 2 ºC.
b) **Hot plate, etc.**: A hot plate or a water bath, etc. which can raise the liquid temperature up to 80 ºC.
c) **Volumetric flask and beaker made of polymer**: A flask and a beaker that are made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the extract procedure in (**4.1**) and in the measurement procedure in (**4.2**).
d) **Filter made of polymer**: A Gooch crucible made of polymer (compatible filter diameter: 25 mm) or a funnel for reduced pressure filtering made of polymer (compatible filter diameter: 21 mm) A Gooch crucible made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the measurement procedure in (**4.2**).

Comment 2 A funnel for reduced pressure filtering made of polymer (compatible filter diameter: 21 mm) is sold under the name Polyethylene Kiriyama Funnel PSB-21.

(**4**) **Test procedure**
(**4.1**) **Extraction**: Conduct extraction as shown below.
a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask made of polymer.
b) Add about 150 mL of sodium hydroxide solution (20 g/L) heated up to about 65 ºC, and heat for 1 hour while shaking to mix at every 10 minutes in a water bath at 65 ºC ± 2 ºC.
c) After immediate cooling is complete, add water up to the marked line
d) Filter with Type 3 filter paper to make a sample solution.

(**4.2**) **Measurement**: Conduct measurement as shown below.
a) Put a predetermined volume (the equivalents of 20 mg - 50 mg as SiO2 and no more than 25 mL of liquid volume) in a 200-mL beaker made of polymer.
b) Add about 10 mL of hydrochloric acid and about 15 mL of potassium fluoride solution, and further add about 2 g of potassium chloride to dissolve, and then cool in a refrigerator for about 30 minutes (3) to form the precipitate of potassium fluoride.
c) Filter under reduced pressure with a filter made of polymer (4) topped with Type 6 filter paper, and wash the container 3 times with a potassium chloride solution, then transfer the whole precipitate into the filter, and further wash 6 - 7 times with a small amount of potassium chloride solution (5).
d) Transfer the precipitate on the filter together with the filter paper into a 300-mL tall beaker with water, and further add water to make about 200 mL and heat it and raise the liquid temperature up to 70 ºC - 80 ºC on a hot plate, etc.
e) Add a few drops of phenolphthalein solution (1 g/100 mL) as an indicator and titrate with the

0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes light red.

f) Calculate base-soluble silicic acid ($S\text{-}SiO_2$) by the following formula.

Base-soluble silicic acid ($S\text{-}SiO_2$) (% (mass fraction)) in an analytical sample
$= V_4 \times C \times f \times (V_5/V_6) \times (15.021/W_2) \times (100/1000)$

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_5 : Constant volume (mL) of the extract in (**4.1**) **c**)
V_6 : Aliquot volume (mL) of the extract in (**4.2**) **a**)
W_2 : Mass (g) of the analytical sample

Note (**3**) To be no more than 10 ºC
(**4**) Filter paper pulp can be stuffed to restrain precipitate from outflowing.
(**5**) Until the filtrate becomes neutral.

Comment 3 Table 1 shows results and analysis results from a collaborative study for testing method validation.

Table 1 Analysis results of the collaborative study for the test method validation of acid and base-soluble silicic acid in silica gel fertilizers

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Silica gel fertilizer 1	8	79.37	0.23	0.3	0.55	0.7
Silica gel fertilizer 2	8	84.68	0.42	0.5	0.85	1.0
Silica gel fertilizer 3	8	89.58	0.4	0.4	0.51	0.6
Silica gel fertilizer 4	8	84.44	0.37	0.4	0.77	0.9
Silica gel fertilizer 5	8	85.77	0.46	0.5	0.59	0.7

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Takeshi HASHIMOTO, Akira SHIMIZU and Kaori OKADA: Validation of a Method of Potassium Fluoride for Determination of Sodium Hydroxide-Soluble Silicic Acid in Silica Gel Fertilizer, Research Report of Fertilizer, **Vol. 3**, p. 19 - 24 (2010)

2) Akira SHIMIZU, Shin ABE and Jun ITO: Determination of Solubility Silicic Acid in Silica gel Fertilizer and Silica gel-including Fertilizer by Potassium Fluoride Method: A Collaborative Study, Research Report of Fertilizer, **Vol. 5**, p. 31 - 40 (2012)

5) **Flow sheet for acid and base-soluble silicic acid**: The flow sheet for acid and base-soluble silicic acid in silica gel fertilizers, etc. is shown below:

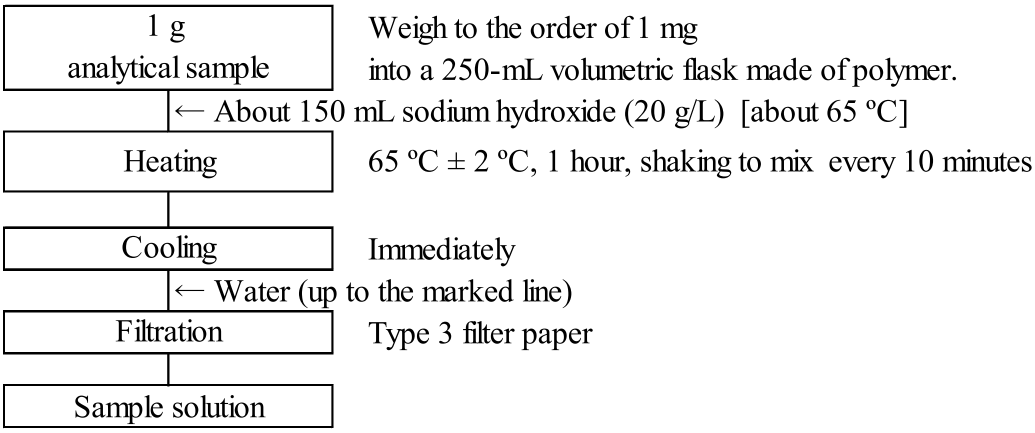


Figure 1 Flow sheet for acid and base-soluble silicic acid in silica gel fertilizers (Extraction procedure)

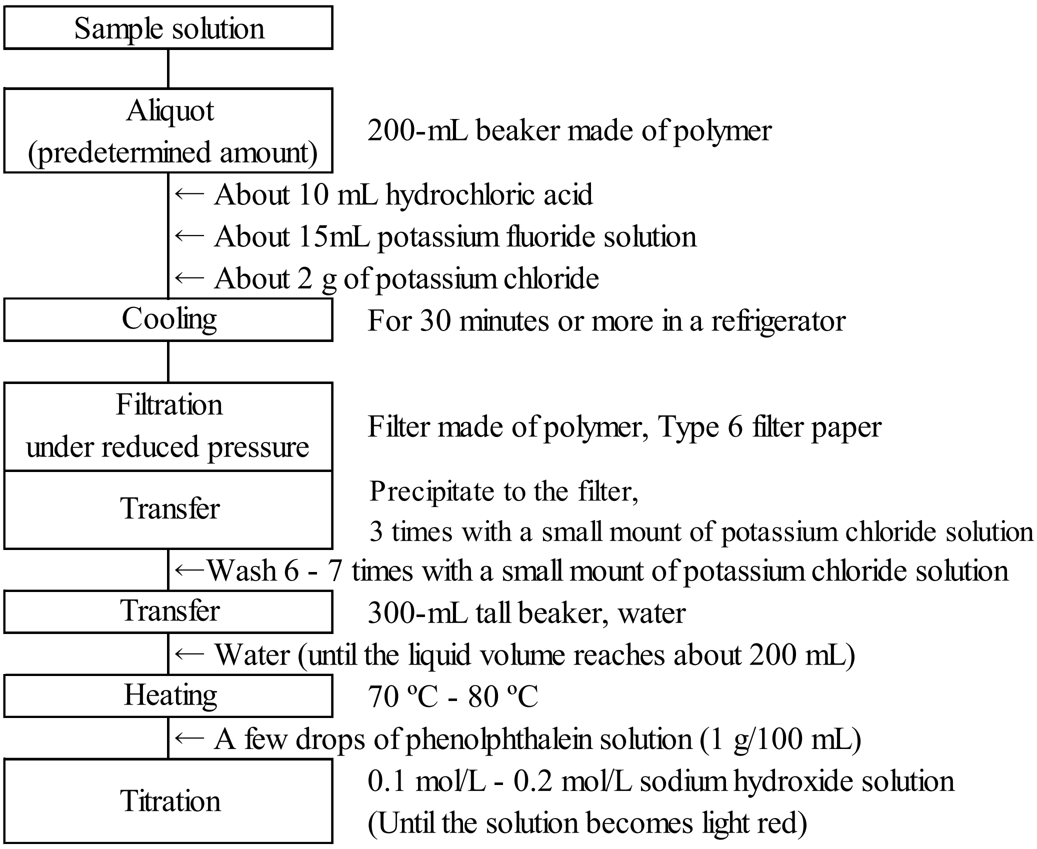


Figure 2 Flow sheet for acid and base-soluble silicic acid in silica gel fertilizers (Measurement procedure)

4.4.1.c Potassium fluoride method (Fertilizers containing silica gel fertilizers)

(1) Summary

This testing method is applicable to fertilizers containing silica gel fertilizers. This testing method is classified as Type B and its symbol is 4.4.1.c-2017 or S-Si.c-1.

Mix the equivalent volumes of the extract which is extracted by adding hydrochloric acid (1+23) to an analytical sample and the liquid by extracting non-dissolved matter on a filter paper with sodium hydroxide (20 g/L), and add hydrochloric acid, a potassium fluoride solution and potassium chloride. Cool it in a refrigerator, and then filter after forming precipitate as potassium silicofluoride (K_2SiF_6). Put the precipitate in water and heat, and titrate dissolved potassium silicofluoride (K_2SiF_6) with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain acid and base-soluble silicic acid (S-SiO2) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(**2**) **Reagent**: Reagents are as shown below.

a) 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C)$$

W_1 : Mass (g) of sulfamic acid sampled
A : Purity (%) of sulfamic acid
V_1 : Aliquot volume (mL) of sulfamic acid solution transferred
V_2 : Constant volume (250 mL) of sulfamic acid solution
V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) Sodium hydroxide: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

c) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

d) Potassium chloride: A JIS Guaranteed Reagent specified in JIS K 8121 or a reagent of equivalent quality.

e) Potassium chloride solution (1): Add 250 mL of ethanol specified in JIS K 8101 to 750 mL of water to mix, and add 150 mL of potassium chloride to dissolve. Add a few drops of methyl red solution (0.1 g/100 mL) as an indicator and add hydrochloric acid drop by drop until the color of the solution becomes red to make it acidic. After leaving at rest for 1 day, neutralize with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution.

f) Potassium fluoride solution (1): Dissolve 58 g of potassium fluoride specified in JIS K 8815 in

1000 mL of water (2).

g) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

h) **Phenolphthalein solution (1 g/100 mL)**: Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store in a container made of polymer that contains no silicon.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

(**3**) **Instruments**: Instruments are as shown below:

a) **Water bath:** A water bath that can be adjusted to 65 ºC ±2 ºC.

b) **Hot plate, etc.**: A hot plate or a water bath, etc. which can raise the liquid temperature up to 80 ºC.

c) **Volumetric flask and beaker made of polymer**: A flask and a beaker that are made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the extract procedure in (**4.1**) and in the measurement procedure in (**4.2**).

d) **Filter made of polymer**: A Gooch crucible made of polymer (compatible filter diameter: 25 mm) or a funnel for reduced pressure filtering made of polymer (compatible filter diameter: 21 mm) A Gooch crucible made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the measurement procedure in (**4.2**).

Comment 2 A funnel for reduced pressure filtering made of polymer (compatible filter diameter: 21 mm) is sold under the name Polyethylene Kiriyama Funnel PSB-21.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 300-mL tall beaker.

b) Add 150 mL of hydrochloric acid (1+23) warmed up to about 30 ºC, and warm it in a water bath at 30 ºC ± 2 ºC while stirring every 10minutes with a glass rod for 1 hour.

c) After immediate cooling is complete, filter with Type 6 filter paper to a 250-mL volumetric flask as an acceptor. Wash the tall beaker with water, then transfer the whole residue on the filter paper and add water up to the marked line to make a sample solution (1).

d) Put the non-dissolved matter on the filter paper together with the filter paper in a 250- mL volumetric flask made of polymer.

e) Add 150 mL of sodium hydroxide solution heated up to about 65 ºC, and heat for 1 hour while shaking to mix at every 10 minutes in a water bath at 65 ºC ± 2 ºC.

f) After immediate cooling is complete, add water up to the marked line and filter with Type 3 filter paper to make a sample solution (2).

(**4.2**) **Measuremen**t: Conduct measurement as shown below.

a) Put a predetermined volume (the equivalents of 20 mg - 50 mg as SiO2) (3) of the sample solution (1) and (2) in a 200-mL beaker made of polymer.

b) Add about 10 mL of hydrochloric acid and about 15 mL of potassium fluoride solution, and further add about 2 g of potassium chloride to dissolve, and then cool in a refrigerator for about 30 minutes or more (4) to form the precipitate of potassium fluoride.

c) Filter under reduced pressure with a filter made of polymer (5) topped with Type 6 filter paper, and wash the container 3 times with a potassium chloride solution, then transfer the whole precipitate into the filter, and further wash 6 - 7 times with a small amount of potassium chloride solution (6).

d) Transfer the precipitate on the filter together with the filter paper into a 300-mL tall beaker with water, and further add water to make about 200 mL and heat it and raise the liquid temperature up to 70 ºC - 80 ºC on a hot plate, etc.

e) Add a few drops of phenolphthalein solution (1 g/100 mL) as an indicator and titrate with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes light red.

f) Calculate acid and base-soluble silicic acid (S-SiO_2) by the following formula.

Acid and base-soluble silicic acid (S-SiO_2) (% (mass fraction)) in an analytical sample

$$= V_4 \times C \times f \times (V_5/V_6) \times (15.021/W_2) \times (100/1000)$$

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C : Estimated concentration (mol/L) of sodium hydroxide solution (0.1 mol/L - 0.2 mol/L)

f : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

V_5 : Constant volume (mL) of the sample solution in (**4.1**) **c**)

V_6 : Aliquot volume (mL) of the sample solution in (**4.2**) **a**)

W_2 : Mass (g) of the analytical sample

Note (**3**) The transferred volume of the sample solution (1) and the sample solution (2) should be equivalent.

(**4**) To be no more than 10 ºC

(**5**) Filter paper pulp can be stuffed to restrain precipitate from outflowing.

(**6**) Until the filtrate becomes neutral.

Comment 3 Table 1 shows results and analysis results from a collaborative study for testing method validation.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.6 % (mass fraction).

Table 1 Analysis results of the collaborative study for the test method validation of acid and base-soluble silicic acid in fertilizers including a silica gel fertilizer

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Mixed phosphorus fertilizer 1	8	24.99	0.16	0.6	0.33	1.3
Mixed phosphorus fertilizer 2	8	34.50	0.26	0.7	0.48	1.4
Compound fertilizer 1	8	30.30	0.13	0.4	0.60	2.0
Compound fertilizer 2	8	33.34	0.13	0.4	0.47	1.4
Compound fertilizer 3	8	15.76	0.11	0.7	0.21	1.3

1) Number of laboratories used in analysis
2) Total mean (n = number of laboratories × number of replication(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Akira SHIMIZU, Jun ITO and Shin ABE: Method Validation of Potassium Fluoride Method for Determination of Acid-Soluble and Base-Soluble Silicic Acid in Fertilizer containing Silica gel, Research Report of Fertilizer, **Vol. 4**, p. 1 - 8 (2011)

2) Akira SHIMIZU: Method Validation of Potassium Fluoride Method for Determination of Acid-Soluble and Base-Soluble Silicic Acid in Fertilizer containing Silica Gel, Research Report of Fertilizer, **Vol. 6**, p. 1 - 8 (2013)

3) Shinji KAWAGUCHI and Akira SHIMIZU: Determination of Soluble Silicic Acid in Fertilizers Containing Silica Gel Fertilizer by Potassium Fluoride Method: A Collaborative Study, Research Report of Fertilizer **Vol. 7**, p. 36 - 42 (2014)

(5) **Flow sheet for acid and base-soluble silicic acid**: The flow sheet for acid and base-soluble silicic acid in fertilizers including silica gel fertilizers is shown below:

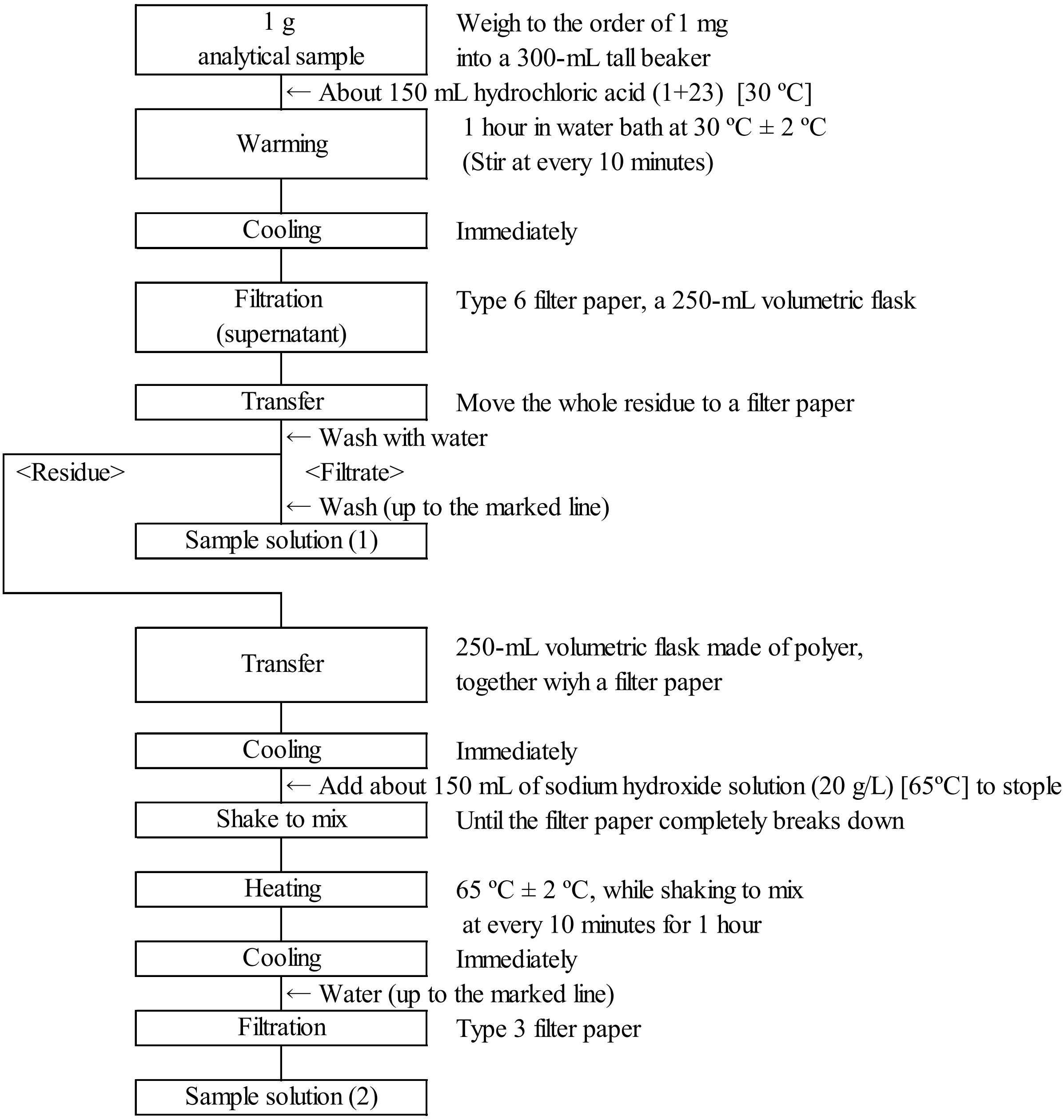


Figure 1 Flow sheet for acid and base-soluble silicic acid in fertilizers
(Extraction procedure)

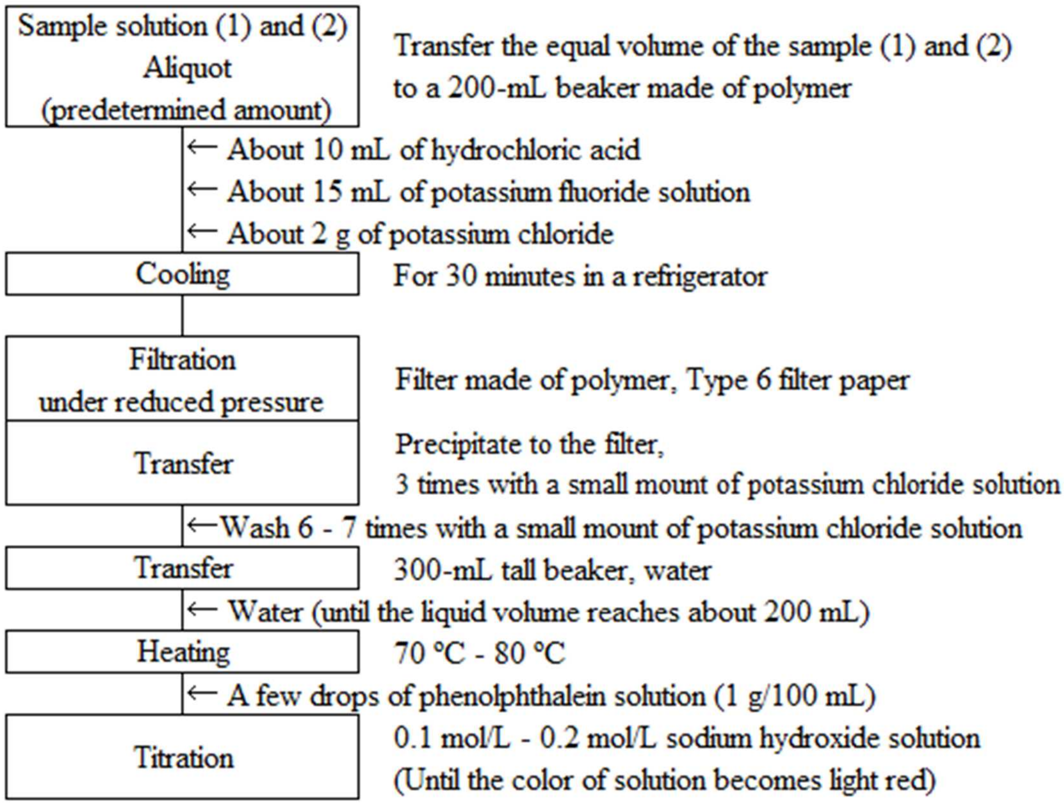


Figure 2 Flow sheet for acid and base-soluble silicic acid in fertilizers
(Measurement procedure)

4.4.1.d Perchloric acid method

(1) Summary

This testing method is applicable to fertilizers containing no silica gel fertilizers. This testing method is classified as Type E and its symbol is 4.4.1.d-2017 or S-Si.d-1.

Extract by adding hydrochloric acid (1+23) to an analytical sample, add perchloric acid and heat, and then measure the formed silicic acid anhydride to obtain hydrochloric acid (1+23) soluble silicic acid (acid and base-soluble silicic acid (S-SiO_2)) in an analytical sample.

(2) Reagents

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Perchloric acid: A JIS Guaranteed Reagent specified in JIS K 8223 or a reagent of equivalent quality.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Constant-temperature vertical rotating mixer: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

b) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

c) Electric furnace: An electric furnace that can be adjusted to 1000 ºC - 1100 ºC.

d) Crucible: After heating a chemical analysis porcelain crucible specified in JIS R 1301 in an electric funnel at 1000 ºC - 1100 ºC, let it stand to cool in a desiccator and measure the mass to the order of 1 mg.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of hydrochloric acid (1+23) heated up to about 30 ºC, and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Comment 1 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(4.2) Measurement: Conduct measurement as shown below.

a) Put a predetermined volume in a 100-mL tall beaker.

b) Add about 10 mL of perchloric acid and heat on a hot plate.

c) When white smoke from the perchloric acid starts evolving, cover with a watch glass, then heat for 15 - 20 minutes to form precipitate of silicone dioxide.

d) After standing to cool, add about 50 mL of hydrochloric acid (1+4) and cover with a watch glass and heat at 70 ºC - 80 ºC on a hot plate for several minutes.

e) Immediately after heating, filter with Type 5-C filter paper, wash the container with heated hydrochloric acid (1+10) and transfer the whole precipitate to a filter paper.

f) Wash the precipitate and the filter paper 2 times with heated hydrochloric acid (1+10), and further wash several times with hot water (1).

g) Put the precipitate together with the filter paper into the crucible.

h) Put the crucible into a drying apparatus and dry at about 120 ºC for 1 hour.

i) After standing to cool, put the crucible into an electric funnel and heat gently to char (2).

j) Ignite at 1000 ºC - 1100 ºC for 1 hour [(2)].
k) After ignition, move the crucible to a desiccator and let it stand to cool.
l) After standing to cool, remove the crucible from the desiccator and measure the mass to the order of 1 mg.
m) Calculate acid and base-soluble silicic acid (S-SiO_2) by the following formula.

Acid and base-soluble silicic acid (S-SiO_2) (% (mass fraction)) in an analytical sample
$= A \times (V_1/V_2)/W \times 100$

A : Mass (g) of the precipitate
W : Mass (g) of the analytical sample
V_1 : Constant volume (mL) of the sample solution in (**4.1**) **c**)
V_2 : Aliquot volume (mL) of the sample solution in (**4.2**) **a**)

Note (**1**) Wash until no reaction by chloride appears in the filtrate.
(**2**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 1000 °C - 1100 °C in 1 to 2 hours.

References
1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.143- 144, Yokendo, Tokyo (1988)

5) **Flow sheet for acid and base-soluble silicic acid**: The flow sheet for acid and base-soluble silicic acid in fertilizers is shown below:

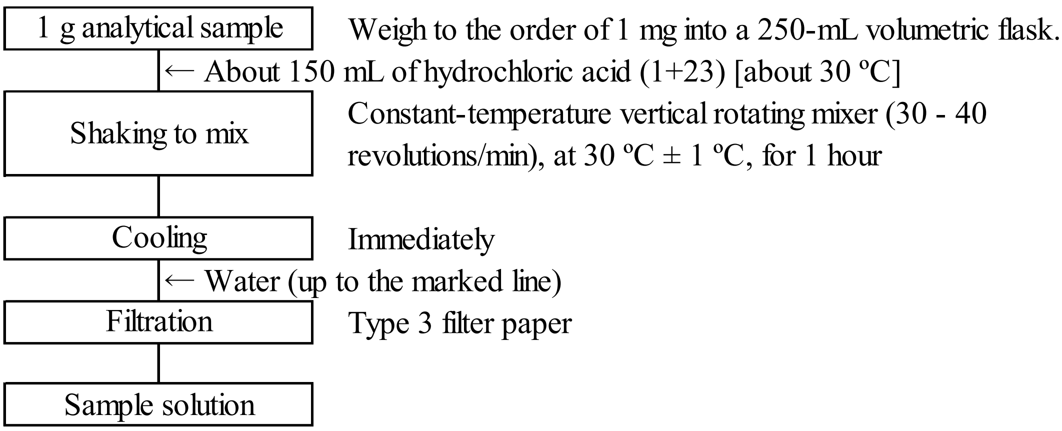


Figure 1 Flow sheet for acid and base-soluble silicic acid in fertilizers (Extraction procedure)

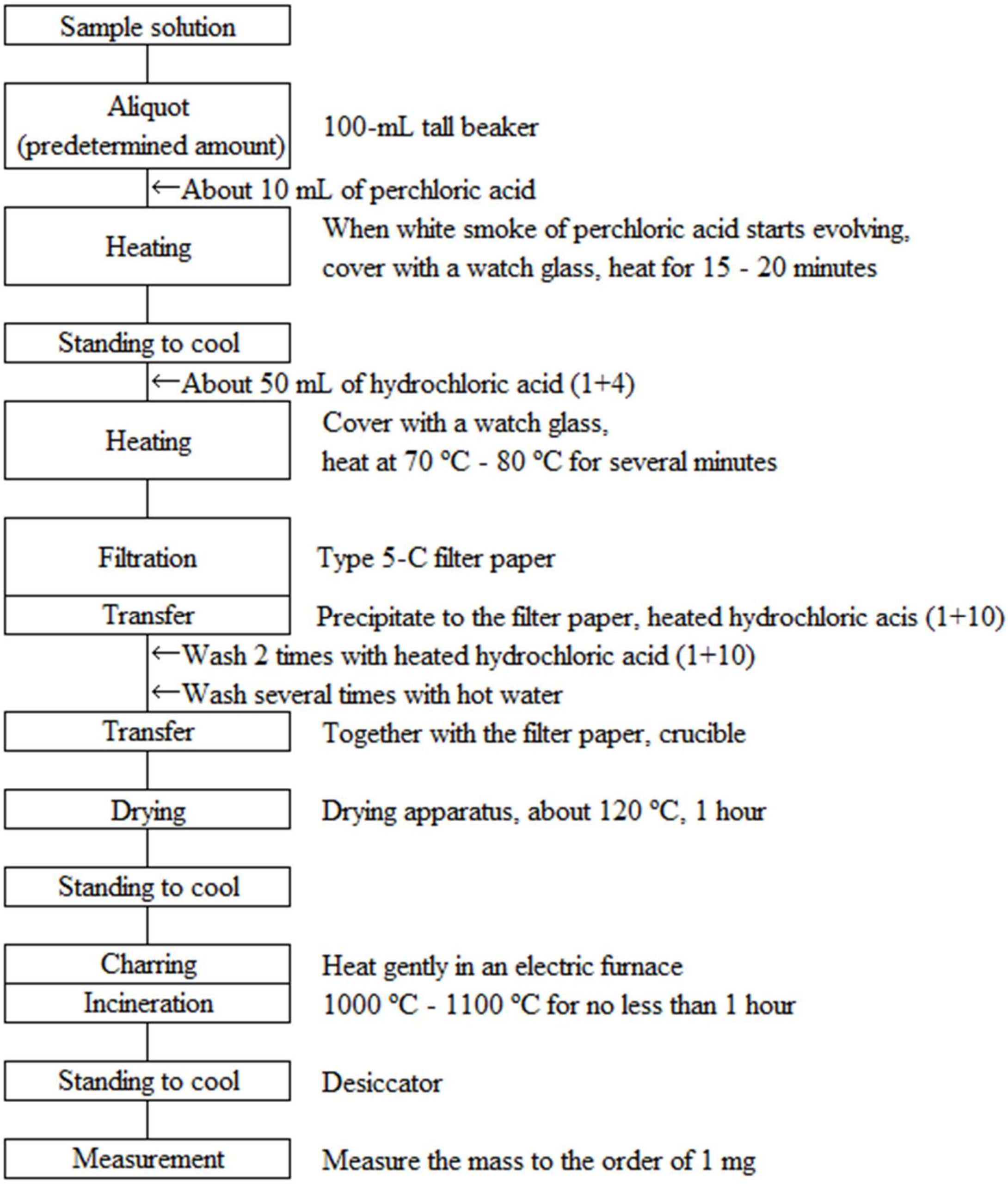


Figure 2 Flow sheet for acid and base-soluble silicic acid in fertilizers (Measurement procedure)

4.4.2 Water-soluble silicic acid
4.4.2.a Potassium fluoride method
(1) Summary

This testing method is applicable to liquid potassium silicate fertilizers. This testing method is classified as Type B and its symbol is 4.4.2.a-2019 or W-Si.a-2.

Extract by adding water to an analytical sample, add hydrochloric acid, a potassium fluoride solution and potassium chloride and cool in a refrigerator, and then filter after forming precipitate as potassium silicofluoride (K_2SiF_6). Put the precipitate in water and heat, and titrate dissolved potassium silicofluoride (K_2SiF_6) with 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain the water-soluble silicic acid (W-SiO_2) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(2) Reagent: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C)$$

W_1 : Mass (g) of sulfamic acid sampled

A : Purity (% (mass fraction)) of sulfamic acid

V_1 : Aliquot volume (mL) of sulfamic acid solution

V_2 : Constant volume (250 mL) of sulfamic acid solution

V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

c) **Potassium chloride**: A JIS Guaranteed Reagent specified in JIS K 8121 or a reagent of equivalent quality.

d) **Potassium chloride solution** (1): Add 250 mL of ethanol specified in JIS K 8101 to 750 mL of water to mix, and add 150 mL of potassium chloride to dissolve. Add a few drops of methyl red solution (0.1 g/100 mL) as an indicator and add hydrochloric acid drop by drop until the color of the solution becomes red to make it acidic. After leaving at rest for 1 day, neutralize with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution.

e) **Potassium fluoride solution** (1): Dissolve 58 g of potassium fluoride specified in JIS K 8815 in 1000 mL of water (2).

f) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.10 g of methyl red specified in JIS K 8896 in

100 mL of ethanol (95) specified in JIS K 8102.

g) **Phenolphthalein solution (1 g/100 mL)**: Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store in a container made of polymer that contains no silicon.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

(**3**) **Instruments**: Instruments are as shown below:

a) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

b) **Hot plate, etc.:** A hot plate or a water bath, etc. which can raise the liquid temperature up to 80 ºC.

c) **Beaker made of polymer**: A beaker made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the extract procedure in (**4.1**) and in the measurement procedure in (**4.2**).

d) **Filter made of polymer**: A Gooch crucible made of polymer (compatible filter diameter: 25 mm) or a funnel for reduced pressure filtering made of polymer (compatible filter diameter: 21 mm) A Gooch crucible made of polyethylene, etc. of a quality of material which prevents silicic acid from eluting in the measurement procedure in (**4.2**).

Comment 2 A funnel for reduced pressure filtering made of polymer (compatible filter diameter: 21 mm) is sold under the name Polyethylene Kiriyama Funnel PSB-21.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Vertical rotating mixer**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 3 In the procedure of (**4.1.1**) **a**) and (**4.1.2**) **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it into a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 4 The procedure in (**4.1.1**) is the same as the procedure in (**4.1.1.1**) in **4.2.4.a**.

(**4.1.2**) **Simple manual extraction**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

(**4.2**) **Measurement**: Conduct measurement as shown below.

a) Put a predetermined volume (the equivalents of 20 mg - 50 mg as SiO_2 and no more than 25 mL of liquid volume) in a 200-mL beaker made of polymer.

b) Add about 10 mL of hydrochloric acid and about 15 mL of potassium fluoride solution, and further add about 2 g of potassium chloride to dissolve, and then cool in a refrigerator for about 30 minutes or more (3) to form the precipitate of potassium fluoride.

c) Filter under reduced pressure with a filter made of polymer (4) topped with Type 6 filter paper, and wash the container 3 times with a potassium chloride solution, then transfer the whole precipitate into the filter, and further wash 6 - 7 times with a small amount of potassium chloride solution (5).

d) Transfer the precipitate on the filter together with the filter paper into a 300-mL tall beaker with water, and further add water to make about 200 mL and heat it and raise the liquid temperature up to 70 ºC - 80 ºC on a hot plate, etc.

e) Add a few drops of phenolphthalein solution (1 g/100 mL) to the sample solution as an indicator and titrate with the 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes light red.

f) Calculate water soluble silicic acid (W-SiO_2) by the following formula.

Water soluble silicic acid (W-SiO_2) (% (mass fraction)) in an analytical sample

$$= V_4 \times C \times f \times (V_5/V_6) \times (15.021/W_2) \times (100/1000)$$

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

V_5 : Constant volume (mL) of the extract in (**4.1**) **c**)

V_6 : Aliquot volume (mL) of the extract in (**4.2**) **a**)

W_2 : Mass (g) of the analytical sample

Note (**3**) To be no more than 10 ºC

(**4**) Filter paper pulp can be stuffed to restrain precipitate from outflowing.

(**5**) Until the filtrate becomes neutral.

Comment 5 The titration procedures in (**2**) **a**) Standardization and (**4.2**) **e**) Titration can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 6 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 30 % (mass fraction) and 12 % (mass fraction) - 20% (mass fraction) were 100.7 % and 99.5 % - 100.5 as water-soluble silicic acid (W-SiO_2) respectively.

The results of the repeatability tests on different days using a liquid potassium silicate fertilizer by extraction with a vertical rotating mixer or simple manual extraction to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.2 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of water-soluble silicic acid

Extraction method	Name of sample	Test days of repeatability (T)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)
Vertical rotating mixer	Liquid potassium silicate fertilizer 1	7	24.01	0.07	0.3	0.08	0.4
	Liquid potassium silicate fertilizer 2	7	16.07	0.03	0.2	0.04	0.3
Simple manual extraction	Liquid potassium silicate fertilizer 1	5	25.28	0.06	0.2	0.13	0.5
	Liquid potassium silicate fertilizer 2	5	15.98	0.12	0.8	0.15	1.0

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table2 Analysis results of the collaborative study for the test method validation of water-soluble silicic acid

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Liquid potassium silicate fertilizer A	9	16.14	0.15	0.9	0.53	1.5
Liquid potassium silicate fertilizer B	9	22.11	0.1	0.3	0.45	2.1
Liquid potassium silicate fertilizer C	9	20.52	0.1	0.6	0.65	2.2
Liquid potassium silicate fertilizer D	9	15.50	0.12	0.7	0.44	2.7
Liquid potassium silicate fertilizer E	9	29.62	0.17	0.6	0.46	1.9

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.144- 146, Yokendo, Tokyo (1988)

2) Shinji KAWAGUCHI: Verification of Performance Characteristics of Testing Method for Water-Soluble Silicic Acid in Fertilizer by Potassium Fluoride Method, Research Report of Fertilizer, **Vol. 8**, p. 174 - 181 (2015)

3) Toshiharu YAGI and Kenta SAKUMA: Extraction Method for the Silicic Acid in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 12**, p. 1 – 9 (2019)

4) Kenta SAKUMA, Tarou MOTOKI and Toshiharu YAGI: Performance Evaluation of Determination Methods for Soluble Silicic Acid and Water-soluble Silicic Acid in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 62 – 75 (2020)

5) **Flow sheet for water-soluble silicic acid**: The flow sheet for water-soluble silicic acid in fertilizers is shown below:

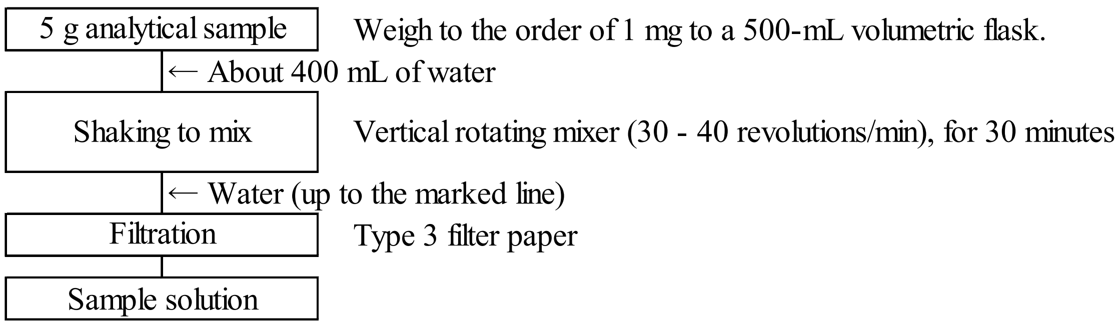


Figure 1-1 Flow sheet for water-soluble silicic acid in fertilizers (Extraction procedure (4.1.1))

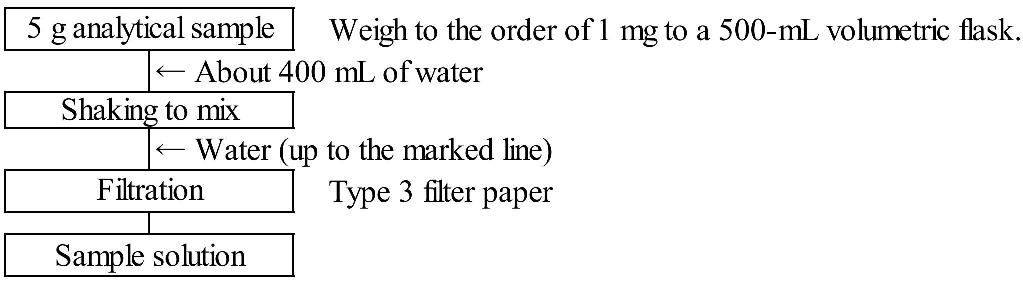


Figure 1-2 Flow sheet for water-soluble silicic acid in fertilizers (Extraction procedure

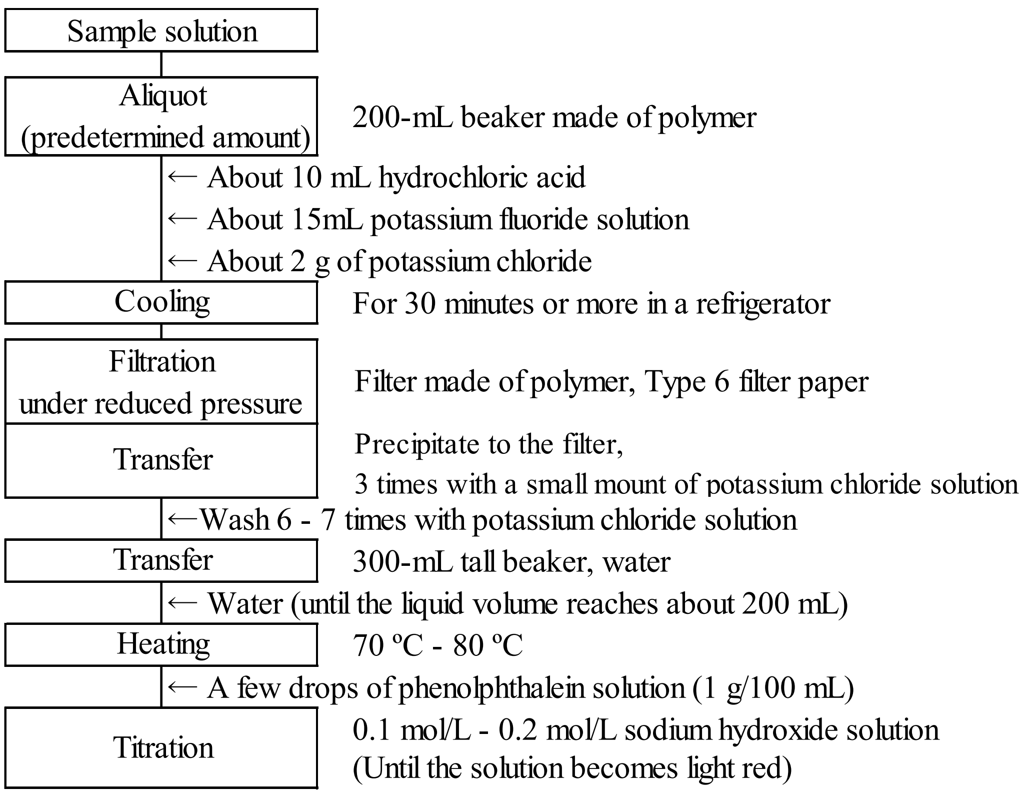


Figure 2 Flow sheet for water-soluble silicic acid in fertilizers (Measurement procedure)

4.5 Lime, calcium and alkalinity
4.5.1 Total lime
4.5.1.a Flame atomic absorption spectrometry
(1) Summary

This testing method is applicable to fertilizers containing organic matters. This testing method is classified as Type B and its symbol is 4.5.1.a-2017 or T-Ca.a-1.

Pretreat an analytical sample with incineration - hydrochloric acid boiling or aqua regia digestion, add an interference suppressor solution, and then spray in an acetylene–air flame, and measure the atomic absorption with calcium at a wavelength of 422.7 nm to quantify total lime (T-CaO) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Interference suppressor solution** (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

c) **Calcium standard solution (CaO 1 mg/mL)** (1): Heat calcium carbonate specified in JIS K 8617 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.785 g into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add 20 mL of hydrochloric acid (1+3) to dissolve, and add water up to the marked line.

d) **Calcium standard solution (CaO 5 µg/mL - 50 µg/mL) for the calibration curve preparation** (1): Put 2.5 mL - 25 mL of a calcium standard solution (CaO 1 mg/mL) in 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and add water up to the marked line (4).

e) **Blank test solution for the calibration curve preparation** (1): Put about 50 mL of interference suppressor solution in a 500-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

(**4**) For storage, use a sealable container made of materials which are not likely to dissolve calcium, such as borosilicate glass-1 specified in JIS R 3503 or Teflon.

Comment 1 Instead of the calcium standard solution in (**2**), a calcium standard solution for the calibration curve preparation can be prepared by using a calcium standard solution (Ca 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate total lime (T-CaO) in the analytical sample by multiplying the concentration (Ca) of the calcium standard solution for calibration curve preparation or a measurement value (Ca) obtained in (**4.2**) by a conversion factor (1.3992).

Comment 2 When using a sample solution obtained in the procedure in (**4.1.2**) **h**) for the measurement of cadmium, nickel, chromium or lead, sulfuric acid and hydrochloric acid in (**2**) should be a reagent of harmful metal analysis grade, microanalysis grade or equivalents.

(**3**) **Instruments**: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A calcium hollow cathode lamp
2) **Gas**: Gas for heating by flame
   (i) Fuel gas: acetylene
   (ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Electric furnace**: An electric furnace that can be adjusted to 550 ºC ± 5 ºC.

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Incineration-hydrochloric acid boiling

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char (5).
c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate (5).
d) After standing to cool, moisten the residue with a small amount of water, gradually add about 10 mL of hydrochloric acid, and further add water to make 20 mL.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.
f) After standing to cool, transfer the solution to a 250-mL - 500-mL volumetric flask with water.
g) Add water up to the marked line.
h) Filter with Type 3 filter paper to make a sample solution.

Note (**5**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 550 °C in 1 to 2 hours.

Comment 3 Do not conduct the procedures in (**4.1.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 4 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Incineration-aqua regia digestion

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char (6).
c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (6).
d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
f) Slightly move the watch glass (7), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (8) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

Note (**6**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.

(**7**) The watch glass can be removed.

(**8**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 5 Do not conduct the procedures in (**4.1.2**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 6 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 422.7 nm

b) **Calibration curve preparation**

1) Spray the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 422.7 nm.

2) Prepare a curve for the relationship between the calcium concentration and the indicated value of the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as CaO) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the calcium content from the calibration curve, and calculate total lime (T-CaO) in the analytical sample.

Comment 7 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 15 % (mass fraction) and 1 % (mass fraction) were 101.8 % and 97.9% as total lime (T-CaO) respectively. In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1.

In addition, the results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 2 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.05 % (mass fraction).

Table 1 Analysis results of the collaborative study for the test method validation of total lime

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Industrial sludge fertilizer	10	36.35	0.50	1.4	0.33	2.4
Compost	9	13.69	0.23	1.7	0.25	2.3
Compound fertilizer	10	10.53	0.09	0.8	0.24	2.4
Composted sludge fertilizer A	8	2.02	0.02	0.9	0.04	2.6
Composted sludge fertilizer B	10	1.55	0.04	2.3	0.03	4.3

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2 Analysis results of the collaborative study to determine total lime of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)	s_R[8)] (%)[3)]	RSD_R[9)] (%)
FAMIC-C-12	11	5.82	0.07	1.2	0.11	2.0	0.29	5.0

1) The number of laboaratories used for analysis conducting flame atomic absorbance spectrometry
2) Mean (the number of laboratory(p)×test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.156- 158, Yokendo, Tokyo (1988)

2) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, **Vol. 3** p. 107 – 116 (2010)

3) Souichi IGARASHI and Yasuharu KIMURA: Verification of Performance Characteristics of Testing Methods for Calcium Content in Fertilizer by Atomic Absorption Spectrometry: Research Report of Fertilizer **Vol. 6**, p. 183 - 192 (2013)

4) Hisanori ARAYA and Kimie KATO: Performance Evaluation of Determination Method for Total Lime and Soluble Lime in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 76 – 86 (2020)

(5) Flow sheet for total lime: The flow sheet for total lime in fertilizers is shown below:

5 g analytical sample	Weigh to the order of 1 mg into a 200-mL- 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 550 ºC ± 5 ºC, no less than 4 hours
Standing to cool	Room temperature
	← Small amount of water, moisten the residue
	← About 10 mL of hydrochloric acid
	← Water (up to about 20 mL)
Heating	Cover with a watch glass, and boil for 5 minutes.
Standing to cool	Room temperature
Transfer	250-mL - 500-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Sample solution	

Figure 1-1 Flow sheet for total lime in fertilizers.
(Incineration-hydrochloric acid boiling prorcedure (4.1.1))

5 g analytical sample	Weigh to the order of 1 mg into a 200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 ºC ± 5 ºC for 8 - 16 hours
Standing to cool	Room temperature
← A small amount of water	
← About 10 mL of nitric acid	
← About 30 mL of hydrochloric acid	
Heating	Cover with a watch glass to digest
Heating	Slightly move the watch glass and remove acid
Standing to cool	Room temperature
← 25 mL - 50 mL of hydrochloric acid (1+5)	
Heating	Cover with a watch glass to dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Sample solution	

Figure 1-2　Flow sheet for total lime in fertilizers
(Incineration-aqua regia digestion procedure (4.1.2))

Sample solution	
Aliquot (predetermined amount)	100-mL volumetric flask
←About 10 mL interference suppressor solution	
← Water (up to the marked line)	
Measurement	Atomic absorption spectrometer

Figure 2　Flow sheet for total lime in fertilizers (Measurement)

4.5.2 Acid-soluble lime

4.5.2.a Flame atomic absorption spectrometry

(1) Summary

This testing method is applicable to fertilizers that guarantee alkalinity. This testing method is classified as Type B and its symbol is 4.5.2.a-2017 or S-Ca.a-1.

Add hydrochloric acid (1+23) to an analytical sample, boil to extract, and add an interference suppressor solution, and then spray in an acetylene–air flame, and measure the atomic absorption with calcium at a wavelength of 422.7 nm to quantify hydrochloric acid (1+23) soluble lime (acid-soluble lime (S-CaO)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Interference suppressor solution** (2): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (1) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

c) **Calcium standard solution (CaO 1 mg/mL)** (2): Put calcium carbonate specified in JIS K 8617 in a drying apparatus, heat at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.785 g into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add 20 mL of hydrochloric acid (1+3) to dissolve, and add water up to the marked line.

d) **Calcium standard solution (CaO 5 µg/mL - 50 µg/mL) for the calibration curve preparation** (2): Put 2.5 mL - 25 mL of a calcium standard solution (CaO 1 mg/mL) in 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and add water up to the marked line (4).

e) **Blank test solution for the calibration curve preparation** (2): Put about 50 mL of interference suppressor solution in a 500-mL volumetric flask (3), and add water up to the marked line (4).

Note (**1**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**2**) This is an example of preparation; prepare an amount as appropriate.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

(**4**) For storage, use a sealable container made of materials which are not likely to dissolve calcium, such as borosilicate glass-1 specified in JIS R 3503 or Teflon.

Comment 1 Instead of the calcium standard solution in (**2**), a calcium standard solution for the calibration curve preparation can be prepared by using a calcium standard solution (Ca 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate acid-soluble lime (S-CaO) in the analytical sample by multiplying the concentration (Ca) of the calcium standard solution for calibration curve preparation or a measurement value (Ca) obtained in (**4.2**) by a conversion factor (1.3992).

(**3**) **Instruments**: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A calcium hollow cathode lamp

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Hot plate** : A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 2 g of an analytical sample to the order of 1 mg, and put it in a 500-mL tall beaker.

b) Add about 20 mL of hydrochloric acid (1+23), cover with a watch glass, and boil on a hot plate for about 5 minutes (5).

c) After immediate cooling is complete, transfer it to a 250-mL - 500-mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make a sample solution.

Note (**5**) Be aware that an analytical sample should not solidify in the bottom of a beaker.

Comment 2 In the case of a by-product magnesia fertilizer or a fertilizer containing a by-product magnesia, if the pH of the sample solution of **d**) is neutral or basic, prepare a sample solution anew by replacing “2 g of an analytical sample” in the procedure in **a**) with “1 g - 1.5 g of an analytical sample”.

Comment 3 In the procedure in **a**), a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. In addition, “cover with a watch glass” in **b**) is replaced by “place a funnel”, and “transfer it to a 250-mL - 500 mL volumetric flask with water” in the procedure in **c**) is skipped.

Comment 4 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 422.7 nm

b) **Calibration curve preparation**

1) Spray the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 422.7 nm.

2) Prepare a curve for the relationship between the calcium concentration and the indicated value of the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as CaO) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the calcium content from the calibration curve, and calculate acid-soluble lime (S-CaO) in the analytical sample.

Comment 5 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 20 % (mass fraction) and 1 % (mass fraction) were 100.9 % and 101.1% as acid-soluble lime (S-CaO) respectively.

In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.05 % (mass fraction).

Table 1 Analysis results of the collaborative study for the test method validation of acid-soluble lime

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Slaked lime	10	65.95	0.70	1.1	2.22	3.4
Calcined phosphorus fertilizer	10	30.31	0.46	1.5	0.79	2.6
Slag silicate fertilizer	10	29.49	0.38	1.3	0.88	3.0
Blended fertilizer	9	7.77	0.08	1.0	0.27	3.5
Mimic fertilizer	8	0.823	0.04	4.6	0.05	6.6

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.167- 169, Yokendo, Tokyo (1988)

2) Souichi IGARASHI and Yasuharu KIMURA: Verification of Performance Characteristics of Testing Methods for Calcium Content in Fertilizer by Atomic Absorption Spectrometry: Research Report of Fertilizer **Vol. 6**, p. 183 - 192 (2013)

4) Hisanori ARAYA and Kimie KATO: Performance Evaluation of Determination Method for Total Lime and Soluble Lime in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 76 – 86 (2020)

(5) Flow sheet for acid-soluble lime: The flow sheet for acid-soluble lime in fertilizers is shown below:

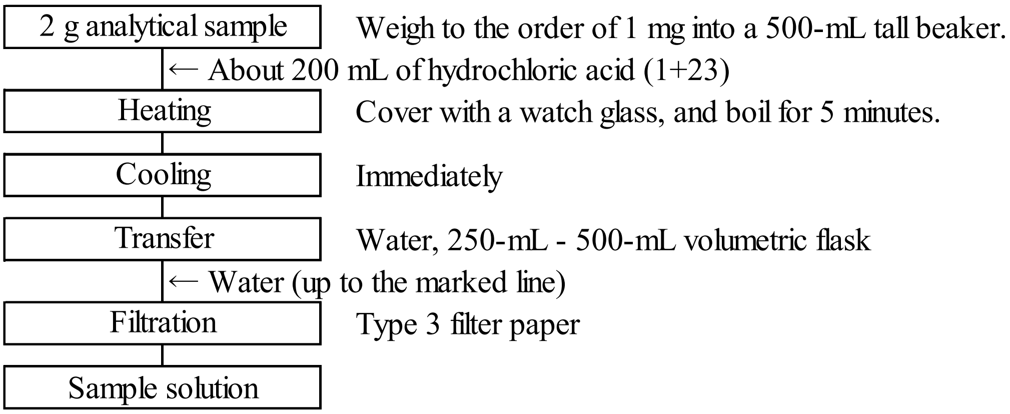


Figure 1 Flow sheet for acid-soluble lime in fertilizers (Extraction procedure)

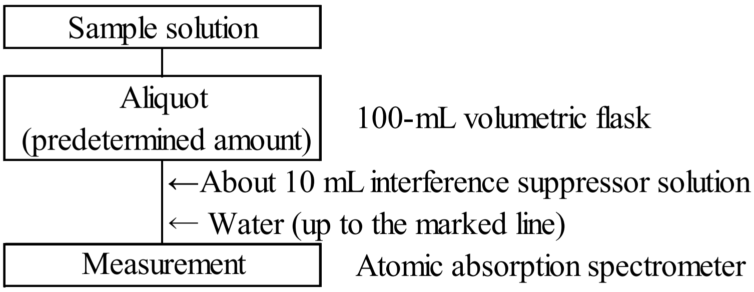


Figure 2 Flow sheet for acid-soluble lime in fertilizers (Measurement procedure)

4.5.3 Citric acid-soluble lime

4.5.3.a Flame atomic absorption spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 4.5.3.a-2020 or C-Ca.a-1.

Extract by adding citric acid solution to an analytical sample, and add an interference suppressor solution, and then spray in an acetylene-air flame, and measure the atomic absorption with calcium at a wavelength of 422.7 nm to quantify citric acid-soluble lime (C-CaO) in an analytical sample.

In addition, the performance of this testing method is shown in **Comment 6.**

(**2**) **Reagent**: Reagents are as shown below.

a) Hydrochloric acid:
A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Citric acid solution (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

c) Interference suppressor solution (1): Weigh 152.1 g of strontium chloride hexahydrate specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

d) Calcium standard solution (CaO 1 mg/mL): Heat calcium carbonate specified in JIS K 8617 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.785 g into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add 20 mL of hydrochloric acid (1+3) to dissolve, and add water up to the marked line.

e) Calcium standard solution (CaO 100 mg/mL) (1): Put 10 mL of calcium standard solution (CaO 1 mg/mL) in a 100-mL flask and add water up to the marked line.

f) Calcium standard solution (CaO 1 µg/mL - 7 µg/mL) for the calibration curve preparation (1): Put 5 mL - 35 mL of a calcium standard solution (CaO 100 µg/mL) in 500-mL volumetric flasks step-by-step, add about 30 mL of a citric acid solution (2) and about 50 mL of an interference suppressor solution (3), and add water up to the marked line (4).

g) Blank test solution for the calibration curve preparation (1): Put about 30 mL of a citric acid solution (2) and about 50 mL of an interference suppressor solution in a 50-mL volumetric flask (3), and add water up to the marked line (4).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Add a citric acid solution that is 6/100 of the volume to be prepared.
(**3**) Add an interference suppressor solution that is 1/10 of the volume to be prepared.
(**4**) For storage, use a sealable container made of materials which are not likely to dissolve calcium, such as borosilicate glass-1 specified in JIS R 3503 or Teflon.

Comment 1 Instead of the calcium standard solution in (**2**), a calcium standard solution for the calibration curve preparation can be prepared by using a calcium standard solution (CaO 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble lime (C-CaO) in the analytical sample by multiplying the concentration (Ca) of the calcium standard solution for calibration curve preparation or a measurement value (Ca) obtained in (**4.2**) by a conversion factor (1.3992).

(**3**) **Instruments**: Instruments are as shown below:

aa) Constant-temperature vertical rotating mixer: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

ab) Horizontal reciprocating water bath shaker: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

b) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A calcium hollow cathode lamp

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Constant-temperature vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (5), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**5**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 2 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Horizontal reciprocating water bath shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (6).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (5), and shake to mix by reciprocating horizontally at 160 times/min with amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**6**) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 3 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 4 For a calcareous fertilizer, etc., if the pH of the sample solution in (**4.1.1**) **d**) and (**4.1.2**) **d**) is neutral or basic, prepare a sample solution anew by replacing “1 g of an analytical sample” in the procedures in (**4.1.1**) **a**) and (**4.1.2**) **a**) with “0.5 g of an analytical sample”.

Comment 5 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, check non-dissolved matters status after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 422.7 nm

b) Calibration curve preparation

1) Spray the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 422.7 nm.

2) Prepare a curve for the relationship between the calcium concentration and the indicated value of the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 0.7 mg as CaO and no more than the equivalents of 6mL of citric acid solution) in a 100-mL volumetric flask.

2) Add the solution to make a citric acid solution equivalent to 6 mL.

3) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

4) Subject to the same procedure as in **b) 1)** to read the indicated value.

5) Obtain the calcium content from the calibration curve, and calculate citric acid-soluble lime (C-CaO) in the analytical sample.

Comment 6 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 30 % (mass fraction), 1 % - 15 % (mass fraction) and 0.2 % (mass fraction) were 99 %, 101 % - 98 % and 96 % as citric acid-soluble lime (C-CaO) respectively.

The results of the repeatability tests on different days using calcium carbonate and designated blended fertilizer (1 brand) to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.2 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of citric acid-soluble lime

Name of sample	Test days of repeatability T 1)	Average 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Calcium carbonate	5	55.77	0.47	0.8	0.47	0.8
Designated blended fertilizer	5	4.756	0.091	1.9	0.114	2.4

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of citric acid-soluble lime

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Designated blended fertilizer	11	3.14	0.07	2.3	0.09	2.7
Composted sludge fertilizer	12	5.82	0.07	2.4	0.14	2.4
Compound fertilizer	12	12.30	0.24	2.0	0.58	4.7
Fish cakes powder	12	15.60	0.25	1.6	0.51	3.2
Gypsum	12	18.84	0.19	1.0	0.75	4.0
Mixed phosphorus fertilizer	11	31.89	0.40	1.3	0.60	1.9
Silicate slag fertilizer	11	40.78	0.18	0.4	0.78	1.9

1) Number of laboratories used in analysis
2) Mean (n = [Number of laboratories] × [Number of samples (2)])
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Souichi IGARASHI and Yasuharu KIMURA: Verification of Performance Characteristics of Testing Methods for Calcium Content in Fertilizer by Atomic Absorption Spectrometry: Research Report of Fertilizer **Vol. 6**, p. 183 – 192 (2013)

2) Madoka KATO, Masayuki YAMANISHI and Yuji SHIRAI: Determination of Citric Acid-Soluble CaO in Fertilizer by AAS and ICP-OES, Research Report of Fertilizer, **Vol. 13**, p. 36 - 49 (2020)

(5) **Flow sheet for citric acid-soluble lime**: The flow sheet for citric acid-soluble lime in fertilizers is shown below:

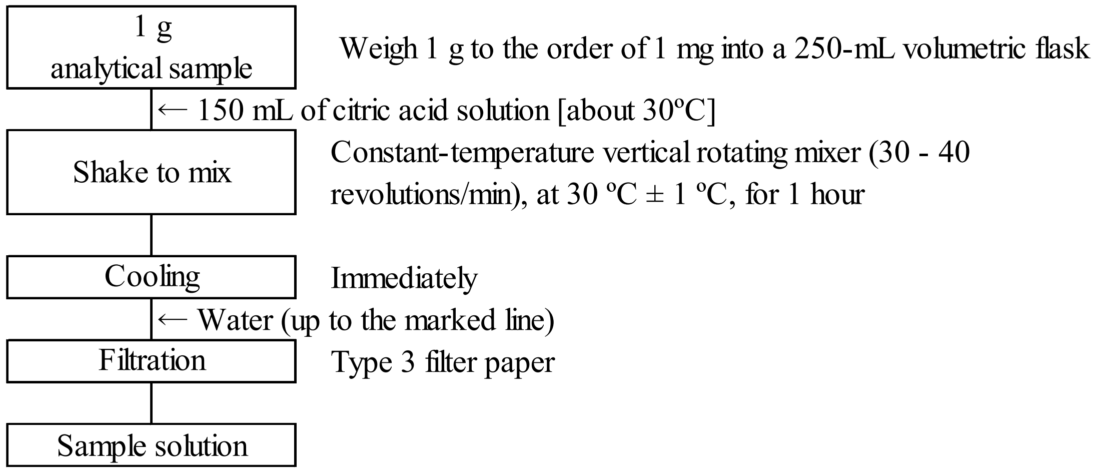


Figure 1-1 Flow sheet for citric acid-soluble lime in fertilizers (Extraction procedure (4.1.1))

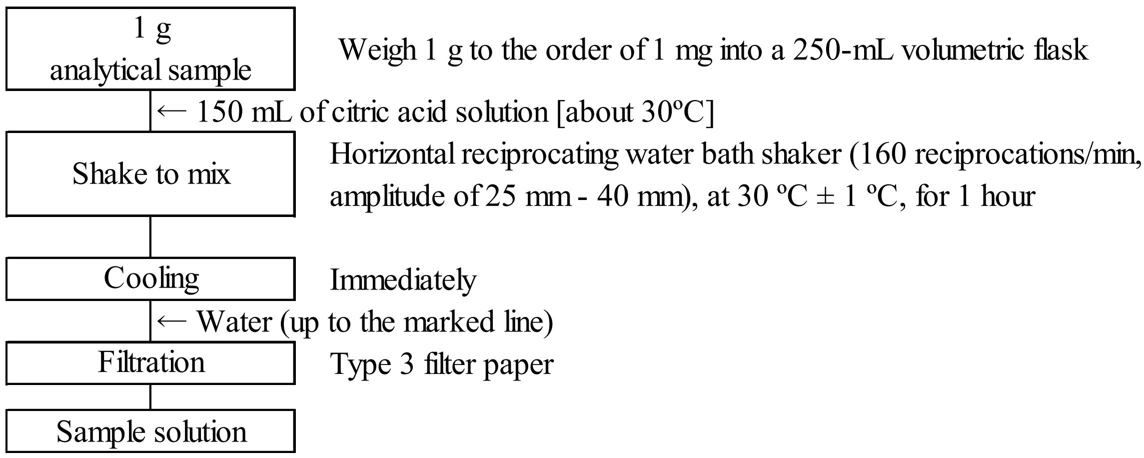


Figure 1-2 Flow sheet for citric acid-soluble lime in fertilizers (Extraction procedure (4.1.2))

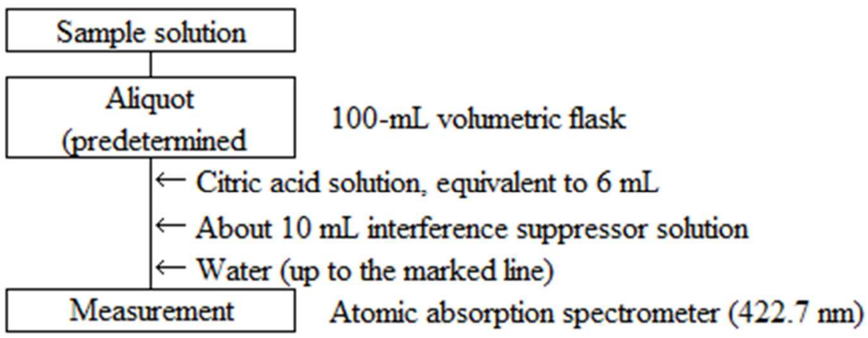


Figure 2 Flow sheet for citric acid-soluble lime in fertilizers (Measurement procedure)

4.5.3.b ICP Optical Emission Spectrometry

(1)Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 4.5.3.b-2020 or C-Ca.b-1.

Extract by adding a citric acid solution to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the calcium at a wavelength of 393.366 nm to obtain citric acid-soluble lime (C-CaO)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL

d) **Calcium standard solution (CaO 1 mg/mL)**: Heat calcium carbonate specified in JIS K 8617 at 110 ºC ± 2 ºC for about 2 hours, let it stand to cool in a desiccator, and weigh 1.785 g into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add 20 mL of hydrochloric acid (1+3) to dissolve, and add water up to the marked line.

e) **Calcium standard solution (CaO 0.1 mg/mL)**: Put 10 mL of calcium standard solution (CaO 1 mg/mL) in a 100-mL flask and add hydrochloric acid (1+23) up to the marked line.

f) **Calcium standard solutions (CaO 1 µg/mL – 20µg /mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of calcium standard solution (CaO 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.

g) **Calcium standard solutions (CaO 0.1 µg/mL – 1 µg /mL) for the calibration curve preparation** (1): Put 1 mL - 10 mL of calcium standard solution (CaO 10 µg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.

h) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**), **f**) and **g**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the calcium standard solution in (**2**), a calcium standard solution for the calibration curve preparation can be prepared by using a calcium standard solution (1 mg/ mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble lime (C-CaO) in the analytical sample by multiplying the concentration (Ca) of the calcium standard solution for calibration curve preparation or a measurement value (Ca) obtained in (**4.2**) by a conversion factor (1.3992).

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

aa) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to

30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

ab) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

b) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Constant-temperature vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (2) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Horizontal reciprocating water bath shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (3).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix by reciprocating horizontally at 160 times/min with amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Note (3) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 5 For a calcareous fertilizer, if the pH of the sample solution in (**4.1.1) d**) and (**4.1.2**) **d**) is neutral or basic, prepare a sample solution anew by replacing "1 g of an analytical sample" in the procedures in (**4.1.1**) **a**) and (**4.1.2**) **a**) with "0.5 g of an analytical sample".

Comment 6 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, check non-dissolved matters status after the procedures of (**4.1.1**) **b)** and (**4.1.2**) **b**).

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometry used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following: Analytical line wavelength: 393.366 nm

b) **Calibration curve preparation**

1) Spray the calcium standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 393.366 nm.

2) Prepare a curve for the relationship between the calcium concentration and the indicated value of the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 14 µg - 1.4 mg as CaO) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b) 1)** to read the indicated value.

4) Obtain the calcium content from the calibration curve, and calculate citric acid-soluble lime (C-CaO) in the analytical sample.

Comment 7 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 8 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 30 % (mass fraction), 1 % - 15 % (mass fraction) and 0.2 % (mass fraction) were 98 %, 103 % - 101 % and 104 % as citric acid-soluble lime (C-CaO) respectively.

The results of the repeatability tests on different days using calcium carbonate and designated blended fertilizer (1 brand) to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.3 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of citric acid-soluble lime

Name of sample	Test days of repeatability T 1)	Average 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Calcium carbonate	5	55.60	0.43	0.8	0.86	1.5
Designated blended fertilizer	5	4.82	0.13	2.7	0.16	3.3

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination Method for Effect-Development Promoting Agent (Ca, Fe, Co, Cu, Zn and Mo) in Liquid Compound Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), Research Report of Fertilizer, **Vol. 9**, p. 1 - 9 (2016)

2) Shingo MATSUO: Simultaneous Determination of Citrate-Soluble Principal Ingredients (C-P2O5, C-K2O, C-MgO, C-MnO and C-B2O3) in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 11**, p. 14 – 28 (2018)

3) Madoka KATO, Masayuki YAMANISHI and Yuji SHIRAI: Determination of Citric Acid-Soluble CaO in Fertilizer by AAS and ICP-OES, Research Report of Fertilizer, **Vol. 13**, p. 36 - 49 (2020)

(5) Flow sheet for the testing method: The flow sheet for citric acid-soluble lime in fertilizers is shown below:

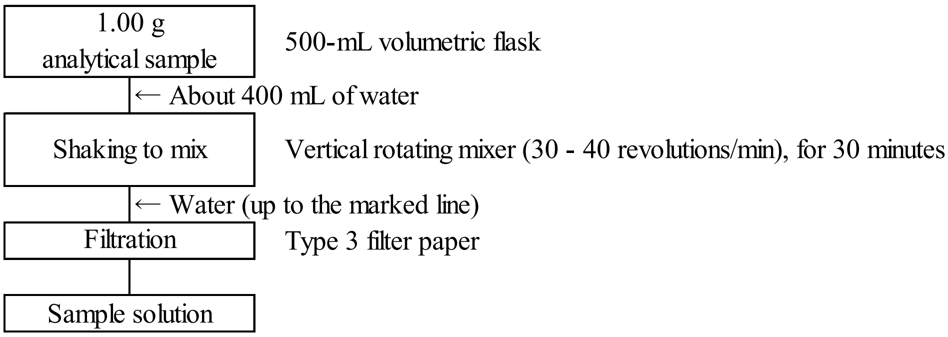


Figure 1-1 Flow sheet for water-soluble calcium in fertilizers (Extraction procedure (4.1.1))

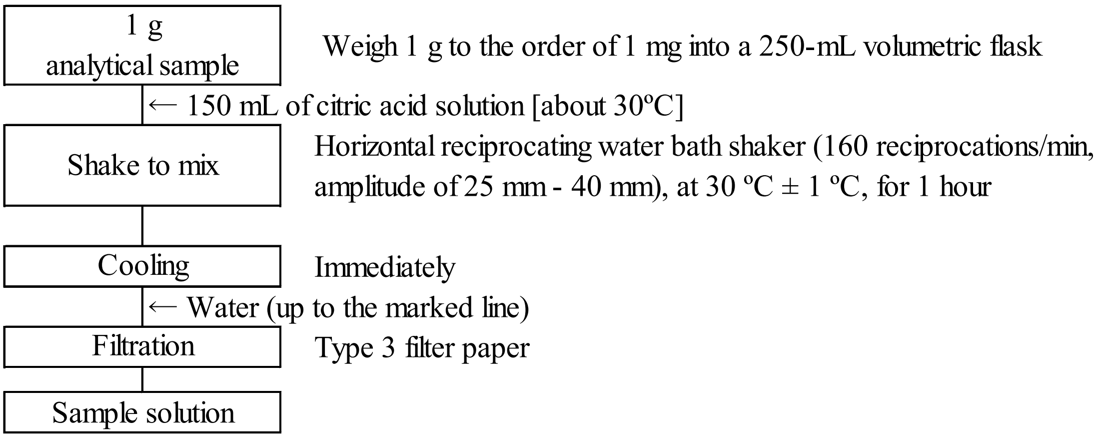


Figure 1-2 Flow sheet for citric acid-soluble lime in fertilizers (Extraction procedure (4.1.2))

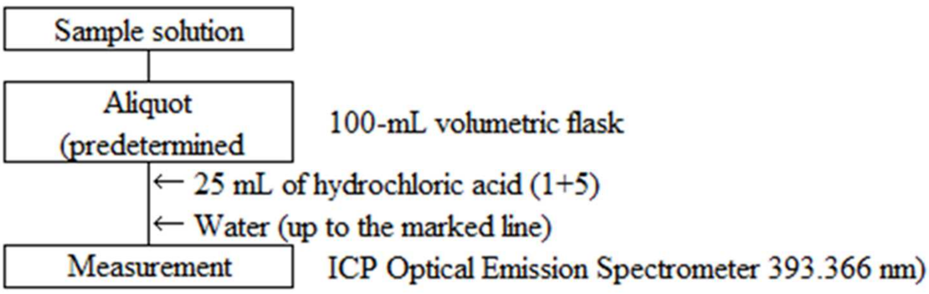


Figure 2 Flow sheet for citric acid-soluble lime in fertilizers (Measurement procedure)

4.5.4 Water-soluble calcium

4.5.4.a Flame atomic absorption spectrometry

(1) Summary

This testing method is applicable to fertilizers that indicate calcium content as a response modifier. This testing method is classified as Type D and its symbol is 4.5.3.a-2017 or W-Ca.a-1.

Extract by adding water to an analytical sample, and add an interference suppressor solution, and then spray in an acetylene‒air flame, and measure the atomic absorption with calcium at a wavelength of 422.7 nm to quantify water-soluble calcium (W-Ca) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**

(**2**) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Interference suppressor solution** (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

c) **Calcium standard solution (Ca 1 mg/mL)**: Calcium standard solution (Ca 1 mg/mL) traceable to National Metrology.

d) **Calcium standard solution (Ca 5 µg/mL - 50 µg/mL) for the calibration curve preparation** (1): Put 2.5 mL - 25 mL of a calcium standard solution (Ca 1 mg/mL) in 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and add water up to the marked line (4).

e) **Blank test solution for the calibration curve preparation** (1): Put about 50 mL of interference suppressor solution in a 500-mL volumetric flask (3), and add water up to the marked line (4).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

(**4**) For storage, use a sealable container made of materials which are not likely to dissolve calcium, such as borosilicate glass-1 specified in JIS R 3503 or Teflon.

Comment 1 Instead of the calcium standard solution in (**2**), a calcium standard solution for the calibration curve preparation can be prepared by using a calcium standard solution (Ca 10 mg/mL) traceable to National Metrology.

Comment 2 Instead of the calcium standard solution in (**2**), a calcium standard solution (CaO 5 µg/mL - 50 µg/mL) for the calibration curve preparation prepared in (**2**) in **4.5.1.a** can be used. In this case, calculate water-soluble calcium (W-Ca) in the analytical sample by multiplying the concentration (CaO) of a calcium standard solution for calibration curve preparation or a measurement value (CaO) obtained in (**4.2**) by a conversion factor (0.7147).

(**3**) **Instruments**: Instruments are as shown below:

a) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

b) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A calcium hollow cathode lamp

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 1.00 g of an analytical sample, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

(4.1.2) Fluid test sample

a) Weigh 1 g of an analytical sample (2) to the order of 1 mg, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, shake to mix and add water up to the marked line.
c) Filter with Type 3 filter paper to make a sample solution.

Note (5) The sampling amount of the analytical sample is 10 g when the content in the analytical sample is less than 0.01 % (mass fraction) as water-soluble calcium.

Comment 3 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 422.7 nm

b) Calibration curve preparation

1) Spray the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 422.7 nm.
2) Prepare a curve for the relationship between the calcium concentration and the indicated value of the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as Ca) in a 100-mL volumetric flask.
2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.
3) Subject to the same procedure as in **b) 1)** to read the indicated value.
4) Obtain the calcium content from the calibration curve, and calculate water-soluble calcium (W-Ca) in the analytical sample.

Comment 3 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rate at the content level of 1 % (mass fraction) - 5 % (mass fraction) was 98.1 % - 101.1% as water-soluble calcium (W-Ca) respectively.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.07 % (mass fraction) for solid fertilizers and about 0.04 % (mass fraction) for fluid fertilizers.

References

1) Souichi IGARASHI and Yasuharu KIMURA: Verification of Performance Characteristics of Testing Methods for Calcium Content in Fertilizer by Atomic Absorption Spectrometry: Research Report of Fertilizer **Vol. 6**, p. 183 - 192 (2013)

(5) **Flow sheet for water-soluble calcium**: The flow sheet for water-soluble calcium in fertilizers is shown below:

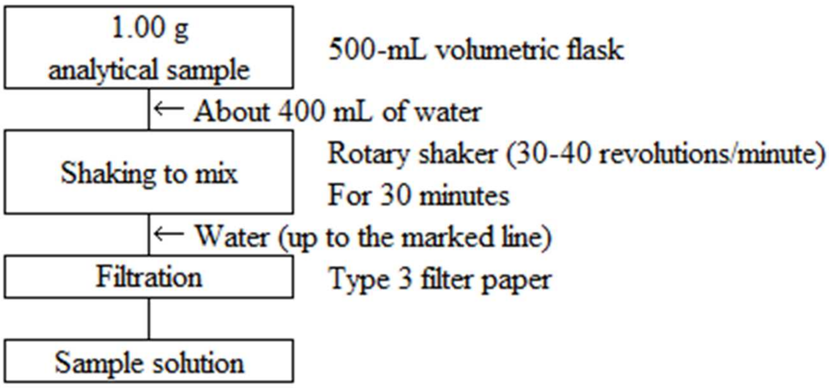


Figure 1-1 Flow sheet for water-soluble calcium in fertilizers (Extraction procedure (4.1.1))

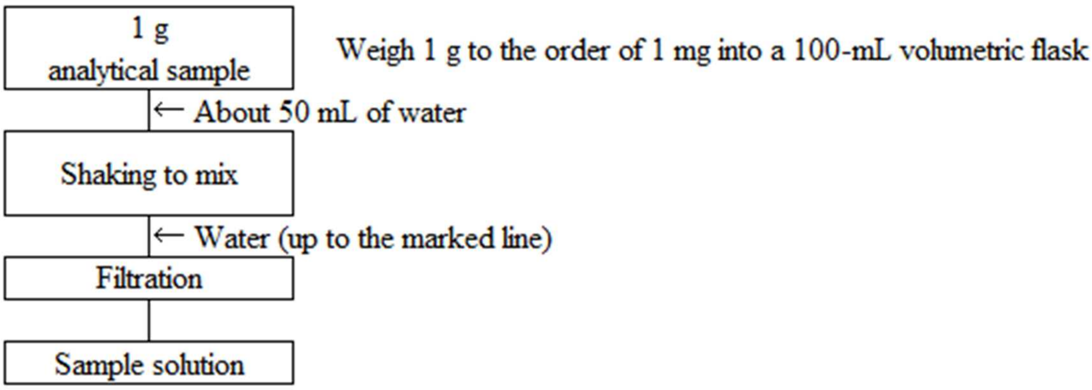


Figure 1-2 Flow sheet for water-soluble calcium in fertilizers (Extraction procedure (4.1.2))

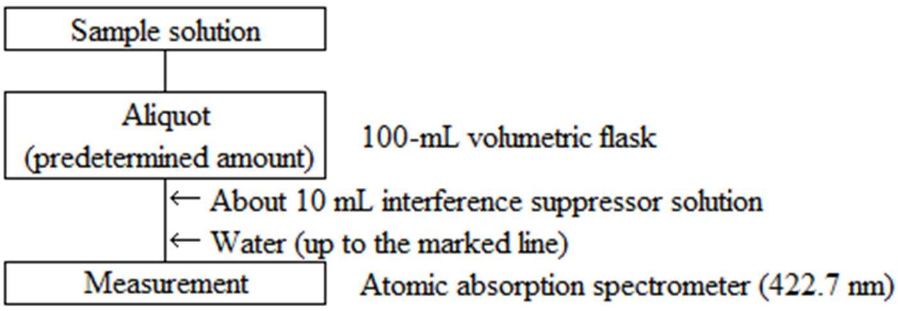


Figure 2 Flow sheet for water-soluble calcium in fertilizers (Measurement procedure)

4.5.4.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fluid compound fertilizers, liquid microelement compound fertilizers and the fluid fertilizers of home garden-use compound fertilizers. This testing method is classified as Type B and its symbol is 4.5.3.b-2017 or W-Ca.b-1.

Add water to an analytical sample to extract, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the calcium at a wavelength of 393.366 nm to obtain water-soluble calcium (W-Ca). In addition, the performance of this testing method is shown in **Comment 5**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Calcium standard solution (Ca 1 mg/mL)**: Calcium standard solution (Ca 1 mg/mL) traceable to National Metrology.

d) **Calcium standard solution (Ca 0.1 mg/mL)** (1): Put 10 mL of calcium standard solution (Ca 1 mg/mL) in a 100-mL flask and add hydrochloric acid (1+23) up to the marked line.

e) **Calcium standard solutions (Ca 1 µg/mL - 20µg /mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of calcium standard solution (Ca 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.

f) **Calcium standard solutions (Ca 0.1 µg/mL - 1 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 10 mL of calcium standard solution (Ca 10 µg/mL) for the calibration curve preparation in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**), **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the calcium standard solution in (**2**), a calcium standard solution for the calibration curve preparation can be prepared by using a calcium standard solution (Ca 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **ICP Optical Emission Spectrometer:** An optical emission spectrometer specified in JIS K 0115

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample (2) to the order of 1 mg, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, shake to mix and add water up to the marked line.

c) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) The sampling amount of the analytical sample is 10 g when the content in the analytical sample is less than 0.01 % (mass fraction) as water-soluble calcium.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following: Analytical line wavelength: 393.366 nm or 317.933 nm (3)

b) **Calibration curve preparation**

1) Spray the calcium standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at an analytical line wavelength.

2) Prepare a curve for the relationship between the calcium concentration and the indicated value of the calcium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 2 mg as Ca) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the calcium content from the calibration curve, and calculate water-soluble calcium (W-Ca) in the analytical sample.

Note (**3**) 317.933 nm can also be used. However, since the intensity of emission obtained is different from the one of 393.366 nm, it is necessary to understand the suitable concentration range of the calibration curve and prepare a standard solution for the calibration curve in advance.

Comment 4 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 5 The comparison of the measurement value (y_i : 0.095 % (mass fraction) - 10.93 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of flame atomic absorbance spectrometry was conducted to evaluate trueness using fluid fertilizers (12 samples). As a result, a regression equation was y=0.005+0.978x, and its correlation coefficient (r) was 0.999. Additionally, additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 0.01 % (mass fraction) and 0.1 % (mass fraction) were 105.9 % and 106.4 % respectively.

The results of the repeatability tests on different days using a fluid compound fertilizer and a home garden-use compound fertilizer to evaluate precision were analyzed by the

one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 2.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.0005 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of water-soluble calcium (Fluid fertilizer)

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluid compound fertilizer	7	2.14	0.02	0.7	0.05	2.1
Home garden-use compound fertilizer (fluid)	7	0.103	0.001	0.9	0.001	1.0

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble calcium

Analytical line wavelength (nm)	Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
393.366	Preparation sample (Liquid) 1	9	0.248	0.003	1.3	0.012	4.9
	Preparation sample (Liquid) 2	9	1.74	0.02	1.2	0.04	2.0
	Preparation sample (Liquid) 3	8	1.02	0.01	0.9	0.02	2.4
	Preparation sample (Liquid) 4	8	0.0540	0.0007	1.3	0.0034	6.4
	Preparation sample (Liquid) 5	8	0.0508	0.0005	1.0	0.0014	2.8
317.933	Preparation sample (Liquid) 1	10	0.246	0.003	1.3	0.011	4.5
	Preparation sample (Liquid) 2	10	1.75	0.01	0.7	0.04	2.1
	Preparation sample (Liquid) 3	10	1.03	0.02	1.5	0.03	2.5
	Preparation sample (Liquid) 4	9	0.0553	0.0006	1.0	0.0047	8.6
	Preparation sample (Liquid) 5	11	0.0551	0.0010	1.9	0.0076	13.9

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination Method for Effect-Development

Promoting Agent (Ca, Fe, Co, Cu, Zn and Mo) in Liquid Compound Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), Research Report of Fertilizer, **Vol. 9**, p. 1 - 9 (2016)

2) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(**5**) **Flow sheet**: The flow sheet for water-soluble calcium of the fluid fertilizers is shown below:

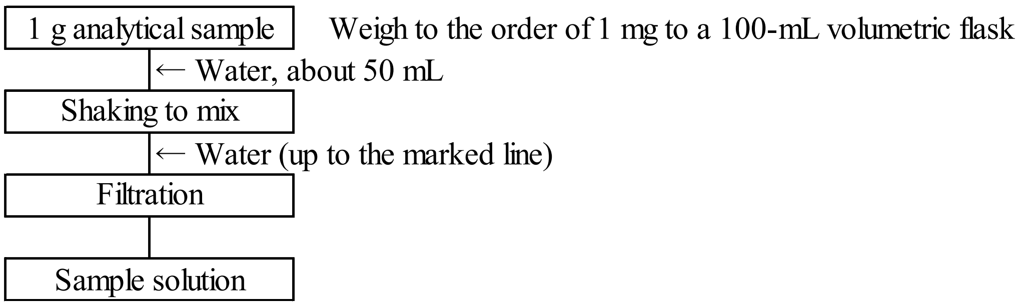


Figure 1 The flow sheet for water-soluble calcium in fluid fertilizers (Extraction procedure)

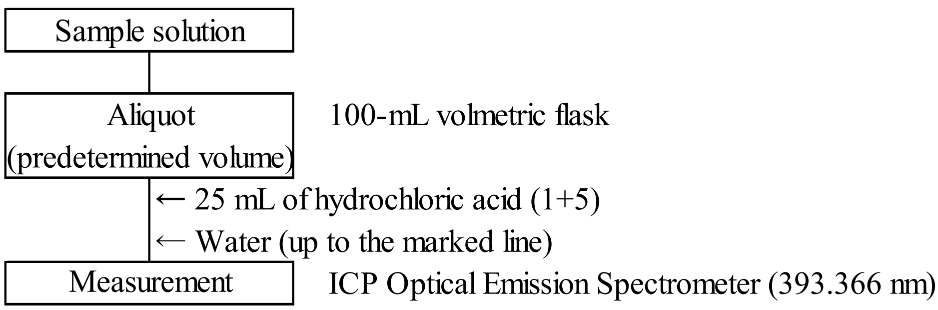


Figure 2 The flow sheet for water-soluble calcium in fluid fertilizers (Measurement procedure)

4.5.5 Alkalinity

4.5.5.a Ethylenediamine tetraacetate method

(1) Summary

This testing method is applicable to fertilizers that guarantee alkalinity. This testing method is classified as Type E and its symbol is 4.5.4.a-2017 or AL.a-1.

Add hydrochloric acid (1+23) to an analytical sample, boil to extract, and mask with 2,2',2"-nitrilotriethanol and a potassium cyanide solution, add a 0.01 mol/L ethylenediamine tetraacetate standard solution, and conduct a chelatometric titration with a 0.01 mol/L magnesium standard solution to obtain alkalinity (AL). Or after masking, conduct a chelatometric titration with an ethylenediamine tetraacetate standard solution to obtain alkalinity (AL) in an analytical sample.

(2) Reagent: Reagents are as shown below.

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Sodium hydroxide: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

c) Ascorbic acid: A JIS Guaranteed Reagent specified in JIS K 9502 or a reagent of equivalent quality.

d) 2,2',2"-nitrilotriethanol (1): A JIS Guaranteed Reagent specified in JIS K 8663 or a reagent of equivalent quality.

e) Acetone: A JIS Guaranteed Reagent specified in JIS K 8034 or a reagent of equivalent quality.

f) Ammonia solution: A JIS Guaranteed (NH_3 28 % (mass fraction)) reagent specified in JIS K 8085 or a reagent of equivalent quality.

g) 0.01 mol/L ethylenediamine tetraacetate standard solution: Dissolve 3.72 g of ethylenediaminetetraacetic acid dihydrogen disodium dihydrate in water to make 1000 mL.

Standardization: Wash zinc reference material for volumetric analysis specified in JIS K 8005 with hydrochloric acid (1+3), water, ethanol (99.5) specified in JIS K 8101, and diethyl ether specified in JIS K 8103 successively, and immediately leave at rest in a desiccator for about 12 hours under no more than 2 kPa to dry, and then weigh about 0.65 mg to the order of 0.1 mg, put it in a 1000-mL volumetric flask, add about 10 mL of hydrochloric acid to dissolve and then add water up to the marked line. Put 25 mL of the solution in a 200-mL - 300-mL Erlenmeyer flask, add about 15 mL of water and about 5 mL of ammonium chloride buffer solution, and titrate with a 0.01 mol/L ethylenediamine tetraacetate standard solution, while adding Eriochrome Black T solution as an indicator, until the color of the solution becomes blue. Calculate the factor of a 0.01 mol/L ethylenediamine tetraacetate standard solution by the following formula.

Factor of 0.01 mol/L ethylenediamine tetraacetate standard solution (f_1)

$$= W_1 \times (A/100) \times (1/65.409) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

$$= W_1 \times A \times (1/65.409) \times (0.25/V_3)$$

W : Mass (g) of d zinc sampled

A : Purity (% (mass fraction)) of zinc

V_1 : Aliquot volume (25 mL) of zinc solution

V_2 : Constant volume (1000 mL) of zinc solution

V_3 : Volume (mL) of 0.01 mol/L ethylenediamine tetraacetate standard solution needed for titration

C_1: Set concentration (0.01 mol/L) of 0.01 mol/L ethylenediamine tetraacetate standard solution

h) **0.01 mol/L magnesium standard solution**: Put 0.24 g of magnesium specified in JIS K 8875 into a 1000-mL beaker, add about 10 mL of hydrochloric acid to dissolve, add a proper amount of water, and while adding a methyl red solution (0.1 g/100 mL) as an indicator, neutralize with an ammonia solution (1+3) until the color of the solution becomes yellow, and then add water to make 1000 mL.

Standardization: Put 25 mL of 0.01 mol/L magnesium standard solution in a 200-mL - 300-mL Erlenmeyer flask, add about 15 mL of water and about 5 mL of ammonium chloride buffer solution, and while adding an Eriochrome Black T solution as an indicator, titrate with 0.01 mol/L ethylenediamine tetraacetate standard solution until the color of the solution becomes blue. Calculate the factor of a 0.01 mol/L magnesium standard solution by the following formula.

Factor of 0.01 mol/L magnesium standard solution (f_2)
$= (C_1 \times f_1 \times V_4) \times (1/V_5) \times (1/C_2)$
$= (f_1 \times V_4) \times (1/V_5)$

C_1 : Set concentration (0.01 mol/L) of 0.01 mol/L ethylenediamine tetraacetate standard solution
C_2 : Set concentration (0.01 mol/L) of 0.01 mol/L magnesium standard solution
f_1 : Factor of 0.01 mol/L ethylenediamine tetraacetate standard solution
V_4 : Volume (mL) of 0.01 mol/L ethylenediamine tetraacetate standard solution needed for titration
V_5 : Aliquot volume (mL) of 0.01 mol/L magnesium standard solution

i) **Ammonium chloride solution**: Dissolve 70 g of ammonium chloride specified in JIS K 8116 and 570 mL of ammonia solution in water to make 1000 mL.
j) **2-aminoethanol solution**: Add 400 mL of water to 150 mL of 2-aminoethanol specified in JIS K 8109, add gradually hydrochloric acid to a pH 10.6.
k) **Potassium cyanide solution**: Dissolve 100 g of potassium cyanide specified in JIS K 8443 in water to make 1000 mL.
l) **Eriochrome Black T solution**: Dissolve 0.5 g of Eriochrome Black T specified in JIS K 8736 and 4.5 g of hydroxyl-ammonium chloride in methanol−water (95+5) to make 100 mL.
m) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.10 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
n) **Methanol**: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.
o) **Citric acid solution** (2): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

Note (**1**) The reagent corresponds to triethanolamine in the Official Methods of Analysis of Fertilizers (1992).
(**2**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of a 0.01 mol/L ethylenediamine tetraacetate standard solution in (**2**) **g**), a 0.1 mol/L ethylenediaminetetraacetic acid dihydrogen disodium solution conforming to ISO/IEC 17025 can be used.

(**3**) **Instruments**: Instruments are as shown below:
a) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 2 g of an analytical sample to the order of 1 mg, and put it in a 500-mL tall beaker.

b) Add about 200mL of hydrochloric acid (1+23), cover with a watch glass, and boil on a hot plate for about 5 minutes (3).

c) After immediate cooling is complete, transfer it to a 250-mL- 500 -mL volumetric flask with water.

d) Immediately add water up to the marked line (4).

e) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) Be aware that an analytical sample should not solidify in the bottom of a beaker.

(**4**) Conduct the procedure in **Comment 5** when there is much manganese content in the fertilizers.

Comment 2 In the case of a by-product magnesia fertilizer, if the pH of the sample solution of **d**) is neutral or basic, prepare a sample solution anew by replacing “2 g of an analytical sample” in the procedure in **a**) with “1 g - 1.5 g of an analytical sample”.

Comment 3 In the procedure in **a**), a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. In addition, “cover with a watch glass” in **b**) is replaced by “place a funnel”, and “transfer it to a 250-mL - 500 mL volumetric flask with water” in the procedure in **c**) is skipped.

Comment 4 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

Comment 5 Put a predetermined volume of filtrate in (**4.1**) **e**) in a 200-mL tall beaker, add a drop of methyl red solution as an indicator and add ammonia water (28 % (mass fraction)) specified in JIS K 8085 drop by drop until the color of the solution changes light yellowish red from purplish red. Add 20 mL of ammonium peroxodisulfate aqueous solution (20 g/100mL) to boil (5). Immediately transfer it to a 100-mL - 200-mL volumetric flask with water. After immediate cooling is complete, add water up to the marked line. Filter with Type 3 filter paper to make a sample solution.

Note (**5**) Precipitation of manganese oxide, etc. is formed.

(4.2) Measurement: Conduct measurement as shown below. Two examples of titration are shown as follows.

(4.2.1) Measurement (A): Titration with a magnesium standard solution (0.01 mol/L)

a) Put a predetermined volume (the equivalents of 5 mg - 20 mg as CaO + MgO) of sample solution in a 200-mL - 300-mL Erlenmeyer flask.

b) Add a proper amount of water, add a drop of methyl red solution as an indicator and add sodium hydroxide solution (5 g/100 mL) drop by drop to neutralize until the color of the solution becomes yellow.

c) Add 0.1 g of ascorbic acid, 1 ml - 10 mL of 2,2’,2”-nitrilotriethanol−water (1+3) and 1 mL - 10 mL of potassium cyanide solution (6).

d) Add a predetermined volume of 0.01 mol/L ethylenediamine tetraacetate standard solution (7).

e) Add 20 mL of ammonium chloride solution or 2-aminoethanol solution.

f) Add several drops of Eriochrome Black T solution, and titrate with a 0.01 mol/L magnesium standard solution until the color of the solution becomes red.

g) Calculate the alkalinity (AL) content in an analytical sample by the following formula.

Alkalinity (AL) (% (mass fraction)) in an analytical sample

$= ((C_1 \times f_1 \times V_6/1000) - (C_2 \times f_2 \times V_7/1000)) \times (56.077/W_2) \times (V_8/V_9) \times 100$

$= ((f_1 \times V_6) - (f_2 \times V_7)) \times (56.077/W_2) \times (V_3/V_4) \times (1/1000)$

C_1 : Set concentration (0.01 mol/L) of 0.01 mol/L ethylenediamine tetraacetate standard solution
C_2 : Set concentration (0.01 mol/L) of 0.01 mol/L magnesium standard solution
f_1 : Factor of 0.01 mol/L ethylenediamine tetraacetate standard solution
f_2 : Factor of 0.01 mol/L magnesium standard solution
V_6 : Additive volume (mL) of 0.01 mol/L ethylenediamine tetraacetate standard solution
V_7 : Volume (mL) of 0.01 mol/L magnesium standard solution needed for titration
V_8 : Constant volume (mL) of the sample solution in (**4.1**) **d**)
V_9 : Aliquot volume (mL) of the sample solution subjected to titration in (**4.2.1**) **a**).
W_2 : Mass (g) of the analytical sample

Note (**6**) If manganese is present, replace "1 mL - 10 mL of potassium cyanide solution" with "1g - 5g of potassium cyanide".
(**7**) Add excess volume since 1.8 mL of ethylenediamine tetraacetate standard solution (0.01 mol/L) is required for 1 mg of CaO.

(**4.2.2**) **Measurement (B)**: Titration with an ethylenediamine tetraacetate standard solution (0.01 mol/L)

a) Put a predetermined volume (the equivalents of 5 mg - 20 mg as CaO + MgO) of sample solution in a 200-mL - 300-mL Erlenmeyer flask.
b) Add a proper amount of water and 5 mL of citric acid solution (8), add a drop of methyl red solution as an indicator and add sodium hydroxide solution (5 g/100 mL) drop by drop to neutralize until the color of the solution becomes yellow.
c) Add 0.1 g of ascorbic acid, 1 ml - 10 mL of 2,2',2"-nitrilotriethanol−water (1+3) and 1 mL - 10 mL of potassium cyanide solution (6).
d) Add 20 mL of ammonium chloride solution or 2-aminoethanol solution.
e) Add several drops of an Eriochrome Black T solution, and immediately titrate with a 0.01 mol/L ethylenediamine tetraacetate standard solution until the color of the solution becomes blue-green.
f) Calculate the alkalinity (AL) content in an analytical sample by the following formula.

Alkalinity (AL) (% (mass fraction)) in an analytical sample

$= (C_1 \times f_1 \times V_{10}/1000) \times (56.077/W_3) \times (V_{11}/V_{12}) \times 100$

$= (f_1 \times V_{10}) \times (56.077/W_3) \times (V_{11}/V_{12}) \times (1/1000)$

C_1 : Set concentration (0.01 mol/L) of 0.01 mol/L ethylenediamine tetraacetate standard solution
f_1 : Factor of 0.01 mol/L ethylenediamine tetraacetate standard solution
V_{10} : Volume (mL) of 0.01 mol/L ethylenediamine tetraacetate standard solution needed for titration
V_{11} : Constant volume (mL) of the sample solution in (**4.1**) **d**)
V_{12} : Aliquot volume (mL) of the sample solution subjected to titration in (**4.2.2**) **a**).
W_3 : Mass (g) of the analytical sample

Note (**8**) When the sample solution does not contain phosphate, silicate, etc., it is not necessary to add citric acid.

Comment 6 Care should be fully taken in the case of using potassium cyanide and its solution in accordance with the Safety Data Sheet (SDS). In addition, observe laws and ordinances concerned such as the Poisonous and Deleterious Substance Control Law.
Criteria of the abolition in the Poisonous and Deleterious Substance Control Law (for reference): Add a sodium hydroxide solution to make it alkalinity more than pH 11, and further add an oxidizer (sodium hypochlorite, bleaching powder) solution to conduct oxidative degradation processes. After dissolving CN ingredient, neutralize with sulfuric acid, and discard it after diluting it with a large amount of water. Take enough time to dissolve the CN ingredient with alkalinity.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.162- 164, Yokendo, Tokyo (1988)

(**5**) **Flow sheet for alkalinity**: The flow sheet for alkalinity in fertilizers is shown below:

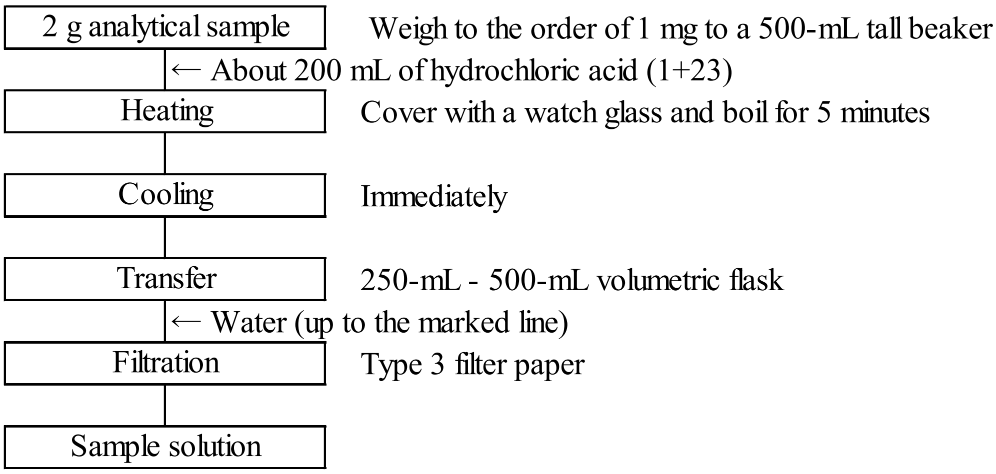


Figure 1 Flow sheet for alkalinity in fertilizers (Extraction procedure)

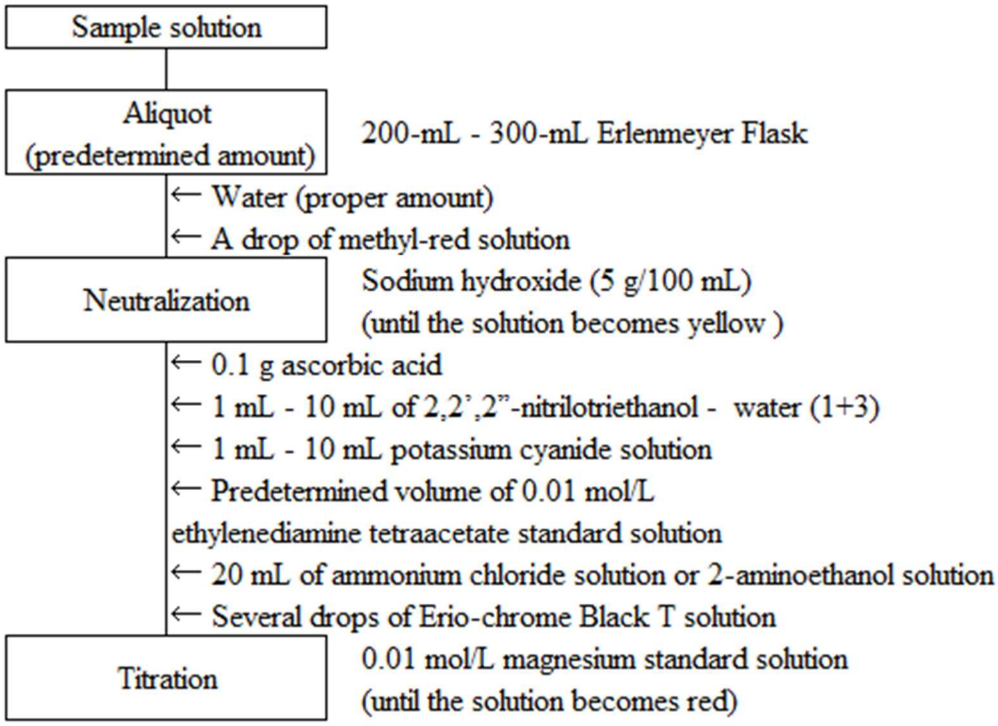


Figure 2-1 Flow sheet for alkalinity in fertilizers (Measurement procedure (4.2.1) (A))

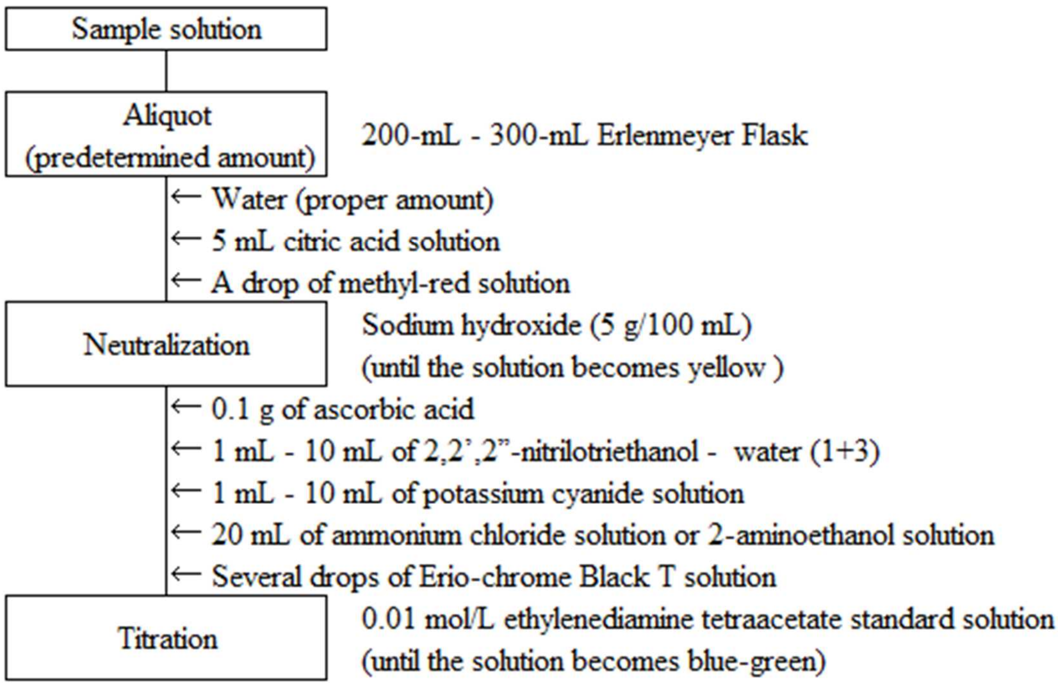


Figure 2-2 Flow sheet for alkalinity in fertilizers (Measurement procedure (4.2.2) (B))

4.5.5.b Calculation with acid-soluble lime and acid-soluble magnesia

(1) Summary

This testing method is applicable to fertilizers that guarantee alkalinity (AL). This testing method is classified as Type A (Def-C) and its symbol is 4.5.4.b-2017 or AL.b-1.

Multiply the acid-soluble magnesia (S-MgO) obtained in **4.6.2** by the factor (1.3934) and add to the acid-soluble lime (S-CaO) obtained in **4.5.2** to calculate alkalinity (AL) in an analytical sample.

(2) Calculation of alkalinity

a) Calculate the alkalinity (AL) in a teat sample by the following formula.

Alkalinity (AL) (% (mass fraction)) in an analytical sample
$= (\text{S-CaO}) + 1.3914 \times (\text{S-MgO})$

S-CaO : Acid-soluble lime (% (mass fraction)) (1) obtained in **4.5.2** in an analytical sample

S-MgO : Acid-soluble magnesia (% (mass fraction)) (1) obtained in **4.6.2** in an analytical sample

Note (**1**) S-CaO and S-MgO use raw data without rounding numerical value

4.6 Magnesia
4.6.1 Total magnesia
4.6.1.a Flame atomic absorption spectrometry
(1) Summary

The testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 4.6.1.a-2021 or T-Mg.a-2.

Pretreat an analytical sample with incineration and hydrochloric acid or nitric acid−hydrochloric acid (1+3), add an interference suppressor solution, and then spray in an acetylene−air flame, and measure the atomic absorption with magnesium at a wavelength of 285.2 nm to quantify total magnesia (T-MgO) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8.**

(**2**) **Reagent, etc**.: Reagents and water are as shown below.

- **a**) **Water**: Water of A3 specified in JIS K 0557.
- **b**) **Nitric acid**: A JIS Guaranteed Reagent specified in JIS K 8541 or a reagent of equivalent quality.
- **c**) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
- **d**) **Interference suppressor solution** (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.
- **e**) **Magnesium standard solution (MgO 1 mg/mL)** (1): Weigh 0.603 g of magnesium (powder) specified in JIS K 8876 into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.
- **f**) **Magnesium standard solution (MgO 0.1 mg/mL)** (1): Put 10 mL of magnesium standard solution (MgO 1 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.
- **g**) **Magnesium standard solution (MgO 1 µg/mL-10 µg/mL) for the calibration curve preparation** (1): Put 2.5 mL-25 mL of magnesium standard solution (MgO 0.1 mg/mL) in 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.
- **h**) **Blank test solution for the calibration curve preparation** (1): Put about 25 mL of interference suppressor solution in a 250-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the magnesium standard solution in (**2**), a magnesium standard solution for the calibration curve preparation can be prepared by using a magnesium standard solution (Mg 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate total magnesia (T-MgO) in the analytical sample by multiplying the concentration (Mg) of the magnesium standard solution for calibration curve preparation or a measurement value (Mg) obtained in (**4.2**) by a conversion factor (1.6583).

Comment 2 When using a sample solution obtained in the procedure in (**4.1.2**) **h**) for the measurement of cadmium, nickel, chromium or lead, sulfuric acid and hydrochloric acid in (**2**) should be a reagent of harmful metal analysis grade, microanalysis grade or equivalents.

(3) Instruments: Instruments are as shown below:

a) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) Light source: A magnesium hollow cathode lamp

2) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) Electric furnace: An electric furnace that can be adjusted to 450 °C ± 5 °C or 550 ºC ± 5 ºC.

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Incineration-hydrochloric acid boiling

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (4).

c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate (4).

d) After standing to cool, moisten the residue with a small amount of water, gradually add about 10 mL of hydrochloric acid, and further add water to make 20 mL.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.

f) After standing to cool, transfer the solution to a 250-mL - 500-mL volumetric flask with water.

g) Add water up to the marked line.

h) Filter with Type 3 filter paper to make a sample solution.

Note (4) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 550 °C in 1 to 2 hours.

Comment 3 Do not conduct the procedures in (**4.1.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 4 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Incineration-aqua regia digestion

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (5).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (5).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (6), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (7) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

Note (**5**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.

(**6**) The watch glass can be removed.

(**7**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in h), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 5 Do not conduct the procedures in (**4.1.2**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 6 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 285.2 nm

b) **Calibration curve preparation**

1) Spray the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 285.2 nm.

2) Prepare a curve for the relationship between the magnesium concentration and the indicated value of the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MgO) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the magnesium content from the calibration curve, and calculate the total magnesia (T-MgO) in the analytical sample.

Comment 7 The analytical line wavelength may be set to 202.5 nm with lower sensitivity. An example preparation of standard solutions for calibration curve preparation for a wavelength of 202.5 nm is 0.07 µg/mL - 5 µg/mL as MgO, and the minimum limit of quantification was estimated to be about 0.07 µg/mL in a measurement solution. However, it is necessary to understand in advance the concentration range of calibration curve suitable for the instrument and prepare standard solutions for calibration curve preparation.

Comment 8 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 5 % (mass fraction), 1 % (mass fraction) and 0.2 % (mass fraction) were 102.4 %, 101.7 % and 103.0 % as total magnesia (T-MgO) respectively.

The results of the repeatability tests on different days using swine manure compost, composted sludge fertilizer and poultry manure ash (1 sample for each) to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.2 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of total magnesia

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Swine manure compost	5	3.14	0.03	1.1	0.05	1.5
Composted sludge fertilizer	5	0.84	0.01	1.2	0.01	1.3
Poultry manure ash	5	3.97	0.03	0.7	0.04	1.1

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of total magnesia

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Compound fertilizer	8	3.58	0.02	0.6	0.07	2.0
Cattle and poultry manure	10	2.66	0.04	1.5	0.13	5.0
Compost	9	1.63	0.02	1.4	0.10	6.2
Composted sludge fertilizer	8	0.649	0.006	0.9	0.012	1.9
Bark compost	10	0.316	0.009	2.7	0.018	5.7

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Erika HIRATA, Hideo SOETA, Hidemi YOSHIMURA, Keiji YAGI: Determination of Total Magnesium in Compost and Sludge Fertilizer by Atomic Absorption Spectrometry - Research Report of Fertilizer **Vol. 11**, p. 29 - 38 (2018)

2) Kenji YAGI, Takuya KOBORI, Hideo SOETA and Hidemi YOSHIMURA: Performance Evaluation of Determination Method for Magnesium in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 87 – 101 (2020)

(5) Flow sheet for total magnesia: The flow sheet for total magnesia in fertilizers is shown below:

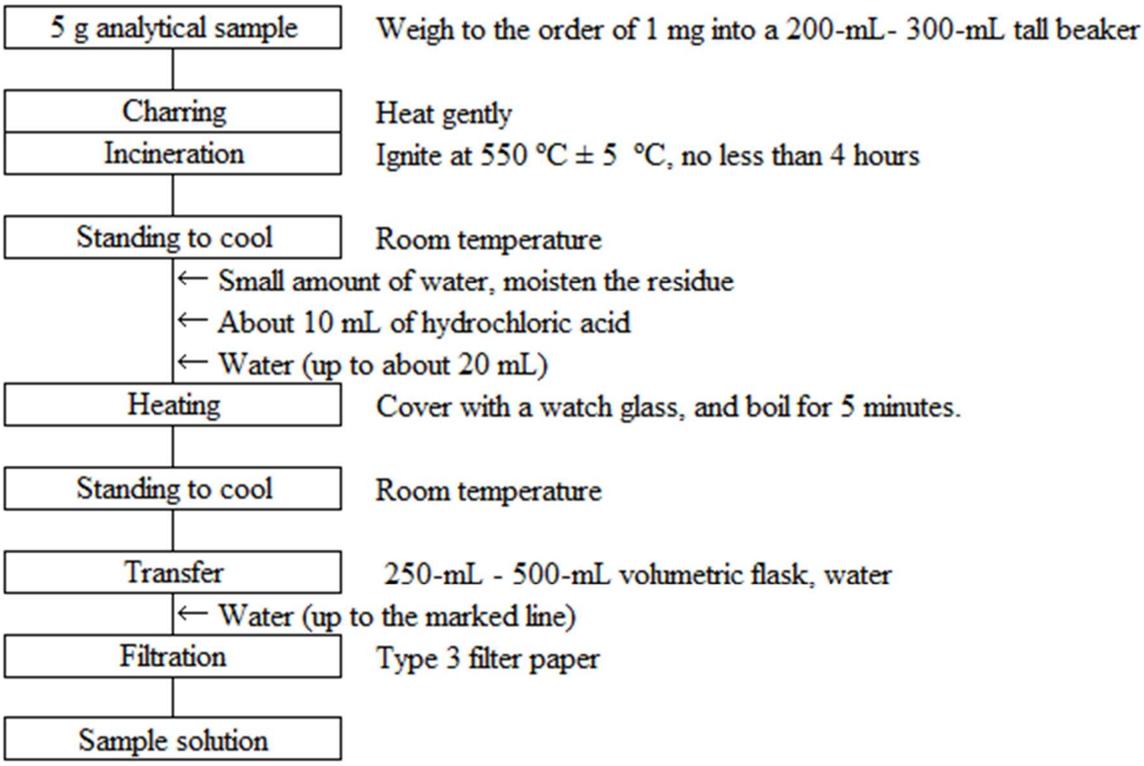


Figure 1-1 Flow sheet for total magnesia in fertilizers.
(Incineration-hydrochloric acid boiling prorcedure (4.1.1))

5 g analytical sample	Weigh to the order of 1 mg into a 200-mL - 300-mL tall beaker
Charring	Heat gently
Incineration	Ignite at 450 °C ± 5 °C for 8 - 16 hours
Standing to cool	Room temperature
	← A small amount of water
	← About 10 mL of nitric acid
	← About 30 mL of hydrochloric acid
Heating	Cover with a watch glass to digest
Heating	Slightly move the watch glass and remove acid
Standing to cool	Room temperature
	← 25 mL - 50 mL of hydrochloric acid (1+5)
Heating	Cover with a watch glass to dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
	← Water (up to the marked line)
Filtration	Type 3 filter paper
Sample solution	

Figure 1-2 Flow sheet for total magnesia in fertilizers
(Incineration-aqua regia digestion procedure (4.1.2))

Sample solution	
Aliquot (predetermined amount)	100-mL volumetric flask
	←About 10 mL interference suppressor solution
	← Water (up to the marked line)
Measurement	Atomic absorption spectrometer

Figure 2 Flow sheet for total magnesia in fertilizers (Measurement procedure)

4.6.2 Acid-soluble magnesia
4.6.2.a Flame atomic absorption spectrometry
(1) Summary

This testing method is applicable to fertilizers containing by-product magnesia fertilizers and fertilizers that guarantee alkalinity. This testing method is classified as Type B and its symbol is 4.6.2.a-2021 or S-Mg.a-2.

Add hydrochloric acid (1+23) to an analytical sample, boil to extract and add an interference suppressor solution, and then spray in an acetylene‒air flame, and measure the atomic absorption with magnesium at a wavelength of 285.2 nm to obtain hydrochloric acid (1+23) soluble magnesia (acid-soluble magnesia (S-MgO)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Interference suppressor solution** (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

c) **Magnesium standard solution (MgO 1 mg/mL)** (1): Weigh 0.603 g of magnesium (powder) specified in JIS K 8876 into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

d) **Magnesium standard solution (MgO 0.1 mg/mL)** (1): Put 10 mL of magnesium standard solution (MgO 1 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

e) **Magnesium standard solution (MgO 1 µg/mL-10 µg/mL) for the calibration curve preparation** (1): Put 2.5 mL-25 mL of magnesium standard solution (MgO 0.1 mg/mL) in 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.

f) **Blank test solution for the calibration curve preparation** (1): Put about 25 mL of interference suppressor solution used in the procedure **e**) in a 250-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the magnesium standard solution in (**2**), a magnesium standard solution for the calibration curve preparation can be prepared by using a magnesium standard solution (Mg 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate acid-soluble magnesia (S-MgO) in the analytical sample by multiplying the concentration (Mg) of a magnesium standard solution for calibration curve preparation or a measurement value (Mg) obtained in (**4.2**) by a conversion factor (1.6583).

(**3**) **Instruments**: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A magnesium hollow cathode lamp

2) **Gas**: Gas for heating by flame
(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Hot plate** : A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 2 g of an analytical sample to the order of 1 mg, and put it in a 500-mL tall beaker.

b) Add about 200mL of hydrochloric acid (1+23), cover with a watch glass, and boil on a hot plate for about 5 minutes (4).

c) After immediate cooling is complete, transfer it to a 250-mL - 500 -mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make a sample solution.

Note (**4**) Be aware that an analytical sample should not solidify in the bottom of a beaker.

Comment 2 In the case of a by-product magnesia fertilizer or a fertilizer containing a by-product magnesia, if the pH of the sample solution of **d**) is neutral or basic, prepare a sample solution anew by replacing “2 g of an analytical sample” in the procedure in **a**) with “1 g - 1.5 g of an analytical sample”.

Comment 3 In the procedure in **a**), a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. In addition, “cover with a watch glass” in **b**) is replaced by “place a funnel”, and “transfer it to a 250-mL - 500 mL volumetric flask with water” in the procedure in **c**) is skipped.

Comment 4 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 285.2 nm

b) **Calibration curve preparation**

1) Spray the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 285.2 nm.

2) Prepare a curve for the relationship between the magnesium concentration and the indicated value of the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MgO) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the magnesium content from the calibration curve, and calculate the acid-soluble magnesia (S-MgO) in the analytical sample.

Comment 5 The analytical line wavelength may be set to 202.5 nm with lower sensitivity. An example preparation of standard solutions for calibration curve preparation for a wavelength of 202.5 nm is 0.07 µg/mL - 5 µg/mL as MgO, and the minimum limit of quantification was estimated to be about 0.07 µg/mL in a measurement solution. However, it is necessary to understand in advance the concentration range of calibration curve suitable for the instrument and prepare standard solutions for calibration curve preparation.

Comment 6 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 15 % (mass fraction) and 1 % (mass fraction) were 101.7 % and 99.5% as acid-soluble magnesia (S-MgO) respectively. In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.2 % (mass fraction) for solid fertilizers and about 0.05 % (mass fraction) for fluid fertilizers.

Table 1 Analysis results of the collaborative study for the test method validation of acid-soluble magnesia

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Slaked lime	11	29.42	0.20	0.5	1.11	3.8
Calcium carbonate	11	22.07	0.28	0.4	1.19	5.4
Mixed phosphorus fertilizer A	10	12.33	0.09	0.9	0.71	5.8
Slag silicate fertilizer A	9	7.30	0.05	0.1	0.21	2.8
Slag silicate fertilizer B	10	4.58	0.03	0.5	0.23	5.0

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.167- 169, Yokendo, Tokyo (1988)

2) Souichi IGARASHI and Yasuharu KIMURA: Verification of Performance Characteristics of Testing Methods for Magnesia Content in Fertilizer by Atomic Absorption Spectrometry: Research Report of Fertilizer **Vol. 6**, p. 193 - 202 (2013)

3) Kenji YAGI, Takuya KOBORI, Hideo SOETA and Hidemi YOSHIMURA: Performance Evaluation of Determination Method for Magnesium in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 87 – 101 (2020)

(5) **Flow sheet for acid-soluble magnesia**: The flow sheet for acid-soluble magnesia in fertilizers is shown below:

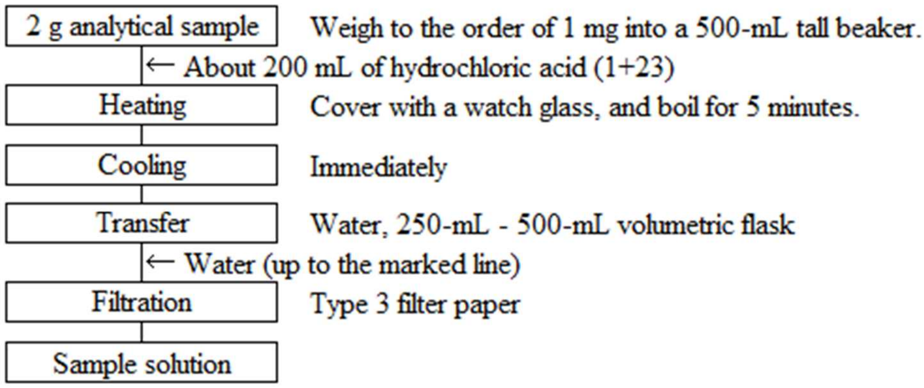


Figure 1 Flow sheet for acid-soluble magnesia in fertilizers (Extraction procedure)

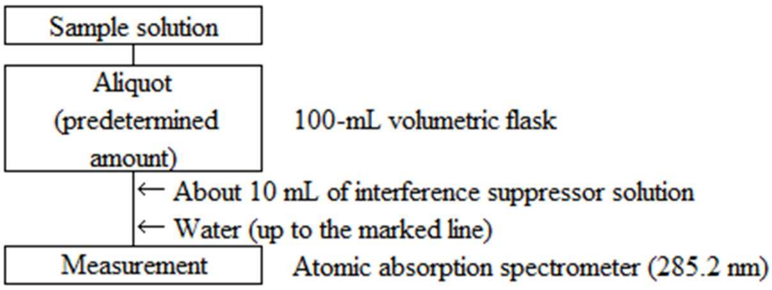


Figure 2 Flow sheet for acid-soluble magnesia in fertilizers (Measurement procedure)

4.6.3 Citric acid-soluble magnesia

4.6.3.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers containing magnesia hydroxide fertilizers, etc. This testing method is classified as Type B and its symbol is 4.6.3.a-2021 or C-Mg.a-3.

Extract by adding a citric acid solution to an analytical sample and add an interference suppressor solution, and then spray in an acetylene−air flame, and measure the atomic absorption with magnesium at a wavelength of 285.2 nm to obtain citric acid-soluble magnesia (C-MgO) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 9**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

c) **Interference suppressor solution** (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

d) **Magnesium standard solution (MgO 1 mg/mL)** (1): Weigh 0.603 g of magnesium (powder) specified in JIS K 8876 into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

e) **Magnesium standard solution (MgO 0.1 mg/mL)** (1): Put 10 mL of magnesium standard solution (MgO 1 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

f) **Magnesium standard solution (MgO 1 µg/mL-10 µg/mL) for the calibration curve preparation** (1): Put 2.5 mL-25 mL of magnesium standard solution (MgO 0.1 mg/mL) in 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Put about 25 mL of interference suppressor solution used in the procedure **f)** in a 250-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the magnesium standard solution in (**2**), a magnesium standard solution for the calibration curve preparation can be prepared by using a magnesium standard solution (Mg 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble magnesia (C-MgO) in the analytical sample by multiplying the concentration (Mg) of a magnesium standard solution for calibration curve preparation or a measurement value (Mg) obtained in (**4.2**) by a conversion factor (1.6583).

(**3**) **Instruments**: Instruments are as shown below:

a) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

aa) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

ab) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

b) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A magnesium hollow cathode lamp

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Constant-temperature vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (4), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (4) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 2 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Horizontal reciprocating water bath shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (5).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (4), and shake to mix by reciprocating at amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) at 160 times/min for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (5) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 3 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 4 For a by-product magnesia fertilizer, if the pH of the sample solution in (**4.1.1**) **d**) and (**4.1.2**) **d**) is neutral or basic, prepare a sample solution anew by replacing "1 g of an analytical sample" in the procedures in (**4.1.1**) **a**) and (**4.1.2**) **a**) with "0.5 g of an analytical sample".

Comment 5 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

Comment 6 In some silicate slag fertilizers, the variation of measurement value of citric acid-soluble magnesia (C-MgO) may be observed according to the time variation of heating state after adding a citric acid solution. Therefore, in the case of silicate slag fertilizers, it is necessary to conduct the procedures of (**4.1.1**) **c**) - **d**) and (**4.1.2**) **c**) - **d**) as quickly as possible after confirming the time of shaking to mix in the procedure in (**4.1.1**) **b**) and (**4.1.2**) **b**).

Comment 7 In the case of a fertilizer containing Kieserite (magnesia sulfate fertilizers), wash the non-dissolved matters with water obtained while preparing the sample solution of water-soluble magnesia in (**4.1**) in **4.6.4.a** to put it in a 250 mL volumetric flask, and then prepare a sample solution by the procedures in (**4.1.1**) **b**) - **d**) and (**4.1.2**) **b**) - **d**). Mix the magnesia obtained in (**4.2**) regarding this sample solution with the water-soluble magnesia in **4.6.4.a** regarding the said fertilizers to make citric acid-soluble magnesia.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 285.2 nm

b) **Calibration curve preparation**

1) Spray the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 285.2 nm.

2) Prepare a curve for the relationship between the magnesium concentration and the indicated value of the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MgO) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the magnesium content from the calibration curve, and calculate the citric acid-soluble magnesia (C-MgO) in the analytical sample.

Comment 8 The analytical line wavelength may be set to 202.5 nm with lower sensitivity. An example preparation of standard solutions for calibration curve preparation for a wavelength of 202.5 nm is 0.07 µg/mL - 5 µg/mL as MgO, and the minimum limit of quantification was estimated to be about 0.07 µg/mL in a measurement solution. However, it is necessary to understand in advance the concentration range of calibration curve suitable for the instrument and prepare standard solutions for calibration curve preparation.

Comment 9 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rate at the content level of 1 % (mass fraction) - 5 % (mass fraction) was 98.9 % - 100.3 % as citric acid-soluble magnesia (C-MgO).
In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1. The results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 2 shows the calculation results of reproducibility, intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to

be about 0.06 % (mass fraction) for solid fertilizers.

Table 1 Analysis results of the collaborative study for the test method validation of citric acid-soluble magnesia

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Magnesia hydroxide	11	38.24	0.42	0.5	1.83	4.8
Compound fertilizer A	11	20.58	0.18	0.4	0.74	3.6
Humic acid magnesia fertilizer	11	10.74	0.11	0.9	0.43	4.0
Compound fertilizer B	11	4.79	0.11	0.1	0.16	3.4
Compound fertilizer C	11	2.42	0.08	0.5	0.13	5.4

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2 Analysis results of the collaborative study to determine citric acid-soluble magnecia of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)	s_R[8)] (%)[3)]	RSD_R[9)] (%)
FAMIC-A-10	11	3.28	0.07	2.0	0.08	2.5	0.11	3.3
FAMIC-A-13	9	3.18	0.03	1.0	0.04	1.4	0.12	3.8

1) The number of laboaratories used for analysis conducting flame atomic absorbance spectrometry
2) Mean (the number of laboratory(p)× test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.167- 169, Yokendo, Tokyo (1988)
2) Souichi IGARASHI and Yasuharu KIMURA: Verification of Performance Characteristics of Testing Methods for Magnesia Content in Fertilizer by Atomic Absorption Spectrometry: Research Report of Fertilizer **Vol. 6**, p. 193 - 202 (2013)
3) Souichi IGARASHI and Yasuharu KIMURA: Effect of Time during Extraction Operation on Measurements of Citric Acid-Soluble Magnesium in Silicate Slag Fertilizer **Vol. 7**, p. 145 - 156 (2014)
4) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)
5) Kenji YAGI, Takuya KOBORI, Hideo SOETA and Hidemi YOSHIMURA: Performance Evaluation of Determination Method for Magnesium in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 87 – 101 (2020)

(5) **Flow sheet for citric acid-soluble magnesia**: The flow sheet for citric acid-soluble magnesia in fertilizers is shown below:

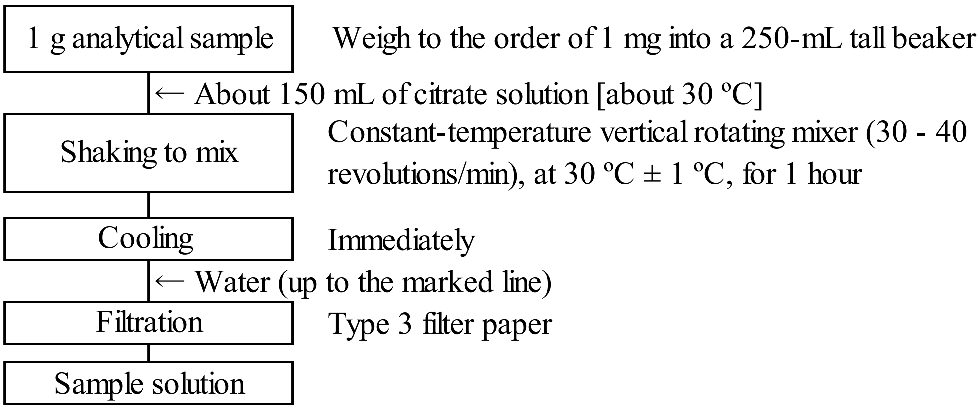


Figure 1-1 Flow sheet for citric acid-soluble magnesia in fertilizers (Extraction procedure (4.1.1))

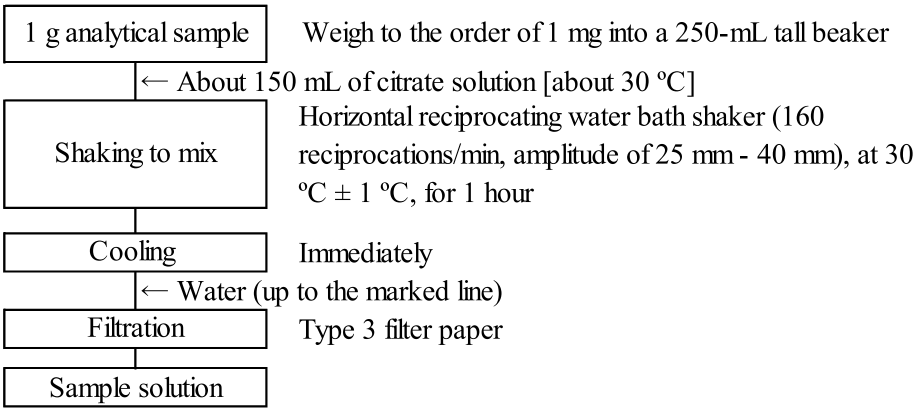


Figure 1-2 Flow sheet for citric acid-soluble magnesia in fertilizers (Extraction procedure (4.1.2))

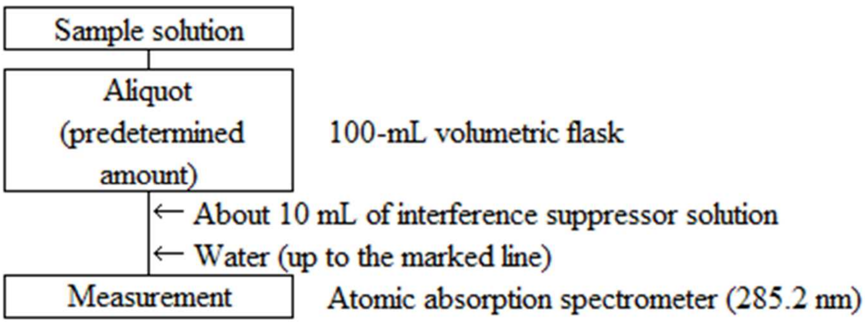


Figure 2 Flow sheet for citric acid-soluble magnesia in fertilizers (Measurement procedure)

4.6.3.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 4.6.3.b-2018 or C-Mg.b-1.

Extract by adding a citric acid solution to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the magnesium at a wavelength of 279.553 nm to obtain citric acid-soluble magnesia (citric acid-soluble magnesia (C-MgO)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 10**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Water:** Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

d) **Magnesium standard solution (MgO 1 mg/mL)** (1): Weigh 0.603 g of magnesium (powder) specified in JIS K 8876 into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

e) **Magnesium standard solution (MgO 0.1 mg/mL)**: Put 10 mL of magnesium standard solution (MgO 1 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.

f) **Magnesium standard solution (MgO 2 µg/mL - 10 µg/mL) for the calibration curve preparation** (1): Put 2 mL-16 mL of magnesium standard solution (MgO 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.

g) **Magnesium standard solution (MgO 0.2 µg/mL - 2 µg/mL) for the calibration curve preparation** (1): Put 2 mL - 20 mL of magnesium standard solution (MgO 10 µg/mL) for the calibration curve preparation in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.

h) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**) - **g**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the magnesium standard solution in (**2**), a magnesium standard solution for the calibration curve preparation can be prepared by using a magnesium standard solution (Mg 1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble magnesia (C-MgO) in the analytical sample by multiplying the concentration (Mg) of a magnesium standard solution for calibration curve preparation or a measurement value (Mg) obtained in (**4.2**) by a conversion factor (1.6583).

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and a spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) **ICP Optical Emission Spectrometry**: An optical emission spectrometer specified in JIS K

0116

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

ba) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

bb) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Constant-temperature vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Horizontal reciprocating water bath shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (3).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix by reciprocating at amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) at 160 times/min for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 5 For a by-product magnesia fertilizer, if the pH of the sample solution in (**4.1.1**) **d**) and (**4.1.2**) **d**) is neutral or basic, prepare a sample solution anew by replacing "1 g of an analytical sample" in the procedures in (**4.1.1**) **a**) and (**4.1.2**) **a**) with "0.5 g of an analytical sample".

Comment 6 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, check the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

Comment 7 In some silicate slag fertilizers, the variation of measurement value of citric acid-soluble magnesia (C-MgO) may be observed according to the time variation of heating state after adding a citric acid solution. Therefore, in the case of silicate slag fertilizers, it is necessary to conduct the procedures of (**4.1.1**) **c**) - **d**) and (**4.1.2**) **c**) - **d**) as quickly as possible after confirming the time of shaking to mix in the procedure in (**4.1.1**) **b**) and (**4.1.2**) **b**).

Comment 8 In the case of a fertilizer containing Kieserite (magnesia sulfate fertilizers), wash the non-dissolved matters with water obtained while preparing the sample solution of water-soluble magnesia in (**4.1**) in 4.6.4.a to put it in a 250 mL volumetric flask, and then prepare a sample solution by the procedures in (**4.1.1**) **b**) - **d**) and (**4.1.2**) **b**) - **d**). Mix the magnesia obtained in (**4.2**) regarding this sample solution with the water-soluble magnesia in **4.6.4.a** regarding the said fertilizers to make citric acid-soluble magnesia.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 279.553 nm

b) **Calibration curve preparation**

1) Spray the magnesium standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 279.553 nm.

2) Prepare a curve for the relationship between the magnesium concentration and the indicated value of the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.02 mg - 1.6 mg as MgO) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the magnesium content from the calibration curve, and calculate the citric acid-soluble magnesia (C-MgO) in the analytical sample.

Comment 9 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 10 The comparison of the measurement value (y_i : 1.59 % (mass fraction) - 15.06 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Flame atomic absorption spectrometry was conducted to evaluate trueness using processed phosphorus fertilizers (2 samples), compound fertilizers (11 samples), silicate slag fertilizer (1 sample), mixed compost compound fertilizer (1 sample), mixed phosphorus fertilizers (2 samples), designated blended fertilizer (1 sample), blended fertilizers (5 samples), byproduct compound fertilizer (1 sample), organic compound fertilizer (1 sample), and fused phosphate fertilizer (1 sample). As a result, a regression equation was y=0.0271+1.0124x, and its correlation coefficient (r) was 0.999. In addition, additive recovery testing was conducted using a preparation sample.

As a result, the mean recovery rate at the additive level of 0.232 % (mass fraction) - 18.81 % (mass fraction) was 94.9 % - 102.7 %.
The results of the repeatability tests on different days using compound fertilizers and blended fertilizers to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.03 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of citric acid-soluble magnecia

Name of sample	Test days of repeatability T[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Blended fertilizer	7	8.41	0.09	1.1	0.10	1.1
Compound fertilizer	7	1.66	0.03	1.6	0.03	1.8

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)

2) Shingo MATSUO: Simultaneous Determination of Citrate-Soluble Principal Ingredients ($C\text{-}P_2O_5$, $C\text{-}K_2O$, C-MgO, C-MnO and $C\text{-}B_2O_3$) in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 11**, p. 14 – 28 (2018)

(5) Flow sheet for citric acid-soluble magnesia: The flow sheet for citric acid-soluble magnesia in fertilizers is shown below:

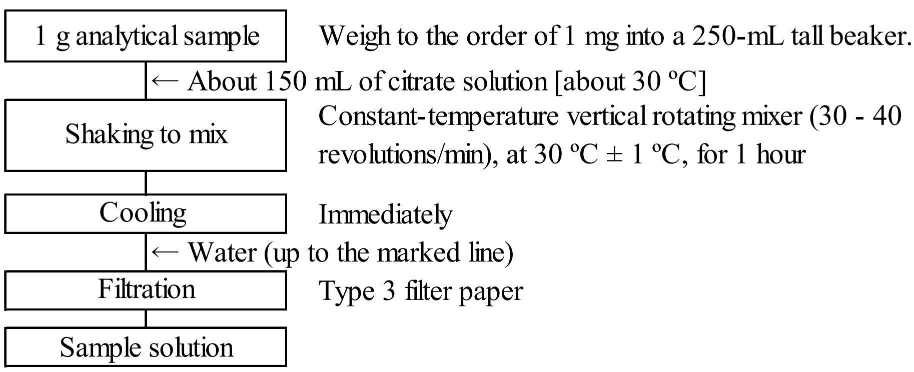


Figure 1-1 Flow sheet for citric acid-soluble magnesia in fertilizers (Extraction procedure (4.1.1))

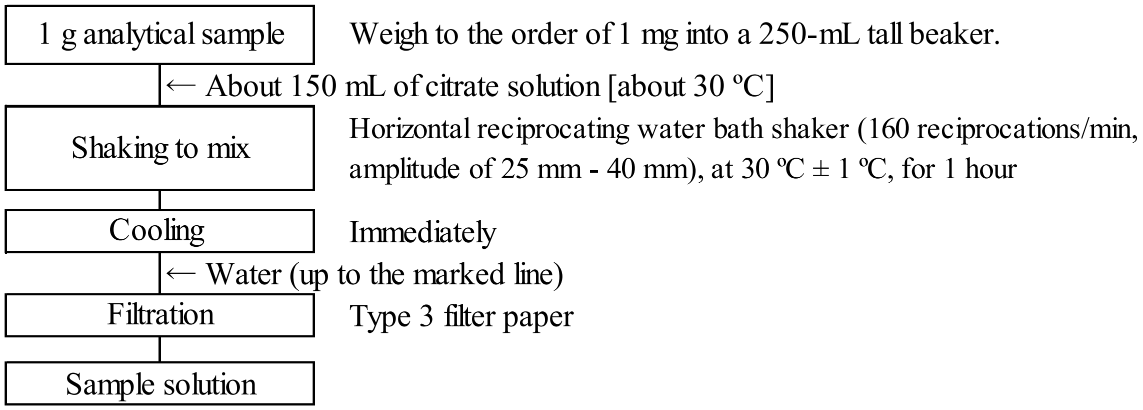


Figure 1-2 Flow sheet for citric acid-soluble magnesia in fertilizers (Extraction procedure (4.1.2))

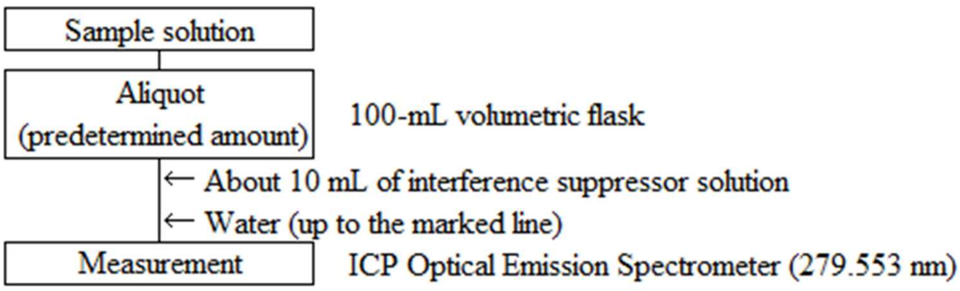


Figure 2 Flow sheet for citric acid-soluble magnesia in fertilizers (Measurement procedure)

4.6.4 Water-soluble magnesia

4.6.4.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers containing magnesia sulfate fertilizers, etc. This testing method is classified as Type B and its symbol is 4.6.4.a-2021 or W-Mg.a-2.

Add water to an analytical sample and boil to extract. Add an interference suppressor solution, then spray in an acetylene−air flame and measure the atomic absorption with magnesium at a wavelength of 285.2 nm to obtain water-soluble magnesia (W-MgO) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(2) Reagent: Reagents are as shown below.

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Interference suppressor solution (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

c) Magnesium standard solution (MgO 1 mg/mL) (1): Weigh 0.603 g of magnesium (powder) specified in JIS K 8876 into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

d) Magnesium standard solution (MgO 0.1 mg/mL) (1): Put 10 mL of magnesium standard solution (MgO 1 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

e) Magnesium standard solution (MgO 1 µg/mL - 10 µg/mL) for the calibration curve preparation (1): Put 2.5 mL-25 mL of magnesium standard solution (MgO 0.1 mg/mL) in 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.

f) Blank test solution for the calibration curve preparation (1): Put about 25 mL of interference suppressor solution used in the procedure **d**) in a 250-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the magnesium standard solution in (**2**), a magnesium standard solution for the calibration curve preparation can be prepared by using a magnesium standard solution (Mg 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble magnesia (W-MgO) in the analytical sample by multiplying the concentration (Mg) of a magnesium standard solution for calibration curve preparation or a measurement value (Mg) obtained in (**4.2**) by a conversion factor (1.6583).

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) Light source: A magnesium hollow cathode lamp

2) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Hot plate** : A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 400-mL tall beaker.

b) Add 400 mL of water, and cover with a watch glass to boil on a hot plate for about 30 minutes (5).

c) After immediate cooling is complete, transfer it to a 500- mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make a sample solution.

Note (**4**) Be aware that an analytical sample should not solidify in the bottom of a beaker.

Comment 2 The procedure in (**4.1.1**) is the same as the procedure in (**4.1.1**) in **4.6.4.b.**

Comment 3 In the procedure in **a**), a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, "cover with a watch glass" in **b**) is replaced by "place a funnel". Skip "transfer it to a 500-mL volumetric flask with water" in the procedure in **c**).

(4.1.2) Fluid test sample

a) Weigh 1 g of an analytical sample (5) to the order of 1 mg, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, shake to mix and add water up to the marked line.

c) Filter with Type 3 filter paper to make a sample solution.

Note (**5**) The sampling amount of the analytical sample is 10 g when there is less magnesia content in the fertilizers such as a home garden-use fertilizer.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 285.2 nm

b) **Calibration curve preparation**

1) Spray the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 285.2 nm.

2) Prepare a curve for the relationship between the magnesium concentration and the indicated value of the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MgO) in a 100-mL volumetric flask.
2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.
3) Subject to the same procedure as in **b**) **1**) to read the indicated value.
4) Obtain the magnesium content from the calibration curve, and calculate the water-soluble magnesia (W-MgO) in the analytical sample.

Comment 5 The analytical line wavelength may be set to 202.5 nm with lower sensitivity. An example preparation of standard solutions for calibration curve preparation for a wavelength of 202.5 nm is 0.07 µg/mL - 5 µg/mL as MgO, and the minimum limit of quantification was estimated to be about 0.07 µg/mL in a measurement solution. However, it is necessary to understand in advance the concentration range of calibration curve suitable for the instrument and prepare standard solutions for calibration curve preparation.

Comment 6 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 1 % (mass fraction) - 5 % (mass fraction) were 100.4 % - 100.9 % as water-soluble magnesia (W-MgO) respectively.

In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.07 % (mass fraction).

Table 1 Analysis results of the collaborative study for the test method validation of water-soluble magnesia

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Magnesia sulfate fertilizer	8	25.70	0.02	0.1	0.44	1.7
Mixed microelement fertilizer	10	15.16	0.31	2.0	0.33	2.1
Mixed phosphorus fertilizer	9	5.56	0.05	0.9	0.15	2.7
Processed magnesia fertilizer	10	3.46	0.05	1.5	0.10	3.0
Humic acid magnesia fertilizer	8	2.38	0.03	1.3	0,04	1.5

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.167- 169, Yokendo, Tokyo (1988)
2) Souichi IGARASHI and Yasuharu KIMURA: Verification of Performance Characteristics of Testing Methods for Magnesia Content in Fertilizer by Atomic Absorption Spectrometry: Research Report of Fertilizer **Vol. 6**, p. 193 - 202 (2013)
3) Kenji YAGI, Takuya KOBORI, Hideo SOETA and Hidemi YOSHIMURA: Performance

Evaluation of Determination Method for Magnesium in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 87 – 101 (2020)

(5) **Flow sheet for water-soluble magnesia**: The flow sheet for water-soluble magnesia in fertilizers is shown below:

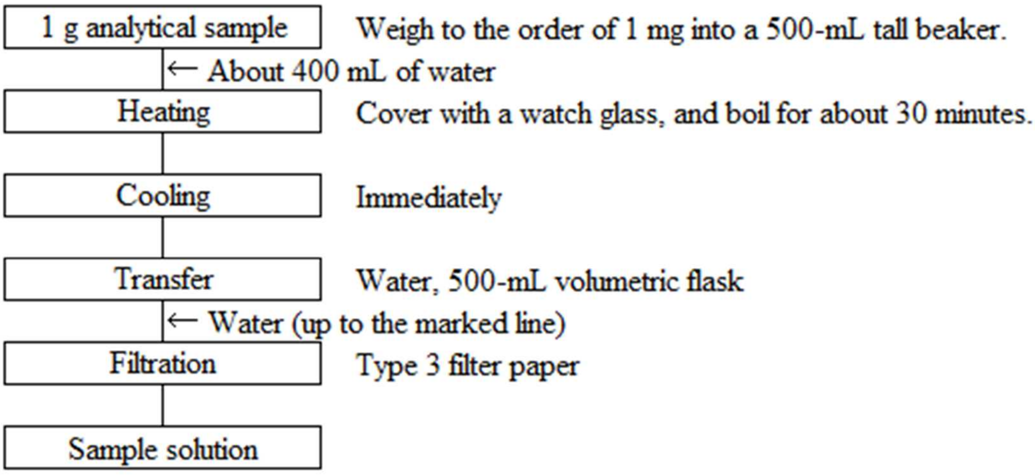


Figure 1-1 Flow sheet for water-soluble magnesia in fertilizers (Extraction procedure (4.1.1))

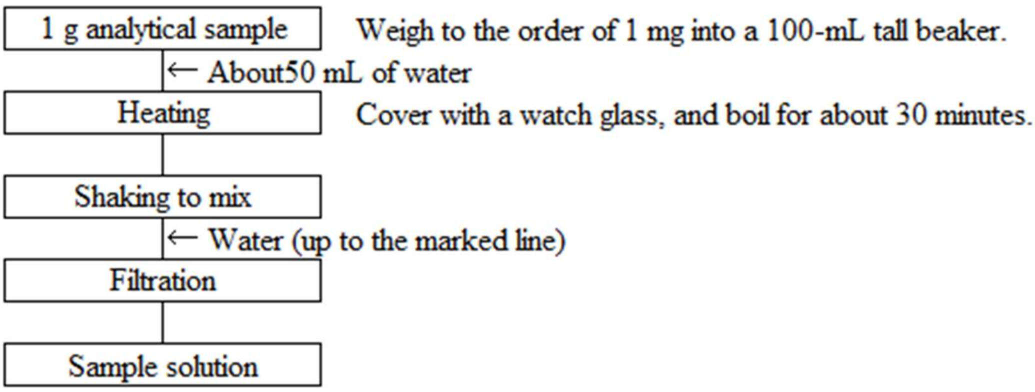


Figure 1-2 Flow sheet for water-soluble magnesia in fertilizers (Extraction procedure (4.1.2))

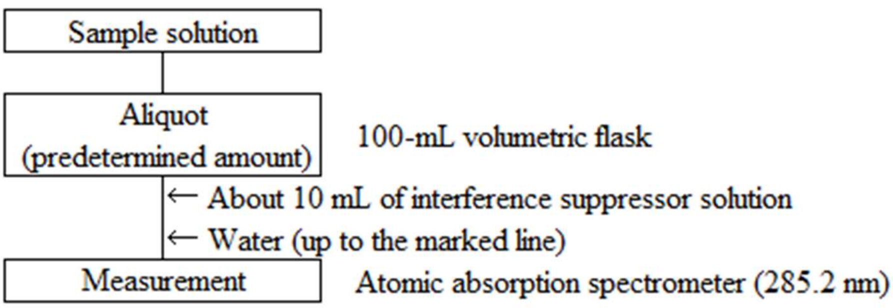


Figure 2 Flow sheet for water-soluble magnesia in fertilizers (Measurement procedure)

4.6.4.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D for solid fertilizers and Type B for fluid fertilizers. Its symbol is 4.6.4.b-2019 or W-Mg.b-2.

Add water to an analytical sample to extract, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the magnesium at a wavelength of 279.553 nm, etc. to obtain water-soluble magnesia (W-MgO) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Magnesium standard solution (MgO 1 mg/mL)** (1): Weigh 0.603 g of magnesium (powder) specified in JIS K 8876 into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

d) **Magnesium standard solution (MgO 0.1 mg/mL)**: Put 10 mL of magnesium standard solution (MgO 1 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.

e) **Magnesium standard solution (MgO 2 µg/mL - 16 µg/mL) for the calibration curve preparation** (1): Put 2 mL-16 mL of magnesium standard solution (MgO 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.

f) **Magnesium standard solution (MgO 0.2 µg/mL - 2 µg/mL) for the calibration curve preparation** (1): Put 2 mL - 20 mL of magnesium standard solution (MgO 10 µg/mL) for the calibration curve preparation in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the magnesium standard solution in (**2**), a magnesium standard solution for the calibration curve preparation can be prepared by using a magnesium standard solution (Mg 1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble magnesia (W-MgO) in the analytical sample by multiplying the concentration (Mg) of a magnesium standard solution for calibration curve preparation or a measurement value (Mg) obtained in (**4.2**) by a conversion factor (1.6583).

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and a spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) **Hot plate:** A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 400-mL tall beaker.
b) Add 400 mL of water, and cover with a watch glass to heat on a hot plate and boil for about 30 minutes (2).
c) After immediate cooling is complete, transfer it to a 500- mL volumetric flask with water.
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Be aware that an analytical sample should not solidify in the bottom of a beaker.

Comment 3 The procedure in (**4.1.1**) is the same as the procedure in (**4.1.1**) in **4.6.4.a.**
Comment 4 In the procedure in **a**), a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, “cover with a watch glass” in **b**) is replaced by “place a funnel”. Skip “transfer it to a 500-mL volumetric flask with water” in the procedure in **c**).

(4.1.2) Fluid test sample

a) Weigh 1 mg of an analytical sample (3) to the order of 1 g, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, shake to mix and add water up to the marked line.
c) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) The sampling amount of the analytical sample is 10 g when there is less magnesia content in the fertilizers such as a home garden-use fertilizer.

Comment 5 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 279.553 nm or 280.270 nm (4)

b) **Calibration curve preparation**

1) Spray the magnesium standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at an analytical line wavelength.
2) Prepare a curve for the relationship between the magnesium concentration and the indicated value of the magnesium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.02 mg - 1.6 mg as MgO) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.
3) Subject to the same procedure as in **b**) **1**) to read the indicated value.
4) Obtain the magnesium content from the calibration curve, and calculate the water-soluble magnesia (W-MgO) in the analytical sample.

Note (**4**) 280.270 nm can also be used. However, since the intensity of emission obtained is different from the one of 279.553 nm, it is necessary to understand the suitable concentration range of the calibration curve and prepare a standard solution for the calibration curve in advance.

Comment 6 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 7 The comparison of the measurement value (y_i : 0.361 % (mass fraction) - 14.51 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of flame atomic absorbance spectrometry was conducted to evaluate trueness using solid fertilizers (28 samples). As a result, a regression equation was $y=-0.0263+1.004x$, and its correlation coefficient (r) was 1.000. Similarly the comparison of the measurement value (y_i : 0.160 % (mass fraction) - 9.36 % (mass fraction)) and the measurement value (x_i) was conducted using fluid fertilizers (12 samples). As a result, a regression equation was $y=-0.006+0.985x$, and its correlation coefficient (r) was 0.999. In addition, additive recovery testing was conducted using preparation fertilizers (5 samples). As a result, the mean recovery rate at the additive level of 0.846 % (mass fraction) - 18.79 % (mass fraction) was 97.7 % - 103.0 %. Additive recovery testing was conducted using a fluid compound fertilizer (1 brand), a home garden-use compound fertilizer (1 brand) and a liquid microelement compound fertilizer (1 sample). As a result, the mean recovery rates at the additive level of 1 % (mass fraction) and 0.15 % (mass fraction) were 98.7 % - 102.8 % and 102.3 % respectively.

The results of the repeatability tests on different days using a mixed microelement fertilizer, a blended fertilizer, a fluid compound fertilizer and a home garden-use compound fertilize (fluid) were analyzed by the one-way analysis of variance to evaluate precision. Table 1-1 and 1-2 show the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study implemented for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.06 % (mass fraction) for solid fertilizers and about 0.002 % (mass fraction) for fluid fertilizers.

Table 1-1 Analysis results of the repeatability tests on different days of water-soluble magnesia (Solid fertilizer)

Name of sample	Test days of repeatability T[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)
Mixed microelement fertilizer	5	7.99	0.22	2.8	0.22	2.8
Blended fertilizer	5	0.986	0.026	2.6	0.026	2.6

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 1-2 Analysis results of the repeatability tests on different days of water-soluble magnesia (Fluid fertilizer)

Name of sample	Test days of repeatability T[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)
Fluid compound fertilizer	7	1.18	0.004	0.3	0.01	1.2
Home garden-use compound fertilizer (Fluid)	7	0.392	0.002	0.5	0.008	2.2

Footnote: Refer to Table 1-1

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble magnesia

Analytical line wavelength (nm)	Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
279.553	Preparation sample (Liquid) 1	8	5.31	0.07	1.4	0.13	2.4
	Preparation sample (Liquid) 2	9	2.13	0.009	0.4	0.17	7.9
	Preparation sample (Liquid) 3	11	1.03	0.01	1.2	0.06	5.5
	Preparation sample (Liquid) 4	8	0.517	0.008	1.5	0.011	2.2
	Preparation sample (Liquid) 5	9	0.0508	0.0005	1.0	0.0025	4.9
280.27	Preparation sample (Liquid) 1	9	5.28	0.07	1.2	0.29	5.5
	Preparation sample (Liquid) 2	11	2.13	0.01	0.6	0.14	6.5
	Preparation sample (Liquid) 3	10	1.00	0.01	1.3	0.03	3.4
	Preparation sample (Liquid) 4	9	0.515	0.008	1.5	0.025	4.8
	Preparation sample (Liquid) 5	10	0.0498	0.0006	1.3	0.0026	5.2

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination of Water-Soluble Principal Ingredients (W-P_2O_5, W-K_2O, W-MgO, W-MnO and W-B_2O_3) in Liquid Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 8**, p. 1 - 9 (2015)

2) Norio FUNAKI: Simultaneous Determination of Water-soluble Principal Ingredients in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 12**, p. 28 – 51 (2019)

3) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(**5**) **Flow sheet**: The flow sheet for water-soluble magnesia in fluid fertilizers is shown below:

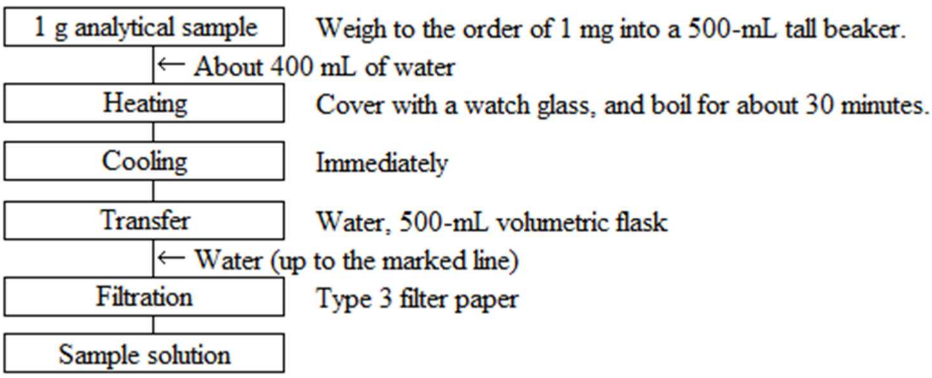


Figure 1-1 Flow sheet for water-soluble magnesia in fertilizers (Extraction procedure (4.1.1))

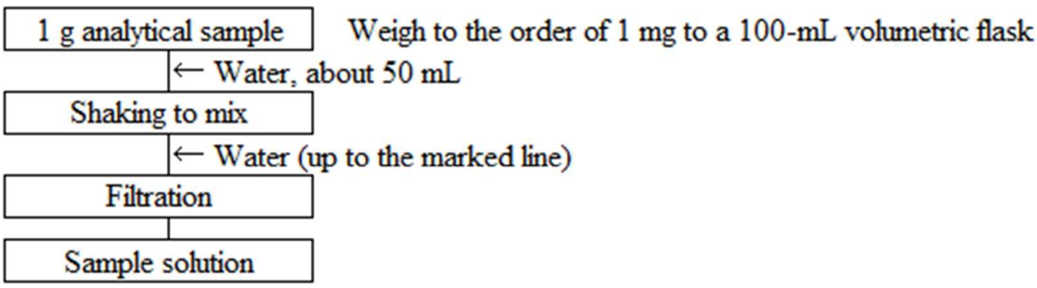


Figure 1-2 Flow sheet for water-soluble magmesia in fertilizers (Extraction procedure (4.1.2))

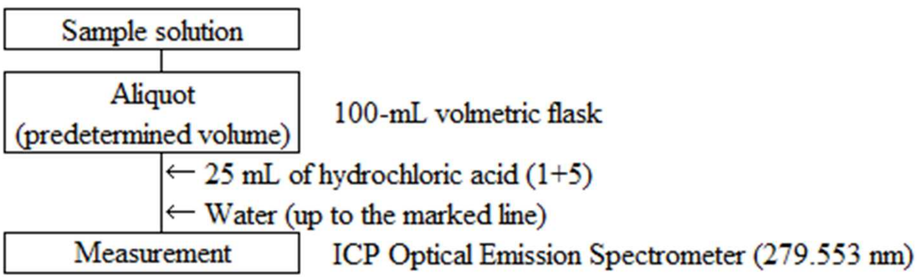


Figure 2 Flow sheet for water-soluble magmesia in fertilizers (Measurement procedure)

4.7 Manganese
4.7.1 Acid-soluble manganese
4.7.1.a Flame atomic absorption spectrometry
(1) Summary

This test method is applicable to fertilizers containing manganese carbonate fertilizers. This testing method is classified as Type D and its symbol is 4.7.1.a-2017 or S-Mn.a-1.

Add hydrochloric acid (1+23) to an analytical sample, boil to extract and add an interference suppressor solution, and then spray into an acetylene−air flame, and measure the atomic absorption with manganese at a wavelength of 279.5 nm to obtain the hydrochloric acid (1+23) soluble manganese (acid-soluble manganese (S-MnO)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 4**.

(2) Reagent: Reagents are as shown below.

a) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) Interference suppressor solution (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

c) Manganese standard solution (MnO 1 mg/mL) (1): Weigh 0.775 g of manganese powder (purity no less than 99 % (mass fraction)) into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

d) Manganese standard solution (MnO 0.1 mg/mL) (1): Put 10 mL of manganese standard solution (MnO 1 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

e) Manganese standard solution (MnO 1 µg/mL-10 µg/mL) for the calibration curve preparation (1): Put 2.5 mL-25 mL of manganese standard solution (MnO 0.1 mg/mL) in 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.

f) Blank test solution for the calibration curve preparation (1): Put about 25 mL of interference suppressor solution used in the procedure **e**) in a 250-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the manganese standard solution in (**2**), a manganese standard solution for the calibration curve preparation can be prepared by using a manganese standard solution (Mn 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate acid-soluble manganese (S-MnO) in the analytical sample by multiplying the concentration (Mn) of a manganese standard solution for calibration curve preparation or a measurement value (Mn) obtained in (**4.2**) by a conversion factor (1.2912).

(3) Instruments: Instruments are as shown below:

a) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A manganese hollow cathode lamp

2) **Gas: Gas for heating by flame**

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Hot plate** : A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 2 g of an analytical sample to the order of 1 mg, and put it in a 500-mL tall beaker.

b) Add about 200mL of hydrochloric acid (1+23), cover with a watch glass, and boil on a hot plate for about 5 minutes (4).

c) After immediate cooling is complete, transfer it to a 250-mL- 500-mL volumetric flask with water.

d) Add water up to the marked line.

e) Filter with Type 3 filter paper to make a sample solution.

Note **(4)** Be aware that an analytical sample should not solidify in the bottom of a beaker.

Comment 2 In the procedure in a), a 500-mL volumetric flask can be used instead of a 500-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. In addition, "cover with a watch glass" in **b**) is replaced by "place a funnel", and "transfer it to a 250-mL - 500 mL volumetric flask with water" in the procedure in **c**) is skipped.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 279.5 nm

b) **Calibration curve preparation**

1) Spray the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 279.5 nm.

2) Prepare a curve for the relationship between the manganese concentration and the indicated value of the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MnO) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the manganese content from the calibration curve, and calculate the acid-soluble manganese (S-MnO) in the analytical sample.

Comment 4 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 5 % (mass fraction) and 0.1 % (mass fraction) were 100.5 % and 101.3 % as acid-soluble manganese (S-MnO) respectively.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.006 % (mass fraction).

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.176- 177, Yokendo, Tokyo (1988)

2) Keiji YAGI, Natuki TOYODOME, Tokiya SUZUKI and Hideo SOETA: Verification of Performance Characteristics of Testing Methods for Manganese Content in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer **Vol. 6**, p. 203 - 212 (2013)

(5) **Flow sheet for acid-soluble manganese**: The flow sheet for acid-soluble manganese in fertilizers is shown below:

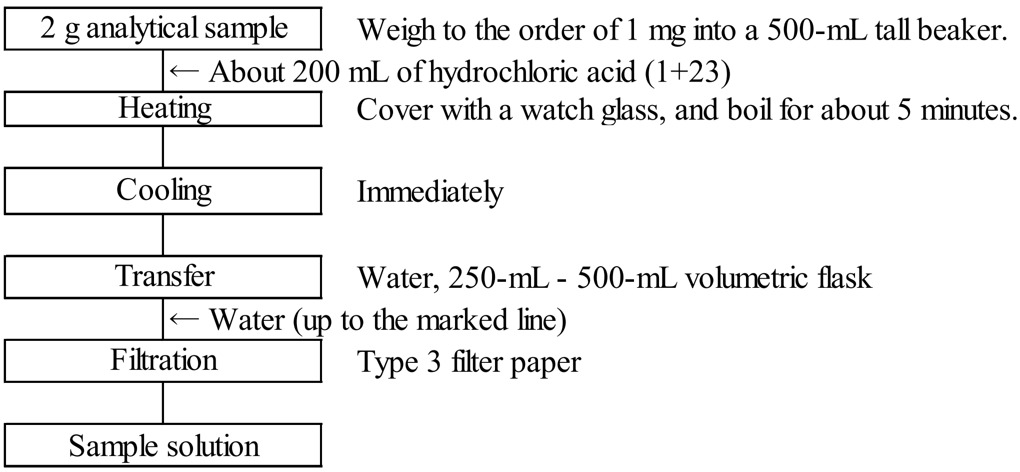


Figure 1 Flow sheet for acid-soluble manganese in fertilizers (Extraction procedure)

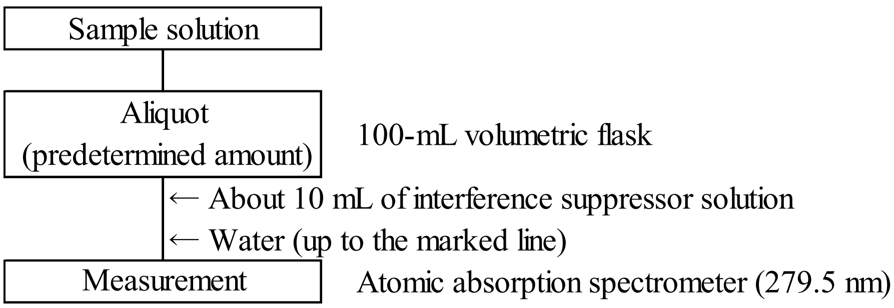


Figure 2 Flow sheet for acid-soluble manganese in fertilizers (Mrasurement procedure)

4.7.2 Citric acid-soluble manganese
4.7.2.a Flame atomic absorption spectrometry
(1) Summary

This test method is applicable to fertilizers containing manganese carbonate fertilizers. This testing method is classified as Type B and its symbol is 4.7.2.a-2021 or C-Mn.a-3.
Extract by adding a citric acid solution to an analytical sample and add an interference suppressor solution, and then spray into an acetylene‒air flame, and measure the atomic absorption with manganese at a wavelength of 279.5 nm to obtain citric acid-soluble manganese (citric acid-soluble manganese(C-MnO)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

c) **Interference suppressor solution** (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

d) **Manganese standard solution (MnO 1 mg/mL)** (1): Weigh 0.775 g of manganese powder (purity no less than 99 % (mass fraction)) into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

e) **Manganese standard solution (MnO 0.1 mg/mL)**: Put 10 mL of manganese standard solution (MnO 1 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

f) **Manganese standard solution (MnO 1 μg/mL - 10 μg/mL) for the calibration curve preparation** (1): Put 2.5 mL-25 mL of manganese standard solution (MnO 0.1 mg/mL) in 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Put about 25 mL of interference suppressor solution used in the procedure f) in a 250-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the manganese standard solution in (**2**), a manganese standard solution for the calibration curve preparation can be prepared by using a manganese standard solution (Mn 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble manganese (C-MnO) in the analytical sample by a multiplying the concentration (Mn) of manganese standard solution for calibration curve preparation or a measurement value (Mn) obtained in (**4.2**) by a conversion factor (1.2912).

(**3**) **Instruments**: Instruments are as shown below:

a) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

aa) Constant-temperature vertical rotating mixer: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

ab) Horizontal reciprocating water bath shaker: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

b) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121.

1) Light source: A manganese hollow cathode lamp

2) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Constant-temperature vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (4), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (4) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 2 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Horizontal reciprocating water bath shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (5).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (4), and shake to mix by reciprocating horizontally at amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) at 160 times/min for 1 hour.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Note (5) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 3 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 4 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 279.5 nm

b) **Calibration curve preparation**

1) Spray the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 279.5 nm.

2) Prepare a curve for the relationship between the manganese concentration and the indicated value of the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MnO) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b) 1)** to read the indicated value.

4) Obtain the manganese content from the calibration curve, and citric acid-soluble manganese (C-MnO) in the analytical sample.

Comment 5 The analytical line wavelength may be set to 403.1 nm with lower sensitivity. An example preparation of standard solutions for calibration curve preparation for a wavelength of 403.1 nm is 0.3 µg/mL - 19 µg/mL as MnO, and the minimum limit of quantification was estimated to be about 0.3 µg/mL in a measurement solution. However, it is necessary to understand in advance the concentration range of calibration curve suitable for the instrument and prepare standard solutions for calibration curve preparation.

Comment 6 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 5 % (mass fraction) and 0.1 % (mass fraction) were 101.9 % and 100.5 % as citric acid-soluble manganese (C-MnO) respectively.

In order to evaluate precision, results from a collaborative study for test method validation and their analysis are shown in Table 1.

Additionally, the results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 2 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.006 % (mass fraction).

Table 1 Analysis results of the collaborative study for the test method validation of citric acid-soluble manganese

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Slag manganese fertilizer	12	32.03	0.36	1.9	0.68	2.1
Calcined microelement mixed fertilizer	12	22.17	0.20	0.9	0.73	3.3
Mixed phosphorus fertilizer	11	1.83	0.02	1.1	0,06	3.2
Compound fertilizers 1	10	0,82	0.01	0.6	0.04	4.3
Compound fertilizers 2	11	0.28	0.00	1.4	0.02	7.4

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

Table 2 Analysis results of the collaborative study to determine citric acid-soluble manganese of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)	s_R[8)] (%)[3)]	RSD_R[9)] (%)
FAMIC-A-10	9	0.403	0.004	1.1	0.01	1.3	0.010	2.4
FAMIC-A-13	10	0.356	0.010	2.7	0.01	3.9	0.018	4.9

1) The number of laboaratories used for analysis conducting flame atomic absorbance spectrometry
2) Mean (the number of laboratory(p) × test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.176- 177, Yokendo, Tokyo (1988)

2) Keiji YAGI, Natuki TOYODOME, Tokiya SUZUKI and Hideo SOETA: Verification of Performance Characteristics of Testing Methods for Manganese Content in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer **Vol. 6**, p. 203 - 212 (2013)

3) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)

4) Toshio HIRABARA, Masahiro ECHI, Ryouto KOBAYASHI: Performance Evaluation of Determination Method for Manganese in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 102 – 111 (2020)

(5) Flow sheet for citric acid-soluble manganese: The flow sheet for citric acid-soluble manganese in fertilizers is shown below:

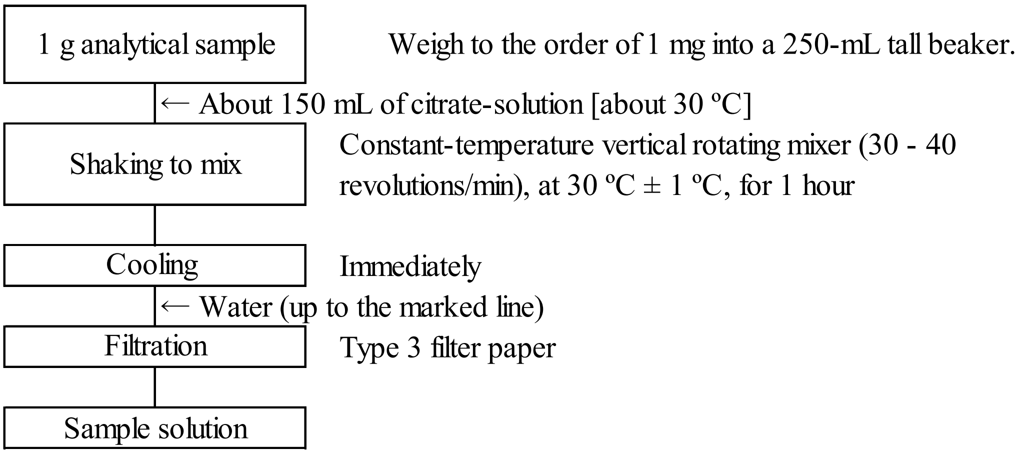


Figure 1-1 Flow sheet for citric acid-soluble manganese in fertilizers
(Extraction procedure (4.1.1))

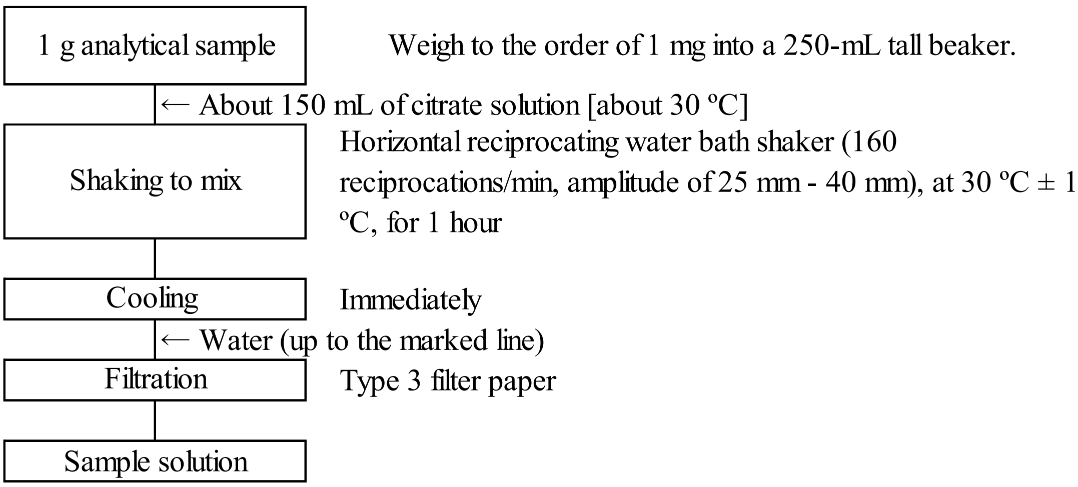


Figure 1-2 Flow sheet for citric acid-soluble manganese in fertilizers
(Extraction procedure (4.1.2))

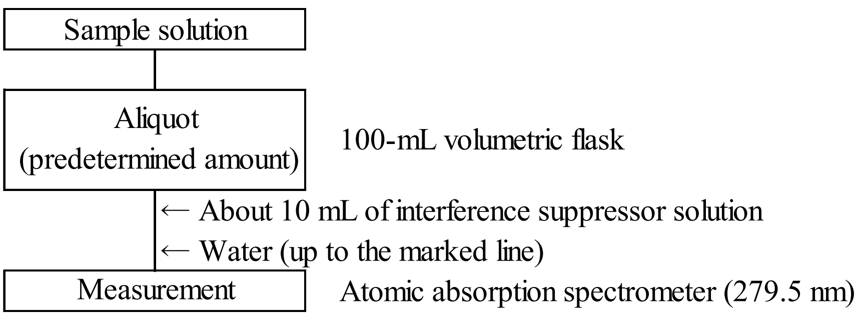


Figure 2 Flow sheet for citric acid-soluble manganese in fertilizers
(Measurement procedure)

4.7.2.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 4.7.2.b-2018 or C-Mn.b-1.

Extract by adding a citric acid solution to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the manganese at a wavelength of 257.610 nm to obtain citric acid-soluble manganese (citric acid-soluble manganese (C-MnO)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

d) **Manganese standard solution (MnO 1 mg/mL)** (1): Weigh 0.775 g of manganese powder (purity no less than 99 % (mass fraction)) into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and further add hydrochloric acid (1+23) up to the marked line.

e) **Manganese standard solution (MnO 0.1 mg/mL)**: Put 10 mL of manganese standard solution (MnO 1 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.

f) **Manganese standard solution (MnO 2 µg/mL - 8 µg/mL) for the calibration curve preparation** (1): Put 2 mL - 8 mL of manganese standard solution (MnO 0.1 mg/mL) in 100-mL volumetric flasks step-by-step and add hydrochloric acid (1+23) up to the marked line.

g) **Manganese standard solution (MnO 0.1 µg/mL - 2 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of manganese standard solution (MnO 10 µg/mL) in 100-mL volumetric flasks step-by-step and add hydrochloric acid (1+23) up to the marked line.

h) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**) and **g**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the manganese standard solution in (**2**), a manganese standard solution for the calibration curve preparation can be prepared by using a manganese standard solution (Mn 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble manganese (C-MnO) in the analytical sample by a multiplying the concentration (Mn) of manganese standard solution for calibration curve preparation or a measurement value (Mn) obtained in (**4.2**) by a conversion factor (1.2912).

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Instruments**: Instruments are as shown below:

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

ba) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

bb) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Constant-temperature vertical rotating mixer**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Horizontal reciprocating water bath shaker**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (3).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix by reciprocating horizontally at amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) at 160 times/min for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 5 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:

Analytical line wavelength: 257.610 nm

b) Calibration curve preparation

1) Spray the manganese standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 257.610 nm.

2) Prepare a curve for the relationship between the manganese concentration and the indicated value of the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 0.8 mg as MnO) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the manganese content from the calibration curve, and calculate the citric acid-soluble manganese (C-MnO) in the analytical sample.

Comment 6 The simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 7 The comparison of the measurement value (y_i : 0.089 % (mass fraction) - 1.88 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Flame atomic absorption spectrometry analysis was conducted to evaluate trueness using compound fertilizers (7 samples), mixed phosphorus fertilizers (2 samples), solid compound fertilizers (2 samples), blended fertilizers (4 samples) and organic blended fertilizer (1 sample). As a result, a regression equation was y=0.0015+0.9988x, and its correlation coefficient (r) was 0.999. In addition, additive recovery testing was conducted using a preparation sample. As a result, the mean recovery rate at the additive level of 0.595 % (mass fraction) - 28.94 % (mass fraction) was 98.5 % - 105.5 %.

The results of the repeatability tests on different days using compound fertilizers and blended fertilizers to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of citric acid-soluble manganese

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Compound fertilzier	7	0.54	0.01	2.3	0.02	3.1
Blended fertilizer	7	0.089	0.002	1.9	0.002	2.4

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)
2) Shingo MATSUO: Simultaneous Determination of Citrate-Soluble Principal Ingredients (C-P_2O_5, C-K_2O, C-MgO, C-MnO and C-B_2O_3) in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 11**, p. 14 – 28 (2018)

(5) **Flow sheet for citric acid-soluble manganese**: The flow sheet for citric acid-soluble manganese in fertilizers is shown below:

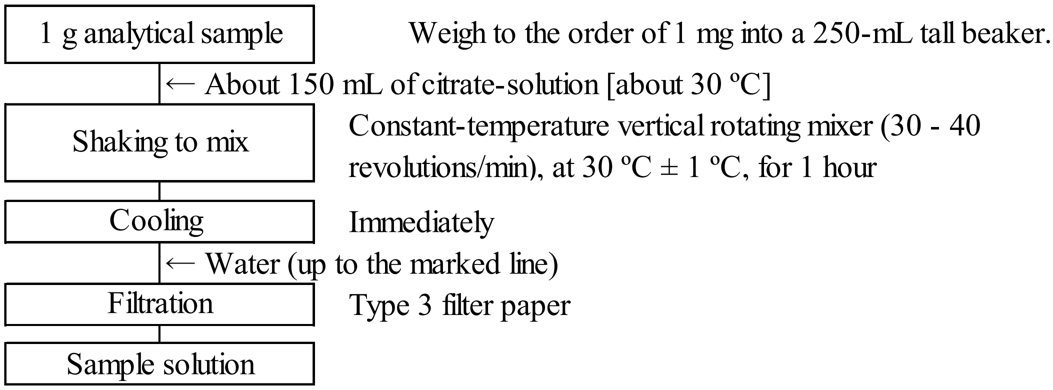


Figure 1-1 Flow sheet for citric acid-soluble manganese in fertilizers (Extraction procedure (4.1.1))

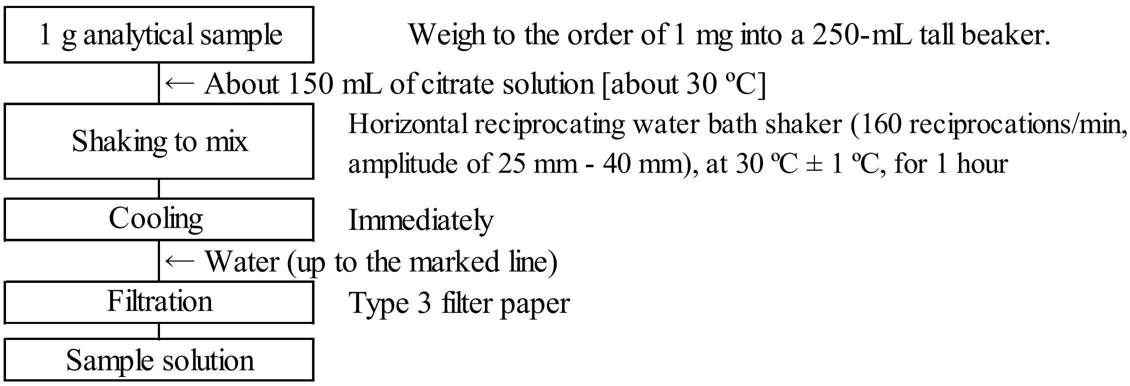


Figure 1-2 Flow sheet for citric acid-soluble manganese in fertilizers (Extraction procedure (4.1.2))

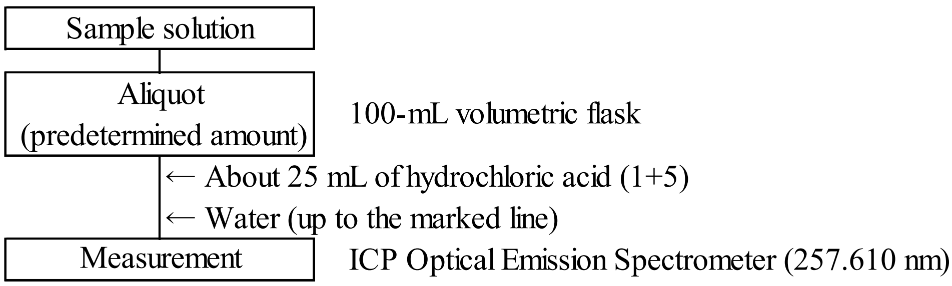


Figure 2 Flow sheet for citric acid-soluble manganese in fertilizers (Measurement procedure)

4.7.3 Water-soluble manganese
4.7.3.a Flame atomic absorption spectrometry
(1) Summary

This test method is applicable to fertilizers containing manganese sulfate fertilizers. This testing method is classified as Type B and its symbol is 4.7.3.a-2021 or W-Mn.a-2.

Extract by adding water to an analytical sample and add an interference suppressor solution, and then spray in an acetylene−air flame, and measure the atomic absorption with manganese at a wavelength of 279.5 nm to obtain water-soluble manganese (W-MnO) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Interference suppressor solution** (1): Weigh 60.9 g - 152.1 g of strontium chloride hexahydrate (2) specified in JIS K 8132 into a 2000-mL beaker, add a small amount of water, add gradually 420 mL of hydrochloric acid to dissolve, and further add water to make 1000 mL.

c) **Manganese standard solution (MnO 1 mg/mL)** (1): Weigh 0.775 g of manganese powder (purity no less than 99 % (mass fraction)) into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and add water up to the marked line.

d) **Manganese standard solution (MnO 0.1 mg/mL)** (1): Put 10 mL of manganese standard solution (MnO 1 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

e) **Manganese standard solution (MnO 1 µg/mL - 10 µg/mL) for the calibration curve preparation** (1): Put 2.5 mL-25 mL of manganese standard solution (MnO 0.1 mg/mL) in 250-mL volumetric flasks step-by-step, add about 25 mL of interference suppressor solution (3), and add water up to the marked line.

f) **Blank test solution for the calibration curve preparation** (1): Put about 25 mL of interference suppressor solution used in the procedure **e**) in a 250-mL volumetric flask (3), and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) 29 g of lanthanum oxide (atomic absorption analysis grade or equivalents) can also be used.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the manganese standard solution in (**2**), a manganese standard solution for the calibration curve preparation can be prepared by using a manganese standard solution (Mn 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble manganese (W-MnO) in the analytical sample by a multiplying the concentration (Mn) of manganese standard solution for calibration curve preparation or a measurement value (Mn) obtained in (**4.2**) by a conversion factor (1.2912).

(**3**) **Instruments**: Instruments are as shown below.

a) **Extractor**: A vertical rotating mixer or vertical reciprocating shaker as described below.

aa) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

ab) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

b) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A manganese hollow cathode lamp

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

(4.1.1.1) Vertical rotating mixer

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Comment 2 In the procedure of (**4.1.1.1**) **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 3 A sample solution obtained in the procedure in (**4.1.1.1**) is also applicable to the components shown in Annex B.

(4.1.1.2) Vertical reciprocating shaker

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add about 200 mL of water, and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Comment 4 A sample solution obtained in the procedure in (**4.1.1.2**) is also applicable to the components shown in Annex B.

(4.1.2) Fluid test sample

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, and shake to mix.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Comment 5 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement

conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 279.5 nm

b) Calibration curve preparation

1) Spray the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 279.5 nm.

2) Prepare a curve for the relationship between the manganese concentration and the indicated value of the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as MnO) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add water up to the marked line.

3) Subject to the same procedure as in **b) 1)** to read the indicated value.

4) Obtain the manganese content from the calibration curve, and water-soluble manganese (W-MnO) in the analytical sample.

Comment 6 The analytical line wavelength may be set to 403.1 nm with lower sensitivity. An example preparation of standard solutions for calibration curve preparation for a wavelength of 403.1 nm is 0.3 µg/mL - 19 µg/mL as MnO, and the minimum limit of quantification was estimated to be about 0.3 µg/mL in a measurement solution. However, it is necessary to understand in advance the concentration range of calibration curve suitable for the instrument and prepare standard solutions for calibration curve preparation.

Comment 7 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 6 % (mass fraction) and 0.1 % (mass fraction) were 101.2 % and 101.1 % as water-soluble manganese (W-MnO) respectively.

The comparison of the measurement value (y_i: 0.0330 % (mass fraction) - 6.18 % (mass fraction)) of extraction by a vertical reciprocating shaker and the measurement value (x_i) of extraction by a vertical rotating mixer was conducted to evaluate trueness of the extraction of solid fertilizers using fertilizers (12 samples). As a result, a regression equation was $y = -0.009 + 1.011x$, and its correlation coefficient (r) was 1.000. The comparison of the measurement value (y_i: 0.0590 % (mass fraction) - 1.27 % (mass fraction)) of simple extraction and the measurement value (x_i) of extraction by a vertical rotating mixer was conducted to evaluate trueness of the extraction of fluid fertilizers using fluid fertilizers (12 samples). As a result, a regression equation was $y = -0.001 + 1.006x$, and its correlation coefficient (r) was 1.000. The results of the repeatability tests on different days using compound fertilizers and mixed microelement fertilizers to evaluate precision were analyzed by one-way analysis of variance. Table 1-1 shows the calculation results of intermediate precision and repeatability. The results of the repeatability tests on different days using a fluid compound fertilizer and a liquid microelement compound fertilizer to evaluate the extract precision of fluid fertilizers were analyzed by the one-way analysis of variance. Table 1-2 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.004 % (mass fraction).

Table 1-1 Analysis results of the repeatability tests on different days of water-soluble manganese(Solid fertilizers)

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Mixed microelement fertilizer	7	3.57	0.03	0.7	0.05	1.5
Compound fertilizer	7	0.226	0.002	1.0	0.004	1.7

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 1-2 Analysis results of the repeatability tests on different days of water-soluble manganese (Fluid fertilizers)

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluidcompound fertilizer	7	1.28	0.01	0.4	0.02	1.3
Liquid microelement compound fertilizer	7	0.230	0.001	0.5	0.003	1.5

Foot note: Refer to Table 1-1

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble manganese

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Manganese sulfate fertilizer	12	11.07	0.17	1.5	0.44	3.9
Mixed microelement fertilizer	12	5.11	0.05	1.0	0.12	2.4
Designated blended fertilizer	12	0.47	0.00	1.0	0.01	2.2
Compound fertilizer	11	0.43	0.00	0.8	0.01	2.1
Magnesia sulfate fertilzer	11	0.11	0.00	1.4	0.10	3.4

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.176- 177, Yokendo, Tokyo (1988)

2) Keiji YAGI, Natuki TOYODOME, Tokiya SUZUKI and Hideo SOETA: Verification of Performance Characteristics of Testing Methods for Manganese Content in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer **Vol. 6**, p. 203 - 212 (2013)

3) Shinji KAWAGUCHI: Simple Extraction Method for Water-Soluble Components in Liquid Compound Fertilizers, Research Report of Fertilizer, **Vol. 9**, p. 10 - 20 (2016)

4) Shinji KAWAGUCHI: Extraction Method for the Water-soluble Principal Ingredients in the Solid Fertilizer using a General-purpose Equipment , Research Report of Fertilizer, **Vol. 10**, p. 1 - 8 (2017)

5) Toshio HIRABARA, Masahiro ECHI, Ryouto KOBAYASHI: Performance Evaluation of Determination Method for Manganese in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 102 – 111 (2020)

(**5**) **Flow sheet for water-soluble manganese**: The flow sheet for water-soluble manganese in fertilizers is shown below:

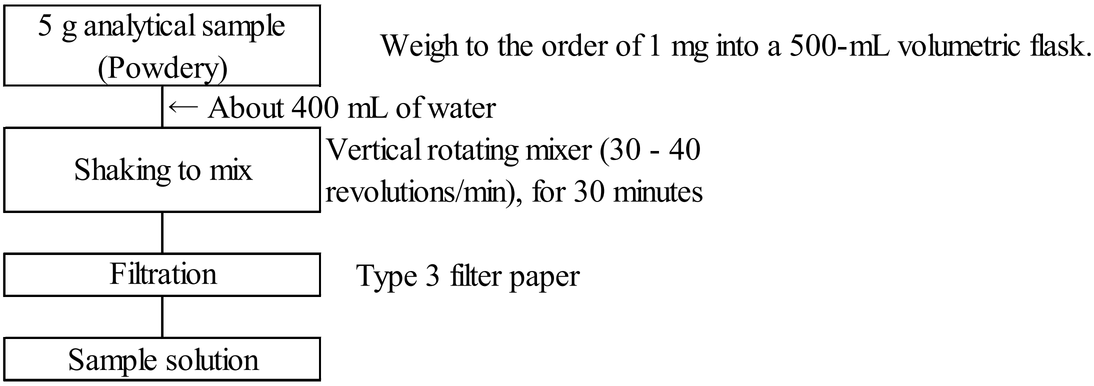


Figure 1-1 Flow sheet for water-soluble manganese in fertilizers (Extraction procedure (4.1.1.1))

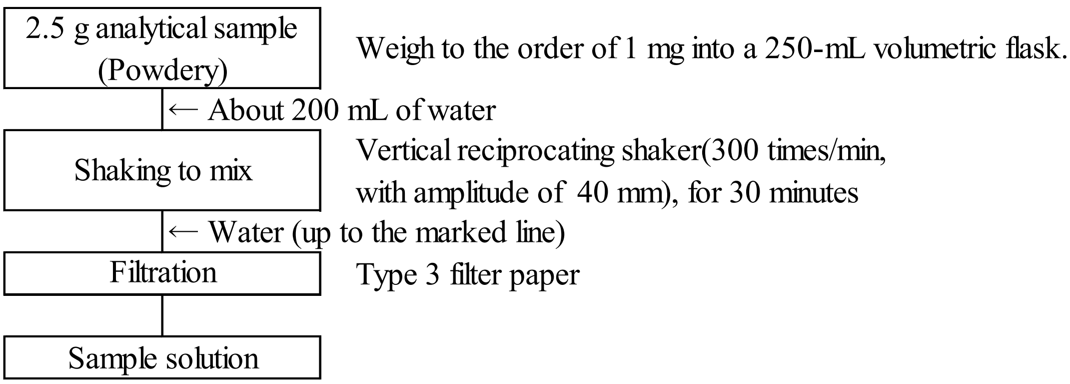


Figure 1-2 Flow sheet for water-soluble manganese in fertilizers (Extraction procedure (4.1.1.2))

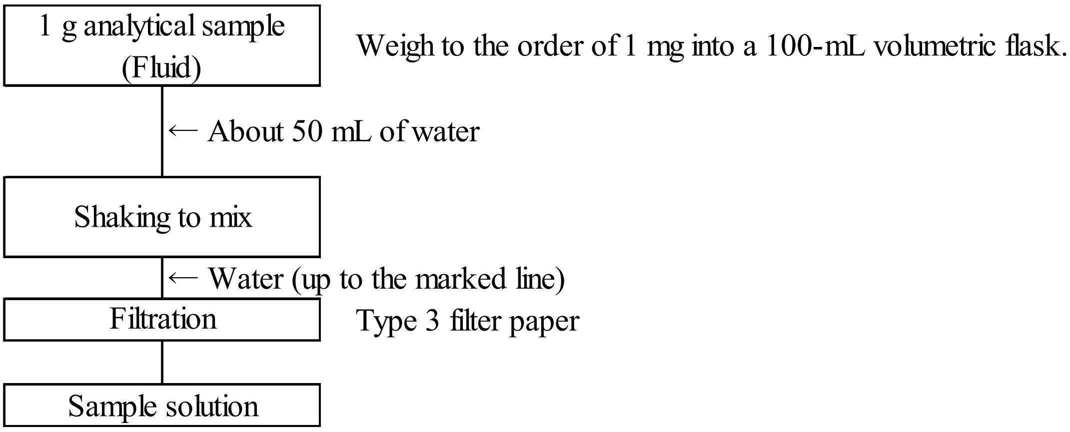


Figure 1-3 Flow sheet for water-soluble manganese in fertilizers
(Extraction procedure (4.1.2))

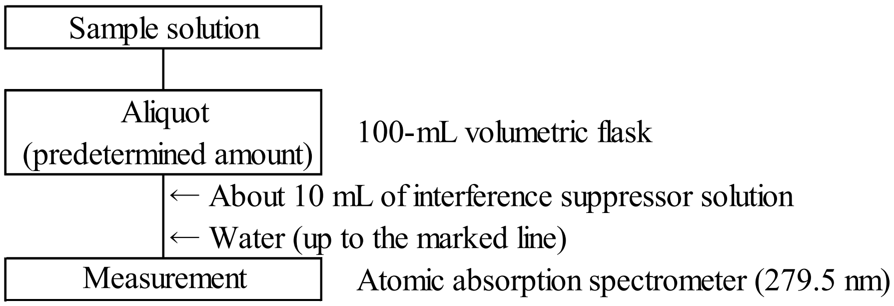


Figure 2 Flow sheet for water-soluble manganese in fertilizers
(Measurement procedure)

4.7.3.b ICP Optical Emission Spectrometry

(1) Summary

The test method is applicable to fertilizers. This testing method is classified as Type D for solid fertilizers and Type B for fluid fertilizers. Its symbol is 4.7.3.b-2019 or W-Mn.b-2.

Add water to an analytical sample to extract, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the manganese at a wavelength of 257.610 nm, etc. to obtain water-soluble manganese (W-MnO) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Manganese standard solution (MnO 1 mg/mL)** (1): Weigh 0.775 g of manganese powder (purity no less than 99 % (mass fraction)) into a weighing dish. Transfer to a 1000-mL volumetric flask with a small amount of water, add about 10 mL of hydrochloric acid to dissolve, and further add hydrochloric acid (1+23) up to the marked line.

d) **Manganese standard solution (MnO 0.1 mg/mL)** (1): Put 10 mL of manganese standard solution (MnO 1 mg/mL) to a 100-mL volumetric flask and add hydrochloric acid (1+23) up in the marked line.

e) **Manganese standard solution (MnO 2 µg/mL - 8 µg/mL) for the calibration curve preparation** (1): Put 2 mL - 8 mL of manganese standard solution (MnO 0.1 mg/mL) in 100-mL volumetric flasks step-by-step and add hydrochloric acid (1+23) up to the marked line.

f) **Manganese standard solution (MnO 0.1 µg/mL - 2 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of manganese standard solution (MnO 10 µg/mL) in 100-mL volumetric flasks step-by-step and add hydrochloric acid (1+23) up to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**), **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the manganese standard solution in (**2**), a manganese standard solution for the calibration curve preparation can be prepared by using a manganese standard solution (Mn 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble manganese (W-MnO) in the analytical sample by a multiplying the concentration (Mn) of manganese standard solution for calibration curve preparation or a measurement value (Mn) obtained in (**4.2**) by a conversion factor (1.2912).

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and a spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Extractor**: A vertical rotating mixer or vertical reciprocating shaker as described below.

aa) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

ab) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

b) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

(4.1.1) Powdery test sample

(4.1.1.1) Vertical rotating mixer

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 3 In the procedure of (**4.1.1.1**) **a**), it is also allowed to weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 4 A sample solution obtained in the procedure in (**4.1.1.1**) is also applicable to the components shown in Annex B.

(4.1.1.2) Vertical reciprocating shaker

a) Weigh 2.5 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.
b) Add about 200 mL of water, and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 5 A sample solution obtained in the procedure in (**4.1.1.2**) is also applicable to the components shown in Annex B.

(4.1.2) Fluid test sample

a) Weigh 1 mg of an analytical sample (2) to the order of 1 mg, and put it in in a 100-mL volumetric flask.
b) Add about 50 mL of water, shake to mix and add water up to the marked line.
c) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) The sampling amount of the analytical sample is 10 g when there is less manganese content in the fertilizers such as a home garden-use fertilizer.

Comment 6 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:

Analytical line wavelength: 257.610 nm or 260.569 nm (3)

b) **Calibration curve preparation**

1) Spray the manganese standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 257.610 nm.

2) Prepare a curve for the relationship between the manganese concentration and the indicated value of the manganese standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 0.8 mg as MnO) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b)** **1)** to read the indicated value.

4) Obtain the manganese content from the calibration curve, and water-soluble manganese (W-MnO) in the analytical sample.

Note (**3**) 260.569 nm can also be used. However, since the intensity of emission obtained is different from the one of 257.610 nm, it is necessary to understand the suitable concentration range of the calibration curve and prepare a standard solution for the calibration curve in advance.

Comment 7 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 8 The comparison of the measurement value (y_i : 0.0145 % (mass fraction) - 0.260 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Flame atomic absorption spectrometry was conducted to evaluate trueness using powdery test fertilizers (14 samples). As a result, a regression equation was $y=-0.0035+0.972x$, and its correlation coefficient (r) was 0.997. The comparison of the measurement value (y_i : 0.027 % (mass fraction) - 1.49 % (mass fraction)) and the measurement value (x_i) was conducted using fluid fertilizers (12 samples). As a result, a regression equation was $y=-0.0013+1.025x$, and its correlation coefficient (r) was 0.999. In addition, additive recovery testing was conducted using preparation fertilizers (6 samples). As a result, the mean recovery rate at the additive level of 0.0907 % (mass fraction) - 41.97 % (mass fraction) was 96.9 % - 101.0 %. Additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand) and a liquid microelement compound fertilizer (1 bland). As a result, the mean recovery rates at the additive level of 0.15 % (mass fraction) - 0.2 % and 0.005 % (mass fraction) were 96.3 % - 96.5 % and 107.0 % respectively.

The results of the repeatability tests on different days using a home garden-use compound fertilize (solid), a blended fertilizer, a fluid compound fertilizer and a home garden-use compound fertilize (fluid) to evaluate precision were analyzed by the one-way analysis of variance. Table 1-1 and 1-2 show the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study implemented for testing method validation and its analysis are shown in Table 2

Note that the minimum limit of quantification of this testing method was estimated to be about 0.005 % (mass fraction) for solid fertilizers and about 0.0002 % (mass fraction) for fluid fertilizers.

Table 1-1 Analysis results of the repeatability tests on different days of water-soluble manganese (Solid fertilizers)

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Home garden-use compound fertilizer (Solid)	5	0.238	0.007	3.1	0.009	3.8
Blended fertilizer	5	0.0649	0.0020	3.0	0.0043	6.7

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 1-2 Analysis results of the repeatability tests on different days of water-soluble manganese (Fluid fertilizers)

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluid compound fertilzier	7	5.69	0.02	0.4	0.06	1.1
Home garden-use compound fertilizer (fluid)	7	2.29	0.02	0.8	0.04	1.6

Footnote:Refert toTable 1-1

Table 2 Analysis results of the collaborative study
for the test method validation of water-soluble manganese

Analytical line wavelength (nm)	Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
257.610	Preparation sample (Liquid) 1	10	0.518	0.004	0.8	0.013	2.5
	Preparation sample (Liquid) 2	10	1.06	0.01	0.8	0.02	2.3
	Preparation sample (Liquid) 3	10	2.11	0.02	0.9	0.08	3.8
	Preparation sample (Liquid) 4	10	0.0518	0.0006	1.2	0.0027	5.2
	Preparation sample (Liquid) 5	11	0.0108	0.0001	1.0	0.0002	2.3
260.569	Preparation sample (Liquid) 1	11	0.518	0.004	0.8	0.015	2.9
	Preparation sample (Liquid) 2	10	1.06	0.01	0.9	0.02	2.2
	Preparation sample (Liquid) 3	11	2.13	0.03	1.2	0.08	3.8
	Preparation sample (Liquid) 4	10	0.0512	0.0006	1.2	0.0014	2.6
	Preparation sample (Liquid) 5	11	0.0108	0.0001	0.7	0.0003	2.5

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination of Water-Soluble Principal Ingredients (W-P_2O_5, W-K_2O, W-MgO, W-MnO and W-B_2O_3) in Liquid Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 8**, p. 1 - 9 (2015)

2) Norio FUNAKI: Simultaneous Determination of Water-soluble Principal Ingredients in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 12**, p. 28 – 51 (2019)

3) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(5) Flow sheet: The flow sheet for water-soluble manganese in fertilizers is shown below:

5 g analytical sample (Powdery) — Weigh to the order of 1 mg to a 500-mL volumetric flask
← Water, about 400 mL
Shaking to mix — Vertical rotating mixer (30 - 40 revolutions/min), for 30 minutes
← Water (up to the marked line)
Filtration — Type 3 filter paper
Sample solution

Figure 1-1 Flow sheet for water-soluble manganese in fertilizers
(Extraction procedure (4.1.1.1))

2.5 g analytical sample (Powdery) — Weigh to the order of 1 mg to a 250-mL volumetric flask
← Water, about 200 mL
Shaking to mix — Vertical reciprocating shaker (300 times/ min, amplitude of 40 mm), 30 minutes
← Water (up to the marked line)
Filtration — Type 3 filter paper
Sample solution

Figure 1-2 Flow sheet for water-soluble manganese in fertilizers
(Extraction procedure (4.1.1.2))

1 g analytical sample (Fluid) — Weigh to the order of 1 mg to a 100-mL volumetric flask
← Water, about 50 mL
Shaking to mix
← Water (up to the marked line)
Filtration — Type 3 filter paper
Sample solution

Figure 1-3 Flow sheet for water-soluble manganese in fertilizers
(Extraction procedure (4.1.2))

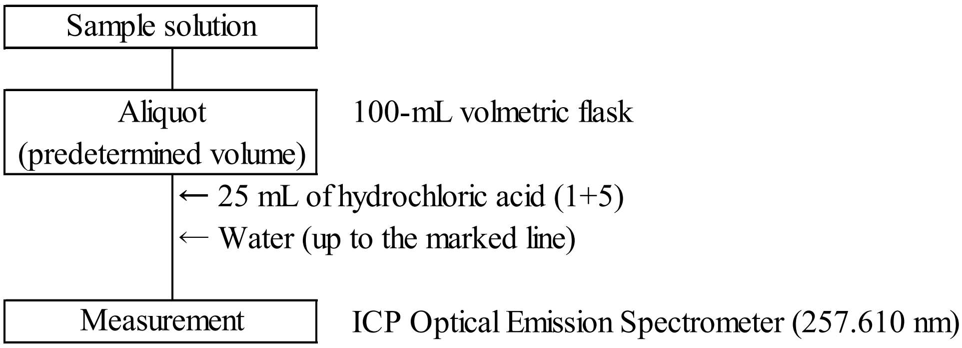


Figure 2 Flow sheet for water-soluble manganese in fertilizers
(Measurement procedure)

4.8 Boron
4.8.1 Citric acid-soluble boron
4.8.1.a Azomethine-H method
(1) Summary

The test method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 4.8.1.a-2019 or C-B.a-3. Extract by adding a citric acid solution to an analytical sample, mask co-existing copper, iron and other salts with ethylenediamine tetraacetate and measure the absorbance with azomethine-H borate formed by the reaction with azomethine-H and correct the absorbance originated in coloring of a sample solution to obtain citric acid-soluble boron (C-B_2O_3) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(2) Reagent: Reagents are as shown below.

a) Citric acid solution (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

b) Ethylenediamine tetraacetate solution (1): Dissolve 37.2 g of ethylenediaminetetraacetic acid dihydrogen disodium dihydrate specified in JIS K 8107 in water to make 1000 mL.

c) Ammonium acetate solution (1): Dissolve 250 g of ammonium acetate specified in JIS K 8359 in water to make 500 mL and adjust pH with sulfuric acid (1+4) to pH 5.2 ± 0.1.

d) Azomethine-H solution: Add water to 0.6 g of azomethine-H and 2 g of L (+) – ascorbic acid specified in JIS K 9502, and heat up to 35 ºC - 40 ºC to dissolve and add water after cooling to make 100 mL.

e) Boron standard solution (B_2O_3 2.5 mg/mL) (1): After leaving boric acid specified in JIS K 8863 at rest in a desiccator for about 24 hours to dry, weigh 4.441 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

f) Boron standard solution (B_2O_3 0.1 mg/mL): Dilute the predetermined volume of boron standard solution (B_2O_3 2.5 mg/mL) with water exactly by a factor of 25.

g) Boron standard solution (B_2O_3 0.01 mg/mL): Dilute the predetermined volume of boron standard solution (B_2O_3 0.1 mg/mL) with water exactly by a factor of 10.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the boron standard solution in (**2**), a boron standard solution for the calibration curve preparation can be prepared by using a boron standard solution (B 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble boron (C-B_2O_3) in the analytical sample by multiplying the concentration (B) of a boron standard solution for calibration curve preparation or a measurement value (B) obtained in (**4.3**) by a conversion factor (3.2199).

(3) Instruments: Instruments are as shown below:

a) Extractor: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

aa) Constant-temperature vertical rotating mixer: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

ab) Horizontal reciprocating water bath shaker: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

b) Spectrophotometer: A spectrophotometer specified in JIS K 0115

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Constant-temperature vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 2 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Horizontal reciprocating water bath shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (3).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (2), and shake to mix by reciprocating horizontally at amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) at 160 times/min for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 3 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 4 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(4.2) Coloring: Conduct coloring as shown below.

a) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg – 0.8 mg as P_2O_5 and no more than the equivalents of 15 mL of citric acid solution) in a 100-mL volumetric flask.

b) Add the solution to make citric acid solution equivalent to 15 mL.

c) Add 25 mL of ethylenediamine tetraacetate solution, and then add 10 mL of ammonium acetate solution.

d) Add 10 mL of azomethine-H solution.

e) After adding water up to the marked line, leave at rest for about 2 hours (4) to make a sample solution for measurement.

f) Conduct the same procedures as **a**) – **c**) and **e**) using another 100-mL volumetric flask to make a sample solution for correction.

Note (**4**) When the solution is muddled, conduct centrifugation with about 1700 × *g* centrifugal force (5) for about five minutes after the procedure in **e**) or filtrate with Type 3 filter paper.

(**5**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

Comment 5 If formaldehyde processed urea, a large quantity of aluminum, copper, iron zinc, organic matters, etc. coexists to affect quantification, put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 0.8 mg as B_2O_3, no more than 10 mL of the solution) in a 100-mL separatory funnel, add 10 mL of hydrochloric acid (1+3), add water to about 20 mL and add 20 mL of 2-ethyl-1,3-hexanediol – 4-methyl-2-pentanone (1+9) to shake to mix with a shaking apparatus for about 1 minute. After allowing to stand still, remove the lower layer (aqueous phase) and add 20 mL of sodium hydroxide (20 g/L) to shake to mix with a shaking apparatus for about 1 minute. After allowing to stand still, transfer the lower layer (aqueous phase) to a 100-mL volumetric flask, add a few drops of phenolphthalein solution (1 g/100mL) to neutralize with hydrochloric acid (1+3) until the color of the solution becomes achromatic, and conduct the procedure in (**4.2**) **b**).

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of spectrophotometer**: Set up the measurement conditions of spectrophotometer considering the following.
Detection wavelength: 415 nm

b) **Calibration curve preparation**

1) Put 1 mL - 8 mL of boron standard solution (B_2O_3 0.1 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Put 1 mL - 10 mL of boron standard solution (B_2O_3 0.01 mg/mL) in 100-mL volumetric flasks step-by-step.

3) Add 15 mL of citric acid solution and conduct the same procedure as (**4.2**) **c**) - **e**) to make the B_2O_3 0.01 mg/100 mL - 0.8 mg/100 mL boron standard solution for the calibration curve preparation.

4) Conduct the same procedures as **3**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

5) Further, put 15 mL of citric acid solution in another 100-mL volumetric flask and conduct the same procedures as **4.2**) **c**) and **e**) to make a test solution for control.

6) Measure absorbance at a wavelength of 415 nm of the blank test solution for the calibration curve preparation and the boron standard solutions for the calibration curve preparation using the test solution for control as the control.

7) Prepare a curve for the relationship between the boron concentration and the absorbance of the boron standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the sample solution for measurement in (**4.2**) **e**) and the sample solution for correction in (**4.2**) **f**), measure absorbance by the same procedure as **b**) **6**).

2) Obtain the boron (B_2O_3) content from the calibration curve using the absorbance obtained by subtracting the absorbance of a sample solution for correction from the absorbance of a sample solution for measurement and calculate citric acid-soluble boron (C-B_2O_3) in the analytical sample.

Comment 6 If it is known in advance, according to the results of quality control analysis, that coloring of a sample solution does not affect the analysis of citric acid-soluble boron in an analytical sample, there is no need to prepare the sample solution for correction ((**4.2**) **f**)).

In that case, read **4.3) c) 2)** as "Obtain the boron (B_2O_3) content from the calibration curve using the absorbance of a sample solution for measurement and calculate citric acid-soluble boron (C-B_2O_3) in the analytical sample".

Comment 7 Recovery testing was conducted using preparation sample to evaluate trueness. As a result, the mean recovery rates at the content level of 1.02 % (mass fraction) - 5.11 % (mass fraction) and 0.20 % (mass fraction) as citric acid-soluble boron (C-B_2O_3) were 99 %, 97 % - 99 % and 106 %.
The results of the repeatability tests on different days using a borate fertilizer and a compound fertilizer (1 brand for each) to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of citric acid-soluble boron

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Borate fertilizer	5	39.35	0.49	1.2	0.68	1.7
Compound fertilizer	5	0.117	0.001	1.0	0.005	4.7

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of citric acid-soluble boron

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Calcined microelement compound fertilizer	9	11.26	0.19	1.7	0.46	4.1
Mixed microelement compound fertilizer	10	1.87	0.05	2.7	0.07	4.0
Compound fertilizers A	10	0.54	0.01	1.3	0.03	5.3
Mixed compost compound fertilizer	8	0.32	0.01	2.7	0.01	4.4
Compound fertilizers B	10	0.12	0.01	5.7	0.01	6.5

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.184- 187, Yokendo, Tokyo (1988)

2) Kimie KATO, Sakiko TAKAHASHI and Yuji SHIRAI: Validation of a Color metric Method for Determination of Nitrogen, Phosphorus and Boron: Evaluation of Calibration curve, Research Report of Fertilizer **Vol. 2**, p. 137 - 144 (2009)

3) Akira SHIMIZU: Verification of Performance Characteristics of Testing Methods for Boron Content by Azomethine H Absorption Photometry, Research Report of Fertilizer **Vol. 6**, p. 174 -182 (2013)

4) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)

5) Masayuki YAMANISHI: Improved Determination Method of Boron (B_2O_3) in Fertilizer, Research Report of Fertilizer **Vol. 22**, p. 10 - 27 (2019)

6) Keisuke AOYAMA: Performance Evaluation of Determination Method for Boron in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 112 – 122 (2020)

(5) **Flow sheet for citric acid-soluble boron**: The flow sheet for citric acid-soluble boron in fertilizers is shown below:

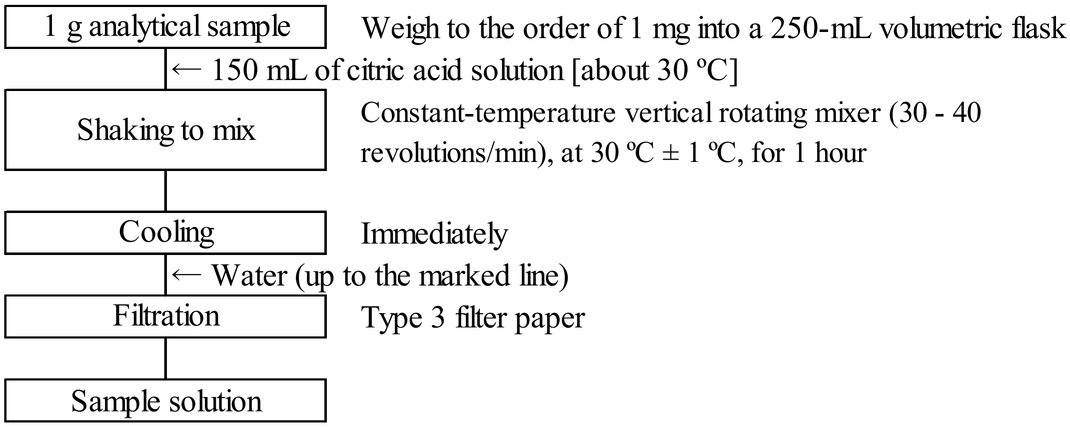


Figure 1-1 Flow sheet for citric acid-soluble boron in fertilizers (Extraction procedure (4.1.1))

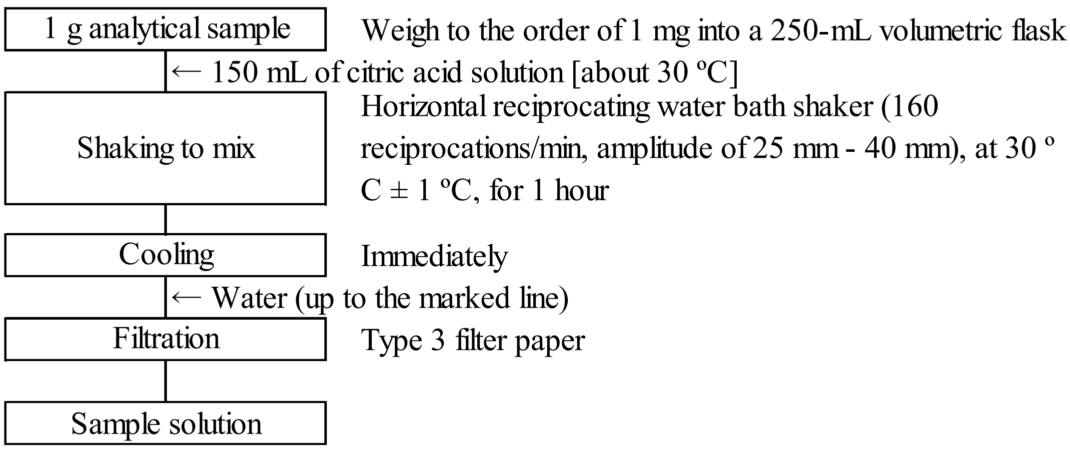


Figure 1-2 Flow sheet for citric acid-soluble boron in fertilizers (Extraction procedure (4.1.2))

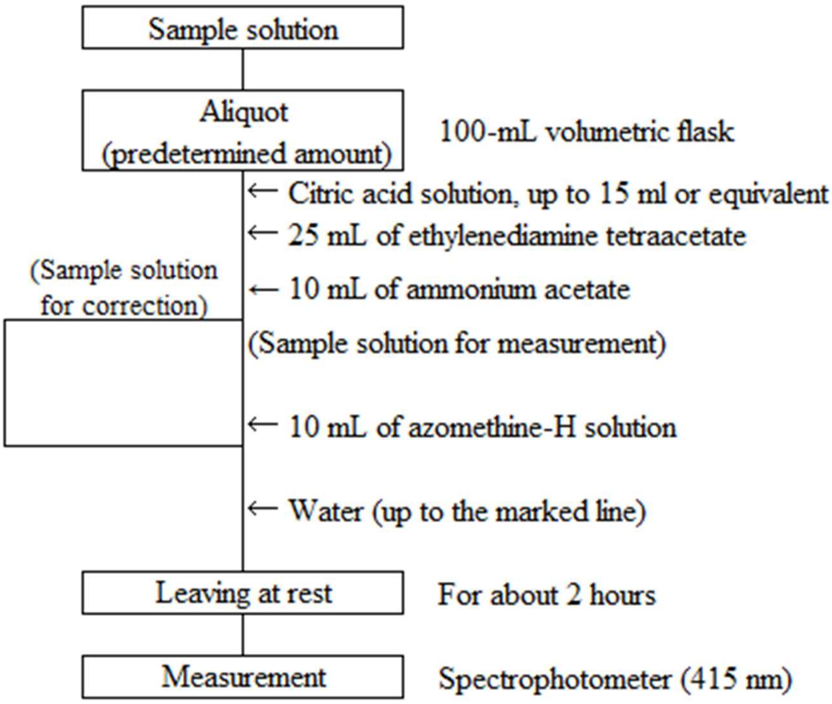


Figure 2-1 Flow sheet for citrate-soluble boron in fertilizers
(Preparation and measurement procedures of sample solution for measurement and sample solution for correction)

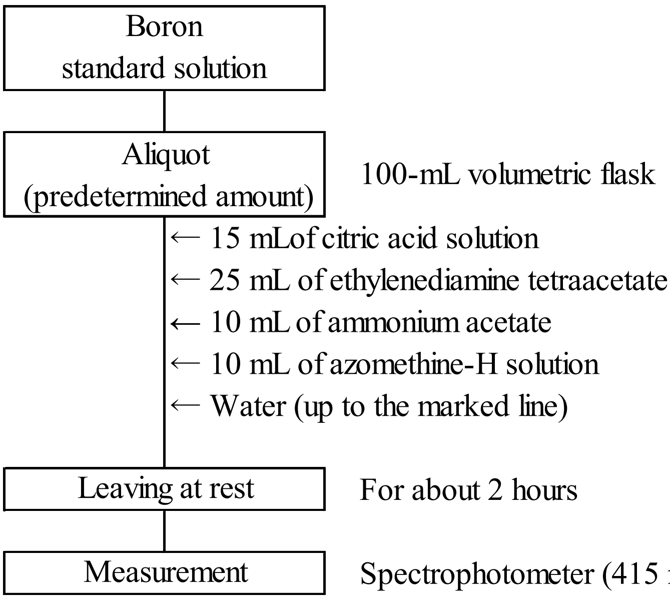


Figure 2-2 Flow sheet for citric acid-soluble boron in fertilizers
(Preparation and measurement procedures of boron standard solution for the calibration curve preparation)

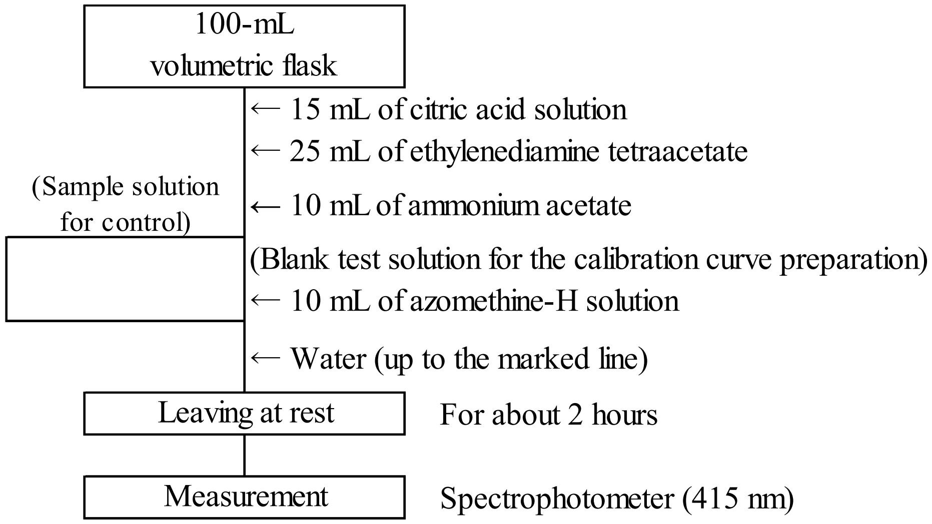


Figure 2-3 Flow sheet for citric acid-soluble boron in fertilizers (Preparation and measurement procedures of test solution for control and blank test solution for the calibration curve preparation)

Reference: A calculation example of citric acid-soluble boron content in an analytical sample is shown below.

a) Reference table 1-1 shows the absorbance (example) of a standard solution for the calibration curve preparation and a blank test solution for the calibration curve preparation using a test solution for control as the control.
In addition, calibration curves are shown in Reference diagram 1 and regression coefficients of the regression equations are in Reference table 1-2.

b) Reference table 2 shows the sampling amount of an analytical sample, the constant volume of extract, the transferred amount of extract and the constant volume of the sample solution for measurement as well as the absorbance (example) of the sample solution for measurement and the sample solution for correction using a test solution for control as the control.

c) Obtain the boron (B_2O_3) content in the sample solution for measurement by formula (1) (Refer to Reference diagram 1) and calculate citric acid-soluble boron (C-B_2O_3) in the analytical sample by formula (2)".

Boron (B_2O_3) content in a sample solution for measurement (C_1)

$=((As-A_b)-a)/b$

$=(Ac-a)/b$ ・・・・・ (1)

Citric acid-soluble boron ($C\text{-}B_2O_3$) in an analytical sample (C_2)

$=C_1\times(V_1/V_2)\times(1/W)\times(100/1000)$ ・・・・・ (2)

C_1 : Boron (B_2O_3) content in 100 mL of a sample solution for measurement (mg)

As : Absorbance of a sample solution for measurement in (**4.2**) **e**) using a test solution for control ((**4.3 b**) **5**)) as the control

A_b : Absorbance of a sample solution for correction in (**4.2**) **f**) using a test solution for control ((**4. 3**) **b**) **5**)) as the control

A_c : Corrected absorbance

a : Intercept of a regression equation of a calibration curve

b : Inclination of a regression equation of a calibration curve

C_2 : Citric acid-soluble boron ($C\text{-}B_2O_3$) in an analytical sample (% (mass fraction))

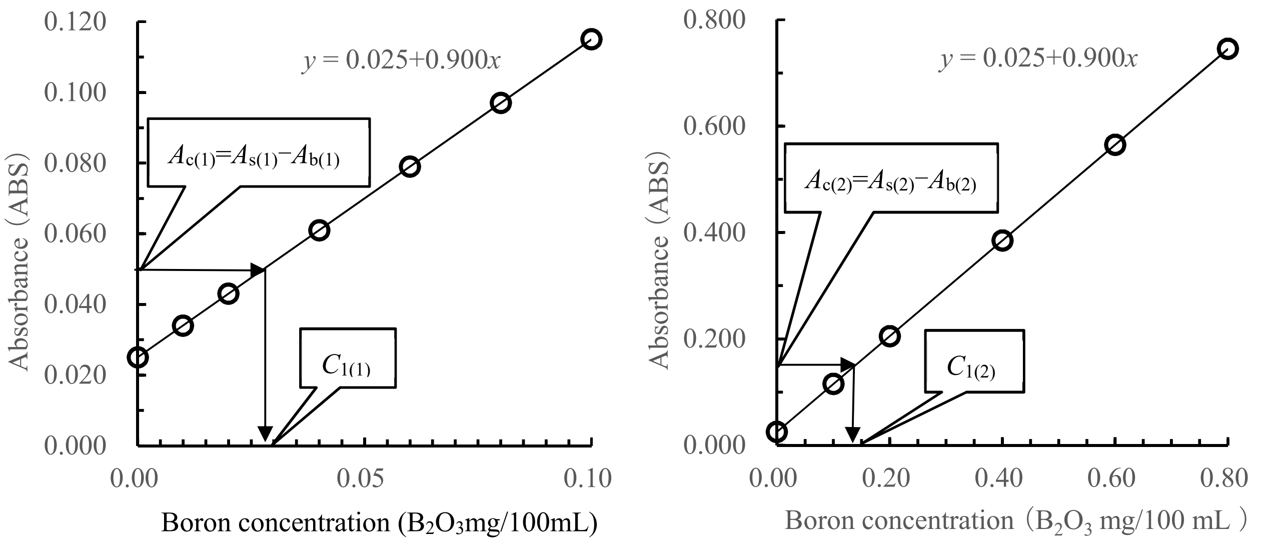
V_1 : Constant volume (mL) of the sample solution in (**4.1.1**) **c**) or (**4.1.2**) **c**)

V_2 : Aliquot volume (mL) of the sample solution in (**4.2**) **a**)

W : Mass (g) of the analytical sample

Reference Table 1-1 Calibration curve (example) of citric acid-soluble boron

Sample name	Calibration curve for low concentration		Calibration curve for moderate or high concentration	
	Boron (B_2O_3) concentration (mg/100 mL)	Absorbance (ABS)	Boron (B_2O_3) concentration (mg/100 mL)	Absorbance (ABS)
Blank test solution for calibration curve	0	0.025	0.0	0.025
Standard solution for calibration curve	0.01	0.034	0.1	0.115
Standard solution for calibration curve	0.02	0.043	0.2	0.205
Standard solution for calibration curve	0.04	0.061	0.4	0.385
Standard solution for calibration curve	0.06	0.079	0.6	0.565
Standard solution for calibration curve	0.08	0.097	0.8	0.745
Standard solution for calibration curve	0.1	0.115		



1) Low concentration range
(B_2O_3 0 mg/100 mL - 0.10 mg/100 mL)

2) High concentration range
(B_2O_3 0 mg/100 mL - 0.80 mg/100 mL)

Reference diagram 1 Calibration curve (example) of citric acid-soluble boron (C-B_2O_3)

Reference table 1-2 Regression equation [1)]
of a calibration curve (example) of citric acid-soluble boron

	Regression coefficient ($y=a+bx$)	
	a	b
Low concentration range	0.025	0.900
High concentration range	0.025	0.900

1) Regression equation calculated by the least squares method

Reference table 2 Measurement (example) of sample solution and calculation of citric acid-soluble boron

	Unit	Blended fertilizer (1)	Compound fertilizer (2)	Borate fertilizer
Sampling amount of analytical sample (W)	g	1	1	1
Predetermined volume of extract (V_1)	mL	250	250	250
Transferred amount of extract (V_2)[1)]	mL	25	10	0.25
Predetermined volume of sampling solution, etc. for measurement (V_3)[1)]	mL	100	100	100
Absorbance of sampling solution for measurement (A_s)	ABS	0.055	0.170	0.400
Absorbance of sampling solution for correction (A_b)	ABS	0.005	0.020	0.000
Corrected absorbance ($A_c=A_s-A_b$)	ABS	0.050	0.150	0.400
Boron concentration in sampling solution for measurement (C_1)[2)]	mg/100 mL	0.028	0.139	0.417
Citrate-soluble boron content in analytical sample (C_2)[3)]	%	0.03	0.35	41.7

1) 2 steps dilutionis is applied to borate fertilizers. Ex: (5 mL→50 mL) (2.5 mL→100 mL)
2) Calculate C_1 by substituting the regression coefficient (interception (a) and gradient (b)) in Table 1-2 for formula (1).
3) Calculate C_2 by substituting C_1, W, V_1 and V_2 for formula 2.

4.8.1.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 4.8.1.b-2018 or C-B.b-1.

Extract by adding a citric acid solution to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the boron at a wavelength of 249.773 nm to obtain citric acid-soluble boron (citric acid-soluble boron acid (C-B_2O_3)) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(2) Reagent: Reagents are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Citric acid solution** (1): Dissolve 20 g of citric acid monohydrate specified in JIS K 8283 in water to make 1000 mL.

d) **Boron standard solution (B_2O_3 2.5 mg/mL)** (1): After leaving boric acid specified in JIS K 8863 at rest in a desiccator for about 24 hours to dry, weigh 4.441 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

e) **Boron standard solution (B_2O_3 0.1 mg/mL)** (1): Put 4 mL of boron standard solution (B_2O_3 2.5 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line (2).

f) **Boron standard solution (B_2O_3 2 µg/mL - 16 µg/mL) for the calibration curve preparation** (1): Put 2 mL - 16 mL of boron standard solution (B_2O_3 0.1 mg/mL) in a 100-mL volumetric flask step by step and add hydrochloric acid (1+23) up to the marked line (2).

g) **Boron standard solution (B_2O_3 0.2 µg/mL - 2 µg/mL) for the calibration curve preparation** (1): Put 2 mL - 20 mL of boron standard solution (B_2O_3 10 µg/mL) in a 100-mL volumetric flask step by step and add hydrochloric acid (1+23) up to the marked line (2).

h) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**) - **g**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) When preserving, use a container, which can be sealed tightly, made of material such as PTFE that boron hardly elutes

Comment 1 Instead of the boron standard solution in (**2**), a boron standard solution for the calibration curve preparation can be prepared by using a boron standard solution (B 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate citric acid-soluble boron (C-B_2O_3) in the analytical sample by multiplying the concentration (B) of a boron standard solution for calibration curve preparation or a measurement value (B) obtained in (**4.2**) by a conversion factor (3.2199).

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and a spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) **ICP Optical Emission Spectrometer**: An Optical Emission Spectrometer specified in JIS K

0116

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) **Extractor**: A constant-temperature vertical rotating mixer or horizontal reciprocating water bath shaker as described below.

ba) **Constant-temperature vertical rotating mixer**: A constant-temperature vertical rotating mixer that can vertically rotate a 250-mL volumetric flask, set up in a thermostat adjustable to 30 ºC ± 1 ºC, at a rate of 30 - 40 revolutions/min.

bb) **Horizontal reciprocating water bath shaker**: A reciprocating water bath shaker that can be adjusted to 30 °C ± 1 °C and horizontally reciprocate a 250-mL volumetric flask, set up vertical to the water surface using a shaking rack, at a rate of 160 reciprocations/min with an amplitude of 25 mm - 40 mm.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Constant-temperature vertical rotating mixer

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask.

b) Add 150 mL of citric acid solution heated up to about 30 ºC (3), and shake to mix at 30 - 40 revolutions/min (30 ºC ± 1 ºC) for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) Shake to mix the volumetric flask gently and disperse an analytical sample into the citric acid solution.

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Horizontal reciprocating water bath shaker

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 250-mL volumetric flask (4).

b) Add 150 mL of citric acid solution heated up to about 30 ºC (3), and shake to mix by reciprocating horizontally at amplitude of 25 mm - 40 mm (30 ºC ± 1 ºC) at 160 times/min for 1 hour.

c) After immediate cooling is complete, add water up to the marked line

d) Filter with Type 3 filter paper to make a sample solution.

Note (**4**) Use a 250-mL flat-bottom volumetric flask to stabilize the shaking.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

Comment 5 The determination may be affected if an analytical sample cakes on the bottom of a 250-mL volumetric flask. Therefore, confirm the status of the non-dissolved matters after the procedures of (**4.1.1**) **b**) and (**4.1.2**) **b**).

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 249.773 nm

b) **Calibration curve preparation**

1) Spray the boron standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 249.773 nm.
2) Prepare a curve for the relationship between the boron concentration and the indicated value of the boron standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.02 mg - 1.6 mg as B_2O_3) in a 100-mL volumetric flask.
2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.
3) Subject to the same procedure as in **b**) **1**) to read the indicated value.
4) Obtain the boron (B_2O_3) content from the calibration curve, and calculate citric acid-soluble boron (C-B_2O_3) in the analytical sample.

Comment 6 Wash the sample injector of an ICP-OES sufficiently with water because boron easily causes the memory effect.

Comment 7 The simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 8 The comparison of the measurement value (y_i : 0.073 % (mass fraction) - 0.51 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Flame atomic absorption spectrometry analysis was conducted to evaluate trueness using compound fertilizers (7 samples), mixed phosphorus fertilizers (1 sample), solid compound fertilizers (2 samples), blended fertilizers (3 samples) and organic compound fertilizer (1 sample). As a result, a regression equation was $y=-0.0408+1.0456x$, and its correlation coefficient (r) was 0.992. In addition, additive recovery testing was conducted using a preparation sample. As a result, the mean recovery rate at the additive level of 0.601 % (mass fraction) - 35.51 % (mass fraction) was 97.0 % - 102.0 %.

The results of the repeatability tests on different days using compound fertilizers and blended fertilizers to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of citric acid-soluble boron

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Compound fertilizer	7	0.38	0.01	1.9	0.01	3.1
Blended fertilizer	7	0.076	0.003	4.2	0.006	7.5

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Yasushi SUGIMURA: Extraction Method for the Citrate-Soluble Principal Ingredients in the Fertilizer using a General-Purpose Equipment, Research Report of Fertilizer, **Vol. 11**, p. 1 – 13 (2018)

2) Shingo MATSUO: Simultaneous Determination of Citrate-Soluble Principal Ingredients (C-P_2O_5, C-K_2O, C-MgO, C-MnO and C-B_2O_3) in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 11**, p. 14 – 28 (2018)

(5) **Flow sheet for citric acid-soluble boron**: The flow sheet for citric acid-soluble boron in fertilizers is shown below:

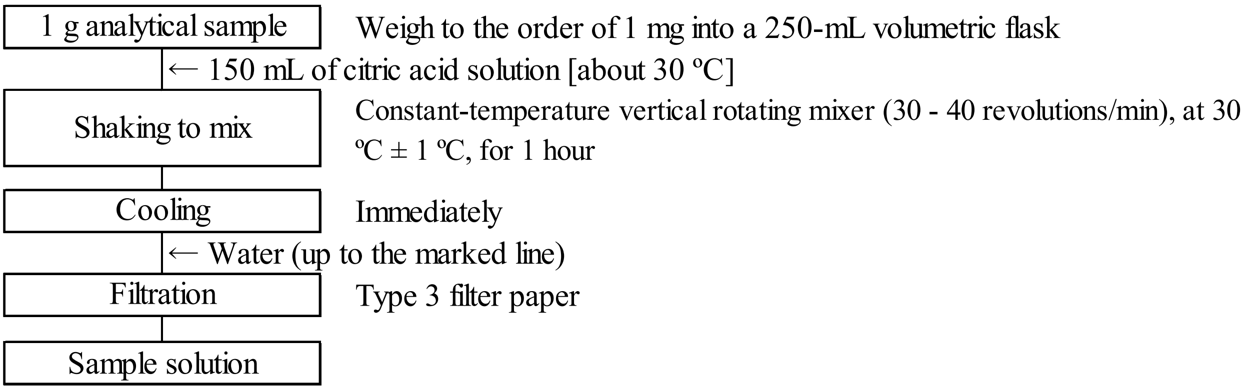


Figure 1-1 Flow sheet for citric acid-soluble boron in fertilizers (Extraction procedure (4.1.1))

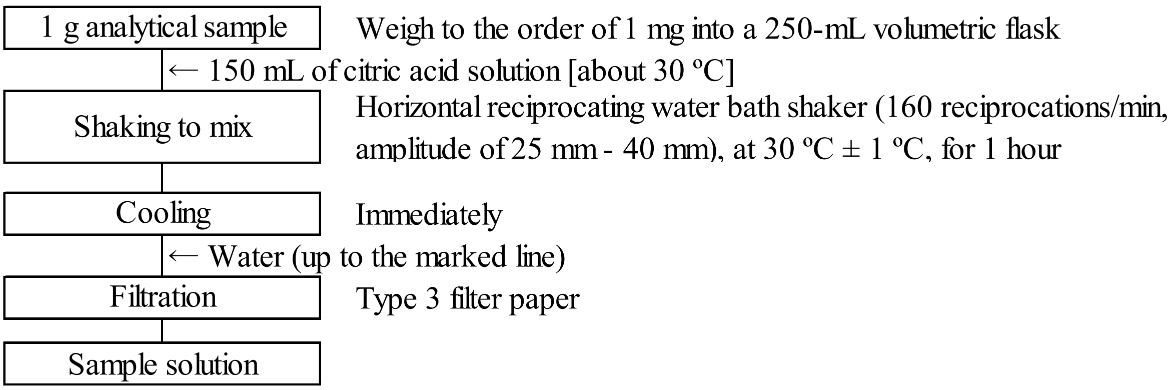


Figure 1-2 Flow sheet for citric acid-soluble boron in fertilizers (Extraction procedure (4.1.2))

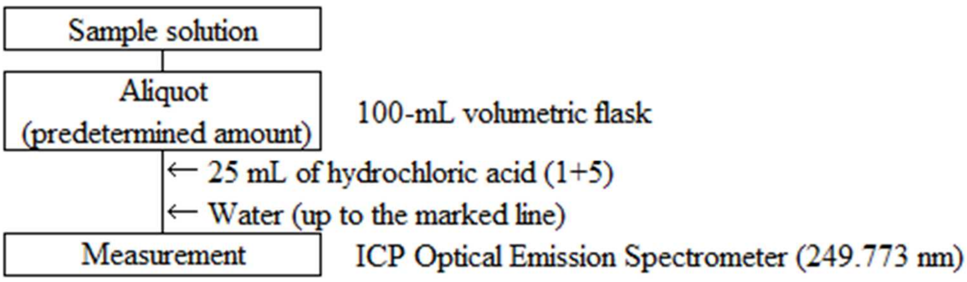


Figure 2 Flow sheet for citric acid-soluble boron in fertilizers (Measurement procedure)

4.8.2 Water-soluble boron

4.8.2.a Azomethine-H method

(1) Summary

This test method is applicable to fertilizers containing borate fertilizers, etc. This testing method is classified as Type B and its symbol is 4.8.2.a-2019 or W-B.a-2.

Extract by adding water to an analytical sample and boiling, mask co-existing copper, iron and other salts with ethylenediamine tetraacetate and measure the absorbance with azomethine-H borate formed by the reaction with azomethine-H and correct the absorbance originated in coloring of a sample solution to obtain water-soluble boron (W-B_2O_3) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 9**.

(2) Reagent: Reagents are as shown below.

a) Ethylenediamine tetraacetate solution (1): Dissolve 37.2 g of ethylenediaminetetraacetic acid dihydrogen disodium dihydrate specified in JIS K 8107 in water to make 1000 mL.

b) Ammonium acetate solution (1): Dissolve 250 g of ammonium acetate specified in JIS K 8359 in water to make 500 mL and adjust pH with sulfuric acid (1+4) to pH 5.2 ± 0.1.

c) Azomethine-H solution (1): Add water to 0.6 g of azomethine-H and 2 g of L (+) – ascorbic acid specified in JIS K 9502, and heat up to 35 ºC - 40 ºC to dissolve and add water after cooling to make 100 mL.

d) Boron standard solution (B_2O_3 2.5 mg/mL) (1): After leaving boric acid specified in JIS K 8863 at rest in a desiccator for about 24 hours to dry, weigh 4.441 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

e) Boron standard solution (B_2O_3 0.1 mg/mL): Dilute the predetermined volume of boron standard solution (B_2O_3 2.5 mg/mL) with water exactly by a factor of 25.

f) Boron standard solution (B_2O_3 0.01 mg/mL): Dilute the predetermined volume of boron standard solution (B_2O_3 0.1 mg/mL) with water exactly by a factor of 10.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the boron standard solution in (**2**), a boron standard solution for the calibration curve preparation can be prepared by using a boron standard solution (B 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble boron (W-B_2O_3) in the analytical sample by multiplying the concentration (B) of a boron standard solution for calibration curve preparation or a measurement value (B) obtained in (**4.3**) by a conversion factor (3.2199).

(3) Instruments: Instruments are as shown below:

a) Spectrophotometer: A spectrophotometer specified in JIS K 0115

b) Hot plate : A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 2.5 g (2) of an analytical sample to the order of 1 mg, and put it in a 300-mL tall beaker.

b) Add about 200 mL of water, and cover with a watch glass and heat on a hot plate to boil for about 15 minutes.

c) After immediate cooling is complete, transfer it to a 250- mL volumetric flask with water.

d) Add water up to the marked line

e) Filter with Type 3 filter paper to make a sample solution.

Note (2) The sampling amount of the analytical sample is 1 g when there is a high amount of boric acid content in the fertilizers such as a borate fertilizer and boric acid fertilizer.

Comment 2 In the procedure in (**4.1.1**) **a**) and (**4.1.1**) **b**), a 250-mL volumetric flask can be used instead of a 300-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, "cover with a watch glass" in **b**) is replaced by "place a funnel". Skip "transfer it to a 250-mL volumetric flask with water" in the procedure in **c**).

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Fluid test sample**

a) Weigh 1 g of an analytical sample to the order of 1 mg, and put it in a 100-mL tall beaker.
b) Add about 50 mL of water, and shake to mix.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Coloring**: Conduct coloring as shown below.

a) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 0.8 mg as B_2O_3) in a 100-mL volumetric flask.
b) Add 25 mL of ethylenediamine tetraacetate solution, and then add 10 mL of ammonium acetate solution.
c) Add 10 mL of azomethine-H solution
d) After adding water up to the marked line, leave at rest for 2 hours (4) to make a sample solution for measurement.
e) Conduct the same procedures as **a**) - **b**) and **d**) using another 100-mL volumetric flask to make a sample solution for correction.

Note (4) When the solution is muddled, conduct centrifugation with about 1700 × *g* centrifugal force (5) for about five minutes after the procedure in **d**) or filtrate with Type 3 filter paper.

(5) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

Comment 5 If formaldehyde processed urea, a large quantity of aluminum, copper, iron zinc, organic matters, etc. coexists to affect quantification, put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 0.8 mg as B_2O_3, no more than 10 mL of the solution) in a 100-mL separatory funnel, add 10 mL of hydrochloric acid (1+3), add water to about 20 mL and add 20 mL of 2-ethyl-1,3-hexanediol–4-methyl-2-pentanone (1+9) to shake to mix with a shaking apparatus for about 1 minute. After allowing to stand still, remove the lower layer (aqueous phase) and add 20 mL of a sodium hydroxide solution (20 g/L) to shake to mix with a shaking apparatus for about 1 minute. After allowing to stand still, transfer the lower layer (aqueous phase) to a 100-mL volumetric flask, add a few drops of phenolphthalein solution (1 g/100mL) to neutralize with hydrochloric acid (1+3) until the color of the solution becomes achromatic, and conduct the procedure in (**4.2**) **b**).

Comment 6 Water-soluble boron can be measured simultaneously with citric acid-soluble boron by adding 15 mL of citric acid solution before the procedure in (**4.2**) **b**).

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of the spectrophotometer**: Set up the measurement conditions of the spectrophotometer considering the following.
Detection wavelength: 415 nm

b) **Calibration curve preparation**

1) Put 1 mL - 8 mL of boron standard solution (B_2O_3 0.1 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Put 1 mL - 10 mL of boron standard solution (B_2O_3 0.01 mg/mL) in 100-mL volumetric flasks step-by-step.

3) Conduct the same procedures as (**4.2**) **b**) - **d**) to make B_2O_3 0.01 mg/100 mL - 0.8 mg/100 mL of the boron standard solutions for the calibration curve preparation.

4) Conduct the same procedures as **3**) for another 100-mL volumetric flask to make the blank test solution for the calibration curve preparation.

5) Further, conduct the same procedures as **4.2 b**) and **d**) for another 100-mL volumetric flask to make the test solution for control.

6) Measure absorbance at a wavelength of 415 nm of the blank test solution for the calibration curve preparation and the boron standard solutions for the calibration curve preparation using the test solution for control as the control.

7) Prepare a curve for the relationship between the boron concentration and absorbance of the boron standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the sample solution for measurement in (**4.2**) **d**) and the sample solution for correction in (**4.2**) **e**), measure absorbance by the same procedure as **b**) **6**).

2) Obtain the boron (B_2O_3) content from the calibration curve using the absorbance obtained by subtracting the absorbance of a sample solution for correction from the absorbance of a sample solution for measurement and calculate water-soluble boron (W-B_2O_3) in the analytical sample.

Comment 7 Water-soluble boron can be measured simultaneously with citric acid-soluble boron by adding 15 mL of citric acid solution before the procedure in (**4.2**) **b**), (**4.3**) **b**) **3**), (**4.3**) **b**) **4**) and (**4.3**) **b**) **5**).

Comment 8 If it is known in advance, according to the results of quality control analysis, that coloring of a sample solution does not affect the analysis of citric acid-soluble boron in an analytical sample, there is no need to prepare the sample solution for correction ((**4.2**) **e**)). In that case, read **4.3**) **c**) **2**) as "Obtain the boron (B_2O_3) content from the calibration curve using the absorbance of a sample solution for measurement and calculate water-soluble boron (W-B_2O_3) in the analytical sample".

Comment 9 Recovery testing was conducted using a powdery preparation sample to evaluate trueness. As a result, the mean recovery rates at the content level of 10.22 % (mass fraction) - 5.11 % (mass fraction) and 0.20 % (mass fraction) as water-soluble boron (W-B_2O_3) were 99 %, 101 % - 103 %, and 102 %.
Recovery testing was conducted using a fluid preparation sample to evaluate trueness. As a result, the mean recovery rates at the content level of 5 % (mass fraction), 0.1 % (mass fraction) and 0.01 % (mass fraction) as water-soluble boron (W-B_2O_3) were

102 %, 99 %, and 93 %.

The results of the repeatability tests on different days using a borate fertilizer, a compound fertilizer and a home garden-use compound fertilizer and a fluid compound fertilizer (one brand for each) to evaluate precision were analyzed by one-way analysis of variance. Table 1-1 and 1-2 show the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 % (mass fraction) for solid fertilizers and about 0.003 % (mass fraction) for fluid fertilizers.

Table 1-1 Analysis results of the repeatability tests on different days of water-soluble boron (Solid fertilizer)

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Borate fertilizer	5	56.25	0.43	0.8	0.43	0.8
Compound fertilizer	5	0.29	0.00	0.7	0.00	0.8

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 1-2 Analysis results of the repeatability tests on different days of water-soluble boron (Fluid fertilizer)

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Home garden-use compound fertilizer	5	4.096	0.03	0.6	0.10	2.4
Fluid compound fertilizer	5	0.018	0.00	1.9	0.00	2.4

Footnote: Refer to Table 1-1

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble boron

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Mixed microelement compound fertilizer 1	8	11.69	0.15	1.3	0.51	4.3
Mixed microelement compound fertilizer 2	9	1.82	0.08	4.4	0.10	5.6
Compound fertilizers A	9	0.52	0.01	1.4	0.02	4.0
Compound fertilizers B	10	0.32	0.01	2.4	0.02	7.5
Mixed compost compound fertilizer	10	0.11	0.00	3.2	0.01	7.4

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.184- 187, Yokendo, Tokyo (1988)

2) Kimie KATO, Sakiko TAKAHASHI and Yuji SHIRAI: Validation of a Color metric Method for Determination of Nitrogen, Phosphorus and Boron: Evaluation of Calibration curve, Research Report of Fertilizer **Vol. 2**, p. 137 - 144 (2009)

3) Akira SHIMIZU: Verification of Performance Characteristics of Testing Methods for Boron Content by Azomethine H Absorption Photometry, Research Report of Fertilizer **Vol. 6,** p. 174 -182 (2013)

4) Masayuki YAMANISHI: Improved Determination Method of Boron(B2O3) in Fertilizer, Research Report of Fertilizer **Vol. 22**, p. 10 - 27 (2019)

5) Keisuke AOYAMA: Performance Evaluation of Determination Method for Boron in Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 112 – 122 (2020)

(5) Flow sheet for water-soluble boron: The flow sheet for water-soluble boron in fertilizers is shown below:

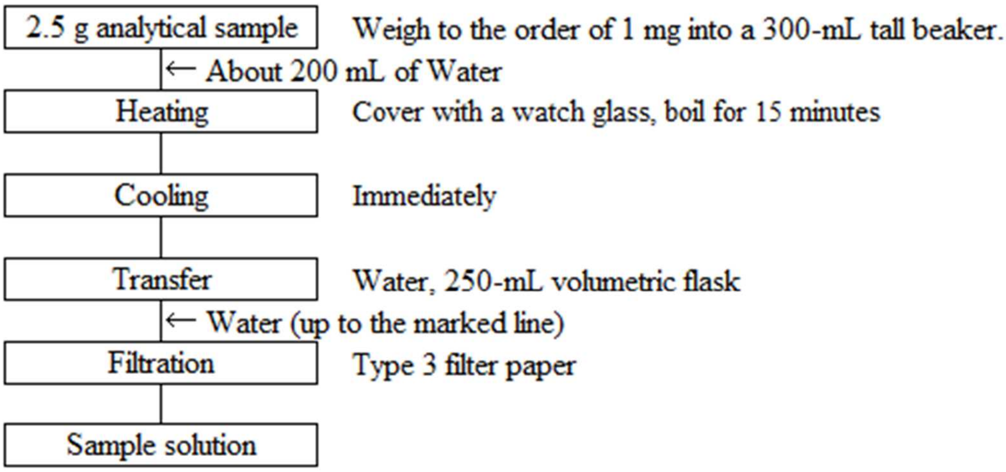


Figure 1-1 Flow sheet for water-soluble boron in fertilizers
(Extraction procedure (4.1.1))

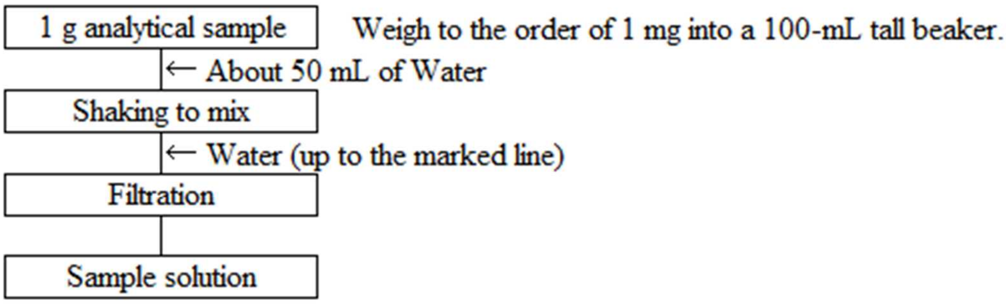


Figure 1-2 Flow sheet for water-soluble boron in fertilizers
(Extraction procedure (4.1.2))

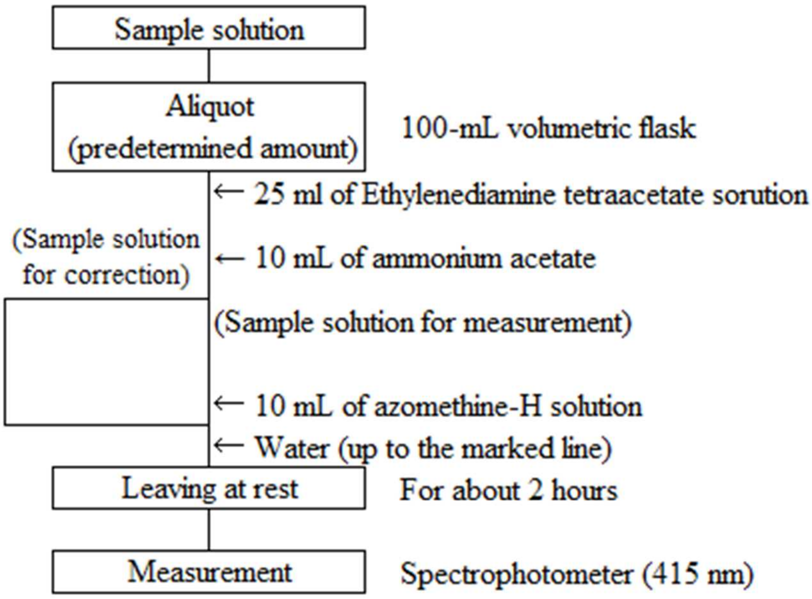


Figure 2-1 Flow sheet for water-soluble boron in fertilizers
(Preparation and measurement procedures of sample solution for measurement and sample solution for correction)

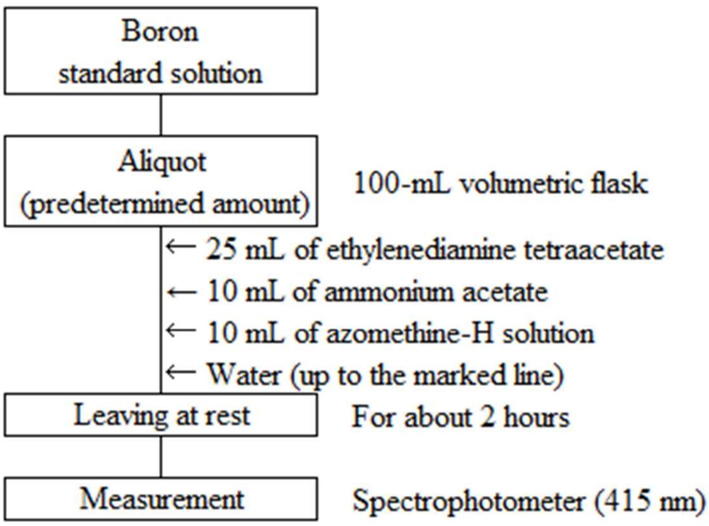


Figure 2-2 Flow sheet for water-soluble boron in fertilizers
(Preparation and measurement procedures of boron standard solution for the calibration curve preparation)

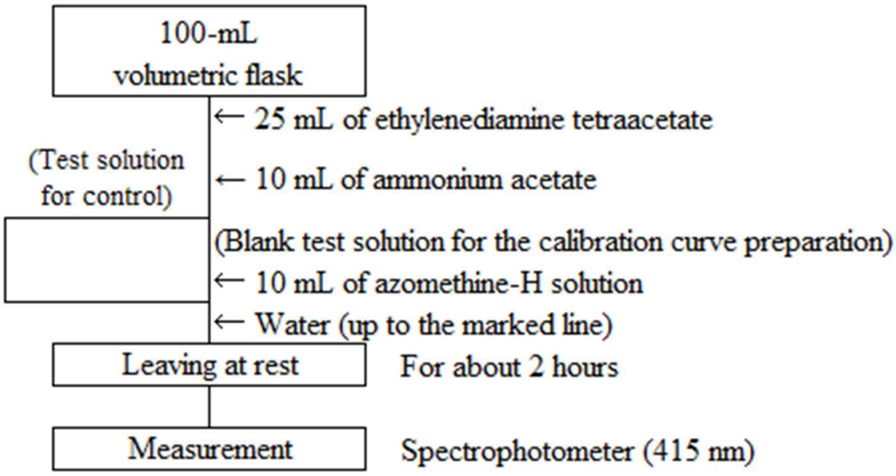


Figure 2-3 Flow sheet for water-soluble boron in fertilizers
(Preparation and measurement procedures of test solution for control and blank test solution for the calibration curve preparation)

Reference: A calculation example of water-soluble boron content in an analytical sample is shown below.

a) Reference table 1-1 shows the absorbance (example) of a standard solution for the calibration curve preparation and a blank test solution for the calibration curve preparation using a test solution for control as the control.
In addition, calibration curves are shown in Reference diagram 1 and regression coefficients of the regression equations are in Reference table 1-2.

b) Reference table 2 shows the sampling amount of an analytical sample, the constant volume of extract, the transferred amount of extract and the constant volume of the sample solution for measurement as well as the absorbance (example) of the sample solution for measurement and the sample solution for correction using a test solution for control as the control.

c) Obtain the boron (B_2O_3) content in the sample solution for measurement by formula (1) (Refer to Reference diagram 1) and calculate water-soluble boron (W-B_2O_3) in the analytical sample by formula (2).

Boron (B_2O_3) content in a sample solution for measurement (C_1)

$=((As-A_b)-a)/b$

$=(Ac-a)/b$ · · · · · · (1)

Water-soluble boron (W-B_2O_3) in an analytical sample (C_2)

$=C_1\times(V_1/V_2)\times(1/W)\times(100/1000)$ · · · · · · (2)

C_1 : Boron (B_2O_3) content in 100 mL of a sample solution for measurement (mg)

As : Absorbance of a sample solution for measurement in (**4.2**) **d**) using a test solution for control ((**4.3 b**) **5**)) as the control

A_b : Absorbance of a sample solution for correction in (**4.2**) **e**) using a test solution for control ((**4. 3**) **b**) **5**)) as the control

A_c : Corrected absorbance

a : Intercept of a regression equation of a calibration curve

b : Inclination of a regression equation of a calibration curve

C_2 : Water-soluble boron (W-B_2O_3) in an analytical sample (% (mass fraction))

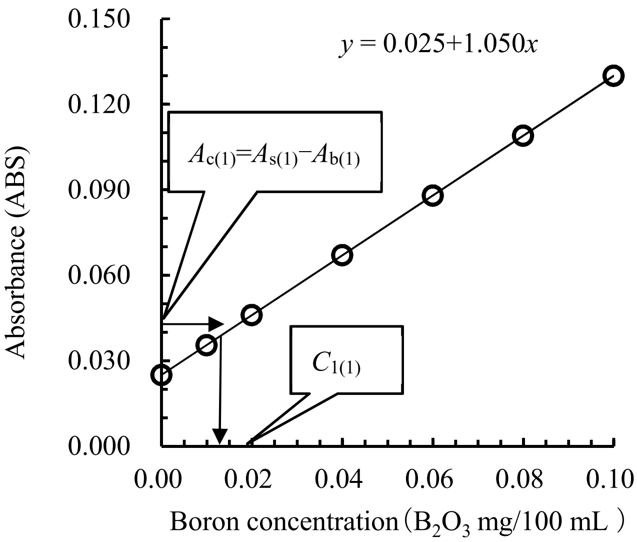
V_1 : Constant volume (mL) of the sample solution in (**4.1.1**) **d**) or (**4.1.2**) **c**)

V_2 : Aliquot (mL) of the sample solution in (**4.2**) **a**)

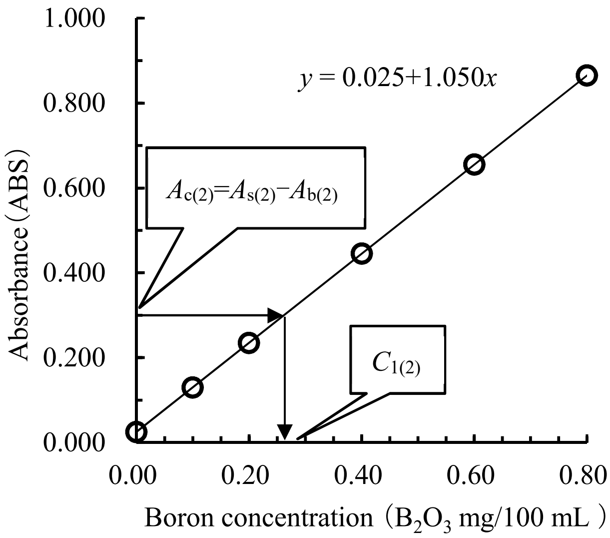
W : Mass (g) of the analytical sample

Reference Table 1-1 Calibration curve (example) of water-soluble boron

Sample name	Calibration curve for low concentration		Calibration curve for high concentration	
	Boron (B_2O_3) concentration (mg/100 mL)	Absorbance (ABS)	Boron (B_2O_3) concentration (mg/100 mL)	Absorbance (ABS)
Blank test solution for calibration curve	0	0.025	0.0	0.025
Standard solution for calibration curve	0.01	0.036	0.1	0.130
Standard solution for calibration curve	0.02	0.046	0.2	0.235
Standard solution for calibration curve	0.04	0.067	0.4	0.445
Standard solution for calibration curve	0.06	0.088	0.6	0.655
Standard solution for calibration curve	0.08	0.109	0.8	0.865
Standard solution for calibration curve	0.1	0.130		



1) Low concentration range
(B_2O_3 0 mg/100 mL - 0.10 mg/100 mL)



2) High concentration range
(B_2O_3 0 mg/100 mL - 0.80 mg/100 mL)

Reference diagram 1 Calibration curve (example) of water-soluble boron (W-B_2O_3)

Reference table 1-2 Regression equation [1)] of a calibration curve (example) of water-soluble boron

	Regression coefficient ($y=a+bx$)	
	a	b
Low concentration range	0.025	1.050
High concentration range	0.025	1.050

1) Regression equation calculated by the least squares method

Reference table 2 Measurement (example) of sample solution and calculation of water-soluble boron

	Unit	Fluid compound fertilizer (1)	Compound fertilizer (2)	Borate fertilizer
Sampling amount of analytical sample (W)	g	1	2.5	2.5
Predetermined volume of extract (V_1)	mL	100	250	250
Transferred amount of extract (V_2)[1)]	mL	25	5	0.05
Predetermined volume of sampling solution, etc. for measurement (V_3)[1)]	mL	100	100	100
Absorbance of sampling solution for measurement (A_s)	ABS	0.045	0.320	0.315
Absorbance of sampling solution for correction (A_b)	ABS	0.005	0.020	0.000
Corrected absorbance ($A_c=A_s-A_b$)	ABS	0.040	0.300	0.315
Boron concentration in sampling solution for measurement (C_1)[2)]	mg/100 mL	0.014	0.262	0.276
Water-soluble boron content in analytical sample (C_2)[3)]	%	0.006	0.52	55.2

1) 2 steps dilutionis is applied to borate fertilizers. Ex:(2 mL→50 mL) (2.5 mL→100 mL)
2) Calculate C_1 by substituting the regression coefficient (interception (a) and gradient (b)) in Table 1-2 for formula (1).
3) Calculate C_2 by substituting C_1, W, V_1 and V_2 for formula 2.

4.8.2.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D for solid fertilizers and Type B for fluid fertilizers. Its symbol is 4.8.2.b-2019 or W-B.b-2.

Extract by adding water to an analytical sample, further dilute the filtered solution, introduce it to an ICP Optical Emission Spectrometer ("ICP-OES") and measure the boron at a wavelength of 249.773 nm, etc. to obtain water-soluble boron (W-B_2O_3) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Boron standard solution (B_2O_3 2.5 mg/mL)** (1): After leaving boric acid specified in JIS K 8863 at rest in a desiccator for about 24 hours to dry, weigh 4.441 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

d) **Boron standard solution (B_2O_3 0.1 mg/mL)** (1): Put 4 mL of boron standard solution (B_2O_3 2.5 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line (2).

e) **Boron standard solution (B_2O_3 2 μg/mL - 16 μg/mL) for the calibration curve preparation** (1): Put 2 mL - 16 mL of boron standard solution (B_2O_3 0.1 mg/mL) in a 100-mL volumetric flask step by step and add hydrochloric acid (1+23) up to the marked line (2).

f) **Boron standard solution (B_2O_3 0.2 μg/mL - 2 μg/mL) for the calibration curve preparation** (1): Put 2 mL - 20 mL of boron standard solution (B_2O_3 10 μg/mL) in a 100-mL volumetric flask step by step and add hydrochloric acid (1+23) up to the marked line (2).

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**), **e**) and **f**) (2).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) When preserving, use a container, which can be sealed tightly, made of material such as PTFE that boron hardly elutes

Comment 1 Instead of the boron standard solution in (**2**), a boron standard solution for the calibration curve preparation can be prepared by using a boron standard solution (B 1 mg/mL or 10 mg/mL) traceable to National Metrology. In this case, calculate water-soluble boron (W-B_2O_3) in the analytical sample by multiplying the concentration (B) of a boron standard solution for calibration curve preparation or a measurement value (B) obtained in (**4.2**) by a conversion factor (3.2199).

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and a spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5% (volume fraction) in purity

b) **Hot plate:**
A hot plate whose surface temperature can be adjusted up to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 2.5 g of an analytical sample (3) to the order of 1 mg, and put it in a 300-mL tall beaker.
b) Add about 200 mL of water, and cover with a watch glass and heat on a hot plate to boil for about 15 minutes.
c) After immediate cooling is complete, transfer it to a 250- mL volumetric flask with water.
d) Add water up to the marked line.
e) Filter with Type 3 filter paper to make a sample solution.

Note (3) The sampling amount of the analytical sample is 1 g when there is a high amount of boric acid content in the fertilizers such as a borate fertilizer and boric acid fertilizer.

Comment 3 In the procedure in (**4.1.1**) **a**), 250-mL volumetric flask can be used instead of a 300-mL tall beaker. However, the volumetric flask used should be distinguished as an extraction flask and should not be used for the other purposes. Additionally, “cover with a watch glass” in **b**) is replaced by “place a funnel”. Skip “transfer it to a 250-mL volumetric flask with water” in the procedure in **c**).

Comment 4 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(4.1.2) Fluid test sample

a) Weigh 1 g of an analytical sample (4) to the order of 1 mg, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, shake to mix and add water up to the marked line.
c) Filter with Type 3 filter paper to make a sample solution.

Note (4) The sampling amount of the analytical sample is 10 g when there is less boron content in the fertilizers such as a home garden-use fertilizer.

Comment 5 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 249.773 nm or 249.678 nm (5)

b) **Calibration curve preparation**

1) Spray the boron standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at an analytical line wavelength.
2) Prepare a curve for the relationship between the boron concentration and the indicated value of the boron standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Put a predetermined amount of the sample solution (the equivalents of 0.02 mg - 1.6 mg as B_2O_3) in a 100-mL volumetric flask.
2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.
3) Subject to the same procedure as in **b) 1)** to read the indicated value.
4) Obtain the boron content from the calibration curve, and calculate water-soluble boron (W-B_2O_3) in the analytical sample.

Note (5) 249.678 nm can also be used. However, since the intensity of emission obtained is different from the one of 249.773 nm, it is necessary to understand the suitable concentration range of the calibration curve and prepare a standard solution for the calibration curve in advance.

Comment 6 Wash the sample injector of an ICP-OES sufficiently with water because boron easily causes the memory effect.

Comment 7 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 8 The comparison of the measurement value (y_i : 0.0165 % (mass fraction) - 0.590 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Azomethine-H method was conducted to evaluate trueness using solid fertilizers (21 samples). As a result, a regression equation was y=0.0002+0.993x, and its correlation coefficient (r) was 0.998. The comparison of the measurement value (y_i : 0.013 % (mass fraction) - 0.530 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of Azomethine-H method was conducted to evaluate trueness using fluid fertilizers (12 samples). As a result, a regression equation was y=−0.0041+0.986x, and its correlation coefficient (r) was 0.999. In addition, additive recovery testing was conducted using preparation fertilizers (6 samples). As a result, the mean recovery rate at the additive level of 0.0912 % (mass fraction) - 56.30 % (mass fraction) was 97.4 % - 101.2 %. Additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand) and a liquid microelement compound fertilizer (1 bland). As a result, the mean recovery rates at the additive level of 0.15 % (mass fraction) - 0.2 % and 0.01 % (mass fraction) were 95.5 % - 99.4 % and 96.5 % respectively.

The results of the repeatability tests on different days using a compound fertilizer, a blended fertilizer, a fluid compound fertilizer and a home garden-use compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study implemented for testing method validation and its analysis are shown in Table 2

Note that the minimum limit of quantification of this testing method was estimated to be about 0.005% (mass fraction) for solid fertilizers and about 0.0005% (mass fraction) for fluid fertilizers.

Table 1-1 Analysis results of the repeatability tests on different days of water-soluble boron (Solid fertilizer)

Name of sample	Test days of repeatability T [1)]	Mean[2)] (%)[3)]	s_r [4)] (%)[3)]	RSD_r [5)] (%)	$s_{I(T)}$ [6)] (%)[3)]	$RSD_{I(T)}$ [7)] (%)
Compound fertilizer	5	0.365	0.008	2.3	0.016	4.3
Blended fertilizer	5	0.0456	0.0019	4.1	0.0028	6.1

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 1-2 Analysis results of the repeatability tests on different days of water-soluble boron (Fluid fertilizer)

Name of sample	Test days of repeatability T [1)]	Mean[2)] (%)[3)]	s_r [4)] (%)[3)]	RSD_r [5)] (%)	$s_{I(T)}$ [6)] (%)[3)]	$RSD_{I(T)}$ [7)] (%)
Fluid compound fertilizer	7	0.166	0.001	0.7	0.002	1.2
Home garden-use compound fertilizer (Fluid)	7	0.0134	0.0001	1.0	0.0001	1.0

Footnote: Refer to Table 1-1

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble boron

Analytical line wavelength (nm)	Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r [4)] (%)[3)]	RSD_r [5)] (%)	s_R [6)] (%)[3)]	RSD_R [7)] (%)
249.773	Preparation sample (Liquid) 1	11	0.515	0.004	0.8	0.008	1.5
	Preparation sample (Liquid) 2	12	1.03	0.02	1.7	0.03	2.2
	Preparation sample (Liquid) 3	11	2.06	0.02	0.9	0.02	1.6
	Preparation sample (Liquid) 4	10	0.0515	0.0008	1.6	0.001	2.0
	Preparation sample (Liquid) 5	12	0.0121	0.0004	3.5	0.0007	6.1
249.678	Preparation sample (Liquid) 1	11	0.515	0.005	0.9	0.007	1.3
	Preparation sample (Liquid) 2	11	1.03	0,02	1.7	0.01	1.4
	Preparation sample (Liquid) 3	12	2.07	0.05	2.2	0.07	3.2
	Preparation sample (Liquid) 4	10	0.0513	0.0008	1.5	0.0011	2.1
	Preparation sample (Liquid) 5	11	0.0118	0.0003	2.7	0.0006	4.9

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination of Water-Soluble Principal

Ingredients (W-P_2O_5, W-K_2O, W-MgO, W-MnO and W-B_2O_3) in Liquid Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 8**, p. 1 - 9 (2015)

2) Norio FUNAKI: Simultaneous Determination of Water-soluble Principal Ingredients in Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Research Report of Fertilizer, **Vol. 12**, p. 28 – 51 (2019)

3) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(**5**) **Flow sheet**: The flow sheet for water-soluble boron in fluid fertilizers is shown below:

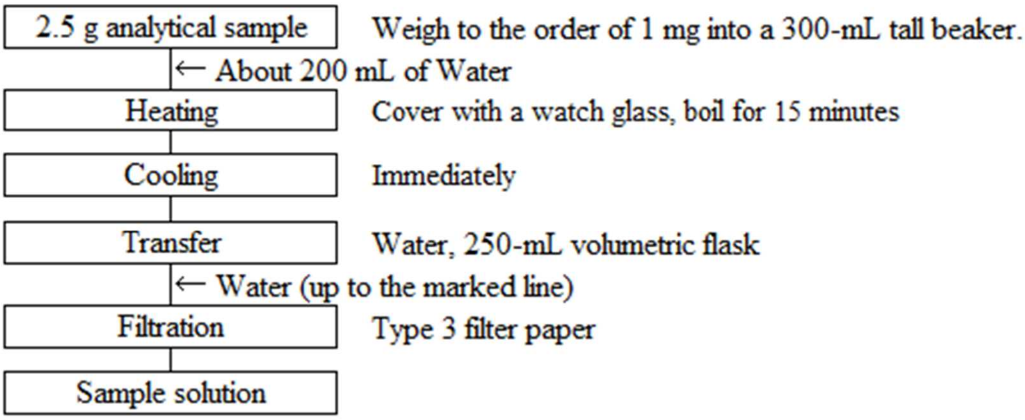


Figure 1-1 Flow sheet for water-soluble boron in fertilizers (Extraction procedure (4.1.1))

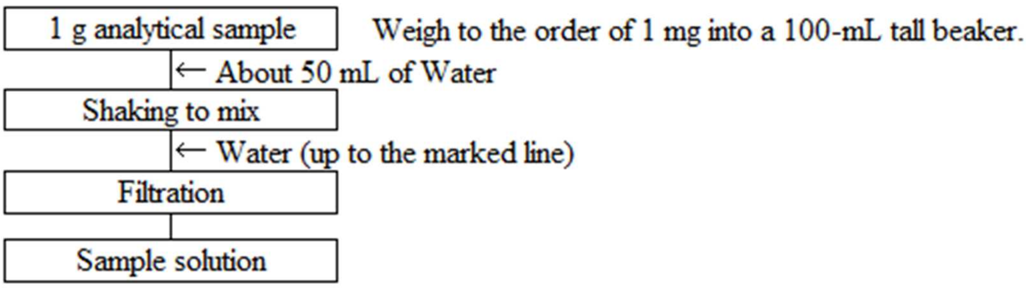


Figure 1-2 Flow sheet for water-soluble boron in fertilizers (Extraction procedure (4.1.2))

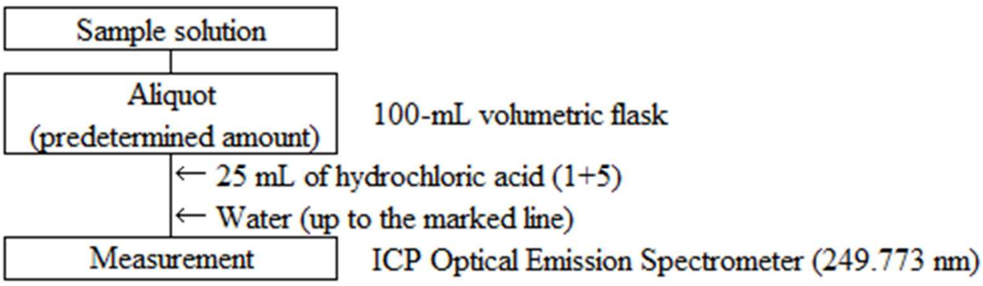


Figure 2 Flow sheet for water-soluble boron in fertilizers (Measurement procedure)

4.9 Zinc
4.9.1 Total zinc
4.9.1.a Flame atomic absorption spectrometry
(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type C and its symbol is 4.9.1.a-2017 or T-Zn.a-1.

Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), and then spray in an acetylene−air flame, and measure the atomic absorption with zinc at a wavelength of 213.9 nm to quantify the total zinc (T-Zn). In addition, the performance of this testing method is shown in **Comment 8**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

c) Nitric acid: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

d) Zinc standard solution (Zn 0.1 mg/mL): A zinc standard solution (Zn 0.1 mg/mL) traceable to National Metrology.

e) Zinc standard solutions (Zn 0.5 µg - 5 µg/mL) for the calibration curve preparation (1): Put 2.5 mL - 25 mL of zinc standard solution (Zn 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

f) Blank test solution for the calibration curve preparation (1): Hydrochloric acid (1+23) used in the procedures in **e**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the zinc standard solution in (**2**), a zinc standard solution for the calibration curve preparation can be prepared by using a zinc standard solution (Zn 1 mg/mL or 10 mg/mL) traceable to National Metrology.

Comment 2 When using a sample solution obtained in the procedure in (**4.1.2**) **h**) for the measurement of cadmium, nickel, chromium or lead, sulfuric acid and hydrochloric acid in (**2**) should be a reagent of harmful metal analysis grade, microanalysis grade or equivalents.

(3) Instruments: Instruments are as shown below:

a) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.

1) **Light source**: A zinc hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) Electric furnace: An electric furnace that can be adjusted to 450 °C±5 °C or 550 ºC ± 5 °C.

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (**2**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure

(**4.1**) Extraction: Conduct extraction as shown below.

(**4.1.1**) Incineration-hydrochloric acid boiling

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (3).

c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate (3).

d) After standing to cool, moisten the residue with a small amount of water, gradually add about 10 mL of hydrochloric acid, and further add water to make 20 mL.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.

f) After cooling is complete, transfer to a 250-mL - 500-mL volumetric flask with water.

g) Add water up to the marked line.

h) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) Example of charring and incineration procedure: After raising from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 550 °C in 1 to 2 hours.

Comment 3 Do not conduct the procedures in (**4.1.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 4 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Incineration-aqua regia digestion**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (4).

c) Ignite at 450 ºC ± 4 ºC for 8 - 16 hours to incinerate (4).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (6) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

Note (**4**) Example of charring and incineration procedure: After raising from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.

(**5**) The watch glass can be removed.

(**6**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 5 Do not conduct the procedures in (**4.1.2**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 6 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 213.9 nm

b) **Calibration curve preparation**

1) Spray the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 213.9 nm.

2) Prepare a curve for the relationship between the zinc concentration and the indicated value of the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Subject the sample solution (7) to the same procedure as in **b**) **1**) to read the indicated value.

2) Obtain the zinc content from the calibration curve, and calculate the total zinc (T-Zn) in the analytical sample.

Note (**7**) If there is a possibility that the zinc concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 7 The zinc concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **c**) **1**) to obtain the zinc content in the blank test solution.

Comment 8 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 1.2 % (mass fraction) and 90 mg/kg were 99.5 % and 97.8 % as the total zinc (T-Zn) respectively.

The results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 1 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 5 mg/kg for solid fertilizers.

Table 1 Analysis results of the collaborative study to determine total zinc of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1)]	Mean[2)] mg/kg	s_r[3)] mg/kg	RSD_r[4)] (%)	$s_{I(T)}$[5)] mg/kg	$RSD_{I(T)}$[6)] (%)	s_R[7)] mg/kg	RSD_R[8)] (%)
FAMIC-C-12	12	992	11	1.1	17	1.7	32	3.3

1) The number of labooaratories used for analysis conducting flame atomic absorbance spectrometry
2) Mean (the number of laboratory(p)× test days(2) ×the number of replicate testing(3))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Intermediate standard deviation
6) Intermediate relative standard deviation
7) Reproducibility standard deviation
8) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.193- 194, Yokendo, Tokyo (1988)
2) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, **Vol. 3** p. 107 - 116 (2010)
3) Shin ABE and Yoshiyuki SUNAGA: Verification of Performance Characteristics of Testing Methods for Zinc in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer **Vol. 6**, p. 156 -164 (2013)

(**5**) **Flow sheet for total zinc**: The flow sheet for total zinc in fertilizers is shown below:

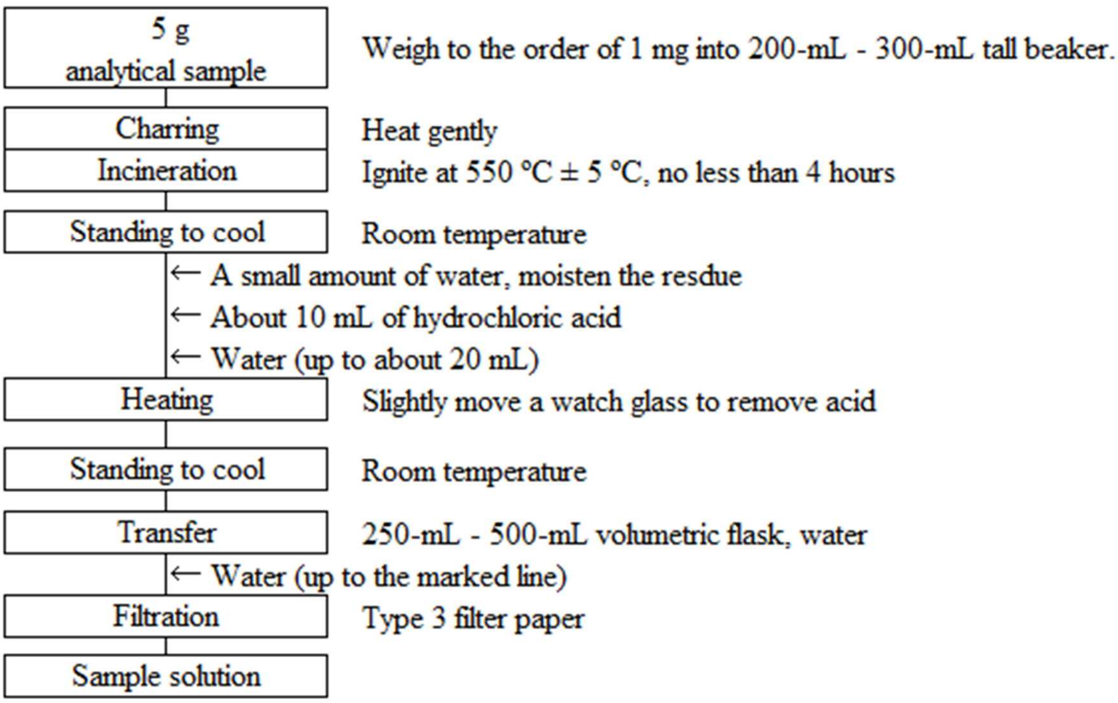


Figure 1-1 Flow sheet for total zinc in fertilizers (Incineration-hydrochloric acid boiling (4.1.1))

5 g analytical sample	Weigh to the order of 1 mg into 200-mL - 300-mL tall beaker.
Charring	Heat gently
Incineration	Ignite at 450 °C ± 5 °C, 8 - 16 hours
Standing to cool	Room temperature
← A small amount of water	
← About 10 mL of nitric acid	
← About 30 mL of hydrochloric acid	
Heating	Cover with a watch glass, and digest
Heating	Slightly move a watch glass to remove acid
Standing to cool	Room temperature
← 25 mL - 50 mL of hydrochloric acid (1+5)	
Heating	Cover with a watch glass, and dissolve
Standing to cool	Room temperature
Transfer	100-mL - 200-mL volumetric flask, water
← Water (up to the marked line)	
Filtration	Type 3 filter paper
Sample solution	

Figure 1-2 Flow sheet for total zinc in fertilizers (Incineration-aqua regia digetion (4.1.2))

Sample solution	
Aliquot (Predetermined volume)	100-mL volumetric flask
← 25 mL of hydrochloric acid (1+5)	
← Water up to the marked line	
Measurement	Atomic absorption spectrometer (213.9 nm)

Figure 2 Flow sheet for total zinc in fertilizers (Measurement procedure)

4.9.1.b ICP Optical Emission Spectrometry

(1) Summary

The testing method is applicable to sludge fertilizers, etc. This testing method is classified as Type D and its symbol is 4.9.1.b-2017 or T-Zn.b-1.

Pretreat an analytical sample with incineration, nitric acid−hydrochloric acid (1+3), introduce it to an ICP Optical Emission Spectrometer ("ICP-OES") and measure the emission with zinc at a wavelength of 206.191 nm to obtain the total zinc (T-Zn) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Nitric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) Hydrochloric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) Zinc standard solution (Zn 0.1 mg/mL): A zinc standard solution (Zn 0.1 mg/mL) traceable to National Metrology.

e) Zinc standard solution (Zn 25 µg/mL) (1): Dilute a predetermined amount of zinc standard solution (Zn 0.1 mg/mL) with hydrochloric acid (1+23) to prepare a zinc standard solution (Zn 25 µg/mL).

Note (1) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the zinc standard solution in (**2**), a zinc standard solution for the calibration curve preparation can be prepared by using a zinc standard solution (Zn 1 mg/mL or 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and a spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) ICP Optical Emission Spectrometer: An optical emission spectrometer specified in JIS K 0116.

1) Gas: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) Electric furnace: An electric furnace that can keep the test temperature at 450 °C ± 5 °C .

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (2).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (2).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (3), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) [(4)] to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**2**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.

(**3**) The watch glass can be removed.

(**4**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 3 Do not conduct the procedures in (**4.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 4 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 206.191 nm

b) **Calibration curve preparation and sample measurement**

1) Put 5mL of sample solution in three 10-mL volumetric flasks respectively.

2) Add 2mL and 4 mL of zinc standard solution (25 µg/mL) to volumetric flasks of **1**) above, then add hydrochloric acid (1+23) up to the marked line to make a sample solution of Standard Addition Method.

3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1**) above to make a sample solution without a standard solution.

4) Spray the sample solution of Standard Addition Method and the sample solution without a standard solution into the induction plasma, and read the indicated value at a wavelength of 206.191 nm.

5) Transfer 5 mL of blank test solution to a 10-mL volumetric flask, conduct the same procedures as in **3**) - **4**) to read the indicated value, and correct the indicated value obtained from the respective sample solutions.

6) Prepare a curve for the relationship between the added zinc concentration and the corrected indicated value of the sample solution for Standard Addition Method and the sample solution without a standard solution.

7) Obtain the zinc content from the intercept of the calibration curve to calculate the total zinc (T-Zn) in the analytical sample.

Comment 5 The total zinc (T-Zn) in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **b**) **1**) - **b**) **4**) and **b**) **6**) - **b**) **7**) to obtain the zinc content in the blank test solution.

Comment 6 The simultaneous measurement of multiple elements is possible with the ICP-OES. In this case, put a pre-determined amount of copper standard solution (Cu 0.1 mg/mL, 1 mg/mL or 10 mg/mL), zinc standard solution (Zn 0.1 mg/mL, 1 mg/mL or 10 mg/mL), cadmium standard solution (Cd 0.1 mg/mL, 1 mg/mL or 10 mg/mL), nickel standard solution (Ni 0.1 mg/mL, 1 mg/mL or 10 mg/mL), chromium standard solution (Cr 0.1 mg/mL, 1 mg/mL or 10 mg/mL) and lead standard solution (Pb 0.1 mg/mL, 1 mg/mL or 10 mg/mL) in a volumetric flask to mix, add hydrochloric acid (1+5) to make acid concentration 0.5 mol/L and further add water up to the marked line to prepare a primary mixed standard solution. Transfer a pre-determined amount of primary mixed standard solution to a volumetric flask, add hydrochloric acid (1+23) up to the marked line to prepare a mixed standard solution for addition within the concentration range in Table 1. The measurement wavelengths of respective elements are according to Table 1.

In addition, the additive amount of mixed standard solution for addition and the additive concentrations of respective elements in a sample solution are shown in the Table below.

Table 1 The preparation concentration of mixed standard solutions for addition, additive concentrations and measurement wavelengths of respective elements in sample solutions

Test item	Concentration of mixed standard solution for addition (µg/mL)	Additive concentration of element in sample solution (µg/mL)			Measurement wavelength (nm)
		Additive amount[1)] 0 mL	Additive amount[1)] 2 mL	Additive amount[1)] 4 mL	
Total zinc	Zn 25	0	5	10	206.191
Total copper	Cu 25	0	5	10	324.754
Cadmium	Cd 0.25	0	0.05	0.1	228.802
Nickel	Ni 2.5	0	0.5	1	231.604
Chromium	Cr 2.5	0	0.5	1	205.552
Lead	Pb 2.5	0	0.5	1	220.351

1) Additive amount of mixed standard solution for addition

Comment 7 The comparison of the measurement value (x_i : 65.0 mg/kg - 3310 mg/kg) of ICP Optical Emission Spectrometry and the measurement value (y_i) of Flame Atomic Absorption Spectrometry was conducted to evaluate trueness using sludge fertilizers (49 samples). As a result, a regression equation was $y=-47.6+1.080x$ and its correlation coefficient (r) was 0.995. Triplicates measurement for each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer, calcined sludge fertilizers and composted sludge fertilizer was conducted. As a result, a repeatability obtained was 0.1 % - 2.3 % as a relative standard deviation.

Note that the minimum limit of quantification of this testing method was estimated to be about 8 mg/kg.

References

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI and Tetuya NOMURA: Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer, **Vol. 4**, p. 30 - 35 (2011)

(**5**) **Flow sheet for total zinc**: The flow sheet for total zinc in fertilizers is shown below:

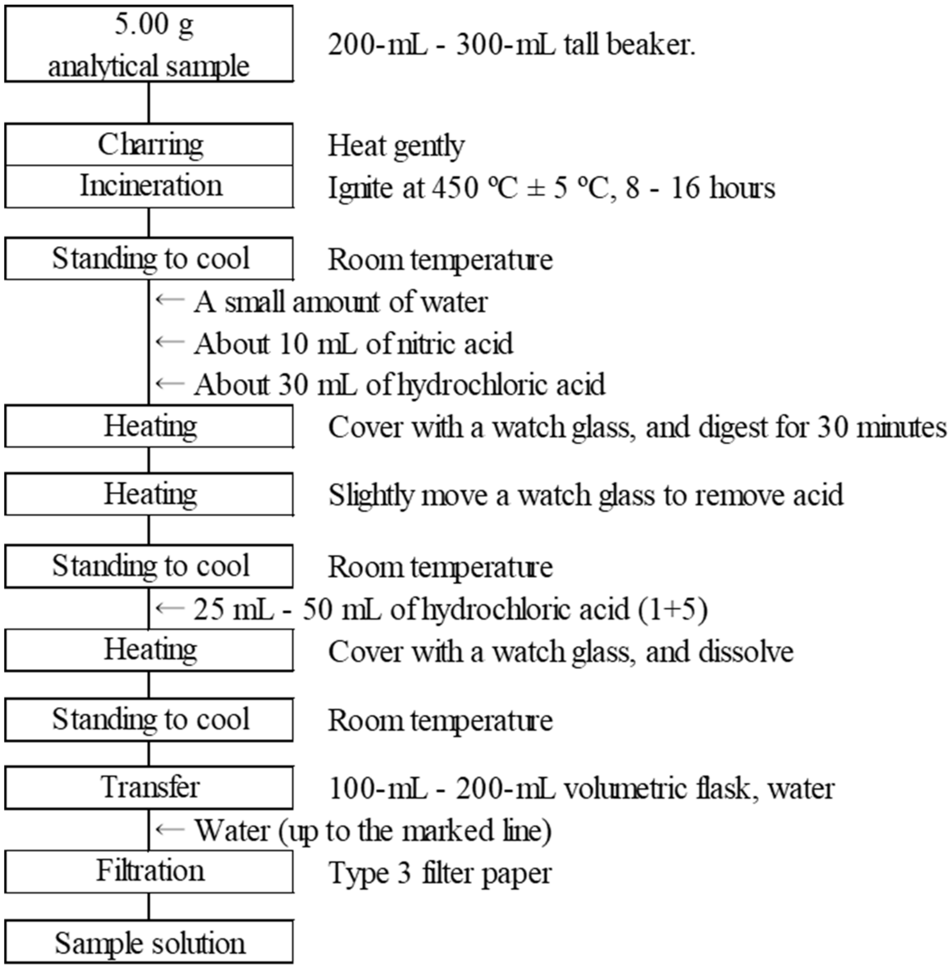


Figure 1 Flow sheet for total zinc in fertilizers (Exrtraction procedure)

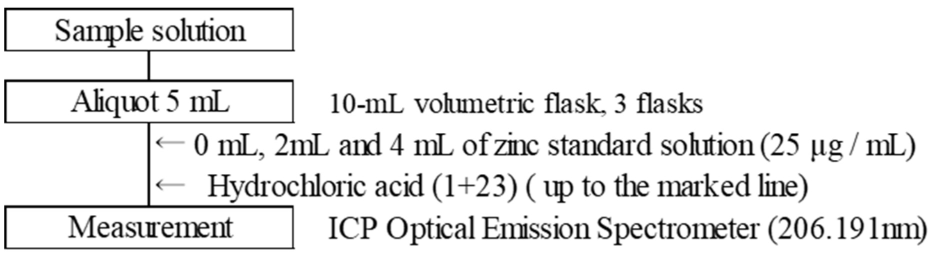


Figure 2 Flow sheet for total zinc in fertilizers (Measurement procedure)

4.9.2 Water-soluble zinc
4.9.2.a Flame atomic absorption spectrometry
(1) Summary
This testing method is applicable to fertilizers that indicate zinc content as a response modifier. This testing method is classified as Type D and its symbol is 4.9.2.a-2017 or W-Zn.a-1.
Extract by adding water to an analytical sample, spray in an acetylene−air flame and measure the atomic absorption with zinc at a wavelength of 213.9 nm to obtain water-soluble zinc (W-Zn) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.
a) **Water**: Water of A3 specified in JIS K 0557.
b) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
c) **Zinc standard solution (Zn 0.1 mg/mL)**: A zinc standard solution (Zn 0.1 mg/mL) traceable to National Metrology.
d) **Zinc standard solutions (Zn 0.5 µg - 5 µg/mL) for the calibration curve preparation** (1): Put 2.5 mL - 25 mL of zinc standard solution (Zn 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.
e) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the zinc standard solution in (**2**), a zinc standard solution for the calibration curve preparation can be prepared by using a zinc standard solution (Zn 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(**3**) **Instruments**: Instruments are as shown below:
a) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.
b) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.
1) **Light source**: A zinc hollow cathode lamp (when the continuous source method as the background correction method is used, the light source is a deuterium lamp.)
2) **Gas**: Gas for heating by flame
(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

Note (**2**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure
(**4.1**) **Extraction**: Conduct extraction as shown below.
(4.1.1) Powdery test sample
a) Weigh 5.00 g of an analytical sample, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 2 In the procedure in (**4.1.1**) **a**), it is also allowed to weigh 2.50 g of the analytical sample and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Fluid test sample**

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 213.9 nm

b) **Calibration curve preparation**

1) Spray the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 213.9 nm.
2) Prepare a curve for the relationship between the zinc concentration and the indicated value of the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.05 mg - 0.5 mg as Zn) in a 100-mL volumetric flask.
2) Add about 25 mL of hydrochloric acid (1+5), and add water up to the marked line.
3) Subject to the same procedure as in **b**) **1**) to read the indicated value.
4) Obtain the zinc content from the calibration curve, and calculate the water-soluble zinc (W-Zn) in the analytical sample.

Comment 5 Recovery testing was conducted to evaluate trueness using a preparation sample (solid). As a result, the mean recovery rates at the content level of 10 % (mass fraction), 2 % (mass fraction) and 0.01 % (mass fraction) were 101.6 %, 101.9 % and 98.9 % as water-soluble zinc (W-Zn) respectively. In addition, recovery testing was conducted using a preparation sample (fluid). As a result, the mean recovery rates at the content level of 1 % (mass fraction), 0.05 % (mass fraction) and 20 mg/kg were 99.6 %, 100.4 % and 100.6 % as water-soluble zinc respectively.
The results of the repeatability tests on different days using a fluid compound fertilizer and a liquid microelement compound fertilizer to evaluate the extract precision of fluid fertilizers were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to be about 10 mg/kg for solid fertilizers and about 0.9 mg/kg for fluid fertilizers.

Table 1 Analysis results of the repeatability tests on different days of water-soluble zinc (Fluid fertilizer)

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluid compound fertilizer	7	1.28	0.01	0.4	0.02	1.3
Liquid microelement compound fertilizer	7	0.230	0.001	0.5	0.003	1.5

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.192- 194, Yokendo, Tokyo (1988)

2) Shin ABE and Yoshiyuki SUNAGA: Verification of Performance Characteristics of Testing Methods for Zinc in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer **Vol. 6**, p. 156 -164 (2013)

3) Shinji KAWAGUCHI: Simple Extraction Method for Water-Soluble Components in Liquid Compound Fertilizers, Research Report of Fertilizer, **Vol. 9**, p. 10 - 20 (2016)

(5) Flow sheet for water-soluble zinc: The flow sheet for water-soluble zinc in fertilizers is shown below:

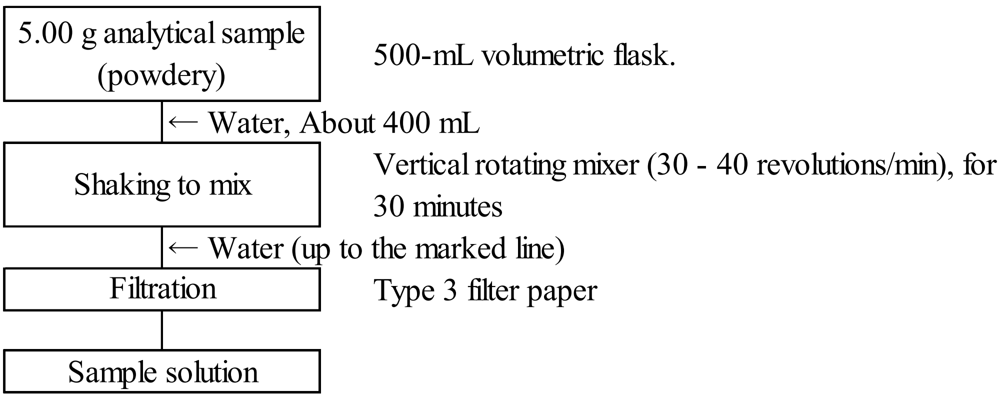


Figure 1-1 Flow sheet for water-soluble zinc in fertilizers (Extraction procedure(4.1.1))

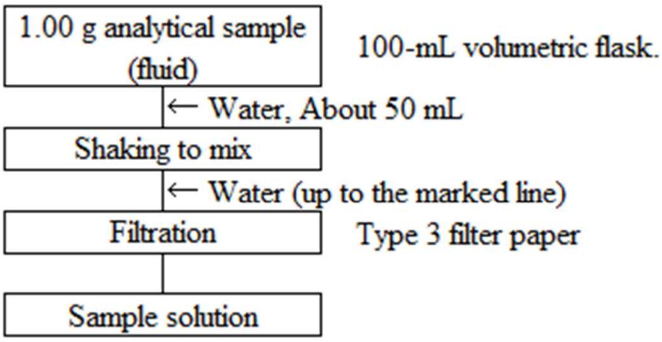


Figure 1-2 Flow sheet for water-soluble zinc in fertilizers
(Extraction procedure(4.1.2))

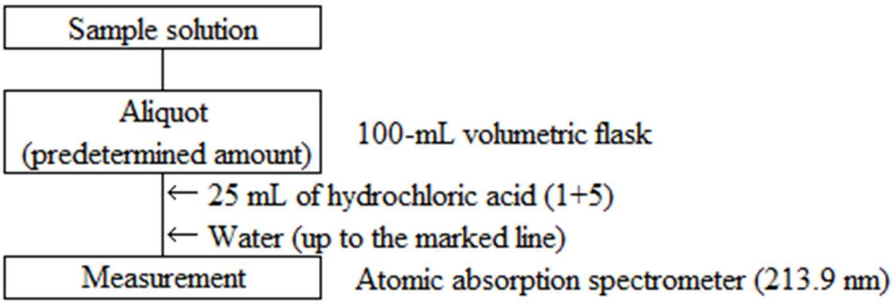


Figure 2 Flow sheet for water-soluble zinc in fertilizers
(Measurement procedure)

4.9.2.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fluid compound fertilizers, liquid microelement compound fertilizers and the fluid fertilizers of home garden-use compound fertilizers. This testing method is classified as Type B and its symbol is 4.9.2.b-2017 or W-Zn.b-1.

Extract by adding water to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the zinc at a wavelength of 213.856 nm, etc. to obtain water-soluble zinc (W-Zn) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Zinc standard solution (Zn 1 mg/mL)**: A zinc standard solution (Zn 1 mg/mL) traceable to National Metrology.

d) **Zinc standard solution (Zn 0.1 mg/mL)** (1): Put 10 mL of zinc standard solution (Zn 1 mg/mL) in a 100-mL flask and add hydrochloric acid (1+23) up to the marked line.

e) **Zinc standard solutions (Zn 1 µg - 20 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of zinc standard solution (Zn 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

f) **Zinc standard solutions (Zn 0.1 µg - 1 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 10 mL of zinc standard solution (Zn 10 µg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**), **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the zinc standard solution in (**2**), a zinc standard solution for the calibration curve preparation can be prepared by using a zinc standard solution (Zn 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and a spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g (2) of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, shake to mix and add water up to the marked line.

c) Filter with Type 3 filter paper to make a sample solution.

Note (2) The sampling amount of the analytical sample is 10 g when the content in the sample is less than 0.01 % (mass fraction) as water-soluble zinc.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following: Analytical line wavelength: 213.856 nm or 206.200 nm (3)

b) **Calibration curve preparation**

1) Spray the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at an analytical line wavelength.

2) Prepare a curve for the relationship between the zinc concentration and the indicated value of the zinc standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 2 mg as Zn) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b) 1)** to read the indicated value.

4) Obtain the zinc content from the calibration curve, and calculate the water-soluble zinc (W-Zn) in the analytical sample.

Note (3) 206.200 nm can also be used. However, since the intensity of emission obtained is different from the one of 213.856 nm, it is necessary to understand the suitable concentration range of the calibration curve and prepare a standard solution for the calibration curve in advance.

Comment 4 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b)** - **c)**, and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 5 The comparison of the measurement value (y_i : 0.0109 % (mass fraction) - 0.0827 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of flame atomic absorbance spectrometry was conducted to evaluate trueness using fluid fertilizers (12 samples). As a result, a regression equation was $y=-0.0007+0.984x$, and its correlation coefficient (r) was 0.998. Additionally, additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 0.01 % (mass fraction) and 0.1 % (mass fraction) were 91.6 % and 95.9 % respectively.

The results of the repeatability tests on different days using a fluid compound fertilizer and a home garden-use compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study implemented for 3333testing method validation and its analysis are shown in Table 2. Note that the minimum limit of quantification of this testing method was estimated to be about 0.0005 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of water-soluble zinc (Fluid fertilizer)

Name of sample	Test days of repeatability T[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)
Fluid compound fertilizer	7	0.0677	0.0004	0.6	0.0005	0.7
Home garden-use compound fertilizer(fluid)	7	0.0107	0.0003	2.3	0.0004	4.2

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble zinc

Analytical line wavelength (nm)	Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
213.856	Preparation sample (Liquid) 1	12	1.07	0.01	1.3	0.03	2.8
	Preparation sample (Liquid) 2	12	2.14	0.02	0.8	0.06	2.9
	Preparation sample (Liquid) 3	11	0.525	0.002	0.4	0.011	2.0
	Preparation sample (Liquid) 4	10	0.106	0.0005	0.5	0.003	2.5
	Preparation sample (Liquid) 5	11	0.0522	0.0005	1.0	0.0012	2.3
206.200	Preparation sample (Liquid) 1	12	1.07	0.02	1.5	0.04	3.6
	Preparation sample (Liquid) 2	12	2.14	0.02	0.9	0.07	3.1
	Preparation sample (Liquid) 3	10	0.530	0.002	0.4	0.011	2.1
	Preparation sample (Liquid) 4	10	0.105	0.0004	0.3	0.003	2.8
	Preparation sample (Liquid) 5	11	0.0517	0.0005	1.1	0.0014	2.7

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination Method for Effect-Development Promoting Agent (Ca, Fe, Co, Cu, Zn and Mo) in Liquid Compound Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), Research Report of Fertilizer, **Vol. 9**, p. 1 - 9 (2016)

2) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(5) **Flow sheet for water-soluble zinc**: The flow sheet for water-soluble zinc in fluid fertilizers is shown below:

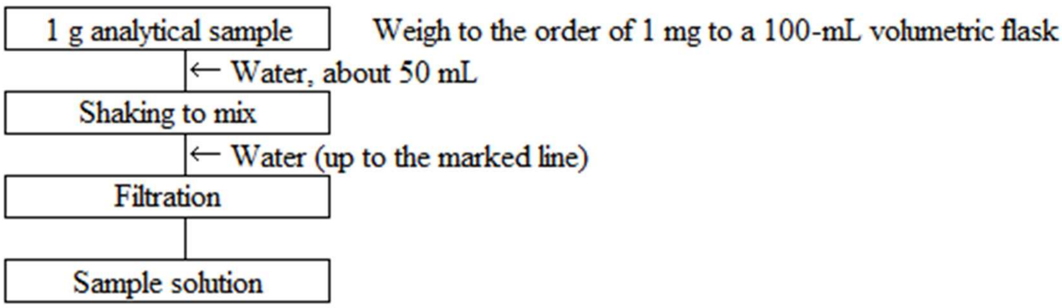


Figure 1 Flow sheet for water-soluble zinc in fluid fertilizers (Extraction procedure)

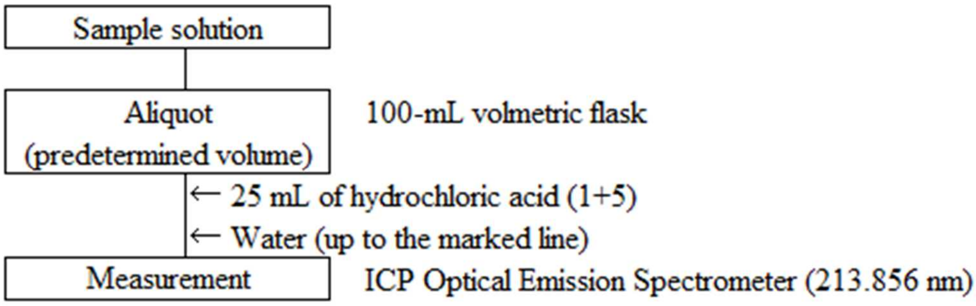


Figure 2 Flow sheet for water-soluble zinc in fluid fertilizers (Measurement procedure)

4.10 Copper
4.10.1 Total copper
4.10.1.a Flame atomic absorption spectrometry
(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type C and its symbol is 4.10.1.a-2017 or T-Cu.a-1.

Pretreat an analytical sample with incineration and nitric acid−hydrochloric acid (1+3), and then spray in an acetylene−air flame, and measure the atomic absorption with copper at a wavelength of 324.8 nm to obtain the total copper (T-Cu) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

c) **Nitric acid**: A JIS Guaranteed Reagent specified in JIS K 8541 (HNO_3 60 % (mass fraction)) or a reagent of equivalent quality.

d) **Copper standard solution (Cu 0.1 mg/mL)**: A copper standard solution (Cu 0.1 mg/mL) traceable to National Metrology.

e) **Copper standard solutions (Cu 0.5 µg - 5 µg/mL) for the calibration curve preparation** (1): Put 2.5 mL - 25 mL of copper standard solution (Cu 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

f) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the copper standard solution in (**2**), a copper standard solution for the calibration curve preparation can be prepared by using a copper standard solution (Cu 1 mg/mL or 10 mg/mL) traceable to National Metrology.

Comment 2 When using a sample solution obtained in the procedure in (**4.1.2**) **h**) for the measurement of cadmium, nickel, chromium or lead, sulfuric acid and hydrochloric acid in (**2**) should be a reagent of harmful metal analysis grade, microanalysis grade or equivalents.

(**3**) **Instruments**: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.

1) **Light source**: A copper hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Electric furnace**: An electric furnace that can keep the test temperature at 450 °C ± 5 °C or 550 ºC ± 5 ºC.

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (**2**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Incineration-hydrochloric acid boiling**

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char (3).
c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate (3).
d) After standing to cool, moisten the residue with a small amount of water, gradually add about 10 mL of hydrochloric acid, and further add water to make 20 mL.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.
f) After cooling is complete, transfer to a 250-mL - 500-mL volumetric flask with water.
g) Add water up to the marked line.
h) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) Example of charring and incineration procedure: After raising from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 550 °C in 1 to 2 hours.

Comment 3 Do not conduct the procedures in (**4.1.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 4 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) Incineration-aqua regia digestion

a) Weigh 5 g of an analytical sample to the order of 1 mg, and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char (4).
c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (4).
d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
f) Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (6) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

Note (**4**) Example of charring and incineration procedure: After raising from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.
(**5**) The watch glass can be removed.
(**6**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 5 Do not conduct the procedures in (**4.1.2**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 6 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 324.8 nm

b) **Calibration curve preparation**

1) Spray the copper standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 324.8 nm.

2) Prepare a curve for the relationship between the copper concentration and the indicated value of the copper standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Subject the sample solution (7) to the same procedure as in **b**) **1**) to read the indicated value.

2) Obtain the copper content from the calibration curve, and calculate the total copper (T-Cu) in the analytical sample.

Note (**7**) If there is a possibility that the copper concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 7 The copper concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **c**) **1**) to obtain the copper content in the blank test solution.

Comment 8 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 0.15 % (mass fraction) and 0.03 % (mass fraction) were 100.4 % and 99.6 % as total copper (T-Cu) respectively.

The results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 1 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 4 mg/kg.

Table 1 Analysis results of the collaborative study to determine total copper of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1]	Mean[2] mg/kg	s_r[3] mg/kg	RSD_r[4] (%)	$s_{I(T)}$[5] mg/kg	$RSD_{I(T)}$[6] (%)	s_R[7] mg/kg	RSD_R[8] (%)
FAMIC-C-12	11	583	7	1.1	11	1.9	22	3.8

1) The number of laboaratories used for analysis conducting flame atomic absorbance spectrometry
2) Mean (the number of laboratory(p) × test days(2) ×the number of replicate testing(3))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Intermediate standard deviation
6) Intermediate relative standard deviation
7) Reproducibility standard deviation
8) Reproducibility relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.254- 255, Yokendo, Tokyo (1988)
2) Kimie KATO, Masayuki YOSHIMOTO and Yuji SHIRAI: Systematization of Determination Methods of Major Components in Sludge Fertilizer, Compost and Organic Fertilizer, Research Report of Fertilizer, **Vol. 3** p. 107 - 116 (2010)
3) Shin ABE and Yoshiyuki SUNAGA: Verification of Performance Characteristics of Testing Methods for Copper in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer, **Vol. 6**, p. 165 - 173 (2013)

(**5**) **Flow sheet for total copper**: The flow sheet for total copper in fertilizers is shown below:

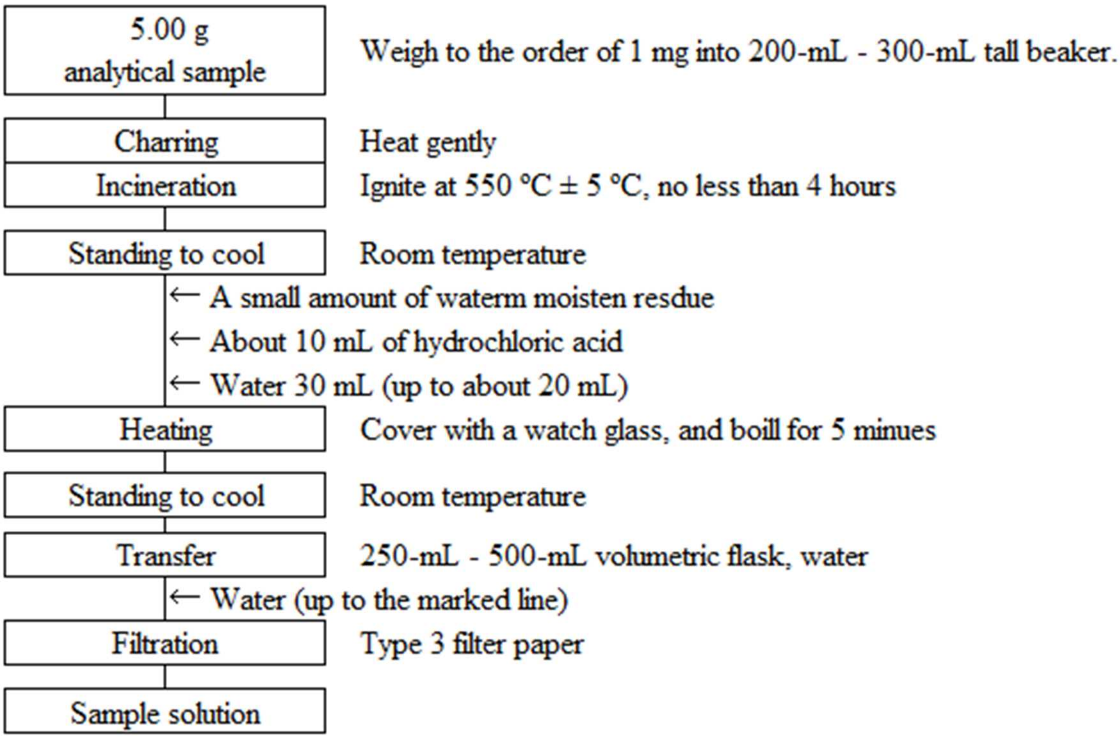


Figure 1-1 Flow sheet for total copper in fertilizers
(Incineration-hydrochloric acid boiling procedure (4.1.1))

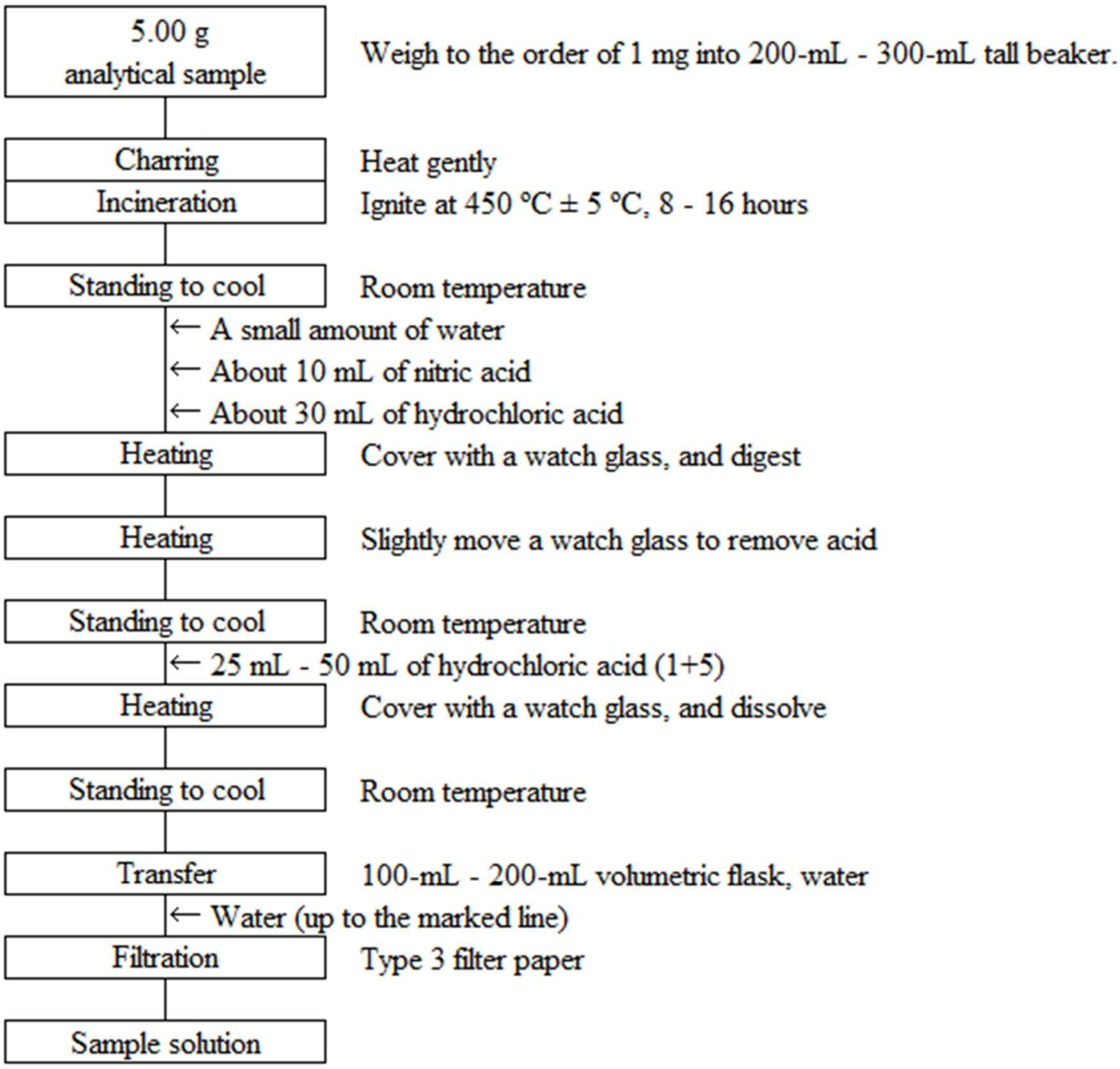


Figure 1-2 Flow sheet for total copper in fertilizers
(Incineration-aqua regia digestion procedure (4.1.2))

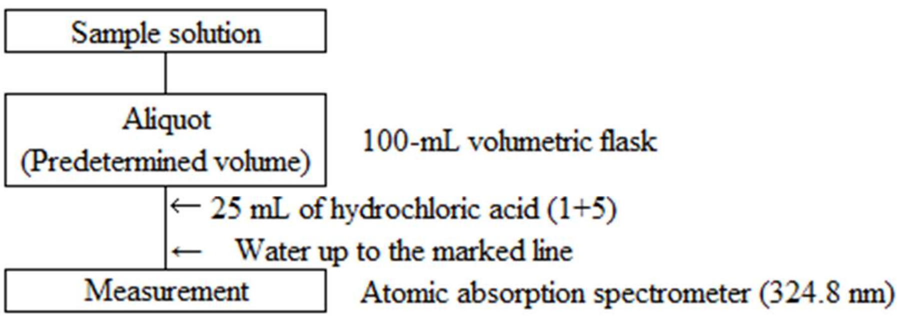


Figure 2 Flow sheet for total copper in fertilizers (Measurement procedure)

4.10.1.b ICP Optical Emission Spectrometry

(1) Summary

The test method is applicable to sludge fertilizers, etc. This testing method is classified as Type D and its symbol is 4.10.1.b-2017 or T-Cu.b-1.

Pretreat an analytical sample with incineration, nitric acid−hydrochloric acid (1+3), introduce it to ICP Optical Emission Spectrometry (ICP-OES) and measure the emission with copper at a wavelength of 324.754 nm to quantify the total copper (T-Cu) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.
b) Nitric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
c) Hydrochloric acid:
d) Copper standard solution (Cu 0.1 mg/mL): A copper standard solution (Cu 0.1 mg/mL) traceable to National Metrology.
e) Copper standard solutions (Cu 25 µg/mL) (1): Dilute a predetermined amount of copper standard solution (Cu 0.1 mg/mL) with hydrochloric acid (1+23) to prepare a copper standard solution (Cu 25 µg/mL).

Note (1) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the copper standard solution in (**2**), a copper standard solution for the calibration curve preparation can be prepared by using a copper standard solution (Cu 1 mg/mL or 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) ICP Optical Emission Spectrometer: An optical emission spectrometer specified in JIS K 0116.
  1) Gas: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity
b) Electric furnace: An electric furnace that can keep the test temperature at 450 °C ± 5 °C .
c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char (2).
c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (2).
d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
f) Slightly move the watch glass (3), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (4) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
i) As a blank test, conduct the procedures in **b)** - **h)** using another tall beaker to prepare a blank test solution.

Note (**2**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.
(**3**) The watch glass can be removed.
(**4**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 3 Do not conduct the procedures in (**4.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.
Comment 4 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.
a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 324.754 nm
b) **Calibration curve preparation and sample measurement**
1) Put 5mL of sample solution in three 10-mL volumetric flasks respectively.
2) Add 2mL and 4 mL of copper standard solution (25 µg/mL) to volumetric flasks of **1**) above, then add hydrochloric acid (1+23) to the marked line to make a sample solution of Standard Addition Method.
3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1**) above to make a sample solution without a standard solution.
4) Spray the sample solution of Standard Addition Method and the sample solution without a standard solution into the induction plasma, and read the indicated value at a wavelength of 324.754 nm.
5) Transfer 5 mL of blank test solution to a 10-mL volumetric flask, conduct the same procedures as in **3**) - **4**) to read the indicated value, and correct the indicated value obtained from the respective sample solutions.
6) Prepare a curve for the relationship between the added copper concentration and the corrected indicated value of the sample solution for Standard Addition Method and the sample solution without a standard solution.
7) Obtain the copper content from the intercept of the calibration curve to calculate the total copper (T-Cu) in the analytical sample.

Comment 5 The copper concentration in the analytical sample can also be corrected by subjecting the blank test solution to the same procedures as in **b**) **1**) - **b**) **4**) and **b**) **6**) - **b**) **7**) to obtain the copper content in the blank test solution.
Comment 6 The simultaneous measurement of multiple elements is possible with the ICP-OES. In

that case, see **4.9.1.b Comment 6**.

Comment 7 The comparison of the measurement value (x_i : 12.0 mg/kg - 1400 mg/kg) of ICP Optical Emission Spectrometry and the measurement value (y_i) of Flame Atomic Absorption Spectrometry was conducted to evaluate trueness using sludge fertilizers (49 samples). As a result, a regression equation was $y=-5.5+1.062x$ and its correlation coefficient (r) was 0.997. Triplicates measurement for each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer, calcined sludge fertilizers and composted sludge fertilizer was conducted. As a result, a repeatability obtained was 0.6 % - 1.8 % as a relative standard deviation.

Note that the minimum limit of quantification of this testing method was estimated to be about 3 mg/kg.

References

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI and Tetuya NOMURA: Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer, **Vol. 4**, p. 30 - 35 (2011)

(5) **Flow sheet for total copper**: The flow sheet for total copper in fertilizers is shown below:

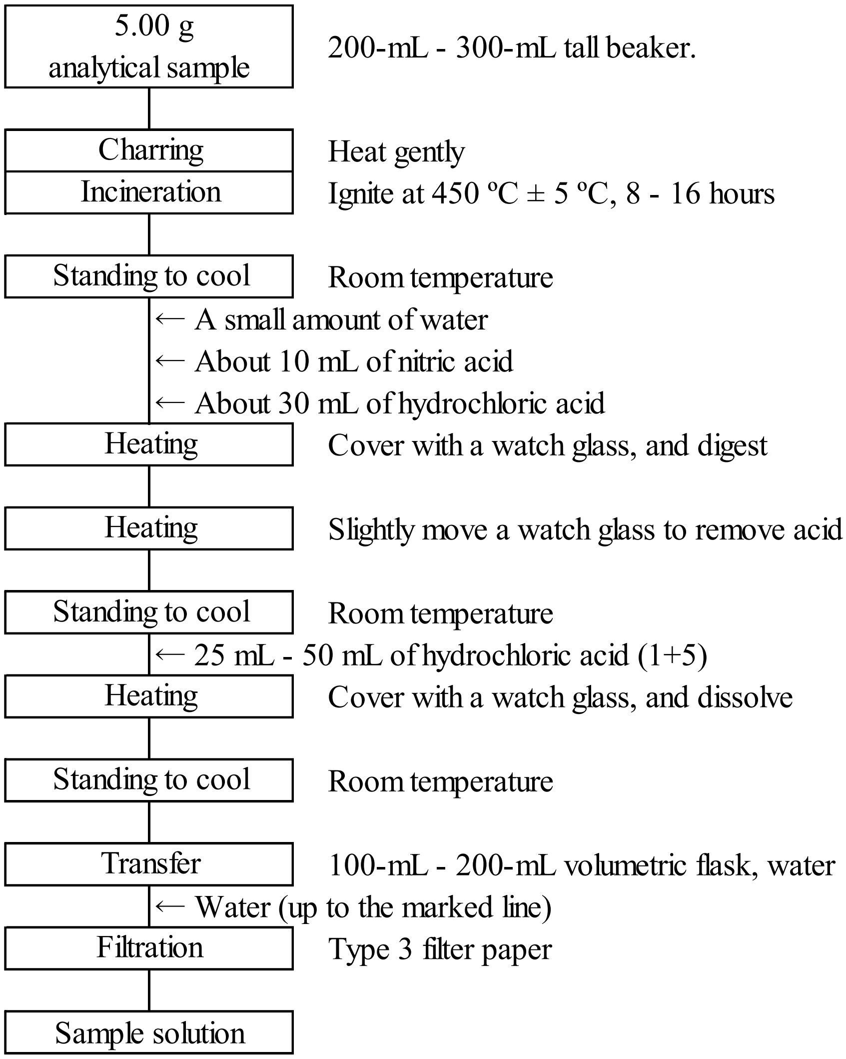


Figure 1 Flow sheet for total copper in fertilizers (Extraction procedure)

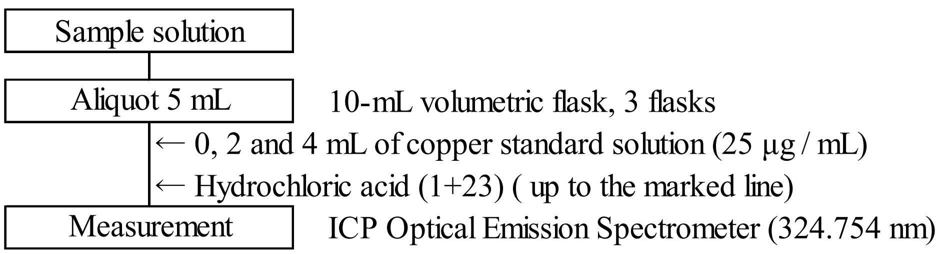


Figure 2 Flow sheet for total copper in fertilizers (Measurement procedure)

4.10.2 Water-soluble copper

4.10.2.a Flame atomic absorption spectrometry

(1) Summary

This testing method is applicable to fertilizers that indicate copper content as a response modifier. This testing method is classified as Type D and its symbol is 4.10.2.a-2017 or W-Cu.a-1.

Extract by adding water to an analytical sample, spray in an acetylene−air flame and measure the atomic absorption with copper at a wavelength of 324.8 nm to obtain water-soluble copper (W-Cu) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

c) Copper standard solution (Cu 0.1 mg/mL): A copper standard solution (Cu 0.1 mg/mL) traceable to National Metrology.

d) Copper standard solutions (Cu 0.5 µg - 5 µg/mL) for the calibration curve preparation (1): Put 2.5 mL - 25 mL of copper standard solution (Cu 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

e) Blank test solution for the calibration curve preparation (1): Hydrochloric acid (1+23) used in the procedures in **d**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the copper standard solution in (**2**), a copper standard solution for the calibration curve preparation can be prepared by using a copper standard solution (Cu 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(3) Instruments: Instruments are as shown below:

a) Vertical rotating mixer: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

b) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.

1) **Light source**: A copper hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

Note (**2**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 5.00 g of an analytical sample, and put it in a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Comment 2 In the procedure in (**4.1.1**) **a**), it is also allowed to weigh 2.50 g of the analytical sample and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Fluid test sample**

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 324.8 nm

b) **Calibration curve preparation**

1) Spray the copper standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 324.8 nm.
2) Prepare a curve for the relationship between the copper concentration and the indicated value of the copper standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.05 mg - 0.5 mg as Cu) in a 100-mL volumetric flask.
2) Add about 25 mL of hydrochloric acid (1+5), and add water up to the marked line.
3) Subject to the same procedure as in **b**) **1**) to read the indicated value.
4) Obtain the copper content from the calibration curve, and calculate the water-soluble copper (W-Cu).

Comment 5 Recovery testing was conducted to evaluate trueness using a preparation sample (solid). As a result, the mean recovery rates at the content level of 10 % (mass fraction), 1 % (mass fraction) and 0.03 % (mass fraction) were 100.7 %, 99.4 % and 102.6 % as water-soluble copper (W-Cu) respectively. In addition, recovery testing was conducted using a preparation sample (fluid). As a result, the mean recovery rates at the content level of 1 % (mass fraction), 0.05 % (mass fraction) and 20 mg/kg were 98.8 %, 99.3 % and 101.4 % as water-soluble copper respectively.

The results of the repeatability tests on different days using a fluid compound fertilizer and a liquid microelement compound fertilizer to evaluate the extract precision of fluid fertilizers were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 10 mg/kg for solid fertilizers and about 3 mg/kg for fluid fertilizers.

Table 1 Analysis results of the repeatability tests on different days of water-soluble copper (Fluid fertilizer)

Name of sample	Test days of repeatability T[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Fluid compound fertilizer	7	0.0540	0.0003	0.6	0.0007	1.3
Liquid microelement compound fertilizer	7	0.0172	0.0001	0.7	0.0003	1.5

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.254- 255, Yokendo, Tokyo (1988)

2) Shin ABE and Yoshiyuki SUNAGA: Verification of Performance Characteristics of Testing Methods for Copper in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer, **Vol. 6**, p. 165 - 173 (2013)

3) Shinji KAWAGUCHI: Simple Extraction Method for Water-Soluble Components in Liquid Compound Fertilizers, Research Report of Fertilizer, **Vol. 9**, p. 10 - 20 (2016)

(5) Flow sheet for water-soluble copper: The flow sheet for water-soluble copper in fertilizers is shown below:

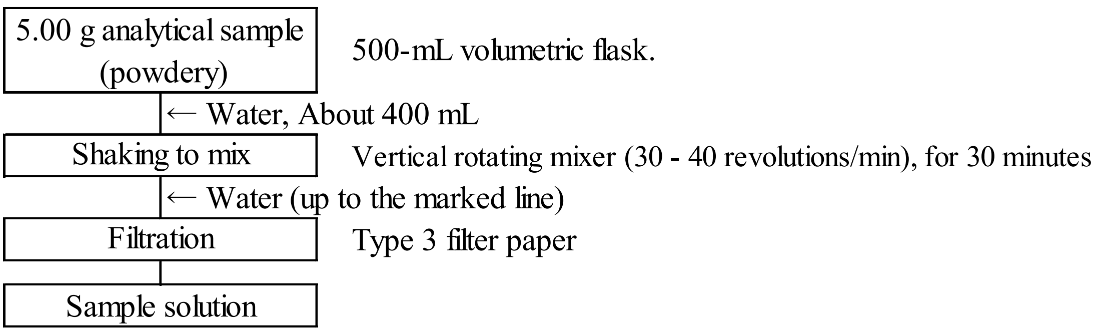


Figure 1-1 Flow sheet for water-soluble copper in fertilizers (Extraction procedure (4.1.1))

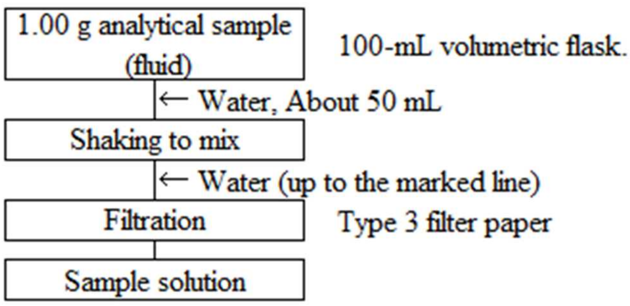


Figure 1-2 Flow sheet for water-soluble copper in fertilizers (Extraction procedure (4.1.2))

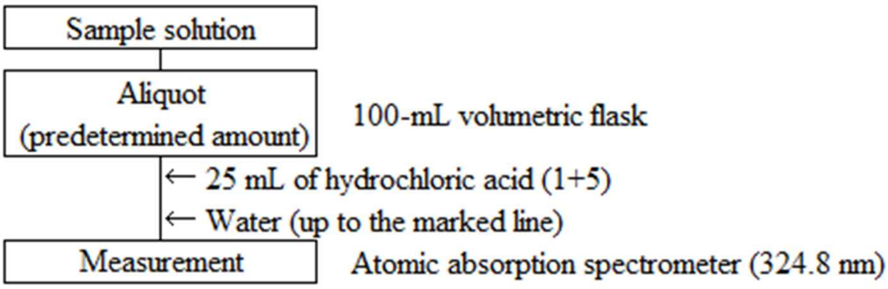


Figure 2 Flow sheet for water-soluble copper in fertilizers (Measurement procedure)

4.10.2.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fluid compound fertilizers, liquid microelement compound fertilizers and the fluid fertilizers of home garden-use compound fertilizers. This testing method is classified as Type B and its symbol is 4.10.2.b-2017 or W-Cu.b-1.

Extract by adding water to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the copper at a wavelength of 327.396 nm, etc. to obtain water-soluble copper (W-Cu) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Copper standard solution (Cu 1 mg/mL)**: A copper standard solution (Cu 1 mg/mL) traceable to National Metrology.

d) **Copper standard solutions (Cu 0.1 mg/mL) for the calibration curve preparation** (1): Put 10 mL of copper standard solution (Cu 1 mg/mL) in 100-mL volumetric flask, and add hydrochloric acid (1+23) to the marked line.

e) **Copper standard solutions (Cu 1 µg - 20 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of copper standard solution (Cu 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

f) **Copper standard solutions (Cu 0.1 µg - 1 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 10 mL of copper standard solution (Cu 10 µg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**), **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the copper standard solution in (**2**), a copper standard solution for the calibration curve preparation can be prepared by using a copper standard solution (Cu 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample (2) to the order of 1 mg, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, shake to mix and add water up to the marked line.

c) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) The sampling amount of the analytical sample is 10 g when the content in the sample is less than 0.01 % (mass fraction) as water-soluble copper.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following: Analytical line wavelength: 327.396 nm, 224.700 nm or 324.754 nm (3)

b) **Calibration curve preparation**

1) Spray the copper standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at an analytical line wavelength.

2) Prepare a curve for the relationship between the copper concentration and the indicated value of the copper standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 2 mg as Cu) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b)** **1)** to read the indicated value.

4) Obtain the copper content from the calibration curve, and calculate the water-soluble copper (W-Cu).

Note (**3**) 224.700 nm or 324.754 nm can also be used. However, since the intensity of emission obtained is different from the one of 327.396 nm, it is necessary to understand the suitable concentration range of the calibration curve and prepare a standard solution for the calibration curve in advance.

Comment 4 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 5 The comparison of the measurement value (y_i : 0.00982 % (mass fraction) - 0.0819 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of flame atomic absorbance spectrometry was conducted to evaluate trueness using fluid fertilizers (12 samples). As a result, a regression equation was $y=-0.0006+0.966x$, and its correlation coefficient (r) was 0.999. Additionally, additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 0.01 % (mass fraction) and 0.1 % (mass fraction) were 93.5 % and 95.3 % respectively.

The results of the repeatability tests on different days using a fluid compound fertilizer

and a home garden-use compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study implemented for testing method validation and its analysis are shown in Table 2.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.0005 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of water-soluble copper (Fluid fertilizer)

Name of sample	Test days of repeatability T 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluid compound fertilizer	7	0.0643	0.0006	0.9	0.0011	1.7
Home garden-use compound fertilizer (fluid)	7	0.00976	0.00006	0.6	0.00033	3.4

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble copper

Analytical line wavelength (nm)	Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
327.396	Preparation sample (Liquid) 1	12	2.14	0.03	1.2	0.06	2.6
	Preparation sample (Liquid) 2	11	0.533	0.006	1.1	0.010	1.8
	Preparation sample (Liquid) 3	11	1.09	0.007	0.6	0.02	1.6
	Preparation sample (Liquid) 4	11	0.113	0.0005	0.4	0.005	4.1
	Preparation sample (Liquid) 5	11	0.0530	0.0005	1.0	0.0010	1.9
224.700	Preparation sample (Liquid) 1	10	2.12	0.01	0.7	0.03	1.2
	Preparation sample (Liquid) 2	10	0.535	0.005	1.0	0.008	1.4
	Preparation sample (Liquid) 3	10	1.09	0.006	0.6	0.01	1.0
	Preparation sample (Liquid) 4	10	0.111	0.0005	0.4	0.002	1.5
	Preparation sample (Liquid) 5	10	0.0527	0.0006	1.1	0.0008	1.5
324.756	Preparation sample (Liquid) 1	12	2.14	0.03	1.2	0.05	2.4
	Preparation sample (Liquid) 2	11	0.534	0.050	0.8	0.011	2.0
	Preparation sample (Liquid) 3	11	1.09	0.007	0.7	0.02	1.7
	Preparation sample (Liquid) 4	11	0.111	0.0011	1.0	0.002	1.8
	Preparation sample (Liquid) 5	11	0.0532	0.0004	0.8	0.0009	1.8

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination Method for Effect-Development Promoting Agent (Ca, Fe, Co, Cu, Zn and Mo) in Liquid Compound Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), Research Report of Fertilizer, **Vol. 9**, p. 1 – 9 (2016)

2) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(**5**) **Flow sheet for water-soluble copper**: The flow sheet for water-soluble copper in fluid fertilizers is shown below:

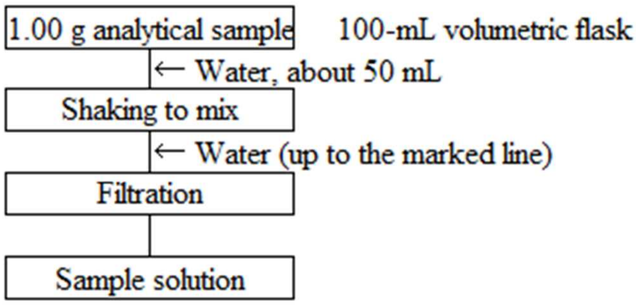


Figure 1 Flow sheet for water-soluble copper in fluid fertilizers (Extraction procedure)

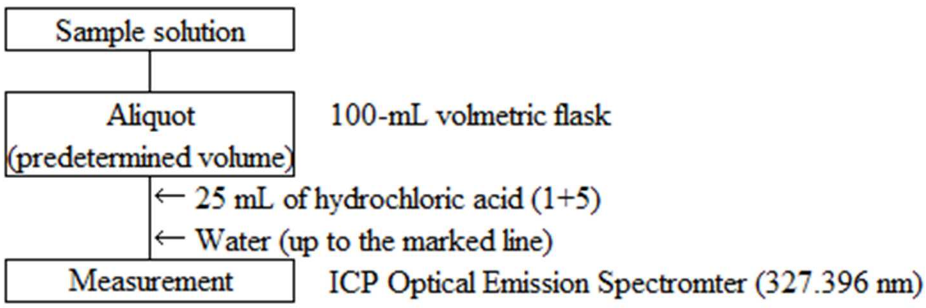


Figure 2 Flow sheet for water-soluble copper in fluid fertilizers (Measurement procedure)

4.11 Organic carbon and carbon-nitrogen ratio
4.11.1 Organic carbon
4.11.1.a Dichromate oxidation
(1) Summary

This test method is applicable to sludge fertilizers and compost, etc. This testing method is classified as Type C and its symbol is 4.11.1.a-2017 or O-C.a-1.

Add a potassium dichromate-sulfuric acid solution to an analytical sample and heat to oxidize organic carbon with potassium dichromate. Quantify unconsumed potassium dichromate by oxidation-reduction titration to obtain organic carbon (O-C). This test method is also referred to as Tyurin's method. In addition, the performance of this testing method is shown in **Comment 2**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.2 mol/L ammonium iron (II) sulfate solution** (1): Weigh 80 g of ammonium iron (II) sulfate hexahydrate specified in JIS K 8979 into a 2000-mL beaker, and add 1000 mL of sulfuric acid (1+50) to dissolve.

Standardization: Grind potassium dichromate reference material for volumetric analysis specified in JIS K 8005 in an agate mortar to powder, heat at 150 ºC ± 2 ºC for 1 hour, let it stand to cool in a desiccator, and then transfer about 1 g to a weighing dish, and weigh the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 100-mL volumetric flask, and add water up to the marked line to make the potassium dichromate standard solution (1) (2). On each day to use a 0.2 mol/L ammonium iron (II) sulfate solution, put 10 mL of the potassium dichromate standard solution in a 100-mL Erlenmeyer flask, add about 5 mL of sulfuric acid (1+2), and then conduct the procedures in (**4.2**) **b**) - **c**), and calculate the factor of a 0.2 mol/L ammonium iron (II) sulfate solution by the following formula:

Factor (f) of 0.2 mol/L ammonium iron (II) sulfate solution

$$= W_1 \times (A/100) \times (6/294.18) \times (V_1/V_2) \times (1000/V_3)/C$$

$$= (W_1 \times A/V_3) \times (30/294.18)$$

W_1 : Mass (g) of potassium dichromate weighed

A : Purity (% (mass fraction)) of potassium dichromate

V_1 : Aliquot volume (10 mL) of potassium dichromate solution

V_2 : Constant volume (100 mL) of potassium dichromate solution

V_3 : Volume (mL) of 0.2 mol/L ammonium iron (II) sulfate solution needed for titration

C : Set concentration (0.2 mol/L) of 0.2 mol/L ammonium iron (II) sulfate solution

d) **Potassium dichromate-sulfuric acid solution** (1): Weigh 40 g of potassium dichromate specified in JIS K 8517 to a 3000-mL beaker. Add 1000 mL of water to dissolve, and further add gradually 1000 mL of sulfuric acid while cooling and mixing.

e) **N-Phenylanthranilic acid solution**: Dissolve 0.2 g of N-phenylanthranilic acid of no less than 98 % (mass fraction) in purity and 0.2 g of sodium carbonate specified in JIS K 8625 in a small amount of water, and add water to make 100 mL.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This corresponds to the standard potassium dichromate solution (0.2 M (1/6 $K_2Cr_2O_7$) solution) in **7.1 B 1**) in the Official Methods of Analysis of Fertilizers (1992).

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

b) Sample digestion flask (3): A 100-mL borosilicate glass volumetric flask 100 mL (180 mm total height, 13 mm mouth diameter)

Note **(3)** Distinguish the volumetric flask used in digestion as a sample digestion flask and do not use it for any other purposes.

(4) Test procedure

(4.1) Dichromate oxidation: Conduct oxidation as follows:

a) Weigh 0.05 g of an analytical sample to the order of 0.1 mg (4), and put it in a sample digestion flask.

b) Add 25 mL of potassium dichromate-sulfuric acid solution.

c) Heat on a hot plate at 200 ºC until organic matters are completely digested (5).

d) After immediate cooling is complete, precisely adjust to 100 mL with water to make a sample solution.

e) As a blank test, conduct the procedures in **b)** and **d)** using another sample digestion flask to prepare a blank test solution.

Note **(4)** Up to about 28 mg as organic carbon (O-C).

(5) Heat for no less than 1 hour after boiling

Comment 1 Sample an analytical sample from a test sample prepared in **2.3.3 Grinding** (**3.1**) by grinding with a mill until it completely passes through a sieve of 500 µm aperture or from a test sample prepared in **2.3.3 Grinding Comment 1.**

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0116 and as shown below.

a) Put 20 mL of the sample solution in a 100-mL Erlenmeyer flask.

b) Add a 0.2 mol/L ammonium iron (II) sulfate solution drop-by-drop until the brown color of dichromate ion almost disappears from the sample solution.

c) Add about a 0.25 mL of N-phenylanthranilic acid solution (6), and titrate with a 0.2 mol/L ammonium iron (II) sulfate solution until the color of the solution changes from dark red-purple to blue-green.

d) Put 20 mL of the blank test solution in a 100-mL Erlenmeyer flask, and conduct the procedures in **b)** - **c)** to titrate.

e) Calculate the organic carbon (O-C) in the analytical sample by the following formula:

Organic carbon (% (mass fraction)) in the analytical sample

$$= (V_4 - V_5) \times C \times f \times (12.011/4)/W_2 \times (100/1000) \times (V_6/V_7)$$

$$= (V_4 - V_5) \times f \times (12.011/40)/W_2$$

V_4 : Volume (mL) of 0.2 mol/L ammonium iron (II) sulfate solution needed for the titration of the blank test solution

V_5 : Volume (mL) of 0.2 mol/L ammonium iron (II) sulfate solution needed for the titration of the sample solution

C : Set concentration (0.2 mol/L) of 0.2 mol/L ammonium iron (II) sulfate solution

f : Factor of 0.2 mol/L ammonium iron (II) sulfate solution

V_6 : Constant volume (100 mL) of the sample solution and the blank test solution in (**4.1**) **d)**

V_7 : Aliquot volume (20 mL) of the sample solution and the blank test solution subjected to titration in (**4.2**) **a**) and (**4.2**) **d**)

W_2 : Mass (g) of the analytical sample

Note (**6**) About 5 drops with a 1-mL - 2-mL Komagome pipette. Add the same volume to the sample solution and the blank test solution.

Comment 2 The results of the collaborative study to determine a certified reference material fertilizer were analyzed using a three-level nesting analysis of variance. Table 1 shows the calculation results of reproducibility, intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to be about 1.5 % (mass fraction).

Table 1 Analysis results of the collaborative study to determine organic carbon of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)	s_R[8)] (%)[3)]	RSD_R[9)] (%)
FAMIC-C-12	12	20.2	0.3	1.5	0.5	2.3	0.6	3.1

1) The number of laboaratories used for analysis conducting Dichromate oxidation
2) Mean (the number of laboratory(p) × test days(2) ×the number of replicate testing(3))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation
8) Reproducibility standard deviation
9) Reproducibility relative standard deviation

References

1) Yuji SHIRAI, Yuko SEKINE, and Toshiaki HIROI: Validation of Determination Method for Organic Form Carbon in Sludge Fertilizer and Compost, Research Report of Fertilizer, **Vol. 3**, p. 117 - 122 (2010)

(5) Flow sheet for organic carbon: The flow sheet for organic carbon in sludge fertilizers and compost, etc. is shown below:

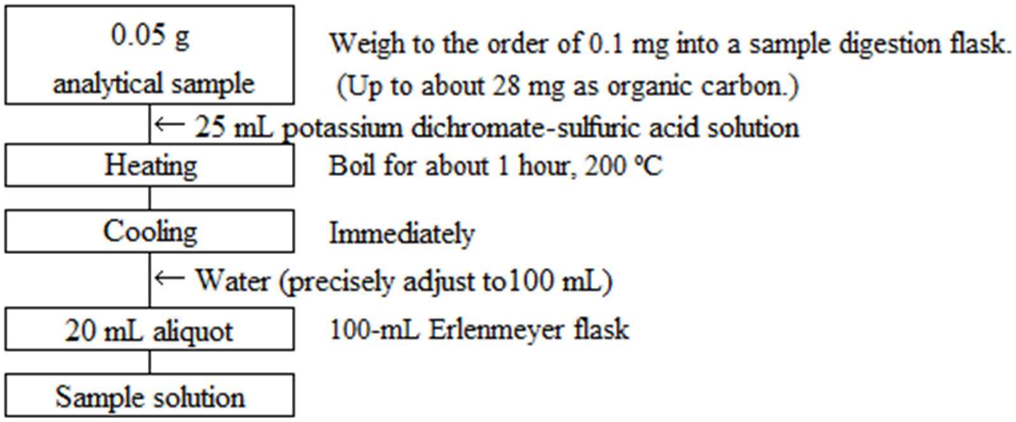


Figure 1 Flow sheet for organic carbon in sludge fertilizers and compost, etc.
(Dichromate oxidation procedure)

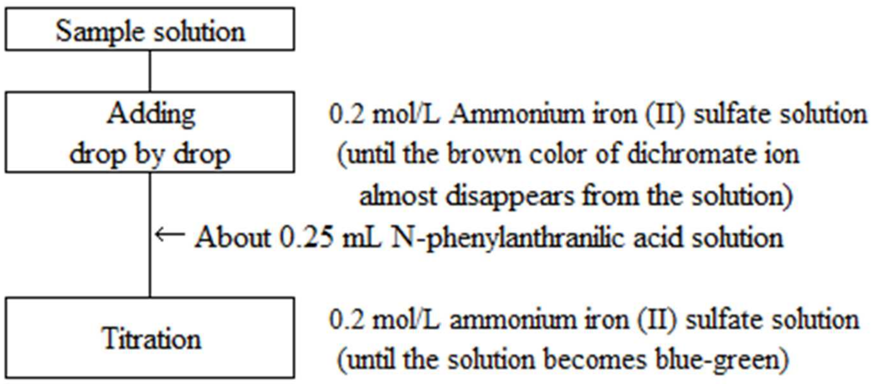


Figure 2 Flow sheet for organic carbon in sludge fertilizers and compost, etc.
(Measurement procedure)

4.11.1.b Combustion method

(1) Summary

This testing method is applicable to compost and sludge fertilizers. This testing method is classified as Type B and its symbol is 4.11.1.b-2017 or O-C.b-1.

Add hydrochloric acid (1+3) drop by drop to an analytical sample and evaporate inorganic carbon as carbon dioxide, then thermally decompose carbon compounds using a total nitrogen-total carbon analyzer by the combustion method to measure carbon dioxide gas with a thermal conductivity detector and obtain organic carbon (O-C) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 4**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Sea sand**: Particle diameter 425 µm - 850 µm

b) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

Comment 1 Sea sand (particle diameter 425 µm - 850 µm) is commercially sold by FUJIFILM Wako Pure Chemical Co., Ltd. and YONEYAMA YAKUHIN KOGYO Co., Ltd.

(**3**) **Instruments**: Instruments are as shown below:

a) **Total nitrogen-total carbon analyzer by the combustion method**: A total nitrogen-total carbon analyzer configured on the basis of the principle of the combustion method (modified Dumas' method).

1) Turn on the total nitrogen-total carbon analyzer by the combustion method (1), and adjust so that stable indicated values can be obtained.

(i) Combustion gas: Oxygen having purity no less than 99.99 % (volume percentage)

(ii) Carrier gas: Helium having purity no less than 99.99 % (volume percentage)

b) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

c) **Drying apparatus**: A drying apparatus that can be adjusted to 105 ºC ± 2 ºC.

Note (**1**) The setup of the program and the parameters of the analyzer are according to the specification and the operation method of the total nitrogen-total carbon analyzer by the combustion method used.

(**4**) **Test procedures**: Conduct measurement as shown below. However, confirm that there is no difference from the measured value of organic carbon obtained in advance according to **4.11.1**.**a** by using an analytical sample.

(**4.1**) **Hydrochloric acid treatment**

a) Weigh 0.05 g of an analytical sample to the order of 0.1 mg, and put it in a container for combustion.

b) Cover the analytical sample with about 0.2 g of sea sand and add a few drops of water drop by drop to moisten the analytical sample.

c) After adding 0.5 mL - 0.7 mL of hydrochloric acid (1+3) little by little (2), add about 0.3 mL of water drop by drop (3) (4).

d) Heat a container for combustion on a hot plate at 100 ºC for 90 minutes to exsiccate it.

e) Put the container for combustion at 105 °C ± 2 °C into a drying apparatus and heat for 30 minutes (5).

f) After heating, let it stand to cool to make a sample for measurement.

Note (**2**) The additive amount of hydrochloric acid (1+3) is merely a target. It is enough to make the whole analytical sample come into contact with hydrochloric acid. Let it stand for a short time in the case of producing bubbles.

(3) In some cases, it is not necessary to add water due to the capacity of a container.

(4) Shake calmly the container for combustion to make the analytical sample come into complete contact with hydrochloric acid.

(5) Remove hydrochloric acid completely

Comment 2 Sample an analytical sample from a test sample prepared in **2.3.3 Grinding** (**3.1**) by grinding with a mill until it completely passes through a sieve of 500 μm aperture or from a test sample prepared in **2.3.3 Grinding Comment 1**.

Comment 3 When it is confirmed that, for example, the volatilization of hydrogen chloride is not detected by using a test paper, etc. and hydrochloric is completely removed in the procedure **d**), the procedure **e**) can be skipped.

(**4.2**) **Measurement**: Specific measurement procedures are according to the operation method of a total nitrogen-total carbon analyzer by the combustion method.

a) **Measurement conditions for the total nitrogen-total carbon analyzer by the combustion method**: Set up the measurement conditions for the total nitrogen-total carbon analyzer considering the following:

Combustion temperature: No less than 870 ºC

b) **Calibration curve preparation**

1) Turn on the total nitrogen-total carbon analyzer by the combustion method (1), and adjust so that stable indicated values can be obtained.

2) Weigh a predetermined amount of the standard for calibration curves (6) to the order of 0.1 mg into a combustion vessel.

3) Insert the combustion vessel into the total nitrogen-total carbon analyzer by the combustion method, and read the indicated value.

4) Conduct the procedure in **3**) for another combustion vessel for a blank test, and read the indicated value.

5) Prepare a curve for the relationship between the carbon content and the indicated value of the standard for calibration curves and the blank test for calibration curves.

c) **Sample measurement**

1) Insert the combustion vessel containing the sample for measurement to the total nitrogen-total carbon analyzer by the combustion method, and read the indicated value.

2) Obtain the carbon content from the calibration curve, and calculate organic carbon in the analytical sample.

Note (**6**) Standard for calibration curves: DL-Aspartic acid (purity no less than 99 % (mass fraction)), EDTA (purity no less than 99 % (mass fraction)), hippuric acid (purity no less than 98 % (mass fraction)) or other reagents having equivalent purity recommended by the total nitrogen-total carbon analyzer by the combustion method used.

Comment 4 The comparison of the measurement value (y_i : 0.21 % (mass fraction) - 45.40 % (mass fraction)) of Combustion method and the measurement value (x_i) of Dichromate oxidation was conducted to evaluate trueness using sludge fertilizers and compost (total 25 samples). As a result, a regression equation was y=0.004+1.009x and its correlation coefficient (r) were 0.999.

Results from a collaborative study for test method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.05 % (mass fraction).

Table 1 Results and statistical analysis results from the collaborative study for the organic carbon method

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Human waste sludge fertilizer	8	34.96	0.07	0.2	0.62	1.8
Industrial sludge fertilizer	8	15.13	0.20	1.3	0.42	2.8
Calcined sludge fertilizer	9	9.45	0.17	1.8	0.38	4.0
Composted sludge fertilizer	9	38.20	0.27	0.7	0.73	1.9
Compost	9	20.50	0.76	3.7	0.94	4.6

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Aiko YANO, Satono AKIMOTO, and Yuji SHIRAI: Determination of Organic Carbon in Sludge Fertilizer and Compost by Hydrochloric Acid-Treated Combustion Method, Research Report of Fertilizer, **Vol. 6**, p. 9 - 19 (2013)
2) Aiko YANO and Yuji SHIRAI: Determination of Organic Carbon in Sludge Fertilizer and Compost by Hydrochloric Acid-Treated Combustion Method: A Collaborative Study, Research Report of Fertilizer, **Vol. 7**, p. 22 - 27 (2014)

(4) **Flow sheet for organic carbon**: The flow sheet for organic carbon in sludge fertilizers and compos is shown below:

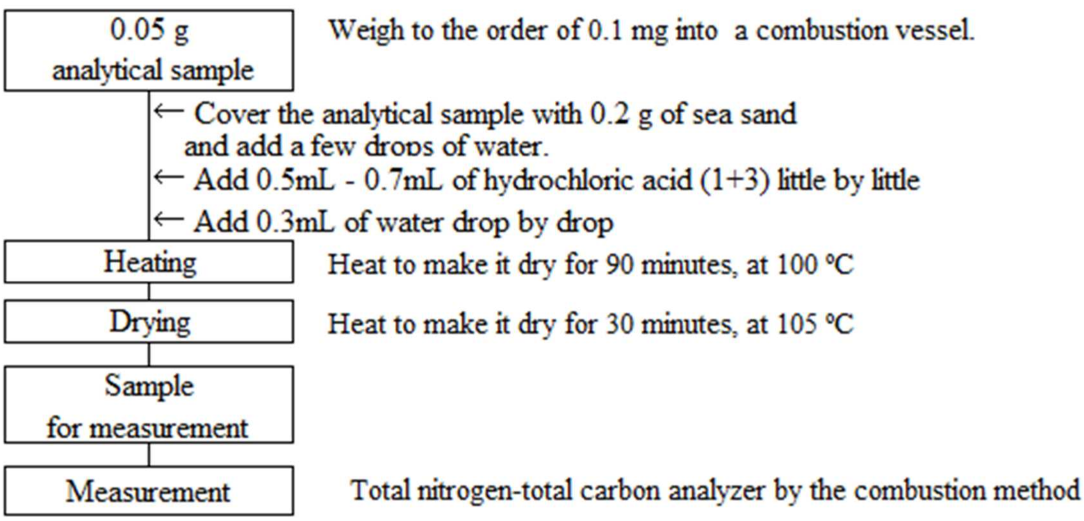
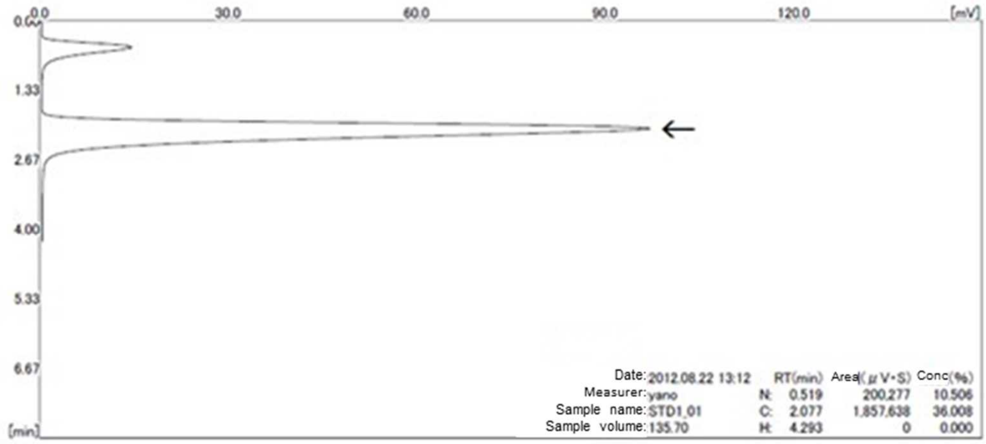
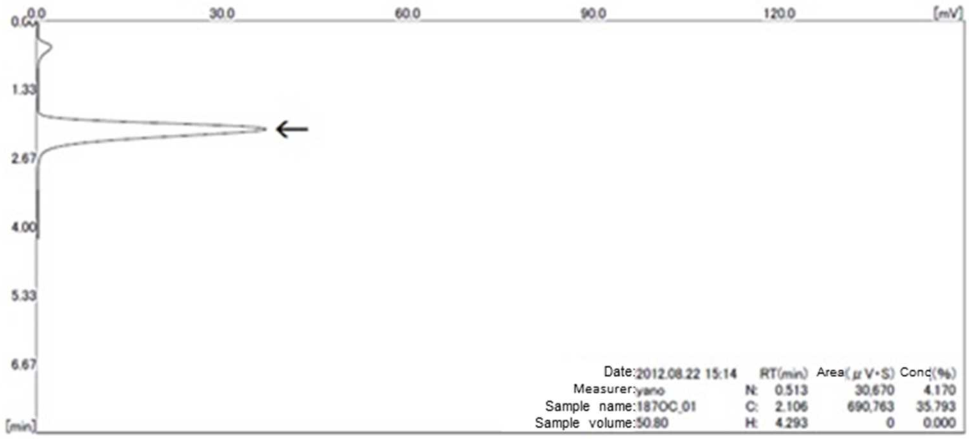


Figure Flow sheet for organic carbon by the combustion method

Reference: Chromatograms of the standard for calibration curves and an analytical sample are shown below:



1) Total carbon in a standard for calibration curve (DL-Aspartic acid)



2) Total carbon in an analytical sample (sludge fertilizer)

Reference figures: Chromatograms of organic carbon.

Measurement conditions for total nitrogen-total carbon analyzer by the combustion method

Combustion gas: Highly pure oxygen, purity no less than 99.99995 % (volume fraction), flow rate 200 mL/min

Carrier gas: Highly pure helium, purity no less than 99.9999 % (volume fraction), flow rate 80 mL/min

Separation column: A silica gel stainless column (length 1m)

Detector: Thermal conductivity detector (TCD)

Measurement cycle: Purge time = 60 seconds, circulation combustion time = 300 seconds, measurement time = 270 seconds

Current value of Detector: 160 mA

Temperature conditions: Reaction furnace temperature: 870 ºC
Reaction furnace temperature: 600 °C
Column oven temperature: 70 °C
Detector temperature: 100 °C

4.11.2 Carbon-nitrogen ratio

4.11.2.a Calculation with organic carbon and total nitrogen

(1) Summary

This test method is applicable to compost and sludge fertilizers. This testing method is classified as Type A (Def-C) and its symbol is 4.11.2.a-2017 or C/N.a-1.

Calculate carbon-nitrogen ratio (CN ratio) by dividing the organic carbon obtained in **4.11.1** by the total nitrogen obtained in **4.1.1**.

(2) The calculation of carbon-nitrogen ratio

a) Calculate the carbon-nitrogen ratio (CN ratio) in an analytical sample by the following formula:

Carbon-nitrogen ratio in an analytical sample
= O-C/T-N

O-C : Organic carbon (% (mass fraction)) [(1)] in the analytical sample obtained in **4.11.1**

T-N : Total nitrogen (% (mass fraction)) [(1)] in the analytical sample obtained in **4.1.1**

Note **(1)** O-C and T-N use raw data without rounding numerical value

4.12 Sulfur
4.12.1 Total sulfur content
4.12.1.a Potassium permanganate analysis

(1) Summary

This testing method is applicable to fertilizers mainly containing ferrous sulfate (iron (II) sulfate) ($FeSO_4$)) among sulfur and its compound. This testing method is classified as Type D and its symbol is 4.12.1.a-2017 or T-S.a-1.

Dissolve an analytical sample in water and diluted sulfuric acid, add phosphoric acid and then titrate ferrous sulfate (iron (II) sulfate) ($FeSO_4$)) by oxidation-reduction with a potassium permanganate solution to obtain the total sulfate (T-SO_3). In addition, the performance of this testing method is shown in **Comment 1**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **Phosphoric acid**: A JIS Guaranteed Reagent specified in JIS K 9005 or a reagent of equivalent quality.

d) **0.02 mol/L potassium permanganate solution**: Dissolve 3.16 g of potassium permanganate specified in JIS K 8247 in about 800 mL of water to boil and add water to make 1000 mL and leave at rest 1 - 2 day(s). Further filter with a funnel type glass filter (G4) and store in a colored bottle. Or, a reagent of equivalent quality (volumetric analysis grade) that is commercially available.

Standardization: Dry sodium oxalate of reference materials for volumetric analysis specified in JIS K 8005 at 200 ºC for 1 hour and let it stand to cool in a desiccator, and then put about 0.3 g into a weighing dish to measure the mass to the order of 0.1 mg. Add about 250 mL of sulfuric acid (1+20) cooled down to 25 ºC - 30 ºC after boiling and dissolve. Add about 40 mL of 0.02 mol/L potassium permanganate solution while gently shaking for about 1 minute. Heat up to 55 ºC - 60 ºC after the red color of a potassium permanganate solution disappears. Titrate with a 0.02 mol/L potassium permanganate solution while keeping the temperature and continue titrating until the color of the solution becomes light red (1). Calculate the factor of a 0.02 potassium permanganate solution by the following formula.

Factor (f) of 0.02 mol/L potassium permanganate solution

$$= W_1 \times (A/100) \times ((2/5)/133.999) \times ((1000/V_1)/C)$$
$$= W_1 \times (A/V_1) \times 1.4925$$

W_1 : Mass (g) of sodium oxalate sampled

A : Purity (% (mass fraction)) of sodium oxalate sampled

V_1 : Volume (mL) of 0.02 mol/L potassium permanganate needed for titration

C : 0.02 mol/L potassium permanganate solution

Note **(1)** The endpoint is reached when the color of solution keeps as it is for 30 seconds after coloring

(3) Instruments: Instruments are as shown below:

a) **Magnetic stirrer**

(4) Test procedure

(4.1) Measurement: Conduct measurement as shown below.

a) Weigh 0.5 g - 1 g of an analytical sample to the order of 0.1 mg, and put it in a 200-mL tall beaker.
b) Add about 50 mL of water and about 15 mL of sulfuric acid (1+5) and shake with a magnetic stirrer to dissolve.
c) Immediately add about 1 mL of phosphate, and then titrate with a 0.02 mol/L potassium permanganate solution until the color of the solution becomes light red (2).
d) As a blank test, conduct the procedures in **b**) - **c**) using another 200-mL tall beaker to titrate (2).
e) Calculate the total sulfur content (T-SO_3) in an analytical sample by the following formula.

$$\text{Total sulfur content (\% (mass fraction))} = (5 \times 0.02 \times f \times (V_2 - V_3)/1000 \times 80.064)/W_2 \times 100$$
$$= (f \times (V_2 - V_3))/W_2 \times 0.80064$$

W_2 : Mass (g) of the sampled analytical sample
V_2 : Volume (mL) of 0.02 mol/L potassium permanganate needed for titration
V_3 : Volume (mL) of 0.02 mol/L potassium permanganate needed for titration
f : Factor of 0.02 mol/L potassium permanganate solution

Note (**2**) Titrate using a brown burette.

Comment 1 Recovery testing was conducted using a reagent (ferrous sulfate heptahydrate); as a result, the mean recovery rate was 29.1 % (mass fraction) as total sulfate content (T-SO_3). The recovery rate to a theoretical value was 101.0 %.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.04 % (mass fraction).

References

1) Yasushi SUGIMURA and Shinjiro IZUKA: Method validation of Redox Titration for Determination of Sulfur content (as sulfur trioxide) in Fertilizers of Ferrous sulfate and its mixture materials, Research Report of Fertilizer, **Vol. 3**, 25 - 29 (2010).
2) JIS K 8978 : Iron (II) sulfate heptahydrate (Reagent) (2008)

(**5**) **Flow sheet for total sulfur content**: The flow sheet for total sulfur content in fertilizers mainly containing ferrous sulfate is shown below.

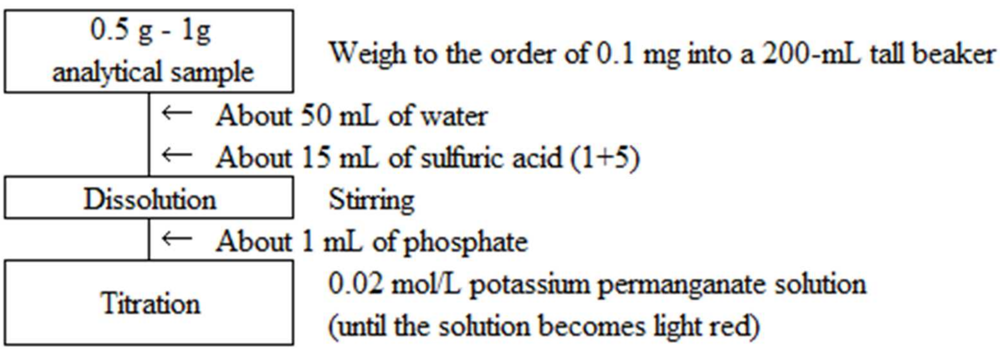


Figure Flow sheet for total sulfur content (raw material: ferrous sulfate)

4.12.1.b Barium chloride gravimetric analysis

(1) Summary

This test method is applicable to fertilizers mainly containing sulfur or sulfuric acid among sulfur and its compound. This testing method is classified as Type B and its symbol is 4.12.1.b-2017 or T-S.b-1. Dissolve an analytical sample in a potassium hydroxide-ethanol solution and add hydrogen peroxide to oxidize, and measure the mass of barium sulfate ($BaSO_4$) formed by reaction with barium chloride to obtain the total sulfate content (T-SO_3). In addition, the performance of this testing method is shown in **Comment 1**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Potassium hydroxide/ethanol solution**: Dissolve 10 g of potassium hydroxide specified in JIS K 8574 in 50 mL of ethanol (95) specified in JIS K 8102, further add 50 mL of water.

b) **Hydrogen peroxide**: A JIS Guaranteed Reagent (30 % (mass fraction)) specified in JIS K 8230 or a reagent of equivalent quality.

c) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

d) **Nitric acid**: A JIS Guaranteed (HNO_3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

e) **Barium chloride solution** (1): Dissolve 100 g of barium chloride dihydrate specified in JIS K 8155 in water to make 1000 mL.

f) **Silver nitrate solution (2 g/100mL)**: Dissolve 2 g of silver nitrate specified in JIS K 8550 in water to make 100 mL.

g) **Phenolphthalein solution (1 g/100 mL)**: Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Hot plate**: A hot plate whose surface temperature can be adjusted up to 250 ºC.

b) **Water bath**: Water bath that can be adjusted to 80 ºC - 90 ºC.

c) **Crucible**: After heating porcelain crucible or platinum crucible in an electric furnace at 800 ºC in advance, let it stand to cool in a desiccator and measure the mass to the order of 0.1 mg.

d) **Drying apparatus:** Drying apparatus that can be adjusted to 110 ºC - 120 ºC.

e) **Electric furnace**: An electric furnace that can be kept at 800 ºC ± 5 ºC.

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as follows (2):

a) Weigh 1 g - 5 g of an analytical sample to the order of 0.1 mg, and put it in a 200-mL tall beaker.

b) Add about 50 mL of potassium hydroxide/ethanol solution, cover with a watch glass and heat on a hot plate to boil (3).

c) After standing to cool, transfer to a 250-mL volumetric flask with water and add water up to the marked line.

d) Filter with Type 3 filter paper (4) to make a sample solution.

Note (**2**) Omit extraction if fluid fertilizers are made from only sulfuric acid and all materials are dissolved

(**3**) Until sulfur content is dissolved. About 5 minutes when raw materials, etc. are not dissolved.

(**4**) Omit the procedures in **d**) when all materials are dissolved.

(**4.2**) **Measurement**: Conduct measurement as shown below.

a) Put a predetermined volume (the equivalents of 30 mg - 170 mg as SO_3) of sample solution in a 300-mL tall beaker. (5)

b) Add about 50 mL of water and about 5 mL of hydrogen peroxide and heat in a water bath at 80 ºC - 90 ºC for about 1 hour while sometimes shaking (6).

c) After standing cool, add 1 - 2 drop(s) of phenolphthalein solution (1 g/100 mL) (7), and add hydrochloric acid (2+1) until the color of the solution disappears (8).

d) Further add hydrochloric acid (2+1), add water to make about 100 mL and boil on a hotplate for about 5 minutes.

e) Immediately add about 6 mL of thermal barium chloride solution (9) while sometimes shaking in a water bath at 80 ºC - 90 ºC (10).

f) After leaving at rest for a few minutes (11), add a few drops of thermal barium chloride solution and check that new precipitate of barium sulfate is no longer formed.

g) Further add about 2 mL of thermal barium chloride solution (100 g/L) while shaking to mix (12).

h) After heating in a water bath at 80 ºC - 90 ºC for about 2 hours, stop the heat source of water bath and let it stand to cool for no less than 4 hours. (6)

i) Filter with filter paper (Type 5 - C), wash a container with water and transfer the whole precipitate to a filter paper.

j) Wash the precipitate and the filter paper (Type 5 - C) with water several times (13).

k) Put the precipitate together with the filter paper into the crucible.

l) Put the crucible into a drying apparatus and dry at about 110 ºC - 120 ºC for 1 hour.

m) After standing to cool, put the crucible into an electric funnel and heat gently to char (14).

n) Ignite at about 800 ºC ± 5 ºC for 2 hours (14).

o) After ignition (15), move the crucible to a desiccator and let it stand to cool (16).

p) Measure the mass of crucible to the order of 0.1 mg.

q) Calculate the total sulfur content (T-SO_3) in an analytical sample by the following formula.

$$\text{Total sulfur content (\% (mass fraction))} = (A \times 0.343)/(W \times V_2/V_1) \times 100$$
$$= 34.3 \times A \times V_1/(W \times V_2)$$

A : Mass (g) of the precipitate in **p**)
W : Mass (g) of the analytical sample
V_1 : Constant volume (mL) of sample solution
V_2 : Volume (mL) of sample solution transferred

Note (5) Weigh 1g - 5g of an analytical sample to the order of 0.1 mg if the fluid fertilizers of the analytical sample are made from only sulfuric acid and all materials are dissolved.

(**6**) It can be suspended after the procedures end.

(**7**) A pH meter can be used for neutralization.

(**8**) Omit the procedures in **c**) if fluid fertilizers are made from only sulfuric acid and all materials are dissolved.

(**9**) Heated up to 70 ºC - 80 ºC in a water bath in advance.

(**10**) Add drop-by-drop.

(**11**) Until precipitate settles.

(**12**) Add a barium chloride solution slightly excessively to reduce the solubility of barium sulfate.

(**13**) Wash the precipitate until a white turbidity is no longer formed when about 5 mL of nitric acid (1+2) and about 1 mL of silver nitrate solution (2 g/100 mL) are added to about 20 mL of washing.

(**14**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 800 °C in 1 to 2 hours.

(15) To prevent a crucible from chipping, it is recommended to let it stand to cool gently in an electric furnace until the temperature of an electric furnace falls to no more than 200 ºC.

(16) Time to let it stand to cool in a desiccator should be constant. In the case of a porcelain crucible, it is about 45 - 60 minutes.

Comment 1 Testing was conducted using sulfur simple substance fertilizers containing no materials (2 samples); as a result, the quantitative value of the total sulfur content (T-SO_3) was 99.9 % - 100.1 % to a theoretical value.

Results from a collaborative study for test method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.4 % (mass fraction).

Table 1 Analysis results of the collaborative study for total sulfur content with the barium chroride gravimetric analysis (analized as surfur (S))

Sample name	Number of laboratories[1)]	Mean[2)] (%)[8)]	Mean[3)] (%)[8)]	s_r[4)] (%)[8)]	RSD_r[5)] (%)	s_R[6)] (%)[8)]	RSD_R[7)] (%)
Surfur material a	8	8.32	3.33	0.02	0.7	0.05	1.4
Surfur material b	10	12.71	5.09	0.03	0.6	0.14	2.8
Surfur material c	9	247.6	99.17	0.24	0.2	1.39	1.4
Surfur material d	8	245.6	98.37	0.18	0.2	0.30	0.3
Surfuric acid material e	8	1.41	0.564	0.002	0.4	0.003	0.6
Surfuric acid material f	9	2.89	1.157	0.001	0.1	0.010	0.9

1) Number of laboratories used in analysis
2) Total mean as surfur trioxid (SO_3) (n =number of laboratories × number of repetition (2))
3) Total mean as surfur (S) obtained by dividing the total mean in 2) by 2.4969
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation
8) Mass fraction

References

1) JIS K 8088 : Sulfur (Regent) (2010)
2) JIS K 8217 : Iron ores - Methods for determination of sulfur content (1994)
3) Edited by KANTO CHEMICAL CO., INC.: Technique on Chemical Analysis of Regent - Practical Basic Technique and Knowledge, p. 112 - 120 (2009)
4) Yasushi SUGIMURA Validation of Gravimetric Analysis for Determination of Sulfur Content (as Sulfur Trioxide) in Sulfur and its Compounds as Fertilizers, Research Report of Fertilizer, **Vol. 4**, P. 9 - 15 (2011)
5) Shin ABE, Chika SUZUKI and Yuji SHIRAI: Testing Method of the Total Amount of Sulfur Content (as Sulfur Trioxide): A Collaborative Study, Research Report of Fertilizer, **Vol. 7**, p. 28 - 35 (2014)

(**5**) **Flow sheet for total sulfur content**: The flow sheet for total sulfur content in fertilizers mainly containing sulfur and sulfuric acid is shown below.

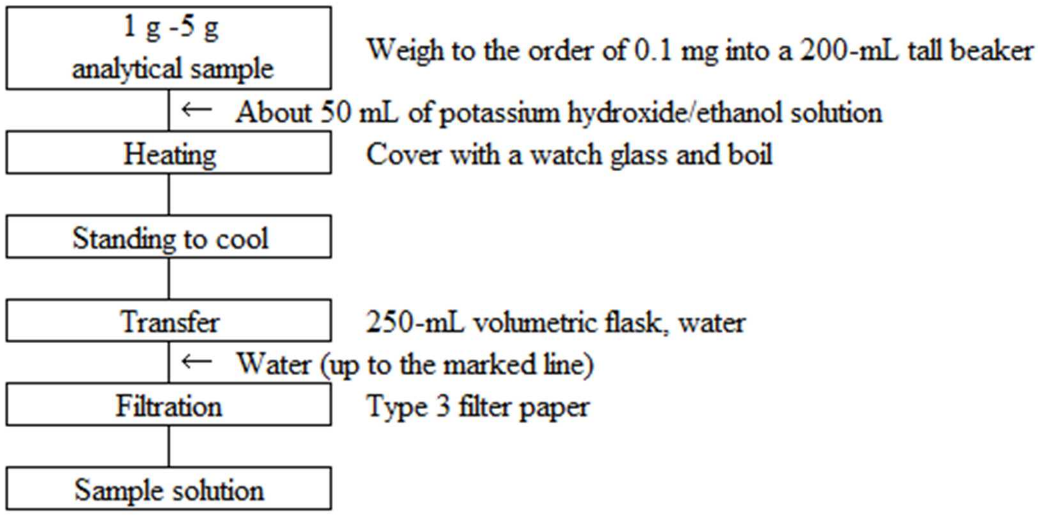


Figure 1 Flow sheet for total sulfur content in fertilizers
(Extraction procedure)

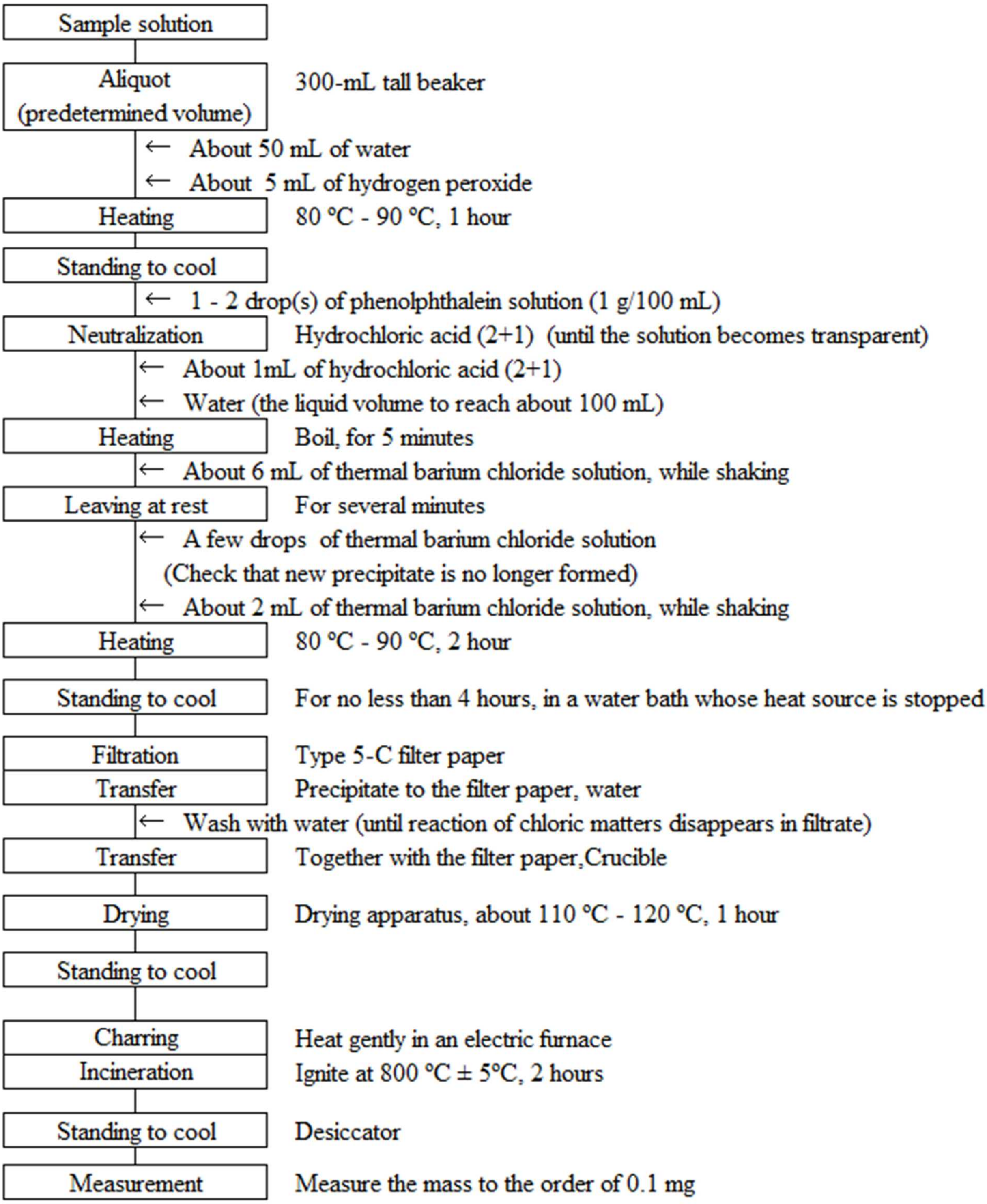


Figure 2 Flow sheet for total sulfur content in fertilizers
(Measurement procedure)

4.12.1.c Transmitted light analysis

(1) Summary

This test method is applicable to fertilizers mainly containing sulfur or sulfuric acid among sulfur and its compound. This testing method is classified as Type D and its symbol is 4.12.1.c-2017 or T-S.c-1. Dissolve an analytical sample in a potassium hydroxide-ethanol solution and add hydrogen peroxide to oxidize, and then measure the intensity of transmitted light of suspension of barium sulfate ($BaSO_4$) formed by the reaction with barium chloride as the absorbance to obtain total sulfur content (T-SO_3) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 2**.

(2) Reagent, etc.: Reagents are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Potassium hydroxide/ethanol solution: Dissolve 10 g of potassium hydroxide specified in JIS K 8574 in 50 mL of ethanol (95) specified in JIS K 8102, further add 50 mL of water.

c) Hydrogen peroxide: A JIS Guaranteed Reagent (H_2O_2 30 % (mass fraction)) specified in JIS K 8230 or a reagent of equivalent quality.

d) Hydrochloric acid: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

e) Glycerin – ethanol solution (1+1): Add 250 mL of ethanol (95) specified in JIS K 8102 to 250 mL of glycerin specified in JIS K 8295.

f) Sodium chloride solution (1): Dilute 240 g of sodium chloride specified on JIS K 8150 in water containing 20 mL of hydrochloric acid specified in JIS K 8180, further add water to make 1000 mL.

g) Barium chloride solution: Sieve barium chloride dihydrate specified in JIS K 8155 to make barium chloride whose particle size is between 500 µm - 710 µm.

h) Sulfate standard solution (SO_3 2 mg/mL) (1): Heat potassium sulfate specified in JIS K 8962 until it becomes constant weight value at 800 ºC in advance, stand to cool it in a desiccator, and then transfer 4.3531 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

i) Sulfate standard solution (SO_3 0.02 mg/mL - 0.1 mg/mL): Put 2mL - 10 mL of sulfate standard solution (SO_3 2 mg/mL) in 200-mL volumetric flasks step by step and add water up to the marked line.

j) Phenolphthalein solution (1 g/100 mL): Dissolve 1 g of phenolphthalein specified in JIS K 8799 in 100 mL of ethanol (95) specified in JIS K 8102.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Hot plate: A hot plate whose surface temperature can be adjusted up to 250 ºC.

b) Water bath: Water bath that can be adjusted to 30 ºC ± 2 °C and 80 ºC - 90 ºC.

c) Magnetic stirrer

d) Spectrophotometer: A spectrophotometer specified in JIS K 0115

(4) Test procedure

(4.1) Extraction: Conduct extraction as follows (2):

a) Weigh 1 g - 2 g of an analytical sample to the order of 0.1 mg, and put it in a 200-mL tall beaker.

b) Add about 50 mL of potassium hydroxide/ethanol solution, cover with a watch glass and heat on a hot plate to boil (3).

c) After standing to cool, transfer to a 250-mL volumetric flask with water and add water up to the marked line.

d) Filter with Type 3 filter paper (4) to make an extract.

Note (**2**) Omit extraction if fluid fertilizers are made from only sulfuric acid and all materials are dissolved
(**3**) Until sulfur content is dissolved. About 5 minutes when raw materials, etc. are not dissolved.
(**4**) Omit the procedures in **d**) when all materials are dissolved.

(**4.2**) **Oxidation**: Conduct oxidation as follows:

a) Put a predetermined volume (the equivalents of 5 mg - 200 mg as SO_3) of sample solution in a 300-mL tall beaker (5).

b) Add about 50 mL of water and about 5 mL of hydrogen peroxide and heat in a water bath at 80 ºC - 90 ºC for about 1 hour while sometimes shaking (6).

c) After standing cool, add 1 - 2 drop(s) of phenolphthalein solution (1 g/100 mL) (7), and add hydrochloric acid (2+1) until the color of the solution disappears (8).

d) After standing to cool, transfer to a 200-mL volumetric flask with water and add water up to the marked line.

e) Filter with 0.3μm glass filter paper.

Note (**5**) Weigh 1g - 5g of an analytical sample to the order of 0.1 mg if the fluid fertilizers of the analytical sample are made from only sulfuric acid and all materials are dissolved.
(**6**) It can be suspended after the procedures end.
(**7**) A pH meter can be used for neutralization.
(**8**) Omit the procedures in **c**) if fluid fertilizers are made from only sulfuric acid and all materials are dissolved.

(**4.3**) **Precipitate formation**: Form precipitate as shown below.

a) Put 50 mL of filtrate in a 100-mL Erlenmeyer flask with screw cap.

b) Add about 10 mL of glycerin – ethanol solution (1+1) and about 5 mL of sodium chloride solution in the Erlenmeyer flask with screw cap.

c) Warm up on a water bath at 30ºC ± 2 ºC.

d) After warming, add 0.30 g of barium chloride and stir with a magnetic stirrer for about 2 minutes.

e) Warm up on a water bath at 30ºC ± 2 ºC for about 4 minutes.

f) After warming, stir with a magnetic stirrer for about 3 minutes to make a sample solution.

g) As a blank test, conduct the procedures in **a**) - **c**) and **f**) using another 100-mL Erlenmeyer flask with screw cap to prepare a blank test solution.

(**4.4**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of the spectrophotometer**: Set up the measurement conditions of the spectrophotometer considering the following.
Detection wavelength: 450 nm

b) **Calibration curve preparation**

1) Put 50 mL of sulfate standard solution (SO_3 0.02 mg/mL - 0.1 mg/mL) in a 100-mL Erlenmeyer flask with screw cap and conduct the procedure (**4.3**) **b**) - **f**) to make an SO_3 1 mg/65 mL - 5 mg/65 mL sulfate standard solution for the calibration curve preparation.

2) Put 50 mL of water in another 100-mL Erlenmeyer flask with screw cap and conduct the same procedures as **1**) to make a blank test solution for the calibration curve preparation.

3) Measure absorbance at a wavelength of 450 nm of the sulfuric acid standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control (8) (9).

4) Prepare the calibration curve of the sulfuric acid concentration and absorbance of the sulfuric acid standard solutions for the calibration curve preparation.

c) Sample measurement

1) For the sample solution, conduct procedures similarly as in **b) 3)** to measure absorbance.

2) For the blank test solution, conduct procedures similarly as in **1)** to measure absorbance, and correct the absorbance obtained for the sample solution.

3) Obtain the sulfate (SO_3) content from the calibration curve, and calculate the total sulfur content (T-SO_3) in the analytical sample.

Note (**8**) Measure right after stirring because barium sulfate easily precipitates.
(**9**) A spectrophotometer with automatic sample introducing device is preferable.

Comment 1 The range of calibration curve with linearity is SO_3 1 mg/65 mL - 5 mg/65 mL and the curve does not pass through the origin.

Comment 2 Testing was conducted using sulfur simple substance fertilizers containing no materials (2 samples); as a result, the quantitative value of the total sulfur content (T-SO_3) was 98.4 % - 99.4 % to a theoretical value.
Note that the minimum limit of quantification of this testing method was estimated to be about 1 % (mass fraction).

References

1) JIS K 8001 : General rule for test methods of reagents (2009)
2) JIS K 8088 : Sulfur (Regent) (2010)
3) JAPAN Sewage Works Association: Sewage Sludge Analysis -2007-, p. 132 - 134 Tokyo (2007)
4) Edited by KANTO CHEMICAL CO., INC.: Technique on Chemical Analysis of Regent - Practical Basic Technique and Knowledge, p. 131 - 135 (2009)
5) Yasushi SUGIMURA: Method Validation of Turbidimetry for Determination of Sulfur Content (as Sulfur Trioxide) in Fertilizers of Sulfur, Sulfuric acid and Its Mixture, Research Report of Fertilizer **Vol. 6**, p. 20 - 26 (2013)

(5) **Flow sheet for total sulfur content**: The flow sheet for total sulfur content in fertilizers mainly containing sulfur and sulfuric acid is shown below.

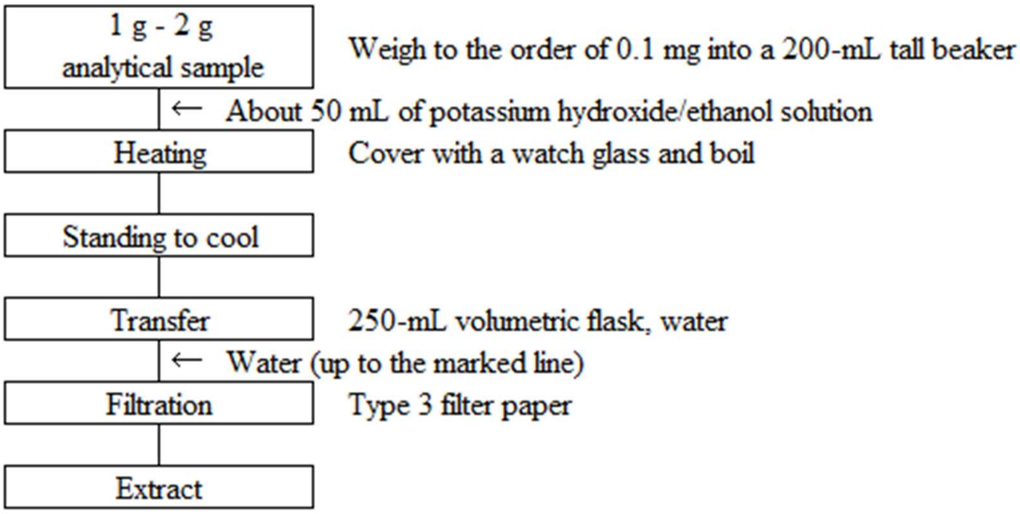


Figure 1 Flow sheet for total sulfur content in fertilizers (Extraction procedure)

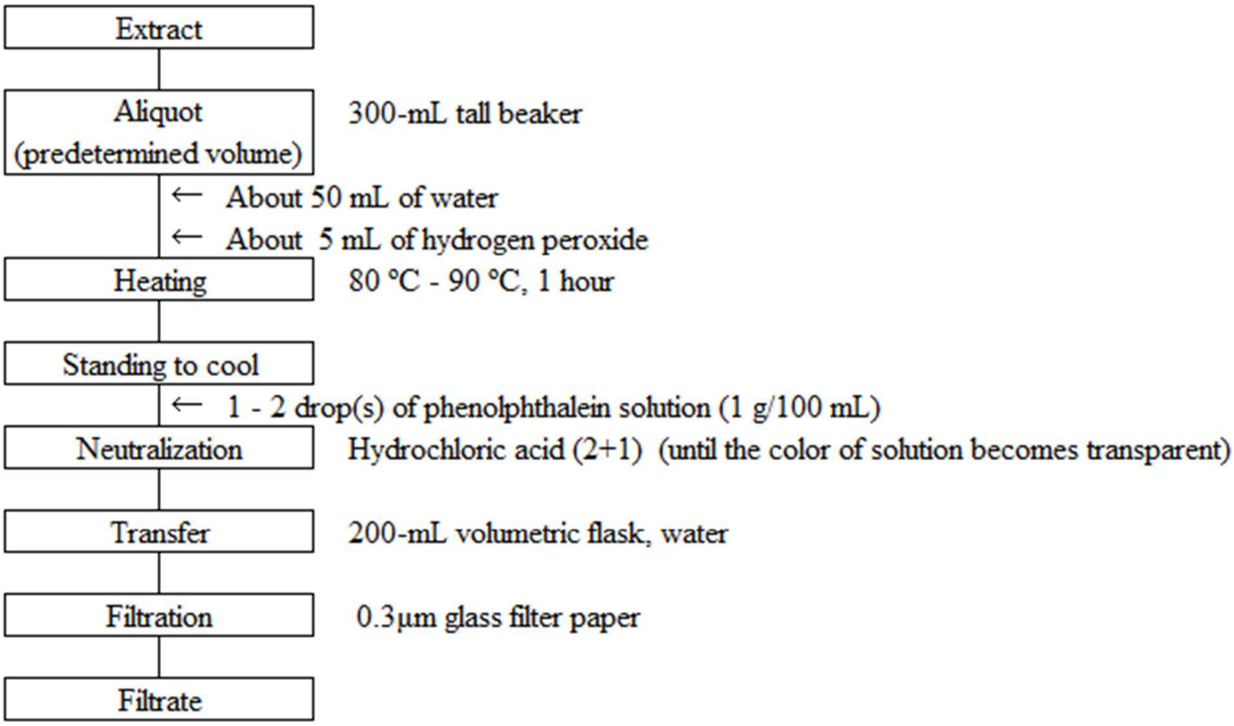


Figure 2 Flow sheet for total sulfur content in fertilizers (Oxidation procedure)

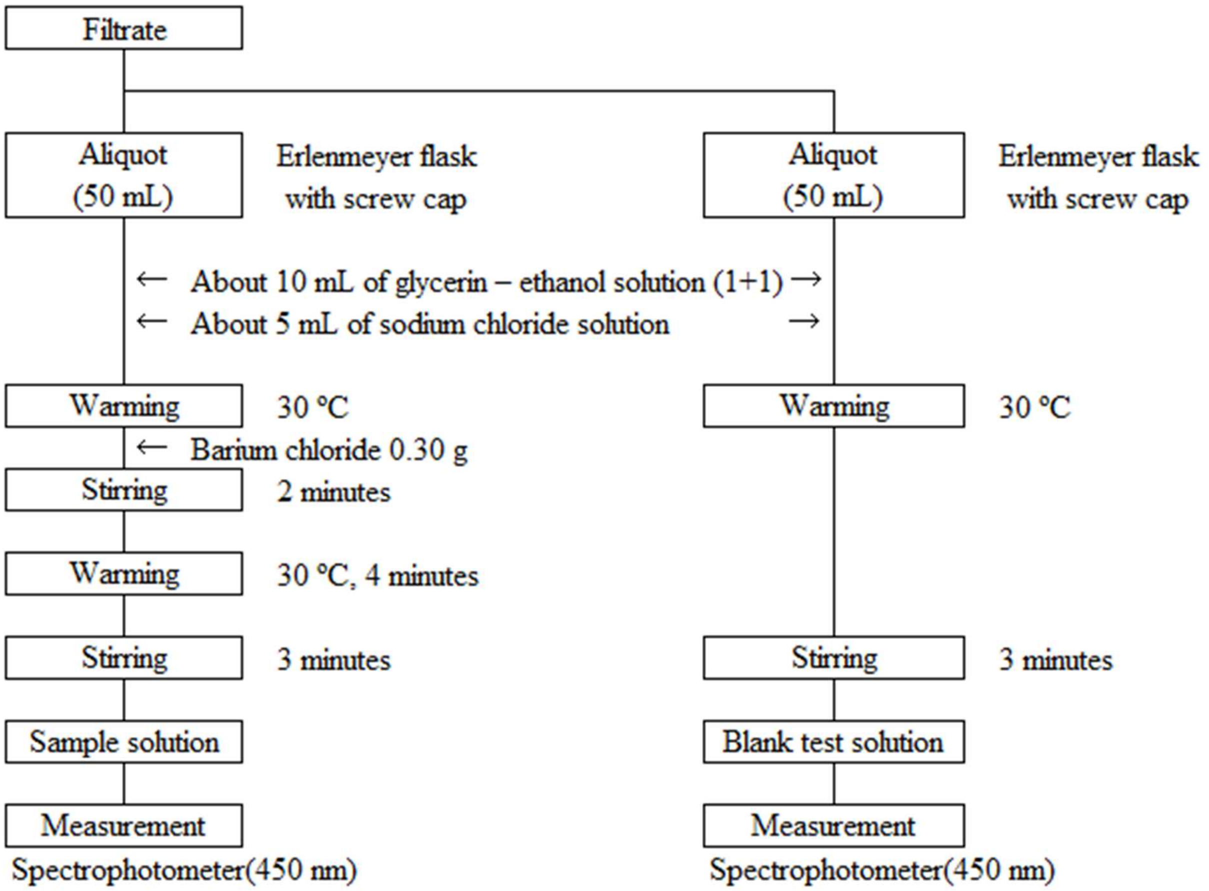


Figure 2 Flow sheet for total sulfur content in fertilizers
(precipitate formation and measurement procedure)

4.12.2 Acid-soluble sulfur

4.12.2.a Ion Chromatography

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 4.12.2.a-2021 or S-S.a-2.

Add hydrochloric acid (1+23) to an analytical sample to extract sulfate ions, introduce them to an Ion Chromatograph (IC) to isolate it with an ion exchange column, and then measure with an electric conductivity detector to obtain the sulfate ion (SO_4^{2-}) content in an analytical sample and calculate acid-soluble sulfur (S-S). Note that the performance of this testing method is shown in **Comment 6**. Examples of columns to be used, measurement conditions, and IC chromatograms are shown in Annex D.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A4 specified in JIS K 0557.

b) **Sodium carbonate**: A JIS Guaranteed Reagent specified in JIS K 8625 or a reagent of equivalent quality.

c) **Sodium hydrogencarbonate**: A JIS Guaranteed Reagent specified in JIS K 8622 or a reagent of equivalent quality.

d) **Eluent** (1): Prepare an eluent with a composition suitable for the column to be used. If it is used for the eluent of ion chromatograph, filter with a membrane filter (pore size: no more than 0.5 μm) made of hydrophilic PTFE.

e) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

f) **Sulfate ion standard solution (SO_4^{2-} 1000 mg/L)**: A sulfate ion standard solution (SO_4^{2-} 1000 mg/L) traceable to National Metrology.

g) **Sulfate ion standard solution (SO_4^{2-} 50 μg/mL)** (1): Put a predetermined amount of sulfate ion standard solution (SO_4^{2-} 1000 mg/mL) in a volumetric flask and add water up to the marked line.

h) **Sulfate ion standard solution (SO_4^{2-} 2 μg/mL - 5 μg/mL) for the calibration curve preparation** (1): Put 4 mL – 10 mL of sulfate ion standard solution (SO_4^{2-} 50 μg/mL) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

i) **Sulfate ion standard solution (SO_4^{2-} 0.2 μg/mL - 1 μg/mL) for the calibration curve preparation** (1): Put 4 mL – 20 mL of sulfate ion standard solution for the calibration curve preparation (SO_4^{2-} 5 μg/mL) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Carbonate buffer solution and hydroxide solution are available as an eluent. A carbonate buffer solution prepared by dissolving 0.191 g of sodium carbonate and 0.143 g of sodium hydrogencarbonate in water in a 1000-mL volumetric flask and adding water to the marked line has pH of approximately 10. One thousand mL of this carbonate buffer solution contains 1.8 mmol of sodium carbonate and 1.7 mmol of sodium hydrogencarbonate.

It is also possible to make an eluent using an automated eluent generation system. Stock solutions used for such systems are sold under the production name EGC-KOH, etc.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 200-mL ground-in stopper Erlenmeyer flask to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

b) **Centrifugal separator**: A centrifugal separator that can work at 1700 × *g*.
c) **Ion Chromatograph**: An ion chromatograph specified in JIS K 0127 that satisfies the following requirements.
1) **Column**: A no less than 4.0-mm inner diameter 7.5-mm long 3.5-μm particle diameter column tube filled with polymer particles, to which quaternary ammonium groups, quaternary alkylamines or quaternary alkanolamines bond (3).
2) **Column bath**
3) **Suppressor**: Cation exchange membrane or resin should be used.
4) **Detection unit**: An electric conductivity detector.
d) **Membrane filters**: Pore size is no more than 0.45 μm, made of hydrophilic PTFE

Comment 2 A column for which a carbonate buffer solution is used as the eluent is sold under the production name Shodex IC SI-90 4E, TSKgel SuperIC-Anion HS, PCI-205, IonPac AS22, Shim-pack IC-SA2, etc., and a column for which a hydroxide solution is used as the eluent is sold under the production name IonPac AS20, etc.

(4) **Test procedure**
(4.1) **Extraction**: Conduct extraction as shown below.
a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.
b) Add about 100 mL of hydrochloric acid (1+23) and shake to mix by using a vertical reciprocating shaker at a rate of about 300 reciprocations/min (amplitude of 40 mm) for about 10 minutes.
c) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube (2).
d) Centrifuge it at about 1700 × *g* centrifugal force for about 10 minutes (3), and use the supernatant as an extract.
e) Get a predetermined amount of the extract, and dilute by a factor of 100 with the eluent or water (4) (5).
f) Filter with a membrane filter (pore size: no more than 0.45 μm) to make a sample solution.

Note **(2)** The vessel should be made of polypropylene, etc. to not affect the measurement.
(3) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.
(4) In case of exceeding the calibration curve, dilute by a factor of more than 100.
(5) Confirm in advance that the sample solution is within the pH range applicable to the column.

Comment 3 An applicable pH range is limited for columns for which a carbonate buffer solution is used as the eluent, and it is desirable to dilute using the eluent in the procedure in **(4.1) e**).

Comment 4 Instead of the procedures in (**4.1**) **f**), it is allowed to put the diluted solution in a 1.5-mL ground-in stopper centrifugal precipitate tube (4), and centrifuge at 8000 × *g* - 10000 × *g* for about 5 minutes to make the supernatant as a sample solution.

(4.2) **Measurement**: Conduct the measurement by using suppressor method as indicated in JIS K 0127 and as shown below. Specific measurement procedures are according to the operation method of the ion chromatograph used in measurement.
a) **Measurement conditions for Ion Chromatograph**: Example measurement conditions are shown in Table 1 of Annex D. Set the actual measurement conditions suitable for the instruments, column, etc. to be used for the items below referencing Annex D.
1) **Column**: An ion exchange column filled with polymer particles to which quaternary

ammonium groups, quaternary alkylamines or quaternary alkanolamines bond.
2) **Column bath temperature**: 25 °C～40 °C
3) **Eluent**: Carbonate buffer solution or hydroxide solution
4) **Flow rate**: 0.8～1.5 mL/min
5) **Detection unit**: An electric conductivity detector

Comment 5 Set the column bath temperature, eluent, flow rate, injection volume, etc. according to the performance of the column to be used.

b) Calibration curve preparation
1) Inject 20 µL (6) of respective standard solutions for the calibration curve preparation to the ion chromatograph, and record the chromatogram of electric conductivity to obtain a peak area or peak height.
2) Prepare a curve for the relationship between the concentration and the peak area or peak height of electric conductivity of respective standard solutions for the calibration curve preparation (7). Prepare a calibration curve when the sample is measured.

Note (**6**) Change the injection volume according to the performance of the column to be used.

c) Sample measurement
1) Subject 20 µL of the sample solution to the same procedure as in **b**) **1**).
2) Obtain a sulfate ion concentration from the calibration curve by the peak area or peak height to calculate the sulfate ion (SO_4^{2-}) in the analytical sample (7).
3) Calculate acid-soluble sulfur (S-S) by the following formula.

Acid-soluble sulfur (S-S) (% (mass fraction)) in an analytical sample

$$= A \times (MW_1/MW_2)$$
$$= A \times 0.3338$$

A : Sulfate ion (SO_4^{2-}) (% (mass fraction)) in the analytical sample
MW_1 : Atomic weight of sulfur (32.066)
MW_2 : Molecular weight of sulfate ion (96.063)

Note (**7**) As for potassium humic acid fertilizers and fertilizers containing potassium humic acid, their peak form sometimes changes depending on the measurement conditions. Therefore, it should be calculated by a peak height.

Comment 6 The comparison of the analysis value (y_i: 3.90 % (mass fraction) - 73.09% (mass fraction)) of this method (6.10.1.a-2021) where extraction is performed using hydrochloric acid (1+23) and the analysis value (x_i) of 6.10.1.a Ion Chromatography of the Testing Methods for Fertilizers (2020) was conducted to evaluate trueness using ammonium sulfate (1 sample), guanylurea sulfate (1 sample), superphosphate of lime (1 sample), double superphosphate of lime (1 sample), potassium sulfate (1 sample), compound fertilizer (1 sample), magnesia sulfate fertilizer (2 samples), humic acid magnesia fertilizer (1 sample), mixed magnesia fertilizer (1 sample), manganese sulfate fertilizer (1 sample), designated blended fertilizer (1 sample), gypsum (1 sample), and 10 % preparation sample (1 sample). As a result, a regression equation was $y = 1.001x + 0.423$, and its correlation coefficient (r) was 0.999.
The results of repeated experiments using potassium sulfate and double superphosphate of lime were analyzed by the one-way analysis of variance. Table 1 shows the estimation results of intermediate precision and repeatability. Additionally, results of statistical analysis using analysis values obtained by conducting an internationally-standardized collaborative study to estimate reproducibility are shown

in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.2 % (mass fraction).

The validity of this testing method was evaluated for sulfate iron (SO_4^{2-}).

Table 1 Estimation results of repeatability and intermediate precision

Sample name	Days of repeatability T 1)	Mean 2) (%) 3)	Repeatability		Intermediate precision	
			s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Potassium sulfate	5	56.69	0.66	1.16	0.97	1.71
Double superphosphate of lime	5	3.74	0.03	0.76	0.05	1.33

1) The number of days conducting duplicate analysis
2) Mean ([Number of days of repeatability (T)] × [Number of duplicates (2)])
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Repeatability and reproducibility estimated from results of harmonized collaborative studies

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	Repeatability		Reproducibility	
			s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Ammonium sulfate	9	71.77	2.14	3.0	2.81	3.9
Gypsum	9	48.80	0.61	1.3	1.35	2.8
Compound fertilizer 1	10	32.96	0.49	1.5	1.89	5.7
Compound fertilizer 2	9	15.99	0.38	2.4	1.34	8.4
Double superphosphate of lime	9	3.64	0.05	1.4	0.35	9.7
Compound fertilizer 3	10	1.90	0.07	3.8	0.27	14.3

1) Number of laboratories used in analysis
2) Mean (n = [Number of laboratories] × [Number of samples (2)])
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Satoko SAKAIDA, Kenji KOZUKA and Yuji SHIRAI: Determination of Sulfate ion by Ion Chromatography, Research Report of Fertilizers, **Vol. 13**, p. 50 – 64 (2020)

(**5**) **Flow sheet for testing method**: The flow sheet for acid-soluble sulfur in fertilizers is shown below:

1.00 g analytical sample — 200-mL ground-in stopper Erlenmeyer flask
← 100 mL of hydrochloric acid (1+23)
Shaking to mix — Vertical reciprocating shaker (300 reciprocations/min, amplitude of 40 mm), for 10 minutes
Centrifugal separation — Ground-in stopper centrifugal precipitate tube, 1700 × g, 10 minutes
Supernatant
Dilution — No less than 100 times, eluent or water
Filtration — Hydrophilic PTFE membrane filter (no more than 0.45 µm)
Measurement — Ion Chromatograph

Figure Flow sheet for acid-soluble sulfur in fertilizers

4.13 Iron

4.13.1 Water-soluble iron

4.13.1.a Flame atomic absorption spectrometry

(1) Summary

This test method is applicable to fertilizers that indicate iron content as a response modifier. This testing method is classified as Type D and its symbol is 4.13.1.a-2017 or W-Fe.a-1.

Extract by adding water to an analytical sample, spray in an acetylene−air flame and measure the atomic absorption with iron at a wavelength of 248.3 nm to obtain water-soluble iron (W-Fe) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Iron standard solution (Fe 0.1 mg/mL)**: An iron standard solution (Fe 0.1 mg/mL) traceable to National Metrology.

d) **Iron standard solutions (Fe 0.5 µg/ mL - 5 µg/ mL) for the calibration curve preparation** (1): Put 2.5 mL - 25 mL of iron standard solution (Fe 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line.

e) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the iron standard solution in (**2**), an iron standard solution for the calibration curve preparation can be prepared by using an iron standard solution (Fe 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(**3**) **Instruments**: Instruments are as shown below:

a) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

b) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.

1) **Light source**: An iron hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

Note (**2**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Powdery test sample**

a) Weigh 5.00 g of an analytical sample, and put it in a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make a sample solution.

Comment 2 In the procedure in (**4.1.1**) **a**), it is also allowed to weigh 2.50 g of the analytical sample and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

(**4.1.2**) **Fluid test sample**

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix**.**
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 4 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 248.3 nm

b) **Calibration curve preparation**
1) Spray the iron standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 248.3 nm.
2) Prepare a curve for the relationship between the iron concentration and the indicated value of the iron standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**
1) Put a predetermined amount of the sample solution (the equivalents of 0.05 mg - 0.5 mg as Fe) in a 100-mL volumetric flask.
2) Add about 25 mL of hydrochloric acid (1+5), and add water up to the marked line.
3) Subject to the same procedure as in **b**) **1**) to read the indicated value.
4) Obtain the iron content from the calibration curve, and calculate the water-soluble iron (W-Fe) in the analytical sample.

Comment 5 Recovery testing was conducted to evaluate trueness using a preparation sample (solid). As a result, the mean recovery rates at the content level of 10 % (mass fraction), 5 % (mass fraction) and 0.05 % (mass fraction) were 101.1 %, 102.8 % and 107.0 % as water-soluble iron (W-Fe) respectively. In addition, recovery testing was conducted using a preparation sample (fluid). As a result, the mean recovery rates at the content level of 1 % (mass fraction), 0.1 % (mass fraction) and 0.01 % (mass fraction) were 103.6 %, 105.7 % and 105.1 % as a water-soluble iron (W-Fe) respectively.
The results of the repeatability tests on different days using a fluid compound fertilizer and a liquid microelement compound fertilizer to evaluate the extract precision of fluid fertilizers were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to

be about 40 mg/kg for solid fertilizers and about 4 mg/kg for fluid fertilizers.

Table 1 Analysis results of the repeatability tests on different days of water-soluble iron (Fluid fertilizer)

Name of sample	Test days of repeatability (T)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Fluid compound fertilizer	7	0.244	0.002	0.6	0.003	1.4
Liquid microelement compound fertilizer	7	0.099	0.001	0.5	0.003	2.9

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.252, Yokendo, Tokyo (1988)

2) Shinei TAKAHASHI, Chika SUZUKI and Noriyuki SASAKI: Verification of Performance Characteristics of Testing Method for Iron in Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer, **Vol. 7**, p. 131 - 137 (2014)

3) Shinji KAWAGUCHI: Simple Extraction Method for Water-Soluble Components in Liquid Compound Fertilizers, Research Report of Fertilizer, **Vol. 9**, p. 10 - 20 (2016)

(5) Flow sheet for water-soluble iron: The flow sheet for water-soluble iron in fertilizers is shown below:

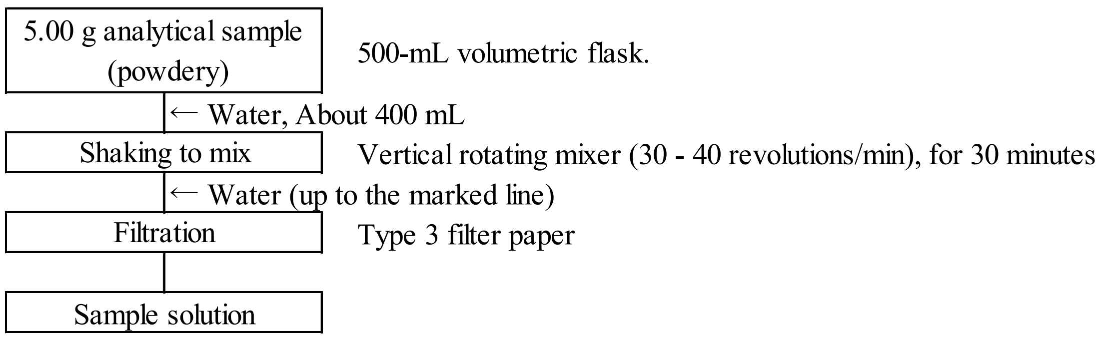


Figure 1-1 Flow sheet for water-soluble iron in fertilizers (Extraction procedure (4.1.1))

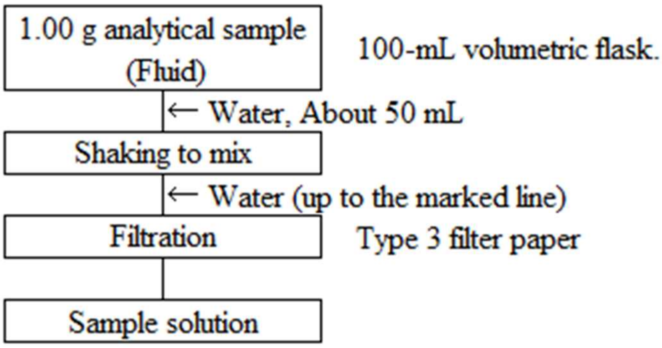


Figure 1-2 Flow sheet for water-soluble iron in fertilizers
(Extraction procedure (4.1.2))

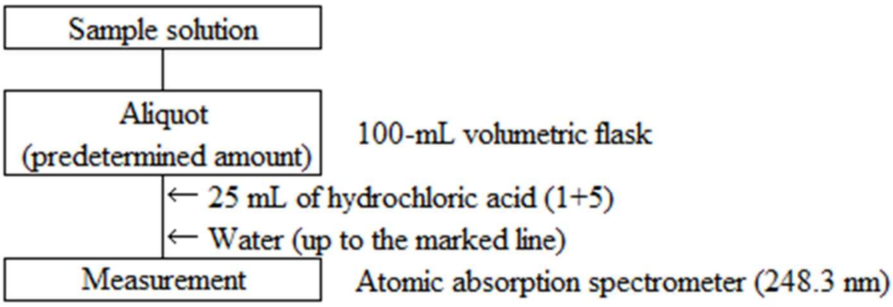


Figure 2 Flow sheet for water-soluble iron in fertilizers
(Measurement procedure)

4.13.1.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fluid compound fertilizers, liquid microelement compound fertilizers and the fluid fertilizers of home garden-use compound fertilizers. This testing method is classified as Type B and its symbol is 4.13.1.b-2017 or W-Fe.b-1.

Extract by adding water to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the iron at a wavelength of 259.940 nm, etc. to obtain water-soluble iron(W-Fe) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

- **a**) **Water**: Water of A3 specified in JIS K 0557.
- **b**) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c**) **Iron standard solution (Fe 1 mg/mL)**: An iron standard solution (Fe 1 mg/mL) traceable to National Metrology.
- **d**) **Iron standard solution (Fe 0.1 mg/mL)** (1): Put 10 mL of iron standard solution (Fe 1 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.
- **e**) **Iron standard solutions (Fe 1 µg - 20 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of iron standard solution (Fe 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.
- **f**) **Iron standard solutions (Fe 0.1 µg - 1 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 10 mL of iron standard solution (Fe 10 µg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.
- **g**) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**), **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the iron standard solution in (**2**), an iron standard solution for the calibration curve preparation can be prepared by using an iron standard solution (Fe 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

- **a**) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116.
- **1**) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

- **a**) Weigh 1 g of an analytical sample (2) to the order of 1 mg, and put it in a 100-mL volumetric flask.
- **b**) Add about 50 mL of water, shake to mix and add water up to the marked line.
- **c**) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) The sampling amount of the analytical sample is 10 g when the content in the sample is less than 0.01 % (mass fraction) as water-soluble iron.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 259.940 nm or 238.204 nm (3)

b) **Calibration curve preparation**

1) Spray the iron standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at an analytical line wavelength.

2) Prepare a curve for the relationship between the iron concentration and the indicated value of the iron standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 2 mg as Fe) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the iron content from the calibration curve, and calculate the water-soluble iron (W-Fe) in the analytical sample.

Note (**3**) 238.204 nm can also be used. However, since the intensity of emission obtained is different from the one of 259.940 nm, it is necessary to understand the suitable concentration range of the calibration curve and prepare a standard solution for the calibration curve in advance.

Comment 4 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 5 The comparison of the measurement value (y_i : 0.0191 % (mass fraction) - 0.517 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of flame atomic absorbance spectrometry was conducted to evaluate trueness using fluid fertilizers (12 samples). As a result, a regression equation was y=0.001+0.968x, and its correlation coefficient (r) was 0.999. Additionally, additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 0.01 % (mass fraction) and 0.1 % (mass fraction) were 96.5 % and 93.9 % respectively.

The results of the repeatability tests on different days using a fluid compound fertilizer

and a home garden-use compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study implemented for testing method validation and its analysis are shown in Table 2.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.0005 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of water-soluble iron (Fluid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluid compound fertilizer	7	0.145	0.001	0.6	0.002	1.1
Home garden-use compound fertilizer (fluid)	7	0.0485	0.0003	0.5	0.0005	0.9

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble iron

Analytical line wavelength (nm)	Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
259.940	Preparation sample (Liquid) 1	11	2.09	0.03	1.2	0.03	1.6
	Preparation sample (Liquid) 2	11	0.511	0.004	0.8	0.008	1.5
	Preparation sample (Liquid) 3	11	1.05	0.007	0.7	0.01	1.3
	Preparation sample (Liquid) 4	11	0.111	0.0008	0.8	0.002	2.2
	Preparation sample (Liquid) 5	11	0.0530	0.0005	1.0	0.0009	1.6
238.204	Preparation sample (Liquid) 1	11	2.08	0.02	1.2	0.03	1.5
	Preparation sample (Liquid) 2	11	0.509	0.005	1.0	0.012	1.3
	Preparation sample (Liquid) 3	11	1.05	0.007	0.7	0.02	1.9
	Preparation sample (Liquid) 4	10	0.110	0.0008	0.7	0.002	1.7
	Preparation sample (Liquid) 5	11	0.0528	0.0006	1.1	0.0010	1.8

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination Method for Effect-Development Promoting Agent (Ca, Fe, Co, Cu, Zn and Mo) in Liquid Compound Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), Research

Report of Fertilizer, **Vol. 9**, p. 1 - 9 (2016)
2) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(**5**) **Flow sheet**: The flow sheet for water-soluble iron in fluid fertilizers is shown below:

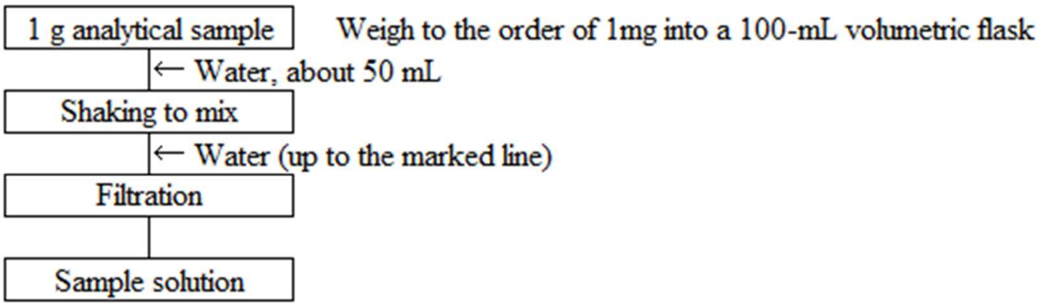


Figure1 The flow sheet for water-soluble iron in fluid fertilizers (Extraction procedure)

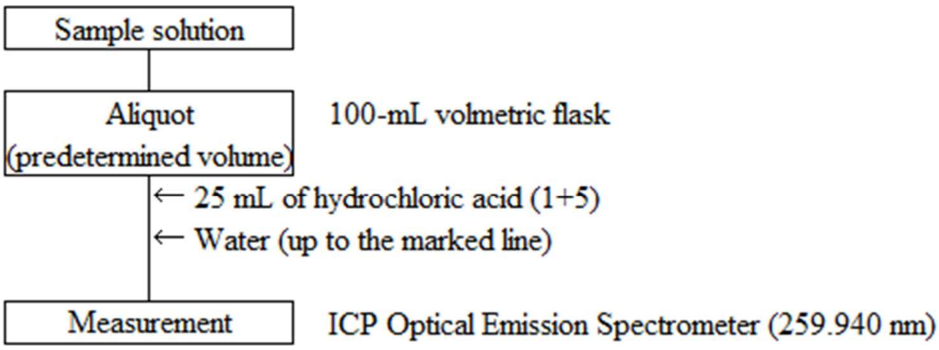


Figure 2 The flow sheet for water-soluble iron in fluid fertilizers (Measurement procedure)

4.14 Molybdenum

4.14.1 Water-soluble molybdenum

4.14.1.a Sodium thiocyanate absorptiometric analysis

(1) Summary

This test method is applicable to fertilizers that indicate molybdenum content as a response modifier. This testing method is classified as Type D and its symbol is 4.14.1.a-2017 or W-Mo.a-1.

Extract by adding water to an analytical sample, add sulfuric acid (1+1) and perchloric acid, further add a sodium thiocyanate solution and a tin (II) chloride solution, and measure the absorbance with thiocyanate complex formed by the reaction of reduced molybdenum (V) with thiocyanate ion to obtain water-soluble molybdenum (W-Mo) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(2) Reagent: Reagents are as shown below.

a) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.
b) **Perchloric acid**: A JIS Guaranteed Reagent specified in JIS K 8223 or a reagent of equivalent quality.
c) **Iron (III) sulfate solution** (1): Dissolve 5 g of iron (III) sulfate specified in JIS K 8981 in about 10 mL of sulfuric acid (1+1) and a proper amount of water, and further add water to make 100 mL.
d) **Sodium thiocyanate solution** (1): Dissolve 50 g of sodium thiocyanate specified in JIS K 9002 in water to make 500 mL.
e) **Tin (II) chloride solution** (1): Dissolve 20 g of tin (II) chloride dihydrate specified in JIS K 8136 in 80 mL of hydrochloric acid (1+1) while heating, then add water to make 200 mL.
f) **Molybdenum standard solution (Mo 1 mg/mL)** (1): After leaving at rest molybdenum (VI) oxide (2) in a desiccator for about 24 hours to dry, put it to a 1,500 g weighing dish. Dissolve in a small amount of water, transfer to a 1000 mL volumetric flask and add about 5 g of sodium hydroxide specified in JIS K 8576 to dissolve and add water up to the marked line.
g) **Molybdenum standard solution (Mo 0.01 mg/mL)**: Dilute a predetermined amount of molybdenum standard solution (Mo 1 mg/mL) precisely by a factor of 100 with water.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) A reagent of no less than 99.5 % (mass fraction) in purity is commercially sold as molybdenum (VI) oxide.

Comment 1 Instead of the molybdenum standard solution in (**2**), a molybdenum standard solution for the calibration curve preparation can be prepared by using a molybdenum standard solution (Mo 0.1 mg/mL, 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(3) Instruments: Instruments are as shown below:

a) **Vertical rotating mixer**: A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.
b) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Powdery test sample**

a) Weigh 5.00 g of an analytical sample, and put it in a 500-mL volumetric flask.
b) Add about 400 mL of water, and shake to mix at 30-40 revolutions/min for about 30 minutes.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 2 In the procedure in (**4.1.1**) **a**), it is also allowed to weigh 2.50 g of the analytical sample and put it in a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 3 A sample solution obtained in the procedure in (**4.1.1**) is also applicable to the components shown in Annex B.

Comment 4 When the sample solution in **d**) contains organic matters that affect the determination, put a predetermined amount of the sample solution to a 100-mL tall beaker, add a small amount of sulfuric acid and nitric acid to heat and digest the organic matters until white smoke of sulfuric acid evolves. After standing to cool, transfer the solution to a 100-mL volumetric flask with water and add water up to the marked line to filter. The filtrate is prepared as the sample solution of (**4.2**) **a**).

(**4.1.2**) **Fluid test sample**

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix.
c) Add water up to the marked line.
d) Filter with Type 3 filter paper to make a sample solution.

Comment 5 A sample solution obtained in the procedure in (**4.1.2**) is also applicable to the components shown in Annex B.

(**4.2**) **Coloring**: Conduct coloring as shown below.

a) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 0.3 mg as Mo) in a 100-mL volumetric flask.
b) Add about 5 mL of sulfuric acid (1+1), about 5 mL of perchloric acid and about 2 mL of iron (III) sulfate solution.
c) Add about 16 mL of sodium thiocyanate solution and about 10 mL of tin (II) chloride solution successively while shaking to mix and further add water up to the marked line (3).

Note (**3**) When the solution becomes muddy, centrifuge after the procedure in **c**). However, if it is presumed that copper (I) thiocyanate caused the muddying, centrifuge after leaving at rest for 1 hour.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of the spectrophotometer**: Set up the measurement conditions of the spectrophotometer considering the following.
Detection wavelength: 460 nm

b) **Calibration curve preparation**

1) Put 1 mL - 30 mL of molybdenum standard solution (Mo 0.01 mg/mL) in 100-mL volumetric flasks step-by-step.

2) Conduct the same procedure as (**4.2**) **b**) - **c**) to make a 0.01 mg/100 mL - 0.3 mg/100 mL molybdenum standard solution for the calibration curve preparation.

3) Conduct the same procedures as **2**) for another 100-mL volumetric flask to make a blank test solution for the calibration curve preparation.

4) Measure absorbance at a wavelength 460 nm of the molybdenum standard solution for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control.

5) Prepare a curve for the relationship between the molybdenum concentration and the absorbance of the molybdenum standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Regarding the solution in (**4.2**) **c**), measure absorbance by the same procedure as **b**) **4**).

2) Obtain the molybdenum content from the calibration curve, and calculate the water-soluble molybdenum (W- Mo).

Comment 6 Recovery testing was conducted to evaluate trueness using a preparation sample. As a result, the mean recovery rates at the content level of 2.5 % (mass fraction) and 0.1 % (mass fraction) were 100.2 % and 100.8 % as water-soluble molybdenum (W-Mo) respectively.

The results of the repeatability tests on different days using a fluid compound fertilizer and a liquid microelement compound fertilizer to evaluate the extract precision of fluid fertilizers were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 60 mg/kg for solid fertilizers and about 6 mg/kg for fluid fertilizers.

Table 1 Analysis results of the repeatability tests on different days of water-soluble molybudenum (Fluid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluid compound fertilizer	7	0.242	0.001	0.4	0.002	1.0
Liquid microelement compound fertilizer	7	0.0228	0.0001	0.4	0.0002	0.8

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.281- 283, Yokendo, Tokyo (1988)

2) Keiji YAGI, Natuki TOYODOME, Tokiya SUZUKI and Hideo SOETA: Verification of Performance Characteristics of Testing Method for Water-soluble Molybdenum Content in Fertilizer by Sodium Thiocyanate Absorptiometric Analysis, Research Report of Fertilizer **Vol. 7**, p. 138 - 144 (2014)

3) Shinji KAWAGUCHI: Simple Extraction Method for Water-Soluble Components in

Liquid Compound Fertilizers, Research Report of Fertilizer, **Vol. 9**, p. 10 - 20 (2016)

(5) **Flow sheet for water-soluble molybdenum**: The flow sheet for water-soluble molybdenum in fertilizers is shown below:

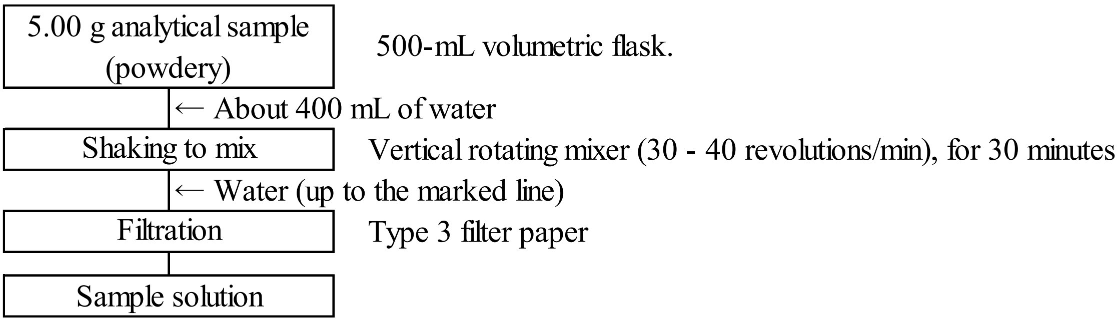


Figure 1-1 Flow sheet for water-soluble molybdenum in fertilizers (Extraction procedure (4.1.1))

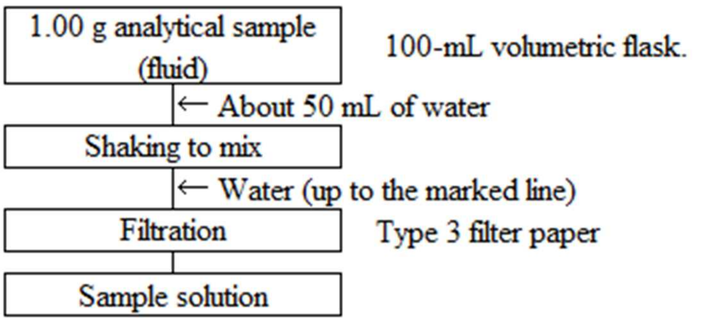


Figure 1-2 Flow sheet for water-soluble molybdenum in fertilizers (Extraction procedure (4.1.2))

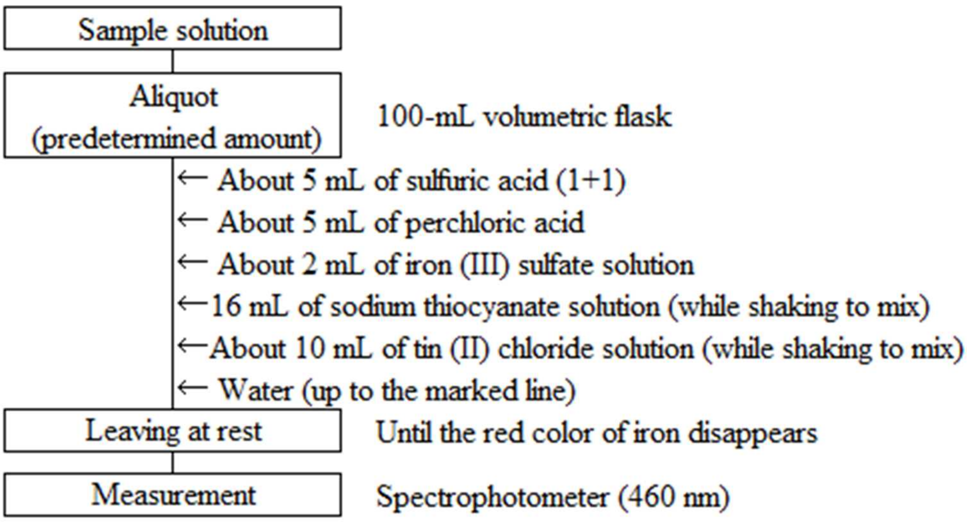


Figure 2 Flow sheet for water-soluble molybdenum in fertilizers (Measurement procedure)

4.14.1.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fluid compound fertilizers, liquid microelement compound fertilizers and the fluid fertilizers of home garden-use compound fertilizers. This testing method is classified as Type B and its symbol is 4.14.1.b-2017 or W-Mo.b-1.
Extract by adding water to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the molybdenum at a wavelength of 202.030 nm, etc. to obtain water-soluble molybdenum (W-Mo) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent, etc**.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Molybdenum standard solution (Mo 1 mg/mL)**: A molybdenum standard solution (Mo 1 mg/mL) traceable to National Metrology.

d) **Molybdenum standard solution (Mo 0.1 mg/mL)** (1): Put 10 mL of molybdenum standard solution (Mo 1 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.

e) **Molybdenum standard solutions (Mo 1 µg - 20 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of molybdenum standard solution (Mo 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

f) **Molybdenum standard solutions (Mo 0.1 µg - 1 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 10 mL of molybdenum standard solution (Mo 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**), **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the molybdenum standard solution in (**2**), a molybdenum standard solution for the calibration curve preparation can be prepared by using a molybdenum standard solution (Mo 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample (2) to the order of 1 mg, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, shake to mix and add water up to the marked line.
c) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) The sampling amount of the analytical sample is 10 g when the content in the sample is less than 0.01 % (mass fraction) as water-soluble molybdenum.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 202.030 nm or 277.540 nm (3)

b) **Calibration curve preparation**

1) Spray the molybdenum standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 202.030 nm.
2) Prepare a curve for the relationship between the molybdenum concentration and the indicated value of the molybdenum standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 2 mg as Mo) in a 100-mL volumetric flask.
2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.
3) Subject to the same procedure as in **b**) **1**) to read the indicated value.
4) Obtain the molybdenum content from the calibration curve, and calculate the water-soluble molybdenum (W- Mo).

Note (**3**) 277.540 nm can also be used. However, since the intensity of emission obtained is different from the one of 202.030 nm, it is necessary to understand the suitable concentration range of the calibration curve and prepare a standard solution for the calibration curve in advance.

Comment 4 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 5 The comparison of the measurement value (y_i : 0.00342 % (mass fraction) - 0.20374 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of the Sodium thiocyanate absorptiometric analysis was conducted to evaluate trueness using fluid fertilizers (12 samples). As a result, a regression equation was y=0.0004+0.982x and its correlation coefficient (r) was 0.999. Additionally, additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 0.01 % (mass fraction) and 0.1 % (mass fraction) were 95.4 % and

97.6 % respectively.

The results of the repeatability tests on different days using a fluid compound fertilizer and a home garden-use compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study implemented for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.0005 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of water-soluble molybdenum (Fluid fertilizer)

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Fluid compound fertilizer	7	0.124	0.001	0.5	0.001	1.2
Home garden-use compound fertilizer (fluid)	7	0.00359	0.00001	0.3	0.00014	4.0

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble molybdenum

Analytical line wavelength (nm)	Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
202.030	Preparation sample (Liquid) 1	12	1.03	0.02	1.5	0.03	2.5
	Preparation sample (Liquid) 2	11	0.499	0.006	1.1	0.012	2.3
	Preparation sample (Liquid) 3	11	1.96	0.02	0.9	0.05	2.3
	Preparation sample (Liquid) 4	11	0.0471	0.0006	1.2	0.0012	2.6
	Preparation sample (Liquid) 5	10	0.0544	0.0003	0.5	0.0009	1.6
277.540	Preparation sample (Liquid) 1	11	1.04	0.01	1.3	0.02	2.4
	Preparation sample (Liquid) 2	10	0.501	0.004	0.8	0.012	2.4
	Preparation sample (Liquid) 3	10	1.95	0.02	1.0	0.04	2.2
	Preparation sample (Liquid) 4	9	0.0478	0.0003	0.7	0.0008	1.6
	Preparation sample (Liquid) 5	10	0.0540	0.0005	1.0	0.0010	1.8

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination Method for Effect-Development

Promoting Agent (Ca, Fe, Co, Cu, Zn and Mo) in Liquid Compound Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), Research Report of Fertilizer, **Vol. 9**, p. 1 - 9 (2016)

2) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(**5**) **Flow sheet**: The flow sheet for water-soluble molybdenum in fluid fertilizers is shown below:

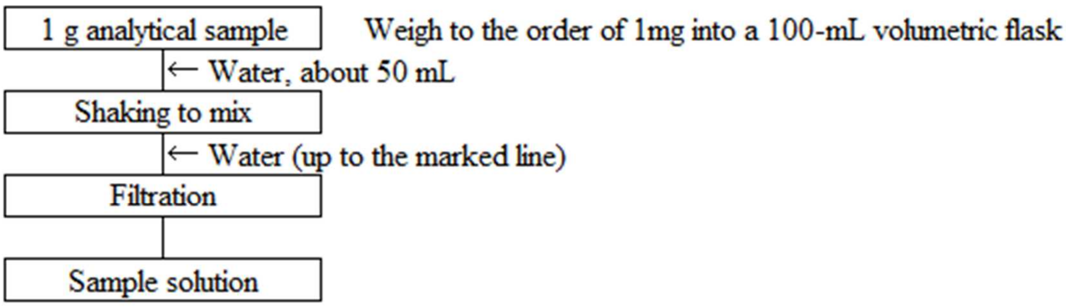


Figure 1 Flow sheet for water-soluble molybdenum in fluid fertilizers
(Extraction procedure)

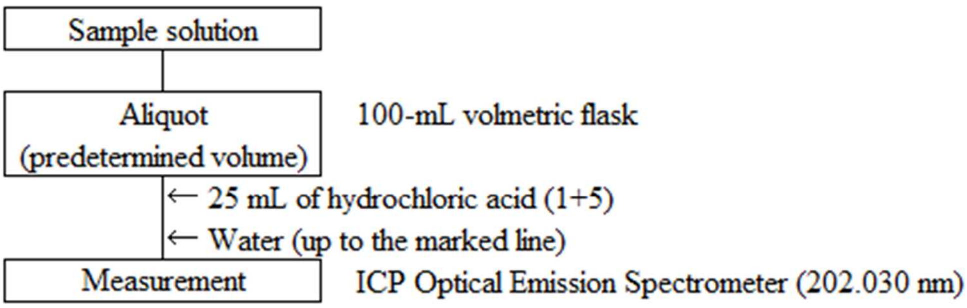


Figure 2 Flow sheet for water-soluble molybdenum in fluid fertilizers
(Measurement procedure)

4.15 Cobalt
4.15.1 Water-soluble cobalt
4.15.1.a Flame atomic absorption spectrometry
(1) Summary

This testing method is applicable to fluid compound fertilizers, liquid microelement compound fertilizers and the fluid fertilizers of home garden-use compound fertilizers. This testing method is classified as Type E and its symbol is 4.15.1.a-2017 or W-Co.a-1.

Extract by adding water to an analytical sample, spray in an acetylene−air flame and measure the atomic absorption with cobalt at a wavelength of 240.7 nm to obtain water-soluble cobalt (W-Co) in an analytical sample.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Cobalt standard solution (Co 0.1 mg/mL)**: A cobalt standard solution (Co 0.1 mg/mL) traceable to National Metrology.

d) **Cobalt standard solutions (Co 0.5 µg - 5 µg/mL) for the calibration curve preparation** (1): Put 2.5 mL - 25 mL of cobalt standard solution (Co 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

e) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the cobalt standard solution in (**2**), a cobalt standard solution for the calibration curve preparation can be prepared by using a cobalt standard solution (Co 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(**3**) **Instruments**: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (2) function.

1) **Light source**: An cobalt hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)

2) **Gas:** Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

Note (**2**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample (3) to the order of 1 mg, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, shake to mix and add water up to the marked line.

c) Filter with Type 3 filter paper to make a sample solution.

Note (**3**) The sampling amount of the analytical sample is 10 g when the content in the sample is less than 0.01 % (mass fraction) as water-soluble cobalt.

Comment 2 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 240.7 nm

b) **Calibration curve preparation**

1) Spray the cobalt standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 240.7 nm.

2) Prepare a curve for the relationship between the cobalt concentration and the indicated value of the cobalt standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 2 mg as Co) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b) 1**) to read the indicated value.

4) Obtain the cobalt content from the calibration curve, and calculate the water-soluble cobalt (W-Co) in the analytical sample.

(**5**) **Flow sheet**: The flow sheet for water-soluble cobalt in fluid fertilizers is shown below:

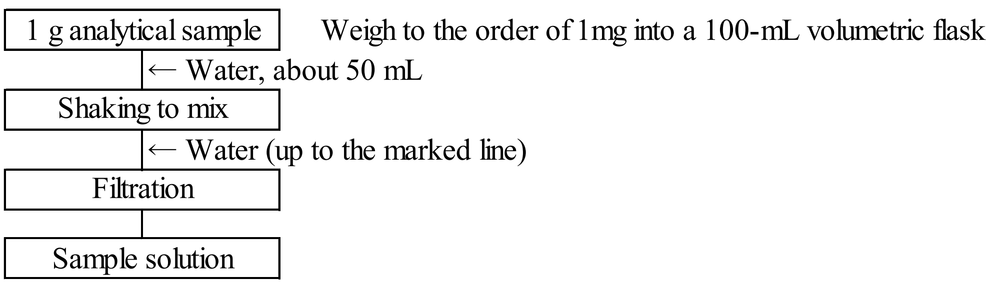


Figure 1 The flow sheet for water-soluble cobalt in fluid fertilizers (Extraction procedure)

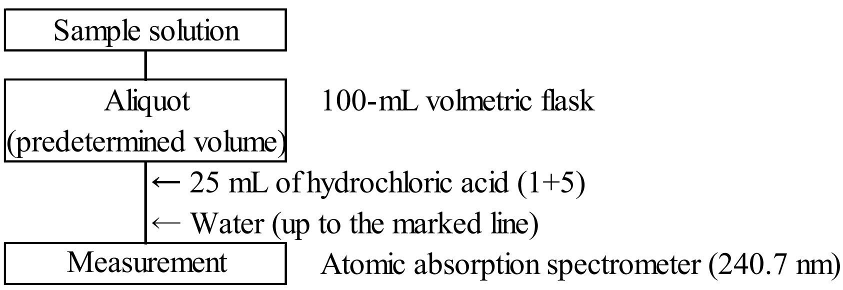


Figure 2 The flow sheet for water-soluble cobalt in fluid fertilizers (Measurement procedure)

4.15.1.b ICP Optical Emission Spectrometry

(1) Summary

This testing method is applicable to fluid compound fertilizers, liquid microelement compound fertilizers and the fluid fertilizers of home garden-use compound fertilizers. This testing method is classified as Type B and its symbol is 4.15.1.b-2017 or W-Co.b-1.

Extract by adding water to an analytical sample, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the cobalt at a wavelength of 228.616 nm to obtain water-soluble cobalt (W-Co) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Cobalt standard solution (Co 1 mg/mL)**: A cobalt standard solution (Co 1 mg/mL) traceable to National Metrology.

d) **Cobalt standard solution (Co 0.1 mg/mL)** (1): Put 10 mL of cobalt standard solution (Co 1 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.

e) **Cobalt standard solutions (Co 1 µg - 20 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of cobalt standard solution (Co 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

f) **Copper standard solutions (Cu 0.1 µg - 1 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 10 mL of copper standard solution (Cu 10 µg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **d**), **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Instead of the cobalt standard solution in (**2**), a cobalt standard solution for the calibration curve preparation can be prepared by using a cobalt standard solution (Co 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample (2) to the order of 1 mg, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, shake to mix and add water up to the marked line.

c) Filter with Type 3 filter paper to make a sample solution.

Note (**2**) The sampling amount of the analytical sample is 10 g when the content in the sample is less than 0.01 % (mass fraction) as water-soluble cobalt.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following: Analytical line wavelength: 228.616 nm

b) **Calibration curve preparation**

1) Spray the cobalt standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 228.616 nm.

2) Prepare a curve for the relationship between the cobalt concentration and the indicated value of the cobalt standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg - 2 mg as Co) in a 100-mL volumetric flask.

2) Add 25 mL of hydrochloric acid (1+5), and add water up to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Obtain the cobalt content from the calibration curve, and calculate the water-soluble cobalt (W-Co) in the analytical sample.

Comment 4 Simultaneous measurement of multiple elements is possible with the ICP Optical Emission Spectrometry. In that case, prepare standard solutions for calibration curve preparation referencing the measurement conditions in Table 1 of Annex C1, conduct procedures similarly as (**4.2**) **b**) - **c**), and multiply the obtained measurement values of the respective element concentrations by conversion factors to calculate respective main components in an analytical sample.

Comment 5 The comparison of the measurement value (y_i : 0.00105 % (mass fraction) - 0.0213 % (mass fraction)) of ICP Optical Emission Spectrometry and the measurement value (x_i) of flame atomic absorbance spectrometry was conducted to evaluate trueness using fluid fertilizers (12 samples). As a result, a regression equation was y=0.0001+0.927x, and its correlation coefficient (r) was 0.996. Additionally, additive recovery testing was conducted using a fluid compound fertilizer (1 brand) and a home garden-use compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 0.01 % (mass fraction) and 0.1 % (mass fraction) were 94.6 % and 98.4 % respectively.

The results of the repeatability tests on different days using a fluid compound fertilizer and a home garden-use compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study implemented for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.0005 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of water-soluble cobalt (Fluid fertilizer)

Name of sample	Test days of repeatability (T)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Fluid compound fertilizer	7	0.0554	0.0010	1.7	0.0024	4.4
Home garden-use compound fertilizer (fluid)	7	0.0105	0.0003	3.3	0.0005	4.8

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of water-soluble cobalt

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Preparation sample (Liquid) 1	11	0.524	0.006	1.2	0.009	1.7
Preparation sample (Liquid) 2	11	1.08	0.01	0.7	0.02	1.9
Preparation sample (Liquid) 3	10	2.15	0.005	0.2	0.03	1.2
Preparation sample (Liquid) 4	10	0.108	0.0003	0.3	0.002	1.4
Preparation sample (Liquid) 5	11	0.0521	0.0005	1.1	0.0014	2.6

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Keisuke AOYAMA: Simultaneous Determination Method for Effect-Development Promoting Agent (Ca, Fe, Co, Cu, Zn and Mo) in Liquid Compound Fertilizer using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), Research Report of Fertilizer, **Vol. 9**, p. 1 - 9 (2016)

2) Masayuki YAMANISHI, Madoka KATOU and Yuji SHIRAI: Performance Evaluation of Determination Method for Effective Ingredients by ICP-OES in Liquid Fertilizer: Harmonized Collaborative Validation, Research Report of Fertilizer, **Vol. 13**, p. 123 – 145 (2020)

(5) Flow sheet: The flow sheet for water-soluble cobalt in fluid fertilizers is shown below:

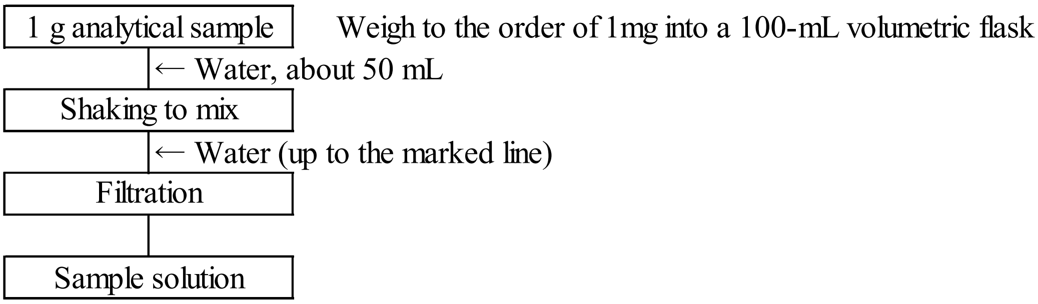


Figure 1 The flow sheet for water-soluble cobalt in fluid fertilizers
(Extraction procedure)

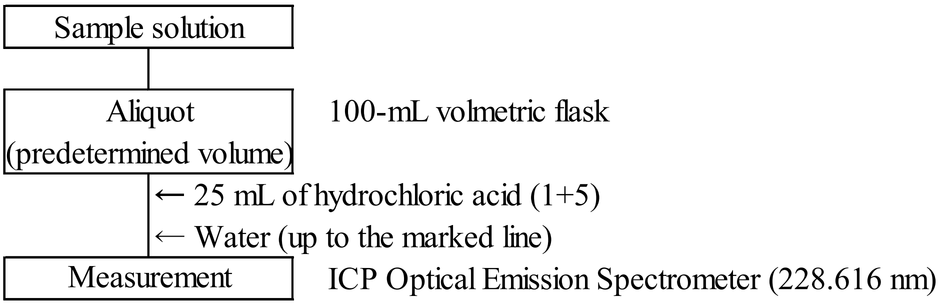


Figure 2 The flow sheet for water-soluble cobalt in fluid fertilizers
(Measurement procedure)

5. Harmful components

5.1 Mercury

5.1.a Cold vapor atomic absorption spectrometry

(1) Summary

The test method is applicable to fertilizers excluding fluid sludge fertilizers. This testing method is classified as Type B and its symbol is 5.1.a-2017 or Hg.a-1.

Pretreat an analytical sample with nitric acid- perchloric acid, and then reduce mercury (II) in the solution with tin (II) chloride. Aerate this solution, and measure the atomic absorption for generated mercury vapor at a wavelength of 253.7 nm to obtain mercury (Hg) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

- **a**) **Water**: Water of A3 specified in JIS K 0557.
- **b**) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c**) **Perchloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d**) **Sulfuric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **e**) **Tin (II) chloride solution**: To 10 g of tin (II) chloride dihydrate (1) specified in JIS K 8136, add 60 mL of sulfuric acid (1+20), and stir while heating to dissolve. After cooling is complete, add water to make 100 mL.
- **f**) **L-cysteine solution**: To 10 mg of L-cysteine ($HSCH_2CH(NH_2)COOH$) of no less than 98.0 % in purity, add 100 mL of water and 2 mL of nitric acid to dissolve, and further add water to make 1000 mL. Store in a refrigerator, and do not use after 6 months after preparation.
- **g**) **Tri-n-butyl phosphate** (2): A reagent of no less than 98 % in purity.
- **h**) **Mercury standard solution (Hg 0.1 mg/mL)**: A mercury standard solution (Hg 0.1 mg/mL) traceable to National Metrology.
- **i**) **Mercury standard solution (Hg 10 µg/mL)** (3) (4): Put 10 mL of mercury standard solution (Hg 0.1 mg/mL) in a 100 mL of volumetric flask and add an L-cysteine solution up to the marked line.
- **j**) **Mercury standard solution (Hg 0.1 µg/mL)** (3) (5): Dilute a predetermined amount of mercury standard solution (Hg 10 µg/mL) with an L-cysteine solution to prepare a mercury standard solution (Hg 0.1 µg/mL).

Note (**1**) Use a reagent with low mercury content, such as for mercury analysis or for harmful metal analysis.
(**2**) Use as an anti-forming agent.
(**3**) This is an example of preparation; prepare an amount as appropriate.
(**4**) Store in a refrigerator, and do not use after 4 months after preparation.
(**5**) Store in a refrigerator, and do not use after 1 months after preparation.

Comment 1 Instead of the mercury standard solution in (**2**), a mercury standard solution for the calibration curve preparation can be prepared by using a mercury standard solution (Hg 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

- **a**) **Mercury atomic absorption spectrometer**: Mercury atomic absorption spectrometer using a method for producing the atomic vapor by reduction specified in JIS K 0121.
  - 1) **Light source**: Low-pressure mercury lamp
- **b**) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can

be set to 180 °C - 200 °C.

c) **Sample digestion flask** (6): A 100-mL borosilicate glass volumetric flask 100 mL (180 mm total height, 13 mm mouth diameter)

Note (**6**) Distinguish the volumetric flask used in digestion as a sample digestion flask and do not use it for any other purposes.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it in a sample digestion flask.

b) Add about 10 mL of nitric acid, and heat on a hot plate or sand bath for a short time (7).

c) After standing to cool, add about 10 mL of perchloric acid, and digest by heating on a hot plate or sand bath at 180 ºC - 200 ºC for about 30 minutes - 1 hour (8).

d) After standing to cool, precisely adjust with water to 100 mL to make a sample solution.

e) As a blank test, conduct the procedures in **b)** - **d)** using another sample digestion flask to prepare a blank test solution.

Note (**7**) If it foams vigorously, leave at rest overnight.

(8) The sample solution and the blank test solution should be stored when they are cooled after the procedure in (**4.1**) **c**). After precisely adjust the volume of the sample solution and the blank test solution with water, immediately conduct the procedure in (**4.2**).

(**4.2**) **Measurement**: Conduct measurement by cold vapor atomic absorption spectrometry specified in JIS K 0121. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used. An example of measurement using a mercury atomic absorption spectrometer is shown below:

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 253.7 nm

b) **Calibration curve preparation**

1) Put 1 mL - 20 mL of mercury standard solution (Hg 0.1 µg/mL) in 100-mL volumetric flasks step-by-step, and add water up to the marked line. Put 5 mL of these solutions in respective reduction vessels, add 1 drop of tri-n-butyl phosphate (9), to make mercury standard solutions for the calibration curve preparation.

2) Put 5 mL of water in another reduction vessel, and add 1 drop of tri-n-butyl phosphate (9), to make the blank test solution for the calibration curve preparation.

3) Connect the reduction vessel to the mercury atomic absorption spectrometer, and introduce sulfuric acid (1+1) and a tin (II) chloride solution, and circulate air.

4) Read the indicated value at a wavelength of 253.7 nm.

5) Prepare a curve for the relationship between the mercury content (µg) and the indicated value of the mercury standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put 5 mL of a sample solution in respective reduction vessels, add 1 drop of tri-n-butyl phosphate (9), and conduct similarly as in **b) 3)** - **4)** to read the indicated value.

2) Put 5 mL of the blank test solution in a reduction vessel, add 1 drop of tri-n-butyl phosphate (9), and conduct similarly as in **b) 3)** - **4)** to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the mercury content (µg) from the calibration curve, and calculate mercury (Hg) in the analytical sample.

Note (**9**) It is not required to add tri-*n*-butyl phosphate if not needed.

Comment 2 Instead of the correction method in **c**) **2**), mercury (Hg) can be corrected by obtaining the mercury content in the blank test.

Comment 3 Recovery testing was conducted to evaluate trueness using an industrial sludge fertilizer (1 sample), composted sludge (3 samples) and a human waste sludge fertilizer (1 sample). As a result, the mean recovery rates at the concentration level of 2 mg/kg and 0.2 mg/kg were 98.7 % - 101.6 % and 100.7 % - 105.4 % as mercury (Hg) respectively. In addition, recovery testing was conducted using soybean meal, rape seed meal, compound fertilizers (2 samples) and blended fertilizers. As a result, the mean recovery rates at the concentration level of 40 mg/kg and 0.5 mg/kg were 98.5 % - 101.5 % and 100.4 % - 103.3 % as mercury (Hg) respectively.

In order to evaluate precision, results from a collaborative study for test method validation and its analysis are shown in Table 1. In addition, the results of the collaborative study to determine a certified reference material fertilizer were analyzed by using a three-level nesting analysis of variance. Table 2 shows the calculation results of reproducibility, intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 mg/kg.

Table 1 Analysis results of the collaborative study for mercury test method validation

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Human waste sludge fertilizer A	11	0.651	5.3	11.6
Human waste sludge fertilizer B	11	1.10	6.3	10.2
Composted sludge fertilizer A	11	0.489	6.8	10.2
Composted sludge fertilizer B	11	0.822	8.1	13.1
Composted sludge fertilizer C	9	0.182	10.6	10.6

1) Number of laboratories used in analysis

2) Mean (*n* = number of laboratories × number of samples (2))

3) Repeatability relative standard deviation

4) Reproducibility relative standard deviation

Table 2 Analysis results of the collaborative study to determine mercury of a certified reference material fertilizer

Name of certified reference material fertilizer	Number of laboratory (p)[1]	Mean[2] mg/kg	s_r[3] mg/kg	RSD_r[4] (%)	$s_{I(T)}$[5] mg/kg	$RSD_{I(T)}$[6] (%)	s_R[7] mg/kg	RSD_R[8] (%)
FAMIC-B-10	10	0.86	0.02	2.4	0.03	4.0	0.05	5.5
FAMIC-C-12	11	0.48	0.02	3.5	0.02	4.6	0.02	5.0

1) The number of laboaratories used for analysis conducting flame atomic absorbance spectrometry
2) Mean (the number of laboratory(p)×test days(2) ×the number of replicate testing(3))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Intermediate standard deviation
6) Intermediate relative standard deviation
7) Reproducibility standard deviation
8) Reproducibility relative standard deviation

References

1) Fumihiro ABE, Takeshi HASHIMOTO and Yasushi SUGIMURA: Determination of mercury in sludge fertilizer - Improved decomposition method -, Research Report of Fertilizer **Vol. 1**, p. 60 - 66 (2008)

2) Fumihiro ABE, Takeshi HASHIMOTO and Norio HIKICHI: Determination of mercury in sludge fertilizer - Collaborative Test Results -, Research Report of Fertilizer **Vol. 1**, p. 67 - 73 (2008)

3) Akira SHIMIZU, Kaori OKADA, Takeshi HASHIMOTO, Yasuto IDE and Toshiaki HIROI: Determination of Mercury in Fertilizer- Expanding the scope of application of improved method -, Research Report of Fertilizer **Vol. 2**, p. 12 - 17 (2009)

(**5**) **Flow sheet for mercury**: The flow sheet for mercury in fertilizers is shown below:

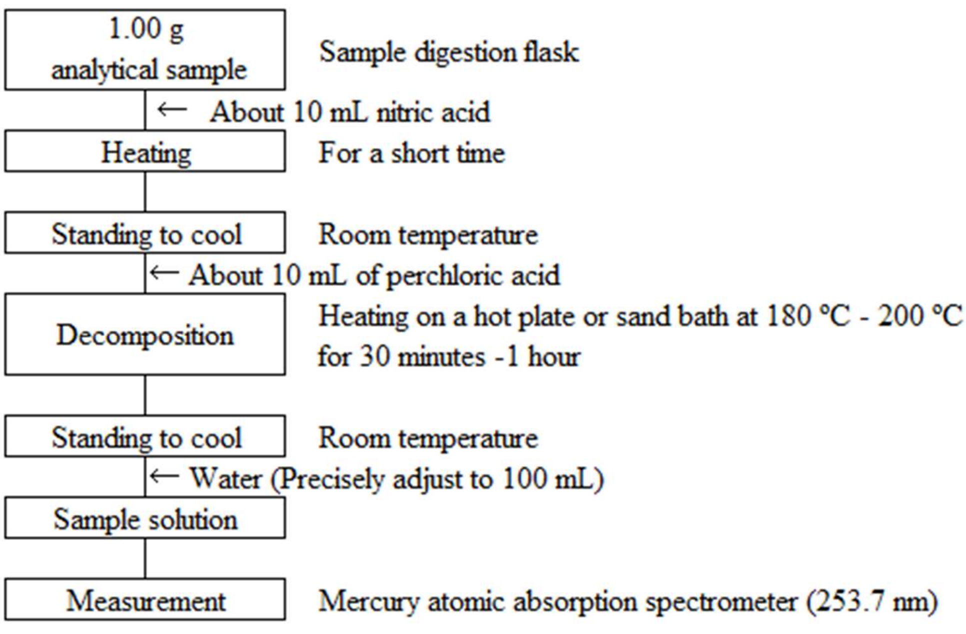


Figure Flow sheet for mercury in fertilizers

5.1.b Cold vapor atomic absorption spectrometry (Fluid sludge fertilizers)

(1) Summary

The test method is applicable to fluid sludge fertilizers. This testing method is classified as Type D and its symbol is 5.1.b-2017 or Hg.b-1.

Pretreat an analytical sample with nitric acid- perchloric acid, and then reduce mercury (II) in the solution with tin (II) chloride. Aerate this solution, and measure the atomic absorption for generated mercury vapor at a wavelength of 253.7 nm to obtain mercury (Hg) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Hydrogen peroxide**: A JIS Guaranteed Reagent specified in JIS K 8230 or a reagent of equivalent quality.

d) **Sulfuric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

e) **Tin (II) chloride solution**: To 10 g of tin (II) chloride dihydrate (1) specified in JIS K 8136, add 60 mL of sulfuric acid (1+20), and stir while heating to dissolve. After cooling is complete, add water to make 100 mL.

f) **L-cysteine solution:** To 10 mg of L-cysteine ($HSCH_2CH(NH_2)COOH$) of no less than 98.0 % in purity, add 100 mL of water and 2 mL of nitric acid to dissolve, and further add water to make 1000 mL. Store in a refrigerator, and do not use after 6 months after preparation.

g) **Mercury standard solution (Hg 0.1 mg/mL)**: A mercury standard solution (Hg 0.1 mg/mL) traceable to National Metrology.

h) **Mercury standard solution (Hg 10 µg/mL)** (2) (3): Put 10 mL of mercury standard solution (Hg 0.1 mg/mL) in a 100 mL of volumetric flask and add an L-cysteine solution up to the marked line.

i) **Mercury standard solution (Hg 0.1 µg/mL)** (2) (4): Dilute a predetermined amount of mercury standard solution (Hg 10 µg/mL) with an L-cysteine solution to prepare a mercury standard solution (Hg 0.1 µg/mL).

Note (**1**) Use a reagent with low mercury content, such as for mercury analysis or for harmful metal analysis.

(**2**) This is an example of preparation; prepare an amount as appropriate.

(**3**) Store in a refrigerator, and do not use after 4 months after preparation.

(**4**) Store in a refrigerator, and do not use after 1 month after preparation.

Comment 1 Instead of the mercury standard solution in (**2**), a mercury standard solution for the calibration curve preparation can be prepared by using a mercury standard solution (Hg 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Mercury atomic absorption spectrometer** : A mercury atomic absorption spectrometer using a method for producing the atomic vapor by reduction specified in JIS K 0121.

1) **Light source**: Low-pressure mercury lamp

b) **Pressure vessel decomposing device**: A device which pressurizes the inside of a vessel by putting acid, etc. to heat in the decomposing vessel, and decomposes a sample by the interaction of heating, pressurizing and acid. The following requirements should be met.

1) **The main part of a decomposing device**: In the case of the microwave heating method, a device should be able to produce a high-frequency wave using a frequency which is permitted

at an industrial frequency facility. It is desirable to be able to monitor pressure and temperature, etc. in the decomposing vessel with a sensor inside the device. The interior of a device should be acid-resistant treated and should have a high temperature durability and a high level of safety.

2) **Exhaust system**: A system which has an exhaust fan of acid-resistant specification, and has an air-cool function inside the device to allow constant airflow, keeping operating temperature below a certain temperature level.

3) **Decomposing vessel**: A vessel which is heat-resistance and pressure-tight and has the durability required to decompose particles, and has resistance to internal contamination. It should have safety functions such as causing overheat prevention valve to work and internal pressure to drop by emitting gas, and preventing gas bumping in the case of exceeding a pressure limitation.

c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 20.0 g (5) of an analytical sample, and put it in an airtight vessel.

b) Gradually add 2.5 mL of nitric acid and 2 mL of hydrogen peroxide.

c) Put the airtight vessel into the main part of a decomposing device and heat using a microwave (6).

d) Ignite at 240 ºC ±5 ºC for no less than 10 minutes (6) to decompose (7).

e) After standing to cool, transfer the solution to a 50-mL volumetric flask (8) with water.

f) Add water up to the marked line and transfer it to a 50-mL ground-in stopper centrifugal precipitate tube (8).

g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (9) and use the supernatant as a sample solution.

h) As a blank test, conduct the procedures in **b**) - **g**) using another airtight vessel to prepare a blank test solution.

Note (**5**) The maximum limit of solid content, which is converted from moisture content, in the sampling volume 20.0 g of an analytical sample is about 0.5 g. If solid content is likely to exceed the limit, reduce sampling volume as necessary.

(**6**) Condition examples for a microwave decomposing device: 0 min (room temperature) → 10min (240 ºC) → 20 min (240 ºC) → 40 min (room temperature), initial output 1400 W

(**7**) When organic matters still remain, for example the digestion solution is colored, repeat the procedures in (**4.1**) **b**) - **c**).

(**8**) The vessel should be made of polypropylene, etc. to not affect the measurement.

(**9**) 16.5-cm of radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**4.2**) **Measurement**: Conduct measurement by cold vapor atomic absorption spectrometry specified in JIS K 0121. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used. An example of the measurement using the mercury atomic absorption spectrometer is shown below:

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 253.7 nm

b) **Calibration curve preparation**

1) Put 0.4 mL - 10 mL of mercury standard solution (Hg 0.1 μg/mL) in 100-mL volumetric flasks step-by-step, and add water up to the marked line. Put 5 mL of these solutions in respective reduction vessels to make mercury standard solutions for the calibration curve preparation.

2) Put 5 mL of water in another reduction vessel to make the blank test solution for the calibration curve preparation.

3) Connect the reduction vessel to the mercury atomic absorption spectrometer, and introduce sulfuric acid (1+1) and a tin (II) chloride solution, and circulate air.

4) Read the indicated value at a wavelength of 253.7 nm.

5) Prepare a curve for the relationship between the mercury content (μg) and the indicated value of the mercury standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put 5 mL of a sample solution in respective reduction vessels, and conduct similarly as in **b**) **3**) - **4**) to read the indicated value.

2) Put 5 mL of the blank test solution in a reduction vessel and conduct similarly as in **b**) **3**) - **4**) to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the mercury content (μg) from the calibration curve, and calculate mercury (Hg) in the analytical sample.

Comment 2 Instead of the correction method in **c**) **2**), mercury (Hg) can be corrected by obtaining the mercury content in the blank test.

Comment 3 Triplicates additive recovery testing was conducted to evaluate trueness using fluid industrial sludge fertilizers (2 samples), and composted sludge fertilizers (6 samples). As a result, the mean recovery rates at the concentration level of 0.2 mg/kg - 0.4 mg/kg, 0.01 mg/kg - 0.09 mg/kg and 0.7μg /kg - 7μg /kg were 100.0 % - 109.1 %, 99.0 % - 114.6 % and 100.4 % - 113.4 % as mercury (Hg) in an actual article respectively.
The results of the repeatability tests on different days using two kinds of fluid sludge fertilizers to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.2 μg/kg.

Table 1 Analysis results of the repeatability tests on different days of mercury
(Fluid fertilizer)

Name of sample	Test days of repeatability (T)[1]	Mean[2] (mg/kg)	s_r[3] (mg/kg)	RSD_r[4] (%)	$s_{I(T)}$[5] (mg/kg)	$RSD_{I(T)}$[6] (%)
Composted sludge fertilizer 1	5	0.0577	0.0009	1.5	0.0014	2.5
Composted sludge fertilizer 2	5	0.0142	0.0002	1.7	0.0003	2.2

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Intermediate standard deviation
6) Intermediate relative standard deviation

References

1) Toshiharu YAGI: Determination of Heavy Metals in Fluid Sludge Fertilizers by ICP-MS and CV-AAS, Research Report of Fertilizer **Vol. 8**, p. 26 - 37 (2015)

(**5**) **Flow sheet for mercury**: The flow sheet for mercury in fluid sludge fertilizers is shown below:

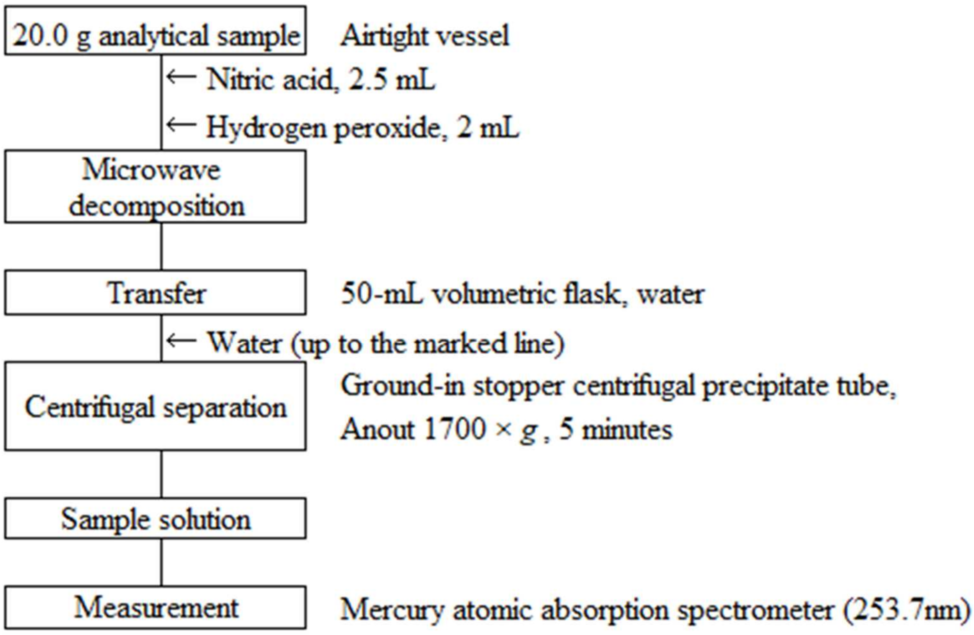


Figure Flow sheet for mercury in fluid sludge fertilizers

5.2 Arsenic

5.2.a Hydride generation atomic absorption spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 5.2.a-2017 or As.a-1.

Pretreat an analytical sample with nitric acid-sulfuric acid-perchloric acid, and then generate arsenic hydride by the addition of sodium tetrahydroborate in the acidic condition with hydrochloric acid, introduce it with argon gas to a heated absorption cell, and measure the atomic absorption with arsenic at a wavelength of 193.7 nm to obtain arsenic (As) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(2) Reagent, etc.: Reagents and water are as shown below.

- **a) Water**: Water of A3 specified in JIS K 0557.
- **b) Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **c) Sulfuric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **d) Perchloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
- **e) Hydrochloric acid**: Hydrochloric acid for arsenic analysis specified in JIS K 8180, or of harmful metal analysis grade, microanalysis grade or equivalents.
- **f) Potassium iodide solution** (1): Dissolve 20 g of potassium iodide specified in JIS K 8913 in water to make 100 mL.
- **g) Sodium hydroxide**: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.
- **h) Sodium tetrahydroborate solution** (1): Dissolve 10 g of sodium tetrahydroborate ($NaBH_4$) for atomic absorption spectrometry in a sodium hydroxide solution (4 g/L) to make 1000 mL.
- **i) Arsenic standard solution (As 0.1 mg/mL)**: An arsenic standard solution (As 0.1 mg/mL) traceable to National Metrology.
- **j) Arsenic standard solution (As 1 μg/mL)** (2) (3): Dilute a predetermined amount of arsenic standard stock solution (As 0.1 mg/mL) accurately with hydrochloric acid (1+100) to prepare an arsenic standard solution (As 1 μg/mL).
- **k) Arsenic standard solution (As 0.1 μg/mL)** (2) (4): Dilute a predetermined amount of arsenic standard solution (As 0.1 μg/mL) with hydrochloric acid (1+100) to prepare an arsenic standard solution (As 0.1 μg/mL).

Note (**1**) The concentrations of a potassium iodide solution and a sodium tetrahydroborate solution vary depending on the instrument used.
(**2**) This is an example of preparation; prepare an amount as appropriate.
(**3**) Store in a refrigerator, and do not use after 6 months after preparation.
(**4**) Store in a refrigerator, and do not use after 1 month after preparation.

Comment 1 Instead of the arsenic standard solution in (**2**), an arsenic standard solution for the calibration curve preparation can be prepared by using an arsenic standard solution (As 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(3) Instruments: Instruments are as shown below:

- **a) Atomic absorption spectrometer** : To an atomic absorption spectrometer specified in JIS K 0121, connect a hydride generator and parts shown below. Also, an atomic absorption spectrometer with a built-in hydride generator can be used.
  - **1) Light source**: An arsenic hollow cathode lamp or an arsenic high-intensity discharge lamp.
  - **2) Atomizer**: Heated absorption cell (5)
  - **3) Gas**: Gases for heating the heated absorption cell.

(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Hydride generator**: A batch-type or continuous-type hydride generator specified in JIS K 0121. For continuous hydride generators, there is a method to introduce a potassium iodide solution on-line in addition to a sample solution, hydrochloric acid, and a sodium tetrahydroborate solution.

1) **Argon:** Argon of grade 2 specified in JIS K 1105 or equivalents.

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 350 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to no less than 300 °C.

Note (**5**) For cell heating, there are a method of electric heating and a method of flame heating.

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1.00 g - 2.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.

b) Add about 10 mL of nitric acid and about 5 mL of sulfuric acid, cover the tall beaker with a watch glass, and leave at rest overnight.

c) Heat mildly on a hot plate or sand bath at 170 ºC - 220 ºC for no less than 30 minutes. After bubbles cease to form, set the temperature of the hot plate or sand bath to no less than 300 ºC, and heat until nitroxide (yellow-brown smoke) is no longer generated (6) (7).

d) After standing to cool, add about 5 mL of perchloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or sand bath at no less than 300 ºC for 2 - 3 hours to digest (8).

f) Slightly move the watch glass (9), and keep on heating on the hot plate or sand bath to concentrate until the liquid volume becomes no more than 2 mL (10).

g) After standing to cool, add about 5 mL of hydrochloric acid (1+10) and about 20 mL of water, cover the tall beaker with a watch glass and heat mildly to dissolve.

h) After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**6**) Carbonization (degradation) of organic matters by sulfuric acid begins by heating when nitric acid no longer remains. In this state, As5+ may be reduced to As3+ and evaporate; therefore, stop heating immediately after the end of the generation of nitroxide (yellow-brown smoke).

(**7**) Oxidation of organic matters by perchloric acid progresses extremely rapidly and explosively. For that reason, add perchloric acid after fully degrading organic matters with nitric acid to avoid danger.

(**8**) When the white smoke of perchloric acid is generated, if the solution is colored such as black-brown or brown, stop heating immediately, and after standing to cool, add nitric acid, and heat again to degrade remaining organic matters.

(**9**) The watch glass can be removed.

(**10**) The generation of arsenic hydride is inhibited by the presence of nitric acid; therefore, remove nitric acid by sufficiently generating the white smoke of sulfuric acid.

Comment 2 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B. However, the sampling amount of the analytical sample in (**4.1**) **a**) in **5.5.c** is 1.00 g.

Comment 3 When the analytical sample solidifies in the procedure in (**4.1**) **b**), moisten the analytical sample with a small amount of water as necessary in advance.

Comment 4 It is not necessary to conduct the procedure in (**4.1**) **b**) "leave at rest overnight" in the case of fertilizers not containing organic matters.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used. Two examples of measurement procedures with a continuous hydride generator are shown below.

(**4.2.1**) **Measurement (A)**: Leaving at rest after adding a potassium iodide solution.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 193.7 nm

b) **Calibration curve preparation**

1) Put 2.5 mL - 10 mL of arsenic standard solution (As 0.1 μg/mL) in 50-mL volumetric flasks step-by-step.

2) Add 5 mL of hydrochloric acid and 5 mL of potassium iodide solution, leave at rest for about 15 minutes, and then add water up to the marked line, to make 5 ng/mL -20 ng/mL arsenic standard solutions for the calibration curve preparation.

3) Conduct the same procedures as **2**) for another 50-mL volumetric flask to make a blank test solution for the calibration curve preparation.

4) While letting argon flow, introduce the arsenic standard solution for the calibration curve preparation for each step and the blank test solution for the calibration curve preparation respectively, further introduce hydrochloric acid (1+1) and a sodium tetrahydroborate solution to the hydride generator to generate arsenic hydride.

5) Separate arsenic hydride and liquid waste, and then introduce the gas containing arsenic hydride to a heated absorption cell, and read the indicated value at a wavelength of 193.7 nm.

6) Prepare a curve for the relationship between the arsenic concentration and the indicated value of the arsenic standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution in a 50-mL volumetric flask, and conduct procedures similarly as in **b**) **2**) and **b**) **4**) - **5**) to read the indicated value.

2) Put a predetermined amount of the blank test solution in a 50-mL volumetric flask, and conduct procedures similarly as in **b**) **2**) and **b**) **4**) - **5**) to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the arsenic content from the calibration curve, and calculate arsenic (As) in the analytical sample.

(**4.2.2**) **Measurement (B)**: On-line introduction of a potassium iodide solution.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 193.7 nm

b) **Calibration curve preparation**

1) Put 5 mL - 25 mL of arsenic standard solution (As 0.1 μg/mL) in 50-mL volumetric flasks step-by-step, add water up to the marked line, to make the 10 ng/mL - 50 ng/mL arsenic standard solution for the calibration curve preparation. Use water as the blank test solution for the calibration curve preparation.

2) While letting argon flow, introduce arsenic standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation, respectively, and further introduce a potassium iodide solution, hydrochloric acid (1+1) and a sodium tetrahydroborate solution to the hydride generator to generate arsenic hydride.

3) Separate arsenic hydride and liquid waste, and then introduce the gas containing arsenic hydride to a heated absorption cell, and read the indicated value at a wavelength of 193.7 nm.

4) Prepare a curve for the relationship between the arsenic concentration and the indicated value of the arsenic standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put a predetermined amount of the sample solution in a 50-mL volumetric flask, and conduct procedures similarly as in **b**) **2**) - **3**) to read the indicated value.

2) Put a predetermined amount of the blank test solution in a 50-mL volumetric flask, add water up to the marked line, and conduct similarly as in **b**) **2**) - **3**) to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the arsenic content from the calibration curve, and calculate arsenic (As) in the analytical sample.

Comment 5 The coexistence of iron, nickel, and cobalt at over 5, 10, 80 folds amount of arsenic, respectively, inhibits the generation of arsenic hydride. However, the inhibition of arsenic hydride generation even in the coexistence of iron at 1000 folds amount can be removed by adding or introducing a potassium iodide solution.

Comment 6 Instead of the correction method in **c**) **2**), the arsenic (As) in the analytical sample can also be corrected by obtaining the arsenic content in the blank test solution.

Comment 7 Recovery testing was conducted using industrial sludge fertilizer, composted sludge fertilizer (3 samples) and human waste sludge fertilizer; as a result, the recovery rates at the concentration level of 50 mg/kg and 5 mg/kg were 94.6 % - 100.6 % and 99.9 % - 103.3 % as arsenic (As), respectively. Also, recovery testing was conducted using processed slug phosphorus fertilizer, soybean meal, rape seed meal, compound fertilizer and magnesia-potassium-sulfate fertilizer; as a result, the recovery rates at the concentration level of 50 mg/kg and 5 mg/kg were 98.5 % - 109.8 % and 103.5 % - 108.6 %, respectively.

Results from a collaborative study for test method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.1 mg/kg.

Table 1 Analysis results of the collaborative study for arsenic test method validation

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Sewage sludge fertilizer	11	6.42	3.5	10.7
Human waste sludge fertilizer	10	4.62	4.9	7.0
Industrial sludge fertilizer	12	0.632	5.7	19.7
Calcined sludge fertilizer	12	5.08	4.1	9.5
Composted sludge fertilizer	10	1.23	6.1	11.4

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Naoki ASAO, Yukie ISHIDA, Shinjiro IZUKA and Masakazu SAIKI: Determination of Arsenic in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer **Vol. 1**, p. 74 - 81 (2008)
2) Naoki ASAO, Shinjiro IZUKA and Norio HIKICHI: Determination of Arsenic in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer **Vol. 1**, p. 82 - 89 (2008)
3) Yasushi SUGIMURA, Naoki ASAO and Shinjiro IZUKA: Determination of Arsenic in Fertilizer- Expanding the scope of application of improved method -, Research Report of Fertilizer **Vol. 2**, p. 18 - 24 (2009)

(5) Flow sheet for arsenic: The flow sheet for arsenic in fertilizers is shown below:

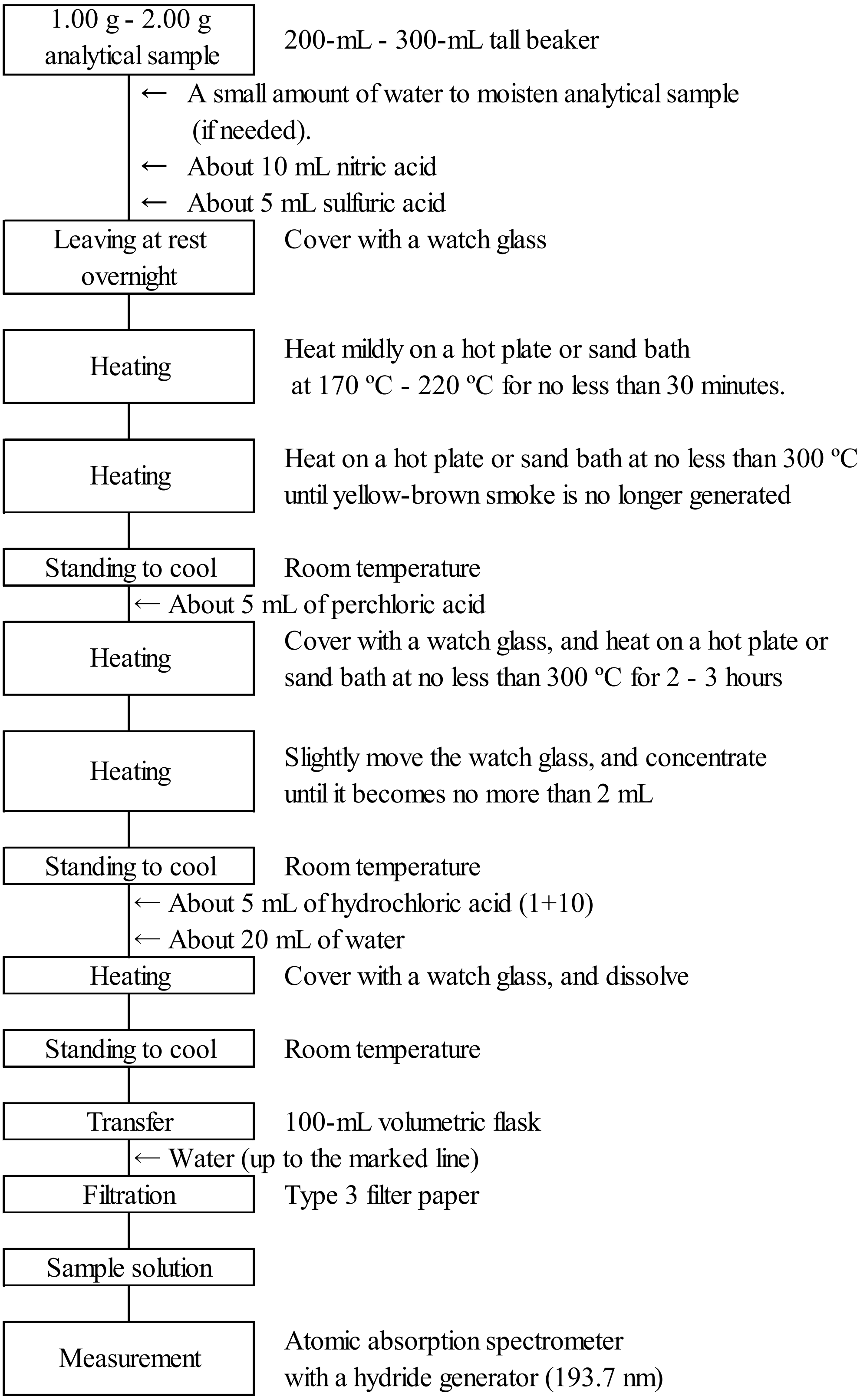


Figure Flow sheet for arsenic in fertilizers

5.2.b Silver diethyl dithiocarbamate absorptiometric analysis

(1) Summary

This test method is applicable to fertilizers other than sulfur and its compound. This testing method is classified as Type E and its symbol is 5.2.b-2017 or As.b-1.

Pretreat an analytical sample with nitric acid - sulfuric acid - perchloric acid, and then put the predetermined volume into an arsenic hydride generation bottle, and generate arsenic hydride by adding a potassium iodide solution, a tin chloride solution and zinc successively in the acidic condition with hydrochloric acid to react with silver diethyl dithiocarbamate in pyridine. Measure the absorbance with the silver diethyl dithiocarbamate solution, the coloring liquid, at a wavelength of 510 nm or 519 nm to obtain arsenic (As) in an analytical sample.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Nitric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) Sulfuric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) Perchloric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

e) Hydrochloric acid: Hydrochloric acid for arsenic analysis specified in JIS K 8180, or of harmful metal analysis grade, microanalysis grade or equivalents.

f) Potassium iodide solution: Dissolve 20 g of potassium iodide specified in JIS K 8913 in water to make 100 mL.

g) Tin (II) chloride solution: Dissolve 15 g of tin (II) chloride dihydrate specified in JIS K 8136 in 100 mL of hydrochloric acid (1+1), add a small amount of granular tin specified in JIS K 8580 then store in a colored bottle.

h) Ascorbic acid: A JIS Guaranteed Reagent specified in JIS K 9502 or a reagent of equivalent quality.

i) Zinc: A reagent of arsenic analysis grade specified in JIS K 8012 or equivalents. (1 mm - 1.5 mm particle diameter)

j) Lead acetate glass wool: Glass wool air-dried after moisturizing with 100 mL of the solution, where 10 g of lead acetate (II) trihydrate specified in JIS K 8374 is dissolved in water.

k) Silver diethyldithiocarbamate solution: Dissolve 0.5 g of silver N, N-diethyl dithiocarbamate specified in JIS K 9512 in 100 ml of pyridine specified in JIS K 8777, then store in cool and dark place

l) Arsenic standard solution (As 0.1 mg/mL): An arsenic standard solution (As 0.1 mg/mL) traceable to National Metrology.

m) Arsenic standard solution (As 1 µg/mL) (1) (2): Dilute a predetermined amount of arsenic standard stock solution (As 0.1 mg/mL) accurately with hydrochloric acid (1+100) to prepare an arsenic standard solution (As 1 µg/mL).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store in a refrigerator, and do not use after 6 months after preparation.

Comment 1 Instead of the arsenic standard solution in (**2**), an arsenic standard solution for the calibration curve preparation can be prepared by using an arsenic standard solution (As 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(3) Instruments: Instruments are as shown below:

a) Arsenic hydride generator: An arsenic hydride generator specified in 61.1 in JIS K 0102 or equivalents.

b) Spectrophotometer: A spectrophotometer specified in JIS K 0115

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 350 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to no less than 300 °C.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g - 2.00 g of an analytical sample, and put it in a 200-mL - 300-mL tall beaker.
b) Add about 10 mL of nitric acid and about 5 mL of sulfuric acid, cover the tall beaker with a watch glass, and leave at rest overnight.
c) Heat mildly on a hot plate or sand bath at 170 ºC - 220 ºC for no less than 30 minutes. After bubbles cease to form, set the temperature of the hot plate or sand bath to no less than 300 ºC, and heat until nitroxide (yellow-brown smoke) is no longer generated (3) (4).
d) After standing to cool, add about 5 mL of perchloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or sand bath at no less than 300 ºC for 2 - 3 hours to digest (5).
f) Slightly move the watch glass (6), and keep on heating on the hot plate or sand bath to concentrate until the liquid volume becomes no more than 2 mL (7).
g) After standing to cool, add about 5 mL of hydrochloric acid (1+10) and about 20 mL of water, cover the tall beaker with a watch glass and heat mildly to dissolve.
h) After standing to cool, transfer the solution to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**3**) Carbonization (degradation) of organic matters by sulfuric acid begins by heating when nitric acid no longer remains. In this state, As^{+5} may be reduced to As^{+3} and evaporate; therefore, stop heating immediately after the end of the generation of nitroxide (yellow-brown smoke).

(**4**) Oxidation of organic matters by perchloric acid progresses extremely rapidly and explosively. For that reason, add perchloric acid after fully degrading organic matters with nitric acid to avoid danger.

(**5**) When the white smoke of perchloric acid is generated, if the solution is colored such as black-brown or brown, stop heating immediately, and after standing to cool, add nitric acid, and heat again to degrade remaining organic matters.

(**6**) The watch glass can be removed.

(**7**) The generation of arsenic hydride is inhibited by the presence of nitric acid; therefore, remove nitric acid by sufficiently generating the white smoke of sulfuric acid.

Comment 2 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

Comment 3 When the analytical sample solidifies in the procedure in (**4.1**) **b**), moisten the analytical sample with a small amount of water as necessary in advance.

Comment 4 It is not necessary to conduct the procedure in (**4.1**) **b**) “leave at rest overnight” in the case of fertilizers not containing organic matters.

(4.2) Reaction: Conduct reaction as shown below.

a) Put a predetermined amount (the equivalents of 1 µg - 20 µg of As and liquid volume is no more than 40 mL) of the sample solution into an arsenic hydride generation bottle.
b) Add water to the solution to make about 40 mL.
c) Add about 10 mL of hydrochloric acid.

d) Add about 2 mL of potassium iodide solution, shake and leave at rest for a few minutes.
e) Add about 1 mL of tin (II) chloride solution, shake and leave at rest for about 10 minutes (8).
f) Connect arsenic hydride generation bottle, glass tube lightly stuffed with lead acetate glass wool in advance and 5 mL of silver diethyl dithiocarbamate solution (9), and quickly put 2.5g of zinc into an arsenic hydride generation bottle.
g) Leave at rest at room temperature (15ºC - 25ºC) for about 45 minutes, and allow generated arsenic hydride to absorb into the silver diethyl dithiocarbamate solution and color.
h) Put a predetermined amount of blank test solution into the arsenic hydride generation bottle, conduct procedures similarly as in **b**) - **g**) to allow generated arsenic hydride to absorb into the silver diethyl dithiocarbamate solution and color.

Note (**8**) When a large amount of iron is contained, add 1 g of ascorbic acid and 2mL of tin (II) chloride solution and shake to mix to leave at rest for about 10 minutes instead of the procedure in **e**).
(**9**) Apply a small amount of silicone grease, etc. to the connecting parts of an arsenic hydride generation bottle, glass tube and arsenic hydride absorption tube to maintain their airtightness.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used.

a) **Measurement conditions of the spectrophotometer**: Set up the measurement conditions of the spectrophotometer considering the following.
Detection wavelength: 510 nm or 519 nm

b) **Calibration curve preparation**

1) Put 2.5 mL - 20 mL of arsenic standard solution (1 µg/mL) in arsenic hydride generation bottles step-by-step.
2) Conduct procedures similarly as in (**4.2**) **b**) - **g**) and allow them to react.
3) For another arsenic hydride generation bottle, the silver diethyl dithiocarbamate solution prepared similarly as in the procedure in **2**) is used as the blank test solution for the calibration curve preparation.
4) Measure the absorbance at a wavelength 510 nm of the silver diethyl dithiocarbamate solution of the arsenic standard solution for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control.
5) Prepare a curve for the relationship between the arsenic concentration and the indicated value of the arsenic standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) For the silver diethyl dithiocarbamate solution of (**4.2**) **g**), conduct procedures similarly as in **b**) **4**) to measure absorbance.
2) For the silver diethyl dithiocarbamate solution of (**4.2**) **h**), conduct procedures similarly as in **b**) **4**) to measure absorbance, and correct the absorbance obtained for the sample solution.
3) Obtain the arsenic content from the calibration curve, and calculate arsenic (As) in the analytical sample.

Comment 5 Instead of the correction method in **c**) **2**), the arsenic (As) in the analytical sample can also be corrected by obtaining the arsenic content in the blank test solution.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers

(Details), p.270- 273, Yokendo, Tokyo (1988)

(**5**) **Flow sheet for arsenic**: The flow sheet for arsenic in fertilizers is shown below:

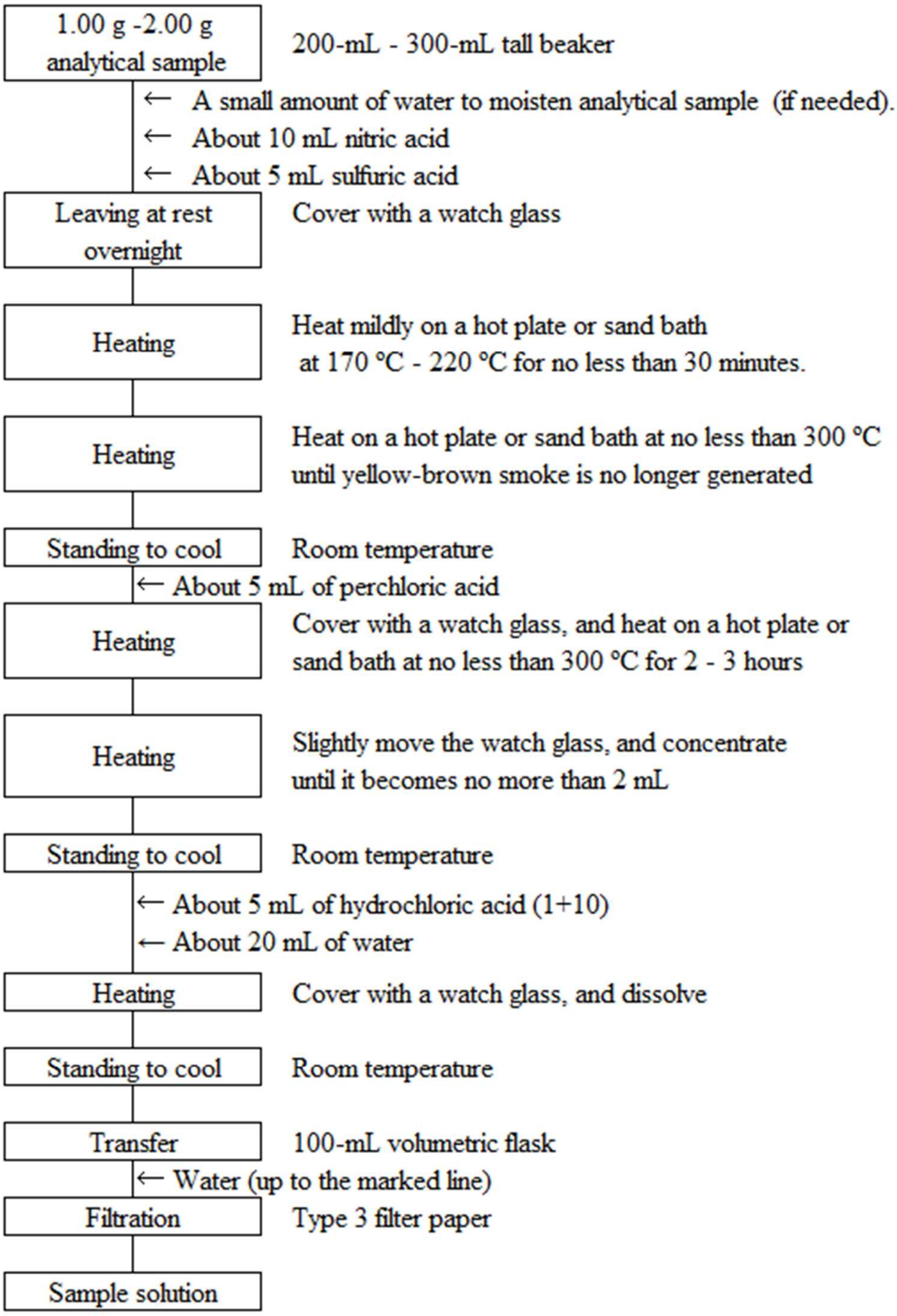


Figure 1 Flow sheet for arsenic in fertilizers
(Extraction procedure)

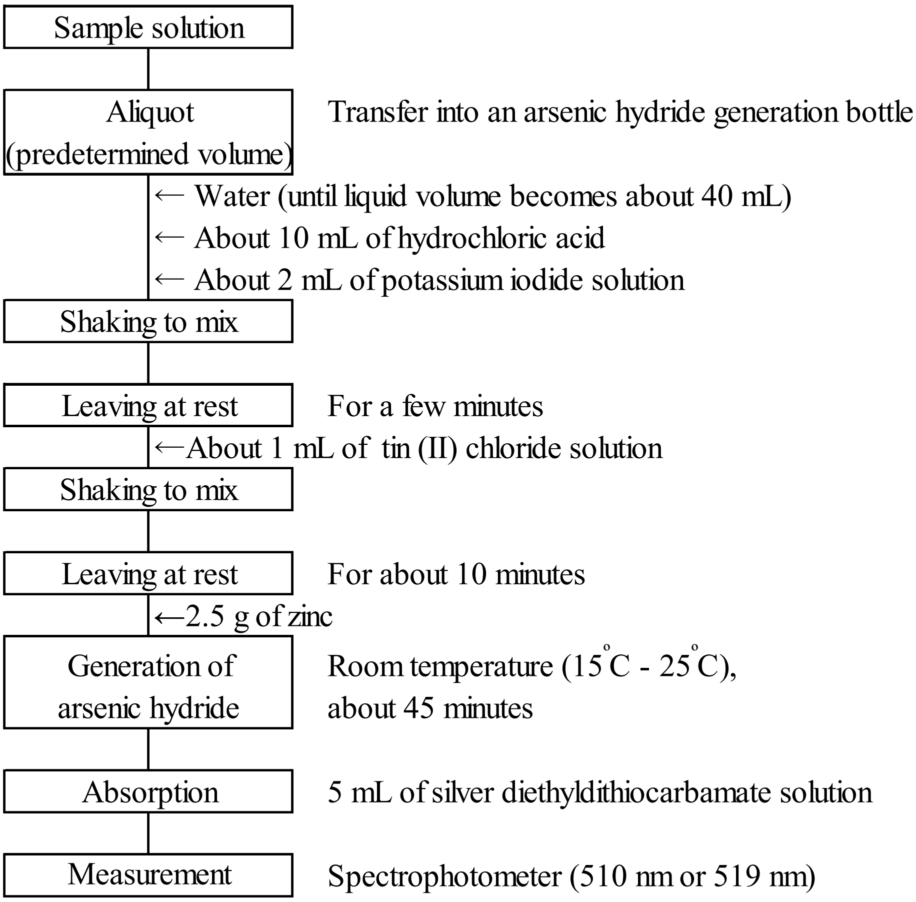


Figure 2 Flow sheet for arsenic in fertilizers
(Reaction and measurement procedure)

5.2.c ICP Mass Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 5.2.c-2021 or As.c-2.

Add nitric acid – hydrogen peroxide to an analytical sample, and heat to decompose by microwave irradiation. Introduce it to an ICP Mass Spectrometer (ICP-MS) and measure the respective indicated values of arsenic and an internal standard element (tellurium) with mass/charge number (*m*/*z*) and obtain arsenic (As) in the analytical sample from the ratio of the indicated value for arsenic and the indicated value for the internal standard element. Note that the performance of this testing method is shown in **Comment 8**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A4 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Nitric acid**: Nitric acid used to dilute a standard solution and a sample solution is a highly pure reagent specified in JIS K 9901.

d) **Hydrogen peroxide**: A JIS Guaranteed Reagent specified in JIS K 8230 or a reagent of equivalent quality.

e) **Tellurium standard solution (Te 1000 mg/L)**: A tellurium standard solution (Te 1000 mg/L) traceable to National Metrology.

f) **Tellurium standard solution (Te 5 μg/mL)** (1) (2) (3): Dilute a predetermined amount of tellurium standard solution (Te 1000 mg/L) with nitric acid (1+19) to prepare a tellurium standard solution (Te 5 μg/mL).

g) **Tellurium standard solution (Te 100 ng/mL)** (1) (3): Dilute a predetermined amount of tellurium standard solution (Te 5 μg/mL) with nitric acid (1+19) to prepare a tellurium standard solution (Te 100 ng/mL).

h) **Arsenic standard solution (As 1000 mg/L)**: An arsenic standard solution (As 1000 mg/L) traceable to National Metrology.

i) **Arsenic standard solution (As 200 ng/mL)** (1) (2) (3): Dilute the arsenic standard solution (As 1000 mg/L) with nitric acid (1+19) to prepare an arsenic standard solution (As 200 ng/mL).

j) **Arsenic standard solutions (As 4 ng/mL - 20 ng/mL) for the calibration curve preparation** (1) (3): Put 2 mL - 10 mL of arsenic standard solution (As 200 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) to the marked line.

k) **Arsenic standard solutions (As 0.2 ng/mL - 2 ng/mL) for the calibration curve preparation** (1) (3): Put 1 mL - 10 mL of arsenic standard solution (As 20 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) to the marked line.

l) **Blank test solution for the calibration curve preparation** (1) (3): Nitric acid (1+19) used in the procedures in f), g), i), j) and k).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store in a cool, dark place, and do not use after 6 months after preparation.

(**3**) For preparation and storage, use sealable containers made of materials such as polypropylene containing no arsenic.

Comment 1 Instead of the tellurium standard solution in (**2**), a tellurium standard solution can be prepared by using a tellurium standard solution (Te 100 mg/L or 10000 mg/L) traceable to National Metrology.

Comment 2 Instead of the arsenic standard solution in (**2**), an arsenic standard solution for the calibration curve preparation can be prepared by using an arsenic standard solution (As 100 mg/L or 10000 mg/L) traceable to National Metrology.

Comment 3 If a sample solution or a standard solution for the calibration curve preparation and an internal standard solution are not simultaneously introduced in the measurement of ICP-MS, when preparing respective solutions in the procedures in **j**), **k**) and **l**), add tellurium standard solution (Te 100 ng/mL) 1/10 of the volume of the solution.

Comment 4 ICP-MS detection methods include a pulse detection method and an analog detection method. There are detection method models that combine them, but when the measurement value is affected by switching, the concentration of the standard solution and the internal standard solution may be changed as appropriate so that measurement can be performed with either detection method.

(**3**) **Instruments**: Instruments are as shown below:

a) **ICP Mass Spectrometer**: A high-frequency plasma mass spectrometer specified in JIS K 0133, that is equipped with a collision/reaction cell.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.995 % in purity

b) **Pressure vessel decomposing device**: A device which pressurizes the inside of a vessel by putting acid, etc. to heat in the decomposing vessel, and decomposes a sample by the interaction of heating, pressurizing and acid. The following requirements should be met.

1) **The main part of a decomposing device**: In the case of the microwave heating method, a device should be able to produce a high-frequency wave using a frequency which is permitted at an industrial frequency facility. It is desirable to be able to monitor pressure and temperature, etc. in the decomposing vessel with a sensor inside the device. The interior of a device should be acid-resistant treated and should have a high temperature durability and a high level of safety.

2) **Exhaust system**: A system which has an exhaust fan of acid-resistant specification, and has an air-cool function inside the device to allow constant airflow, keeping operating temperature below a certain temperature level.

3) **Decomposing vessel**: A vessel which is heat-resistance and pressure-tight and has the durability required to decompose particles, and has resistance to internal contamination. It should have safety functions such as causing overheat prevention valve to work and internal pressure to drop by emitting gas, and preventing gas bumping in the case of exceeding a pressure limitation.

c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Fluid sludge fertilizer**

a) Weigh 20.0 g (4) of an analytical sample, and put it into a decomposing vessel.

b) Gradually add 2.5 mL of nitric acid and 2 mL of hydrogen peroxide.

c) Seal the decomposing vessel, put it into the main part of a decomposing device and heat using a microwave.

d) Ignite and pressurize at 180 ºC - 220 ºC for no less than 10 minutes (5) to decompose (6).

e) After standing to cool, transfer the solution to a 50-mL volumetric flask (7) with water.

f) Add water up to the marked line and put it in a 50-mL ground-in stopper centrifugal precipitate tube (7).

g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (8) and use the supernatant as a sample solution.

h) As a blank test, conduct the procedures in **b**) - **g**) using another decomposing vessel to prepare a blank test solution.

(**4.1.2**) **Fertilizers except fluid sludge fertilizers**

a) Weigh 0.20 g of an analytical sample, and put it into a decomposing vessel.

b) Gradually add 10 mL of nitric acid and 1 mL of hydrogen peroxide.
c) Seal the decomposing vessel, put it into the main part of a decomposing device and heat using a microwave.
d) Ignite and pressurize at 180 ºC - 220 ºC for no less than 10 minutes [(5)] to decompose [(6)].
e) After standing to cool, transfer the solution to a 50-mL volumetric flask [(7)] with water.
f) Add water up to the marked line and transfer it to a 50-mL ground-in stopper centrifugal precipitate tube [(7)].
g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes [(8)] and use the supernatant as a sample solution.
h) As a blank test, conduct the procedures in **b**) - **g**) using another decomposing vessel to prepare a blank test solution.

Note (**4**) The maximum limit of solid content, which is converted from moisture content, in the sampling volume 20.0 g of an analytical sample is about 0.5 g. If solid content is likely to exceed the limit, reduce sampling volume as necessary.
(**5**) Example setting conditions of a microwave decomposing device are as shown in Table 1.

Table 1 Example setting conditions of a microwave decomposing device

Time (min)	Temperature (°C)	Output (kW)
0	-	0
20	200 (temperature rising)	1400
10	200	1400
40	Room temperature	0

(**6**) When organic matters, for example colored precipitate, etc., still remain, add 2 mL of nitric acid and 1 mL of hydrogen peroxide and repeat the procedures in (**4.1**) **c**) – **d**).
(**7**) The vessel should be made of polypropylene, etc. to not affect the measurement.
(**8**) 16.5-cm of radius and 3000 rpm of revolutions makes about 1700 × g centrifugal force.

Comment 5 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Internal Standard Method) according to JIS K 0113 and as shown below. Specific measurement procedures are according to the operation method of the ICP Mass Spectrometer used in measurement.
a) **Measurement conditions for the ICP Mass Spectrometer**: Set up the measurement conditions for the ICP Mass Spectrometer considering the following:
Arsenic: mass/charge number (*m*/*z*): 75
Tellurium: mass/charge number (*m*/*z*): 125
Collision cell: He-KED (Kinetic Energy Discrimination) mode [(9)]
b) **Calibration curve preparation**
1) Spray the arsenic standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation together with tellurium standard solution (Te 100 ng/mL) into the inductively coupling plasma [(10)], and read the ratio of the indicated values for the respective mass/charge number of an element subjected to measurement and an internal

standard element.

2) Prepare a calibration curve for the relationship between the concentration and the ratio of the indicated value of an element subjected to measurement.

c) **Sample measurement**

1) Put no more than 2.5 mL of the sample solution in a 50-mL volumetric flask [(7)], add nitric acid to be nitric acid (1+19), and add water to the marked line [(11)].

2) Subject to the same procedure as in **b**) **1**) to read the ratio of the indicated values.

3) For the blank test solution, conduct procedures similarly as in **1**) - **2**) to correct the ratio of the indicated value obtained for the measurement solution.

4) Obtain the cadmium content from the calibration curve, and calculate cadmium (Cd) in the analytical sample.

Note (**9**) Note that, when He-H_2 mixture gas is used, H_2 may react with As and the indicated value of As becomes lower, affecting the measurement.

(**10**) Simultaneously introduce an internal standard solution 1/9 of the volume of the standard solution for the calibration curve preparation or the blank test solution for the calibration curve preparation.

(**11**) If there is a possibility that the arsenic concentration in the sample solution will exceed the maximum limit of the calibration curve, reduce the sampling amount for the sample solution or dilute it with nitric acid (1+19).

Comment 6 Instead of the correction method in **c**) 3), the arsenic concentration in the analytical sample can also be corrected by obtaining the arsenic concentration in the blank test solution.

Comment 7 Divalent ions ^{150}Sm and ^{150}Nd cause spectrum interference to ^{75}As. Spectrum interference affects analysis values depending on the resolution of the instrument. Therefore, ascertain in advance the resolution of the instrument and the Sm and Nd concentrations that affect analysis values, and perform analysis using other methods such as hydride generation atomic absorption spectrometry if the sample is known to have Sm and Nd concentrations higher than the interfering concentrations.

Comment 8 Triplicates additive recovery testing was conducted to evaluate trueness using mixed compost compound fertilizers and fluid composted sludge fertilizers. As a result, the mean recovery rates at the concentration level of 1 mg/kg - 50 mg/kg were 102 % - 112 % as arsenic (As).

The comparison of the analysis value (y_i: 0.06 mg/kg - 40.2 mg/kg) of ICP-MS and the analysis value (x_i) of hydride generation flame atomic absorption spectrometer was conducted using sewage sludge fertilizer (2 samples), human waste sludge fertilizer (5 samples), industrial sludge fertilizer (1 sample), mixed sludge fertilizer (1 sample), calcined sludge fertilizer (3 samples), composted sludge fertilizer (12 samples), composted seafood byproduct fertilizer (1 sample), superphosphate of lime (1 sample), double superphosphate of lime (1 sample), processed phosphorus fertilizer (2 samples), byproduct animal fertilizer (1 sample), compound fertilizer (13 samples), blended fertilizer (3 samples), fluid compound fertilizer (1 sample), coating compound fertilizer (2 samples), solid compound fertilizer (2 samples), byproduct compound fertilizer (2 samples), mixed compost compound fertilizer (1 samples), silicate slag fertilizer (1 sample), byproduct magnesia fertilizer (1 sample), mixed microelement fertilizer (1 sample), and distribution fertilizer (58 samples). As a result, a regression equation was $y = -0.0982 + 0.9987x$, and its correlation coefficient (r) was 0.993.

The results of repeated analyses on different days using a composted sludge fertilizer and standard solution-added compound fertilizer were analyzed by the one-way analysis of variance. Table 2 shows the estimation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 mg/kg for fluid sludge fertilizers and about 1 mg/kg for other fertilizers.

Table 2 Estimation results of repeatability and intermediate precision

Sample name	Days of repeatability T[1)]	Mean[2)] (mg/kg)[3)]	Repeatability		Intermediate precision	
			s_r[4)] (mg/kg)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (mg/kg)[3)]	$RSD_{I(T)}$[7)] (%)
Composted sludge fertilizer	5	3	0.1	3.0	0.2	5.8
Compound fertilizer	5	53	0.7	1.3	0.7	1.3

1) The number of days conducting duplicate analysis
2) Mean ([Number of days of repeatability (T)] × [Number of duplicates (2)])
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Comment 9 The simultaneous measurement of multiple elements is possible with the ICP-MS. In that case, prepare standard solutions, etc. referencing Table 1 of Annex C2, conduct procedures similarly as (**4.2**) **b**) - **c**), and calculate respective element concentrations in an analytical sample.

Note that the concentration of a standard solution and an internal standard solution can be changed as appropriate according to **Comment 4**.

References

1) Toshiharu YAGI: Determination of Heavy Metals in Fluid Sludge Fertilizers by ICP-MS and CV-AAS, Research Report of Fertilizer **Vol. 8**, p. 26 - 37 (2015)

2) Toshiharu YAGI, Kenta SAKUMA and Yoshimi HASHIMOTO: Determination of Heavy Metals in Sludge Fertilizers using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Research Report of Fertilizer, **Vol. 9**, p. 21 – 32 (2016)

3) Satoko SAKAIDA, Mayu OSHIMA, Keisuke AOYAMA and Yuji SHIRAI: Determination of Harmful Components in Fertilizers using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Research Report of Fertilizer, **Vol. 12**, p. 52 – 68 (2019)

(**5**) **Flow sheet for arsenic**: The flow sheet for arsenic in fluid sludge fertilizers is shown below:

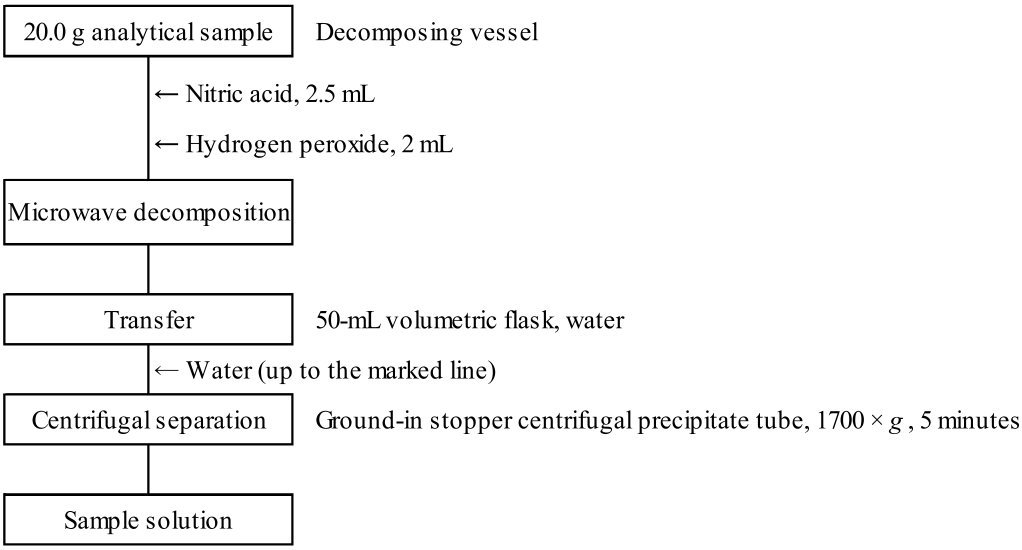


Figure 1 Flow sheet for arsenic in fluid sludge fertilizers (Extraction procedure)

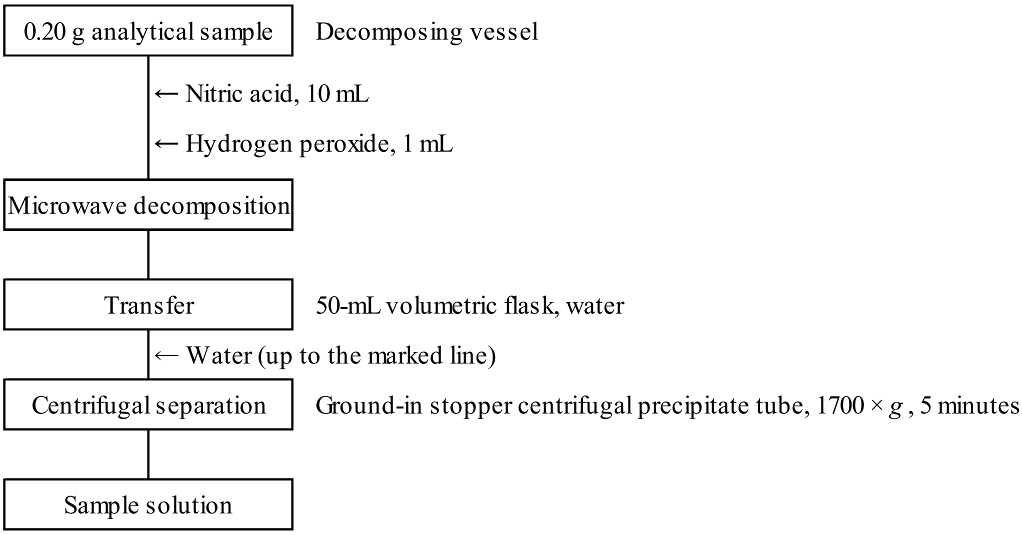


Figure 2 Flow sheet for arsenic in fertilizers except fluid sludge fertilizers (Extraction procedure)

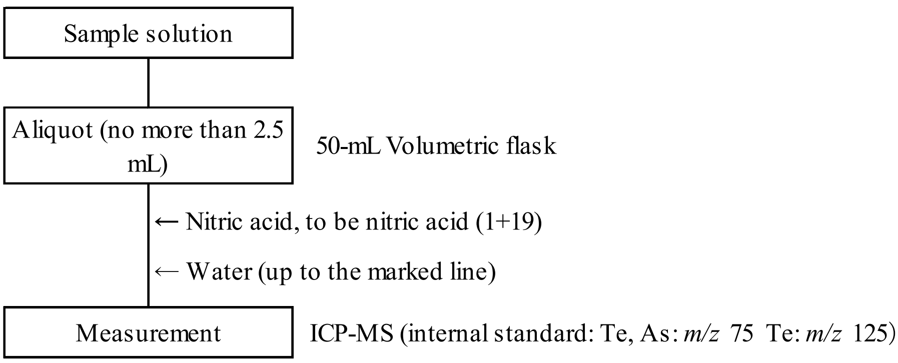


Figure 3 Flow sheet for arsenic in fertilizers (Measurement procedure)

5.3 Cadmium

5.3.a Flame atomic absorption spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 5.3.a-2017 or Cd.a-1.

Pretreat an analytical sample with incineration and nitric acid-hydrochloric acid (1+3), spray into an acetylene-air flame, and measure the atomic absorption with cadmium at a wavelength of 228.8 nm to obtain cadmium (Cd) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) **Cadmium standard solution (Cd 0.1 mg/mL)**: A cadmium standard solution (Cd 0.1 mg/mL) traceable to National Metrology.

e) **Cadmium standard solution (Cd 10 µg/mL)**: Put 10 mL of cadmium standard solution (Cd 0.1 mg/mL) in a 100-mL volumetric flask, and add hydrochloric acid (1+23) up to the marked line.

f) **Cadmium standard solutions (Cd 0.05 µg - 0.5 µg/mL) for the calibration curve preparation** (1) (2): Put 2.5 mL - 25 mL of cadmium standard solution (Cd 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

g) **Blank test solution for the calibration curve preparation** (1) (2): Hydrochloric acid (1+23) used in the procedures in **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store at room temperature, and do not use after 6 months after preparation.

Comment 1 Instead of the cadmium standard solution in (**2**), a cadmium standard solution for the calibration curve preparation can be prepared by using a cadmium standard solution (Cd 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(**3**) **Instruments**: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (3) function.

1) **Light source**: A cadmium hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Electric furnace**: An electric furnace that can keep the test temperature at 450 °C ± 5 °C .

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (**3**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and pit it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (4).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (4).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (6) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**4**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.

(**5**) The watch glass can be removed.

(**6**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 2 Do not conduct the procedures in (**4.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 228.8 nm

b) **Calibration curve preparation**

1) Spray the cadmium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 228.8 nm.

2) Prepare a curve for the relationship between the cadmium concentration and the indicated value of the cadmium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Subject the sample solution (7) to the same procedure as in **b**) **1**) to read the indicated value.

2) Subject the blank test solution to the same procedure as in **b**) **1**) to read the indicated value, and correct the indicated value obtained for the sample solution.

3) Obtain the cadmium content from the calibration curve, and calculate cadmium (Cd) in the analytical sample.

Note (**7**) If there is a possibility that the cadmium concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 4 Instead of the correction method in **c**) **2**), the cadmium (Cd) in the analytical sample can also be corrected by obtaining the cadmium content in the blank test solution.

Comment 5 Recovery testing was conducted using industrial sludge fertilizer and composted sludge fertilizer (5 samples); as a result, the recovery rates at the concentration level of 5 mg/kg and 0.5 mg/kg were 97.5 % - 99.2 % and 96.7 % - 99.7 %, respectively.
Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 1.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.1 mg/kg.

Table 1 Analysis results of the collaborative study for cadmium test method validation

Sample name	Number of laboratories[1]	Mean[2] (mg/kg)	RSD_r[3] (%)	RSD_R[4] (%)
Sewage sludge fertilizer a	10	1.50	5.5	6.4
Sewage sludge fertilizer b	10	3.35	1.2	4.2
Composted sludge fertilizer a	10	1.96	1.0	4.4
Composted sludge fertilizer b	11	3.81	1.9	3.2
Composted sludge fertilizer c	10	1.80	3.5	4.9

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Yoshinari SAKAKIBARA, Manabu MATSUZAKI and Tadao AMANO: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer **Vol. 1**, p. 41 - 49 (2008)

2) Yoshinari SAKAKIBARA and Manabu MATSUZAKI: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer **Vol. 1**, p. 50 - 59 (2008)

3) Hisanori ARAYA and Yoshimi TAKEBA: Validation of atomic absorption spectrometry for determination of cadmium, lead, nickel and chromium in calcined sludge fertilizer - Research Report of Fertilizer **Vol. 3**, p. 30 - 42 (2010)

(5) **Flow sheet for cadmium**: The flow sheet for cadmium in fertilizers is shown below:

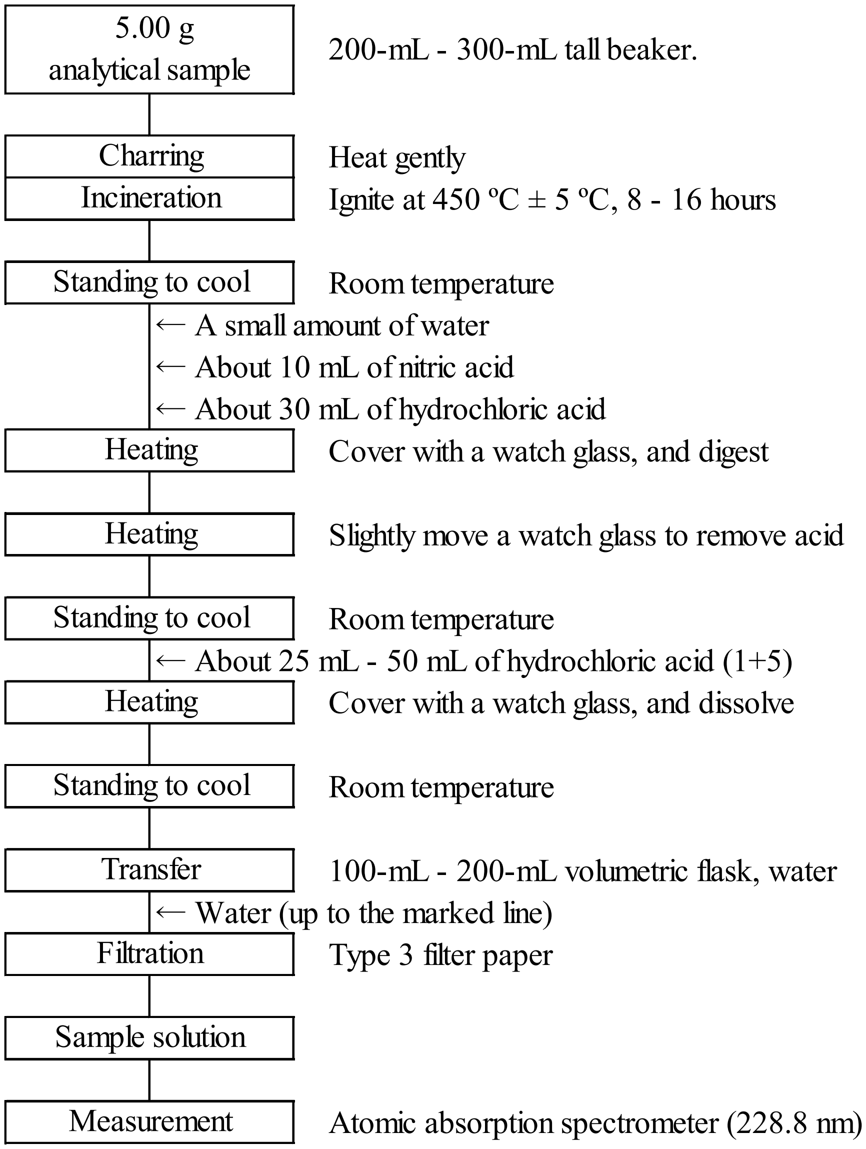


Figure Flow sheet for nickel in fertilizers

5.3.b ICP Optical Emission Spectrometry

(1) Summary

The test method is applicable to sludge fertilizers, etc. This testing method is classified as Type D and its symbol is 5.3.b-2017 or Cd.b-1.

Pretreat an analytical sample with incineration, nitric acid - hydrochloric acid (1+3), introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the emission with cadmium at a wavelength of 228.802 nm to quantify the cadmium (Cd) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) **Cadmium standard solution (Cd 0.1 mg/mL**): A cadmium standard solution (Cd 0.1 mg/mL) traceable to National Metrology.

e) **Cadmium standard solution (Cd 0.25μg/mL)** (1) (2): Dilute a predetermined amount of cadmium standard solution (Cd 0.1 mg/mL) with hydrochloric acid (1+23) to prepare a cadmium standard solution (Cd 0.25 μg/mL)

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store at room temperature, and do not use after 6 months after preparation.

Comment 1 Instead of the cadmium standard solution in (**2**), a cadmium standard solution for the calibration curve preparation can be prepared by using a cadmium standard solution (Cd 1 mg/mL or 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(**3**) **Instruments**: Instruments are as shown below:

a) **ICP Optical Emission Spectrometer:** An optical emission spectrometer specified in JIS K 0116.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity.

b) **Electric furnace**: An electric furnace that can keep the test temperature at 450 °C ± 5 °C.

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (3).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (3).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (4), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (5) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer the solution to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**3**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.
(**4**) The watch glass can be removed.
(**5**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 3 Do not conduct the procedures in (**4.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.
Comment 4 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 228.802 nm

b) **Calibration curve preparation and sample measurement**

1) Put 5mL of sample solution in three 10-mL volumetric flasks respectively.
2) Add 2mL and 4 mL of cadmium standard solution (0.25 µg/mL) to volumetric flasks of **1**) above, and further add hydrochloric acid (1+23) to the marked line to make a sample solution of Standard Addition Method.
3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1**) above to make a sample solution without a standard solution.
4) Spray the sample solution of Standard Addition Method and the sample solution without a standard solution into the induction plasma, and read the indicated value at a wavelength of 228.802 nm.
5) Put 5 mL of blank test solution in a 10-mL volumetric flask, conduct the same procedures as in **3**) - **4**) to read the indicated value, and correct the indicated value obtained from the respective sample solutions.
6) Prepare a curve for the relationship between the added cadmium concentration and the corrected indicated value of the sample solution for Standard Addition Method and sample solution without a standard solution.
7) Obtain the cadmium content from the intercept of the calibration curve to calculate cadmium (Cd) in the analytical sample

Comment 5 Instead of the correction method in **b**) **5**), the cadmium (Cd) in the analytical sample can also be corrected by obtaining the cadmium content in the blank test solution.

Comment 6 Coexistence of As in large quantities causes interference. Ascertain in advance the As concentration that causes interference for the instrument, and perform analysis using other methods such as flame atomic absorption spectrometry if the sample is known to have an As concentrations higher than the interfering concentration.

Comment 7. The simultaneous measurement of multiple elements is possible with the ICP-OES. In that case, **see 4.9.1.b Comment 6**.

Comment 8 The comparison of the measurement value (x_i : 0.003 mg/kg - 3.32 mg/kg) of ICP Optical Emission Spectrometry and the measurement value (y_i) of Flame Atomic Absorption Spectrometry was conducted to evaluate trueness using sludge fertilizers (49 samples). As a result, a regression equation was $y=-0.03+1.009x$ and its correlation coefficient (r) was 0.996. Triplicates measurement for each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer, calcined sludge fertilizers and composted sludge fertilizer was conducted. As a result, a repeatability obtained was 0.8 % - 4.1 % as a relative standard deviation.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.2 mg/kg.

References

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI and Tetuya NOMURA: Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer, **Vol. 4**, p. 30 - 35 (2011)

(**5**) **Flow sheet for cadmium**: The flow sheet for cadmium in fertilizers is shown below:

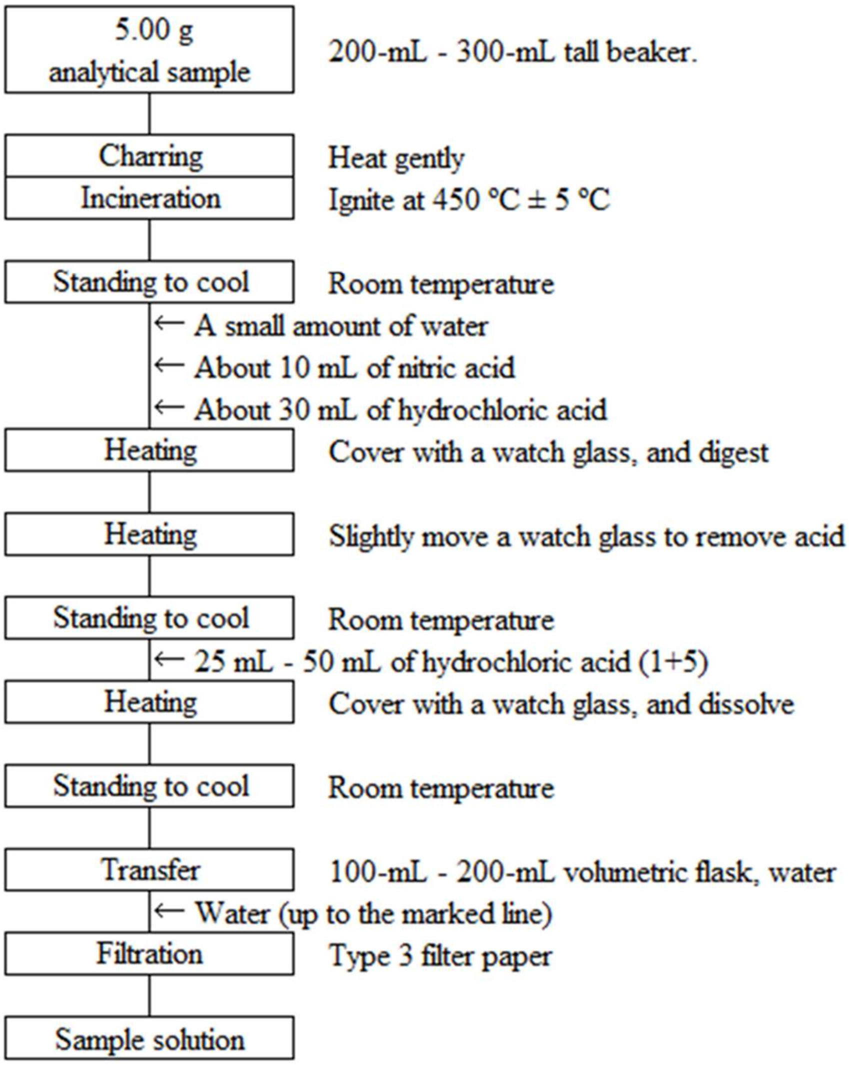


Figure 1 Flow sheet for cadmium in sludge fertilizers
(Extraction procedure)

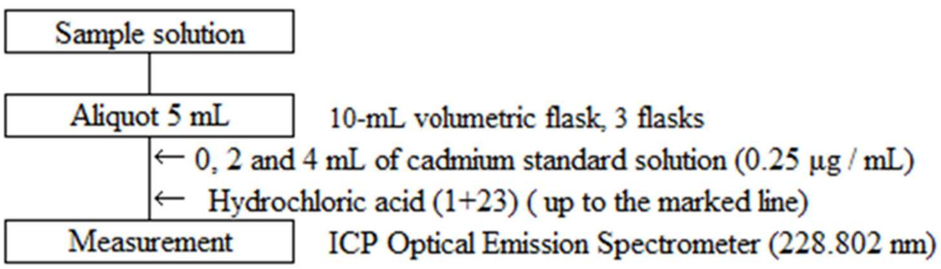


Figure 2 Flow sheet for cadmium in sludge fertilizers
(Measurement procedure)

5.3.c ICP Mass Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 5.3.c-2021 or Cd.c-2.

Add nitric acid–hydrogen peroxide to an analytical sample, and heat to decompose by microwave irradiation. Introduce it to an ICP Mass Spectrometer (ICP-MS) and measure the respective indicated values of cadmium and an internal standard element (rhodium) with mass/charge number (*m*/*z*) and obtain cadmium (Cd) in the analytical sample from the ratio of the indicated value for cadmium and the indicated value for the internal standard element. Note that the performance of this testing method is shown in **Comment 7**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A4 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Nitric acid**: Nitric acid used to dilute a standard solution and a sample solution is a highly pure reagent specified in JIS K 9901.

d) **Hydrogen peroxide**: A JIS Guaranteed Reagent specified in JIS K 8230 or a reagent of equivalent quality.

e) **Rhodium standard solution (Rh 1000 mg/L)**: A rhodium standard solution (Rh 1000 mg/L) traceable to National Metrology.

f) **Rhodium standard solution (Rh 5 µg/mL)** (1) (2) (3): Dilute a predetermined amount of rhodium standard solution (Rh 1000 mg/L) with nitric acid (1+19) to prepare a rhodium standard solution (Rh 5 µg/mL).

g) **Rhodium standard solution (Rh 50 ng/mL)** (1) (3): Dilute a predetermined amount of rhodium standard solution (Rh 5 µg/mL) with nitric acid (1+19) to prepare a rhodium standard solution (Rh 50 ng/mL).

h) **Cadmium standard solution (Cd 1000 mg/L)**: A cadmium standard solution (Cd 1000 mg/L) traceable to National Metrology.

i) **Cadmium standard solution (Cd 50 ng/mL)** (1) (2) (3): Dilute a predetermined amount of cadmium standard solution (Cd 1000 mg/L) with nitric acid (1+19) to prepare a cadmium standard solution (Cd 50 ng/mL).

j) **Cadmium standard solutions (Cd 1 ng/mL - 5 ng/mL) for the calibration curve preparation** (1) (2) (3): Put 2 mL - 10 mL of cadmium standard solution (Cd 50 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) up to the marked line.

k) **Cadmium standard solutions (Cd 0.05 ng/mL - 0.5 ng/mL) for the calibration curve preparation** (1) (3): Put 1 mL - 10 mL of cadmium standard solution (Cd 5 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) up to the marked line.

l) **Blank test solution for the calibration curve preparation** (1) (3): Nitric acid (1+19) used in the procedures in f), g), i), j) and k).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store in cool and dark place, and do not use after 6 month after preparation.

(**3**) For preparation and storage, use sealable containers made of materials such as polypropylene containing no cadmium.

Comment 1 Instead of the rhodium standard solution in (**2**), a rhodium standard solution for the calibration curve preparation can be prepared by using a rhodium standard solution (Rh 100 mg/L or 10000 mg/L) traceable to National Metrology.

Comment 2 Instead of the cadmium standard solution in (**2**), a cadmium standard solution for the calibration curve preparation can be prepared by using a cadmium standard solution (Cd 100 mg/L or 1000 mg/L) traceable to National Metrology.

Comment 3 When a sample solution or a standard solution for the calibration curve preparation and an internal standard solution are not simultaneously introduced in the measurement of ICP-MS, add 1/10 volume of rhodium standard solution (Rh 50 ng/mL) to the volume of the solution when preparing respective solutions in the procedures in **j**), **k**) and **l**).

Comment 4 ICP-MS detection methods include a pulse detection method and an analog detection method. There are detection method models that combine them, but when the measurement value is affected by switching, the concentration of the standard solution and the internal standard solution may be changed as appropriate so that measurement can be performed with either detection method.

(**3**) **Instruments**: Instruments are as shown below:

a) **ICP Mass Spectrometer**: A high-frequency plasma mass spectrometer specified in JIS K 0133, that is equipped with a collision/reaction cell.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.995 % in purity.

b) **Pressure vessel decomposing device**: A device which pressurizes the inside of a vessel by putting acid, etc. to heat in the decomposing vessel, and decomposes a sample by the interaction of heating, pressurizing and acid. The following requirements should be met.

1) **The main part of a decomposing device**: In the case of the microwave heating method, a device should be able to produce a high-frequency wave using a frequency which is permitted at an industrial frequency facility. It is desirable to be able to monitor pressure and temperature, etc. in the decomposing vessel with a sensor inside the device. The interior of a device should be acid-resistant treated and should have a high temperature durability and a high level of safety.

2) **Exhaust system**: A vessel which is heat-resistance and pressure-tight and has the durability required to decompose particles, and has resistance to internal contamination. It should have safety functions such as causing overheat prevention valve to work and internal pressure to drop by emitting gas, and preventing gas bumping in the case of exceeding a pressure limitation.

3) **Decomposing vessel**: A vessel which is heat-resistance and pressure-tight and has the durability required to decompose particles, and has resistance to internal contamination. It should have safety functions such as causing overheat prevention valve to work and internal pressure to drop by emitting gas, and preventing gas bumping in the case of exceeding a pressure limitation.

c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Fluid sludge fertilizer**

a) Weigh 20.0 g (4) of an analytical sample, and put it into a decomposing vessel.

b) Gradually add 2.5 mL of nitric acid and 2 mL of hydrogen peroxide.

c) Seal the decomposing vessel, put it into the main part of a decomposing device and heat using a microwave.

d) Ignite and pressurize at 180 ºC - 220 ºC for no less than 10 minutes (5) to decompose (6).

e) After standing to cool, transfer the solution to a 50-mL volumetric flask (7) with water.

f) Add water up to the marked line and put it in a 50-mL ground-in stopper centrifugal precipitate tube (7).

g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (8) and use the supernatant as a sample solution.

h) As a blank test, conduct the procedures in **b**) - **g**) using another decomposing vessel to prepare a blank test solution.

(**4.1.2**) **Fertilizers except fluid sludge fertilizers**

a) Weigh 0.20 g of an analytical sample, and put it into a decomposing vessel.
b) Gradually add 10 mL of nitric acid and 1 mL of hydrogen peroxide.
c) Seal the decomposing vessel, put it into the main part of a decomposing device and heat using a microwave.
d) Ignite and pressurize at 180 ºC - 220 ºC for no less than 10 minutes (5) to decompose (6).
e) After standing to cool, transfer the solution to a 50-mL volumetric flask (7) with water.
f) Add water up to the marked line and transfer it to a 50-mL ground-in stopper centrifugal precipitate tube (7).
g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (8) and use the supernatant as a sample solution.
h) As a blank test, conduct the procedures in **b**) - **g**) using another decomposing vessel to prepare a blank test solution.

Note (4) The maximum limit of solid content, which is converted from moisture content, in the sampling volume 20.0 g of an analytical sample is about 0.5 g. If solid content is likely to exceed the limit, reduce sampling volume as necessary.
(5) Example setting conditions of a microwave decomposing device are as shown in Table 1.

Table 1 Example setting conditions of a microwave decomposing device

Time (min)	Temperature (°C)	Output (kW)
0	-	0
20	200 (temperature rising)	1400
10	200	1400
40	Room temperature	0

(6) When organic matters, for example colored precipitate, etc., still remain, add 2 mL of nitric acid and 1 mL of hydrogen peroxide and repeat the procedures in (**4.1**) **c**) – **d**).
(7) The vessel should be made of polypropylene, etc. to not affect the measurement.
(8) 16.5-cm of radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

Comment 5 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Internal Standard Method) according to JIS K 0113 and as shown below. Specific measurement procedures are according to the operation method of the ICP Mass Spectrometer used in measurement.

a) **Measurement conditions for the ICP Mass Spectrometer**: Set up the measurement conditions for the ICP Mass Spectrometer considering the following:
Cadmium: mass/charge number (*m*/*z*): 111
Rhodium: mass/charge number (*m*/*z*): 103
Collision cell: He-KED (Kinetic Energy Discrimination) mode (9) (10)

b) **Calibration curve preparation**

1) Spray the cadmium standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation together with rhodium standard solution (Rh 50 ng/mL) into the inductively coupling plasma (11), and read the ratio of the indicated values for the respective mass/charge number of an element subjected to measurement and an internal standard element.

2) Prepare a curve for the relationship between the concentration and the ratio of the ion count number of an element subjected to measurement.

c) **Sample measurement**

1) Put no more than 2.5 mL of the sample solution in a 50-mL volumetric flask (7), add nitric acid to be nitric acid (1+19), and add water to the marked line (12).

2) Subject to the same procedure as in **b**) **1**) to read the ratio of the indicated values (13).

3) For the blank test solution, conduct procedures similarly as in **1**) - **2**) to correct the ratio of the indicated value obtained for the measurement solution.

4) Obtain the cadmium content from the calibration curve, and calculate cadmium (Cd) in the analytical sample.

Note (**9**) Note that, when He-H_2 mixture gas is used, H_2 may react with Rh and the indicated value of Rh becomes lower, affecting the measurement.

(**10**) For MoO, etc. that causes spectrum interference to ^{111}Cd, its generation can be suppressed by reducing the oxide ion generation ratio. Adjust the instrument conditions to reducing the oxide ion generation ratio prior to the measurement. (Example: The CeO/Ce ratio is less than 1%.)

(**11**) Simultaneously introduce an internal standard solution 1/9 of the volume of the standard solution for the calibration curve preparation or the blank test solution for the calibration curve preparation.

(**12**) If there is a possibility that the cadmium concentration in the sample solution will exceed the maximum limit of the calibration curve, reduce the sampling amount for the sample solution or dilute it with nitric acid (1+19).

(**13**) In the case of a fertilizer in which raw materials or a fertilizer using Mo are used secondarily, correct the indicated value of ^{111}Cd using the correction formula (a) below.

$$^{111}\mathrm{Cd} = 1.000\times{}^{111}\mathrm{M} - 1.073({}^{108}\mathrm{M} - 0.712\times{}^{106}\mathrm{M}) \quad \cdots \quad (a)$$

^{111}Cd : Indicated value of ^{111}Cd after correction

^{111}M: Indicated value of ^{111}Cd before correction

^{108}M : Indicated value of ^{108}Cd

^{106}M: Certified indicated value of ^{106}Cd

Comment 6 Instead of the correction method in **c**) **3**), the cadmium concentration in the analytical sample can also be corrected by obtaining the cadmium concentration in the blank test solution.

Comment 7 Triplicates additive recovery testing was conducted to evaluate trueness using mixed compost compound fertilizers and fluid composted sludge fertilizers. As a result, the mean recovery rates at the concentration level of 0.1 mg/kg - 5 mg/kg were 98.9 % - 111 % as cadmium (Cd).

The comparison of the analysis value (y_i: 0.09 mg/kg – 5.52 mg/kg) of ICP Mass Spectrometry and the analysis value (x_i) of Flame Atomic Absorption Spectrometry was conducted using sludge fertilizers (20 samples). As a result, a regression equation was $y = -0.0084 + 0.98x$, and its correlation coefficient (r) was 0.999. Similarly, the comparison of the analysis value (y_i: 0.10 mg/kg - 5.55 mg/kg) of ICP Mass Spectrometry and the analysis value (x_i) of Flame Atomic Absorption Spectrometry was conducted using superphosphate of lime (1 sample), double superphosphate of lime (1 sample), processed phosphorus fertilizer (2 samples), byproduct animal fertilizer (1 sample), compound fertilizer (11 samples), blended fertilizer (1 sample), coating compound fertilizer (2 samples), solid compound fertilizer (2 samples), byproduct compound fertilizer (1 sample), mixed compost compound fertilizer (6 samples), byproduct magnesia fertilizer (1 sample), and mixed microelement fertilizer (1 sample). As a result, a regression equation was $y = 0.0199 + 1.0147x$, and its correlation coefficient (r) was 0.998.

The results of repeated analyses using two types of composted sludge fertilizer and compound fertilizer were analyzed by the one-way analysis of variance. Table 2 shows the estimation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.002 mg/kg for fluid sludge fertilizers and about 0.2 mg/kg for other fertilizers.

Table 2 Estimation results of repeatability and intermediate precision

Sample name	Days of repeatability T 1)	Mean 2) (mg/kg) 3)	Repeatability s_r 4) (mg/kg) 3)	Repeatability RSD_r 5) (%)	Intermediate precision $s_{I(T)}$ 6) (mg/kg) 3)	Intermediate precision $RSD_{I(T)}$ 7) (%)
Composted sludge fertilizer 1	5	0.1	0.003	3.0	0.004	3.5
Composted sludge fertilizer 2	5	4.6	0.07	1.6	0.1	2.7
Compound fertilizer 1	5	0.6	0.05	9.1	0.06	9.9
Compound fertilizer 2	5	5.6	0.1	2.2	0.1	2.6

1) The number of days conducting duplicate analysis
2) Mean ([Number of days of repeatability (T)] × [Number of duplicates (2)])
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Comment 8 The simultaneous measurement of multiple elements is possible with the ICP-MS. In that case, prepare standard solutions, etc. referencing Table 1 of Annex C2, conduct procedures similarly as (**4.2**) **b**) - **c**), and calculate respective element concentrations in an analytical sample.

Note that the concentration of a standard solution and an internal standard solution can be changed as appropriate according to **Comment 4**.

References

1) Toshiharu YAGI: Determination of Heavy Metals in Fluid Sludge Fertilizers by ICP-MS and CV-AAS, Research Report of Fertilizer, **Vol. 8**, p. 26 - 37 (2015)

2) Satoko SAKAIDA, Mayu OSHIMA, Keisuke AOYAMA and Yuji SHIRAI: Determination of Harmful Components in Fertilizers using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Research Report of Fertilizer, **Vol. 12**, p. 52 – 68 (2019)

(5) **Flow sheet for cadmium**: The flow sheet for cadmium in fluid sludge fertilizers is shown below:

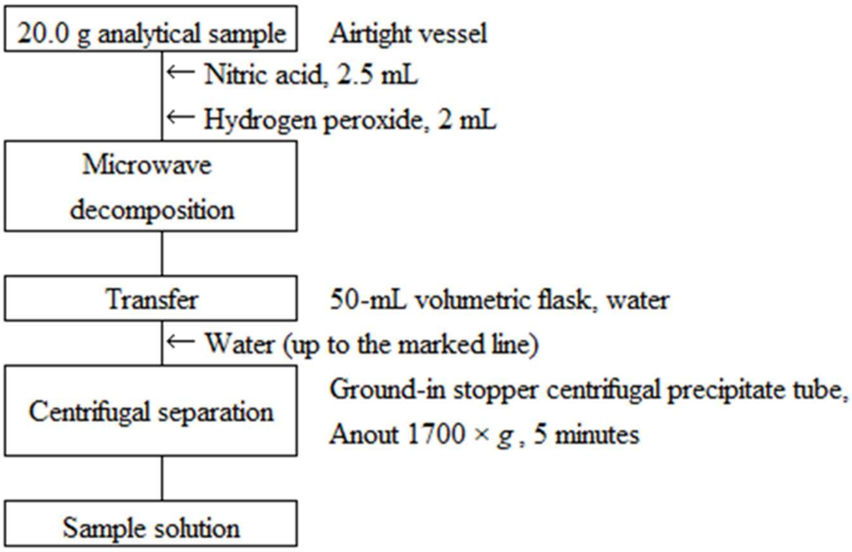


Figure 1 Flow sheet for cadmium in fluid sludge fertilizers (Extraction procedure)

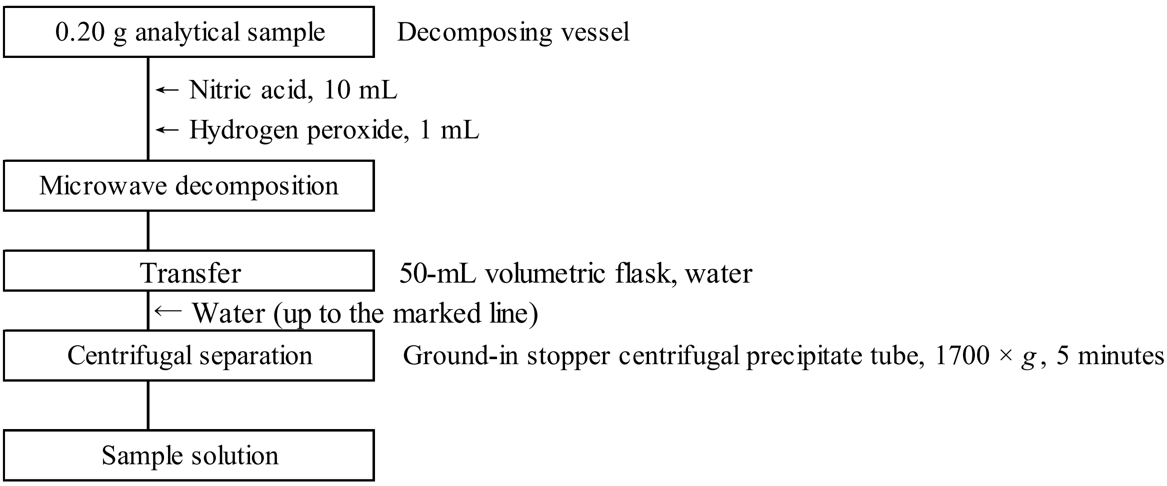


Figure 2 Flow sheet for cadmium in fertilizers except fluid sludge fertilizers (Extraction procedure)

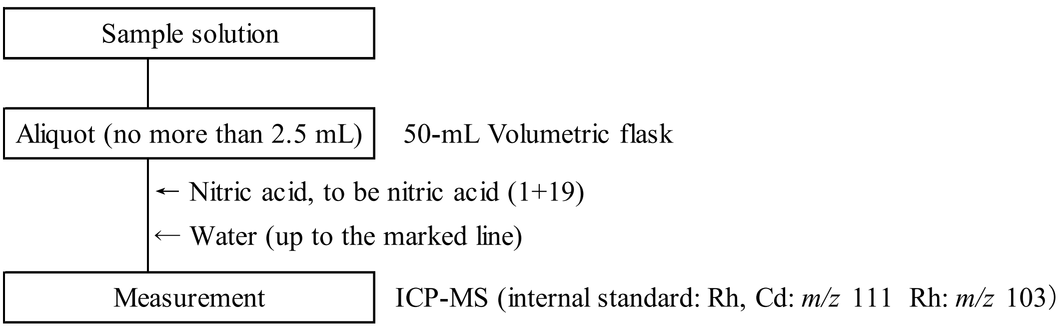


Figure 3 Flow sheet for cadmium in fertilizers (Measurement procedure)

5.3.d

5.4 Nickel

5.4.a Flame atomic absorption spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 5.4.a-2017 or Ni.a-1.

Pretreat an analytical sample with incineration and nitric acid‒hydrochloric acid (1+3), spray into an acetylene‒air flame, and measure the atomic absorption with nickel at a wavelength of 232.0 nm to obtain nickel (Ni) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) **Nickel standard solution (Ni 0.1 mg/mL)**: A nickel standard solution (Ni 0.1 mg/mL) traceable to National Metrology.

e) **Nickel standard solutions (Ni 0.5 µg/mL - 5 µg/mL) for the calibration curve preparation** (1) (2): Put 2.5 mL - 25 mL of nickel standard solution (Ni 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

f) **Blank test solution for the calibration curve preparation** (1) (2): Hydrochloric acid (1+23) used in the procedures in **e**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store at room temperature, and do not use after 6 months after preparation.

Comment 1 Instead of the nickel standard solution in (**2**), a nickel standard solution for the calibration curve preparation can be prepared by using a nickel standard solution (Ni 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(**3**) **Instruments**: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (3) function.

1) **Light source**: A nickel hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Electric furnace**: An electric furnace that can keep the test temperature at 450 °C ± 5 °C .

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (**3**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (4).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (4).
d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
f) Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (6) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer the solution to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**4**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.
(**5**) The watch glass can be removed.
(**6**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 2 Do not conduct the procedures in (**4.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.
Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 232.0 nm

b) **Calibration curve preparation**
1) Spray the nickel standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 232.0 nm.
2) Prepare a curve for the relationship between the nickel concentration and the indicated value of the nickel standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**
1) Subject the sample solution (7) to the same procedure as in **b**) **1**) to read the indicated value.
2) Subject the blank test solution to the same procedure as in **b**) **1**) to read the indicated value, and correct the indicated value obtained for the sample solution.
3) Obtain the nickel content from the calibration curve, and calculate nickel (Ni) in the analytical sample.

Note (**7**) If there is a possibility that the nickel concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 4 Instead of the correction method in **c**) **2**), the nickel (Ni) in the analytical sample can also be corrected by obtaining the nickel content in the blank test solution.

Comment 5 Recovery testing was conducted using industrial sludge fertilizer and composted sludge fertilizer (5 samples); as a result, the recovery rates at the concentration level of 300 mg/kg and 30 mg/kg were 98.5 % - 100.3 % and 97.1 % - 99.9 %, respectively. Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 1 mg/kg.

Table 1 Analysis results of the collaborative study for nickel test method validation

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Sewage sludge fertilizer a	11	56.9	1.1	4.6
Sewage sludge fertilizer b	11	21.8	2.2	3.9
Composted sludge fertilizer a	11	28.9	1.3	6.4
Composted sludge fertilizer b	11	28.5	1.8	4.4
Composted sludge fertilizer c	12	58.3	1.6	4.4

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Yoshinari SAKAKIBARA, Manabu MATSUZAKI and Tadao AMANO: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer **Vol. 1**, p. 41 - 49 (2008)

2) Yoshinari SAKAKIBARA and Manabu MATSUZAKI: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer **Vol. 1**, p. 50 - 59 (2008)

3) Hisanori ARAYA and Yoshimi TAKEBA: Validation of atomic absorption spectrometry for determination of cadmium, lead, nickel and chromium in calcined sludge fertilizer - Research Report of Fertilizer **Vol. 3**, p. 30 - 42 (2010)

(5) Flow sheet for nickel: The flow sheet for nickel in fertilizers is shown below:

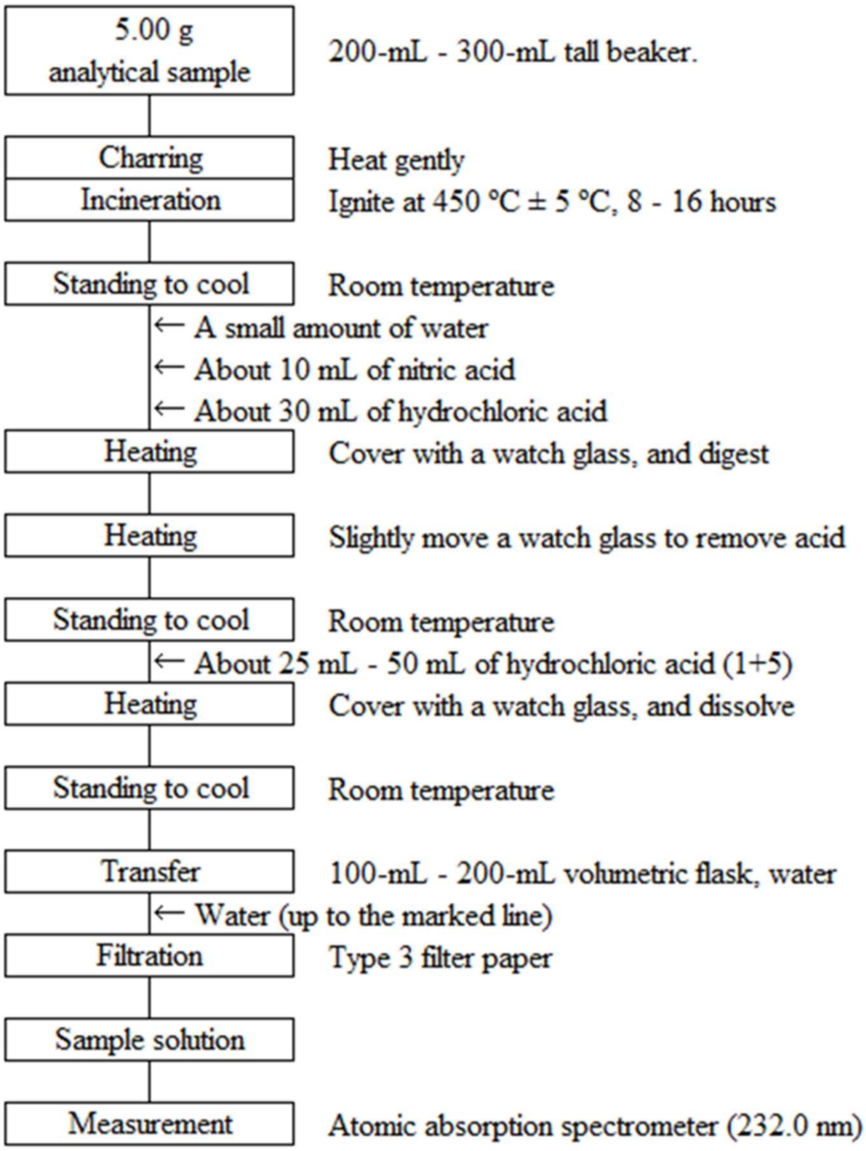


Figure Flow sheet for nickel in fertilizers

5.4.b ICP Optical Emission Spectrometry

(1) Summary

The test method is applicable to sludge fertilizers, etc. This testing method is classified as Type D and its symbol is 5.4.b-2017 or Ni.b-1.

Pretreat an analytical sample with incineration and nitric acid–hydrochloric acid (1+3), and then introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the emission with nickel at a wavelength of 231.604 nm to obtain nickel (Ni) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Nitric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) Hydrochloric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) Nickel standard solution (Ni 0.1 mg/mL): A nickel standard solution (Ni 0.1 mg/mL) traceable to National Metrology.

e) Nickel standard solutions (Ni 2.5 µg/mL) (1) (2): Dilute a predetermined amount of nickel standard solution (Ni 0.1 mg/mL) with hydrochloric acid (1+23) to prepare a nickel standard solution (Ni 2.5 µg/mL)

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store at room temperature, and do not use after 6 months after preparation.

Comment 1 Instead of the nickel standard solution in (**2**), a nickel standard solution for the calibration curve preparation can be prepared by using a nickel standard solution (Ni 1 mg/mL or 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) ICP Optical Emission Spectrometer: An optical emission spectrometer specified in JIS K 0116.

1) Gas: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) Electric furnace: An electric furnace that can keep the test temperature at 450 °C ± 5 °C .

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (3).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (3).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass [(4)], and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) [(5)] to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer the solution to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**3**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.
(**4**) The watch glass can be removed.
(**5**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 3 Do not conduct the procedures in (**4.1**) **b**) - **c**) in the case of fertilizers not containing organic matters.
Comment 4 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.
a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following: Analytical line wavelength: 231.604 nm
b) **Calibration curve preparation and sample measurement**
1) Put 5mL of sample solution in three 10-mL volumetric flasks respectively.
2) Add 2 mL and 4 mL (2.5 µg/mL) of nickel standard solution to volumetric flasks of **1**) above, then add hydrochloric acid (1+23) to the marked line to make a sample solution of Standard Addition Method.
3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1**) above to make a sample solution without a standard solution.
4) Spray the sample solution of Standard Addition Method and the sample solution without a standard solution into the induction plasma, and read the indicated value at a wavelength of 231.604 nm.
5) Put 5 mL of blank test solution in a 10-mL volumetric flask, conduct the same procedures as in **3**) - **4**) to read the indicated value, and correct the indicated value obtained from the respective sample solutions.
6) Prepare a curve for the relationship between the added nickel concentration and the corrected indicated value of the sample solution for Standard Addition Method and the sample solution without a standard solution.
7) Obtain the nickel content from the intercept of the calibration curve to calculate nickel (Ni) concentration in the analytical sample.

Comment 5 Instead of the correction method in **b**) **5**), the nickel (Ni) in the analytical sample can also be corrected by obtaining the nickel content in the blank test solution.

Comment 6 The simultaneous measurement of multiple elements is possible with the ICP-OES. In that case, see **4.9.1.b Comment 6**.

Comment 7 The comparison of the measurement value (x_i : 8.4 mg/kg - 129 mg/kg) of ICP Optical Emission Spectrometry and the measurement value (y_i) of Flame Atomic Absorption Spectrometry was conducted to evaluate trueness using sludge fertilizers (49 samples). As a result, a regression equation was $y=-0.96+1.010x$ and its correlation coefficient (r) was 0.995. Triplicates measurement for each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer, calcined sludge fertilizers and composted sludge fertilizer was conducted. As a result, a repeatability obtained was1.0 % - 2.6 % as a relative standard deviation.
Note that the minimum limit of quantification of this testing method was estimated to be about 8 mg/kg.

References

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI and Tetuya NOMURA: Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer, **Vol. 4**, p. 30 - 35 (2011)

(5) Flow sheet for nickel: The flow sheet for nickel in fertilizers is shown below:

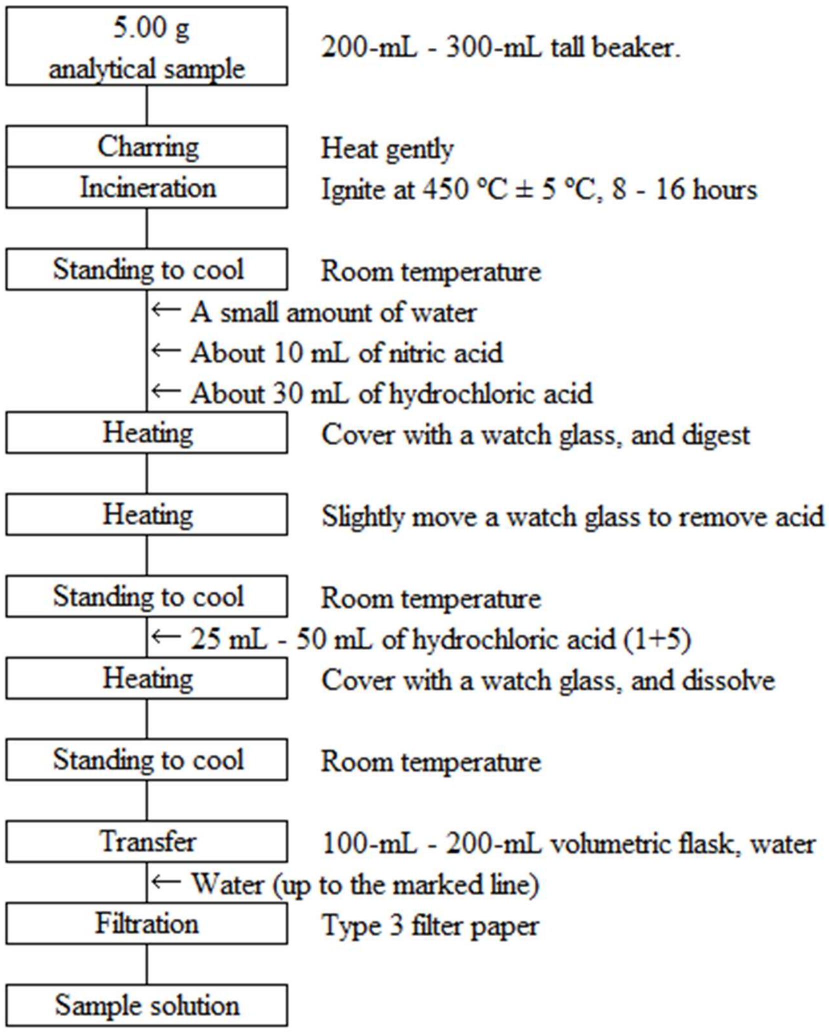


Figure 1 Flow sheet for nickel in sludge fertilizers, etc. (Extraction procedure)

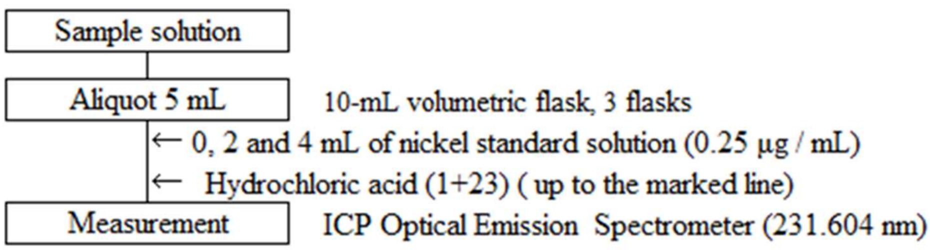


Figure 2 Flow sheet for nickel in sludge fertilizers, etc. (Measurement procedure)

5.4.c ICP Mass Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 5.4.c-2021 or Ni.c-2.

Add nitric acid – hydrogen peroxide to an analytical sample, and heat to decompose by microwave irradiation. Introduce it to an ICP Mass Spectrometer (ICP-MS) and measure the respective indicated values of nickel and an internal standard element (rhodium) with mass/charge number (*m*/*z*) and obtain nickel (Ni) in the analytical sample from the ratio of the indicated value for nickel and the indicated value for an internal standard element. Note that the performance of this testing method is shown in **Comment 7**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A4 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Nitric acid**: Nitric acid used to dilute a standard solution and a sample solution is a highly pure reagent specified in JIS K 9901.

d) **Hydrogen peroxide**: A JIS Guaranteed Reagent specified in JIS K 8230 or a reagent of equivalent quality.

e) **Rhodium standard solution (Rh 1000 mg/L)**: A rhodium standard solution (Rh 1000 mg/L) traceable to National Metrology.

f) **Rhodium standard solution (Rh 5 μg/mL)** [(1)(2)(3)]: Dilute a predetermined amount of rhodium standard solution (Rh 1000 mg/L) with nitric acid (1+19) to prepare a rhodium standard solution (Rh 5 μg/mL).

g) **Rhodium standard solution (Rh 50 ng/mL)** [(1) (3)]: Dilute a predetermined amount of rhodium standard solution (Rh 5 μg/mL) with nitric acid (1+19) to prepare a rhodium standard solution (Rh 50 ng/mL).

h) **Nickel standard solution (Ni 1000 mg/L)**: A nickel standard solution (Ni 1000 mg/L) traceable to National Metrology.

i) **Nickel standard solution (Ni 500 ng/mL)** [(1) (2) (3)]: Dilute a predetermined amount of nickel standard solution (Ni 1000 mg/L) with nitric acid (1+19) to prepare a nickel standard solution (Ni 500 ng/mL).

j) **Nickel standard solutions (Ni 10 ng/mL - 50 ng/mL) for the calibration curve preparation** [(1) (3)]: Put 2 mL - 10 mL of nickel standard solution (Ni 500 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) up to the marked line.

k) **Nickel standard solutions (Ni 0.5 ng/mL - 5 ng/mL) for the calibration curve preparation** [(1) (3)]: Put 1 mL - 10 mL of nickel standard solution (Ni 50 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) up to the marked line.

l) **Blank test solution for the calibration curve preparation** [(1) (3)]: Nitric acid (1+19) used in the procedures in f), g), i), j) and k).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store in cool and dark place, and do not use after 6 month after preparation.

(**3**) For preparation and storage, use sealable containers made of materials such as polypropylene containing no nickel.

Comment 1 Instead of the rhodium standard solution in (**2**), a rhodium standard solution for the calibration curve preparation can be prepared by using a rhodium standard solution (Rh 100 mg/L or 10000 mg/L) traceable to National Metrology.

Comment 2 Instead of the nickel standard solution in (**2**), a nickel standard solution for the calibration curve preparation can be prepared by using a nickel standard solution (Ni 100 mg/L or 10000 mg/L) traceable to National Metrology.

Comment 3 When a sample solution or a standard solution for the calibration curve preparation and an internal standard solution are not simultaneously introduced in the measurement of ICP-MS, add 1/10 volume of rhodium standard solution (Rh 50 ng/mL) to the volume of the solution when preparing respective solutions in the procedures in **j**), **k**) and **l**).

Comment 4 ICP-MS detection methods include a pulse detection method and an analog detection method. There are detection method models that combine them, but when the measurement value is affected by switching, the concentration of the standard solution and the internal standard solution may be changed as appropriate so that measurement can be performed with either detection method.

(3) Instruments: Instruments are as shown below:

a) ICP Mass Spectrometer: A high-frequency plasma mass spectrometer specified in JIS K 0133, that is equipped with a collision/reaction cell.

1) Gas: Argon gas specified in JIS K 1105 of no less than 99.995 % in purity

b) Pressure vessel decomposing device: A device which pressurizes the inside of a vessel by putting acid, etc. to heat in the decomposing vessel, and decomposes a sample by the interaction of heating, pressurizing and acid. The following requirements should be met.

1) The main part of a decomposing device: In the case of the microwave heating method, a device should be able to produce a high-frequency wave using a frequency which is permitted at an industrial frequency facility. It is desirable to be able to monitor pressure and temperature, etc. in the decomposing vessel with a sensor inside the device. The interior of a device should be acid-resistant treated and should have a high temperature durability and a high level of safety.

2) Exhaust system: A system which has an exhaust fan of acid-resistant specification, and has an air-cool function inside the device to allow constant airflow, keeping operating temperature below a certain temperature level.

3) Decomposing vessel: A vessel which is heat-resistance and pressure-tight and has the durability required to decompose particles, and has resistance to internal contamination. It should have safety functions such as causing overheat prevention valve to work and internal pressure to drop by emitting gas, and preventing gas bumping in the case of exceeding a pressure limitation.

c) Centrifugal separator: A centrifugal separator that can work at about 1700 × *g*.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Fluid sludge fertilizer

a) Weigh 20.0 g (4) of an analytical sample, and put it into an airtight vessel.

b) Gradually add 2.5 mL of nitric acid and 2 mL of hydrogen peroxide.

c) Seal the decomposing vessel, put it into the main part of a decomposing device and heat using a microwave.

d) Ignite and pressurize at 180 ºC - 220 ºC for no less than 10 minutes (5) to decompose (6).

e) After standing to cool, transfer the solution to a 50-mL volumetric flask (7) with water.

f) Add water up to the marked line and put it in a 50-mL ground-in stopper centrifugal precipitate tube (7).

g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (8) and use the supernatant as a sample solution.

h) As a blank test, conduct the procedures in **b) - g)** using another decomposing vessel to prepare a blank test solution.

(4.1.2) Fertilizers except fluid sludge fertilizers

a) Weigh 0.20 g of an analytical sample, and put it into a decomposing vessel.
b) Gradually add 10 mL of nitric acid and 1 mL of hydrogen peroxide.
c) Seal the decomposing vessel, put it into the main part of a decomposing device and heat using a microwave.
d) Ignite and pressurize at 180 ºC - 220 ºC for no less than 10 minutes (5) to decompose (6).
e) After standing to cool, transfer the solution to a 50-mL volumetric flask (7) with water.
f) Add water up to the marked line and transfer it to a 50-mL ground-in stopper centrifugal precipitate tube (7).
g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (8) and use the supernatant as a sample solution.
h) As a blank test, conduct the procedures in **b)** - **g)** using another decomposing vessel to prepare a blank test solution.

Note (**4**) The maximum limit of solid content, which is converted from moisture content, in the sampling volume 20.0 g of an analytical sample is about 0.5 g. If solid content is likely to exceed the limit, reduce sampling volume as necessary.
(**5**) Example setting conditions of a microwave decomposing device are as shown in Table 1.

Table 1 Example setting conditions of a microwave decomposing device

Time (min)	Temperature (°C)	Output (kW)
0	-	0
20	200 (temperature rising)	1400
10	200	1400
40	Room temperature	0

(**6**) When organic matters, for example colored precipitate, etc., still remain, add 2 mL of nitric acid and 1 mL of hydrogen peroxide and repeat the procedures in (**4.1**) **c**) – **d**).
(**7**) The vessel should be made of polypropylene, etc. to not affect the measurement.
(**8**) 16.5-cm of radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

Comment 5 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Internal Standard Method) according to JIS K 0113 and as shown below. Specific measurement procedures are according to the operation method of the ICP Mass Spectrometer used in measurement.

a) **Measurement conditions for the ICP Mass Spectrometer**: Set up the measurement conditions for the ICP Mass Spectrometer considering the following:

Nickel: mass/charge number (m/z): 60

Rhodium: mass/charge number (m/z): 103

Collision cell: He-KED (Kinetic Energy Discrimination) mode (9)

b) **Calibration curve preparation**

1) Spray the nickel standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation together with rhodium standard solution (Rh 50 ng/mL) into the inductively coupling plasma [(10)], and read the ratio of the indicated values for the respective mass/charge number of an element subjected to measurement and an internal standard element.

2) Prepare a calibration curve for the relationship between the concentration and the ratio of the indicated value of an element subjected to measurement.

c) **Sample measurement**

1) Put no more than 2.5 mL of the sample solution in a 50-mL volumetric flask [(7)], add nitric acid to be nitric acid (1+19), and add water to the marked line [(11)].

2) Subject to the same procedure as in **b**) **1**) to read the ratio of the indicated values.

3) For the blank test solution, conduct procedures similarly as in **1**) - **3**) to correct the ratio of the indicated value obtained for the measurement solution.

4) Obtain the nickel content from the calibration curve, and calculate nickel (Ni) in the analytical sample.

Note (**9**) Note that, when He-H_2 mixture gas is used, H_2 may react with Rh and the indicated value of Rh becomes lower, affecting the measurement.

(**10**) Simultaneously introduce an internal standard solution 1/9 of the volume of the standard solution for the calibration curve preparation or the blank test solution for the calibration curve preparation.

(**11**) If there is a possibility that the nickel concentration in the sample solution will exceed the maximum limit of the calibration curve, reduce the sampling amount for the sample solution or dilute it with nitric acid (1+19).

Comment 6 Instead of the correction method in **c**) **4**), the nickel (Ni) in the analytical sample can also be corrected by obtaining the nickel content in the blank test solution.

Comment 7 Triplicates additive recovery testing was conducted to evaluate trueness using mixed compost compound fertilizers and fluid composted sludge fertilizers. As a result, the mean recovery rates at the concentration level of 6 mg/kg - 300 mg/kg were 102 % - 109 % as nickel (Ni).

The comparison of the measurement value (x_i: 3.67 mg/kg – 305 mg/kg) of ICP-MS and the measurement value (y_i) of Flame Atomic Absorption Spectrometry was conducted using sludge fertilizers (16 samples). As a result, a regression equation was $y = 0.7617 + 0.97x$, and its correlation coefficient (r) was 1.000. Similarly, the comparison of the analysis value (y_i: 1.88 mg/kg - 320 mg/kg) of ICP Mass Spectrometry and the analysis value (x_i) of Flame Atomic Absorption Spectrometry was conducted using superphosphate of lime (1 sample), double superphosphate of lime (1 sample), processed phosphorus fertilizer (2 samples), mixed phosphorus fertilizer (1 sample), compound fertilizer (13 samples), blended fertilizer (1 sample), coating compound fertilizer (2 samples), solid compound fertilizer (2 samples), byproduct compound fertilizer (2 samples), mixed compost compound fertilizer (6 samples), byproduct magnesia fertilizer (1 sample), and mixed microelement fertilizer (1 sample). As a result, a regression equation was $y = 0.873 + 1.0032x$, and its correlation coefficient (r) was 0.999.

The results of repeated analyses using two types of human waste sludge fertilizer and compound fertilizer were analyzed by the one-way analysis of variance. Table 2 shows the estimation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to

be about 0.03 mg/kg for fluid sludge fertilizers and about 3 mg/kg for other fertilizers.

Table 2 Estimation results of repeatability and intermediate precision

Sample name	Days of repeatability T 1)	Mean 2) (mg/kg) 3)	Repeatability		Intermediate precision	
			s_r 4) (mg/kg) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (mg/kg) 3)	$RSD_{I(T)}$ 7) (%)
Human waste sludge fertilizer 1	5	13	0.3	2.5	0.5	4.1
Human waste sludge fertilizer 2	5	236	5	2.2	5	2.0
Compound fertilizer 1	5	13	0.5	3.6	0.5	3.8
Compound fertilizer 2	5	336	4	1.1	6	1.7

1) The number of days conducting duplicate analysis
2) Mean ([Number of days of repeatability (T)]× [Number of duplicates (2)])
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Comment 8 The simultaneous measurement of multiple elements is possible with the ICP-MS. In that case, prepare standard solutions, etc. referencing Table 1 of Annex C2, conduct procedures similarly as (**4.2**) **b**) - **c**), and calculate respective element concentrations in an analytical sample.

Note that the concentration of a standard solution and an internal standard solution can be changed as appropriate according to **Comment 4**.

References

1) Toshiharu YAGI: Determination of Heavy Metals in Fluid Sludge Fertilizers by ICP-MS and CV-AAS, Research Report of Fertilizer **Vol. 8**, p. 26 - 37 (2015)

2) Toshiharu YAGI, Kenta SAKUMA and Yoshimi HASHIMOTO: Determination of Heavy Metals in Sludge Fertilizers using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Research Report of Fertilizer, **Vol. 9**, p. 21 – 32 (2016)

3) Satoko SAKAIDA, Mayu OSHIMA, Keisuke AOYAMA and Yuji SHIRAI: Determination of Harmful Components in Fertilizers using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Research Report of Fertilizer, **Vol. 12**, p. 52 – 68 (2019)

(**5**) **Flow sheet for nickel**: The flow sheet for nickel in fluid sludge fertilizers is shown below:

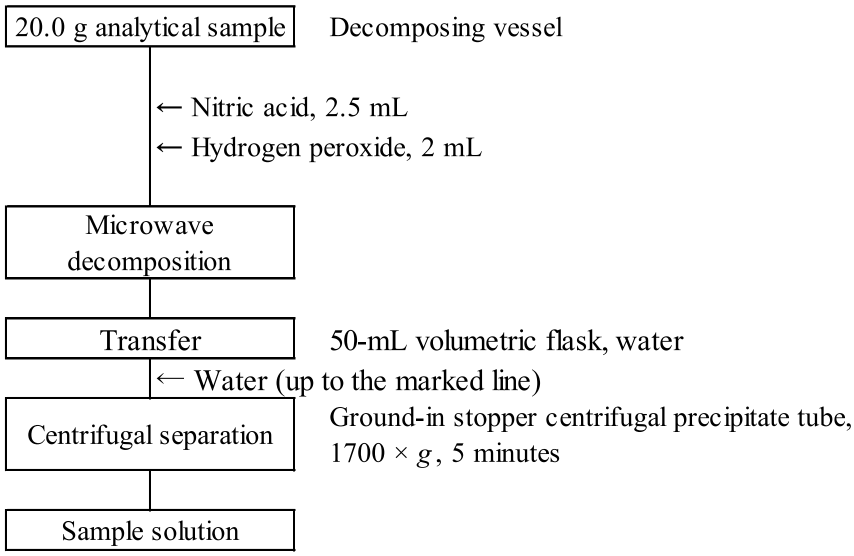


Figure 1　Flow sheet for nickel in fluid sludge fertilizers (Extraction procedure)

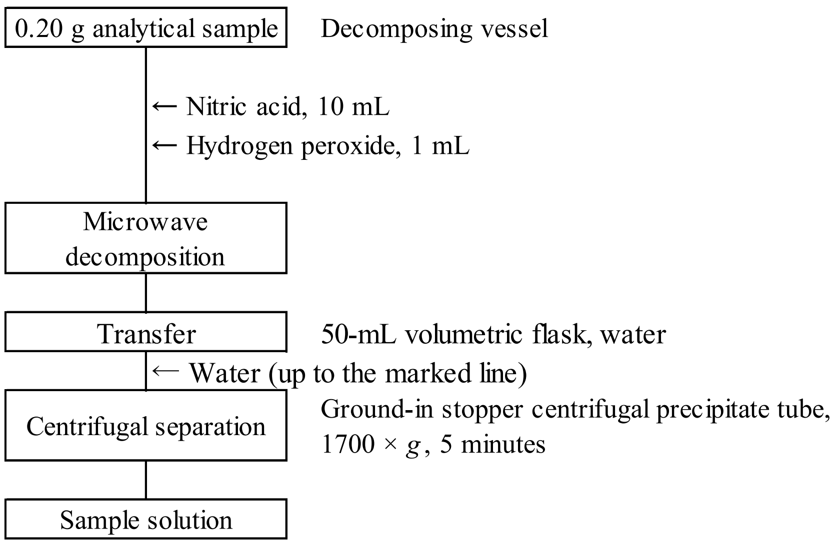


Figure 2　Flow sheet for nickel in fertilizers except fluid sludge fertilizers (Extraction procedure)

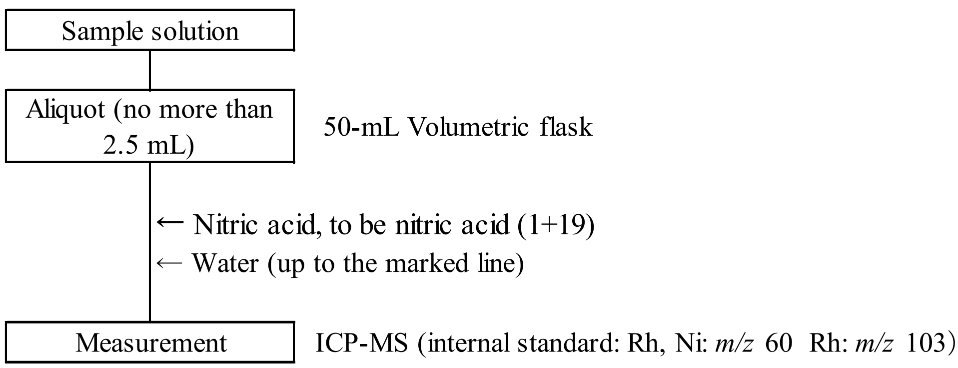


Figure 3　Flow sheet for nickel in fertilizers (Measurement procedure)

5.4.d

5.5 Chromium

5.5.a Flame atomic absorption spectrometry (Fertilizers containing organic matters)

(1) Summary

This testing method is applicable to fertilizers containing organic matters. This testing method is classified as Type B and its symbol is 5.5.a-2017 or Cr.a-1.

Pretreat an analytical sample with incineration and nitric acid−hydrochloric acid (1+3), spray into an acetylene−air flame, and measure the atomic absorption with chromium at a wavelength of 357.9 nm or 359.3 nm to obtain chromium (Cr) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) **Interference suppressor solution** (1): Dissolve 100 g of potassium disulfate specified in JIS K 8783 in water to make 1000 mL.

e) **Chromium standard solution (Cr 0.1 mg/mL)**: A chromium standard solution (Cr 0.1 mg/mL) traceable to National Metrology

f) **Chromium standard solutions (Cr 0.5 µg/mL - 5 µg/mL) for the calibration curve preparation** (1) (2): Put 2.5 mL - 25 mL of chromium standard solution (Cr 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and further add hydrochloric acid (1+23) to the marked line.

g) **Blank test solution for the calibration curve preparation** (1) (2): Put about 50 mL of interference suppressor solution (3) in a 500-mL volumetric flask, and add hydrochloric acid (1+23) used in the procedure in **f**) to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store at room temperature, and do not use after 6 months after preparation.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the chromium standard solution in (**2**), a chromium standard solution for the calibration curve preparation can be prepared by using a chromium standard solution (Cr 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(**3**) **Instruments**: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (4) function.

1) **Light source**: A chromium hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Electric furnace**: An electric furnace that can keep the test temperature at 450 °C ± 5 °C .

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (**4**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (5).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (5).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (6), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.

g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (7) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**5**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.

(**6**) The watch glass can be removed.

(**7**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 2 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 357.9 nm or 359.3 nm (8)

b) **Calibration curve preparation**

1) Spray the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame (9), and read the indicated value at a wavelength of 357.9 nm or 359.3 nm (8).

2) Prepare a curve for the relationship between the chromium concentration and the indicated value of the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put 25 mL of the sample solution (10) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add hydrochloric acid (1+23) to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Subject the blank test solution to the same procedure as in **1**) - **2**) and **b**) **1**) to read the indicated value, and correct the indicated value obtained for the sample solution.

5) Obtain the chromium content from the calibration curve, and calculate chromium (Cr) in the analytical sample.

Note (**8**) When background correction is conducted by the Zeeman method, 359.3 nm is recommended as the analytical line wavelength.

(**9**) Use low-fuel acetylene−air flame. Acetylene−nitrous oxide flame can also be used.

(**10**) If there is a possibility that the chromium concentration in the sample solution will exceed the maximum limit of the calibration curve, decrease the amount to be transferred.

Comment 3 In an acetylene−air flame, sensitivity is enhanced in high-fuel flame, but interference by coexisting substances such as iron and nickel will also be enhanced.
In an acetylene−nitrous oxide flame, such interference hardly affects the result.

Comment 4 Instead of the correction method in **c**) **4**), the chromium (Cr) in the analytical sample can also be corrected by obtaining the chromium content in the blank test solution.

Comment 5 Recovery testing was conducted using industrial sludge fertilizer and composted sludge fertilizer (5 samples); as a result, the recovery rates at the concentration level of 500 mg/kg and 50 mg/kg were 97.5 % - 100.0 % and 95.9 % - 101.9 %, respectively.
Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 1.
Note that the minimum limit of quantification of this testing method was estimated to be about 1 mg/kg.

Table 1 Analysis results of the collaborative study for chromium test method validation

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Sewage sludge fertilizer a	12	33.6	5.3	15.6
Sewage sludge fertilizer b	12	26.3	4.9	18.7
Composted sludge fertilizer a	11	41.3	2.1	11.0
Composted sludge fertilizer b	12	30.2	5.5	13.8
Composted sludge fertilizer c	12	85.0	6.4	12.5

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (3))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Yoshinari SAKAKIBARA, Manabu MATSUZAKI and Tadao AMANO: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer **Vol. 1**, p. 41 - 49 (2008)

2) Yoshinari SAKAKIBARA and Manabu MATSUZAKI: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer **Vol. 1**, p. 50 - 59 (2008)

3) Yoshinari SAKAKIBARA, Chie INOUE: Validation of determination methods for chromium in sludge fertilizer - Evaluation of measurement procedure, Research Report of Fertilizer **Vol. 2**, p. 130 - 136 (2009)

(5) Flow sheet for chromium: The flow sheet for chromium in fertilizers containing organic matters is shown below:

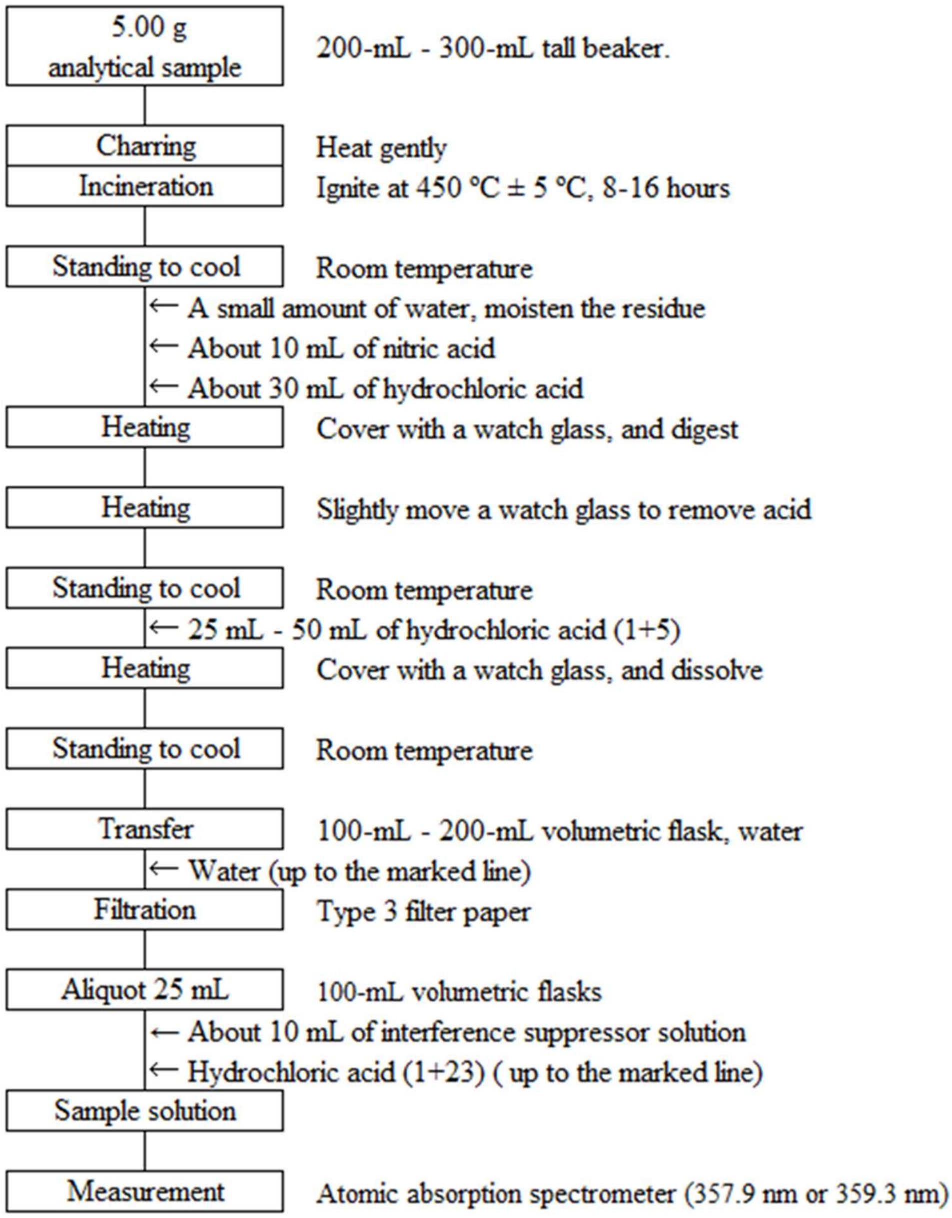


Figure Flow sheet for chromium in fertilizers containing organic matters

5.5.b Flame atomic absorption spectrometry (Fertilizers mainly containing fused matters, slag, etc.)

(1) Summary

This testing method is applicable to fertilizers mainly containing fused matters, slag, etc. This testing method is classified as Type B and its symbol is 5.5.b-2017 or Cr.b-1.

Add ammonium sulfate to prevent the bumping of an analytical sample, pretreat the analytical sample with nitric acid−sulfuric acid−perchloric acid, and then spray into an acetylene−air flame. Measure the atomic absorption with chromium at a wavelength of 357.9 nm or 359.3 nm to obtain chromium (Cr) in an analytical sample. The performance of this testing method is shown in **Comment 4**.

In addition, fertilizers containing no organic matters can also be measured by the method of **5.5.c**. However, this method should be used for fertilizers that bump when heating. (Fertilizers made of fused matters, slag, etc. may bump in many cases).

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.
b) **Ammonium sulfate**: A reagent of atomic absorption analysis grade or equivalents
c) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
d) **Sulfuric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
e) **Perchloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
f) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
g) **Interference suppressor solution** (1): Dissolve 100 g of potassium disulfate specified in JIS K 8783 in water to make 1000 mL.
h) **Chromium standard solution (Cr 0.1 mg/mL):** A chromium standard solution (Cr 0.1 mg/mL) traceable to National Metrology
i) **Chromium standard solutions (Cr 0.01 mg/mL) for the calibration curve preparation** (1): Put 10 mL of chromium standard solution (Cr 0.1 mg/mL) in 100-mL volumetric flask, and add hydrochloric acid (1+23) to the marked line.
j) **Chromium standard solutions (Cr 0.5 µg/mL - 5 µg/mL) for the calibration curve preparation** (1) (2): Put 2.5 mL - 25 mL of chromium standard solution (Cr 0.1 mg/mL) or chromium standard solution (Cr 0.01 mg/mL) in 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and further add hydrochloric acid (1+23) to the marked line.
k) **Blank test solution for the calibration curve preparation** (1) (2): Put about 50 mL of interference suppressor solution (3) in a 500-mL volumetric flask, and add hydrochloric acid (1+23) used in the procedure in **i**) and **j**) to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store at room temperature, and do not use after 6 months after preparation.
(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the chromium standard solution in (**2**), a chromium standard solution for the calibration curve preparation can be prepared by using a chromium standard solution (Cr 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(3) Instruments: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (4) function.

1) **Light source**: A chromium hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)
2) **Gas**: Gas for heating by flame
(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 350 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to no less than 300 °C.

Note (**4**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample and put it in a 300-mL tall beaker.
b) Add 4 g of ammonium sulfate and moisten the analytical sample with a small amount of water.
c) Add about 10 mL of nitric acid, about 5 mL of sulfuric acid and about 5 mL of perchloric acid. Cover the tall beaker with a watch glass, heat on a hot plate or sand bath gently at 170 °C - 220 °C for no less than 1 hour, and then warm to raise temperature gradually to no less than 300 °C taking no less than 30 minutes (5) and further heat at no less than 300 °C for 2-3 hours.
d) Slightly move the watch glass (6), and keep on heating on the hot plate or sand bath to concentrate until the liquid volume becomes about 3 mL (7).
e) After standing to cool, add about 5 mL of hydrochloric acid (1+10) and about 20 mL of water, cover the tall beaker with a watch glass and heat mildly for about 10 minutes to dissolve (8).
f) After standing to cool, transfer the solution to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
g) As a blank test, conduct the procedures in **b**) - **f**) using another tall beaker to prepare a blank test solution. However, the heating procedure after adding acid in the procedures in **c**) should be conducted as shown below. Cover the tall beaker with a watch glass, heat on a hot plate or sand bath at about 170 °C for a while (8), slightly move the watch glass (6) and then heat gently for no less than 1 hour to vapor nitric acid. After concentrating to about 15 mL (9), heat and raise the temperature gradually to no less than 300 °C in more than 30 minutes (5). When white smoke starts evolving, cover the tall beaker with a watch glass and further heat at no less than 300 °C for 2-3 hours.

Note (**5**) Sudden temperature rise may cause bumping. Therefore, increase the temperature gradually.
(**6**) If there is no possibility of bumping, the watch glass can be removed.
(**7**) Exsiccating it may cause the chromium to become insoluble. Therefore, care should be taken not to concentrate too much.
(**8**) Heating at high temperature may cause bumping. Therefore, it is recommended to increase the temperature gradually from about 170 °C to the temperature at which it boils slightly.
(**9**) If much nitric acid remains, care should be taken because bumping can occur easily when heating temperature rises.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement

conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 357.9 nm or 359.3 nm (10)

b) Calibration curve preparation

1) Spray the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a low-fuel acetylene-air flame (11), and read the indicated value at a wavelength of 357.9 nm or 359.3 nm (10).

2) Prepare a curve for the relationship between the chromium concentration and the indicated value of the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement

1) Put 25 mL of the sample solution (12) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add hydrochloric acid (1+17) to the marked line.

3) Subject to the same procedure as in **b**) **1**) to read the indicated value.

4) Subject the blank test solution to the same procedure as in **1**) - **2**) and **b**) **1**) to read the indicated value, and correct the indicated value obtained for the sample solution.

5) Obtain the chromium content from the calibration curve, and calculate chromium (Cr) in the analytical sample.

Note (**10**) When background correction is conducted by the Zeeman method, 359.3 nm is recommended as the analytical line wavelength.

(**11**) Acetylene−nitrous oxide flame can also be used.

(**12**) If there is a possibility that the concentration of chromium in the sample solution exceeds the maximum limit of the calibration curve, put no more than 10 mL of the sample solution in a 100-mL volumetric flask and add about 10 mL of interference suppressor solution and about 67 mL of hydrochloric acid (1+17), and add water up to the marked line.

Comment 2 In an acetylene−air flame, sensitivity is enhanced in high-fuel flame, but interference by coexisting substances such as iron and nickel will also be enhanced.
In an acetylene−nitrous oxide flame, such interference hardly affects the result.

Comment 3 Instead of the correction method in **c**) **4**), the chromium (Cr) in the analytical sample can also be corrected by obtaining the chromium content in the blank test solution.

Comment 4 The comparison of the measurement value (y_i : 54 mg/kg - 4649 mg/kg) of this testing method and the measurement value (x_i) of 5.8 Chromium 5.8.2 Atomic Absorption Spectrometry in Official Methods of Analysis of Fertilizers (1992) was conducted to evaluate trueness using fertilizers (29 samples). As a result, a regression equation was $y = -6.842 + 0.998x$ and its correlation coefficient (r) was 0.999.

The results of the repeatability tests on different days using fused silicic phosphate fertilizer, silicate slag fertilizer and compound fertilizer (1 sample for each) to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 6 mg/kg.

Table 1 Analysis results of the repeatability tests on different days of chromium

Name of sample	Test days of repeatability (T) 1)	Mean 2) (mg/kg)	s_r 3) (mg/kg)	RSD_r 4) (%)	$s_{I(T)}$ 5) (mg/kg)	$RSD_{I(T)}$ 6) (%)
Fused silicic phosphate fertilizer	5	4628	37	0.8	175	3.8
Compound fertilizer	5	545	5.9	1.1	7.3	1.3
Silicate slag fertilizer	5	319	3.8	1.2	5.7	1.8

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) × the number of duplicate testing(2))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Intermediate standard deviation
6) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of chromium

Sample name	Number of laboratories 1)	Mean 2) (mg/kg)	s_r 3) (mg/kg)	RSD_r 4) (%)	s_R 5) (mg/kg)	RSD_R 6) (%)
Silicate slag fertilizer	12	63.75	2.02	3.2	3.87	6.1
Mixed phosphorus fertilizer	12	912.9	13.0	1.4	37.3	4.1
Slag manganese fertilizer	12	2962	74	2.5	176	5.9
Fused silicic phosphate fertilizer	10	4662	135	2.9	166	3.6
Compound fertilizers	10	543.6	10.2	1.9	15.4	2.8

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Reproducibility standard deviation
6) Reproducibility relative standard deviation

References

1) Toshiaki HIROI, Fumika TAKATSU: Evaluation of Digest Method for Determination of Chromium in Inorganic Fertilizer by Atomic Absorption Spectrometry, Research Report of Fertilizer **Vol. 10**, p. 9 - 28 (2017)

2) Toshio HIRABARA, Toshiaki HIROI, Tomomi ISIKAWA: Evaluation of Digest Method for Determination of Chromium in Inorganic Fertilizer by Atomic Absorption Spectrometry: A Collaborative Study, Research Report of Fertilizer **Vol. 11**, p. 39 - 46 (2018)

(5) **Flow sheet for chromium**: The flow sheet for chromium in fertilizers mainly containing fused matters and slag is shown below:

1.00 g analytical sample — 300-mL tall beaker.
← 4 g of ammonium sulfate
← A small amount of water, moisten the analytical sample
← About 10 mL of nitric acid
← About 5 mL of sulfuric acid
← About 5 mL of perchloric acid

Heating — Cover with a watch glass, and heat mildly on a hot plate or sand bath at 170 ºC - 220 ºC for no less than 1 hour

Heating — Raise temperature gradually to no less than 300 °C in more than 30 minutes

Heating — Digest on a hot plate or sand bath at no less than 300 °C for 2 -3 hours

Heating — Slightly move a watch glass and concentrate until the liquid volume becomes about 3 mL

Standing to cool — Room temperature
← About 5 mL of hydrochloric acid (1+5)
← About 20 mL of water

Heating — Cover with a watch glass, and dissolve (heat for about 10 minutes)

Standing to cool — Room temperature

Transfer — 100-mL volumetric flask
← Water (up to the marked line)

Filtration — Type 3 filter paper

Sample solution

Figure 1 Flow sheet for chromium in fertilizers mainly containing fused matters and slag, etc. (Extraction procedure)

Sample solution

Aliquot — 100-mL volumetric flasks
← About 10 mL of interference suppressor solution
← Hydrochloric acid (1+17) (up to the marked line)

Measurement — Atomic absorption spectrometer (357.9 nm or 359.3 nm)

Figure 2 Flow sheet for chromium in fertilizers mainly containing fused matters and slag, etc. (Measurement procedure)

5.5.c Flame atomic absorption spectrometry (Fertilizers not containing organic matters)

(1) Summary

This testing method is applicable to fertilizers not containing organic matters (including calcined sludge fertilizer). This testing method is classified as Type B and its symbol is 5.5.c-2017 or Cr.c-1.

In addition, for fertilizers mainly made of fused matters, slag, etc., care should be taken because they may bump while heating. **5.5.b** applies to fertilizers that may bump.

Pretreat an analytical sample with nitric acid−sulfuric acid−perchloric acid, and then spray into an acetylene−air flame, and measure the atomic absorption with chromium at a wavelength of 357.9 nm or 359.3 nm to obtain chromium (Cr) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Sulfuric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) **Perchloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

e) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

f) **Interference suppressor solution** (1): Dissolve 100 g of potassium disulfate specified in JIS K 8783 in water to make 1000 mL.

g) **Chromium standard solution (Cr 0.1 mg/mL)**: A chromium standard solution (Cr 0.1 mg/mL) traceable to National Metrology

h) **Chromium standard solutions (Cr 0.01 mg/mL) for the calibration curve preparation** (1): Put 10 mL of chromium standard solution (Cr 0.1 mg/mL) in 100-mL volumetric flask, and add hydrochloric acid (1+23) to the marked line.

i) **Chromium standard solutions (Cr 0.5 µg/mL - 5 µg/mL) for the calibration curve preparation** (1) (2): Put 2.5 mL - 25 mL of chromium standard solution (Cr 0.1 mg/mL) or chromium standard solution (Cr 0.01 mg/mL) in 500-mL volumetric flasks step-by-step, add about 50 mL of interference suppressor solution (3), and further add hydrochloric acid (1+23) to the marked line.

j) **Blank test solution for the calibration curve preparation** (1) (2): Put about 50 mL of interference suppressor solution (3) in a 500-mL volumetric flask, and add hydrochloric acid (1+23) used in the procedure in **h**) and **i**) to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store at room temperature, and do not use after 6 months after preparation.

(**3**) Add an interference suppressor solution that is 1/10 volume of the volume to be prepared.

Comment 1 Instead of the chromium standard solution in (**2**), a chromium standard solution for the calibration curve preparation can be prepared by using a chromium standard solution (Cr 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(3) Instruments: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (4) function.

1) **Light source**: A chromium hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene
(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 350 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to no less than 300 °C.

Note (**4**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.
b) Add about 10 mL of nitric acid and about 5 mL of sulfuric acid, cover the tall beaker with a watch glass, and leave at rest overnight.
c) Heat mildly on a hot plate or sand bath at 170 ºC - 220 ºC for no less than 30 minutes. After bubbles cease to form, set the temperature of the hot plate or sand bath to no less than 300 ºC (5), and heat until nitroxide (yellow-brown smoke) is no longer generated (6).
d) After standing to cool, add about 5 mL of perchloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or sand bath at no less than 300 ºC for 2 - 3 hours to digest (7).
f) Slightly move the watch glass (8), and keep on heating on the hot plate or sand bath to concentrate until the liquid volume becomes no more than 2 mL (9).
g) After standing to cool, add about 5 mL of hydrochloric acid (1+10) and about 20 mL of water, cover the tall beaker with a watch glass and heat mildly to dissolve.
h) After standing to cool, transfer the solution to a 100-mL - 200-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**5**) If there is vigorous bumping, increase the temperature gradually.
(**6**) Oxidation of carbides by perchloric acid progresses extremely rapidly and explosively. For that reason, add perchloric acid after fully degrading carbides with nitric acid to avoid danger.
(**7**) When the white smoke of perchloric acid is generated, if the solution is colored such as black-brown or brown, stop heating immediately, and after standing to cool, add nitric acid, and heat again to degrade remaining carbides.
(**8**) If there is no possibility of bumping, the watch glass can be removed.
(**9**) When exsiccating it, chromium may not dissolve completely in **g**) and the concentration may become a low value.

Comment 2 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.
Comment 3 When the analytical sample solidifies in the procedure in (**4.1**) **b**), moisten the analytical sample with a small amount of water as necessary in advance.
Comment 4 It is not necessary to conduct the procedures in (**4.1**) **b**) “leave at rest overnight” because the range subjected to analysis does not contain organic matters.
Comment 5 In some cases, about 10 minutes heating in procedure in (**4.1**) **g**) is required.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below.

Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the atomic absorption spectrometer**: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 357.9 nm or 359.3 nm (10)

b) **Calibration curve preparation**

1) Spray the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a low-fuel acetylene−air flame (11), and read the indicated value at a wavelength of 357.9 nm or 359.3 nm (10).

2) Prepare a curve for the relationship between the chromium concentration and the indicated value of the chromium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Put 25 mL of the sample solution (12) in a 100-mL volumetric flask.

2) Add about 10 mL of interference suppressor solution (3), and add hydrochloric acid (1+17) to the marked line.

3) Subject to the same procedure as in **b) 1)** to read the indicated value.

4) Subject the blank test solution to the same procedure as in **1) - 2)** and **b) 1)** to read the indicated value, and correct the indicated value obtained for the sample solution.

5) Obtain the chromium content from the calibration curve, and calculate chromium (Cr) in the analytical sample.

Note (**10**) When background correction is conducted by the Zeeman method, 359.3 nm is recommended as the analytical line wavelength.

(**11**) Acetylene−nitrous oxide flame can also be used.

(**12**) If there is a possibility that the concentration of chromium in the sample solution exceeds the maximum limit of the calibration curve, put no more than 10 mL of the sample solution in a 100-mL volumetric flask and add about 10 mL of interference suppressor solution and about 67 mL of hydrochloric acid (1+17), and add water up to the marked line.

Comment 6 In an acetylene-air flame, sensitivity is enhanced in high-fuel flame, but interference by coexisting substances such as iron and nickel will also be enhanced.
In an acetylene−nitrous oxide flame, such interference hardly affects the result.

Comment 7 Instead of the correction method in **c) 4)**, the chromium (Cr) in the analytical sample can also be corrected by obtaining the chromium content in the blank test solution.

Comment 8 The comparison of the measurement value (y_i : 52 mg/kg - 4052 mg/kg) of this testing method and the measurement value (x_i) of 5.8 Chromium 5.8.2 Atomic Absorption Spectrometry in Official Methods of Analysis of Fertilizers (1992) was conducted to evaluate trueness using fertilizers (27 samples). As a result, a regression equation was $y=-0.405+0.994x$ and its correlation coefficient (r) was 0.999.
The results of the repeatability tests on different days using mixed phosphate fertilizer, compound fertilizer and silicate slag fertilizer (1 sample for each) to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.
Additionally, results from a collaborative study for testing method validation using calcined sludge fertilizers and its analysis are shown in Table 2.
The minimum limit of quantification of this testing method was estimated to be about 6 mg/kg.

Table 1 Analysis results of the repeatability tests on different days of chromium

Name of sample	Test days of repeatability (T) [1]	Mean[2] (mg/kg)	s_r [3] (mg/kg)	RSD_r [4] (%)	$s_{I(T)}$ [5] (mg/kg)	$RSD_{I(T)}$ [6] (%)
Mixed phosphorus fertilizer	5	3966	96	2.4	107	2.7
Compound fertilizer	5	542	6	1.1	9	1.6
Silicate slag fertilizer	5	288	7	2.4	13	4.4

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Intermediate standard deviation
6) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of chromium

Sample name	Number of laboratories[1]	Mean[2] (mg/kg)	RSD_r [3] (%)	RSD_R [4] (%)
Calcined sludge fertilizer 1	10	107	5.0	9.7
Calcined sludge fertilizer 2	9	136	3.4	3.6
Calcined sludge fertilizer 3	9	182	1.1	2.6
Calcined sludge fertilizer 4	9	213	1.1	3.9
Calcined sludge fertilizer 5	9	117	1.8	4.0

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Hisanori ARAYA, Yoshimi TAKEBA and Toshiaki HIROI: Evaluation of Digest Method for Determination of Chromium in Calcined Sludge Fertilizer by Atomic Absorption Spectrometry - Research Report of Fertilizer **Vol. 4**, p. 23 - 29 (2011)
2) Takeshi UCHIYAMA and Yuji SHIRAI: Evaluation of Digest Method for Determination of Chromium in Calcined Sludge Fertilizer by Atomic Absorption Spectrometry: A Collaborative Study. Research Report of Fertilizer **Vol. 5**, p. 41 - 47 (2012)

(5) **Flow sheet for chromium**: The flow sheet for chromium in calcined sludge fertilizer and fertilizers not containing organic matters is shown below: However, fertilizers that may bump are excluded from the scope of application.

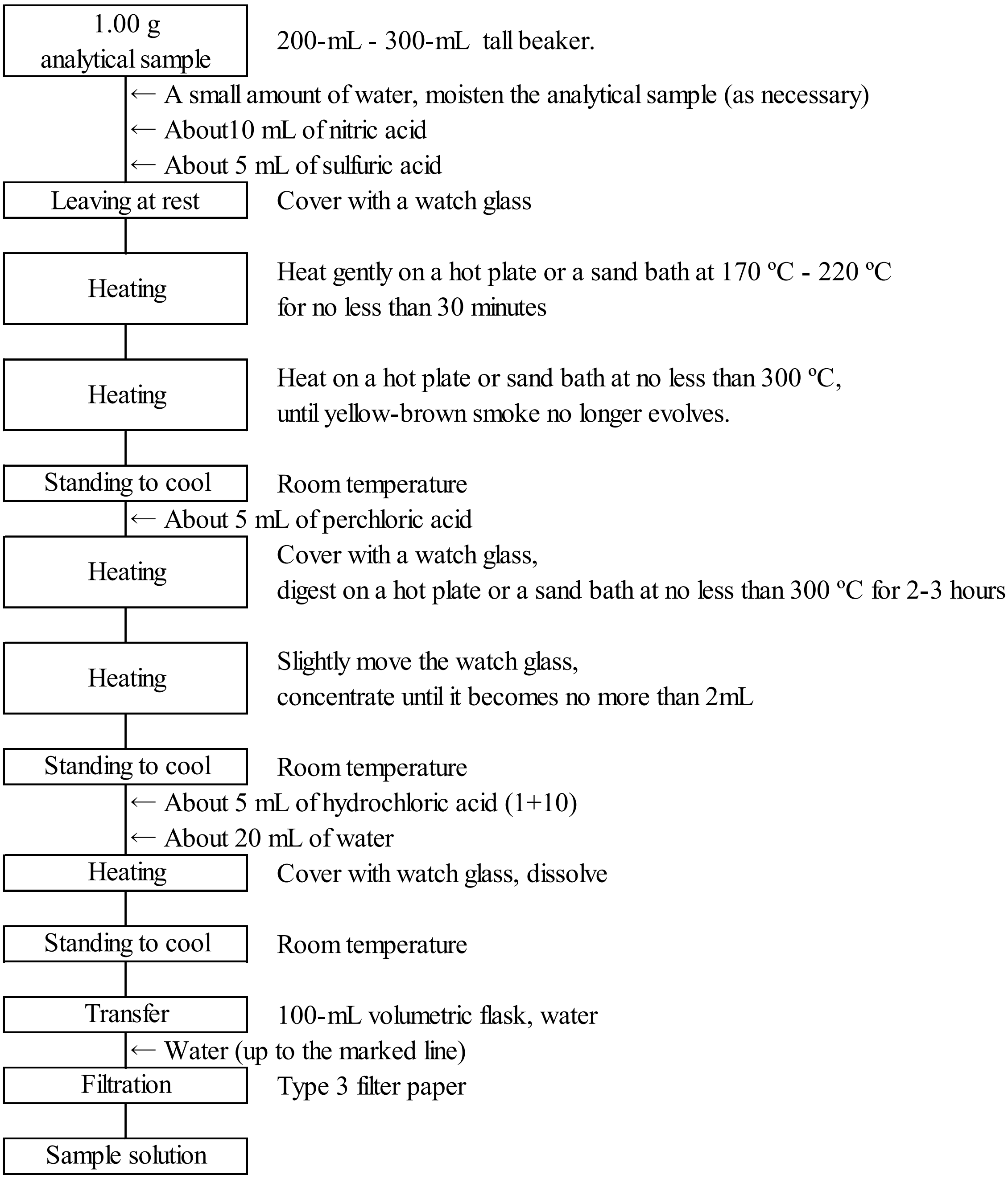


Figure 1 Flow sheet for chromium in calcined sludge fertilizer and fertilizers not containing organic matters (Extraction procedure)
(Excluding fertilizers which may bump while heating)

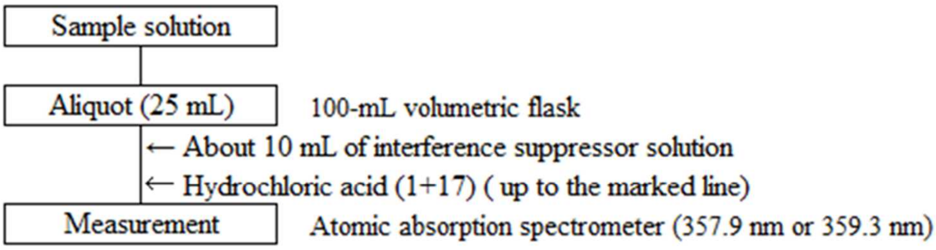


Figure 2 Flow sheet for chromium in calcined sludge fertilizer and fertilizers not containing organic matters (Measurement procedure)
(Excluding fertilizers which may bump while heating)

5.5.d ICP Optical Emission Spectrometry

(1) Summary

The testing method is applicable to sludge fertilizers, etc. (except for calcined sludge fertilizer) This testing method is classified as Type D and its symbol is 5.5.d-2017 or Cr.d-1.

Pretreat an analytical sample with incineration, nitric acid−hydrochloric acid (1+3), introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the emission with chromium at a wavelength of 205.552 nm to quantify the chromium (Cr) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Hydrochloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) **Chromium standard solution (Cr 0.1 mg/mL)**: A chromium standard solution (Cr 0.1 mg/mL) traceable to National Metrology

e) **Chromium standard solutions (Cr 2.5 μg/mL)** (1) (2): Dilute a predetermined amount of chromium standard solution (Cr 0.1 mg/mL) with hydrochloric acid (1+23) to prepare a chromium standard solution (Cr 2.5 μg/mL)

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store at room temperature, and do not use after 6 months after preparation.

Comment 1 Instead of the chromium standard solution in (**2**), a chromium standard solution for the calibration curve preparation can be prepared by using a chromium standard solution (Cr 1 mg/mL or 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) **Electric furnace**: An electric furnace that can keep the test temperature at 450 °C ± 5 °C .

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (3).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (3).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass (4), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (5) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer the solution to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
i) As a blank test, conduct the procedures in **b**) - **h**) using another tall beaker to prepare a blank test solution.

Note (**3**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.
(**4**) The watch glass can be removed.
(**5**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.
a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following: Analytical line wavelength: 205.552 nm
b) **Calibration curve preparation and sample measurement**
1) Put 5mL of sample solution in three 10-mL volumetric flasks respectively.
2) Add 2mL and 4 mL of chromium standard solution (2.5 µg/mL) to volumetric flasks of **1**) above, then add hydrochloric acid (1+23) to the marked line to make a sample solution of Standard Addition Method.
3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1**) above to make a sample solution without a standard solution.
4) Spray the sample solution of Standard Addition Method and the sample solution without a standard solution into the induction plasma, and read the indicated value at a wavelength of 205.552 nm.
5) Put 5 mL of blank test solution in a 10-mL volumetric flask, conduct the same procedures as in **3**) - **4**) to read the indicated value, and correct the indicated value obtained from the respective sample solutions.
6) Prepare a curve for the relationship between the added chromium concentration and the corrected indicated value of the sample solution for Standard Addition Method and the sample solution without a standard solution.
7) Obtain the chromium content from the intercept of the calibration curve to calculate chromium (Cr) in the analytical sample.

Comment 4 Instead of the correction method in **b**) **5**), the chromium (Cr) in the analytical sample can also be corrected by obtaining the chromium content in the blank test solution.
Comment 5 The simultaneous measurement of multiple elements is possible with the ICP-OES. In that case, see **4.9.1.b Comment 6**.

Comment 6 The comparison of the measurement value (x_i : 12.9 mg/kg - 193 mg/kg) of ICP Optical Emission Spectrometry and the measurement value (y_i) of Flame Atomic Absorption Spectrometry was conducted to evaluate trueness using sludge fertilizers (49 samples). As a result, a regression equation was y=1.74+0.971x and its correlation coefficient (r) was 0.991. Triplicates measurement for each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer and composted sludge fertilizer was conducted. As a result, a repeatability obtained was 0.9 % - 2.5 % as a relative standard deviation.
Note that the minimum limit of quantification of this testing method was estimated to be about 4 mg/kg.

References

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI and Tetuya NOMURA: Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer, **Vol. 4**, p. 30 - 35 (2011)

(**5**) **Flow sheet for chromium**: The flow sheet for chromium in fertilizers is shown below:

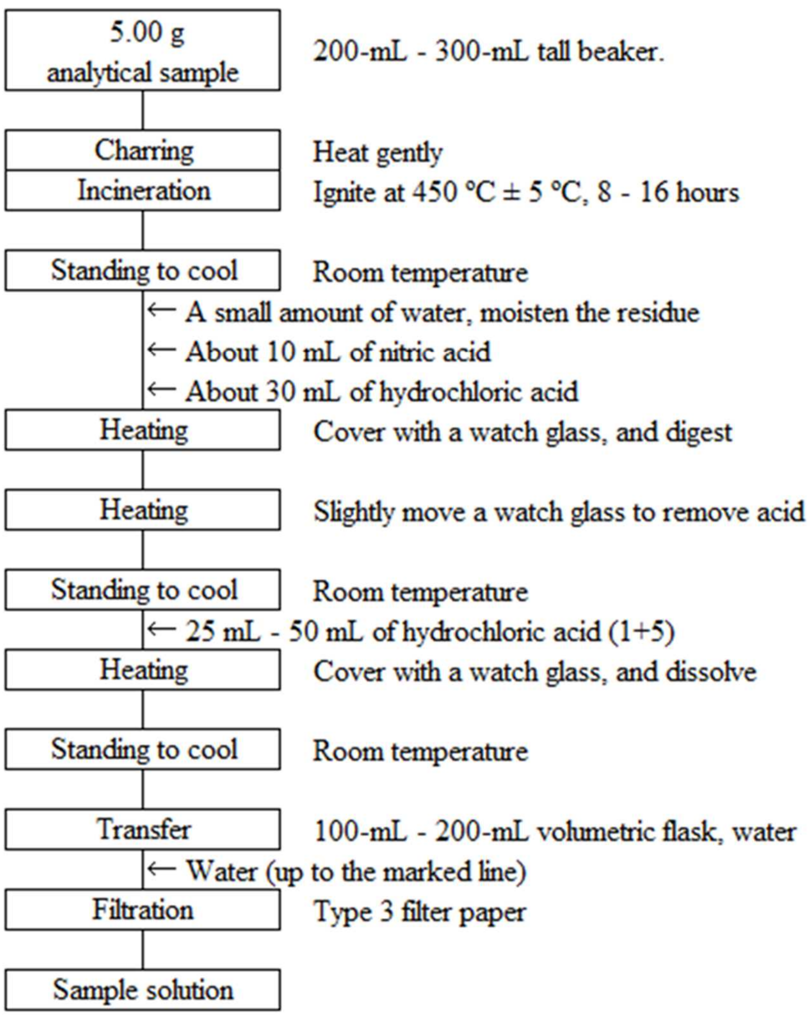


Figure 1 Flow sheet for chromium in sludge fertilizers (Extraction procedure)

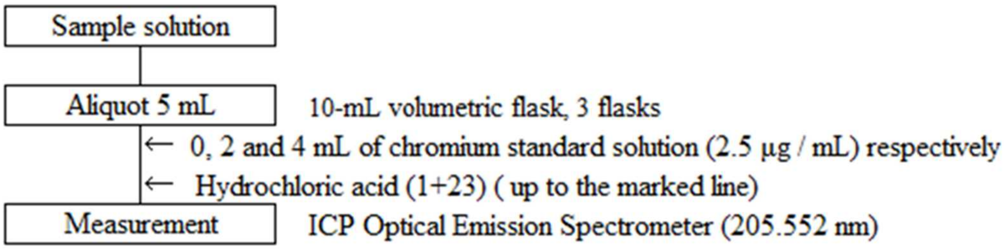


Figure 2 Flow sheet for chromium in sludge fertilizers (Measurement procedure)

5.5.e ICP Mass Spectrometry

(1) Summary

This testing method is applicable to fertilizers containing organic matters. This testing method is classified as Type D and its symbol is 5.5.e-2021 or Cr.e-2.

Add nitric acid – hydrogen peroxide to an analytical sample, and heat to decompose by microwave irradiation. Introduce it to an ICP Mass Spectrometer (ICP-MS) and measure the respective indicated values of chromium and an internal standard element (scandium) with mass/charge number (*m*/*z*) and obtain chromium (Cr) in the analytical sample from the ratio of the indicated value for chromium and the indicated value for an internal standard element. Note that the performance of this testing method is shown in **Comment 7**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A4 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Nitric acid**: Nitric acid used to dilute a standard solution and a sample solution is a highly pure reagent specified in JIS K 9901.

d) **Hydrogen peroxide**: A JIS Guaranteed Reagent specified in JIS K 8230 or a reagent of equivalent quality.

e) **Scandium standard solution (Sc 1000 mg/L)**: A scandium standard solution (Sc 1000 mg/L) traceable to National Metrology.

f) **Scandium standard solution (Sc 25 µg/mL)** (1) (2) (3): Dilute a predetermined amount of scandium standard solution (Sc 1000 mg/L) with nitric acid (1+19) to prepare a scandium standard solution (Sc 25 µg/mL).

g) **Scandium standard solution (Sc 500 ng/mL)** (1) (3): Dilute a predetermined amount of scandium standard solution (Sc 25 µg/mL) with nitric acid (1+19) to prepare a scandium standard solution (Sc 500 ng/mL).

h) **Chromium standard solution (Cr 1000 mg/L)**: A chromium standard solution (Cr 1000 mg/L) traceable to National Metrology.

i) **Chromium standard solution (Cr 1000 ng/mL)** (1) (2) (3): Dilute a predetermined amount of chromium standard solution (Cr 1000 mg/L) with nitric acid (1+19) to prepare a chromium standard solution (Cr 1000 ng/mL).

j) **Chromium standard solutions (Cr 20 ng/mL - 100 ng/mL) for the calibration curve preparation** (1) (3): Put 2 mL - 10 mL of chromium standard solution (Cr 1000 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) up to the marked line.

k) **Chromium standard solutions (Cr 1 ng/mL - 10 ng/mL) for the calibration curve preparation** (1) (3): Put 1 mL - 10 mL of chromium standard solution (Cr 100 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) up to the marked line.

l) **Blank test solution for the calibration curve preparation** (1) (3): Nitric acid (1+19) used in the procedures in f), g), i), j) and k).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store in cool and dark place, and do not use after 6 month after preparation.

(**3**) For preparation and storage, use sealable containers made of materials such as polypropylene containing no chromium.

Comment 1 Instead of the scandium standard solution in (**2**), a scandium standard solution can be prepared by using a scandium standard solution (Sc 100 mg/L or 10000 mg/L) traceable to National Metrology.

Comment 2 Instead of the chromium standard solution in (**2**), a chromium standard solution for the calibration curve preparation can be prepared by using a chromium standard solution (Cr 100 mg/L or 10000 mg/L) traceable to National Metrology.

Comment 3 When a sample solution or a standard solution for the calibration curve preparation and an internal standard solution are not simultaneously introduced in the measurement of ICP-MS, add 1/10 volume of scandium standard solution (Sc 500 ng/mL) to the volume of the solution when preparing respective solutions in the procedures in **j**), **k**) and **l**).

Comment 4 ICP-MS detection methods include a pulse detection method and an analog detection method. There are detection method models that combine them, but when the measurement value is affected by switching, the concentration of the standard solution and the internal standard solution may be changed as appropriate so that measurement can be performed with either detection method.

(**3**) **Instruments**: Instruments are as shown below:

a) **ICP Mass Spectrometer**: A high-frequency plasma mass spectrometer specified in JIS K 0133, that is equipped with a collision/reaction cell.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.995 % in purity

b) **Pressure vessel decomposing device**: A device which pressurizes the inside of a vessel by putting acid, etc. to heat in the decomposing vessel, and decomposes a sample by the interaction of heating, pressurizing and acid. The following requirements should be met.

1) **The main part of a decomposing device**: In the case of the microwave heating method, a device should be able to produce a high-frequency wave using a frequency which is permitted at an industrial frequency facility. It is desirable to be able to monitor pressure and temperature, etc. in the decomposing vessel with a sensor inside the device. The interior of a device should be acid-resistant treated and should have a high temperature durability and a high level of safety.

2) **Exhaust system**: A system which has an exhaust fan of acid-resistant specification, and has an air-cool function inside the device to allow constant airflow, keeping operating temperature below a certain temperature level.

3) **Decomposing vessel**: A vessel which is heat-resistance and pressure-tight and has the durability required to decompose particles, and has resistance to internal contamination. It should have safety functions such as causing overheat prevention valve to work and internal pressure to drop by emitting gas, and preventing gas bumping in the case of exceeding a pressure limitation.

c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Fluid sludge fertilizer**

a) Weigh 20.0 g (4) of an analytical sample, and put it into an airtight vessel.

b) Gradually add 2.5 mL of nitric acid and 2 mL of hydrogen peroxide.

c) Put the decomposing vessel into the main part of a decomposing device and heat using a microwave.

d) Ignite and pressurize at 180 ºC - 220 ºC for no less than 10 minutes (5) to decompose (6).

e) After standing to cool, transfer the solution to a 50-mL volumetric flask (7) with water.

f) Add water up to the marked line and put it in a 50-mL ground-in stopper centrifugal precipitate tube (7).

g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (8) and use the supernatant as a sample solution.

h) As a blank test, conduct the procedures in **b**) - **g**) using another decomposing vessel to prepare a blank test solution.

(**4.1.2**) **Fertilizers containing organic matters except fluid sludge fertilizers**

a) Weigh 0.20 g of an analytical sample, and put it into a decomposing vessel.

b) Gradually add 10 mL of nitric acid and 1 mL of hydrogen peroxide.

c) Seal the decomposing vessel, put it into the main part of a decomposing device and heat using a microwave.

d) Ignite and pressurize at about 180 ºC - 220 ºC for no less than 10 minutes (5) to decompose (6).

e) After standing to cool, transfer the solution to a 50-mL volumetric flask (7) with water.

f) Add water up to the marked line and transfer it to a 50-mL ground-in stopper centrifugal precipitate tube (7).

g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (8) and use the supernatant as a sample solution.

h) As a blank test, conduct the procedures in **b**) - **g**) using another airtight vessel to prepare a blank test solution.

Note (**4**) The maximum limit of solid content, which is converted from moisture content, in the sampling volume 20.0 g of an analytical sample is about 0.5 g. If solid content is likely to exceed the limit, reduce sampling volume as necessary.

(**5**) Example setting conditions of a microwave decomposing device are as shown in Table 1.

Table 1 Example setting conditions of a microwave decomposing device

Time (min)	Temperature (°C)	Output (kW)
0	-	0
20	200 (temperature rising)	1400
10	200	1400
40	Room temperature	0

(**6**) When organic matters, for example colored precipitate, etc., still remain, add 2 mL of nitric acid and 1 mL of hydrogen peroxide and repeat the procedures in (**4.1**) **c**) – **d**).

(**7**) The vessel should be made of polypropylene, etc. to not affect the measurement.

(**8**) 16.5-cm of radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

Comment 5 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Internal Standard Method) according to JIS K 0113 and as shown below. Specific measurement procedures are according to the operation method of the ICP Mass Spectrometer used in measurement.

a) **Measurement conditions for ICP Mass Spectrometer**: Set up the measurement conditions for the ICP Mass Spectrometer considering the following:
Chromium: mass/charge number (m/z): 52
Scandium: mass/charge number (m/z): 45
Collision cell: He-KED (Kinetic Energy Discrimination) mode

b) **Calibration curve preparation**

1) Spray the chromium standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation together with scandium standard solution (Sc 500 ng/mL) into the inductively coupling plasma (9), and read the ratio of the indicated values for the respective mass/charge number of an element subjected to measurement and an internal standard element.

2) Prepare a calibration curve for the relationship between the concentration and the ratio of the indicated value of an element subjected to measurement.

c) **Sample measurement**

1) Put no more than 2.5 mL of the sample solution in a 50-mL volumetric flask (7), add nitric acid to be nitric acid (1+19), and add water to the marked line (10).

2) Subject to the same procedure as in **b**) **1**) to read the ratio of the indicated values.

3) For the blank test solution, conduct procedures similarly as in **1**) - **2**) to correct the ratio of the indicated value obtained for the measurement solution.

4) Obtain the chromium content from the calibration curve, and calculate chromium (Cr) in the analytical sample.

Note (**9**) Simultaneously introduce an internal standard solution 1/10 of the volume of the standard solution for the calibration curve preparation or the blank test solution for the calibration curve preparation.

Note (**10**) If there is a possibility that the chromium concentration in the sample solution will exceed the maximum limit of the calibration curve, reduce the sampling amount for the sample solution or dilute it with nitric acid (1+19).

Comment 6 Instead of the correction method in **c**) **4**), the chromium (Cr) in the analytical sample can also be corrected by obtaining the chromium content in the blank test solution.

Comment 7 Triplicates additive recovery testing was conducted to evaluate trueness using mixed compost compound fertilizers and fluid composted sludge fertilizers. As a result, the mean recovery rates at the concentration level of 10 mg/kg - 500 mg/kg were 93.9 % - 103 % as chromium (Cr).

The comparison of the measurement value (x_i: 4.43 mg/kg – 428 mg/kg) of ICP-MS and the measurement value (y_i) of Flame Atomic Absorption Spectrometry was conducted using sludge fertilizers (15 samples). As a result, a regression equation was $y = 5.1849 + 0.98x$, and its correlation coefficient (r) was 0.999. Similarly, the comparison of the measurement value (y_i: 16.0 mg/kg – 499 mg/kg) of ICP Mass Spectrometry and the measurement value (x_i) of Flame Atomic Absorption Spectrometry was conducted using compound fertilizer (5 samples), solid compound fertilizer (2 samples), and mixed compost compound fertilizer (6 samples). As a result, a regression equation was $y = 15.028 + 1.0014x$, and its correlation coefficient (r) was 0.999.

The results of repeated analyses using two types of human waste sludge fertilizer and compound fertilizer were analyzed by the one-way analysis of variance. Table 2 shows the estimation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.04 mg/kg for fluid sludge fertilizers and about 4 mg/kg for other fertilizers.

Table 2 Estimation results of repeatability and intermediate precision

Sample name	Days of repeatability T 1)	Mean 2) (mg/kg) 3)	Repeatability s_r 4) (mg/kg) 3)	Repeatability RSD_r 5) (%)	Intermediate precision $s_{I(T)}$ 6) (mg/kg) 3)	Intermediate precision $RSD_{I(T)}$ 7) (%)
Human waste sludge fertilizer 1	5	17	0.6	3.6	0.8	4.5
Human waste sludge fertilizer 2	5	451	5	1.1	12	2.7
Compound fertilizer 1	5	58	2	3.3	3	5.2
Compound fertilizer 2	5	556	4	0.7	8	1.5

1) The number of days conducting duplicate analysis
2) Mean ([Number of days of repeatability (T)] × [Number of duplicates (2)])
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Comment 8 The simultaneous measurement of multiple elements is possible with the ICP-MS. In that case, prepare standard solutions, etc. referencing Table 1 of Annex C2, conduct procedures similarly as (**4.2**) **b**) - **c**), and calculate respective element concentrations

in an analytical sample.
Note that the concentration of a standard solution and an internal standard solution can be changed as appropriate according to **Comment 4**.

References

1) Toshiharu YAGI: Determination of Heavy Metals in Fluid Sludge Fertilizers by ICP-MS and CV-AAS, Research Report of Fertilizer **Vol. 8**, p. 26 - 37 (2015)

2) Toshiharu YAGI, Kenta SAKUMA and Yoshimi HASHIMOTO: Determination of Heavy Metals in Sludge Fertilizers using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Research Report of Fertilizer, **Vol. 9**, p. 21 – 32 (2016)

3) Satoko SAKAIDA, Mayu OSHIMA, Keisuke AOYAMA and Yuji SHIRAI: Determination of Harmful Components in Fertilizers using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Research Report of Fertilizer, **Vol. 12**, p. 52 – 68 (2019)

(**5**) **Flow sheet for chromium**: The flow sheet for chromium in fluid sludge fertilizers is shown below:

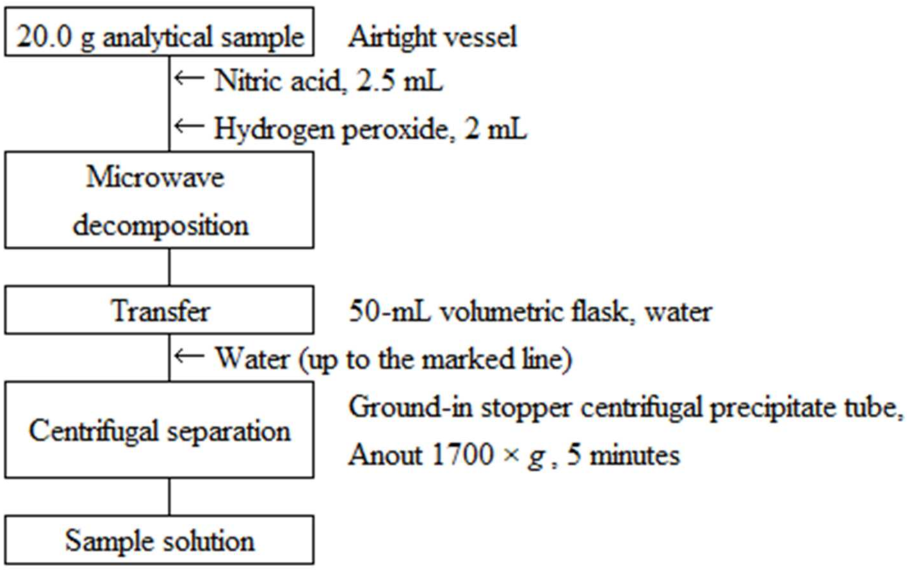


Figure 1 Flow sheet for chromium in fluid sludge fertilizers (Extraction procedure)

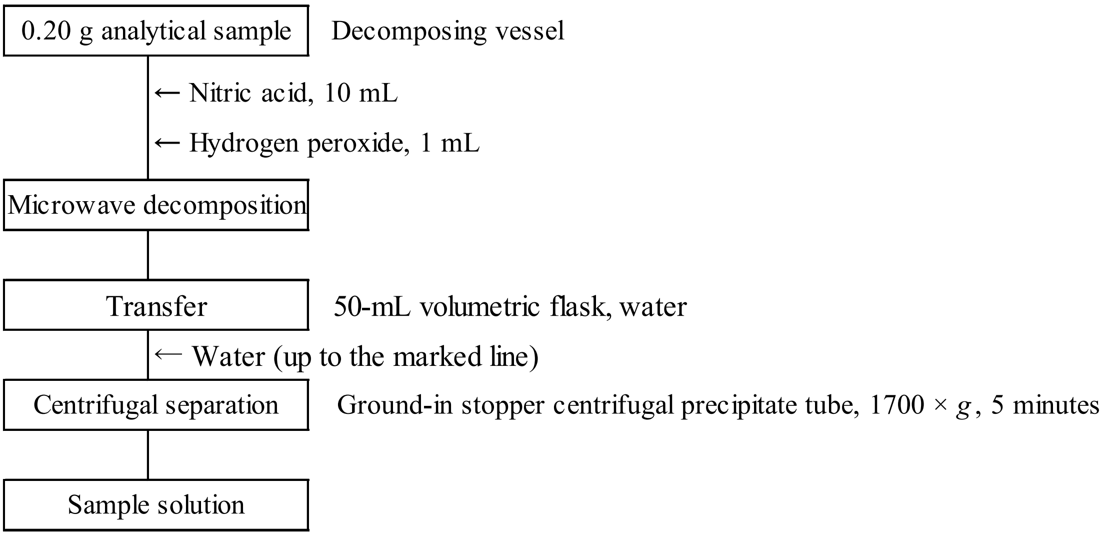


Figure 2 Flow sheet for chromium in fertilizers containing organic matters except fluid sludge fertilizers (Extraction procedure)

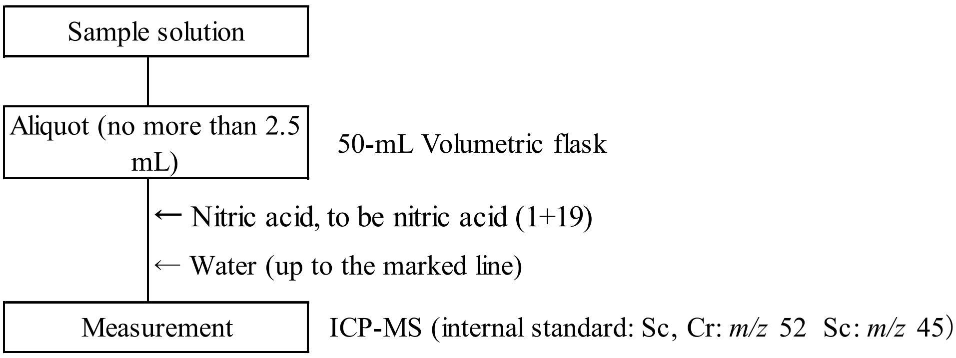


Figure 3 Flow sheet for chromium in fertilizers containing organic matters (Measurement procedure)

5.5.f

5.6 Lead
5.6.a Flame atomic absorption spectrometry
(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 5.6.a-2017 or Pb.a-1.

Pretreat an analytical sample with incineration and nitric acid−hydrochloric acid (1+3), spray into an acetylene−air flame, and measure the atomic absorption with lead at a wavelength of 217.0 nm or 283.3 nm to obtain the lead (Pb) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Nitric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) Hydrochloric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) Lead standard solution (Pb 0.1 mg/mL): A lead standard solution (Pb 0.1 mg/mL) traceable to National Metrology.

e) Lead standard solutions (Pb 0.5 µg - 5 µg/mL) for the calibration curve preparation (1) (2): Put 2.5 mL - 25 mL of lead standard solution (Pb 0.1 mg/mL) in 500-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

f) Blank test solution for the calibration curve preparation (1) (2): Hydrochloric acid (1+23) used in the procedures in **e**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store at room temperature, and do not use after 6 months after preparation.

Comment 1 Instead of the lead standard solution in (**2**), a lead standard solution for the calibration curve preparation can be prepared by using a lead standard solution (Pb 1 mg/mL or 10 mg/mL) traceable to National Metrology.

(3) Instruments: Instruments are as shown below:

a) Flame atomic absorption spectrometer: An atomic absorption spectrometer specified in JIS K 0121 with the background correction (3) function.

1) Light source: A lead hollow cathode lamp (In case of background correction system using continuous spectrum source, the light source is a deuterium lamp.)

2) Gas: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) Electric furnace: An electric furnace that can keep the test temperature at 450 °C ± 5 °C .

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

Note (**3**) There are the continuous source method, the Zeeman method, the non-resonance spectrum method, and the self-reversal method, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char (4).
c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (4).
d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.
f) Slightly move the watch glass (5), and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.
g) After standing to cool, add 25 mL - 50 mL of hydrochloric acid (1+5) (6) to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.
h) After standing to cool, transfer to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare a blank test solution.

Note **(4)** Example of charring and incineration procedure: After raising the temperature from room temperature to about 250°C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.
(5) The watch glass can be removed.
(6) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 2 Do not conduct the procedures in (**4.1**) **b**) **- c**) in the case of fertilizers not containing organic matters.
Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the atomic absorption spectrometer used in measurement.

a) Measurement conditions for the atomic absorption spectrometer: Set up the measurement conditions for the atomic absorption spectrometer considering the following:
Analytical line wavelength: 217.0 nm or 283.3 nm

b) Calibration curve preparation
1) Spray the lead standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 217.0 nm or 283.3 nm.
2) Prepare a curve for the relationship between the lead concentration and the indicated value of the lead standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) Sample measurement
1) Subject the sample solution (7) to the same procedure as in **b**) **1**) to read the indicated value.
2) Subject the blank test solution to the same procedure as in **b**) **1**) to read the indicated value, and correct the indicated value obtained for the sample solution.
3) Obtain the lead content from the calibration curve, and calculate lead (Pb) in the analytical sample.

Note (**7**) If there is a possibility that the lead concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount with hydrochloric acid (1+23).

Comment 4 Instead of the correction method in **c**) **2**), the lead (Pb) in the analytical sample can also be corrected by obtaining the lead content in the blank test solution.

Comment 5 Recovery testing was conducted using industrial sludge fertilizer and composted sludge fertilizer (5 samples); as a result, the recovery rates at the concentration level of 100 mg/kg and 10 mg/kg were 99.1 % **-** 100.6 % and 97.5 % **-** 99.6 %, respectively. Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 1 mg/kg.

Table 1 Analysis resultsof the collaborative study for the test method validation of lead

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	RSD_r[3)] (%)	RSD_R[4)] (%)
Sewage sludge fertilizer a	10	25.2	4.6	3.9
Sewage sludge fertilizer b	11	29.4	3.7	4.3
Composted sludge fertilizer a	10	18.6	3.2	5.0
Composted sludge fertilizer b	10	22.2	1.8	7.0
Composted sludge fertilizer c	11	86.8	1.3	4.0

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Repeatability relative standard deviation
4) Reproducibility relative standard deviation

References

1) Yoshinari SAKAKIBARA, Manabu MATSUZAKI and Tadao AMANO: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Improved Decomposition Method - Research Report of Fertilizer **Vol. 1**, p. 41 - 49 (2008)

2) Yoshinari SAKAKIBARA and Manabu MATSUZAKI: Determination of Cadmium, Lead, Nickel and Chromium in Sludge Fertilizer - Collaborative Test Results - Research Report of Fertilizer **Vol. 1**, p. 50 - 59 (2008)

3) Hisanori ARAYA and Yoshimi TAKEBA: Validation of atomic absorption spectrometry for determination of cadmium, lead, nickel and chromium in calcined sludge fertilizer - Research Report of Fertilizer **Vol. 3**, p. 30 - 42 (2010)

(**5**) **Flow sheet for lead**: The flow sheet for lead in fertilizers is shown below:

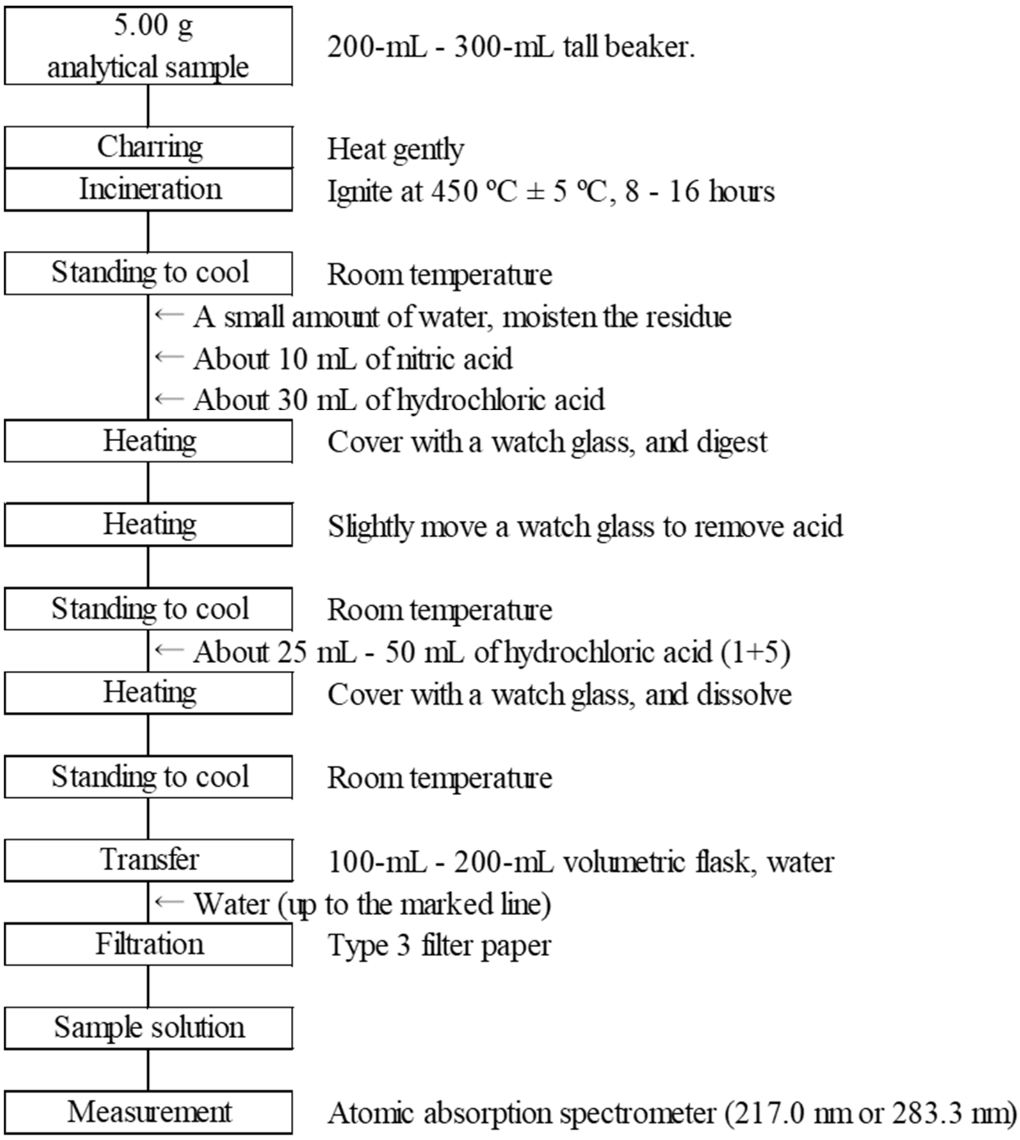


Figure Flow sheet for lead in fertilizers

5.6.b ICP Optical Emission Spectrometry

(1) Summary

The test method is applicable to sludge fertilizers, etc. This testing method is classified as Type D and its symbol is 5.6.b-2017 or Pb.b-1.

Pretreat an analytical sample with incineration, nitric acid−hydrochloric acid (1+3), introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the emission with lead at a wavelength of 220.351 nm to obtain the lead (Pb) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Nitric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) Hydrochloric acid: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

d) Lead standard solution (Pb 0.1 mg/mL): A lead standard solution (Pb 0.1 mg/mL) traceable to National Metrology.

e) Lead standard solutions (Pb 2.5 µg/mL) (1) (2): Dilute a predetermined amount of lead standard solution (Pb 0.1 mg/mL) with hydrochloric acid (1+23) to prepare a lead standard solution (Pb 2.5 µg/mL).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store at room temperature, and do not use after 6 months after preparation.

Comment 1 Instead of the lead standard solution in (**2**), a lead standard solution for the calibration curve preparation can be prepared by using a lead standard solution (Pb 1 mg/mL or 10 mg/mL) traceable to National Metrology.

Comment 2 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) ICP Optical Emission Spectrometer: An optical emission spectrometer specified in JIS K 0116.

1) Gas: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) Electric furnace: An electric furnace that can keep the test temperature at 450 °C ± 5 °C .

c) Hot plate or sand bath: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.

b) Put the tall beaker in an electric furnace, and heat gently to char (3).

c) Ignite at 450 ºC ± 5 ºC for 8 - 16 hours to incinerate (3).

d) After standing to cool, moisten the residue with a small amount of water, and add about 10 mL of nitric acid and about 30 mL of hydrochloric acid.

e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to digest.

f) Slightly move the watch glass [(4)], and continue heating on the hot plate or sand bath to concentrate until nearly exsiccated.

g) After standing to cool, add 25 mL **-** 50 mL of hydrochloric acid (1+5) [(5)] to the digest, cover the tall beaker with the watch glass, and heat quietly to dissolve.

h) After standing to cool, transfer the solution to a 100-mL - 200 mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.

i) As a blank test, conduct the procedures in **b) - h)** using another tall beaker to prepare a blank test solution.

Note (**3**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 450 °C in 1 to 2 hours.

(**4**) The watch glass can be removed.

(**5**) Add hydrochloric acid (1+5) so that the hydrochloric acid concentration of the sample solution will be hydrochloric acid (1+23). For example, when a 100-mL volumetric flask is used in the procedure in **h**), about 25 mL of hydrochloric acid (1+5) should be added.

Comment 3 Do not conduct the procedures in (**4.1**) **b**) **- c**) in the case of fertilizers not containing organic matters.

Comment 4 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Standard Addition Method) according to JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 220.351 nm

b) **Calibration curve preparation and sample measurement**

1) Put 5mL of sample solution in three 10-mL volumetric flasks respectively.

2) Add 2 mL and 4 mL of lead standard solution (0.25 µg/mL) to volumetric flasks of **1**) above, then add hydrochloric acid (1+23) to the marked line to make a sample solution of Standard Addition Method.

3) Add hydrochloric acid (1+23) to the marked line of the remaining volumetric flask of **1**) above to make a sample solution without a standard solution.

4) Spray the sample solution of Standard Addition Method and the sample solution without a standard solution into the induction plasma, and read the indicated value at a wavelength of 220.351 nm.

5) Put 5 mL of blank test solution in a 10-mL volumetric flask, conduct the same procedures as in **3**) **- 4**) to read the indicated value, and correct the indicated value obtained from the respective sample solutions.

6) Prepare a curve for the relationship between the added lead concentration and the corrected indicated value of the sample solution for Standard Addition Method and the sample solution without a standard solution.

7) Obtain the lead content from the intercept of the calibration curve, and calculate lead (Pb) in the analytical sample.

Comment 5 Instead of the correction method in **b**) **5**), the lead (Pb) in the analytical sample can also be corrected by obtaining the lead content in the blank test solution.

Comment 6 The simultaneous measurement of multiple elements is possible with the ICP-OES. In that case, see **4.9.1.b Comment 6**.

Comment 7 The comparison of the measurement value (x_i : 1.1 mg/kg - 69.0 mg/kg) of ICP Optical Emission Spectrometry and the measurement value (y_i) of Flame Atomic Absorption Spectrometry was conducted to evaluate trueness using sludge fertilizers (49 samples). As a result, a regression equation was $y=-0.31+1.045x$ and its correlation coefficient (r) was 0.993. Triplicates measurement for each one sample of sewage sludge fertilizer, human waste sludge fertilizer, industrial sludge fertilizer, mixed sludge fertilizer, calcined sludge fertilizers and composted sludge fertilizer was conducted. As a result, a repeatability obtained was 0.9 % - 3.3 % as a relative standard deviation.

Note that the minimum limit of quantification of this testing method was estimated to be about 5 mg/kg.

References

1) Masahiro ECHI, Tomoe INOUE, Megumi TABUCHI and Tetuya NOMURA: Simultaneous Determination of Cadmium, Lead, Nickel, Chromium, Copper and Zinc in Sludge Fertilizer using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Research Report of Fertilizer, **Vol. 4**, p. 30 - 35 (2011)

(5) Flow sheet for lead: The flow sheet for lead in fertilizers is shown below:

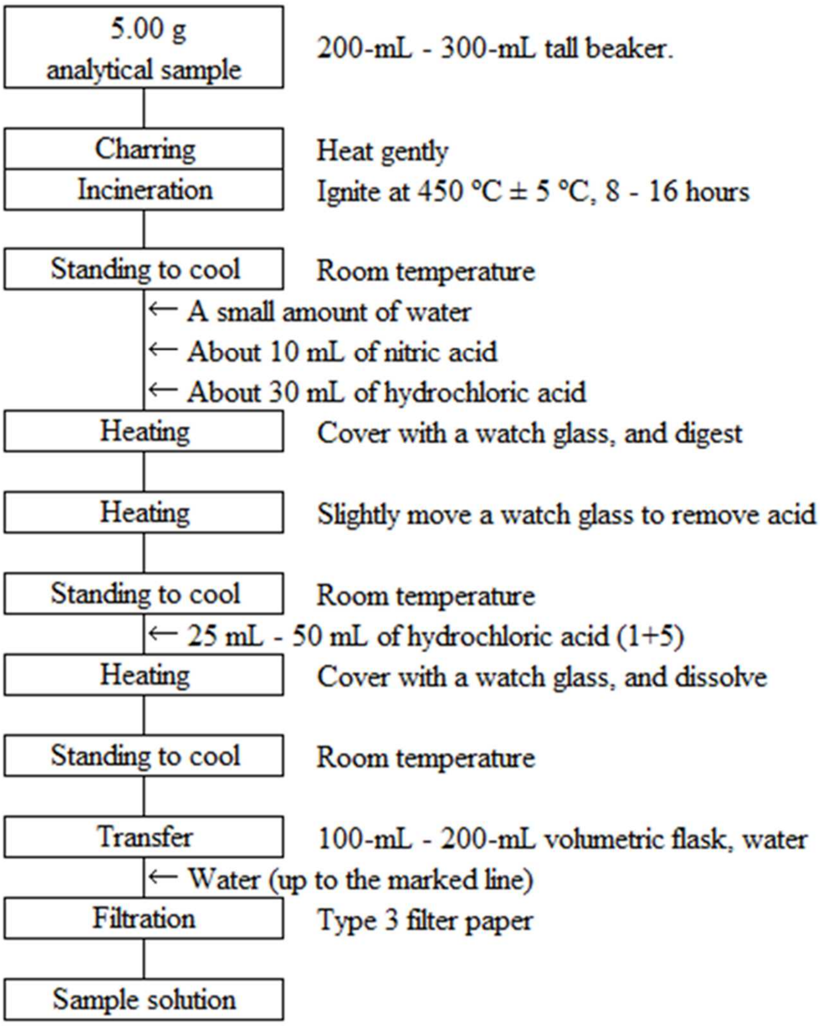


Figure 1 Flow sheet for lead in sludge fertilizers (Extraction procedure)

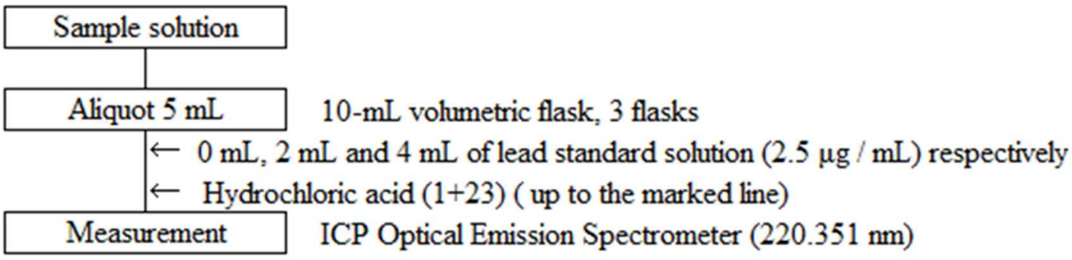


Figure 2 Flow sheet for lead in sludge fertilizers (Measurement procedure)

5.6.c ICP Mass Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 5.6.c-2021 or Pb.c-2.

Add nitric acid – hydrogen peroxide to an analytical sample, and heat to decompose by microwave irradiation. Introduce it to an ICP Mass Spectrometer (ICP-MS) and measure the respective indicated values of lead and an internal standard element (thallium) with mass/charge number (*m*/*z*) and obtain lead (Pb) in the analytical sample from the ratio of the indicated value for lead and the indicated value for an internal standard element. Note that the performance of this testing method is shown in **Comment 7**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A4 specified in JIS K 0557.

b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.

c) **Nitric acid**: Nitric acid used to dilute a standard solution and a sample solution is a highly pure reagent specified in JIS K 9901.

d) **Hydrogen peroxide**: A JIS Guaranteed Reagent specified in JIS K 8230 or a reagent of equivalent quality.

e) **Thallium standard solution (Tl 1000 mg/L)**: A thallium standard solution (Tl 1000 mg/L) traceable to National Metrology.

f) **Thallium standard solution (Tl 2.5 μg/mL)** (1) (2) (3): Dilute a predetermined amount of thallium standard solution (Tl 1000 mg/L) with nitric acid (1+19) to prepare a thallium standard solution (Tl 2.5 μg/mL).

g) **Thallium standard solution (Tl 50 ng/mL)** (1) (3): Dilute a predetermined amount of thallium standard solution (Tl 2.5 μg/mL) with nitric acid (1+19) to prepare a thallium standard solution (Tl 50 ng/mL).

h) **Lead standard solution (Pb 1000 mg/L)**: A lead standard solution (Pb 1000 mg/L) traceable to National Metrology.

i) **Lead standard solution (Pb 100 ng/mL)** (1) (2) (3): Dilute a predetermined amount of lead standard solution (Pb 1000 mg/L) with nitric acid (1+19) to prepare a lead standard solution (Pb 100 ng/mL).

j) **Lead standard solutions (Pb 2 ng/mL - 10 ng/mL) for the calibration curve preparation** (1) (3): Put 2 mL - 10 mL of lead standard solution (Pb 100 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) to the marked line.

k) **Lead standard solutions (Pb 0.1 ng/mL - 1 ng/mL) for the calibration curve preparation** (1) (3): Put 1 mL - 10 mL of lead standard solution (Pb 10 ng/mL) in 100-mL volumetric flasks step-by-step, and add nitric acid (1+19) to the marked line.

l) **Blank test solution for the calibration curve preparation** (1) (3): Nitric acid (1+19) used in the procedures in f), g), i), j) and k).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) Store in cool and dark place, and do not use after 6 month after preparation.

(**3**) For preparation and storage, use sealable containers made of materials such as polypropylene containing no lead.

Comment 1 Instead of the thallium standard solution in (**2**), a thallium standard solution can be prepared by using a thallium standard solution (Tl 100 mg/L or 10000 mg/L) traceable to National Metrology.

Comment 2 Instead of the lead standard solution in (**2**), a lead standard solution for the calibration curve preparation can be prepared by using a lead standard solution (Pb 100 mg/L or 10000 mg/L) traceable to National Metrology.

Comment 3 When a sample solution or a standard solution for the calibration curve preparation and an internal standard solution are not simultaneously introduced in the measurement of ICP-MS, add 1/10 volume of thallium standard solution (Tl 50 ng/mL) to the volume of the solution when preparing respective solutions in the procedures in **j**), **k**) and **l**).

Comment 4 ICP-MS detection methods include a pulse detection method and an analog detection method. There are detection method models that combine them, but when the measurement value is affected by switching, the concentration of the standard solution and the internal standard solution may be changed as appropriate so that measurement can be performed with either detection method.

(**3**) **Instruments**: Instruments are as shown below:

a) **ICP Mass Spectrometer**: A high-frequency plasma mass spectrometer specified in JIS K 0133, that is equipped with a collision/reaction cell.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.995 % in purity

b) **Pressure vessel decomposing device**: A device which pressurizes the inside of a vessel by putting acid, etc. to heat in the decomposing vessel, and decomposes a sample by the interaction of heating, pressurizing and acid. The following requirements should be met.

1) **The main part of a decomposing device**: In the case of the microwave heating method, a device should be able to produce a high-frequency wave using a frequency which is permitted at an industrial frequency facility. It is desirable to be able to monitor pressure and temperature, etc. in the decomposing vessel with a sensor inside the device. The interior of a device should be acid-resistant treated and should have a high temperature durability and a high level of safety.

2) **Exhaust system**: A system which has an exhaust fan of acid-resistant specification, and has an air-cool function inside the device to allow constant airflow, keeping operating temperature below a certain temperature level.

3) **Decomposing vessel**: A vessel which is heat-resistance and pressure-tight and has the durability required to decompose particles, and has resistance to internal contamination. It should have safety functions such as causing overheat prevention valve to work and internal pressure to drop by emitting gas, and preventing gas bumping in the case of exceeding a pressure limitation.

c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Fluid sludge fertilizer**

a) Weigh 20.0 g (4) of an analytical sample, and put it into an airtight vessel.

b) Gradually add 2.5 mL of nitric acid and 2 mL of hydrogen peroxide.

c) Put the decomposing vessel into the main part of a decomposing device and heat using a microwave.

d) Ignite and pressurize at 180 ºC - 220 ºC for no less than 10 minutes (5) to decompose (6).

e) After standing to cool, transfer the solution to a 50-mL volumetric flask (7) with water.

f) Add water up to the marked line and put it in a 50-mL ground-in stopper centrifugal precipitate tube (7).

g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (8) and use the supernatant as a sample solution.

h) As a blank test, conduct the procedures in **b**) - **g**) using another decomposing vessel to prepare a blank test solution.

(**4.1.2**) **Fertilizers except fluid sludge fertilizers**

a) Weigh 0.20 g of an analytical sample, and put it into a decomposing vessel.
b) Gradually add 10 mL of nitric acid and 1 mL of hydrogen peroxide.
c) Seal the decomposing vessel, put it into the main part of a decomposing device and heat using a microwave.
d) Ignite and pressurize at 180 ºC - 220 ºC for no less than 10 minutes (5) to decompose (6).
e) After standing to cool, transfer the solution to a 50-mL volumetric flask (7) with water.
f) Add water up to the marked line and transfer it to a 50-mL ground-in stopper centrifugal precipitate tube (7).
g) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (8) and use the supernatant as a sample solution.
h) As a blank test, conduct the procedures in **b)** - **g)** using another decomposing vessel to prepare a blank test solution.

Note (**4**) The maximum limit of solid content, which is converted from moisture content, in the sampling volume 20.0 g of an analytical sample is about 0.5 g. If solid content is likely to exceed the limit, reduce sampling volume as necessary.
(**5**) Example setting conditions of a microwave decomposing device are as shown in Table 1.

Table 1Example setting conditions of a microwave decomposing device

Time (min)	Temperature (°C)	Output (kW)
0	-	0
20	200 (temperature rising)	1400
10	200	1400
40	Room temperature	0

(**6**) When organic matters, for example colored precipitate, etc., still remain, add 2 mL of nitric acid and 1 mL of hydrogen peroxide and repeat the procedures in (**4.1**) **c**) – **d**).
(**7**) The vessel should be made of polypropylene, etc. to not affect the measurement.
(**8**) 16.5-cm of radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

Comment 5 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct measurement (Internal Standard Method) according to JIS K 0113 and as shown below. Specific measurement procedures are according to the operation method of the ICP Mass Spectrometer used in measurement.
a) **Measurement conditions for ICP Mass Spectrometer**: Set up the measurement conditions for the ICP Mass Spectrometer considering the following:
Lead: mass/charge number (m/z): 208, 206, 207
Thallium: mass/charge number (m/z): 205
Collision cell: He-KED (Kinetic Energy Discrimination) mode
b) **Calibration curve preparation**
1) Spray the lead standard solution for the calibration curve preparation and the blank test solution for the calibration curve preparation together with thallium standard solution (Tl 50 ng/mL) into the inductively coupling plasma (9), and read the ratio of the indicated values for the respective mass/charge number of an element subjected to measurement and an internal standard element (10).
2) Prepare a calibration curve for the relationship between the concentration and the ratio of the indicated value of an element subjected to measurement.

c) Sample measurement

1) Put no more than 2.5 mL of the sample solution in a 50-mL volumetric flask (7), add nitric acid to be nitric acid (1+19), and add water to the marked line (11).

2) Subject to the same procedure as in **b) 1)** to read the ratio of the indicated values.

3) For the blank test solution, conduct procedures similarly as in **1) - 3)** to correct the ratio of the indicated value obtained for the measurement solution.

4) Obtain the lead content from the calibration curve, and calculate lead (Pb) in the analytical sample.

Note (**9**) Simultaneously introduce an internal standard solution 1/9 of the volume of the standard solution for the calibration curve preparation or the blank test solution for the calibration curve preparation.

(**10**) If there is a possibility that the lead concentration in the sample solution will exceed the maximum limit of the calibration curve, reduce the sampling amount for the sample solution or dilute it with nitric acid (1+19).

Comment 6 Instead of the correction method in **c**) **3**), the lead (Pb) in the analytical sample can also be corrected by obtaining the lead content in the blank test solution.

Comment 7 Triplicates additive recovery testing was conducted to evaluate trueness using mixed compost compound fertilizers and fluid composted sludge fertilizers. As a result, the mean recovery rates at the concentration level of 2 mg/kg - 100 mg/kg were 96.5 % - 101 % as lead (Pb).

The comparison of the measurement value (x_i: 2.00 mg/kg – 101 mg/kg) of ICP-MS and the measurement value (y_i) of Flame Atomic Absorption Spectrometry was conducted using sludge fertilizers (14 samples). As a result, a regression equation was $y = -0.4586 + 0.98x$, and its correlation coefficient (r) was 0.999. Similarly, the comparison of the analysis value (y_i: 3.41 mg/kg - 108 mg/kg) of ICP Mass Spectrometry and the analysis value (x_i) of Flame Atomic Absorption Spectrometry was conducted using superphosphate of lime (1 sample), double superphosphate of lime (1 sample), mixed phosphorus fertilizer (1 sample), compound fertilizer (3 samples), solid compound fertilizer (2 samples), mixed compost compound fertilizer (5 samples), and byproduct magnesia fertilizer (1 sample). As a result, a regression equation was $y = -0.7161 + 0.9923x$, and its correlation coefficient (r) was 0.999.

The results of repeated analyses using two types of human waste sludge fertilizer and compound fertilizer were analyzed by the one-way analysis of variance. Table 2 shows the estimation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 mg/kg for fluid sludge fertilizers and about 1 mg/kg for other fertilizers.

Table 2 Estimation results of repeatability and intermediate precision

Sample name	Days of repeatability T 1)	Mean 2) (mg/kg) 3)	Repeatability		Intermediate precision	
			s_r 4) (mg/kg) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (mg/kg) 3)	$RSD_{I(T)}$ 7) (%)
Human waste sludge fertilizer 1	5	12	0.7	6.1	0.7	5.7
Human waste sludge fertilizer 2	5	100	2	1.8	3	2.8
Compound fertilizer 1	5	4	0.1	3.0	0.2	5.0
Compound fertilizer 2	5	101	1	1.1	1	1.4

1) The number of days conducting duplicate analysis
2) Mean ([Number of days of repeatability (T)] × [Number of duplicates (2)])
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Comment 8 The simultaneous measurement of multiple elements is possible with the ICP-MS. In that case, prepare standard solutions, etc. referencing Table 1 of Annex C2, conduct procedures similarly as (**4.2**) **b**) - **c**), and calculate respective element concentrations in an analytical sample.
Note that the concentration of a standard solution and an internal standard solution can be changed as appropriate according to **Comment 4**.

References

1) Toshiharu YAGI: Determination of Heavy Metals in Fluid Sludge Fertilizers by ICP-MS and CV-AAS, Research Report of Fertilizer **Vol. 8**, p. 26 - 37 (2015)

2) Toshiharu YAGI, Kenta SAKUMA and Yoshimi HASHIMOTO: Determination of Heavy Metals in Sludge Fertilizers using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Research Report of Fertilizer, **Vol. 9**, p. 21 – 32 (2016)

3) Satoko SAKAIDA, Mayu OSHIMA, Keisuke AOYAMA and Yuji SHIRAI: Determination of Harmful Components in Fertilizers using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Research Report of Fertilizer, **Vol. 12**, p. 52 – 68 (2019)

(**5**) **Flow sheet for lead**: The flow sheet for lead in fluid sludge fertilizers is shown below:

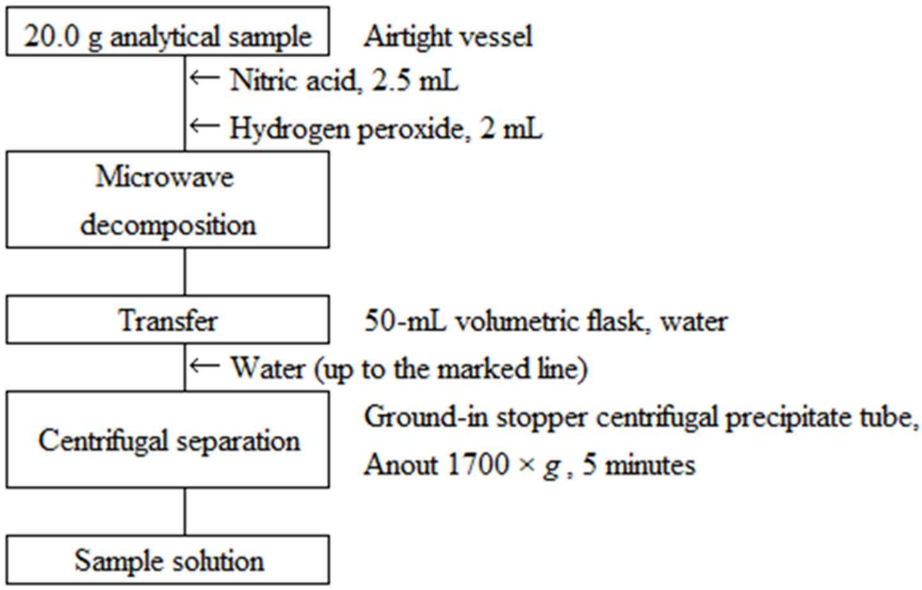


Figure 1 Flow sheet for lead in fluid sludge fertilizers (Extraction procedure)

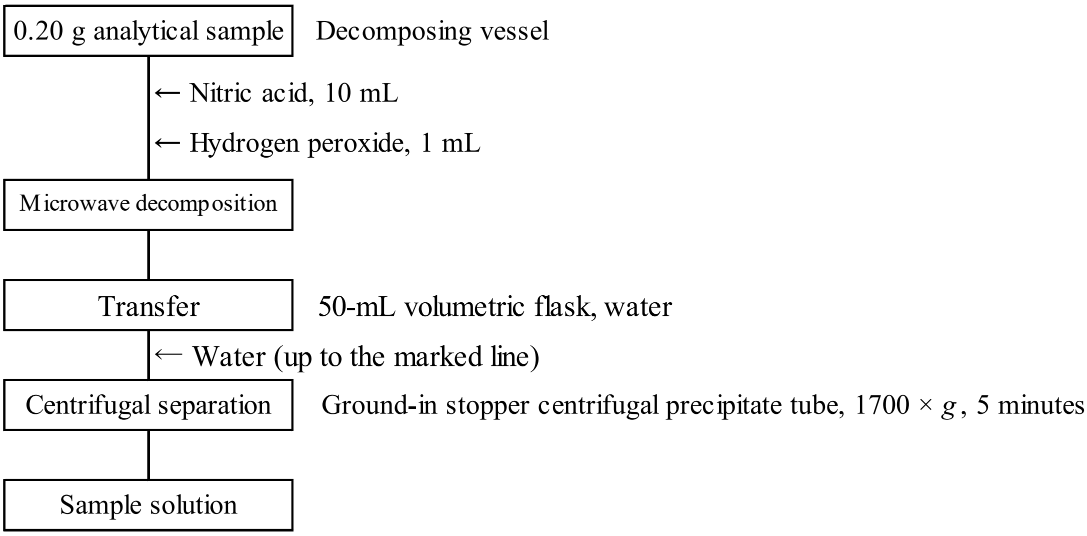


Figure 2 Flow sheet for lead in fertilizers except fluid sludge fertilizers (Extraction procedure)

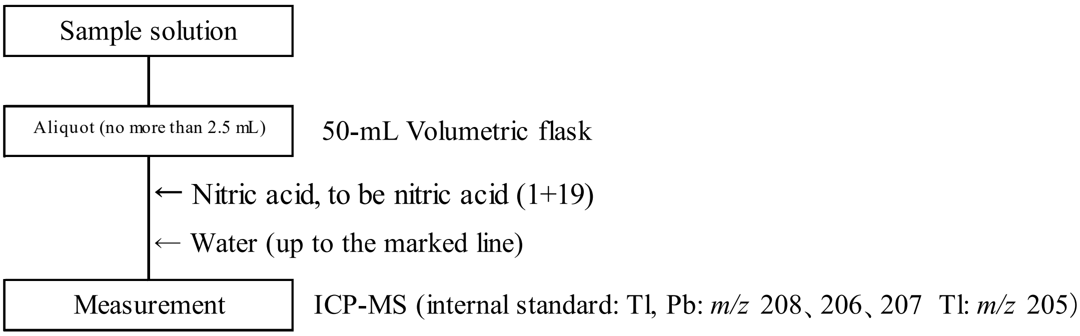


Figure 3 Flow sheet for lead in fertilizers (Measurement procedure)

5.6.d

5.7 Sulfamic acid (amidosulfuric acid)

5.7.a Ion Chromatography

(1) Summary

The testing method is applicable to ammonium sulfate. This testing method is classified as Type D and its symbol is 5.7.a-2017 or AS-acid.a-1.

Add water to an analytical sample to extract sulfamic acid, introduce it to an Ion Chromatograph (IC) or a High-Performance Liquid Chromatograph (HPLC) to isolate it with an ion exchange column, then measure the sulfamic acid with an electric conductivity detector to obtain sulfamic acid (amidosulfuric acid) in an analytical sample. Note that the performance of this testing method is shown in **Comment 5**.

Sulfamic acid and sulfurized cyanide (ammonium thiocyanate) can be simultaneously quantified by using this method. (Refer to **Comment 4**).

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Phthalic acid**: A reagent of no less than 98 % (mass fraction) in purity.

c) ***p*-hydroxybenzoic acid**: A reagent of no less than 95 % (mass fraction) in purity.

d) **1-sodium octane sulfonate**: A reagent of no less than 98 % (mass fraction) in purity.

e) **1-sodium hexane sulfonate**: A reagent of no less than 98 % (mass fraction) in purity.

f) **Boric acid**: A JIS Guaranteed Reagent specified in JIS K 8863 or a reagent of equivalent quality.

g) **Eluent** (1) (2): Weigh 0.083 g of phthalic acid, 0.552 g of *p*-hydroxybenzoic acid, 0.195 g of 1-sodium octane sulfonate, 0.376 g of 1-sodium hexane sulfonate and 6.183 g of boric acid to a 1000-mL volumetric flask, add about 500 mL of water to dissolve and add water up to the marked line. Filter with a membrane filter (aperture diameter: no more than 0.5 μm) made of hydrophilic PTFE.

h) **Sulfamic acid standard solution (1000 mg/L)** (1): Put 0.1 g of sulfamic acid, reference material for volumetric analysis ($HOSO_2NH_2$: dried for 48 hours in a silica gel desiccator), in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.

i) **Sulfamic acid standard solution (10 mg/L)** (1): At the time of usage, put 2.5 mL of sulfamic acid standard solution (1000 mg/L) in a 250-mL volumetric flask and add water up to the marked line.

j) **Sulfamic acid standard solution for the calibration curve preparation (0.3 mg/L - 3 mg/L)**: At the time of usage, put 3 mL - 30 mL of sulfamic acid standard solution (10 mg/L) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) The concentration of prepared solutions is phthalic acid 0.5 mmol/L, *p*-hydroxybenzoic acid 4.0 mmol/L, 1-sodium octane sulfonate 0.9 mmol/L, 1-sodium hexane sulfonate 2.0 mmol/L and boric acid 100 mmol/L.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **Ion Chromatograph or High-Performance Liquid Chromatograph**: An ion chromatograph specified in JIS K 0127 or a high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 4-mm inner diameter 100-mm long stainless-steel column tube filled with hydrophilic methacrylate-gel, to which 5-μm particle diameter class 4 ammonium group chemically bonds (3).

2) **Column bath**: A column bath whose temperature can be adjusted to 55 ºC - 60 ºC.

3) **Detection unit**: An electric conductivity detector

b) **Membrane filters**: Pore size is no more than 0.5 µm, made of hydrophilic PTFE

Note (**3**) A column is commercially sold under the name Shodex IC NI-424, etc.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water and shake to dissolve, and then add water up to the marked line.

c) Put a predetermined amount of the solution, and dilute exactly by a factor of 12.5 with water.

d) Filter with a membrane filter (pore size: no more than 0.5 µm) to make a sample solution.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0127 or JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the Ion Chromatograph or the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the Ion Chromatograph or High-Performance Liquid Chromatograph**: An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) **Column**: A hydrophilic methacrylate-gel column (4-mm inner diameter, 100-mm long, 5-µm particle diameter) to which quaternary ammonium group chemically bonds.

2) **Column bath temperature:** 58 °C

3) **Eluent**: Prepared by the procedures in (**2**) **g**)

4) **Flow rate**: 1 mL/min

5) **Injection volume**: 20 µL

6) **Detection unit**: An electric conductivity detector

b) **Calibration curve preparation**

1) Inject 20 µL of respective standard solutions for the calibration curve preparation to the Ion Chromatograph or the High-Performance Liquid Chromatograph, and record the chromatogram of electric conductivity to obtain a peak area.

2) Prepare a curve for the relationship between the concentration and the peak area of electric conductivity of respective standard solutions for the calibration curve preparation.

Prepare a calibration curve when the sample is measured.

Comment 1 In the measurement of a sample solution, there is a possibility that the recovery rate becomes lower than actual due to the influence of matrix if the concentration is calculated with the peak height. Therefore, prepare a calibration curve using the peak area.

c) **Sample measurement**

1) Subject 20 µL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain the sulfamic acid content from the calibration curve by the peak area to calculate the sulfamic acid (amidosulfuric acid) in the analytical sample.

Comment 2 Calculate the concentration by the peak area similarly as the calibration curve preparation to prevent the influence of matrix.

Comment 3 Note that it takes time to stabilize the baseline due to the usage of the ion-pairing reagent in the elute. It is recommended to take about 120 minutes for stabilization time before starting measurement.

Comment 4 It is possible for the simultaneous measurement of sulfamic acid and ammonium thiocyanate in this testing method. In that case, mix a predetermined amount of sulfamic acid standard solution (1000 mg/L) and ammonium thiocyanate standard solution (1000 mg/L), dilute with water to prepare a mixture standard solution (10 mg/L) (1) and use it instead of a sulfamic acid standard solution (10 mg/L) in (**2**) **i**). After that, conduct the same procedure in (**4.2**) **b**) to calculate the respective concentrations of materials subjected to measurement in the analytical sample.

Comment 5 Recovery testing of ammonium sulfate (3 brands) was conducted. As a result, the mean recovery rates at additive level of 0.25 % (mass fraction) and 0.075 % (mass fraction) were 94.4 % **-** 103.5 % and 94.4 % **-** 100.8 %.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.04 % (mass fraction).

References

1) Toshiaki HIROI and Yuji SHIRAI: Simultaneous Determination of Sulfamic Acid and Ammonium Thiocyanate in Ammonium Sulfate by Non-suppressed Ion Chromatography, Research Report of Fertilizer, **Vol. 5**, p. 1 - 23 (2012)

(**5**) **Flow sheet for testing method**: The flow sheet for sulfamic acid in ammonium sulfate is shown below:

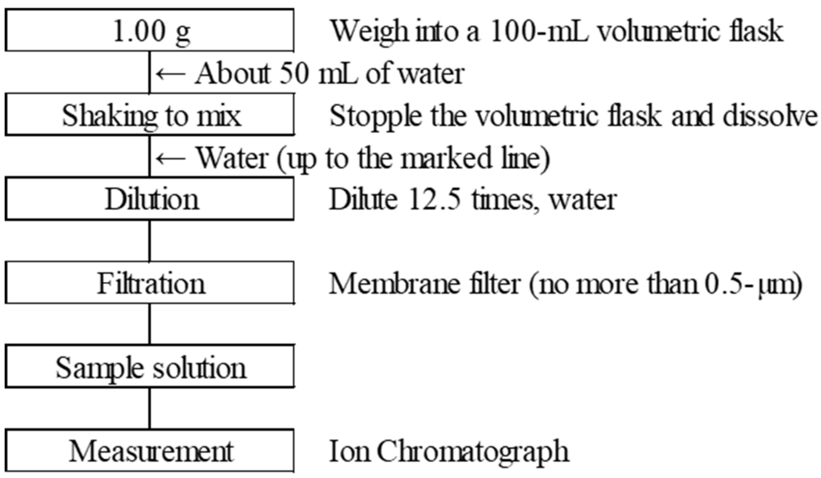
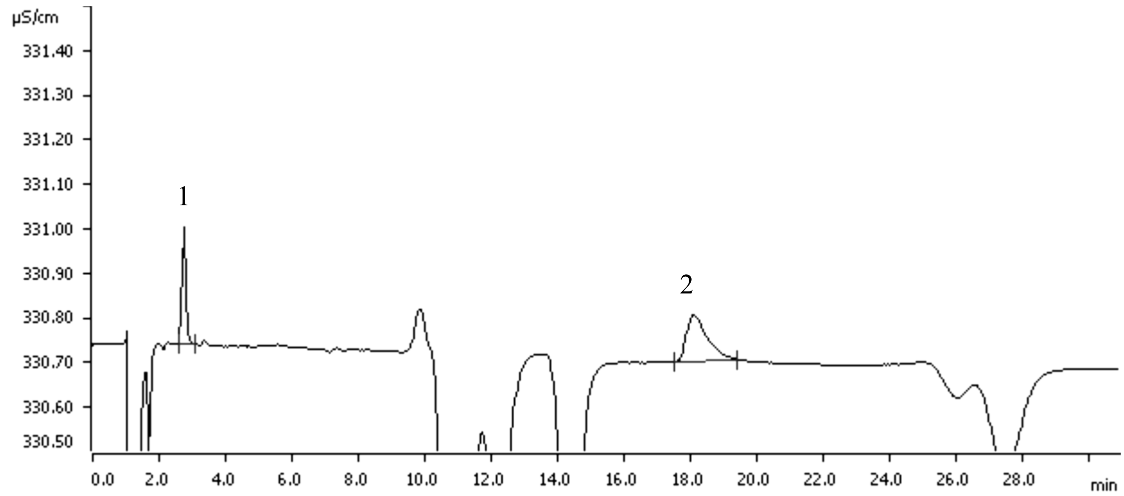
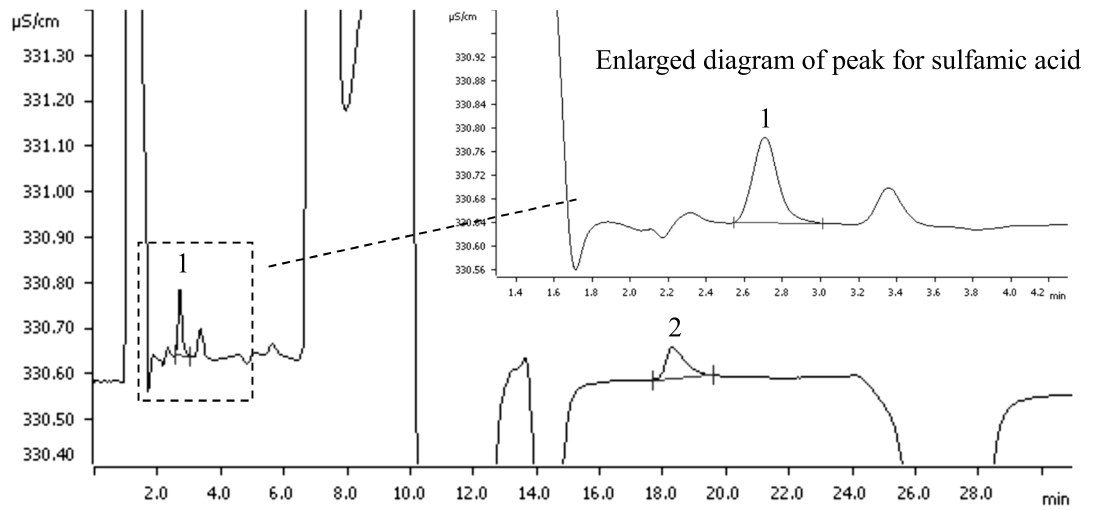


Figure Flow sheet for sulfamic acid in ammonium sulfate

Reference: Examples of the IC chromatograms of sulfamic acid and thiocyanic acid of the standard solution for the calibration curve preparation and sample solution (ammonium sulfate) are shown below.



(A) Mixture standard solution (the equivalents of 60 ng (3 mg/L, 20 µL) as sulfamic acid and ammonium thiocyanate, respectively)



(B) Sample solution (the equivalents of 0.25 % (mass fraction) (2500 µg/g) as sulfamic acid and ammonium thiocyanate added in ammonium sulfate, respectively)

Reference diagram: IC chromatogram of sulfamic acid and thiocyanic acid.
(Peak: 1. Sulfamic acid, 2. Ammonium thiocyanate)

IC measurement conditions

Column: Shodex IC NI-424 (4.6-mm inner diameter, 100-mm long, 5µm-particle diameter)

Other conditions are according to the example of measurement conditions in (**4.2**) **a**)

5.7.b High-Performance Liquid Chromatograph Mass Spectrometry

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 5.7.b-2017 or AS-acid.b-1.

Add water to an analytical sample to extract sulfamic acid, introduce it to a High-Performance Liquid Chromatograph Mass Spectrometer (LC-MS) to isolate it with a silica gel column, to which crosslink-type diol chemically bonds, and measure with a Selected Ion Monitoring (SIM) method to obtain sulfamic acid (amidosulfuric acid) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557. Note that water of A4 or equivalent quality should be used as the eluent which is introduced to LC-MS.
b) **Acetonitrile**: A reagent of LC-MS analysis grade or equivalents.
c) **Formic acid**: A reagent of LC-MS analysis grade or equivalents.
d) **Ammonium formate buffer solution (pH 3.2)**: Dilute 3.153 g of ammonium formate (no less than 95 % (mass fraction) in purity) with water to make 500 mL and adjust to pH 3.2 with formic acid.
e) **Sulfamic acid standard solution (1 mg/L)**: Put 0.1 g of sulfamic acid, reference material for volumetric analysis specified in JIS K 8005, in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.
f) **Sulfamic acid standard solution (10 µg/L)** (1): At the time of usage, put 2.5 mL of standard solution (1 mg/L) in a 250-mL volumetric flask and add water up to the marked line.
g) **Sulfamic acid standard solution (200 ng/L)** (1): At the time of usage, put 5 mL of standard solution (10 µg/L) in a 250-mL volumetric flask and add water up to the marked line.
h) **Sulfamic acid standard solution for the calibration curve preparation (10 ng/L - 600 ng/L)**: At the time of usage, put 2.5 mL - 6 mL of sulfamic acid standard solution (10 µg/L) in 100-mL volumetric flasks step-by-step and add water up to the marked line. Similarly put 5 mL - 50 mL of sulfamic acid standard solution (200 ng/L) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **High-Performance Liquid Chromatograph/ Mass Spectrometer**: A high-performance liquid chromatograph/mass spectrometer specified in JIS K 0136 that satisfies the following requirements.
 1) **High-Performance Liquid Chromatograph**
  (i) Column bath: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
  (ii) Column: A 2-mm - 3-mm inner diameter 100-mm - 150 mm long stainless-steel column tube filled with silica gel to which 5-µm crosslink-type diol chemically bonds or polyhydroxymethacrylate. This should comply with the specification of a Mass Spectrometer.
 2) **Mass Spectrometer**
  (i) Ionization method: Electro-Spray Ionization (ESI) method
  (ii) Ion detection method: Selected Ion Monitoring (SIM) method
b) **Magnetic stirrer**
c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.
d) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000

× *g*.

Comment 1 A High-Performance Liquid Chromatograph/Mass Spectrometer (LC-MS/MS) can be used instead of an LC-MS. In this case, set up the measurement conditions considering (**4.3**) **a**) The measurement conditions of High-Performance Liquid Chromatograph/Mass spectrometer and confirm that a calibration curve can be prepared by the procedure in **b**) in advance.

Comment 2 A column is sold under the production name LUNA HILIC, Shodex ODP2 HP-2D, etc.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Powdery test sample**

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.

d) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (2) and use the supernatant as an extract.

Note (**2**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

Comment 3 Instead of the procedures in (**4.1.1**) **c**) and **d**), it is allowed to filter with Type 3 filter paper and the filtrate can be the extract.

(**4.1.2**) **Fluid test sample**

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, and shake to mix.

c) Add water to the marked line to make an extract

(**4.2**) **Dilution**: Conduct dilution of the extract as shown below.

a) Put 2 mL of the sample solution in a 200-mL Erlenmeyer flask.

b) Add water up to the marked line and put it in a 1.5-mL ground-in stopper centrifugal precipitate tube (3).

c) Centrifuge it at 8000 × *g* **-** 10000 × *g* centrifugal force for about five minutes (4) and use the supernatant as a sample solution.

Note (**3**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(**4**) 7.2-cm **-** 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* **-** 10000 × *g* centrifugal force.

Comment 4 Instead of the procedures in (**4.2**) **b**) and **c**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5 μm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0136 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph Mass Spectrometer used in measurement.

a) The measurement conditions of the High-Performance Liquid Chromatograph/Mass spectrometer: An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) High-Performance Liquid Chromatograph

(i) Column: A silica gel column to which crosslink-type diol chemically bonds or polyhydroxymethacrylate (2-mm - 3-mm inner diameter, 100-mm - 150-mm long, 5-µm particle diameter).

(ii) Flow rate: 0.2 mL/min

(iii) Eluent: Ammonium formate buffer solution - Acetonitrile (1+9)

(iv) Temperature of column bath: 40 °C

(v) Injection rate: 1 µL

(vi) Measurement time: About 20 minutes

2) Mass Spectrometer

(i) Ionization method: Electro-Spray Ionization (ESI) method

(ii) Mode: Negative

(iii) Monitor ion: *m/z* 95.9

b) Calibration curve preparation

1) Inject 1 µL of respective standard solutions for the calibration curve preparation to a High-Performance Liquid Chromatograph/Mass Spectrometer, and record the chromatogram of monitor ion (*m/z*) to obtain a peak area.

2) Prepare a curve for the relationship between the sulfamic acid concentration and the peak area of monitor ion of respective standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 1 µL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain the sulfamic acid content from the calibration curve to calculate the sulfamic acid (amidosulfuric acid) in the analytical sample.

Comment 5 Recovery testing was conducted using samples that sulfamic acid equivalent to 1/5 - 4 times of permissible content are added to ammonium sulfate fertilizer (1 brand), by-product nitrogen fertilizer (1 brand), by-product compound fertilizer (1 brand), compound fertilizer (1 brand) and fluid compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 0.1 % (mass fraction), 0.025 % (mass fraction) and 0.005 % (mass fraction) were 97.6 % - 104.2 %, 95.2 % - 107.0 % and 96.4 % - 111.2 % respectively.

The results of the repeatability tests on different days using ammonium sulfate fertilizer, by-product nitrogen fertilizer and compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Table 2 shows results and analysis results from a collaborative study for testing method validation. Note that sufficient reproducibility by sulfamic acid concentration 0.0116 % (mass fraction) was not obtainable. But sufficient reproducibility was obtained in the scope of sulfamic acid concentration 0.0386 % (mass fraction) - 0.401 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of sulfamic acid

Name of sample	Test days of repeatability (T)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)
Ammonium sulfate	5	0.0974	0.0011	1.1	0.0027	2.7
By-product nitrogen fertilizer	5	0.0656	0.0014	2.1	0.0017	2.6
Compound fertilizer	5	0.00510	0.00012	2.4	0.00029	5.8

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for sulfamic acid testing method validation

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Ammonium sulfate	9	0.203	0.021	10.4	0.024	11.9
By-product nitrogen fertilizer	9	0.401	0.030	7.5	0.035	8.8
Compound fertilizer	7	0.0957	0.0043	4.5	0.0043	4.5
By-product compound	9	0.0166	0.0028	16.8	0.0048	29.1
Fluid compound fertilizer 1	9	0.0381	0.0022	5.8	0.0049	12.8
Fluid compound fertilizer 2	9	0.243	0.011	4.5	0.018	7.6

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Kohei ITO, Mariko FUJITA, Yoshimi HASHIMOTO and Yuji SHIRAI: Determination of Sulfamic Acid in Fertilizer by Liquid Chromatography/Mass Spectrometry (LC-MS), Research Report of Fertilizer **Vol. 8**, p. 38 - 49 (2015)

2) Tomoharu NOZAKI Determination of Sulfamic Acid in Fertilizers using Liquid Chromatography-Mass Spectrometer (LC-MS): A Collaborative Study, Research Report of Fertilizer **Vol. 9**, p. 69 - 76 (2016)

3) Kenji KOZUKA, Kohei ITO, Nobuhito NAKAMURA and Yuji SHIRAI: Determination of Sulfamic Acid in Fertilizer by Liquid Chromatography/Mass Spectrometry (LC-MS): A Collaborative Study, Research Report of Fertilizer **Vol. 11**, p. 47 – 53 (2018)

(**5**) **Flow sheet for testing method**: The flow sheet for sulfamic acid in fertilizers is shown below:

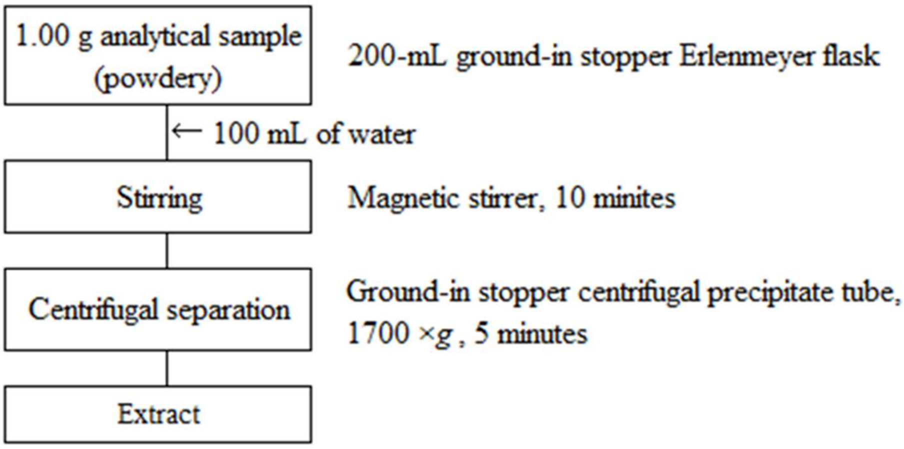


Figure 1-1 Flow sheet for sulfamic acid in fertilizers (Extraxction procedure (4.1.1))

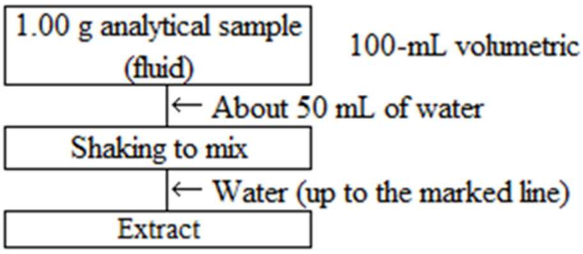


Figure 1-2 Flow sheet for sulfamic acid in fertilizers (Extraxction procedure (4.1.2))

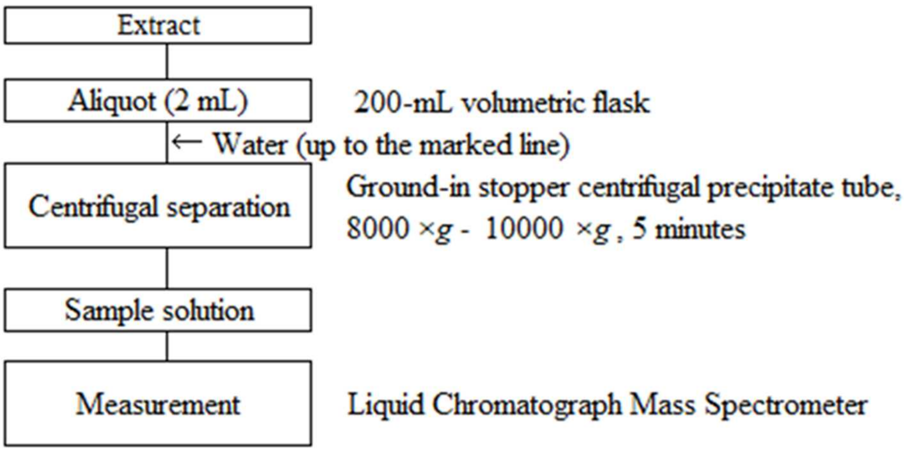
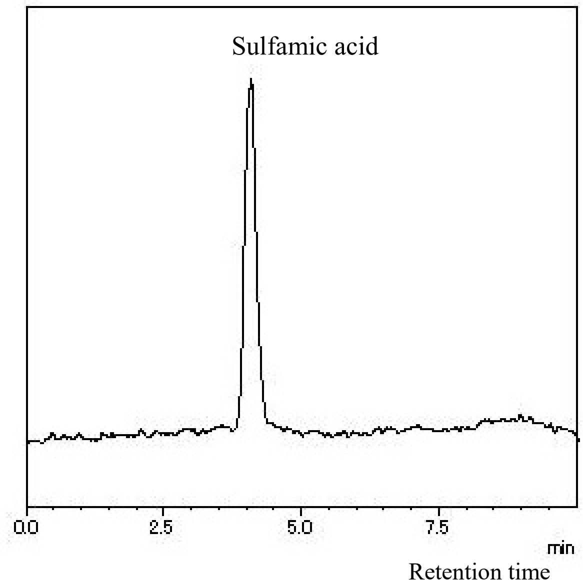


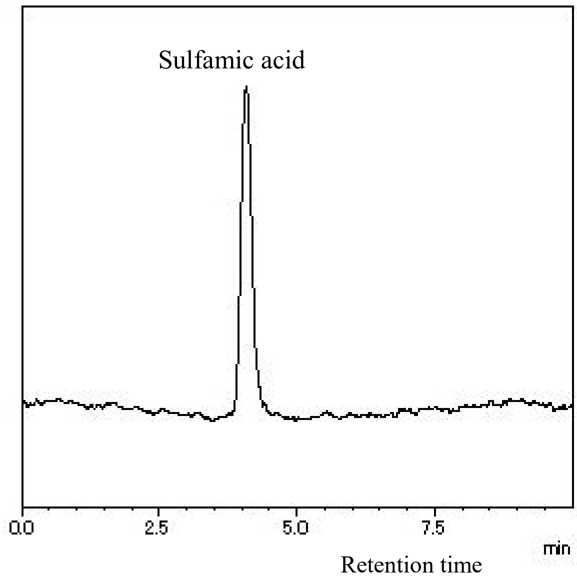
Figure 2 Flow sheet for sulfamic acid in fertilizers (Dilution and measurement procedure)

Reference: An example of the chromatogram of sulfamic acid for the calibration curve preparation is shown below.



(A) Standard solution

(The equivalent of 0.6 ng as sulfamic acid)



(B) Sample solution

(The equivalent of 0.1 % of mass fraction as sulfamic acid is added to a compound fertilizer)

Reference diagram: Chromatogram of sulfamic acid

LC-MS measurement conditions

Column: LUNA HILIC (2.0-mm inner diameter, 100-mm long, 5-µm particle diameter)

Capillary voltage: - 3.5 kv

Temperature of ion source: 300 °C

Nebulizer gas rate: 1.5 L/min

Desolvation temperature: 250 °C

Other conditions are according to the example of LC-MS measurement conditions in (**4.3**) **a**)

5.8 Ammonium thiocyanate (Sulfurized cyanide)
5.8.a Ion Chromatography
(1) Summary

The testing method is applicable to ammonium sulfate. This testing method is classified as Type D and its symbol is 5.8.a-2017 or SCN.a-1.

Add water to an analytical sample to extract ammonium thiocyanate (sulfurized cyanide), introduce it to an Ion Chromatograph (IC) or a High-Performance Liquid Chromatograph (HPLC) to isolate it with an ion exchange column, then measure the thiocyanic acid with an electric conductivity detector to obtain ammonium thiocyanate (sulfurized cyanide) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

Sulfamic acid and ammonium thiocyanate (sulfurized cyanide) can be simultaneously quantified by using this method. (Refer to **Comment 4**).

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Phthalic acid: A reagent of no less than 98 % (mass fraction) in purity.

c) *p*-hydroxybenzoic acid: A reagent of no less than 95 % (mass fraction) in purity.

d) 1-sodium octane sulfonate: A reagent of no less than 98 % (mass fraction) in purity.

e) 1-sodium hexane sulfonate: A reagent of no less than 98 % (mass fraction) in purity.

f) Boric acid: A JIS Guaranteed Reagent specified in JIS K 8863 or a reagent of equivalent quality.

g) Eluent (1) (2): Weigh 0.083 g of phthalic acid, 0.552 g of *p*-hydroxybenzoic acid, 0.195 g of 1-sodium octane sulfonate, 0.376 g of 1-sodium hexane sulfonate and 6.183 g of boric acid to a 1000-mL volumetric flask, add about 500 mL of water to dissolve and add water up to the marked line. Filter with a membrane filter (aperture diameter: no more than 0.5 µm) made of hydrophilic PTFE.

h) Ammonium thiocyanate standard solution (1000 mg/L) (1): Put 0.1 g of ammonium thiocyanate (3), specified in JIS K 9000 in weighing dish, and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.

i) Ammonium thiocyanate standard solution (10 mg/L) (1): At the time of usage, put 10 mL of ammonium thiocyanate standard solution (1000 mg/L) in a 100-mL volumetric flask and add water up to the marked line.

j) Ammonium thiocyanate standard solution for the calibration curve preparation (0.3 mg/L - 3 mg/L): At the time of usage, put 3 mL - 30 mL of ammonium thiocyanate solutions (10 mg/L) in 100-mLvolumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) The concentration of prepared solutions is phthalic acid 0.5 mmol/L, *p*-hydroxybenzoic acid 4.0 mmol/L, 1-sodium octane sulfonate 0.9 mmol/L, 1-sodium hexane sulfonate 2.0 mmol/L and boric acid 100 mmol/L.

(3) It is recommended to store in a desiccator because of deliquescence.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Ion Chromatograph or High-Performance Liquid Chromatograph: An ion chromatograph specified in JIS K 0127 or a high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 4-mm inner diameter 100-mm long stainless steel column tube filled with hydrophilic methacrylate-gel, to which 5-µm particle diameter class 4 ammonium group chemically bonds (4).

2) **Column bath**: A column bath whose temperature can be adjusted to 55 ºC - 60 ºC.
3) **Detection unit**: An electric conductivity detector

b) **Membrane filters**: Pore size is no more than 0.5 μm, made of hydrophilic PTFE

Note (**4**) A column is commercially sold under the name Shodex IC NI-424, etc.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water and shake to dissolve, and then add water up to the marked line**.**

c) Transfer a predetermined amount of the solution, and dilute exactly by a factor of 12.5 with water.

d) Filter with a membrane filter (pore size: no more than 0.5 μm) to make a sample solution.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0127 and as shown below. Specific measurement procedures are according to the operation method of the Ion Chromatograph or the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the Ion Chromatograph or the High-Performance Liquid Chromatograph**: An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) **Column**: A hydrophilic methacrylate-gel column (4-mm inner diameter, 100-mm long, 5-μm particle diameter) to which quaternary ammonium group chemically bonds.
2) **Column bath temperature**: 58 °C
3) **Eluen**t: Prepared by the procedures in (**2**) **g**)
4) **Flow rate**: 1 mL/min
5) **Injection volume**: 20 μL
6) **Detection unit**: An electric conductivity detector

b) **Calibration curve preparation**

1) Inject 20 μL of respective standard solutions for the calibration curve preparation to the Ion Chromatograph or the High-Performance Liquid Chromatograph, and record the chromatogram of electric conductivity to obtain a peak area.
2) Prepare a curve for the relationship between the concentration and the peak area of electric conductivity of respective standard solutions for the calibration curve preparation.
   Prepare a calibration curve when the sample is measured.

Comment 1 In the measurement of a sample solution, there is a possibility that the recovery rate becomes lower than actual due to the influence of matrix if the concentration is calculated with a peak height. Therefore, prepare a calibration curve using a peak area.

c) **Sample measurement**

1) Subject 20 μL of the sample solution to the same procedure as in **b**) **1**)
2) Obtain the ammonium thiocyanate content from the calibration curve by the peak area to calculate ammonium thiocyanate (sulfurized cyanide) in the analytical sample.

Comment 2 Calculate the concentration by the peak area similarly as the calibration curve preparation to prevent the influence of matrix.

Comment 3 Note that it takes time to stabilize the baseline due to the usage of the ion-pairing reagent in the elute. It is recommended to take about 120 minutes for stabilization time before starting measurement.

Comment 4 It is possible for the simultaneous measurement of sulfamic acid and ammonium thiocyanate (sulfurized cyanide) in this testing method. In that case, mix a predetermined amount of sulfamic acid standard solution (1000 mg/L) and ammonium thiocyanate standard solution (1000 mg/L), dilute with water to prepare a mixture standard solution (10 mg/L) [(1)] and use it instead of an ammonium thiocyanate standard solution (10 mg/L) in (**2**) **i**). After that, conduct the same procedure in (**4.2**) **b**) to calculate the concentration of ammonium thiocyanate in the analytical sample.

Comment 5 Recovery testing of ammonium sulfate (3 brands) was conducted. As a result, the mean recovery rates at the additive level of 0.25 % (mass fraction) and 0.075 % (mass fraction) were 101.8 % **-** 103.7 % and 93.9 % **-** 97.4 %.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.04 % (mass fraction).

References

1) Toshiaki HIROI and Yuji SHIRAI: Simultaneous Determination of Sulfamic Acid and Ammonium Thiocyanate in Ammonium Sulfate by Non-suppressed Ion Chromatography, Research Report of Fertilizer, **Vol. 5**, p. 1 - 23 (2012)

(**5**) **Flow sheet for testing method**: The flow sheet for ammonium thiocyanate in ammonium sulfate is shown below:

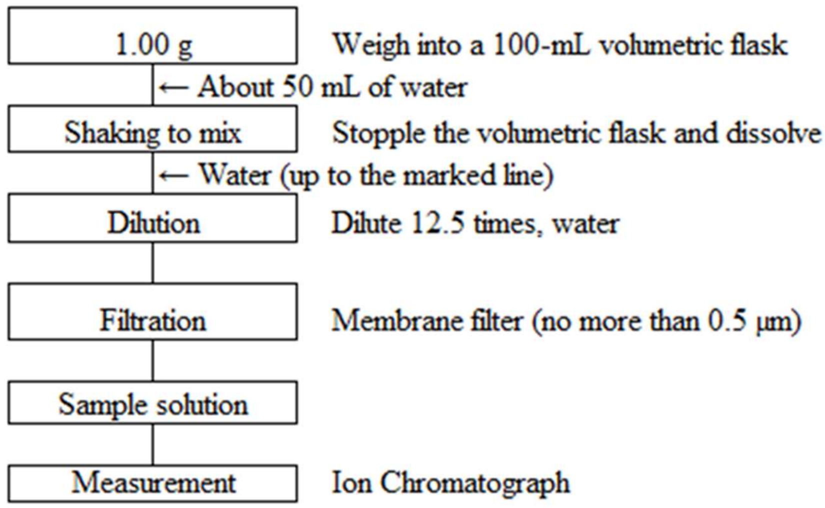
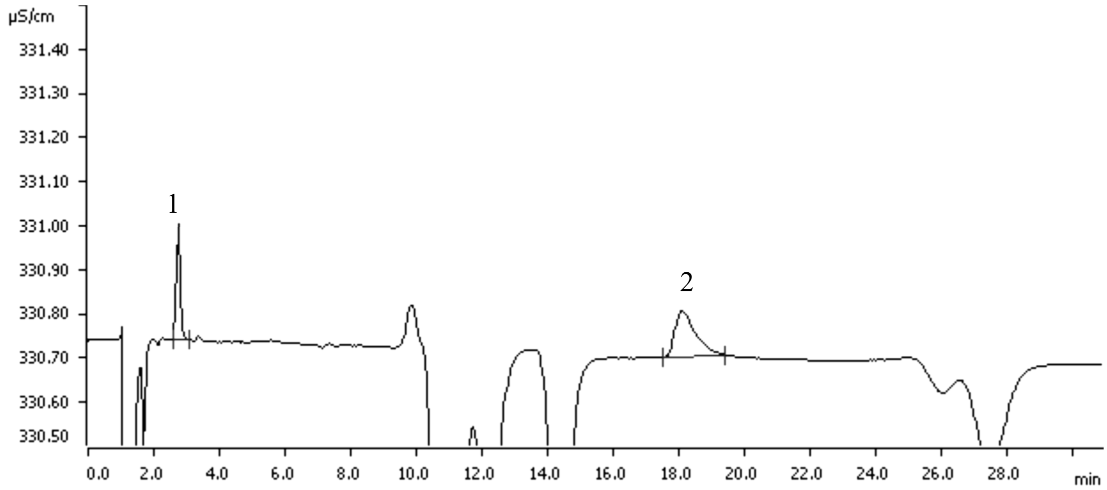
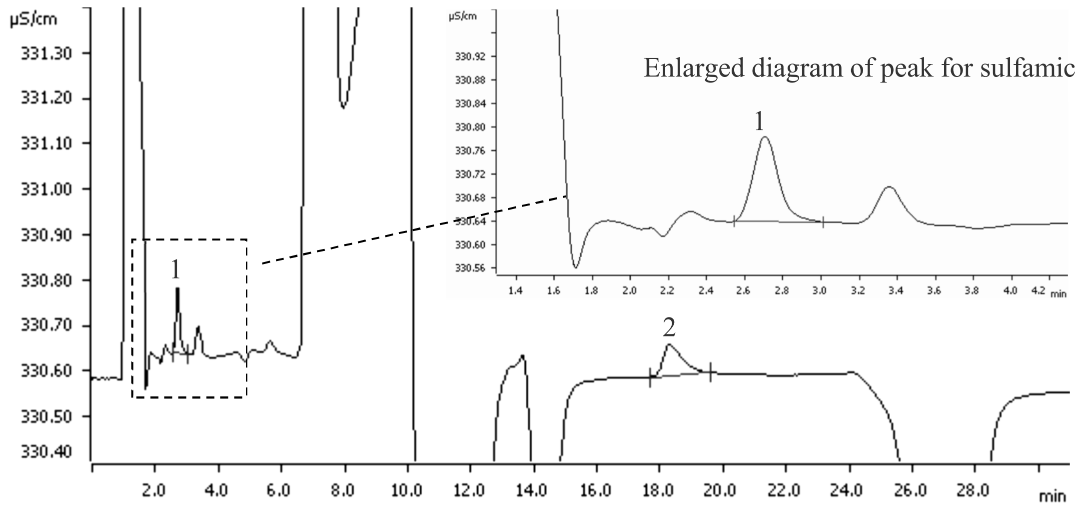


Figure Flow sheet for ammonium thiocyanate in ammonium sulfate

Reference: Examples of the IC chromatogram of sulfamic acid and thiocyanic acid of the standard solution for the calibration curve preparation and sample solution (ammonium sulfate) are shown below.



(A) Mixture standard solution (the equivalents of 60 ng (3 mg/L, 20 µL) as sulfamic acid and ammonium thiocyanate, respectively)



(B) Sample solution (the equivalents of 0.25 % (mass fraction) (2500 µg/g) as sulfamic acid and ammonium thiocyanate added in ammonium sulfate, respectively)

Reference diagram: IC chromatogram of sulfamic acid and thiocyanic acid.
(Peak: 1. Sulfamic acid, 2. Thiocyanic acid)

IC measurement conditions

Column: Shodex IC NI-424 (4.6-mm inner diameter, 100-mm long, 5-µm particle diameter)

Other conditions are according to the example of HPLC measurement conditions in **(4.2) a)**

5.8.b High-Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 5.8.b-2017 or SCN.b-1.

Add water to an analytical sample, extract ammonium thiocyanate (sulfurized cyanide) and adjust pH as necessary. Introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with a silica gel column to which amino group chemically bonds or a vinyl alcohol polymer column to which amino group chemically bonds, and measure at a wavelength of 210 nm to obtain ammonium thiocyanate (sulfurized cyanide) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

Nitrous acid and ammonium thiocyanate (sulfurized cyanide) can be simultaneously quantified by using this method. (Refer to **Comment 4**).

(2) Reagent, etc.: Reagents and water are as shown below.

- **a) Water**: Water of A3 specified in JIS K 0557.
- **b) Sodium hydroxide**: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.
- **c) Disodium hydrogen phosphate dodecahydrate**: A JIS Guaranteed Reagent specified in JIS K 9019 or a reagent of equivalent quality.
- **d) Sodium dihydrogen phosphate dihydrate**: A JIS Guaranteed Reagent specified in JIS K 9009 or a reagent of equivalent quality.
- **e) Sodium perchlorate monohydrate**: A JIS Guaranteed Reagent specified in JIS K 8227 or a reagent of equivalent quality.
- **f) Ammonium thiocyanate standard solution (1 mg/L)** (1): Put 0.1 g of ammonium thiocyanate specified in JIS K 9000 in weighing dish, and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.
- **g) Ammonium thiocyanate standard solution (10 mg/L)** (1): At the time of usage, put 10 mL of ammonium thiocyanate standard solution (1 mg/L) in a 100-mL volumetric flask and add water up to the marked line.
- **h) Ammonium thiocyanate standard solution for the calibration curve preparation (1 µg/mL - 20 µg/mL)**: At the time of usage, put 1 mL - 20 mL of ammonium thiocyanate standard solution (100 µg/mL) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

- **a) High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.
  - **1) Column**: A 4-mm to 6-mm inner diameter 150-mm to 250-mm long stainless steel column tube filled with poly vinyl alcohol or silica gel (2) , to which 5-µm particle diameter amino group chemically bonds.
  - **2) Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
  - **3) Detection unit**: An absorptiometric detector that can measure at a wavelength around 210 nm.
- **b) Magnetic stirrer**
- **c) Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.
- **d) High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

e) **pH test paper**: A pH test paper infilled with indicator and dried, which can measure the value from pH 1 to pH 11 and a color change chart with the pH interval value 1 is attached.

Note (**2**) Remaining silanol group of silica gel affects the measurement of ion in some cases. Therefore, use a column which does not affect the measurement of sodium thiocyanate by treating the silanol group. As an example of the treatment, silica gel is to be entirely covered with the uniform membrane of silicone polymer.

Comment 1 A column is sold under the names Asahipak NH2P-50 4E, CAPCELL PAK NH2 UG80, etc.
Comment 2 pH test paper is sold under the name UNIV Test Paper, etc.

(**4**) **Test procedure**
(**4.1**) **Extraction**: Conduct extraction as shown below.
(**4.1.1**) **Powdery test sample**
a) Weigh 1.00 g of an analytical sample, and put it in a 200-mL volumetric flask.
b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.
c) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.
d) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (3) and use the supernatant as an extract.

Note (**3**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**4.1.2**) **Fluid test sample**
a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix.
c) Add water to the marked line to make an extract

(**4.2**) **pH adjustment**: Conduct pH adjustment of the extract as shown below.
a) Get a small amount of the extract to confirm pH value using a pH-test paper.
b) If the pH value in **a**) is pH 5 or more, put the extract in a 1.5-mL ground-in stopper centrifugal precipitate tube (4) and conduct the procedure in **f**) to prepare a sample solution.
c) If the pH value in a) is pH 4 or less, put 40 mL of the extract in a 100-mL beaker.
d) Add a sodium hydroxide solution (5 mg/mL), adjust it to pH 5 to pH 7 with a pH meter and transfer to a 50-mL volumetric flask with water.
e) Add water up to the marked line and put it in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).
f) Centrifuge it at 8000 × *g* **-** 10000 × *g* centrifugal force for about five minutes (5) and use the supernatant as an extract.

Note (**4**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.
(**5**) 7.2-cm **-** 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* **-** 10000 × *g* centrifugal force.

Comment 3 Instead of procedures in (**4.2**) **b**) and **e**) **- f**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.3) Measurement: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) Measurement conditions for the High-Performance Liquid Chromatograph: An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) Column: A vinyl alcohol polymer column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino group chemically bonds or a silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino group chemically bonds.

2) Column bath temperature: 30 °C - 40 °C

3) Eluent (1): Dissolve 1.79 g of disodium hydrogen phosphate dodecahydrate, 0.78 g of sodium dihydrogen phosphate dihydrate and 14.04 g of sodium perchlorate monohydrate in water to make 1000 mL. Filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.

4) Flow rate: 0.9 mL/min - 1.0 mL/min

5) Injection volume: 10 µL

6) Detection unit: An absorptiometric detector, a measurement wavelength: 210 nm

b) Calibration curve preparation

1) Inject 10 µL of respective standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 210 nm and obtain a peak area.

2) Prepare a curve for the relationship between the concentration and the peak area at a wavelength 210 nm of the respective standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**

2) Obtain the ammonium thiocyanate content from the calibration curve by the peak area to calculate ammonium thiocyanate (sulfurized cyanide) in the analytical sample.

Comment 4 This testing method enables the simultaneous measurement of nitrous acid and ammonium thiocyanate (sulfurized cyanide). In this case, mix a predetermined amount of nitrous acid standard solution (1 mg/mL) and ammonium thiocyanate standard solution (1 mg/mL), dilute with water to prepare a mixture standard solution (100 µg/mL) (1) and use it instead of **(2) g)** ammonium thiocyanate standard solution (100 µg/mL). After that, conduct the same procedure in **(4.3) b)** to calculate the respective concentrations of materials subjected to measurement in the analytical sample.

Comment 5 Recovery testing was conducted using samples that ammonium thiocyanate equivalent to 1/5 - 5 times of permissible content are added to an ammonium sulfate fertilizer (1 brand), a coating nitrogen fertilizer (1 brand), a blended fertilizer (2 brands), a compound fertilizer (1 brand) and a fluid compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 0.025 % (mass fraction), 0.01 % (mass fraction), 0.005 % (mass fraction) and 0.0025 % (mass fraction) were 95.4 % - 100.5 %, 94.7 % - 103.8 %, 83.8 % - 109.0 % and 87.2 % - 103.3 % respectively.

Additionally, results from a collaborative study for test method validation and its analysis are in Table 1. Ammonium thiocyanate had sufficient reproducibility in the range from 0.00476 % (mass fraction) to 0.204 %. (mass fraction).

Note that the minimum limit of quantification of this testing method was estimated to be about 0.002 % (mass fraction).

Table 1 Statistical analysis results of the collaborative study for the ammonium thiocyanate method validation

Sample name	Number of laboratories[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	s_R[6] (%)[3]	RSD_R[7] (%)
Home garden-use compound fertilizer 1	10	0.00476	0.00019	4.1	0.00060	12.7
Home garden-use compound fertilizer 2	9	0.00976	0.00029	2.9	0.00050	4.7
Home garden-use compound fertilizer 3	9	0.0506	0.0019	3.7	0.0022	4.3
Compound fertilizer 1	10	0.101	0.002	2.3	0.003	2.6
Compound fertilizer 2	11	0.204	0.006	2.7	0.008	3.7
Compound fertilizer 3	9	0.00989	0.00037	3.8	0.00060	6.5

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Kohei ITO, Yasuharu KIMURA, Masanori HASEGAWA and Yuji SHIRAI: Simultaneous Determination of Nitrous Acid and Thiocyanate in Fertilizer by High-Performance Liquid Chromatography (HPLC), Japanese Journal of Soil Science and Plant Nutrition, **Vol. 87** (2), p.120 - 124 (2016)

2) Masanori HASEGAWA and Yasuharu KIMURA: Determination of Nitrous Acid and Ammonium Thiocyanate in Fertilizer by High-Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer **Vol. 8**, p. 70 - 78 (2015)

(5) Flow sheet for testing method: The flow sheet for ammonium thiocyanate in fertilizers is shown below:

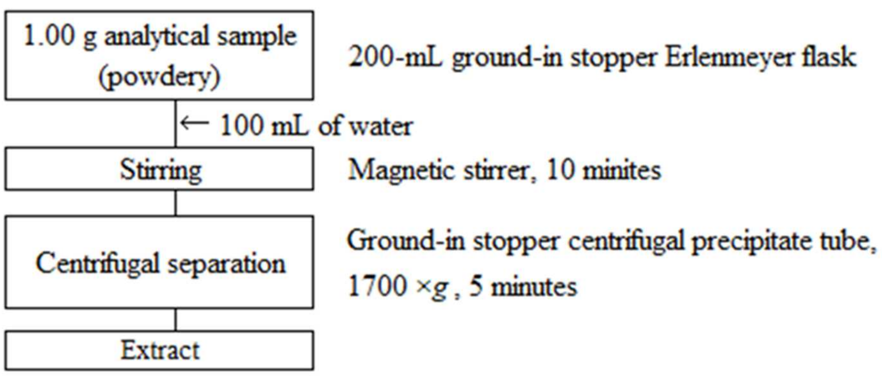


Figure 1-1 Flow sheet for ammonium thiocyanate in fertilizers (Extraction procedure (4.1.1))

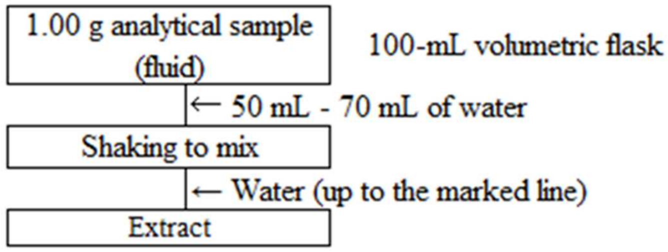


Figure 1-2 Flow sheet for ammonium thiocyanate in fertilizers (Extraction procedure (4.1.2))

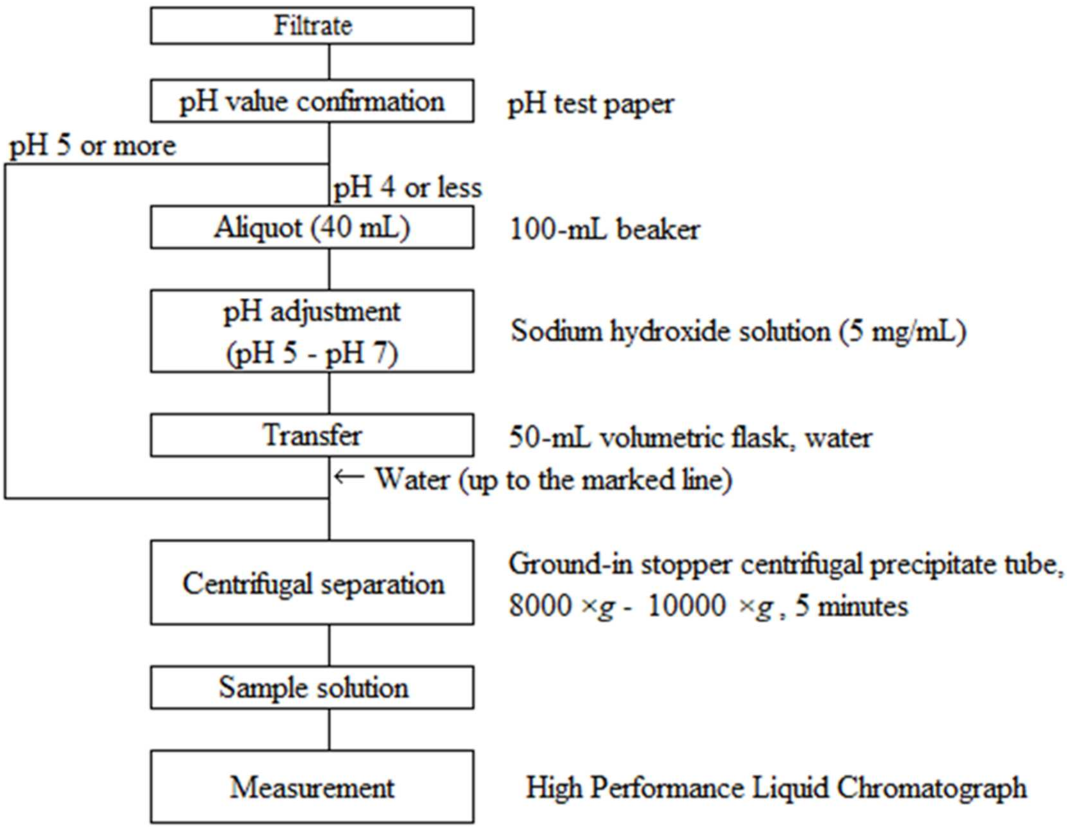
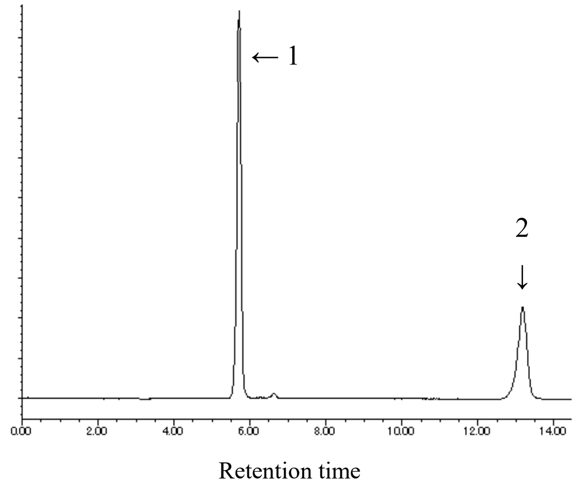
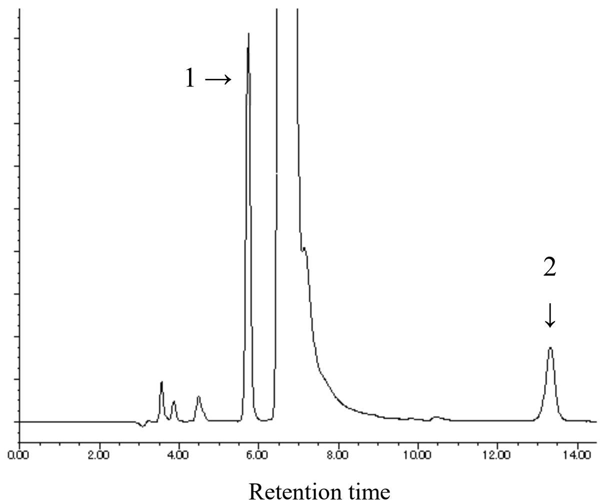


Figure 2 Flow sheet for ammonium thiocyanate in fertilizers (pH adjustment and measurement procedure)

Reference: Examples of the HPLC chromatogram of nitrous acid and ammonium thiocyanate are shown below.



(A) Mixture standard solution
(The equivalents of 100 ng (10 µg/mL, 10 µL) as nitrous acid and ammonium thiocyanate, respectively)



(B) Sample solution
(The equivalents of 0.1 % (mass fraction) as nitrous acid and ammonium thiocyanate added in blended fertilizer respectively)

Reference diagram: HPLC chromatogram of nitrous acid and ammonium thiocyanate
(Peak: 1. Nitrous acid, 2. Thiocyanate)

Measurement conditions for HPLC
Column: CAPCELL PAK NH2 UG80 (4.6-mm inner diameter, 250-mm long, 5-µm particle diameter)
Other conditions are according to the example of HPLC measurement conditions in **(4.3) a)**

5.9 Nitrous acid

5.9.a High-Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 5.9.a-2017 or NO2.a-1.

Add water to an analytical sample, extract nitrous acid and adjust pH as necessary. Introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with a silica gel column to which amino group chemically bonds or a vinyl alcohol polymer column to which amino group chemically bonds, and measure at a wavelength 210 nm to obtain nitrous acid in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

Nitrous acid and ammonium thiocyanate (sulfurized cyanide) can be simultaneously quantified by using this method. (Refer to **Comment 4**).

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Sodium hydroxide**: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

c) **Disodium hydrogen phosphate dodecahydrate**: A JIS Guaranteed Reagent specified in JIS K 9019 or a reagent of equivalent quality.

d) **Sodium dihydrogen phosphate dihydrate**: A JIS Guaranteed Reagent specified in JIS K 9009 or a reagent of equivalent quality.

e) **Sodium perchlorate monohydrate**: A JIS Guaranteed Reagent specified in JIS K 8227 or a reagent of equivalent quality.

f) **Nitrous Acid standard solution (1 mg/mL)** (1): Put 0.147 g of sodium nitrite specified in JIS K 8019 in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.

g) **Nitrous acid standard solution (100 µg/mL)** (1): At the time of usage, put 10 mL of nitrous acid standard solution (1 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

h) **Nitrous acid standard solution for the calibration curve preparation (1 µg/mL - 20 µg/mL)**: At the time of usage, put 1 mL - 20 mL of nitrous acid standard solution (100 µg/mL) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **High-Performance Liquid Chromatograph**: a high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 4-mm **-** 6-mm inner diameter 150-mm **-** 250-mm long stainless steel column tube filled with poly vinyl alcohol or silica gel (2), to which 5-µm particle diameter amino group chemically bonds.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC **-** 45 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 210 nm.

b) **Magnetic stirrer**

c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

d) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* **-** 10000 × *g*.

e) **pH test paper**: A pH test paper infilled with indicator and dried, which can measure the value from pH 1 - pH 11 and a color change chart with the pH interval value 1 is attached.

Note (2) Remaining silanol group of silica gel affects the measurement of ion in some cases. Therefore, use a column which does not affect the measurement of nitrous acid by treating the silanol group. As an example of the treatment, silica gel is to be entirely covered with the uniform membrane of silicone polymer.

Comment 1 A column is sold under the names Asahipak NH2P-50 4E, CAPCELL PAK NH2 UG80, etc.

Comment 2 pH test paper is sold under the name UNIV Test Paper, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.

d) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (3) and use the supernatant as an extract.

Note (3) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(4.1.2) Fluid test sample

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, and shake to mix.

c) Add water to the marked line to make an extract

(4.2) pH adjustment: Conduct pH adjustment of the extract as shown below.

a) Get a small amount of the extract to confirm pH value using a pH-test paper.

b) If the pH value in **a**) is pH 5 or more, put the extract in a 1.5-mL ground-in stopper centrifugal precipitate tube (4) and conduct the procedure in **f**) to prepare a sample solution.

c) If the pH value in **a**) is pH 4 or less, put 40 mL of the extract in a 100-mL beaker.

d) Add a sodium hydroxide solution (5 mg/mL), adjust it to pH 5 to pH 7 with a pH meter and transfer to a 50-mL volumetric flask with water.

e) Add water up to the marked line and put it in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

f) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (5) and use the supernatant as a sample solution.

Note (4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(5) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 3 Instead of procedures in (**4.2**) **b**) and **e**) - **f**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) **Column**: A vinyl alcohol polymer column (4-mm **-** 6-mm inner diameter, 150-mm **-** 250-mm long, 5-µm particle diameter) to which amino group chemically bonds or a silica gel column (4-mm **-** 6-mm inner diameter, 150-mm **-** 250-mm long, 5-µm particle diameter) to which amino group chemically bonds.

2) **Column bath temperature**: 30 °C **-** 40 °C

3) **Eluent** (1): Dissolve 1.79 g of disodium hydrogen phosphate dodecahydrate, 0.78 g of sodium dihydrogen phosphate dihydrate and 14.04 g of sodium perchlorate monohydrate in water to make 1000 mL. Filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.

4) **Flow rate**: 0.9 mL/min **-** 1.0 mL/min

5) **Injection volume**: 10 µL

6) **Detection unit**: An absorptiometric detector, a measurement wavelength: 210 nm

b) **Calibration curve preparation**

1) Inject 10 µL of respective standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 210 nm and obtain a peak area.

2) Prepare a curve for the relationship between the concentration and the peak area at a wavelength 210 nm of the respective standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 10 µL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain the nitrous acid content by the peak area using the calibration curve to calculate the concentration of nitrous acid in the analytical sample.

Comment 4 This testing method enables the simultaneous measurement of nitrous acid and ammonium thiocyanate (sulfurized cyanide). In this case, mix a predetermined amount of nitrous acid standard solution (1 mg/mL) and ammonium thiocyanate standard solution (1 mg/mL), dilute with water to prepare a mixture standard solution (100 µg/mL) (1) and use it instead of (**2**) **g**) nitrous acid standard solution (100 µg/mL). After that, conduct the same procedure in (**4.3**) **b**) to calculate the respective concentrations of materials subjected to measurement in the analytical sample.

Comment 5 Recovery testing was conducted using samples that nitrous acid equivalent to 1/5 - 5 times of permissible content are added to ammonium sulfate fertilizer (1 brand), coating nitrogen fertilizer (1 brand), blended fertilizer (2brands), compound fertilizer (1 brand) and fluid compound fertilizer (1 brand). As a result, the mean recovery rates at the additive level of 0.1 % (mass fraction), 0.04 % (mass fraction), 0.02 % (mass fraction) and 0.01 % (mass fraction) were 99.0 % **-** 100.8 %, 100.4 % **-** 102.0 %, 103.1 % **-** 106.6 % and 101.2 % **-** 105.9 % respectively.

Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 1. Nitrous acid had sufficient reproducibility in the range from 0.0255 % (mass fraction) to 0.291 %. (mass fraction).

Note that the minimum limit of quantification of this testing method was estimated to be about 0.0003 % (mass fraction).

Table 1 Statistical analysis results of the collaborative study for the nitrous acid method validation

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Home garden-use compound fertilizer 1	10	0.0502	0.0005	1.1	0.0009	1.7
Home garden-use compound fertilizer 2	11	0.0255	0.0007	2.6	0.0009	3.5
Home garden-use compound fertilizer 3	9	0.150	0.004	2.9	0.005	3.6
Compound fertilizer 1	10	0.202	0.004	1.9	0.004	2.2
Compound fertilizer 2	10	0.291	0.004	1.3	0.005	1.7
Compound fertilizer 3	10	0.0498	0.0007	1.4	0.0010	2.0

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Kohei ITO, Yasuharu KIMURA, Masanori HASEGAWA and Yuji SHIRAI: Simultaneous Determination of Nitrous Acid and Thiocyanate in Fertilizer by High-Performance Liquid Chromatography (HPLC), Japanese Journal of Soil Science and Plant Nutrition, **Vol. 87** (2), p.120 **-** 124 (2016)

2) Masanori HASEGAWA and Yasuharu KIMURA: Determination of Nitrous Acid and Ammonium Thiocyanate in Fertilizer by High-Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer **Vol. 8**, p. 70 **-** 78 (2015)

(**5**) **Flow sheet for testing method**: The flow sheet for nitrous acid in fertilizers is shown below:

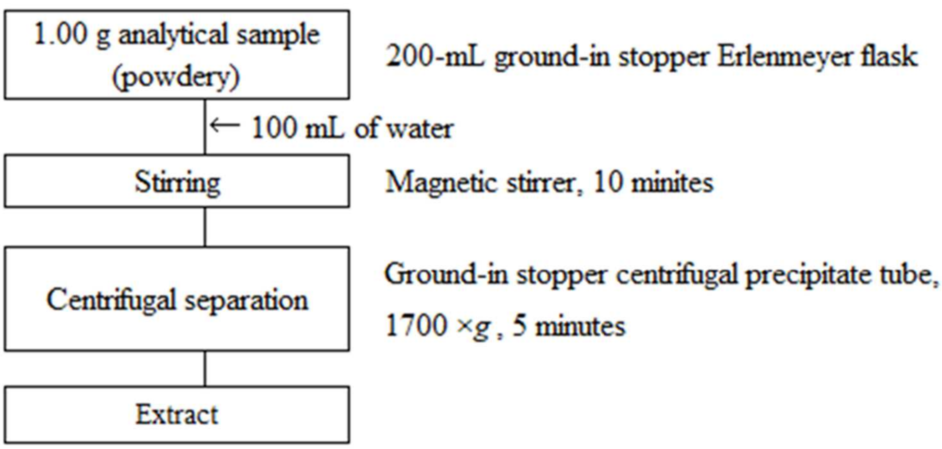


Figure 1-1 Flow sheet for nitrous acid in fertilizers (Extraction procedure (4.1.1))

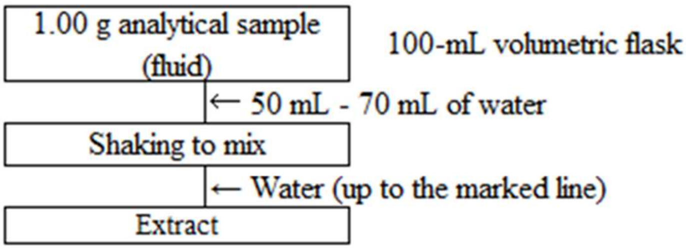


Figure 1-2 Flow sheet for nitrous acid in fertilizers
(Extraction procedure (4.1.2))

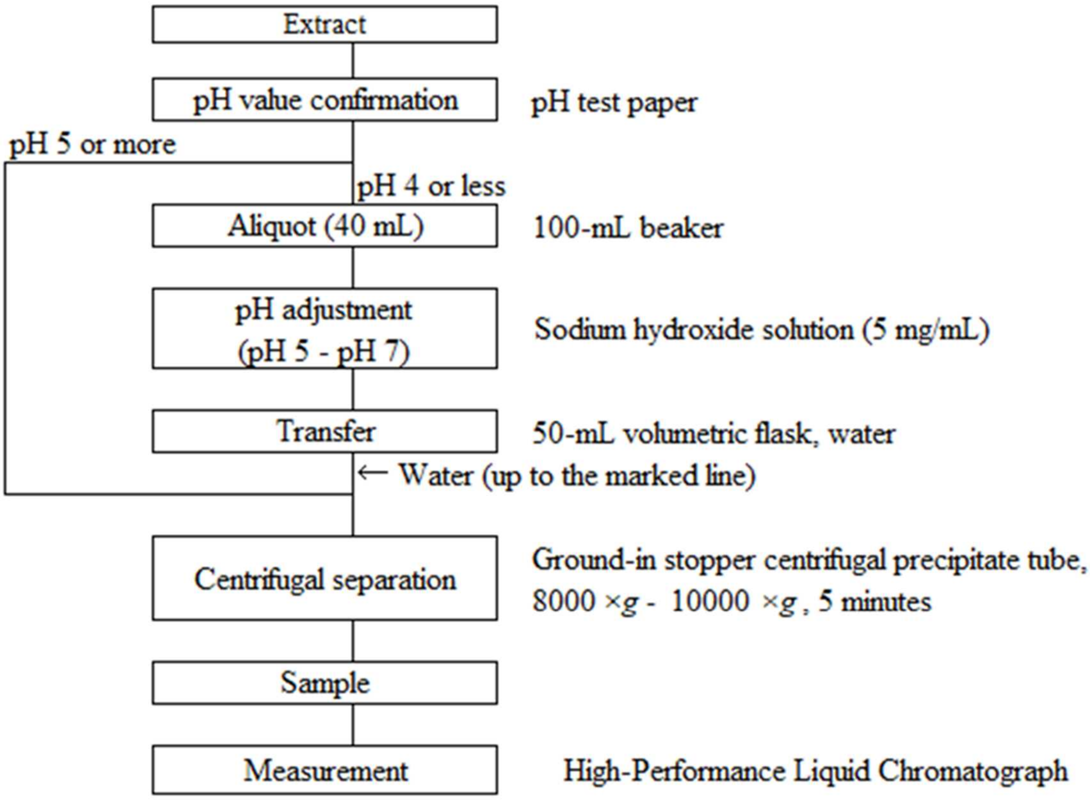
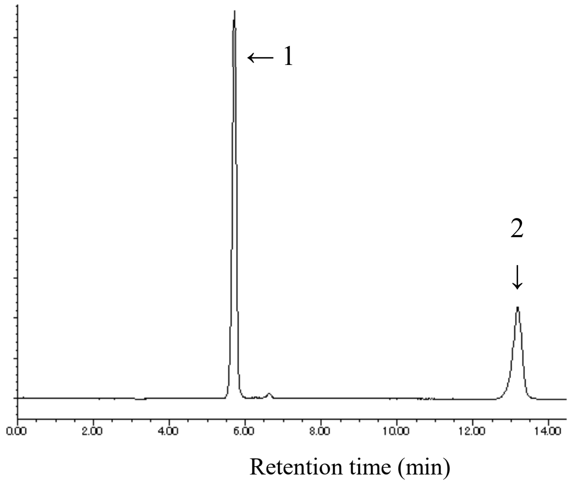
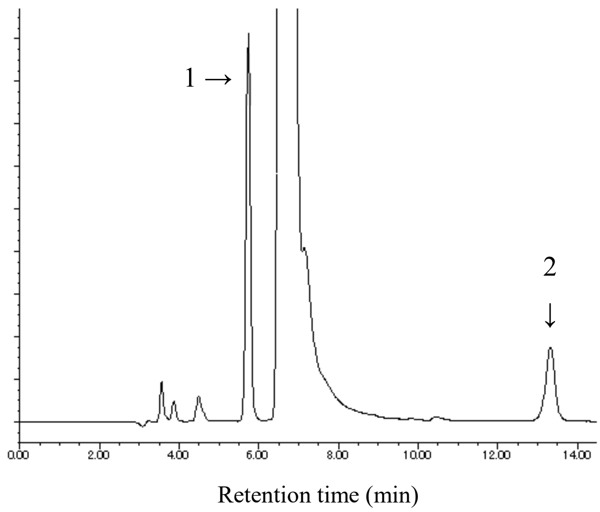


Figure 2 Flow sheet for nitrous acid in fertilizers
(pH adjustment and measurement procedure)

Reference: Examples of the HPLC chromatogram of nitrous acid and ammonium thiocyanate are shown below.



(A) Mixture standard solution
(The equivalents of 100 ng (10 µg/mL, 10 µL) as nitrous acid and ammonium thiocyanate, respectively)



(B) Sample solution
(The equivalents of 0.1 % (mass fraction) as nitrous acid and ammonium thiocyanate added in blended fertilizer respectively)

Reference diagram: HPLC chromatogram of nitrous acid and ammonium thiocyanate

Measurement conditions for HPLC
Column: CAPCELL PAK NH2 UG80 (4.6-mm inner diameter, 250-mm long, 5-µm particle diameter)
Other conditions are according to the example of HPLC measurement conditions in **(4.3) a)**

5.10 Biuret nitrogen
5.10.a High-Performance Liquid Chromatography
(1) Summary

The testing method is applicable to nitrolime and fertilizers containing no nitrolime. This testing method is classified as Type B and its symbol is 5.10.a-2017 or B-N.a-1.

Add water to an analytical sample to extract biuret, introduce it to a High-Performance Liquid Chromatograph (HPLC) to isolate it with a weak acid ion-exchange column, and then measure at a wavelength 190 nm to obtain biuret nitrogen (B-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

Dicyandiamide nitrogen (Dd-N), urea nitrogen (U-N), guanidine nitrogen (Gd-N) and guanylurea nitrogen (GU-N) and can be simultaneously quantified by using this method. (Refer to **Comment 5**).

(2) Reagent: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Potassium dihydrogen phosphate**: A JIS Guaranteed Reagent specified in JIS K 9007 or a reagent of equivalent quality.

c) **Phosphoric acid**: A JIS Guaranteed Reagent specified in JIS K 9005 or a reagent of equivalent quality.

d) **Biuret nitrogen standard solution (B-N 2 mg/mL)** (1): Put 0.491 g of biuret [$C_2H_5N_3O_2$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water, transfer to a 100-mLvolumetric flask and warm up to 50ºC to dissolve. After standing to cool (3), add water up to the marked line (3).

e) **Biuret nitrogen standard solution (B-N 200 µg/mL)**: Put 10 mL of biuret nitrogen standard solution (B-N 2 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

f) **Biuret nitrogen standard solution (B-N 50 µg/mL - 100 µg/mL)**: Put 25 mL - 50 mL of biuret nitrogen standard solution (B-N 200 µg/mL) in 100-mL volumetric flasks and add water up to the marked line.

g) **Biuret nitrogen standard solution for the calibration curve preparation (B-N 1 µg/mL - 50 µg/mL)**: At the time of usage, put 1 mL - 50 mL of biuret nitrogen standard solution (B-N 100 µg/mL) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 97 % (mass fraction) in purity is commercially sold as biuret.

(3) If biuret nitrogen standard solution (B-N 2 mg/mL) is stored in a refrigerator, precipitates may appear. Therefore, it is recommended to store it at room temperature. In addition, sudden cooling should be avoided.

Comment 1 Biuret is sold by FUJIFILM Wako Pure Chemical Co., Ltd., Kanto Chemical Co., Inc. and Tokyo Chemical Industry Co., Ltd.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 7.5-mm inner diameter 100-mm long stainless steel column tube filled with weak acid ion-exchange resin.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 190 nm.

b) **Magnetic stirrer**

c) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000

× *g*.

Comment 2 A column is sold under the production name Asahipak ES-502C 7C, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, put a supernatant solution (4) in a 1.5-mL ground-in stopper centrifugal precipitate tube (5).

d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (6) and use the supernatant as a sample solution.

Note (**4**) If there is a possibility that the biuret nitrogen (B-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of supernatant with water.

(**5**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(**6**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

(4.1.2) Fluid test sample

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, and shake to mix.

c) Add water up to the marked line (7) and put it in a 1.5-mL ground-in stopper centrifugal precipitate tube (5).

d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (6) and use the supernatant as a sample solution.

Note (**7**) If there is a possibility that the biuret nitrogen (B-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of precisely adjusted solution with water.

Comment 3 Instead of procedures in (**4.1.1**) **c) - d**) or (**4.1.2**) **c) - d**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) **Column**: A weak acid ion-exchange resin column (4.00-mm - 7.5-mm inner diameter, 100-mm - 150-mm long, 5-µm – 10-µm particle diameter)

2) **Column bath temperature**: 40 °C

3) **Eluent** (1): Dissolve 3.92 g of potassium dihydrogen phosphate and 0.12 g of phosphoric acid in water to make 1000 mL. Filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.

4) **Flow rate**: 0.6 mL/min
5) **Injection volume**: 10 μL
6) **Detection unit**: An absorptiometric detector, a measurement wavelength: 190 nm

Comment 4 Eluent can be prepared as follows. Dissolve 19.6 g of potassium dihydrogen phosphate and 0.584 g of phosphoric acid with water to make 500 mL and store in a refrigerator. At the time of usage, dilute a predetermined volume of the solution by a factor of 10 and filter with a membrane filter (aperture diameter: no more than 0.5-μm) made of hydrophilic PTFE.

b) **Calibration curve preparation**

1) Inject 10 μL of respective standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 190 nm and obtain a peak height.

2) Prepare a curve for the relationship between the biuret nitrogen (B-N) concentration and the peak height at a wavelength 190 nm of the respective standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 10 μL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain the biuret nitrogen (B-N) content from the peak height using the calibration curve to calculate the biuret nitrogen (B-N) in the analytical sample.

Comment 5 This testing method enables the simultaneous measurement of biuret nitrogen (B-N), dicyandiamide nitrogen (Dd-N), urea nitrogen (U-N), guanidine nitrogen (Gd-N) and a guanylurea nitrogen standard solution (GU-N). In this case, mix a predetermined amount of biuret nitrogen (B-N 1 mg/mL), a urea nitrogen standard solution (U-N 2 mg/mL), a dicyandiamide nitrogen standard solution (Dd-N 2 mg/mL), a guanidine nitrogen standard solution (Gd-N 2 mg/mL) and a guanylurea nitrogen standard solution (GU-N 2 mg/mL), dilute with water to prepare a mixture standard solution (200 μg/mL) (1) and use it instead of (**2**) **e**) a biuret nitrogen standard solution (B-N 200 μg/mL). After that, conduct the same procedure in (**4.2**) **b**) to calculate the respective concentrations of materials subjected to measurement in the analytical sample.

Comment 6 Additive recovery testing was conducted using one brand of an acetaldehyde condensed urea fertilizer, a compound fertilizer, a blended fertilizer, a fluid compound fertilizer and a home garden-use compound fertilizer respectively. As a result, the mean recovery rates at the additive level of 0.2 % (mass fraction) and 0.1 % (mass fraction) and 0.02 % (mass fraction) were 87.0 % **-** 95.1 %, 90.6 % **-** 101.1 % and 91.2 % **-** 105 5 % respectively.

The results of the repeatability tests on different days using a blended fertilizer, a compound fertilizer and a home garden-use compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2. Note that the minimum limit of quantification of this testing method was estimated to be about 0.005 % (mass fraction).

Comment 7 Note that nitrolime has ingredients which affect the measurement of biuret nitrogen (B-N).

Table 1 Analysis results of the repeatability tests on different days of biuret nitrogen

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Blended fertilizer	5	0.204	0.0006	0.3	0.0017	0.9
Compound fertilzier	5	0.0969	0.0006	0.6	0.0016	1.6
Home garden-use compound fertilizer	5	0.0103	0.0001	0.9	0.0001	0.9

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Statistical analysis results of the collaborative study for the test method validation of biuret nitrogen method

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Compound fertilizer 1	9	0.00963	0.00030	3.1	0.00029	3.1
Compound fertilizer 2	10	0.0201	0.0003	1.6	0.0007	3.4
Compound fertilizer 3	12	0.114	0.013	11.7	0.017	15.3
Compound fertilizer 4	11	0.212	0.017	7.8	0.026	12.4
Urea	12	0.832	0.050	6.0	0.086	10.3

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masahiro ECHI, Yasuharu KIMURA and Yuji SHIRAI: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography: A Single-Laboratory Validation, Research Report of Fertilizer, **Vol. 10**, p. 72 **-** 85 (2017)

2) Norio FUNAKI and Yasuharu KIMURA: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer, **Vol. 10**, p. 86 **-** 100 (2017)

(5) Flow sheet for testing method: The flow sheet for biuret nitrogen in fertilizers is shown below:

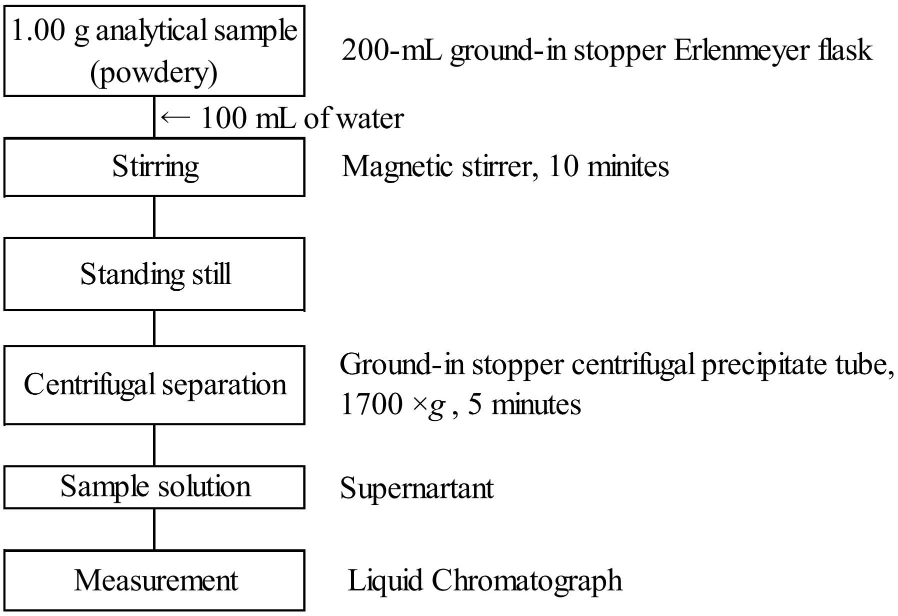


Figure 1 Flow sheet for biuret nitrogen in fertilizers
(Extraction procedure (4.1.1) and measurement)

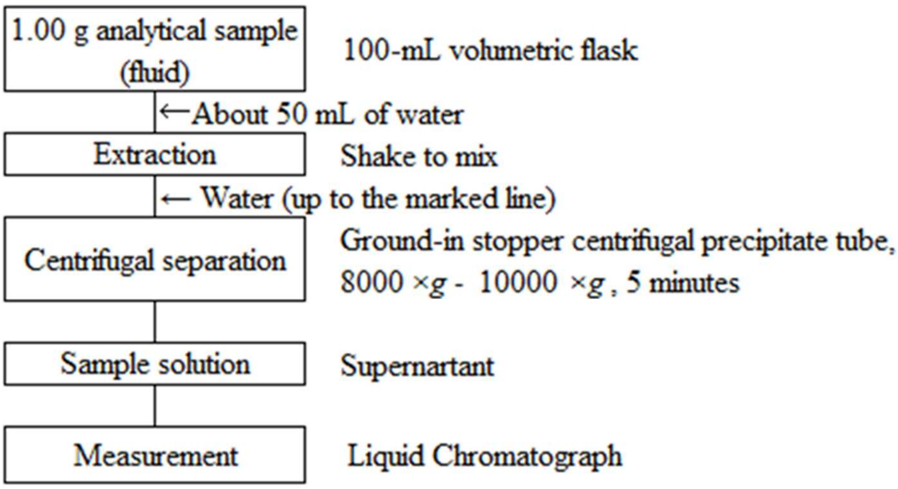
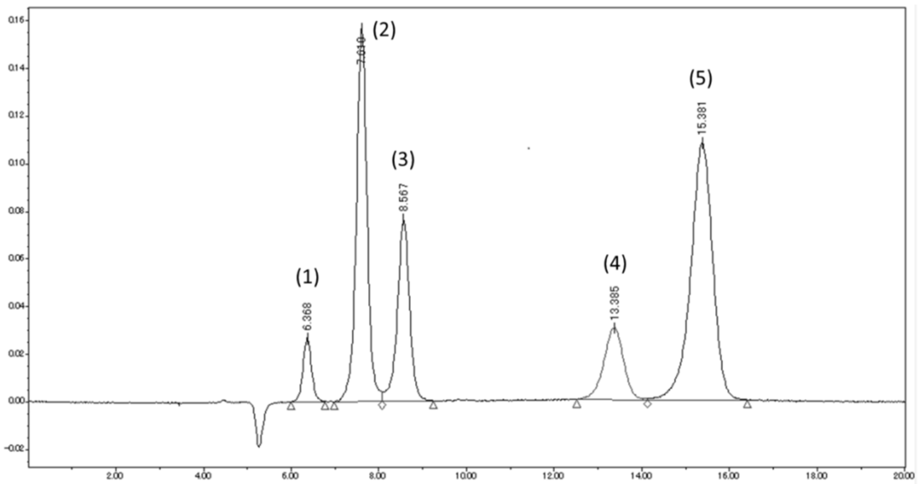


Figure 2 Flow sheet for biuret nitrogen in fertilizers
(Extraction procedure (4.1.2) and measurement)

Reference: An example of the chromatogram of the standard solution of biuret nitrogen for the calibration curve preparation is shown below.



Reference diagram: HPLC chromatogram of the mixture standard solutions for the calibration curve preparation (10 mg/L for each)

Peak name

(1) Urea nitrogen (2) Biuret nitrogen (3) Dicyandiamide nitrogen

(4) Guanidine nitrogen (5) Guanylurea nitrogen

Measurement conditions for HPLC

Column: Asahipak ES-502C 7C (7.5-mm inner diameter, 100-mm long, 9-µm particle diameter)

Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**)

5.11 Titanium
5.11.a ICP Optical Emission Spectrometry (1)
(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 5.11.a-2017 or Ti.a-1.

Pretreat an analytical sample with nitric acid - sulfuric acid - perchloric acid, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the emission with titanium at a wavelength of 334.941 nm to obtain the titanium (Ti) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 4**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.
b) **Nitric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
c) **Sulfuric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
d) **Perchloric acid**: A reagent of harmful metal analysis grade, microanalysis grade or equivalents.
e) **Hydrochloric acid**: A reagent of harmful metal analysis grade or microanalysis grade specified in JIS K 8180, or equivalents.
f) **Titanium standard solution (Ti 1 mg/mL)**: A titanium standard solution (Ti 1 mg/mL) traceable to National Metrology.
g) **Titanium standard solution (Ti 0.1 mg/mL)** (1): Put 10 mL of titanium standard solution (Ti 1 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.
h) **Titanium standard solutions (Ti 0.1 µg - 20 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of titanium standard solution (Ti 0.1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116
  1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity
b) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 350 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to no less than 300 °C.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.
b) Add about 10 mL of nitric acid and about 5 mL of sulfuric acid, cover the tall beaker with a watch glass, and leave at rest overnight.
c) Heat mildly on a hot plate or sand bath at 170 ºC **-** 220 ºC for no less than 30 minutes. After bubbles cease to form, set the temperature of the hot plate or sand bath to no less than 300 ºC, and heat until nitroxide (yellow-brown smoke) is no longer generated (2).

d) After standing to cool, add about 5 mL of perchloric acid.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or sand bath at no less than 300 ºC for 2 - 3 hours to digest (3).
f) Slightly move the watch glass (4), and keep on heating on the hot plate or sand bath to concentrate until the liquid volume becomes no more than 2 mL.
g) After standing to cool, add about 5 mL of hydrochloric acid (1+10) and about 20 mL of water, cover the tall beaker with a watch glass and heat mildly to dissolve.
h) After standing to cool, transfer to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper to make a sample solution.
i) As a blank test, conduct the procedures in **b)** **-** **h)** using another tall beaker to prepare a blank test solution.

Note (**2**) Oxidation of organic matters by perchloric acid progresses extremely rapidly and explosively. For that reason, add perchloric acid after fully degrading organic matters with nitric acid to avoid danger.
(**3**) When the white smoke of perchloric acid is generated, if the solution is colored such as black-brown or brown, stop heating immediately, and after standing to cool, add nitric acid, and heat again to degrade remaining organic matters.
(**4**) The watch glass can be removed.

Comment 2 When the analytical sample solidifies in the procedure in (**4.1**) **b**), moisten the analytical sample with a small amount of water as necessary in advance.
Comment 3 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Atomic Emission Spectrometer**: Set up the measurement conditions for the ICP Atomic Emission Spectrometer considering the following:
Analytical line wavelength: 334.941 nm

b) **Calibration curve preparation**
1) Spray the titanium standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 334.941 nm.
2) Prepare a curve for the relationship between the titanium concentration and the indicated value of the titanium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**
1) Put a predetermined amount of the sample solution (the equivalents of 0.01 mg **-** 2 mg as titanium) in a 100-mL volumetric flask.
2) Add 25 mL of hydrochloric acid (1+23) to the marked line.
3) Subject to the same procedure as in **b)** **1**) to read the indicated value.
4) Obtain the titanium content from the calibration curve, and calculate titanium (Ti) in the analytical sample.

Comment 4 Additive recovery testing was conducted using an autoclaved lightweight concrete powdery fertilizer, a mixed phosphorus fertilizer, a compound fertilizer, a slag manganese fertilizer, a solid compound fertilizer, a fluid compound fertilizer, a mixed compost compound fertilizer and a blended fertilizer respectively. As a result, the mean recovery rate at the additive level of 0.01 % (mass fraction) - 0.5 % (mass fraction) was 92.9 % - 99.5 %.

The results of the repeatability tests on different days using a mixed phosphate fertilizer and a compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.001 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of titanium

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Mixed phosphorus fertilizer	7	0.950	0.013	1.7	0.031	4.3
Compound fertilizer	7	0.130	0.002	1.4	0.006	3.2

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Keisuke AOYAMA: Determination of Titanium in Fertilizer using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) , Research Report of Fertilizer, **Vol. 10**, p. 29 - 40 (2017)

(5) **Flow sheet for testing method**: The flow sheet for titanium in fertilizers is shown below:

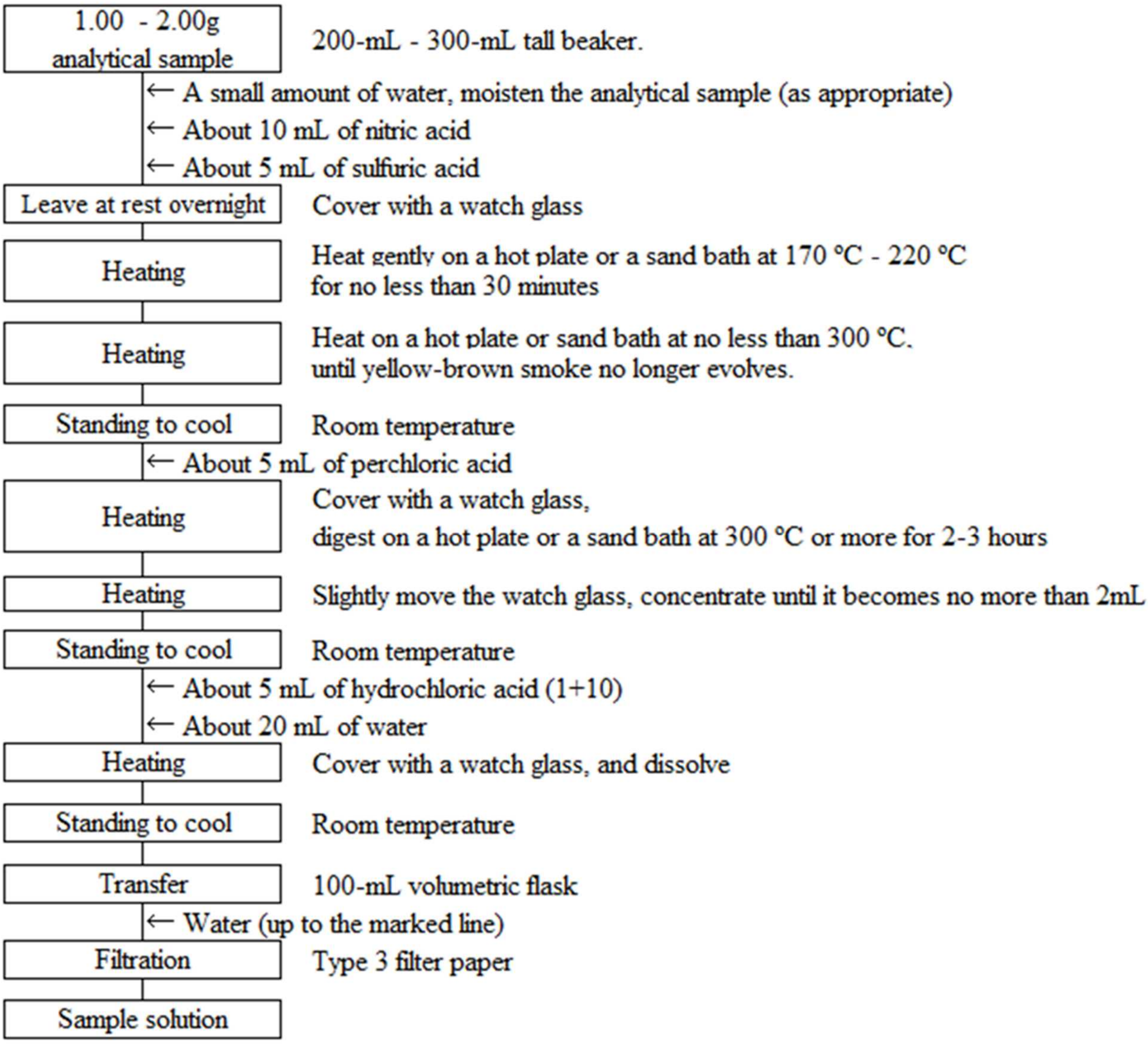


Figure 1 Flow sheet for titanium in fertilizers (Extraction procedure)

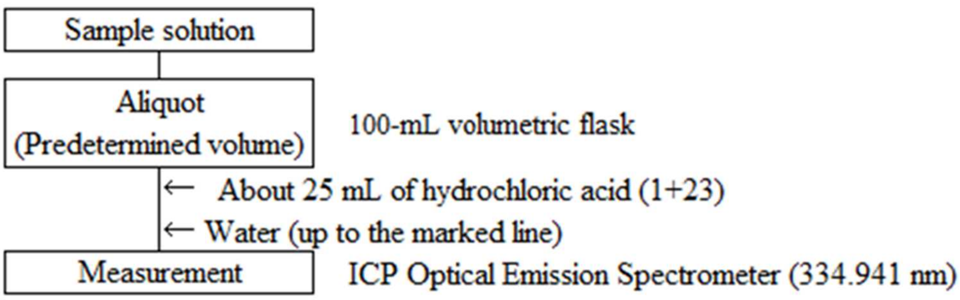


Figure 2 Flow sheet for titanium in fertilizers (Measurement procedure)

5.11.b ICP Optical Emission Spectrometry (2)

(1) Summary

The testing method is applicable to silicate slag fertilizers. This testing method is classified as Type D and its symbol is 5.11.b-2017 or Ti.b-1.

Melt an analytical sample with ammonium hydrogensulfate, introduce it to an ICP Optical Emission Spectrometer (ICP-OES) and measure the emission with titanium at a wavelength of 334.941 nm to obtain the titanium (Ti) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 2**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Ammonium hydrogensulfate**: A reagent of no less than 98 % (mass fraction) in purity.

c) **Hydrochloric acid**: A reagent of harmful metal analysis grade or microanalysis grade specified in JIS K 8180 or equivalents.

d) **Titanium standard solution (Ti 1 mg/mL)**: A titanium standard solution (Ti 1 mg/mL) traceable to National Metrology.

e) **Titanium standard solution (Ti 0.1 mg/mL)** (1): Put 10 mL of titanium standard solution (Ti 1 mg/mL) in a 100-mL volumetric flask and add hydrochloric acid (1+23) up to the marked line.

f) **Titanium standard solutions (Ti 0.1 µg - 20 µg/mL) for the calibration curve preparation** (1): Put 1 mL - 20 mL of titanium standard solution (Ti 1 mg/mL) in 100-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) to the marked line.

g) **Blank test solution for the calibration curve preparation** (1): Hydrochloric acid (1+23) used in the procedures in **e**) and **f**).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

Comment 1 Since the indicated value at an arbitrary wavelength of an ICP-OES varies depending on the observation mode (horizontal and axial mode) of emission and the spectrometer type, the concentration range of the calibration curve suitable for the equipment used differs. Therefore, it is recommended the concentration range of the calibration curve suitable to the equipment used and a standard solution prepared for the calibration curve should be understood in advance.

(3) Instruments: Instruments are as shown below:

a) **ICP Optical Emission Spectrometer**: An optical emission spectrometer specified in JIS K 0116.

1) **Gas**: Argon gas specified in JIS K 1105 of no less than 99.5 % (volume fraction) in purity

b) **Hot plate** : A hot plate whose surface temperature can be adjusted up to 400 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample and put it in a 200-mL tall beaker.

b) Add about 10 g of ammonium hydrogensulfate (2).

c) Heat on a hot plate at no less than 350 ºC and make the melted ammonium hydrogensulfate come into complete contact with the analytical sample (3).

d) Cover with a watch glass, and heat at no less than 350 ºC for 1 hour.

e) After standing to cool, add about 25 mL of hydrochloric acid (1+5), cover the tall beaker with a watch glass and heat mildly to dissolve.

f) After standing to cool, transfer to a 100-mL volumetric flask with water, add water up to the marked line, and filter with Type 3 filter paper.

g) Put 10 mL of the filtrate in another 100-mL volumetric flask and add hydrochloric acid (1+23) to the marked line to make a sample solution.

h) As a blank test, conduct the procedures in **b) - f)** using another tall beaker to prepare a blank test solution.

Note (**2**) Ammonium hydrogensulfate is corrosive. Therefore, use a dispensing spoon made of resin.

(**3**) After the ammonium hydrogensulfate melts, make it come into contact with the analytical sample by tilting the tall beaker or conducting a similar procedure.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0116 and as shown below. Specific measurement procedures are according to the operation method of the ICP Optical Emission Spectrometer used in measurement.

a) **Measurement conditions for the ICP Optical Emission Spectrometer**: Set up the measurement conditions for the ICP Optical Emission Spectrometer considering the following:
Analytical line wavelength: 334.941 nm

b) **Calibration curve preparation**

1) Spray the titanium standard solution for the calibration curve preparation and blank test solution for the calibration curve preparation into inductively coupled plasma, and read the indicated value at a wavelength of 334.941 nm.

2) Prepare a curve for the relationship between the titanium concentration and the indicated value of the titanium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**

1) Subject the sample solution to the same procedure as in **b) 1)** to read the indicated value.

2) Obtain the titanium content from the calibration curve, and calculate titanium (Ti) in the analytical sample.

Comment 2 Additive recovery testing was conducted to evaluate trueness using silicate slag fertilizers (2 samples). As a result, the mean recovery rate at the additive level of 0.1 % (mass fraction) - 0.2 % (mass fraction) was 95.1 % - 98.2 %.
The results of the repeatability tests on different days using silicate slag fertilizers (2 samples) to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.02 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of titanium

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Slag silicate fertilizer 1	7	0.525	0.005	1.0	0.005	1.0
Slag silicate fertilizer 2	7	0.112	0.002	1.6	0.003	2.4

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Keisuke AOYAMA: Determination of Titanium in Fertilizer using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) , Research Report of Fertilizer, **Vol. 10**, p. 29 - 40 (2017)

(**5**) **Flow sheet for testing method**: The flow sheet for titanium in fertilizers is shown below:

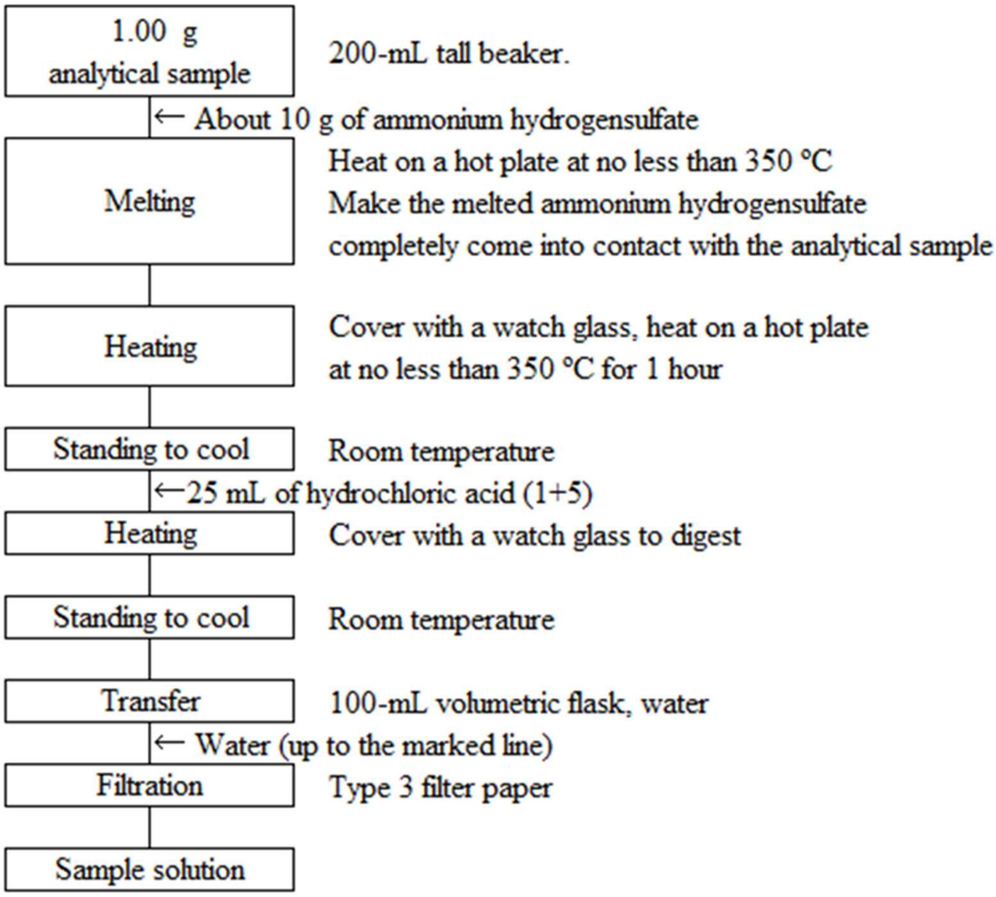


Figure 1 Flow sheet for titanium in fertilizers (Extraction procedure)

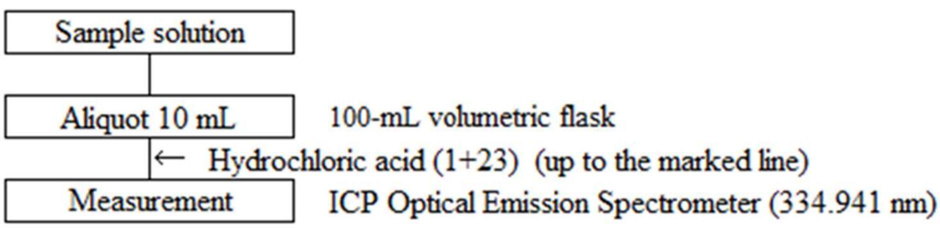


Figure 2 Flow sheet for titanium in fertilizers (Measurement procedure)

5.12 Sulfurous Acid

5.12.a This method is according to 5.3 Sulfurous acid analysis in “The Official Methods of Analysis of Fertilizers 1992”.

References

1) National Institute for Agro-Environmental Sciences, the Ministry of Agriculture, Forestry and Fisheries: The Official Methods of Analysis of Fertilizers 1992, p.78 - 79, Japan Fertilizers Analysis Association, Tokyo (1992)

2) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p. 196 - 197, Yokendo, Tokyo (1988)

6. Testing relating to the other limitations
6.1 Dicyandiamide nitrogen
6.1.a High-Performance Liquid Chromatography (1)
(1) Summary

The testing method is applicable to nitrolime and fertilizers containing nitrolime. This testing method is classified as Type B and its symbol is 6.1.a-2017 or Dd-N.a-1.

Add methanol to an analytical sample to extract dicyandiamide (Dd), introduce it to a High-Performance Liquid Chromatograph (HPLC), isolate with an amino propyl silica gel column and measure at a wavelength 215 nm to obtain dicyandiamide nitrogen (Dd-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 4**.

(2) **Reagent**: Reagents are as shown below.

a) **Methanol**: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

b) **Methanol**: Methanol used in the eluent of a High-Performance Liquid Chromatograph is a regent of High Performance Liquid Chromatograph analysis grade or equivalents.

c) **Acetonitrile**: A reagent of High Performance Liquid Chromatograph analysis grade or equivalents.

d) **Dicyandiamide standard solution (1 mg/mL)** (1): Put 0.1 g of dicyandiamide [$C_2H_4N_4$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of methanol to dissolve, transfer to a 100-mL volumetric flask and add the solvent up to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

e) **Dicyandiamide standard solution (0.1 mg/mL)**: Put 10 mL of dicyandiamide standard solution (1 mg/ mL) in a 100-mL volumetric flask and add methanol up to the marked line.

f) **Dicyandiamide standard solution (10 µg/ mL - 50 µg/ mL) for the calibration curve preparation**: At the time of usage, put 5 mL - 25 mL of dicyandiamide standard solution (0.1 mg/ mL) in 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

g) **Dicyandiamide standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation**: In the case of usage, put 2.5 mL - 25 mL of dicyandiamide standard solution for the calibration curve preparation (20 µg/mL) in 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.
(2) A reagent of no less than 98 % (mass fraction) in purity as dicyandiamide is commercially sold.

Comment 1 Dicyandiamide is commercially sold as dicyanodiamide by FUJIFILM Wako Pure Chemical Co., Ltd. and Kanto Chemical Co., Inc.

(3) **Instruments**: Instruments are as shown below:

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A column of 4 mm - 6 mm inner diameter and 150 mm - 250 mm long stainless steel column tube filled with silica gel, to which amino or amino propyl chemically bonds.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 215 nm.

b) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

c) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 2 A column is sold under production names such as Hibar LiChrosorb NH_2, Inertsil NH_2, Unison UK-Amino, Mightysil NH_2, Shim-pack CLC-NH_2, Shodex NH-5A, Unisil Q NH_2, etc.

(4) **Test procedures**

(4.1) **Extraction**: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL - 200-mL ground-in stopper Erlenmeyer flask.

b) Immediately add 100 mL of methanol (3) and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 10 minutes.

c) After allowing to stand still, put a supernatant solution in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

d) Centrifuge at 8000 × *g* - 10000 × *g* for about five minutes (5).

e) 1 mL of the supernatant is used as a sample solution.

Note (3) Add methanol immediately as the determined value becomes higher than usual if it is left in air.

(4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement

(5) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 3 Instead of the procedures in (4.1) c) - e), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of PTFE and the filtrate can be the sample solution.

(4.2) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions for the High-Performance Liquid Chromatograph is shown below. Set up the measurement conditions considering it:

1) **Column**: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which amino or amino propyl chemically bonds.

2) **Column bath temperature**: 30 °C - 40 °C

3) **Eluent**: Acetonitrile - methanol (6+1)

4) **Flow rate**: 1 mL/min

5) **Detection unit**: An absorptiometric detector, a measurement wavelength: 215 nm

b) **Calibration curve preparation**

1) Inject 10 µL of respective dicyandiamide standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 215 nm, and obtain a peak area or height.

2) Prepare a curve for the relationship between the concentration and the peak area or height at a wavelength 215 nm of the respective dicyandiamide standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 10 μL of the sample solution to the same procedure as in **b**) **1**)
2) Obtain dicyandiamide (Dd) content from the calibration curve to calculate the concentration of dicyandiamide (Dd) in the analytical sample.
3) Calculate the dicyandiamide nitrogen (Dd-N) by the following formula.

Dicyandiamide nitrogen (Dd-N) (% (mass fraction)) in an analytical sample

$$= A \times (MW_1/MW_2)$$
$$= A \times 0.6664$$

A : Dicyandiamide (Dd) (% (mass fraction)) in an analytical sample
MW_1 : 4 atomic weight of nitrogen (56.027)
MW_2 : Molecular weight of dicyandiamide (84.080)

Comment 4 Recovery testing was conducted using nitrolime (3 samples) and a blended fertilizer containing nitrolime (2 samples), as a result, the recovery rates of dicyandiamide at the concentration level of 6 % (mass fraction) and 0.6 % (mass fraction) were 94.9 % - 105.1 % and 95.6 % - 103.5 % respectively.
Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 1.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 % (mass fraction).

Table 1 Analysis results of the collaborative study for the test method validation of dicyandiamide nitrogen

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Nitrolime 1	9	0.0321	0.0010	3.2	0.0012	3.8
Nitrolime 2	10	0.159	0.002	1.3	0.006	3.8
Nitrolime 3	11	0.245	0.002	0.7	0.008	3.3
Blended fertilizer 1	11	0.124	0.001	0.7	0.002	2.0
Blebded fertilizer 2	11	0.410	0.007	1.6	0.008	1.9

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masakazu SAIKI and Miyuki ASAO: Validation of High-Performance Liquid Chromatography for Determination of Dicyandiamide in Nitrolime, Research Report of Fertilizers, **Vol. 2**, p. 25 - 31 (2009)
2) Masakazu SAIKI and Masayuki YOSHIMOTO: Determination of Dicyandiamide in Nitrolime by High-Performance Liquid Chromatography: A Collaborative Study, Research Report of Fertilizers, **Vol. 2**, p. 32 - 37 (2009)

(**5**) **Flow sheet for dicyandiamide nitrogen**: The flow sheet for dicyandiamide nitrogen in nitrolime and fertilizers containing nitrolime is shown below:

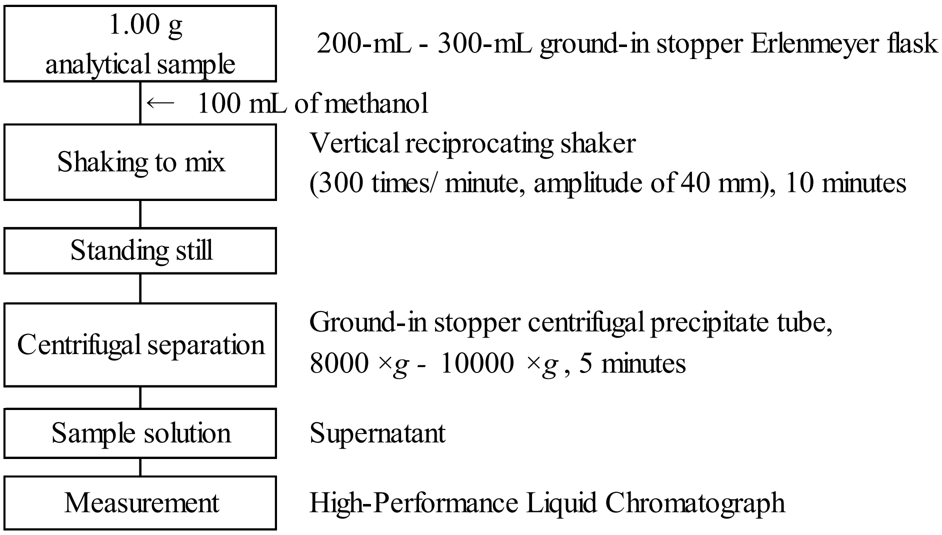
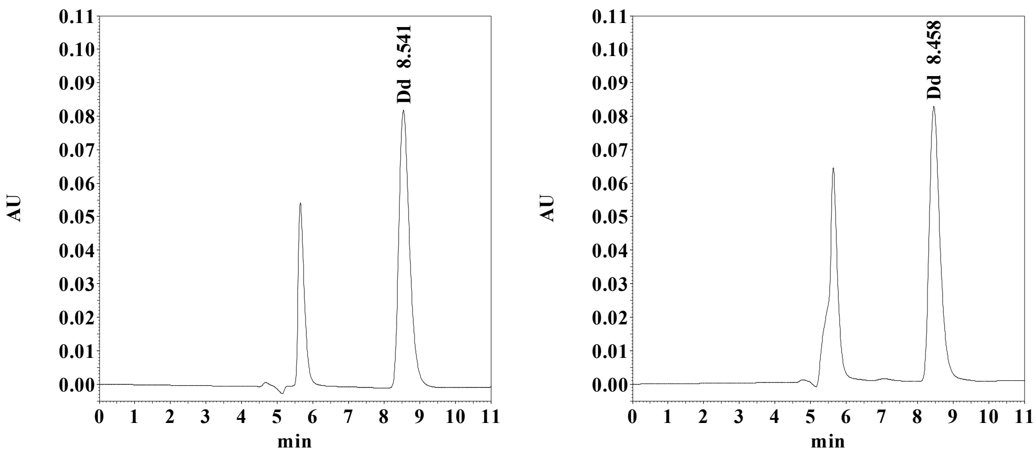


Figure Flow sheet for dicyandiamide nitrogen in nitrolime and fertilizers containing nitrolime

Reference: Examples of the HPLC chromatogram of dicyandiamide standard solution and sample solution (nitrolime) for the calibration curve preparation are shown below.



1) Standard solution 2) Sample solution

Reference diagram: HPLC chromatogram of dicyandiamide

1) Dicyandiamide standard solution (the equivalents of 100 ng (10 µg/ mL, 10 µL) of dicyandiamide)
2) Sample solution (nitrolime)

Measurement conditions for HPLC

Column: Hibar LiChrosorb NH_2 (4.6-mm inner diameter, 25-cm long, 5-µm particle diameter)

Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**)

6.1.b High-Performance Liquid Chromatography (2)

(1) Summary

This testing method is applicable to fertilizers. In addition, nitrolime is excluded from the scope of application. This testing method is classified as Type B and its symbol is 6.1.b-2017 or Dd-N.b-1.

Add water to an analytical sample to extract dicyandiamide, introduce it to a High-Performance Liquid Chromatograph (HPLC) to isolate it with a weak acid ion-exchange column, and then measure at a wavelength 190 nm to obtain dicyandiamide nitrogen (Dd-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

Biuret nitrogen (B-N), urea nitrogen (U-N), guanidine urea (Gd-N) and guanylurea nitrogen (GU-N) can be simultaneously quantified by using this method. (Refer to **Comment 5**).

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Potassium dihydrogen phosphate**: A JIS Guaranteed Reagent specified in JIS K 9007 or a reagent of equivalent quality.

c) **Phosphoric acid**: A JIS Guaranteed Reagent specified in JIS K 9005 or a reagent of equivalent quality.

d) **Dicyandiamide nitrogen standard solution (Dd-N 2 mg/mL)** (1): Put 0.300 g of dicyandiamide [$C_2H_4N_4$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.

e) **Dicyandiamide nitrogen standard solution for the calibration curve preparation (Dd-N 200 µg/mL)** (1): Put 10 mL of dicyandiamide nitrogen standard solution (Dd-N 2 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

f) **Dicyandiamide nitrogen standard solution (Dd-N 50 µg/mL - 100 µg /mL)** (1): Put 25 mL - 50 mL of dicyandiamide nitrogen standard solution (Dd-N 200 µg/mL) in 100-mL volumetric flasks and add water up to the marked line.

g) **Dicyandiamide nitrogen standard solution for the calibration curve preparation (Dd-N 1 µg/mL - 50 µg/mL)** (1): At the time of usage, put 1 mL - 50 mL of dicyandiamide nitrogen standard solution (Dd-N 100 µg/mL) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % (mass fraction) in purity as dicyandiamide is commercially sold.

Comment 1 Dicyandiamide is sold by Tokyo Chemical Industry Co., Ltd. In addition, dicyandiamide is commercially sold as dicyanodiamide by FUJIFILM Wako Pure Chemical Co., Ltd. and Kanto Chemical Co., Inc.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 7.5-mm inner diameter 100-mm long stainless steel column tube filled with weak acid ion-exchange resin.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 190 nm.

b) **Magnetic stirrer**

c) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 2 A column is sold under the production name Asahipak ES-502C 7C, etc.

(4) **Test procedure**
(4.1) **Extraction**: Conduct extraction as shown below.
(4.1.1) **Powdery test sample**
a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.
b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.
c) After allowing to stand still, put a supernatant solution (3) in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).
d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (5) and use the supernatant as a sample solution.

Note (3) If there is a possibility that the dicyandiamide nitrogen (Dd-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of supernatant with water.
(4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.
(5) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

(4.1.2) **Fluid test sample**
a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix.
c) Add water up to the marked line (6) and put it in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).
d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (5) and use the supernatant as a sample solution.

Note (6) If there is a possibility that the dicyandiamide nitrogen (Dd-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of precisely adjusted solution with water.

Comment 3 Instead of procedures in (**4.1.1**) **c**) - **d**) or (**4.1.2**) **c**) - **d**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.2) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.
a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions is shown below. Set up the measurement conditions considering it:
1) **Column**: A weak acid ion-exchange resin column (4.0-mm - 7.5-mm inner diameter, 100-mm - 150-mm long, 5-µm – 10-µm particle diameter)
2) **Column bath temperature:** 40 °C
3) **Eluent** (1): Dissolve 3.92 g of potassium dihydrogenphosphate and 0.12 g of phosphoric

acid in water to make 1000 mL. Filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.

4) **Flow rate**: 0.6 mL/min
5) **Injection volume**: 10 µL
6) **Detection unit**: An absorptiometric detector, a measurement wavelength: 190 nm

Comment 4 Eluent can be prepared as follows. Dissolve 19.6 g of potassium dihydrogenphosphate and 0.584 g of phosphoric acid with water to make 500 mL and store in a refrigerator. At the time of usage, dilute a predetermined volume of the solution by a factor of 10 and filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE**.**

b) Calibration curve preparation

1) Inject 10 µL of respective standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 190 nm and obtain a peak height.
2) Prepare a curve for the relationship between the dicyandiamide nitrogen (Dd-N) concentration and the peak height at a wavelength 190 nm of the respective standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b**) **1**)
2) Obtain the dicyandiamide nitrogen (Dd-N) content from the peak height using the calibration curve to calculate the dicyandiamide nitrogen (Dd-N) in the analytical sample.

Comment 5 This testing method enables the simultaneous measurement of biuret nitrogen (B-N), urea nitrogen (U-N), dicyandiamide nitrogen (Dd-N), guanidine urea (Gd-N) and guanylurea nitrogen (GU-N). In that case, see **5.10.a Comment 5**.

Comment 6 Additive recovery testing was conducted using one brand of an acetaldehyde condensed urea fertilizer, a compound fertilizer, a blended fertilizer, a fluid compound fertilizer and a home garden-use compound fertilizer respectively. As a result, the mean recovery rates at the additive level of 3 % (mass fraction) and 1.5 % (mass fraction) and 0.3 % (mass fraction) were 96.3 % - 96.3 %, 94.5 % - 99.7 % and 88.9 % - 100.6 % respectively.

The results of the repeatability tests on different days using a blended fertilizer, a compound fertilizer and a home garden-use compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of dicyandiamide nitrogen

Name of sample	Test days of repeatability (T)[1)]	Mean[2)] (%)[3)]	s_{r}[4)] (%)[3)]	RSD_{r}[5)] (%)	$s_{\mathrm{I(T)}}$[6)] (%)[3)]	$RSD_{\mathrm{I(T)}}$[7)] (%)
Blended fertilizer	5	3.03	0.02	0.5	0.02	0.5
Compound fertilizer	5	1.45	0.01	0.9	0.02	1.6
Home garden-use compound fertilizer	5	0.145	0.001	0.9	0.003	2.3

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of dicyandiamide nitrogen

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_{r}[4)] (%)[3)]	RSD_{r}[5)] (%)	s_{R}[6)] (%)[3)]	RSD_{R}[7)] (%)
Compound fertilizer 1	10	0.0464	0.0023	5.0	0.0148	31.9
Compound fertilizer 2	12	0.206	0.011	5.1	0.031	15.2
Compound fertilizer 3	12	1.69	0.05	2.8	0.12	7.0
Compound fertilizer 4	10	2.76	0.07	2.6	0.12	4.4

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masahiro ECHI, Yasuharu KIMURA and Yuji SHIRAI: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography: A Single-Laboratory Validation, Research Report of Fertilizer, **Vol. 10**, p. 72 - 85 (2017)

2) Norio FUNAKI and Yasuharu KIMURA: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer, **Vol. 10**, p. 86 - 100 (2017)

(5) Flow sheet for testing method: The flow sheet for dicyandiamide nitrogen in fertilizers is shown below:

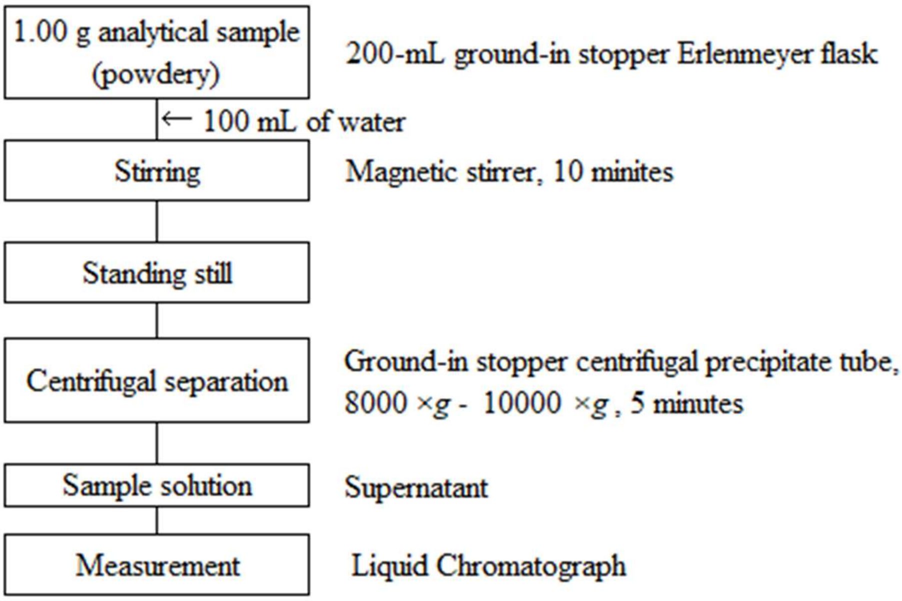


Figure 1 Flow sheet for dicyandiamide nitrogen in fertilizers
(Extraction procedure (4.1.1) and measurement)

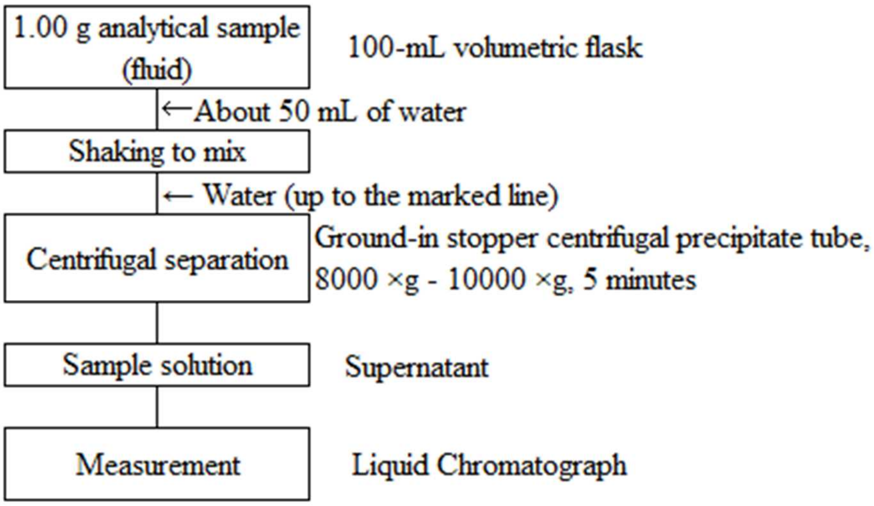
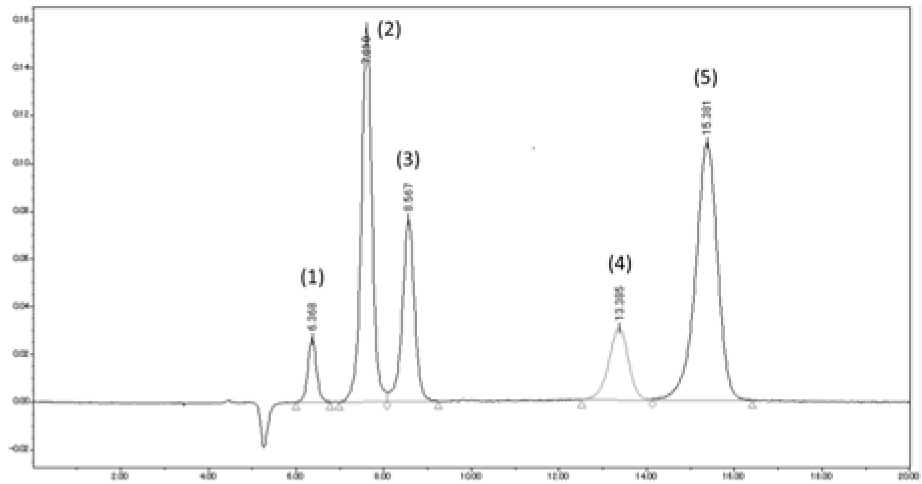


Figure 2 Flow sheet for dicyandiamide nitrogen in fertilizers
(Extraction procedure (4.1.2) and measurement)

Reference: An example of the chromatogram of the standard solution for calibration curve preparation of dicyandiamide nitrogen is shown below.



Reference diagram HPLC chromatogram of the mixture standard solutions (10 mg/L for each) for calibration curve preparation

Peak name
(1) Urea nitrogen (2) Biuret nitrogen (3) Dicyandiamide nitrogen
(4) Guanidine nitrogen (5) Guanylurea nitrogen

Measurement conditions for HPLC
Column: Asahipak ES-502C 7C (7.5-mm inner diameter, 100-mm long, 9-µm particle diameter)
Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**)

6.2 Chlorine
6.2.a Ion Chromatography
(1) Summary

The testing method is applicable to potassium sulfate, potassium bicarbonate, magnesium potassium sulfate, fish cakes powder, fish cakes and compost. This testing method is classified as Type D and its symbol is 6.2.a-2017 or Cl.a-1.

Add water to an analytical sample to extract chloride ions, introduce them to an Ion Chromatograph (IC) to isolate it with an ion exchange column, and then measure with an electric conductivity detector to obtain chlorine (Cl) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A4 specified in JIS K 0557.

b) 1 mol/L sodium carbonate solution: Solution for ion chromatography.

c) Phthalic acid: A reagent of no less than 98 % (mass fraction) in purity.

d) 6-aminohexanic acid (1): A reagent of no less than 97 % (mass fraction) in purity.

e) Phenylboronic acid: A reagent of no less than 97 % (mass fraction) in purity.

f) Chloride ion standard solution (Cl^- 1 mg/mL): A chloride ion standard solution (Cl^- 1000 mg/mL) traceable to National Metrology.

g) Chloride ion standard solution (Cl^- 100 µg/mL) (2): Put a predetermined amount of chloride ion standard solution (Cl^- 1 mg/mL) in a volumetric flask and add water up to the marked line.

h) Chloride ion standard solution (Cl^- 5 µg/ mL - 50 µg/ mL) for the calibration curve preparation (2): Put 5 mL - 50 mL of Chloride ion standard solution (Cl^- 100 µg/mL) in a 100-mL volumetric flask and add water up to the marked line.

i) Chloride ion standard solution (Cl^- 1 µg/ mL - 2 µg/ mL) for the calibration curve preparation (2): Put 5 mL - 10 mL of Chloride ion standard solution (Cl^- 20 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

j) Eluent for suppressor method (2): Put 6.4 mL of 1 mol/L sodium carbonate solution in a 1000-mL volumetric flask and add water up to the marked line. Then filter with membrane type filter (pore size: no more than 0.5 µm) made of hydrophilic PTFE (2).

k) Eluent for non-suppressor method (2): Put 0.349 g of phthalic acid, 0.380 g of 6-aminohexanic acid and 0.732 g of phenylboronic acid in a 1000-mL volumetric flask and add about 500 mL of water to dissolve. Add water up to the marked line and filter with membrane type filter (pore size: no more than 0.5 µm) made of hydrophilic PTFE (3).

Note (**1**) It is also known as "6-amino-*n*-caprotic acid".

(**2**) This is an example of preparation; prepare an amount as appropriate.

(**3**) A solution concentrated by a factor of 10 can be prepared in advance and the solution can be diluted by a factor of 10 at each usage.

Comment 1 Instead of the chloride ion standard solution in (**2**), a chloride ion standard solution for the calibration curve preparation can be prepared by using a chloride ion standard solution (Cl^- 0.1 mg/mL) traceable to National Metrology.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Magnetic stirrer

b) Centrifugal separator: A centrifugal separator that can work at 1700 × *g*.

c) Ion Chromatograph: An ion chromatograph specified in JIS K 0127 that satisfies the following requirements.

1) **Column**: In case of a suppressor method, a 4-mm inner diameter 250-mm long 5-µm particle diameter column tube filled with poly vinyl alcohol porous particles, to which quaternary ammonium group chemically bonds (4).
In case of non-suppressor method, a 4.6-mm inner diameter 100-mm long column tube filled with hydrophilic polymethacrylate-gel, to which quaternary ammonium group chemically bonds (5).
2) **Column bath**: A column bath whose temperature can be adjusted to 40 ºC.
3) **Suppressor**: Cation exchange membrane or resin should be used.
4) **Detection unit**: An electric conductivity detector

d) **Membrane filters**: Pore size is no more than 0.45 µm, made of hydrophilic PTFE

Note (**4**) A column is commercially sold under the name Shodex IC SI-52 4E, etc.
(**5**) A column is commercially sold under the name Shodex IC NI-424, etc.

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.
b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.
c) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.
d) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (6) and use the supernatant as an extract.
e) Transfer a predetermined amount of the solution, and dilute exactly by a factor of 20 with water (7).
f) Filter with a membrane filter (pore size: no more than 0.45 µm) to make a sample solution.

Note (**6**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.
(**7**) In case of exceeding the calibration curve, dilute by a factor of more than 20.

Comment 2 Instead of the procedures in (**4. 1**) **c**) and **d**), it is allowed to filter with Type 3 filter paper and the filtrate can be the extract.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0127 and as shown below. Specific measurement procedures are according to the operation method of the Ion Chromatograph used in measurement.

a) **Measurement conditions for the Ion Chromatograph**: An example of measurement conditions is shown below. Set up the measurement conditions considering it:

aa) **Suppressor method**

1) **Column**: A polyvinyl alcohol porous particles column (4-mm inner diameter 250-mm long 5-µm particle diameter) to which quaternary ammonium group chemically bonds.
2) **Column bath temperature**: 40 °C
3) **Eluent**: Prepared in the procedure in (**2**) **j**).
4) **Flow rate**: 0.8 mL/min
5) **Injection volume**: 20 µL
6) **Detection unit**: An electric conductivity detector

ab) **Non-suppressor method**

1) **Column**: A hydrophilic polymethacrylate-gel column (4.6-mm inner diameter 100-mm long) to which quaternary ammonium group chemically bonds.
2) **Column bath temperature**: 40 °C
3) **Eluent**: Prepared in the procedure in (**2**) **k**).
4) **Flow rate**: 1.0 mL/min
5) **Injection volume**: 20 µL
6) **Detection unit**: An electric conductivity detector

b) Calibration curve preparation

1) Inject 20 µL of respective standard solutions for the calibration curve preparation to the ion chromatograph and record a chromatogram of electric conductivity to obtain a peak area.
2) Prepare a curve for the relationship between the concentration and the peak area of electric conductivity of respective standard solutions for the calibration curve preparation.
   Prepare a calibration curve when the sample is measured.

c) Sample measurement

1) Subject 20 µL of the sample solution to the same procedure as in **b**) **1**)
2) Obtain the chloride ion concentration from the calibration curve by the peak area to calculate the chlorine (Cl) in the analytical sample.

Comment 3 Additive recovery testing was conducted with a suppressor method using samples that 1.8 % (mass fraction) – 33.4 % (mass fraction) of sodium chloride as chlorine is added to potassium sulfate, magnesia potassium sulfate, potassium bicarbonate, cow dung compost and fish cakes powder. As a result, the mean recovery rates at the chlorine additive level of 33.4 % (mass fraction), 10 % (mass fraction) - 13.4 % (mass fraction) and 1.8 % (mass fraction) - 9.1 % (mass fraction) were 100.8 %, 98.6 % - 101.1 % and 96.2 % - 103.2 % respectively. With a non-suppressor method, they are 100.2 %, 96.4 % - 97.2 % and 93.3 % - 101.4 %.

The results of the repeatability tests on different days using potassium sulfate, magnesia potassium sulfate, potassium bicarbonate, cow dung compost and fish cakes powder to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.1 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of chlorine

Measurement method	Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Suppressor method	Pottasium sulfate	5	9.93	0.01	0.1	0.03	0.3
	Fish cakes powder	5	6.13	0.03	0.5	0.07	1.1
Non-supprresor method	Pottasium sulfate	5	4.86	0.01	0.2	0.08	1.7
	Magnesia pottasium sulfate	5	4.89	0.02	0.4	0.06	1.2
	Pottasium bicarbonate	5	4.85	0.02	0.4	0.06	1.3
	Cow dung compost	5	13.15	0.04	0.3	0.16	1.2

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Satoko SAKAIDA, Mariko FUJITA and Yuji SHIRAI: Determination of Chloride in Fertilizers by Ion Chromatography (IC), Research Report of Fertilizers, **Vol. 8**, p. 50 - 60 (2015)

(**5**) **Flow sheet for testing method**: The flow sheet for chlorine in fertilizers is shown below:

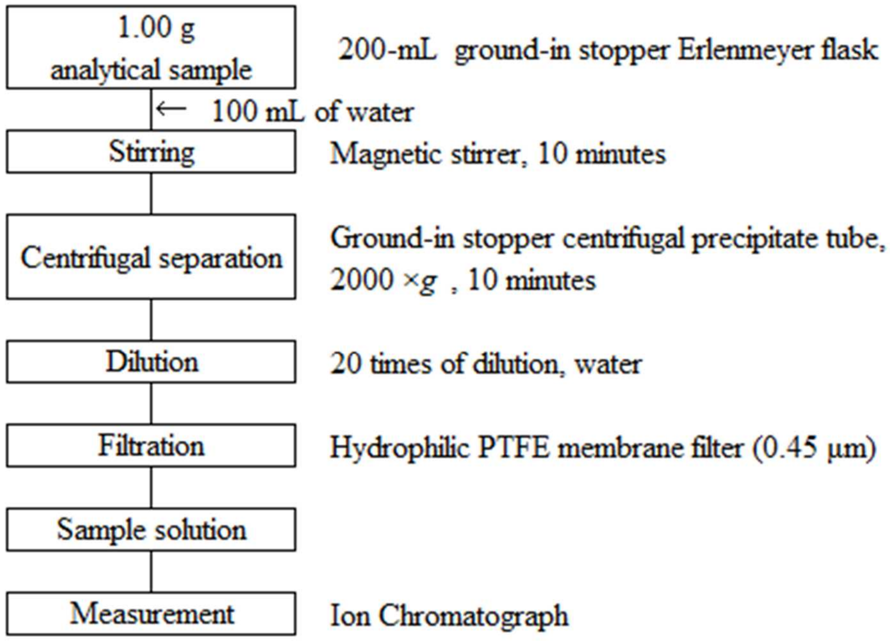
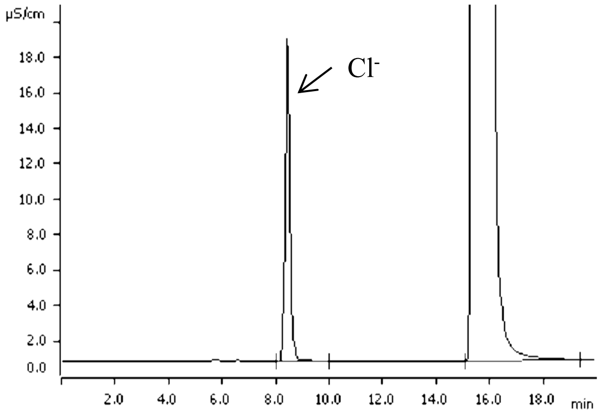
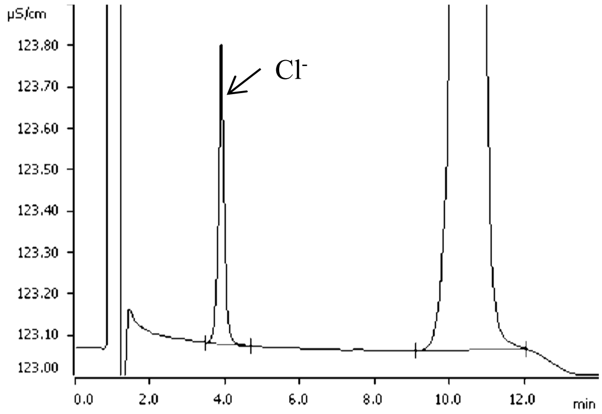


Figure Flow sheet for chlorine in fertilizers

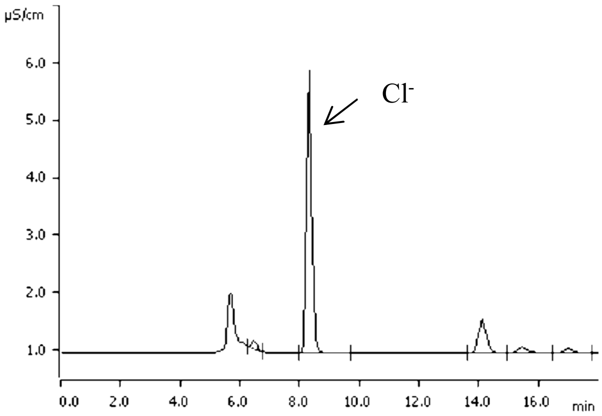
Reference: Examples of the IC chromatograms of the sample solutions (magnesia potassium sulfate and fish cakes powder) are shown below.



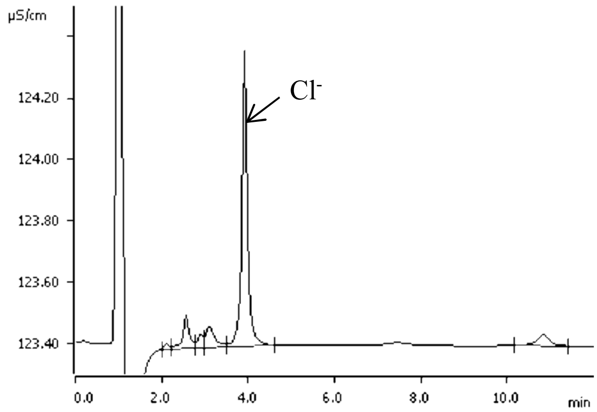
(A) Chromatogram of magnesia potassium sulfate (Suppressor method)



(B) Chromatogram of magnesia potassium sulfate (Non-suppressor method)



(C) Chromatogram of fish cakes powder (Suppressor method)



(D) Chromatogram of fish cakes powder (Non-suppressor method)

Reference diagram: IC chromatogram of chloride ion
(Peak : 1.chloride ion (Cl^-))

6.2.b Silver nitrate method

(1) Summary

The test method is applicable to potassium sulfate, potassium bicarbonate and magnesium potassium sulfate. This testing method is classified as Type E and its symbol is 6.2.b-2017 or Cl.b-1.

Add water to an analytical sample to extract chloride ions and titrate (precipitate) with a 0.1 mol/L silver nitrate standard solution to obtain chlorine (Cl) in an analytical sample.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Nitric acid**: A JIS Guaranteed (HNO3 60 % (mass fraction)) Reagent specified in JIS K 8541 or a reagent of equivalent quality.

c) **0.1 mol/L silver nitrate solution** (1): Weigh 17 g of silver nitrate specified in JIS K 8550 to a 2000-mL beaker, add 1000 mL of water to dissolve and store in a colored bottle.

Standardization: Heat a sodium chloride reference material for volumetric analysis specified in JIS K 8005 at 600 ºC ± 25 °C for 1 hour, let it stand to cool in a desiccator, and then put about 1.5 g in a weighing dish, and weigh the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line to make the sodium chloride solution (1). On each day to use a 0.1 mol/L silver nitrate solution, put 10 mL of the sodium chloride solution in a 200-mL Erlenmeyer flask, add a few drops of potassium chromate solution (5 g/100 mL) as an indicator, and titrate with a 0.1 mol/L silver nitrate solution until the color of the solution becomes reddish brown. Calculate the factor of a 0.1 mol/L silver nitrate solution by the following formula:

Factor (f) of 0.1 mol/L silver nitrate solution

$$= W_1 \times (A/100) \times (1/58.44) \times (V_1/V_2 \times (1000/V_3)) \times (1/C)$$
$$= (W_1 \times A/V_3) \times (4/58.44)$$

W_1 : Mass (g) of sodium chloride weighed
A : Purity (% (mass fraction)) of sodium chloride
V_1 : Aliquot volume (10 mL) of sodium chloride solution
V_2 : Constant volume (250 mL) of sodium chloride solution
V_3 : Volume (mL) of 0.1 mol/L silver nitrate solution needed for titration
C : Set concentration (0.1 mol/L) of 0.1 mol/L silver nitrate solution

d) **Potassium chromate (5 g/ 100 mL)** (1): Dissolve 5 g of potassium chromate in 100 mL of water.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **Magnetic stirrer**

b) **pH test paper**: A pH test paper infiltrated with indicator and dried, which can measure the value from pH 1 to pH 11 and a color change chart with the pH interval value 1 is attached.

Comment 1 pH test paper is sold under the name UNIV Test Paper, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask**.**

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) Filter with Type 3 filter paper to make a sample solution.

(4.2) Measurement: Conduct measurement as shown below.

a) Put 25 mL of sample solution in a 200-mL tall beaker.

b) Confirm the pH of the solution with a pH-test paper. If the pH is basic, neutralize with nitric acid (1+10).

c) Add a few drops of potassium chromate solution (5 g/100 mL) to the sample solution as an indicator and titrate with a 0.1 mol/L silver nitrate solution until the color of the solution becomes reddish brown.

d) Calculate chlorine (Cl) by the following formula.

Chlorine (% (mass fraction)) in the analytical sample

$$= V_4 \times C \times f \times (35.45)/W_2 \times (100/1000) \times (V_5/ V_6)$$

$$= V_4 \times f \times (35.45/25)/W_2$$

V_4 : Volume (mL) of the 0.1 mol/L silver nitrate solution required for titration

C : Set concentration (0.1 mol/L) of 0.1 mol/L silver nitrate solution

f : Factor of 0.1 mol/L silver nitrate solution

V_5 : Liquid volume of water (100 mL) subjected to extraction in (**4.1**) **b**).

V_6 : Aliquot volume (25 mL) of the sample solution subjected to titration in (**4.2**) **a**).

W_2 : Mass (g) of the analytical sample

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.199- 201, Yokendo, Tokyo (1988)

(**5**) **Flow sheet for testing method**: The flow sheet for chlorine in potassium sulfate, etc. is shown below:

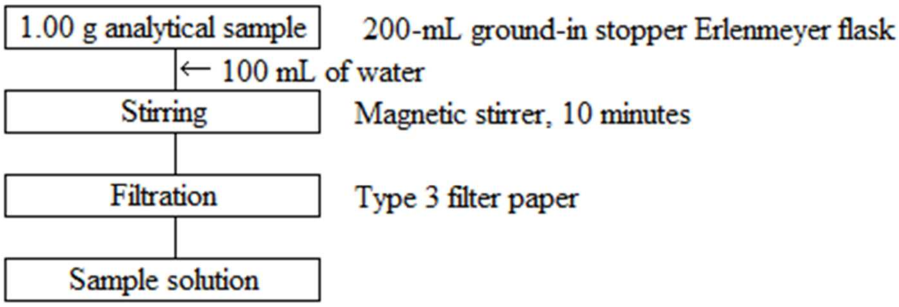


Figure 1 Flow sheet for chroline in potassium sulfate, etc. (Extraction procedure)

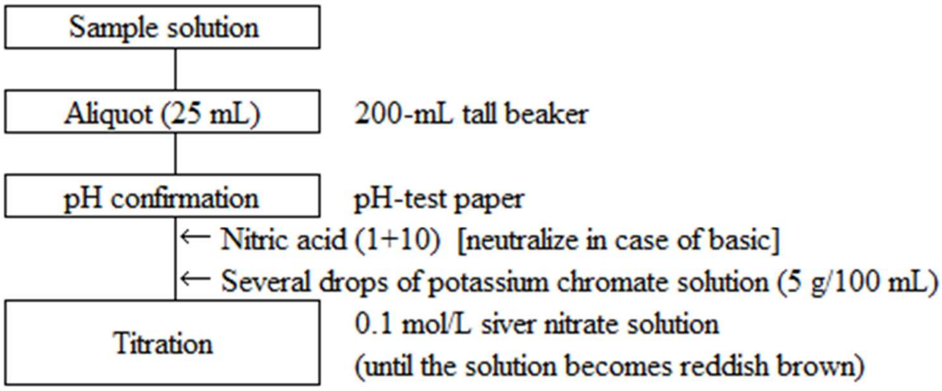


Figure 2 Flow sheet for chroline in potassium sulfate, etc. (Measurement procedure)

6.3 Urea nitrogen

6.3.a Urease method

(1) Summary

The test method is applicable to fertilizers containing urea or urea compounds such as acetaldehyde condensed urea, etc. However, in some cases, it is not applicable to the fertilizers that contain such compounds as nitrolime that digests by heating. This testing method is classified as Type D and its symbol is 6.3.a-2017 or U-N.a-1.

Add water to an analytical sample to extract and add urease to a predetermined amount of the extract to hydrolyze urea into ammonium ion. Add sodium hydroxide to alkalize the solution and subject it to steam distillation. Collect isolated ammonia with 0.25 mol/L sulfuric acid and titrate (neutralize) surplus sulfuric acid using 0.1 mol/L - 0.2 mol/L sodium hydroxide solution and correct separately the titers of urease blank test and urease undigested blank test to obtain urea nitrogen (U-N) in the analytical sample. Or collect isolated ammonia with a boric acid solution and titrate (neutralize) the ammonium ion using 0.25 mol/L sulfuric acid and similarly correct to obtain urea nitrogen (U-N) in the analytical sample. In addition, the performance of this testing method is shown in **Comment 11**.

(2) Reagent: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

W_1 : Mass (g) of sulfamic acid sampled

A : Purity (% (mass fraction)) of sulfamic acid

V_1 : Aliquot volume (mL) of sulfamic acid solution

V_2 : Constant volume (250 mL) of sulfamic acid solution

V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Magnesium oxide**: A JIS Guaranteed Reagent specified in JIS K 8432 or a reagent of equivalent quality.

c) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

d) **0.25 mol/L sulfuric acid** (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Transfer a predetermined amount [(3)] of 0.25 mol/L sulfuric acid to a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L -0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green [(4)]. Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula [(1)]. Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$= V_4/V_5$ ……… (1)

Factor of 0.25 mol/L sulfuric acid (f_2)

$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ ……… (2)

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

e) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.

f) **Urease**: A reagent which completely digests 0.25 g of urea by 0.5 g of urease.

g) **Sodium hydroxide solution (5 g/L)** [(1)]: Dissolve 5 g of sodium hydroxide specified in JIS K 8576 in water to make 1000 mL.

h) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

i) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.

j) **Methyl red solution (0.1 g/100 mL):** Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

k) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.

l) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).

m) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.

n) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).

(**3**) 5 mL -10 mL

(**4**) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

Comment 2 0.25 mol/L sulfuric acid in (**2**) **d**) can be replaced with 0.25 mol/L sulfuric acid conforming to ISO/IEC 17025.

Comment 3 Refined products of urease made from sword beans are commercially sold. The degree of activation may degrade even if stored in a refrigerator. Therefore, it is recommended to test similarly and confirm activation by using urea specified in JIS K 8731 in advance.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Extractor:** A vertical rotating mixer or magnetic stirrer as described below

aa) **Vertical rotating mixer:** A vertical rotating mixer that can vertically rotate a 500-mL volumetric flask at a rate of 30 - 40 revolutions/min.

ab) **Magnetic stirrer**

b) **Steam distillation apparatus**

c) **Distillation flask**: A Kjeldahl flask or a round bottom flask that can be connected to a steam distillation apparatus.

d) **Water bath**: Water bath that can be adjusted to 40 ºC - 45 ºC.

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Vertical rotating mixer**

a) Weigh 5.00 g of an analytical sample, and put it in a 500-mL volumetric flask.

b) Add about 400 mL of water, and shake to mix at 30 - 40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make an extract.

Comment 4 In the procedure in (**4.1.1**) **a**), it is also allowed to weigh 2.50 g of the analytical sample and transfer to a 250-mL volumetric flask. In that case, add about 200 mL of water in the procedure in b).

Comment 5 The procedure in (**4.1.1**) is the same as the procedure in (**4.1.1.1**) in **4.2.4.a**.

(**4.1.2**) **Magnetic stirrer**

a) Weigh 1.00 g of an analytical sample, and put it into a 200- mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) Filter with Type 3 filter paper to make an extract.

Comment 6 The procedure in (**4.1.2**) is the same as the procedure in (**4.1**) in **6.3.c**.

(**4.2**) **Hydrolysis by urease**: Conduct hydrolysis as shown below.

a) Put a predetermined amount of the extract (the equivalents of 10 mg or more as U- N, the equivalents of 10 mg - 100 mg as N) in a 300-mL distillation flask.

b) Add water to the solution to make about 50 mL.

c) Add a few drops of methyl red solution (0.1 g/100 mL), and add a sodium hydroxide solution (5 g/L) or hydrochloric acid (1+200) until the color of the solution becomes light yellowish red (6).

d) Add a sufficient amount of urease to digest urea in the extract (7) (8), seal tightly and heat in the water bath at 40 ºC - 45 ºC.

e) Let it stand to cool to make a sample solution.

f) As an extract blank test, conduct the procedures in **a**) (9) using another distillation flask to prepare an undigested test solution.

g) As a urease blank test, conduct the procedures in **b**), **d**) and **e**) (8) (10) using another distillation flask to prepare a blank test solution.

Note (**6**) pH 5.6 - pH 5.8
(**7**) An example of the additive volume of urease is shown in **Comment 10**.
(**8**) Wash urease off with a small amount of water if it adheres to the surface of a vessel.
(**9**) Aliquot the same amount of the extract as the prepared sample solution.
(**10**) Add the same amount of urease as the preparation of the sample solution used.

(**4.3**) **Distillation**: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Put a predetermined amount (11) of 0.25 mol/L sulfuric acid in an acceptor (12), add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (11) of boric acid solution (40 g/L) in an acceptor (12), add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Add 2 g - 3 g of magnesium oxide to a distillation flask containing the sample solution (13), and connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

f) Subject the undigested test solution to the same procedure as in **a**) - **e**) to obtain the distillate from the undigested test solution.

g) Subject the blank test solution to the same procedure as in **a**) - **e**) to obtain the distillate from the blank test solution.

Note (**11**) 5 mL - 20 mL
(**12**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).
(**13**) Add a small amount of silicone oil as necessary.

Comment 7 Immediately conduct the procedure in (**4.3**) **b**) not to emit ammonia gas in a vessel.

(**4.4**) **Measurement**: Conduct measurement as shown below.

(**4.4.1**) When 0.25 mol/L sulfuric acid is used in (**4.3**):

a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Subject the distillate from the undigested test solution to the same procedure as in **a**) to titrate.

c) Subject the distillate from the blank test solution to the same procedure as in **a**) to titrate.

d) Calculate the urea nitrogen (U-N) in an analytical sample by the following formula:

Urea nitrogen (U-N) (% (mass fraction)) in the analytical sample
$$= ((B \times V_6 - V_7) - (B \times V_6 - V_8) - (B \times V_6 - V_9)) \times C_1 \times f_1 \times (V_{10}/V_{11}) \times (14.007/W_2) \times (100/1000)$$

$= (-(B \times V_6) - (V_7 - V_8 - V_9)) \times C_1 \times f_1 \times (V_{10}/V_{11}) \times (1.4007/W_2)$

B : Volume of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
V_6 : Volume (mL) of 0.25 mol/L sulfuric acid transferred to the acceptor in (**4.3**) **a**)
V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration in (**4.4.1**) **a**)
V_8 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration in (**4.4.1**) **b**)
V_9 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration in (**4.4.1**) **c**)
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_{10} : Constant volume (mL) of the extract in (**4.1.1**) **c**) or additive volume (mL) of water in (**4.1.2**) **b**)
V_{11} : Aliquot volume (mL) of the extract subjected to hydrolysis in (**4.2**) **a**)
W_2 : Mass (g) of the analytical sample in (**4.1.1**) **a**) or (**4.1.2**) **a**)

(**4.4.2**) When a boric acid solution (40 g/L) is used in (**4.3**):

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (14).
b) Subject the distillate from the undigested test solution to the same procedure as in **a**) to titrate.
c) Subject the distillate from the blank test solution to the same procedure as in **a**) to titrate.
d) Calculate the urea nitrogen (U-N) in an analytical sample by the following formula:

Urea nitrogen (U-N) (% (mass fraction)) in the analytical sample
$= (V_{12} - V_{13} - V_{14}) \times C_2 \times 2 \times f_2 \times (V_{10}/V_{11}) \times (14.007/W_2) \times (100/1000)$
$= (V_{12} - V_{13} - V_{14}) \times C_2 \times f_2 \times (V_{10}/V_{11}) \times (2.8014/W_2)$

V_{12} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration in (**4.4.2**) **a**)
V_{13} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration in (**4.4.2**) **b**)
V_{14} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration in (**4.4.2**) **c**)
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid
f_2 : Factor of 0.25 mol/L sulfuric acid
V_{10} : Constant volume (mL) of the extract in (**4.1.1**) **c**) or additive volume (mL) of water in (**4.1.2**) **b**)
V_{11} : Aliquot volume (mL) of the extract subjected to hydrolysis in (**4.2**) **a**)
W_2 : Mass (g) of the analytical sample in (**4.1.1**) **a**) or (**4.1.2**) **a**)

Note (**14**) The endpoint is reached when the color changes from green to light red.

Comment 8 If it is hard to confirm the endpoint due to the carbon dioxide resulting from carbonate in the extract when magnesium oxide is used, it is recommended to boil the extract for 1-2 minute(s) after distilling and cool, and then titrate.

Comment 9 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **d**) **Standardization** and (**4.4**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

Comment 10 An example of the additive volume and titter of urease is shown below.
If the urea content can be estimated, calculate the volume of urea in the aliquot volume of the extract in (**4.2**) **a**) by the following formula.

Estimated urea content (mg) in the transferred volume of the extract
$= (D_1/100) \times W_2 \times (V_{11}/ V_{10})$

D_1 : Estimated urea content (% (mass fraction)) in the analytical sample
V_{10} : Constant volume (mL) of the extract in (**4.1.1**) **c**) or (**4.1.2**) **c**)
V_{11} : Aliquot volume (mL) of the extract subjected to hydrolysis in (**4.2**) **a**)
W_2 : Mass (g) of the analytical sample in (**4.1.1**) **a**) or (**4.1.2**) **a**)

If the urea content cannot be estimated, subtract ammoniacal nitrogen and nitrate nitrogen from the total nitrogen of the permissible content or the display component content of the urea nitrogen in a urea compound to obtain nitrogen content. The obtained nitrogen content is calculated as around the maximum content of urea nitrogen (U-N). In this case, calculate the volume of urea in the transferred volume of the extract in (**4.1.1**) or (**4.2**) **a**) after the procedures in (**4.1.1**) by the following formula.

Estimated urea content (mg) in the transferred volume of the extract
$= (D_2/100) \times (60.056/ (14.007 \times 2)) \times W_2 \times (V_{11}/ V_{10})$
$= (D_2/100) \times 2.1438 \times W_2 \times (V_{11}/ V_{10})$

D_2 : Estimated urea nitrogen (U-N) content (% (mass fraction)) in the analytical sample
V_{10} : Constant volume (mL) of the extract in (**4.1.1**) **c**) or (**4.1.2**) **c**)
V_{11} : Aliquot volume (mL) of the extract subjected to hydrolysis in (**4.2**) **a**)
W_2 : Mass (g) of the analytical sample in (**4.1.1**) **a**) or (**4.1.2**) **a**)

The specification of urease shows that “No more than 0.5 g of urease completely digests 0.25 g of urea”. Therefore about 2 mg of urease is required to digest 1 mg of urea. Assuming that the estimated urea content or the calculated urea content in the transferred volume of the extract is about 43 mg (about 20 mg as urea nitrogen), about 86 mg of urease is required. When adding 0.2 g of urease (potency 130 unit - 150 unit), the extract containing the equivalents of 10 mg - 100 mg as urea nitrogen (U-N) can be hydrolyzed.

In addition, when about 20 mg aliquot is taken as urea nitrogen, the differences between the titer of the distillate from the sample solution ((**4.4.1**) **a**) or (**4.4.2**) **a**)) and the titer of the distillate from the undigested test solution ((**4.4.1**) **b**) or (**4.4.2**) **b**)) are estimated as follows: About 14 mL, when a 0.1 mol/L sodium hydroxide solution is used as a titrant, about 7 mL, when 0.2 mol/L sodium hydroxide is used as a titrant and about 3 mL, when 0.25 mol/L sulfuric acid is used as a titrant.

Comment 11 Additive recovery testing was conducted to evaluate trueness using samples prepared by adding urea to sulfur, a compound fertilizer, isobutyraldehyde condensed urea, a compound fertilizer containing formaldehyde processed urea (one sample for each). As a result, the mean recovery rate at the additive level of 1.58 % (mass fraction) - 39.96 % (mass fraction) was 98.7 % to 103.7 %
The results of the repeatability tests on different days using urea and a compound fertilizer containing UF (one sample for each), to evaluate precision were analyzed

by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

The minimum limit of quantification of this testing method was estimated to be about 0.4 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of urea nitrogen

Name of sample	Test days of repeatability (T)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Urea	5	43.17	0.36	0.8	0.43	1.0
Compound fertilizer containing UF	5	2.39	0.07	2.9	0.12	5.2

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.56- 59, Yokendo, Tokyo (1988)

2) Toshifumi FUJITA, Kimie KATO, Toyokazu NIIMI, Yasuharu KIMURA, Kohei ITO and Yuji SHIRAI: Determination Method for Urea Nitrogen in Fertilizer by Urease, Research Report of Fertilizer, **Vol. 10**, p. 195 - 207 (2017)

(5) **Flow sheet for urea nitrogen testing method**: The flow sheets for urea nitrogen test method in fertilizers are shown below:

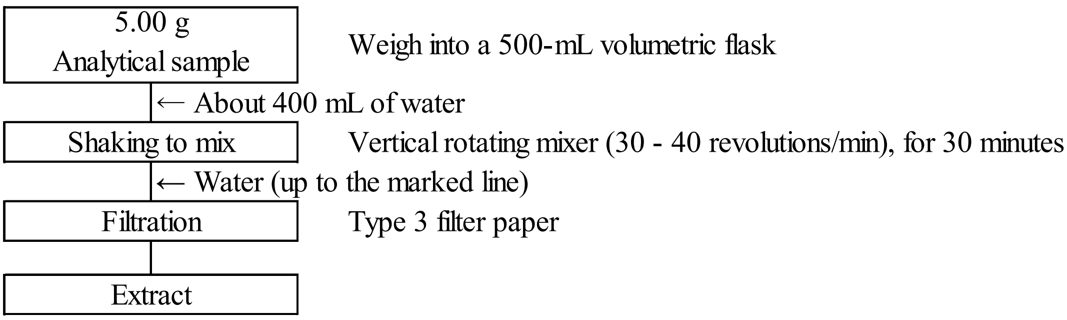


Figure 1-1 Flow sheet for urea nitrogen (Extraction procedure (4.1.1))

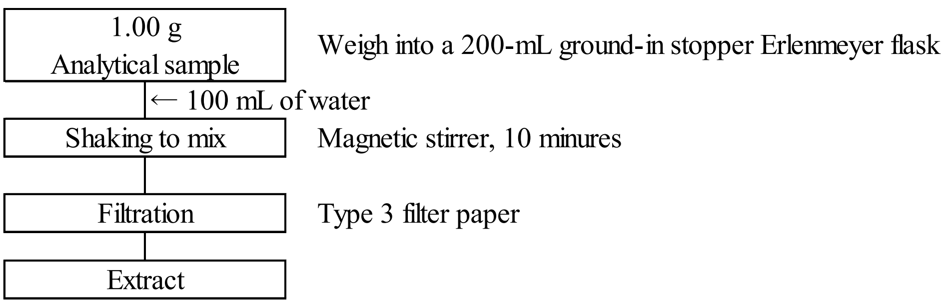


Figure 1-2 Flow sheet for urea nitrogen (Extraction procedure (4.1.2))

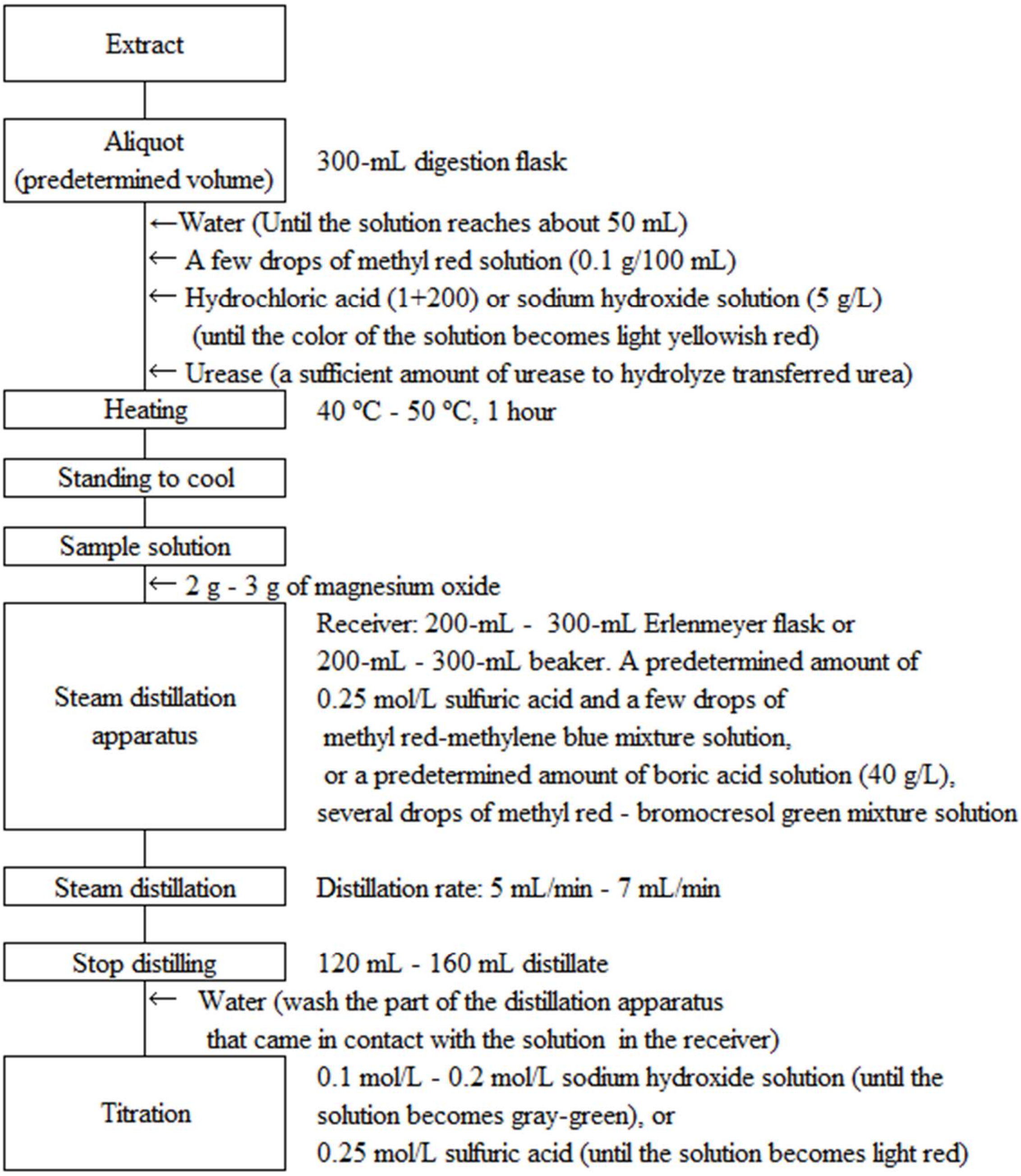


Figure 2 Flow sheet for urea nitrogen
(Hydrolysis by urease, distillation and measurement procedure)

6.3.b High-Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 6.3.b-2017 or U-N.b-1.

Add water to an analytical sample to extract urea, introduce it to a High-Performance Liquid Chromatograph (HPLC) to isolate it with a weak acid ion-exchange column, and then measure at a wavelength 190 nm to obtain urea nitrogen (U-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

Biuret nitrogen (B-N), dicyandiamide nitrogen (Dd-N), guanidine urea (Gd-N) and guanylurea nitrogen (GU-N) can be simultaneously quantified by using this method. (Refer to **Comment 4**).

(2) Reagent, etc.: Reagents and water are as shown below.

- **a) Water**: Water of A3 specified in JIS K 0557.
- **b) Potassium dihydrogen phosphate**: A JIS Guaranteed Reagent specified in JIS K 9007 or a reagent of equivalent quality.
- **c) Phosphoric acid**: A JIS Guaranteed Reagent specified in JIS K 9005 or a reagent of equivalent quality.
- **d) Urea nitrogen standard solution (U-N 2 mg/mL)** (1): Put 0.429 g of urea specified in JIS K 8731 in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.
- **e) Urea nitrogen standard solution for the calibration curve preparation (U-N 200 µg/mL)**: Put 10 mL of urea nitrogen standard solution (U-N 2 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.
- **f) Urea nitrogen standard solution (U-N 50 µg/mL - 100 µg/mL)**: Put 25 mL - 50 mL of urea nitrogen standard solution (U-N 200 µg/mL) in 100-mL volumetric flasks and add water up to the marked line.
- **g) Urea nitrogen standard solution for the calibration curve preparation (U-N 1 µg/mL - 50 µg/mL)**: At the time of usage, put 1 mL - 50 mL of urea nitrogen standard solution (U-N 0.1 mg/mL) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

- **a) High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.
  - **1) Column**: A 7.5-mm inner diameter 100-mm long stainless steel column tube filled with weak acid ion-exchange resin.
  - **2) Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45ºC.
  - **3) Detection unit**: An absorptiometric detector that can measure at a wavelength around 190 nm.
- **b) Magnetic stirrer**
- **c) High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 1 A column is sold under the production name Asahipak ES-502C 7C, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL-ground-in stopper Erlenmeyer flask.
b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.
c) After allowing to stand still, put a supernatant solution (2) in a 1.5-mL ground-in stopper centrifugal precipitate tube (3).
d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (4) and use the supernatant as a sample solution.

Note **(2)** If there is a possibility that the urea nitrogen (U-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of supernatant with water.
(3) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.
(4) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

(4.1.2) Fluid test sample

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.
b) Add about 50 mL of water, and shake to mix.
c) Add water up to the marked line (5) and put it in a 1.5-mL ground-in stopper centrifugal precipitate tube (3).
d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (4) and use the supernatant as a sample solution.

Note **(5)** If there is a possibility that the urea nitrogen (U-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of precisely adjusted solution with water.

Comment 2 Instead of procedures in (**4.1.1**) **c**) - **d**) or (**4.1.2**) **c**) - **d**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions is shown below. Set up the measurement conditions considering it:
1) **Column**: A weak acid ion-exchange resin column (4.0-mm - 7.5-mm inner diameter, 100-mm - 150-mm long, 5-µm – 10-µm particle diameter)
2) **Column bath temperature**: 40 °C
3) **Eluent** (1): Dissolve 3.92 g of potassium dihydrogen phosphate and 0.12 g of phosphoric acid in water to make 1000 mL. Filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.
4) **Flow rate**: 0.6 mL/min
5) **Injection volume**: 10 µL
6) **Detection unit**: An absorptiometric detector, a measurement wavelength: 190 nm

Comment 3 The eluent can be prepared as follows. Dissolve 19.6 g of potassium dihydrogen phosphate and 0.584 g of phosphoric acid with water to make 500 mL and store in a refrigerator. At the time of usage, dilute a predetermined volume of the solution by a factor of 10 and filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.

b) Calibration curve preparation

1) Inject 10 µL of respective standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 190 nm and obtain a peak height.

2) Prepare a curve for the relationship between the urea nitrogen (U-N) concentration and the peak height at a wavelength 190 nm of the respective standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain the urea nitrogen (U-N) content from the peak height using the calibration curve to calculate the urea nitrogen (U-N) in the analytical sample.

Comment 4 This testing method enables the simultaneous measurement of biuret nitrogen (B-N), urea nitrogen (U-N), dicyandiamide nitrogen (Dd-N), guanidine urea (Gd-N) and guanylurea nitrogen (GU-N). In that case, see **5.10.a Comment 5**.

When Asahipak ES-502C 7C is used as a column for HPLC to analyze urea nitrogen (U-N) in a compound fertilizer containing formaldehyde processed urea (UF), the peak of the urea nitrogen (U-N) and the peak of the impurity components originating from UF are not separated, so that urea nitrogen (U-N) cannot be measured. In this case, the peak of the urea nitrogen (U-N) and the peak of the impurity components originating from UF are separated by using PRP-X200 as a column for HPLC instead to be able to analyze the urea nitrogen(U-N) in the compound fertilizer containing UF. However, when the PRP-X200 column is used as a column for HPLC, the simultaneous measurement of urea nitrogen (U-N) and biuret nitrogen (B-N), etc. is impossible.

Comment 5 Additive recovery testing was conducted using one brand of an acetaldehyde condensed urea fertilizer, a compound fertilizer, a blended fertilizer, a fluid compound fertilizer and a home garden-use compound fertilizer respectively. As a result, the mean recovery rates at the additive level of 6 % (mass fraction) and 3 % (mass fraction) and 0.6 % (mass fraction) were 98.3 % - 102.9 %, 98.9 % - 105.2 % and 92.3 % - 99.9 % respectively.

The results of the repeatability tests on different days using a blended fertilizer, a compound fertilizer and a home garden-use compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.03 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of urea nitrogen

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Blended fertilizer	5	6.24	0.03	0.5	0.05	0.8
Compound fertilzier	5	3.01	0.03	0.7	0.04	1.4
Home garden-use compound fertilizer	5	0.315	0.003	0.9	0.005	0.9

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Statistical analysis results of the collaborative study for the test method validation of urea nitrogen

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Compound fertilizer 1	8	0.296	0.011	3.6	0.012	4.1
Compound fertilizer 2	10	0.589	0.015	2.6	0.024	4.1
Compound fertilizer 3	10	3.08	0.04	1.1	0.06	2.0
Compound fertilizer 4	10	6.03	0.11	1.7	0.20	3.4
Compound fertilizer 5	10	46.5	0.6	1.4	1.3	2.8

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masahiro ECHI, Yasuharu KIMURA and Yuji SHIRAI: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography: A Single-Laboratory Validation, Research Report of Fertilizer, **Vol. 10**, p. 72 - 85 (2017)

2) Norio FUNAKI and Yasuharu KIMURA: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer, **Vol. 10**, p. 86 - 100 (2017)

(**5**) **Flow sheet for testing method**: An example of the flow sheet for urea nitrogen in fertilizers is shown below:

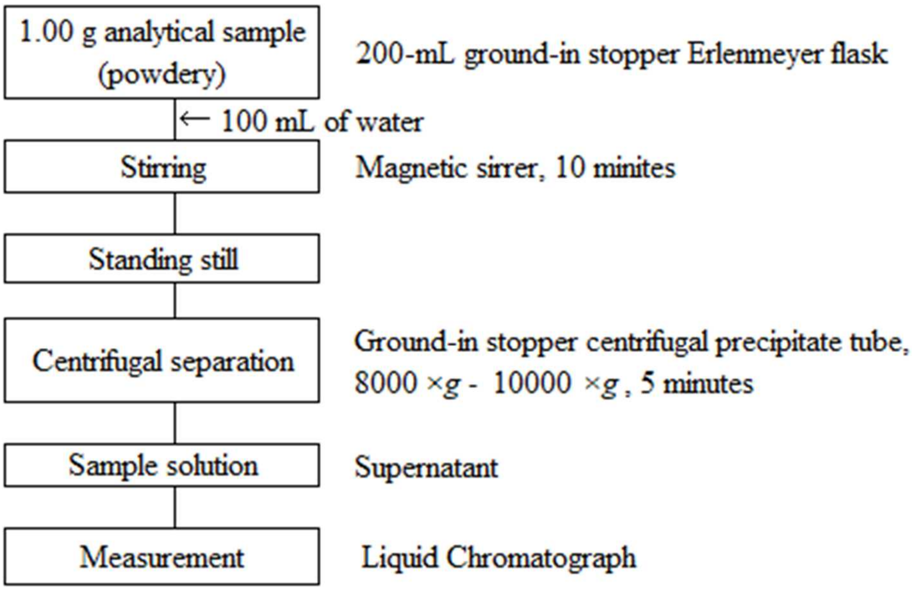


Figure 1 Flow sheet for urea nitrogen in fertilizers (Extraction procedure (4.1.1) and measurement)

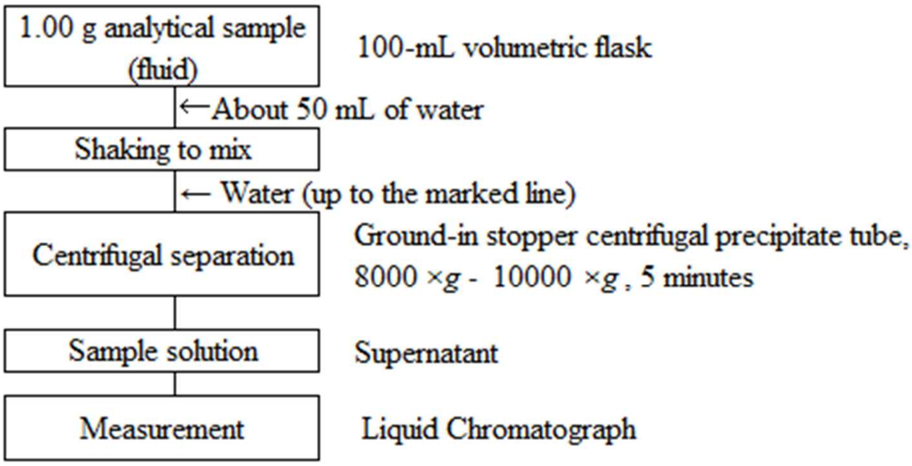
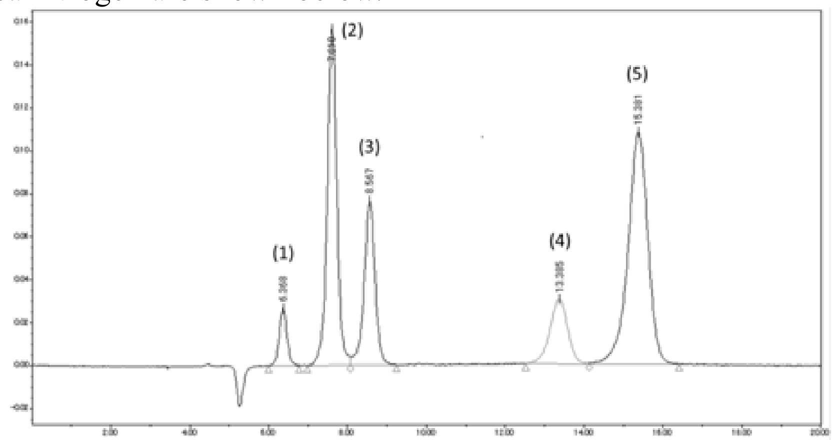


Figure 2 Flow sheet for urea nitrogen in fertilizers (Extraction procedure (4.1.2) and measurement)

Reference: The chromatograms of the standard solution for calibration curve preparation of urea nitrogen are shown below.



Reference diagram 1 HPLC chromatogram of the mixture standard solutions (10 mg/L for each) for calibration curve preparation

Peak name

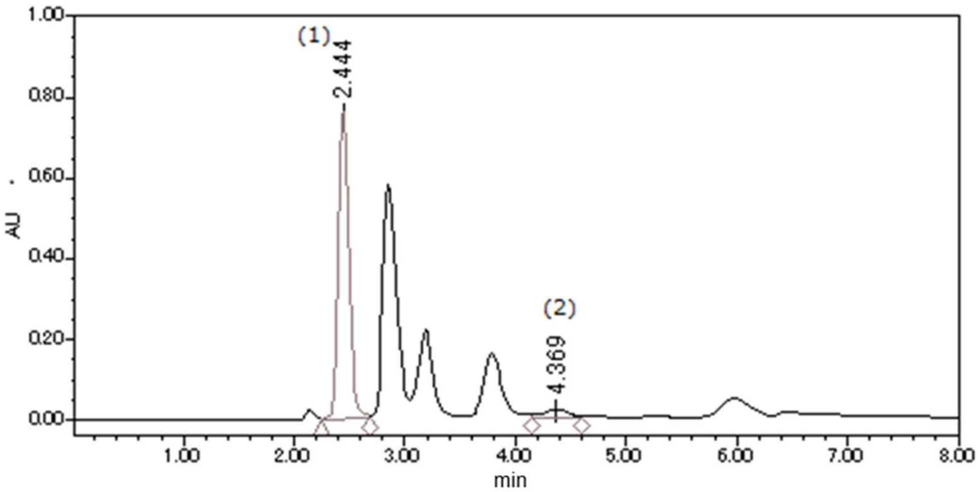
(1) Urea nitrogen (2) Biuret nitrogen (3) Dicyandiamide nitrogen

(4) Guanidine nitrogen (5) Guanylurea nitrogen

Measurement conditions for HPLC

Column: Asahipak ES-502C 7C (7.5-mm inner diameter, 100-mm long, 9-µm particle diameter)

Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**)



Reference diagram 2 HPLC chromatogram of the urea nitrogen standard solutions (20 mg/L) and a formaldehyde processed urea fertilizer for calibration curve preparation

Peak name

(1) Urea nitrogen (2) Biuret nitrogen

Measurement conditions for HPLC

Column: PRP-X200 (4.1-mm inner diameter, 150-mm long, 10-µm particle diameter)

Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**)

6.3.c *p*-dimethylaminobenzaldehide absorptiometry

(1) Summary

The testing method is applicable to fertilizers containing urea nitrogen. However, isobutyraldehyde condensed urea fertilizers, formaldehyde processed urea fertilizers, nitrolime, sludge fertilizers, etc., and special fertilizers are excluded. This testing method is classified as Type D and its symbol is 6.3.c-2018 or U-N.c-1.

Add water to an analytical sample to extract urea and measure the coloration formed by the reaction with dimethylaminobenzaldehyde by absorbance to obtain urea nitrogen (U-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Coloring reagent solution** (1): Dissolve 20 g of *p*-dimethylaminobenzaldehyde specified in JIS K 8101 in 1000 mL of ethanol (99.5) specified in JIS K 8101 and 100 mL of hydrochloric acid specified in JIS K 8180 and leave at rest overnight (2).

c) **Urea nitrogen standard solution (U-N 2 mg/mL)** (1): Put 0.429 g of urea specified in JIS K 8731 in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.

d) **Urea nitrogen standard solution for the calibration curve preparation (U-N 200 µg/mL)** (1): Put 10 mL of urea nitrogen standard solution (U-N 2 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) Store in an amber bottle.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Spectrophotometer**: A spectrophotometer specified in JIS K 0115

b) **Magnetic stirrer**

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200-mL Erlenmeyer flask.

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, filter with Type 3 filter paper to make a sample solution.

Comment 1 The procedure in (**4.1**) is the same as the procedure in (**4.1.2**) of **6.3.a**.

Comment 2 When the determination is affected by the coloring of the sample solution of (**4.1**) **c**), add about 0.5 g of active carbon and filter with Type 3 filter paper to make a sample solution from which the coloring disappears.

(**4.2**) **Coloring**: Conduct coloring as shown below.

a) Put a predetermined amount of the sample solution (the equivalents of 0.5 mg - 5 mg as U-N) in a 50-mL volumetric flask.

b) Add 20 mL of coloring reagent solution, and further add water up to the marked line, and then leave at rest for about 30 minutes.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0115 and as shown below. Specific measurement procedures are according to the operation method of the spectrophotometer used for the measurement.

a) **Measurement conditions of the spectrophotometer**: Set up the measurement conditions of the spectrophotometer considering the following.
Detection wavelength: 450 nm

b) **Calibration curve preparation**
1) Put 2.5 mL - 25 mL of urea nitrogen standard solution (U-N 200 µg/mL) in 50-mL volumetric flasks step-by-step.
2) Conduct the same procedures as (**4.2**) **b**) to make U-N 0.5 mg/50 mL - 5 mg/50 mL of urea nitrogen standard solutions for the calibration curve preparation.
3) Conduct the same procedures as **2**) for another 50-mL volumetric flask to make a blank test solution for the calibration curve preparation.
4) Measure absorbance at a wavelength of 450 nm of the urea nitrogen standard solutions for the calibration curve preparation using the blank test solution for the calibration curve preparation as the control.
5) Prepare a curve for the relationship between the urea nitrogen (U-N) concentration and the absorbance of the urea nitrogen standard solutions for the calibration curve preparation.

c) **Sample measurement**
1) Regarding the solution in (**4.2**) **b**), measure absorbance by the same procedure as **b**) **4**).
2) Obtain the urea nitrogen (U-N) content from the calibration curve to calculate the urea nitrogen (U-N) in the analytical sample.

Comment 3 Additive recovery testing was conducted using a compound fertilizer, crustacea grade fertilizer powdery and a preparation sample. As a result, the mean recovery rates at the additive level of 20 % (mass fraction), 10 % (mass fraction) and 3 % (mass fraction) were 100.0 % - 102.4 %, 100.5 % - 102.0 % and 98.0 % - 103.3 % as urea nitrogen (U-N) respectively.
The results of the repeatability tests on different days using urea, a designated blended fertilizer and a compound fertilizer to evaluate precision were analyzed by the one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method is about 0.2 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of urea nitrogen

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Urea	7	45.9	0.89	1.9	0.91	2.0
Designated blended fertilizer	7	7.45	0.16	2.1	0.20	2.7
Compound fertilizer	7	1.12	0.02	2.2	0.03	2.9

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.60- 62, Yokendo, Tokyo (1988)

2) Shinei TAKAHASHI: Determination of urea nitrogen in Fertilizer by Spectrometry, Research Report of Fertilizer, Vol. 11, p. 54 – 62 (2018)

(**5**) **Flow sheet for testing method**: The flow sheet for urea nitrogen in fertilizers is shown below:

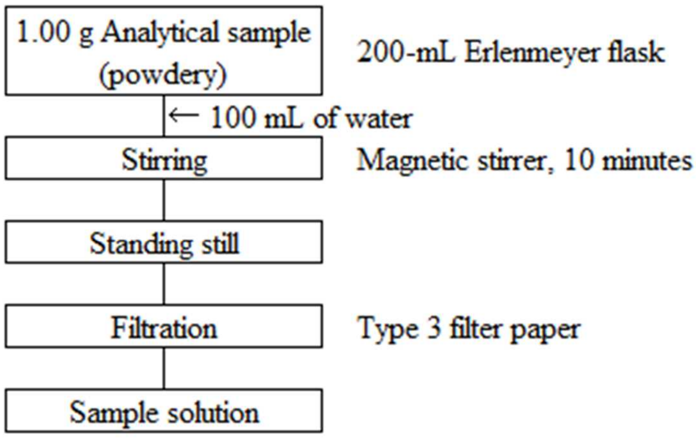


Figure 1 Flow sheet for urea nitrogen in fertilizers (Extraction procedure)

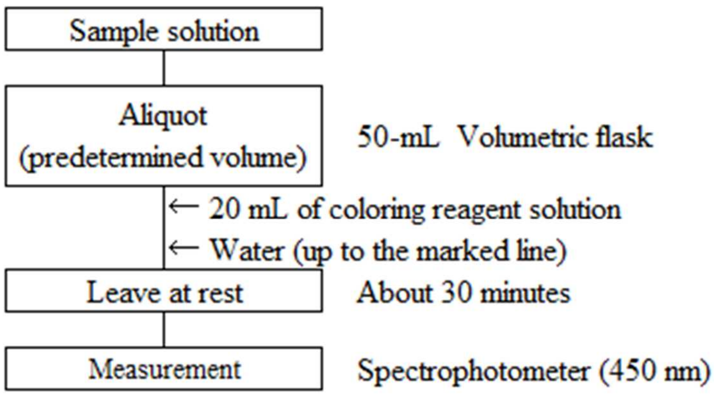


Figure 2 Flow sheet for urea nitrogen in fertilizers (Measurement procedure)

6.4 Guanidine nitrogen

6.4.a High-Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 6.4.a-2017 or Gd-N.a-1.

Add water to an analytical sample to extract guanidine, introduce it to a High-Performance Liquid Chromatograph (HPLC) to isolate it with a weak acid ion-exchange column, and then measure at a wavelength 190 nm to obtain guanidine nitrogen (Gd-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

Biuret nitrogen (B-N), dicyandiamide nitrogen (Dd-N), urea nitrogen(U-N) and guanylurea nitrogen (GU-N) can be simultaneously quantified by using this method. (Refer to **Comment 5**).

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Potassium dihydrogen phosphate**: A JIS Guaranteed Reagent specified in JIS K 9007 or a reagent of equivalent quality.

c) **Phosphoric acid**: A JIS Guaranteed Reagent specified in JIS K 9005 or a reagent of equivalent quality.

d) **Guanidine nitrogen standard solution (Gd-N 2 mg/mL)** (1): Put 0.515 g of guanidine sulfate [$C_2H_{10}N_6 \cdot H_2SO_4$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.

e) **Guanidine nitrogen standard solution for the calibration curve preparation (Gd-N 200 µg/mL)** (1): Put 10 mL of guanidine nitrogen standard solution (Gd-N 2 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

f) **Guanidine nitrogen standard solution (Gd-N 50 µg/mL - 100 µg/mL)** (1): Put 25 mL - 50 mL of guanidine nitrogen standard solution (Gd-N 200 µg/mL) in 100-mL volumetric flasks and add water up to the marked line.

g) **Guanidine nitrogen standard solution for the calibration curve preparation (Gd-N 1 µg/mL - 50 µg/mL)** (1): At the time of usage, put 1 mL - 50 mL of guanidine nitrogen standard solution (Gd-N 100 µg/mL) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % (mass fraction) in purity as guanidine sulfate is commercially sold.

Comment 1 Guanidine sulfate is commercially sold by FUJIFILM Wako Pure Chemical Co., Ltd., Kanto Chemical Co., Inc. and Tokyo Chemical Industry Co., Ltd.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 7.5-mm inner diameter 100-mm long stainless steel column tube filled with weak acid ion-exchange resin.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 190 nm.

b) **Magnetic stirrer**

c) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* -

10000 × *g*.

Comment 2 A column is sold under the production name Asahipak ES-502C 7C, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, put a supernatant solution (3) in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (5) and use the supernatant as a sample solution.

Note **(3)** If there is a possibility that the guanidine nitrogen (Gd-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of supernatant with water.

(4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(5) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

(4.1.2) Fluid test sample

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water (6), and shake to mix.

c) Add water up to the marked line and put it in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

d) Centrifuge it at 8000 × g - 10000 × g centrifugal force for about five minutes (5) and use the supernatant as a sample solution.

Note **(6)** If there is a possibility that the guanidine nitrogen (Gd-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of precisely adjusted solution with water.

Comment 3 Instead of procedures in **(4.1.1) c) - d)** or **(4.1.2) c) - d)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5 µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) **Column**: A weak acid ion-exchange resin column (4.0-mm - 7.5-mm inner diameter, 100-mm - 150-mm long, 5-µm – 10-µm particle diameter)

2) **Column bath temperature**: 40 °C

3) **Eluent** (1): Dissolve 3.92 g of potassium dihydrogen phosphate and 0.12 g of phosphoric acid in water to make 1000 mL. Filter with a membrane filter (aperture diameter: no more than 0.5 µm) made of hydrophilic PTFE.

4) **Flow rate**: 0.6 mL/min

5) **Injection volume**: 10 µL

6) **Detection unit**: An absorptiometric detector, a measurement wavelength: 190 nm

Comment 4 Eluent can be prepared as follows. Dissolve 19.6 g of potassium dihydrogen phosphate and 0.584 g of phosphoric acid with water to make 500 mL and store in a refrigerator. At the time of usage, dilute a predetermined volume of the solution by a factor of 10 and filter with a membrane filter (aperture diameter: no more than 0.5 µm) made of hydrophilic PTFE.

b) Calibration curve preparation

1) Inject 10 µL of respective standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 190 nm and obtain a peak height.

2) Prepare a curve for the relationship between the guanidine nitrogen (Gd-N) concentration and the peak height at a wavelength 190 nm of the respective standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b**) **1**).

2) Obtain the guanidine nitrogen (Gd-N) content from the peak height using the calibration curve to calculate the guanidine nitrogen (Gd-N) in the analytical sample.

Comment 5 This testing method enables the simultaneous measurement of biuret nitrogen (B-N), urea nitrogen (U-N), dicyandiamide nitrogen (Dd-N), guanidine nitrogen (Gd-N) and guanylurea nitrogen (GU-N). In that case, see **5.10.a Comment 5**.

Comment 6 Additive recovery testing was conducted using a preparation sample for a guanylurea fertilizer (one brand). As a result, the mean recovery rates at the additive level of 3.71 % (mass fraction), 1.85 % (mass fraction) and 0.371 % (mass fraction) were 91.2 %, 94.0 % and 100.0 % respectively.

The results of the repeatability tests on different days using a guanylurea fertilizer to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.02 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of guanidine nitrogen

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Guanylurea fertilizer	5	1.81	0.01	0.8	0.04	2.0

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Results and analysis results from a collaborative study for the test method validation of guanidine nitrogen

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Compound fertilizer 1	12	4.91	0.18	3.7	0.29	5.8
Compound fertilizer 2	12	3.94	0.16	4.2	0.27	6.8
Compound fertilizer 3	11	3.03	0.06	2.0	0.12	4.0
Compound fertilizer 4	11	2.05	0.05	2.6	0.09	4.2
Guanylurea fertilizer	11	5.13	0.21	4.0	0.19	3.6

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masahiro ECHI, Yasuharu KIMURA and Yuji SHIRAI: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography: A Single-Laboratory Validation, Research Report of Fertilizer, **Vol. 10**, p. 72 - 85 (2017)

2) Norio FUNAKI and Yasuharu KIMURA: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer, **Vol. 10**, p. 86 - 100 (2017)

(**5**) **Flow sheet for testing method**: An example of the flow sheet for guanidine nitrogen in fertilizers is shown below:

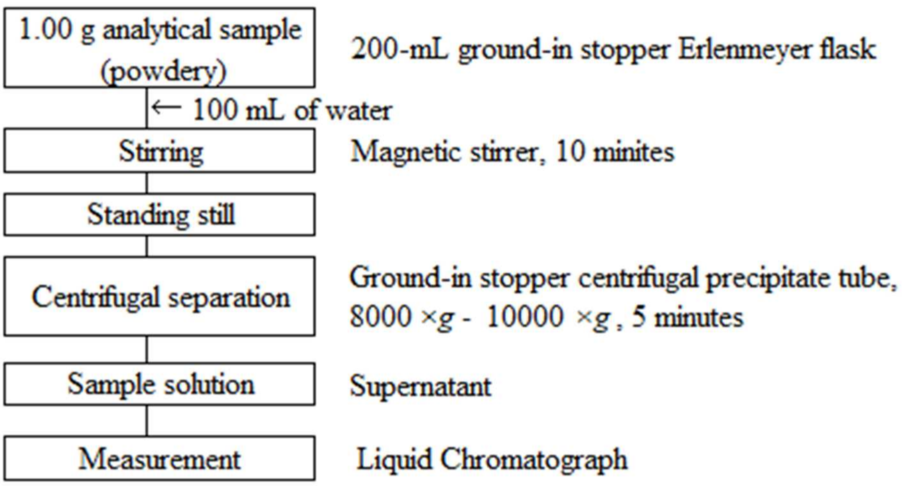


Figure 1 Flow sheet for guanidine nitrogen in fertilizers (Extraction procedure (4.1.1) and measurement)

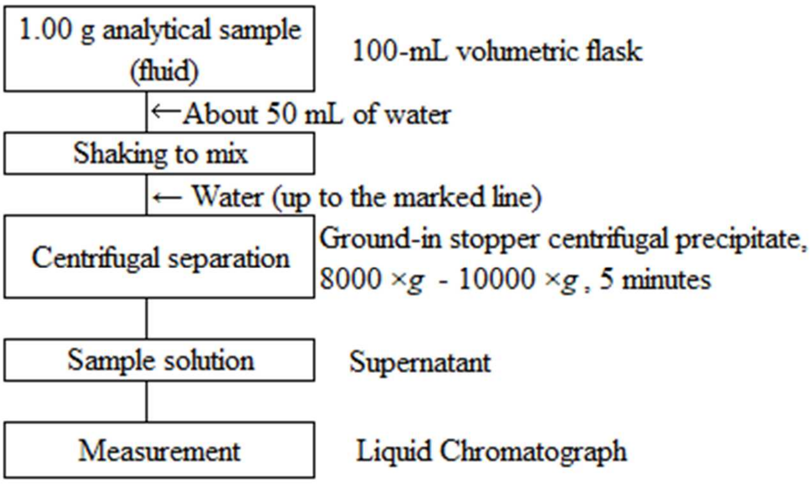
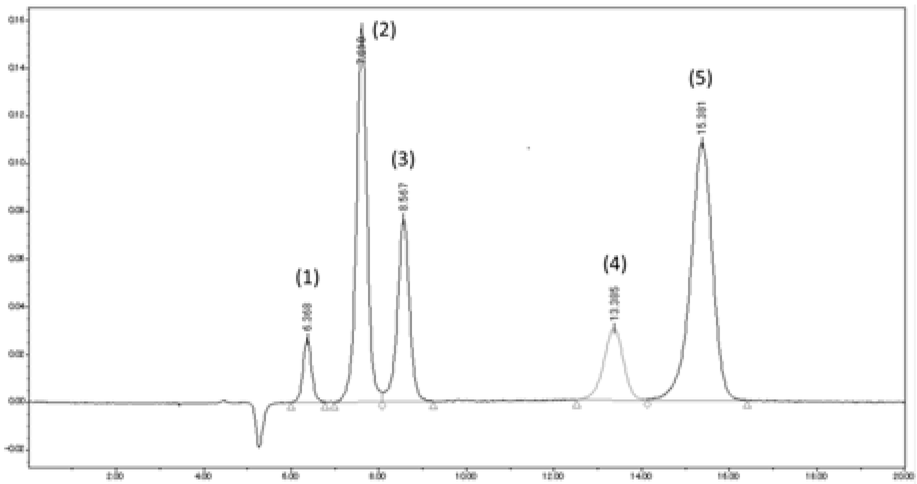


Figure 2 Flow sheet for guanidine nitrogen in fertilizers (Extraction procedure (4.1.2) and measurement)

Reference: The chromatogram of the standard solution for calibration curve preparation of guanidine nitrogen is shown below.



Reference diagram HPLC chromatogram of the mixture standard solutions (10 mg/L for each)

Peak name
(1) Urea nitrogen (2) Biuret nitrogen (3) Dicyandiamide nitrogen
(4) Guanidine nitrogen (5) Guanylurea nitrogen

Measurement conditions for HPLC
Column: Asahipak ES-502C 7C (7.5-mm inner diameter, 100-mm long, 9-µm particle diameter)
Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**)

6.5 Cold buffer solution soluble nitrogen (water-soluble nitrogen)
6.5.a Cold buffer solution method
(1) Summary

The test method is applicable to formaldehyde processed urea fertilizers. This testing method is classified as Type A (Def-M) and its symbol is 6.5.a-2017 or Buf(C)-N.a-1.

Add a phosphate solution (cold buffer solution) to an analytical sample to extract, add copper (II) sulfate pentahydrate, sulfuric acid and potassium sulfate, pretreat by the Kjeldahl method to change cold buffer solution soluble nitrogen to ammonium ion and add a sodium hydroxide solution to subject to steam distillation. Collect isolated ammonia with 0.25 mol/L sulfuric acid and measure surplus sulfuric acid by (neutralization) titration using a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain cold buffer solution soluble nitrogen (water-soluble nitrogen) in the analytical sample. Or collect isolated ammonia with a boric acid solution and measure ammonium ion by (neutralization) titration using 0.25 mol/L sulfuric acid to obtain cold buffer solution soluble nitrogen (water-soluble nitrogen) in the analytical sample.

(2) Reagent: Reagents are as shown below.

a) 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

- W_1 : Mass (g) of sulfamic acid sampled
- A : Purity (% (mass fraction)) of sulfamic acid
- V_1 : Aliquot volume (mL) of sulfamic acid solution
- V_2 : Constant volume (250 mL) of sulfamic acid solution
- V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
- C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) Sulfuric acid: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) 0.25 mol/L sulfuric acid (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Put a predetermined amount (3) of 0.25 mol/L sulfuric acid in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L -0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4). Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$= V_4/V_5$ ………. (1)

Factor of 0.25 mol/L sulfuric acid (f_2)

$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2)$ ………. (2)

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.
e) **Sodium hydroxide solution (200 g/L - 500 g/L)** (1): Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.
f) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.
g) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.
h) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.
i) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).
j) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.
k) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).
l) **Phosphate solution**: Dissolve 3.63 g of potassium dihydrogen phosphate specified in JIS K 9007 and 5.68 g of disodium hydrogen phosphate specified in JIS K 9020 in 1000 mL of water (5). When it is used, adjust the liquid temperature at about 25 ºC (cold buffer solution).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).
(**3**) 5 mL -10 mL
(**4**) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.
(**5**) pH 7.0 ± pH 0.2

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

Comment 2 0.25 mol/L sulfuric acid in (**2**) **c**) can be replaced with 0.25 mol/L sulfuric acid conforming to ISO/IEC 17025.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) Vertical rotating mixer: A vertical rotating mixer that can vertically rotate a 250-mL volumetric flask at a rate of 30 - 40 revolutions/min.

b) Steam distillation apparatus

c) Digestion flask: A Kjeldahl flask which can be connected to a steam distillation apparatus.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample (6), and put it into a 250 mL- Erlenmeyer flask.

b) Add about 200 mL of phosphate solution and shake to mix at the rate of 30 - 40 revolutions/min for about 30 minutes.

c) Add water up to the marked line.

d) Filter with Type 3 filter paper to make an extract.

Note (**6**) Prepare the test sample according to **Comment 3**.

Comment 3 Crush a laboratory sample with a mortar or a pestle, etc. until it completely passes through an 850 μm aperture sieve.

Comment 4 If there is no possibility for an analytical sample to hydrolyze, it is allowed to use water instead of a phosphoric acid solution.

Comment 5 The temperature of a solution in the procedure in (**4.1**) **b**) - **d**) should be no more than 26 ºC.

(**4.2**) **Kjeldahl digestion**: Conduct digestion as shown below.

a) Put a predetermined amount of the sample solution (the equivalents of 0.5 g or less as cold buffer solution soluble nitrogen) in a 300-mL distillation flask.

b) Add 0.1 g of copper (II) sulfate pentahydrate (7) specified in JIS K 8962, and further add 5 mL of sulfuric acid, shake to mix and heat gently to evaporate moisture.

c) After standing to cool, add 1 g of potassium sulfate specified in JIS K 8962 and heat to digest.

d) Ignite further for 30 minutes.

e) After standing to cool, add water while shaking to mix until liquid volume reaches about 30 mL and cool it to make a digestion solution.

Note (**7**) Crush into powder as appropriate.

(4.3) Distillation: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Put a predetermined amount (8) of 0.25 mol/L sulfuric acid in an acceptor, (9) add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (8) of boric acid solution (40 g/L) in an acceptor, (9) add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Add a proper amount of sodium hydroxide solution (200 g/L - 500 g/L) (10), and immediately connect this digestion flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (**8**) 5 mL - 20 mL

(**9**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).

(**10**) An amount sufficient to make the solution strong alkalinity. A blue color will appear.

(**4.4**) **Measurement**: Conduct measurement as shown below.

(**4.4.1**) When 0.25 mol/L sulfuric acid is used in (**4.3**):

a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Calculate the cold buffer solution soluble nitrogen in the analytical sample by the following formula:

Cold buffer solution soluble nitrogen (% (mass fraction)) in the analytical sample

$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (14.007/W_2) \times (100/1000)$$

$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (1.4007/W_2)$$

B : Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

V_6 : Volume (mL) of 0.25 mol/L sulfuric acid put in the acceptor in (**4.2**) **a**)

V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

V_8 : Constant volume (mL) of the extract in (**4.1**) **c**)

V_9 : Aliquot volume (mL) of the extract subjected to the Kjeldahl digestion in (**4.2**) **a**)

W_2 : Mass (g) of the analytical sample

(**4.4.2**) When a boric acid solution (40 g/L) is used in (**4.3**):

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (11).

b) Calculate the cold buffer solution soluble nitrogen in the analytical sample by the following formula:

Cold buffer solution soluble nitrogen (% (mass fraction)) in the analytical sample

$$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_2) \times (100/1000)$$

$$= V_{10} \times C_2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_2)$$

V_{10} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration

C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

f_2 : Factor of 0.25 mol/L sulfuric acid

V_{11} : Constant volume (mL) of the extract in (**4.1**) **c**)

V_{12} : Aliquot volume (mL) of the extract subjected to the Kjeldahl digestion in (**4.2**) **a**)

W_2 : Mass (g) of the analytical sample

Note (**11**) The endpoint is reached when the color changes from green to light red.

Comment 6 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **c**) **Standardization** and (**4.4**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.67- 68, Yokendo, Tokyo (1988)

(**5**) **Flow sheet for cold buffer solution soluble nitrogen testing method**: The flow sheets for cold buffer solution soluble nitrogen testing method in formaldehyde processed urea fertilizers are shown below:

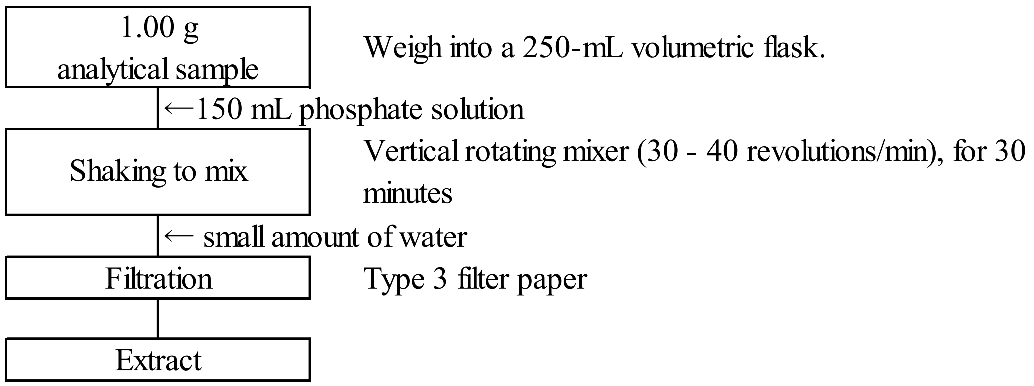


Figure 1 Flow sheet for cold buffer solution soluble nitrogen testing method in formaldehyde processed urea fertilizers
(Extraction procedure)

Extract

Aliquot (predetermined volume) — 300-mL digestion flask

← 0.1 g copper (II) sulfate pentahydrate

← 5 mL sulfuric acid

Heating — Gently

Standing to cool

← 1 g potassium sulfate

Heating — After digestion, ignite further for 30 minutes

Standing to cool

← Water (until liquid volume reaches about 30 mL)

Cooling

Digestion solution

Figure 2 Flow sheet for cold buffer solution soluble nitrogen testing method in formaldehyde processed urea fertilizers (Kjeldahl digestion procedure)

Digestion solution

← Sodium hydroxide solution (200 g/L - 500 g/L)

Steam distillation apparatus — Receiver: 200-mL - 300 - mL Erlenmeyer flask or beaker
A predetermined amount of 0.25 mol/L sulfuric acid and a few drops of methyl red-methylene blue mixture solution, or a predetermined amount of boric acid solution (40 g/L), several drops of methyl red - bromocresol green mixture solution

Steam distillation — Distillation rate: 5 mL/min -7 mL/min

Stop distilling — 120 mL – 160 mL distillate

← Water (wash the part of the distillation apparatus that came in contact with the solution in the receiver)

Titration — 0.1 mol/L-0.2 mol/L sodium hydroxide solution (until the solution becomes gray-green), or 0.25 mol/L sulfuric acid (until the solution becomes light red)

Figure 3 Flow sheet for cold buffer solution soluble nitrogen testing method in formaldehyde processed urea fertilizers (Distillation and measurement procedure)

6.6 Heat buffer solution soluble nitrogen (hot-water soluble nitrogen)

6.6.a Heat buffer solution method

(1) Summary

The testing method is applicable to methylolurea polymerization fertilizers. This testing method is classified as Type A(Def-M) and its symbol is 6.6.a-2017 or Buf(H)-N.a-1.

Add a heat phosphate solution (heat buffer solution) to an analytical sample to elute heat buffer solution soluble nitrogen, add copper (II) sulfate pentahydrate, sulfuric acid and potassium sulfate, pretreat non-dissolved matter by Kjeldahl method to change heat buffer solution soluble nitrogen to ammonium ion and add a sodium hydroxide solution to subject to steam distillation. Collect isolated ammonia with 0.25 mol/L sulfuric acid and measure surplus sulfuric acid by (neutralization) titration using a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain the nitrogen which is insoluble in a heat buffer solution in the analytical sample. Or collect isolated ammonia with a boric acid solution and measure ammonium ion by (neutralization) titration using 0.25 mol/L sulfuric acid to obtain the nitrogen which is insoluble in a heat buffer solution in the analytical sample. Subtract the nitrogen which is insoluble in a heat buffer solution from the separately obtained total nitrogen (T-N) according to the method in **4.1.1** to calculate the nitrogen which is soluble in a heat buffer solution (hot water-soluble nitrogen).

(2) Reagent: Reagents are as shown below.

a) 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4 - 5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

W_1 : Mass (g) of sulfamic acid sampled
A : Purity (% (mass fraction)) of sulfamic acid
V_1 : Aliquot volume (mL) of sulfamic acid solution
V_2 : Constant volume (250 mL) of sulfamic acid solution
V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) Sulfuric acid: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) 0.25 mol/L sulfuric acid (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Put a predetermined amount (3) of 0.25 mol/L sulfuric acid in a 200-mL

- 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L -0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green [(4)]. Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula (1). Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$$= V_4/V_5 \quad \cdots\cdots\cdots \quad (1)$$

Factor of 0.25 mol/L sulfuric acid (f_2)

$$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2) \quad \cdots\cdots\cdots \quad (2)$$

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.

e) **Catalyst** [(5)]: Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate [(6)] specified in JIS K 8983 in the ratio of 9 to 1.

f) **Sodium hydroxide solution (200 g/L - 500 g/L)** [(1)]: Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.

g) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.

h) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

i) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.

j) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).

k) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.

l) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

m) **Heat phosphate solution**: Dissolve 3.63 g of potassium dihydrogen phosphate specified in JIS K 9007 and 5.68 g of disodium hydrogen phosphate specified in JIS K 9020 in 1000 mL of water [(7)]. When it is used, heat until it reaches boiling point (heat buffer solution).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).

(**3**) 5 mL - 10 mL

(**4**) The endpoint is reached when the color becomes gray-green via dark blue from blue-purple.

(**5**) A tablet is commercially available.

(**6**) Crush into powder as appropriate.

(**7**) pH 7.0 ± pH 0.2

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.

Comment 2 0.25 mol/L sulfuric acid in (**2**) **c**) can be replaced with 0.25 mol/L sulfuric acid conforming to ISO/IEC 17025.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Water bath**: A water bath which can boil water.

b) **Steam distillation apparatus**

c) **Digestion flask**: A Kjeldahl flask which can be connected to a steam distillation apparatus.

d) **Distillation flask**: A Kjeldahl flask or round bottom flask that can be connected to a steam distillation apparatus.

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample (8) and put it in a 300-mL tall beaker.

b) Add 100 mL of heat phosphate solution and stir it gently.

c) Cover the tall beaker with a watch glass, and heat in a boiling water bath while stirring at ten-minute intervals for about 30 minutes.

d) Immediately filter with Type 3 filter paper, transfer the whole non-dissolved matter in a vessel on the filter paper with 100 mL of heat phosphate solution and wash the non-dissolved matter with hot water.

Note (**8**) Prepare the test sample according to **Comment 3**.

Comment 3 Crush a laboratory sample with a mortar or a pestle, etc. until it completely passes through an 850 μm aperture sieve.

(**4.2**) **Kjeldahl digestion**: Conduct digestion as shown below.

a) Put the non-dissolved matter in (**4.1**) **d**) together with the filter paper into a 300-mL - 500-mL digestion flask.

b) Add 5 g - 10 g of catalyst, and further add 20 mL - 40 mL of sulfuric acid, shake to mix and heat gently.

c) After bubbles cease to form, heat until white smoke of sulfuric acid evolves.

d) Ignite until organic matters are completely digested (9).

e) After standing to cool, add a small amount of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water (10), and further mix by shaking.

f) After standing to cool, add water up to the marked line to make a digestion solution.

Note (**9**) When the solution has finished changing color, heat further for no less than 2 hours.

(**10**) When the entire sample solution volume is used in measurement, it is not necessary to transfer it to a volumetric flask.

(**4.3**) **Distillation**: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Put a predetermined amount (11) of 0.25 mol/L sulfuric acid in an acceptor (12), add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (11) of boric acid solution (40 g/L) in an acceptor (12), add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Put a predetermined amount of the digestion solution in a 300-mL distillation flask, add a proper amount of sodium hydroxide solution (200 g/L - 500 g/L) (13), and immediately connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (**11**) 5 mL - 20 mL

(**12**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).

(**13**) An amount sufficient to make the solution strong alkalinity. A blue color will appear.

(**4.4**) **Measurement**: Conduct measurement as shown below.

(**4.4.1**) When 0.25 mol/L sulfuric acid is used in (**4.3**):

a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Calculate the heat buffer solution soluble nitrogen in the analytical sample by the following formula:

c) Subtract the nitrogen which is insoluble in a heat buffer solution from the separately obtained total nitrogen (T-N) according to the method in **4.1.1** to calculate the nitrogen which is soluble in a heat buffer solution (14).

Heat buffer solution soluble nitrogen (% (mass fraction)) in the analytical sample

$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (14.007/W_2) \times (100/1000)$$
$$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (1.4007/W_2)$$

B : Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

V_6 : Volume (mL) of 0.25 mol/L sulfuric acid put in the acceptor in (**4.2**) **a**)

V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

V_8 : Constant volume (mL) of the digestion solution in (**4.2**) **f**)

V_9 : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.3**) **b**)

W_2 : Mass (g) of the analytical sample

Note (**14**) The total nitrogen (T-N) and the nitrogen which is insoluble in a heat buffer solution use raw data without rounding numerical value.

(**4.4.2**) When a boric acid solution (40 g/L) is used in (**4.3**):

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (15).

b) Calculate the heat buffer solution soluble nitrogen in the analytical sample by the following formula:

c) Subtract the nitrogen which is insoluble in a heat buffer solution from the separately obtained total nitrogen (T-N) according to the method in **4.1.1** to calculate the nitrogen which is soluble in a heat buffer solution

Heat buffer solution soluble nitrogen (% (mass fraction)) in the analytical sample

$$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_2) \times (100/1000)$$

$$= V_{10} \times C_2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_2)$$

V_{10} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration

C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

f_2 : Factor of 0.25 mol/L sulfuric acid

V_{11} : Constant volume (mL) of the digestion solution in (**4.2**) **f**)

V_{12} : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.3**) **b**)

W_2 : Mass (g) of the analytical sample

Note (**15**) The endpoint is reached when the color changes from green to light red.

Comment 4 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **c**) **Standardization** and (**4.4**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.68- 69, Yokendo, Tokyo (1988)

(5) **Flow sheet for heat buffer solution soluble nitrogen testing method**: The flow sheets for heat buffer solution soluble nitrogen testing method in methylolurea polymerization fertilizers are shown below:

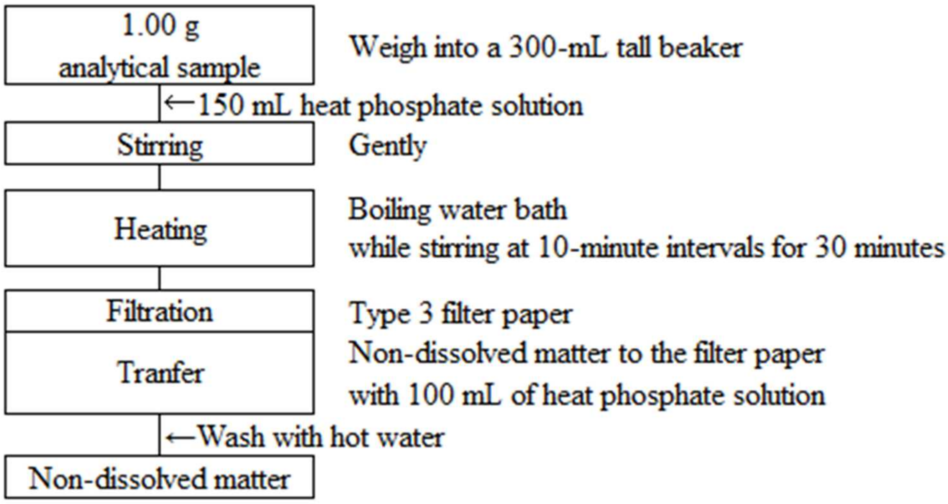


Figure 1 Flow sheet of the testing method for heat buffer solution soluble nitrogen in methylolurea polymerization fertilizers (Extraction procedure)

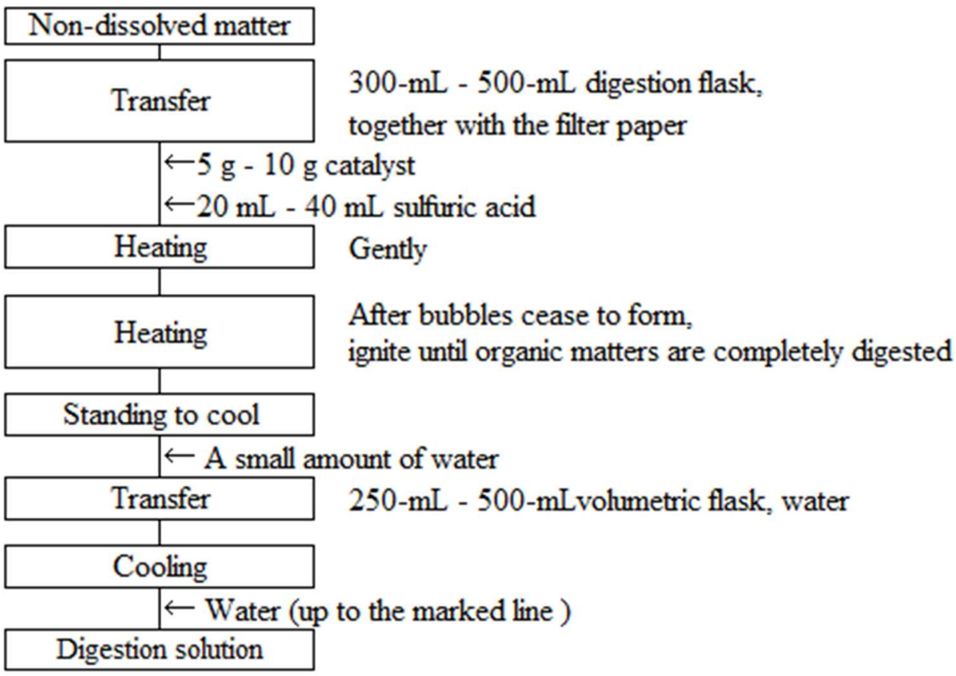


Figure 2 Flow sheet of the testing method for heat buffer solution soluble nitrogen in methylolurea polymerization fertilizers (Kjeldahl digestion procedure)

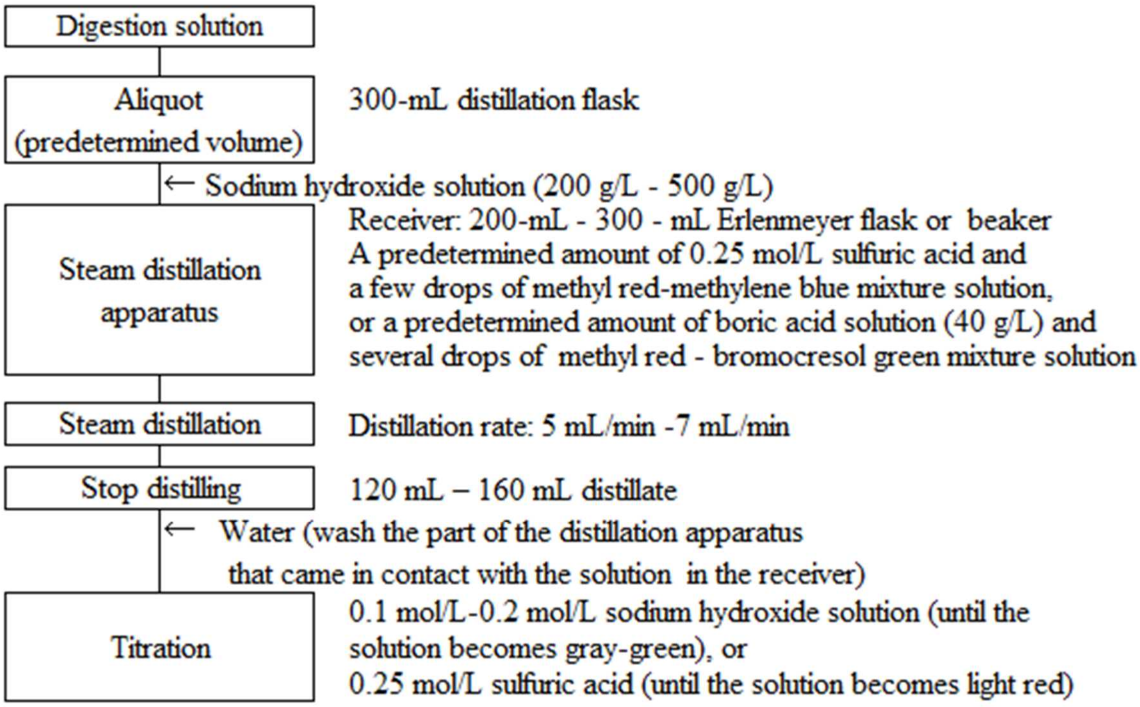


Figure 3 Flow sheet of the testing method for heat buffer solution soluble nitrogen in methylolurea polymerization fertilizers (Distillation and measurement procedure)

6.7 Activity coefficient of nitrogen

6.7.a Buffer solution method

(1) Summary

The testing method is applicable to formaldehyde processed urea fertilizers. This testing method is classified as Type A(Def-M) and its symbol is 6.7.a-2017 or AI-N.a-1.

Add water to an analytical sample to elute cold water-soluble nitrogen, add potassium sulfate copper (II) sulfate pentahydrate and sulfuric acid, pretreat non-dissolved matter by Kjeldahl method to change cold water-soluble nitrogen to ammonium ion and add a sodium hydroxide solution to subject to steam distillation. Collect isolated ammonia with 0.25 mol/L sulfuric acid and measure surplus sulfuric acid by (neutralization) titration using a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution to obtain cold water insoluble nitrogen in the analytical sample. Or collect isolated ammonia with a boric acid solution and measure ammonium ion by (neutralization) titration using 0.25 mol/L sulfuric acid to obtain cold water-soluble nitrogen in the analytical sample. Separately add a heat phosphate solution (heat buffer solution) to an analytical sample to elute the nitrogen which is soluble in a heat buffer solution and conduct the same procedures as above to obtain the nitrogen which is insoluble in a heat buffer solution in the analytical sample. Subtract the nitrogen which is insoluble in a heat buffer solution from cold water non-dissolved matter and calculate the activity coefficient of nitrogen by dividing the subtracted value by the cold water non-dissolved matter.

(2) Reagent: Reagents are as shown below.

a) **0.1 mol/L - 0.2 mol/L sodium hydroxide solution** (1): Put about 30 mL of water in a polyethylene bottle, dissolve about 35 g of sodium hydroxide specified in JIS K 8576 by adding in small portions while cooling, seal tightly and leave at rest for 4-5 days. Put 5.5 mL -11 mL of the supernatant in a ground-in stoppered storage container, and add 1000 mL of water.

Standardization: Dry sulfamic acid reference material for volumetric analysis specified in JIS K 8005 by leaving at rest in a desiccator at no more than 2 kPa for about 48 hours, then put about 2.5 g in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve in a small amount of water, transfer to a 250-mL volumetric flask, and add water up to the marked line (1). Put a predetermined amount of the solution in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of bromothymol blue solution (0.1 g/100 mL) as an indicator, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes green. Calculate the factor of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution by the following formula:

Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution (f_1)

$$= (W_1 \times A \times 0.01/97.095) \times (V_1/V_2) \times (1000/V_3) \times (1/C_1)$$

- W_1 : Mass (g) of sulfamic acid sampled
- A : Purity (% (mass fraction)) of sulfamic acid
- V_1 : Aliquot volume (mL) of sulfamic acid solution
- V_2 : Constant volume (250 mL) of sulfamic acid solution
- V_3 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
- C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

b) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.

c) **0.25 mol/L sulfuric acid** (1) (2): Add about 14 mL of sulfuric acid to a beaker containing 100 mL of water in advance, stir well, and add water to make 1000 mL.

Standardization: Put a predetermined amount [(3)] of 0.25 mol/L sulfuric acid in a 200-mL - 300-mL Erlenmeyer flask, add a few drops of methyl red−methylene blue mixture solution, and titrate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green [(4)]. Calculate the volume of a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid by the following formula [(1)]. Or, calculate the factor of 0.25 mol/L sulfuric acid by the following formula (2):

Volume (B) of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid

$$= V_4/V_5 \qquad \cdots\cdots\cdots \quad (1)$$

Factor of 0.25 mol/L sulfuric acid (f_2)

$$= (f_1 \times C_1 \times V_4/V_5)/(C_2 \times 2) \qquad \cdots\cdots\cdots \quad (2)$$

V_4 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration

V_5 : Volume (mL) of 0.25 mol/L sulfuric acid subjected to standardization

C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution

C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid

d) **Boric acid solution (40 g/L)**: Dissolve 40 g of boric acid specified in JIS K 8863 in water to make 1000 mL.

e) **Catalyst** [(5)]: Mix potassium sulfate specified in JIS K 8962 and copper (II) sulfate pentahydrate [(6)] specified in JIS K 8983 in the ratio of 9 to 1.

f) **Sodium hydroxide solution (200 g/L - 500 g/L)** [(1)]: Dissolve 100 g - 250 g of sodium hydroxide specified in JIS K 8576 in water to make 500 mL.

g) **Bromothymol blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of bromothymol blue specified in JIS K 8842 in 20 mL of ethanol (95) specified in JIS K 8102, add water to make 100 mL.

h) **Methyl red solution (0.1 g/100 mL)**: Dissolve 0.1 g of methyl red specified in JIS K 8896 in 100 mL of ethanol (95) specified in JIS K 8102.

i) **Methylene blue solution (0.1 g/100 mL)**: Dissolve 0.1 g of methylene blue specified in JIS K 8897 in 100 mL of ethanol (95) specified in JIS K 8102.

j) **Methyl red−methylene blue mixture solution**: To 2 volumes of methyl red solution (0.1 g/100 mL), add 1 volume of methylene blue solution (0.1 g/100 mL).

k) **Bromocresol green solution (0.5 g/100 mL)**: Dissolve 0.5 g of bromocresol green specified in JIS K 8840 in 100 mL of ethanol (95) specified in JIS K 8102.

l) **Methyl red−bromocresol green mixture solution**: To a methyl red solution (0.1 g/100 mL), add equal volume of bromocresol green solution (0.5 g/100 mL).

m) **Heat phosphate solution**: Dissolve 1.43 g of potassium dihydrogen phosphate specified in JIS K 9007 and 9.10 g of dipotassium hydrogen phosphate specified in JIS K 9020 in 1000 mL of water [(7)]. When it is used, heat until it reaches boiling point (heat buffer solution).

Note **(1)** This is an example of preparation; prepare an amount as appropriate.

(2) This corresponds to the standard sulfuric acid solution 0.5 M (1/2 sulfuric acid) solution in the Official Methods of Analysis of Fertilizers (1992).

(3) 5 mL -10 mL

(4) The endpoint is reached when the color becomes gray-green via dark blue from blue-

purple.
(**5**) A tablet is commercially available.
(**6**) Crush into powder as appropriate.
(**7**) pH 7.5 ± pH 0.2

Comment 1 A 0.1 mol/L - 0.2 mol/L sodium hydroxide solution in (**2**) **a**) can be replaced with a 0.1 mol/L sodium hydroxide solution or a 0.2 mol/L sodium hydroxide solution conforming to ISO/IEC 17025.
Comment 2 0.25 mol/L sulfuric acid in (**2**) **c**) can be replaced with 0.25 mol/L sulfuric acid conforming to ISO/IEC 17025.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.
a) **Water bath**: A water bath which can boil water.
b) **Steam distillation apparatus**
c) **Digestion flask**: A Kjeldahl flask which can be connected to a steam distillation apparatus.
d) **Distillation flask**: A Kjeldahl flask or round bottom flask that can be connected to a steam distillation apparatus.

(**4**) **Test procedures**
(**4.1**) **Extraction**: Conduct extraction as shown below. Subject the non-dissolved matters of (**4.1.1**) **f**) and (**4.1.2**) **d**) to (**4.2**) **Kjeldahl digestion** respectively.
(**4.1.1**) **Extraction by cold water**
a) Weigh 1.00 g of an analytical sample (8) and put it in a 50-mL beaker.
b) Add a small amount of ethanol specified in JIS K 8101 to moisten and add 20 mL of water at 25ºC ± 2ºC to stir.
c) Leave at rest for 15 minutes while stirring at 5-minute intervals.
d) Filter the supernatant with Type 2 filter paper
e) Wash non-dissolved matter with water at 25ºC ± 2ºC five times and filter the supernatant.
f) Transfer the whole non-dissolved matter on the filter paper using water at 25ºC ± 2ºC and wash the non-dissolved matter using water at the same temperature until filtrate reaches 250 mL.

Note (**8**) Prepare the test sample according to **Comment 3**.

Comment 3 Crush a laboratory sample with a mortar or a pestle, etc. until it completely passes through an 850 µm aperture sieve.

(**4.1.2**) **Extraction by heat phosphate solution**
a) Weigh an analytical sample (8) equivalent to 0.12 g of cold non-resolved matter and put it in a 200-mL tall beaker.
b) Add 100 mL of heat phosphate solution and stir it.
c) Cover the tall beaker with a watch glass, and heat in a boiling water bath while stirring at ten-minute intervals for about 30 minutes.
d) Immediately filter with Type 2 filter paper (9), transfer the whole non-dissolved matter in a vessel on the filter paper using boiling water and wash the non-dissolved matter using 100 mL of boiling water.

Note (**9**) If it takes 4 minutes or more for filtrating, conduct extracting anew according to **Comment 4**.

Comment 4 After conducting the procedures in (**4.1.2**) **a**) - **c**), add 1 g of diatomaceous earth to stir and conduct the procedure in (**4.1.2**) **d**).

(**4.2**) **Kjeldahl digestion**: Conduct digestion as shown below.

a) Put the non-dissolved matter in (**4.1.1**) **f**) and (**4.1.2**) **d**) together with the filter paper into respective 300-mL - 500-mL digestion flasks.

b) Add 5 g - 10 g of catalyst, and further add 20 mL - 40 mL of sulfuric acid, shake to mix and heat gently.

c) After bubbles cease to form, heat until white smoke of sulfuric acid evolves.

d) Ignite until organic matters are completely digested (10).

e) After standing to cool, add a small amount of water, mix well by shaking, transfer to a 250-mL - 500-mL volumetric flask with water (11), and further mix by shaking.

f) After cooling is complete, add water up to the marked line to make a digestion solution.

Note (**10**) When the solution has finished changing color, heat further for no less than 2 hours.

(**11**) When the entire sample solution volume is used in measurement, it is not necessary to transfer it to a volumetric flask.

(**4.3**) **Distillation**: Conduct distillation as shown below. Specific distillation procedures are according to the operation method of the steam distillation apparatus used in measurement.

a) Put a predetermined amount (12) of 0.25 mol/L sulfuric acid in an acceptor (13), add a few drops of methyl red−methylene blue mixture solution, and connect this acceptor to a steam distillation apparatus. Or, put a predetermined amount (12) of boric acid solution (40 g/L) in an acceptor (13), add a few drops of methyl red−bromocresol green mixture solution, and connect this acceptor to a steam distillation apparatus.

b) Put a predetermined amount of the digestion solution in a 300-mL distillation flask, add a proper amount of sodium hydroxide solution (200 g/L - 500 g/L) (14), and immediately connect this distillation flask to the steam distillation apparatus.

c) Send steam to the distillation flask to heat the solution in the distillation flask, and distill at a distillation rate of 5 mL/min - 7 mL/min.

d) Stop distilling when the distillate has reached 120 mL - 160 mL.

e) Wash the part of the steam distillation apparatus that came in contact with the solution in the acceptor with a small amount of water, and pool the washing with the distillate.

Note (**12**) 5 mL - 20 mL

(**13**) As an acceptor, use a 200-mL - 300-mL Erlenmeyer flask or a 200-mL - 300-mL beaker with which the distillate outlet of the steam distillation apparatus can be immersed in 0.25 mol/L sulfuric acid or a boric acid solution (40 g/L).

(**14**) An amount sufficient to make the solution strong alkalinity. A blue color will appear.

(**4.4**) **Measurement**: Conduct measurement as shown below.

(**4.4.1**) When 0.25 mol/L sulfuric acid is used in (**4.3**):

a) Titrate the distillate with a 0.1 mol/L - 0.2 mol/L sodium hydroxide solution until the color of the solution becomes gray-green (4).

b) Calculate the cold-water insoluble nitrogen (N_1) and the nitrogen which is insoluble in a heat buffer solution (N_2) in the analytical sample by the following formula (3):

c) Obtain the activity coefficient of nitrogen in the analytical sample by the following formula (4) (15).

Cold water insoluble nitrogen (N_1) or the nitrogen which is insoluble in a heat buffer

solution (N_2) (% (mass fraction)) in the analytical sample
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (14.007/W_2) \times (100/1000)$
$= (B \times V_6 - V_7) \times C_1 \times f_1 \times (V_8/V_9) \times (1.4007/W_2)$ ・・・・・ (3)

B : Volume of 0.1 mol/L -0.2 mol/L sodium hydroxide solution equivalent to 1 mL of 0.25 mol/L sulfuric acid
V_6 : Volume (mL) of 0.25 mol/L sulfuric acid put in the acceptor in (**4.2**) **a**)
V_7 : Volume (mL) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution needed for titration
C_1 : Set concentration (mol/L) of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
f_1 : Factor of 0.1 mol/L - 0.2 mol/L sodium hydroxide solution
V_8 : Constant volume (mL) of the digestion solution in (**4.2**) **f**)
V_9 : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.3**) **b**)
W_2 : Mass (g) of the analytical sample

Activity coefficient of nitrogen (%)
$= ((N_1 - N_2)/N_1) \times 100$ ・・・・・ (4)

N_1 : Cold water insoluble nitrogen (% (mass fraction))
N_2 : Heat buffer solution insoluble nitrogen (% (mass fraction))

Note (**15**) Cold water insoluble nitrogen (N_1) or the nitrogen which is insoluble in a heat buffer solution (N_2) uses raw data without rounding numerical value.

(**4.4.2**) When a boric acid solution (40 g/L) is used in (**4.3**):

a) Titrate the distillate with 0.25 mol/L sulfuric acid until the color of the solution becomes light red (16).
b) Calculate the cold-water insoluble nitrogen (N_1) and the nitrogen which is insoluble in a heat buffer solution (N_2) in the analytical sample by the following formula (5):
c) Obtain the activity coefficient of nitrogen in the analytical sample by the formula (4) in (**4.4.1**) (14).

Cold water insoluble nitrogen (N_1) or the nitrogen which is insoluble in a heat buffer solution (N_2) (% (mass fraction)) in the analytical sample
$= V_{10} \times C_2 \times 2 \times f_2 \times (V_{11}/V_{12}) \times (14.007/W_2) \times (100/1000)$
$= V_{10} \times C_2 \times f_2 \times (V_{11}/V_{12}) \times (2.8014/W_2)$ ・・・・・ (3)

V_{10} : Volume (mL) of 0.25 mol/L sulfuric acid needed for titration
C_2 : Set concentration (0.25 mol/L) of 0.25 mol/L sulfuric acid
f_2 : Factor of 0.25 mol/L sulfuric acid
V_{11} : Constant volume (mL) of the digestion solution in (**4.2**) **f**)
V_{12} : Aliquot volume (mL) of the digestion solution subjected to distillation in (**4.3**) **b**)
W_2 : Mass (g) of the analytical sample

Note (**16**) The endpoint is reached when the color changes from green to light red.

Comment 5 The titration procedures in (**2**) **a**) **Standardization**, (**2**) **c**) **Standardization** and

(**4.4**) can be conducted by an automatic titrator. The setup of the titration program, the determination parameter for the endpoint and vessels such as acceptors are according to the specification and the operation method of the automatic titrator used.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.68- 69, Yokendo, Tokyo (1988)

(5) **Flow sheet for the activity coefficient of nitrogen testing method**: The flow sheets for the activity coefficient of nitrogen testing method in formaldehyde processed urea fertilizers are shown below:

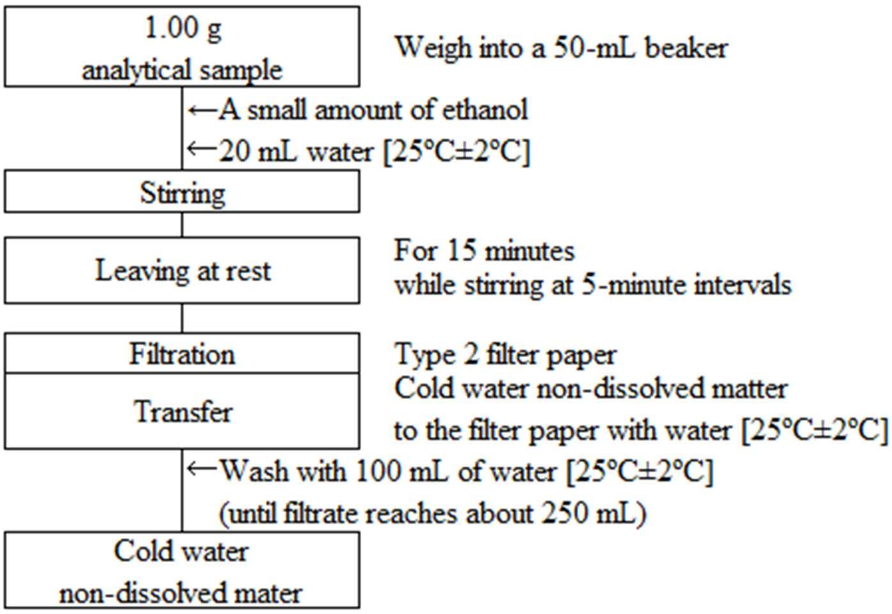


Figure 1-1 Flow sheet of the testing method for the activity coefficient of nitrogen in formaldehyde processed urea fertilizers (Extraction procedure by cold water (4.1.1))

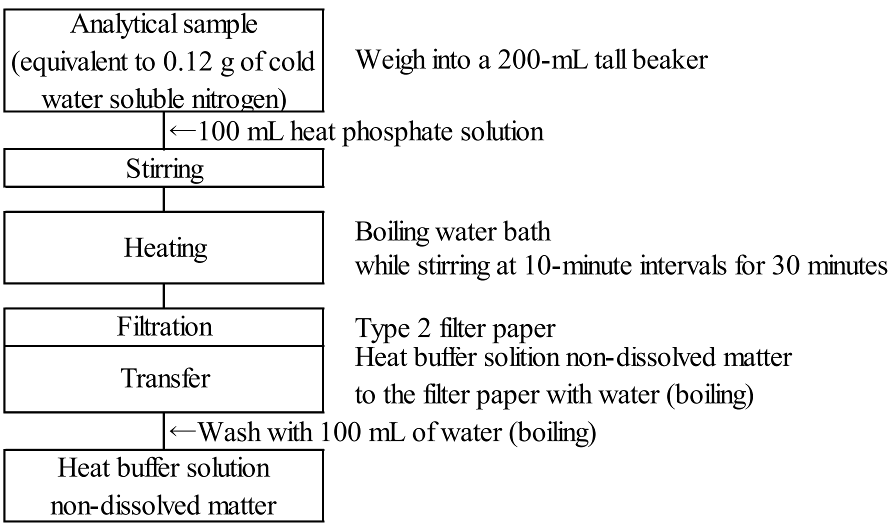


Figure 1-2 Flow sheet of the testing method for the activity coefficient of nitrogen in formaldehyde processed urea fertilizers (Extraction procedure by heat phosphate solution (4.1.2))

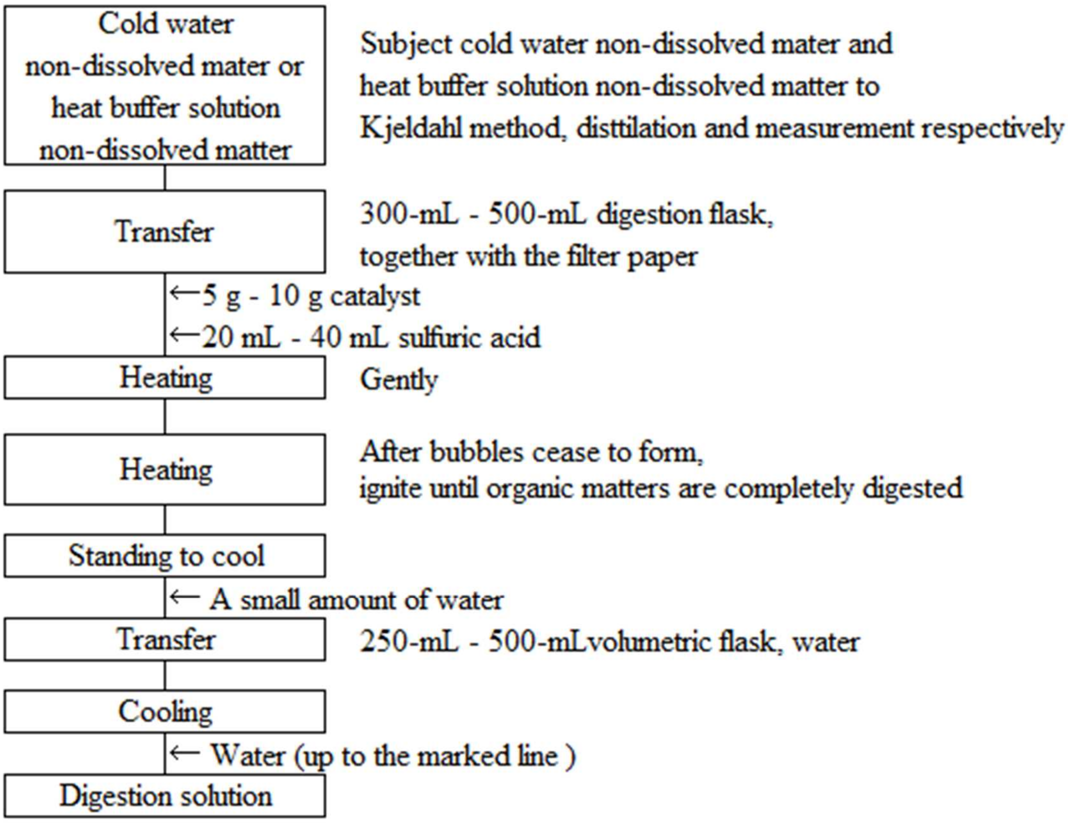


Figure 2 Flow sheet of the testing method for the activity coefficient of nitrogen in formaldehyde processed urea fertilizers (Kjeldahl digestion procedure)

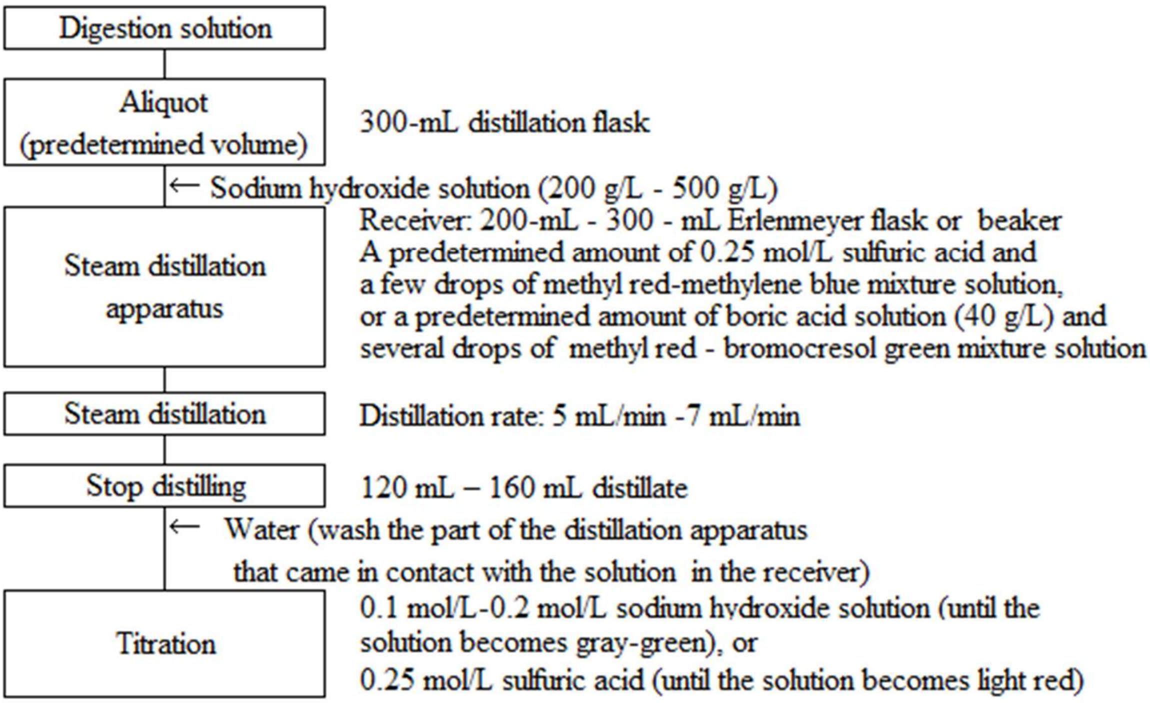


Figure 3 Flow sheet of the testing method for the activity coefficient of nitrogen in formaldehyde processed urea fertilizers (Distillation and measurement procedure)

6.8 Initial elution rate

6.8.a Standing-in-water method

(1) Summary

The testing method is applicable to coated fertilizer. This testing method is classified as Type A(Def-E) and its symbol is 6.8.a-2017 or SDR.a-1.

An initial elution rate is a quick- acting component of coated fertilizers. Target components include total nitrogen (T-N), ammoniacal nitrogen (A-N), nitrate nitrogen (N-N), water-soluble phosphoric acid (W-P_2O_5), water- soluble potassium (W-K_2O) and water-soluble magnesia (W-MgO).

Add water to a laboratory sample, leave it at rest while maintaining in water at 30ºC for 24 hours and obtain the initial elution flow of a target component. Separately measure the corresponding component content by **4.1.1**, **4.1.2**, **4.1.3**, **4.2.4**, **4.3.3** or **4.6.3**. Calculate an initial elution rate dividing the initial elution flow of a target component by the corresponding component content.

(2) Reagent: Reagents are as shown below.

- **a) Reagent solutions for total nitrogen**: Reagents corresponding to clauses in **4.1.1** when determining total nitrogen.
- **b) Reagent solutions for ammoniacal nitrogen**: Reagents corresponding to clauses in **4.1.2** when determining ammoniacal nitrogen.
- **c) Reagent solutions for nitrate nitrogen**: Reagents corresponding to clauses in **4.1.3** when determining nitrate nitrogen.
- **d) Reagent solutions for water-soluble phosphoric acid**: Reagents corresponding to clauses in **4.2.4** when determining water-soluble phosphoric acid.
- **e) Reagent solutions for water-soluble potassium**: Reagents corresponding to clauses in **4.3.3** when determining water-soluble potassium.
- **f) Reagent solutions for water-soluble magnesia**: Reagents corresponding to clauses in **4.6.4** when determining water-soluble magnesia.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

- **a) Incubator**: An incubator whose temperature is 30 ºC ± 1 ºC.
- **b) Total nitrogen**: Apparatus and instruments corresponding to clauses in **4.1.1** when determining total nitrogen.
- **c) Ammoniacal nitrogen**: Apparatus and instruments corresponding to clauses in **4.1.2** when determining ammoniacal nitrogen.
- **d) Nitrate nitrogen**: Apparatus and instruments corresponding to clauses in **4.1.3** when determining nitrate nitrogen.
- **e) Water-soluble phosphoric acid**: Apparatus and instruments corresponding to clauses in **4.2.4** when determining water-soluble phosphoric acid.
- **f) Water-soluble potassium**: Apparatus and instruments corresponding to clauses in **4.3.3** when determining water-soluble potassium.
- **g) Water-soluble magnesia**: Apparatus and instruments corresponding to clauses in **4.6.4** when determining water-soluble magnesia.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

- **a)** Weigh 12.5 g of an analytical sample, and put it into a 300 mL- ground-in stopper Erlenmeyer flask (1).
- **b)** Add 250 mL of water at 30ºC ± 1ºC, put it into an incubator at 30ºC ± 1ºC and leave it at rest for 24 hours (2).

c) Filter with Type 3 filter paper (3) and shake to mix the filtrate to make a sample solution.

Note (**1**) Since no grinding is conducted and inhomogeneous laboratory samples are used, it is desirable to heighten the reliability of determined values by conducting tests using 3 - 5 laboratory samples.
(**2**) Since an initial elution flow is estimated to be higher than usual if the laboratory sample vibrates in water, water should be added gently. Do not shake to mix the sample solution until the filtering in **c**) is completed.
(**3**) Filter most of the solution so that non-dissolved matter remains in the Erlenmeyer flask.

(**4.2**) **Measurement**: Conduct the measurements of the initial elution flow of a target component as specified in respective clauses in **a**) - **f**). In addition, specific measurement procedure for each component is carried out according to a corresponding clause.

a) **Total nitrogen**: Get a predetermined amount of sample solution and quantitate the total nitrogen according to the respective clauses in **4.1.1** to make initial elution flow.

b) **Ammoniacal nitrogen**: Get a predetermined amount of sample solution and quantitate ammoniacal nitrogen according to respective clauses in **4.1.2** to make the initial elution flow.

c) **Nitrate nitrogen**: Get a predetermined amount of sample solution and quantitate nitrate nitrogen according to respective clauses in **4.1.3** to make the initial elution flow.

d) **Water-soluble phosphoric acid**: Get a predetermined amount of sample solution and quantitate water-soluble phosphoric acid according to respective clauses in **4.2.4** to make the initial elution flow.

e) **Water-soluble potassium**: Get a predetermined amount of sample solution and quantitate water-soluble potassium according to respective clauses in **4.3.3** to make the initial elution flow.

f) **Water-soluble magnesia**: Get a predetermined amount of sample solution and quantitate water-soluble magnesia according to respective clauses in **4.6.4** to make the initial elution flow.

(5) Calculation of initial elution rate

a) Calculate an initial elution rate (%) by the following formula using the initial elution flow of a target component obtained in (**4.2**) and separately determine (4) the corresponding component content (5).

Initial elution rate (%)

$= (C_1/C_2) \times 100$

C_1 : Initial elution rate of a target component (% (mass fraction))

C_2 : Corresponding component content (% (mass fraction))

Note (**4**) Determine total nitrogen (T-N), ammoniacal nitrogen (A-N), nitrate nitrogen (N-N), water-soluble phosphoric acid (W-P_2O_5), water-soluble potassium (W-K_2O) or water-soluble magnesia (W-MgO) in **4.1.1**, **4.1.2**, **4.1.3**, **4.2.4**, **4.3.3** or **4.6.4** using test samples prepared in **2.3 Preparation of test samples.**
(**5**) The initial elution flow and corresponding component content use raw data without rounding numerical value.

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers

(Details), p.288- 290, Yokendo, Tokyo (1988)

(**6**) **Initial elution rate testing method**: The flow sheet for initial elution rate testing method in coated fertilizers is shown below:

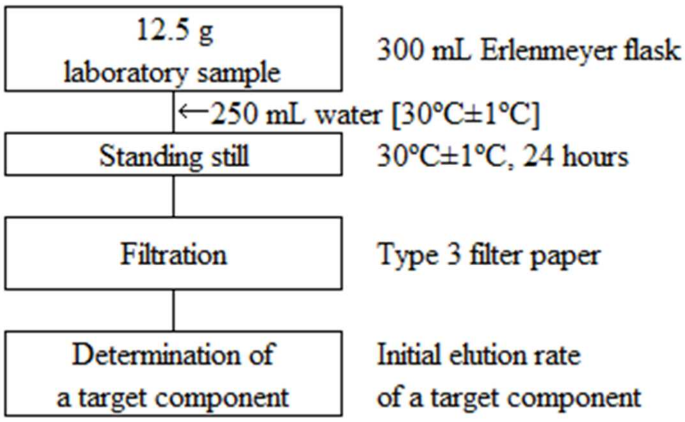


Figure Flow sheet of the testing method for initial elution rate of coated fertilizers

6.9 Humic acid (Acid insoluble - alkali soluble component)
6.9.a Gravimetric analysis
(1) Summary

The testing method is applicable to humic acid chloride fertilizers. This testing method is classified as Type A(Def-M) and its symbol is 6.9.a-2017 or H-acid.a-1.

Add hydrochloric acid (1+9) to elute acid dissolved matter, filter non-dissolved matter and measure the mass of non-dissolved matter to obtain acid non-dissolved matter in the analytical sample. Separately add hydrochloric acid (1+9) to elute acid dissolved matter, add a sodium hydroxide solution (10 g/L) to non-dissolved matter to elute alkali dissolved matter, and filter the non-dissolved matter to obtain acid non-dissolved alkali non-dissolved matter in the analytical sample. Subtract acid non-dissolved alkali non-dissolved matter from acid dissolved matter to calculate humic acid (acid insoluble alkali soluble component).

(2) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Sodium hydroxide**: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

(3) **Instruments**: Instruments are as shown below:

a) **Shaking apparatus**

b) **Drying apparatus**: A drying apparatus that can be adjusted to 105 ºC - 110 ºC.

c) **Crucible type glass filter**: A crucible type glass filter 1G4 specified in JIS R 3503. Let it stand to cool in a desiccator after heating at 105 ºC - 110 ºC in advance and measure the mass to the order of 1 mg.

d) **Ground-in stopper weighing bottles** (1): Low-form weighing bottles, 50 mm × 30 mm, specified in JIS R 3503. Let it stand to cool in a desiccator after heating at 105 ºC - 110 ºC in advance and measure the mass to the order of 1 mg.

Note (1) Aluminum weighing dishes described in the Handbook of the Feed Analysis Standards -2009- can also be used.

(4) Test procedures

(4.1) Acid non-dissolved matter

(4.1.1) **Extraction**: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample to the order of 1 mg and put it in a 100-mL ground-in stopper centrifugal precipitate tube.

b) Add 50 mL of hydrochloric acid (1+9) and shake to mix by using a shaking apparatus (2) for about 1 hour.

c) Centrifuge at about 1700 × g for about five minutes (3) to remove supernatant (4).

d) Add water to stir (5) and centrifuge at about 1700 × *g* for about five minutes (3) to remove supernatant (4).

e) Repeat the procedure in **d**) 3 times.

Note (2) When using a vertical rotating mixer, it should be adjusted to 30 - 40 revolutions/min.

(3) 16.5-cm of radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(4) Remove using Komagome pipet, etc.

(5) Stir with a glass rod, wash non-dissolved matter which may have adhered to the

glass rod with water and add wash to a centrifugal precipitate tube.

(**4.1.2**) **Measurement**: Conduct measurement as shown below.

a) Transfer the whole non-dissolved matter in (**4.1.1**) **e**) to a crucible type glass filter and filter under reduced pressure.

b) Put the non-dissolved matter together with the crucible type glass filter into a drying apparatus and heat at 105 ºC - 110 ºC for 3 hours.

c) As soon as heating is complete, transfer it to a desiccator and let it stand to cool.

d) After standing to cool, remove the crucible type glass filter from the desiccator and measure the mass to the order of 1 mg.

(4.2) Acid non-dissolved - alkali non-dissolved matter

(**4.2.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1 g of an analytical sample to the order of 1 mg and put it in a 100-mL ground-in stopper centrifugal precipitate tube.

b) Add 50 mL of hydrochloric acid (1+9) and shake to mix by using a shaking apparatus (2) for about 1 hour.

c) Centrifuge at about 1700 × *g* for about five minutes (3) to remove supernatant (4).

d) Add water to stir (5) and centrifuge at about 1700 × *g* for about five minutes (3) to remove supernatant (4).

e) Repeat the procedure in **d**) 3 times.

f) Add 50 mL of sodium hydroxide (10 g/L) and shake to mix by using a shaking apparatus (2) for about 1 hour.

g) Centrifuge at about 1700 × *g* for about five minutes (3) to remove supernatant (4).

h) Add water to stir (5) and centrifuge at about 1700 × *g* for about five minutes (3) to remove supernatant (4).

i) Repeat the procedure in **h**) 3 times.

(**4.2.2**) **Measurement**: Conduct measurement as shown below.

a) Put the non-dissolved matter together with a ground-in stopper weighing bottle into a drying apparatus and heat (6).

b) After standing to cool, transfer the non-dissolved matter into the ground-in stopper weighing bottle.

c) Put the non-dissolved matter together with a ground-in stopper weighing bottle into a drying apparatus and heat at 105 ºC - 110 ºC for 3 hours.

d) After heating, fit the stopper into the ground-in stoppered weighing bottle, and immediately transfer to a desiccator to let it stand to cool.

e) After standing to cool, remove the ground-in stoppered weighing bottle from the desiccator, and measure the mass to the order of 1 mg.

Note (**6**) Heat at the temperature to enable the procedure in (**4.2.2**) **b**).

(5) **Calculation of humic acid**

a) Calculate humic acid by the following formula

Humic acid (% (mass fraction))

$$= (A_1/W_1) \times 100 - (A_2/W_2) \times 100 \quad \cdots\cdots \quad (1)$$

A_1 : Mass (g) of the acid non-dissolved matter determined in (**4.1.2**) **d**)

W_1 : Mass (g) of the analytical sample sampled in (**4.1.1**)**a**)

A_2 : Mass (g) of the acid non-dissolved - alkali non-dissolved matter determined in (**4.2.2**) **e**)

W_2 : Mass (g) of the analytical sample sampled in (**4.2.1**)**a**)

References

1) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.316- 317, Yokendo, Tokyo (1988)

(**6**) **Humic acid testing method**: The flow sheet for humic acid testing method is shown below:

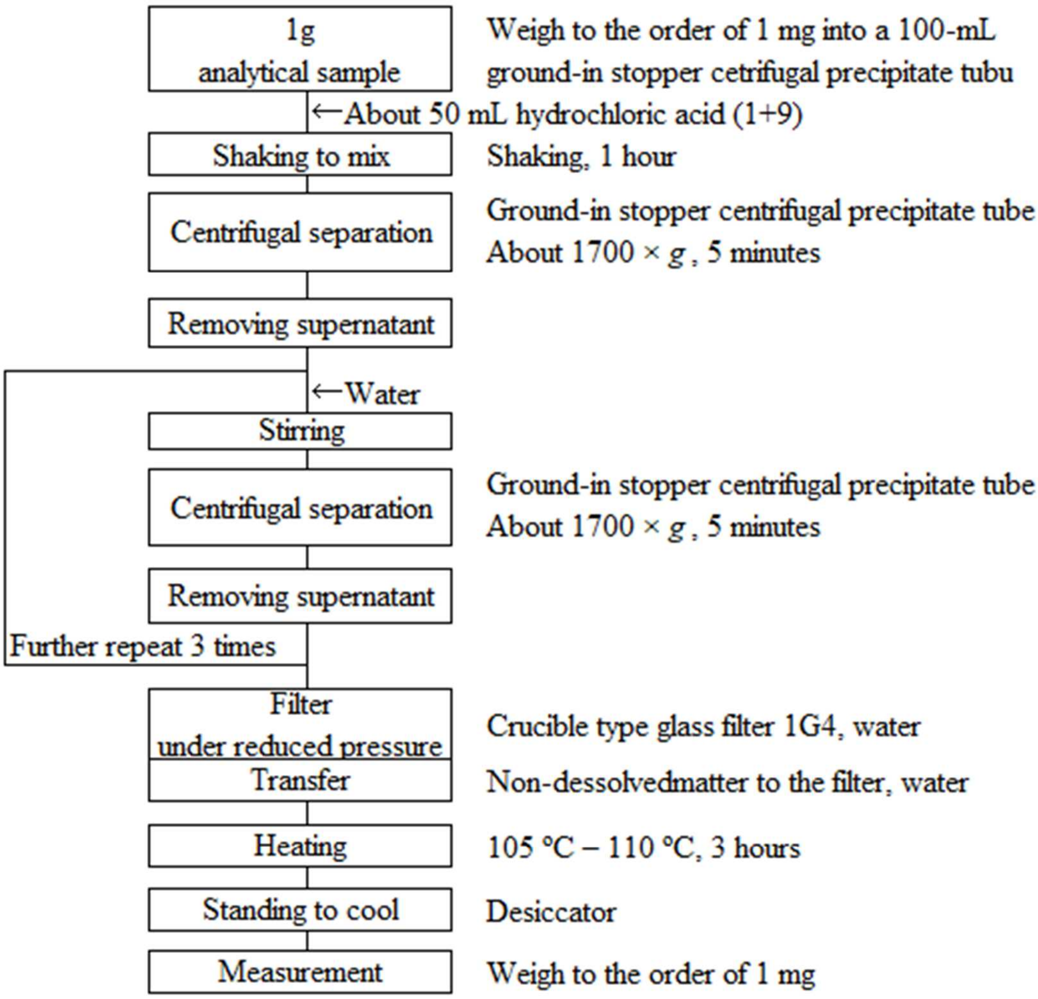


Figure 1 Flow sheet of the testing method for humic acid in humic acid salt fertilizers (Measurement procedure of acid non-dissolved matter (4.1))

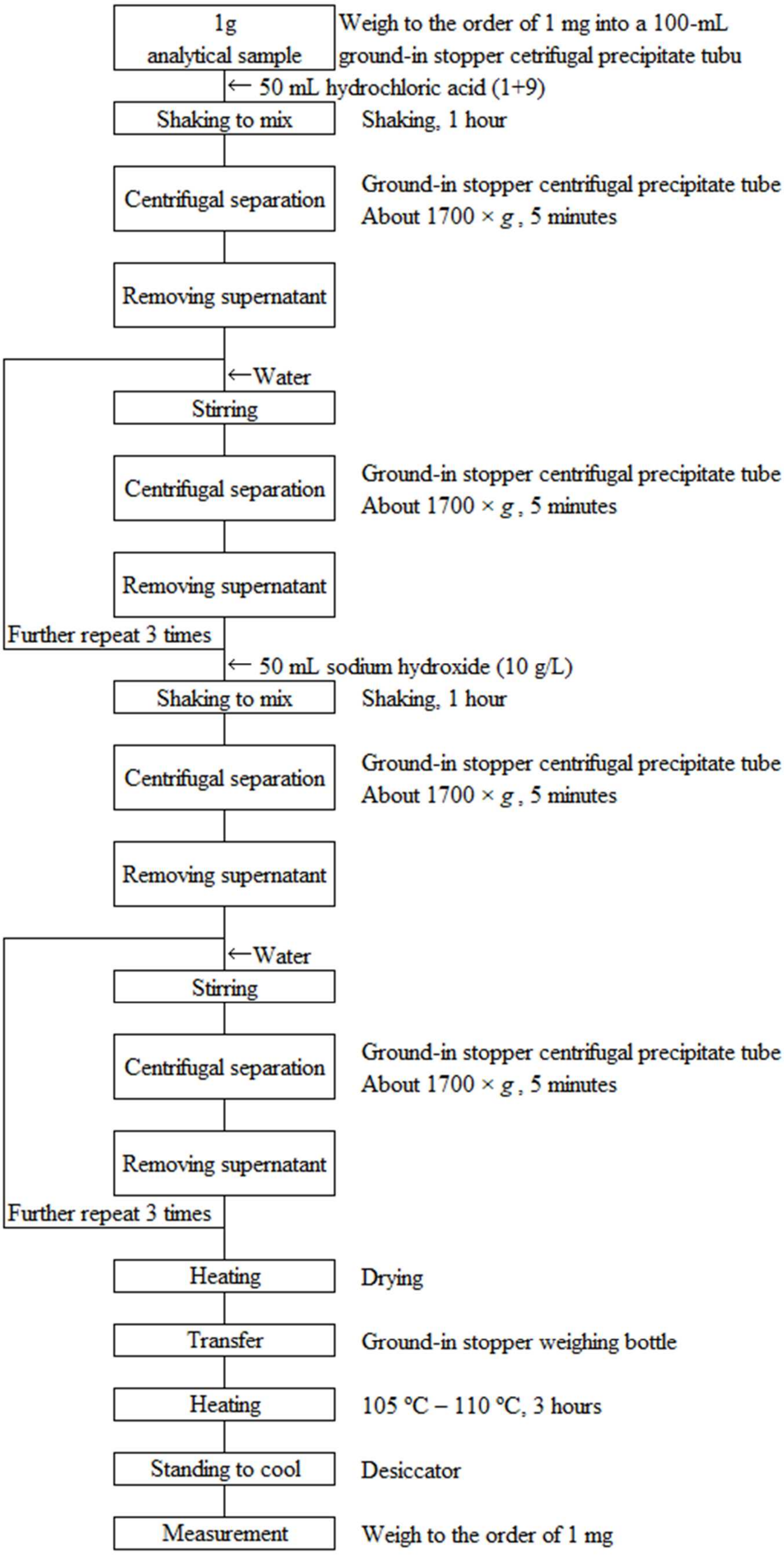


Figure 2 Flow sheet of the testing method for humic acid in humic acid salt fertilizers (Testing procedure of acid insoluble - alkali non-dissolved matter (4.2))

6.10 Sulfuric acid
6.10.1

6.10.2 Sulfate

6.10.2.a This method is according to 5.29.2 Sulfate analysis in "The Official Methods of Analysis of Fertilizers 1992".

References

1) National Institute for Agro-Environmental Sciences, the Ministry of Agriculture, Forestry and Fisheries: The Official Methods of Analysis of Fertilizers 1992, p.145 - 147, Japan Fertilizers Analysis Association, Tokyo (1992)

2) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.285- 286, Yokendo, Tokyo (1988)

6.11 Carbon dioxide

6.11.a This method is according to 5.20 Carbon dioxide analysis in "The Official Methods of Analysis of Fertilizers 1992".

References

1) National Institute for Agro-Environmental Sciences, the Ministry of Agriculture, Forestry and Fisheries: The Official Methods of Analysis of Fertilizers 1992, p.121 - 123, Japan Fertilizers Analysis Association, Tokyo (1992)

2) Masayoshi KOSHINO: Second Revision of The Methods of Analysis of Fertilizers (Details), p.259- 261, Yokendo, Tokyo (1988)

7. Nitrification inhibitor

7.1 2-amino-4-chloro-6-methylpyrimidine (AM)

7.1.a High-Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing 2-amino-4-chloro-6-methylpyrimidine (AM). This testing method is classified as Type C and its symbol is 7.1.a-2017 or AM.a-1.

Add methanol − water (1+1) to an analytical sample to extract 2-amino-4-chloro-6-methylpyrimidine, introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with an octadecyl silylation silica gel column, and measure at a wavelength 295 nm to obtain 2-amino-4-chloro-6-methylpyrimidine (AM) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Methanol**: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

c) **Methanol**: Methanol used in the eluent of a High-Performance Liquid Chromatograph is a regent of High-Performance Liquid Chromatograph analysis grade or equivalents.

d) **2-amino-4-chloro-6-methylpyrimidine standard solution (1 mg/mL)** (1): Put 0.1 g of 2-amino-4-chloro-6-methylpyrimidine [$C_5H_6ClN_3$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add methanol - water (1+1) to dissolve, transfer to a 100-mL volumetric flask and add the same solvent up to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

e) **2-amino-4-chloro-6-methylpyrimidine standard solution (0.1 mg/mL)**: In the case of usage, put 10 mL of 2-amino-4-chloro-6-methylpyrimidine standard solution (1 mg/mL) in a 100-mL volumetric flask and add methanol - water (1+1) up to the marked line.

f) **2-amino-4-chloro-6-methylpyrimidine standard solution (10 µg/mL - 50 µg/mL) for the calibration curve preparation**: In the case of usage, put 5 mL - 25 mL of 2-amino-4-chloro-6-methylpyrimidine standard solution (0.1 mg/mL) for the calibration curve preparation in 50-mL volumetric flasks step-by-step and add methanol - water (1+1) up to the marked line.

g) **2-amino-4-chloro-6-methylpyrimidine standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation**: In the case of usage, put 2.5 mL - 25 mL of 2-amino-4-chloro-6-methylpyrimidine standard solution (20 µg/mL) for the calibration curve preparation in 50-mL volumetric flasks step-by-step and add methanol - water (1+1) up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % (mass fraction) in purity is commercially sold as 2-amino-4-chloro-6-methylpyrimidine.

Comment 1 2-amino-4-chloro-6-methylpyrimidine is commercially sold by FUJIFILM Wako Pure Chemical Co., Ltd. and Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below:

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which octadecyl chemically bonds.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 295 nm.

- **b)** **Magnetic stirrer**
- **c)** **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.
- **d)** **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.
- **e)** **Acidic alumina cartridge column**: Link a 10-mL cylinder to a column (3) that is filled with 500 mg - 1 g of acidic alumina, put 3 mL of methanol and let it flow down.

Note (**3**) A cartridge with a 3-mL - 6-mL column filled with 500 mg - 1 g of silica gel can be used.

Comment 2 A column is sold under production names such as Inertsil ODS, Mightysil RP-18, L-column ODS, Shim-pack VP-ODS, Silica C_{18}M 4D, Puresil C18, COSMOSIL 5C18-MS-II, etc.

Comment 3 An acidic alumina cartridge is commercially sold under production names such as Bond Elut AL-A, Sep-Pak Alumina-A and Supelclean LC-Alumina-A.

(4) Test procedures

(**4.1**) **Extraction**: Conduct extraction as shown below.

- **a)** Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.
- **b)** Add 100 mL of methanol - water (1+1) and stir it by using a magnetic stirrer for about 30 minutes.
- **c)** After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.
- **d)** Centrifuge it at about 1700 × *g* centrifugal force for about five minutes (4) and use the supernatant as an extract (5).

Note (**4**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**5**) If there is a possibility that the 2-amino-4-chloro-6-methylpyrimidine in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of extract with methanol water (1+1).

(**4.2**) **Cleanup**: Conduct cleanup as shown below:

- **a)** Transfer the extract to an acidic alumina cartridge column.
- **b)** Dispose of about the first 3 mL of effluent and then put about the next 2 mL in a test tube.
- **c)** Put the effluent in a 1.5-mL ground-in stopper centrifugal precipitate tube (6).
- **d)** Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (7) and use the supernatant as a sample solution.

Note (**6**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(**7**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 4 Instead of the procedures in (**4.2**) **c**) - **d**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of PTFE and the filtrate can be the sample solution.

Comment 5 The test is possible by the following procedures in the case of fertilizers not containing organic matters.

The procedures in (**4.1**) **c**) - **d**) and (**4.2**) **a**) - **b**) are omitted and "Put the effluent" in (**4.2**) **c**) is replaced with the "After allowing to stand still, put the supernatant" to operate.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph (HPLC) used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions for the High-Performance Liquid Chromatograph is shown below. Set up the measurement conditions considering it:

1) **Column**: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which octadecyl chemically bonds.
2) **Column bath temperature**: 30 °C - 40 °C
3) **Eluent**: Methanol - water (4+6)
4) **Flow rate**: 1 mL/min
5) **Detection unit**: An absorptiometric detector, a measurement wavelength: 295 nm

b) **Calibration curve preparation**

1) Inject 10 µL of respective 2-amino-4-chloro-6-methylpyrimidine standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 295 nm and obtain a peak area or height.
2) Prepare a curve for the relationship between the concentration and the peak area or height at a wavelength 295 nm of the respective 2-amino-4-chloro-6-methylpyrimidine standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 10 µL of the sample solution to the same procedure as in **b**) **1**)
2) Obtain 2-amino-4-chloro-6-methylpyrimidine content from the calibration curve to calculate 2-amino-4-chloro-6-methylpyrimidine (AM) in the analytical sample.

Comment 6 Recovery testing was conducted using compound fertilizer (1 sample) and blended fertilizer (2 samples), as a result, the mean recovery rates of 2-amino-4-chloro-6-methylpyrimidine at the concentration level of 1.0 % (mass fraction), 0.4 % (mass fraction) and 0.1 % (mass fraction) were 99.1 % - 100.5 %, 99.3 % - 101.6 % and 100.2 % - 100.7 %.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.005 % (mass fraction).

References

1) Yuji SHIRAI: The volumetric analysis of 2-amino-4-chloro-6-methylpyrimidine in fertilizers with High-Performance Liquid Chromatograph, Validation Report of Fertilizers (in Japanese), **44** (**3**), p. 26 - 41(1991)

(5) Flow sheet for 2-amino-4-chloro-6-methylpyrimidine (AM):
The flow sheet for 2-amino-4-chloro-6-methylpyrimidine in fertilizers is shown below:

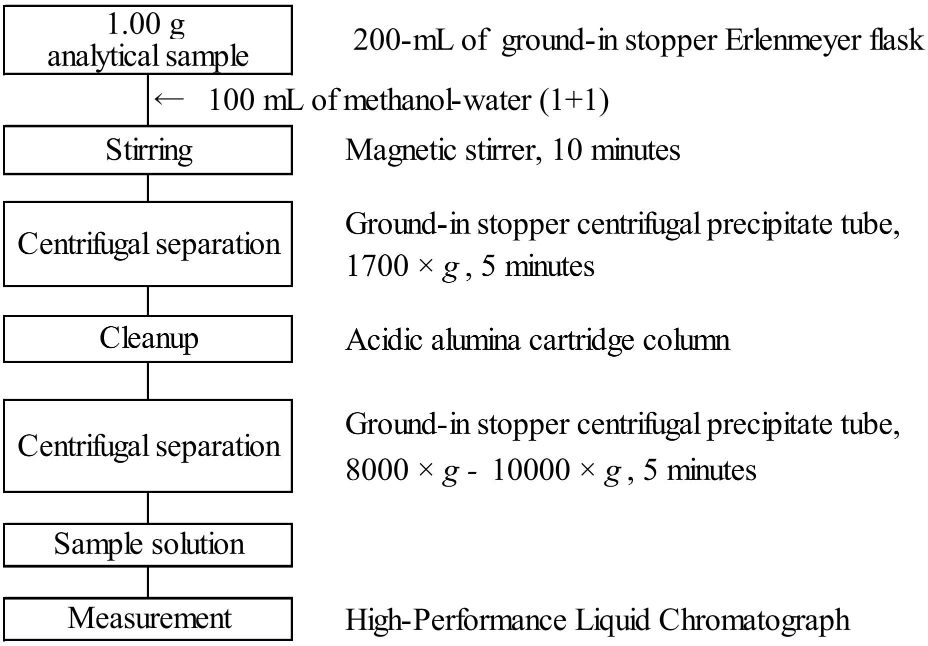
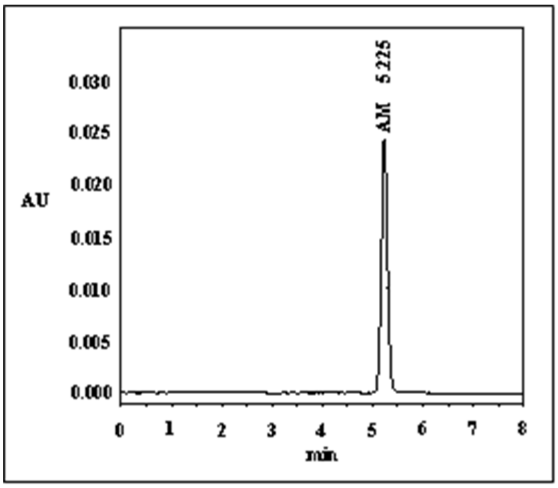


Figure Flow sheet for 2-amino-4-chloro-6-methylprimidine (AM) in fertilizers.

Reference: An example of the HPLC chromatogram of 2-amino-4-chloro-6-methylpyrimidine (AM) standard solution for the calibration curve preparation is shown below.



Reference diagram: HPLC chromatogram of 2-amino-4-chloro-6-methylpyrimidine (AM) standard solution

Measurement conditions for HPLC

Column: Mightysil RP-18 (4.6-mm inner diameter, 150-mm long, 5-µm particle diameter)

2-amino-4-chloro-6-methylpyrimidine standard solution (the equivalents of 100 ng)

Other conditions are according to the example of HPLC measurement conditions in **(4.3) a)**

7.2 1-amidino-2-thiourea (ASU)
7.2.a High-Performance Liquid Chromatography
(1) Summary

This testing method is applicable to fertilizers containing 1-amidino-2-thiourea (ASU). This testing method is classified as Type B and its symbol is 7.2.a-2017 or ASU.a-1.

Add water to an analytical sample to extract 1-amidino-2-thiourea, introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with an octadecyl silylation silica gel column, and measure at a wavelength 262 nm to obtain 1-amidino-2-thiourea (ASU) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 4**.

(2) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Methanol**: Methanol used in the eluent of a High-Performance Liquid Chromatograph is a regent of High-Performance Liquid Chromatograph analysis grade or equivalents.

c) **1-sodium hexasulfonate**: A reagent of ion pair chromatography analysis grade or equivalents.

d) **Acetic acid**: A regent of High-Performance Liquid Chromatograph analysis grade or equivalents.

e) **1-amidino-2-thiourea standard solution (1 mg/mL)** (1): Put 0.1 g of 1-amidino-2-thiourea [$C_2H_6N_4S$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add water to dissolve, transfer to a 100-mL volumetric flask and add water up to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

f) **1-amidino-2-thiourea standard solution (0.1 mg/mL)**: In the case of usage, put 10 mL of 1-amidino-2-thiourea standard solution (1 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

g) **1-amidino-2-thiourea standard solution (10 µg/mL - 50 µg/mL) for the calibration curve preparation**: In the case of usage, put 5 mL - 25 mL of 1-amidino-2-thiourea standard solution (0.1 mg/mL) in 50-mL volumetric flasks step-by-step and add water up to the marked line.

h) **1-amidino-2-thiourea standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation**: In the case of usage, put 2.5 mL - 25 mL of 1-amidino-2-thiourea standard solution (20 µg/mL) for the calibration curve preparation in 50-mL volumetric flasks step-by-step and add water up to the marked line.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % (mass fraction) in purity is commercially sold as 1-amidino-2-thiourea.

Comment 1 1-amidino-2-thiourea is sold under the name guanylthiourea by Tokyo Chemical Industry Co., Ltd, and under the name amidino thiourea by Kanto Chemical Co., Inc.

(3) **Instruments**: Instruments are as shown below:

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which octadecyl chemically bonds.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 262 nm.

b) **Magnetic stirrer**

c) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 2 A column is sold under production names such as Inertsil ODS, Mightysil RP-18, L-column ODS, Shim-pack VP-ODS, Silica C18M 4D, Puresil C_{18} and COSMOSIL 5C18-MS-II.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, put a supernatant solution (3) in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (5) and use the supernatant as a sample solution.

Note **(3)** If there is a possibility that the 1-amidino-2-thiourea concentration in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of supernatant with water.

(4) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement

(5) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 3 Instead of the procedures in (**4.1**) **c**) - **d**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-μm) made of PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions for the High-Performance Liquid Chromatograph (HPLC) is shown below. Set up the measurement conditions considering it:

1) **Column**: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-μm particle diameter) to which octadecyl chemically bonds.

2) **Column bath temperature**: 30 °C - 45 °C

3) **Eluent**: Dissolve 0.94 g of sodium 1-hexasulfonic acid in 1000 mL of methanol water (2+8), adjust to pH 3.15 with acetic acid and filter with a membrane filter (aperture diameter: no more than 0.5-μm) made of hydrophilic PTFE (1).

4) **Flow rate**: 1 mL/min

5) **Detection unit**: An absorptiometric detector, a measurement wavelength: 262 nm

b) **Calibration curve preparation**

1) Inject 10 μL of respective 1-amidino-2-thiourea standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 262 nm and obtain a peak area or height.

2) Prepare a curve for the relationship between the concentration and the peak area or height at a wavelength 262 nm of the respective 1-amidino-2-thiourea standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 10 μL of the sample solution to the same procedure as in **b**) **1**)
2) Obtain the 1-amidino-2-thiourea content from the calibration curve to calculate 1-amidino-2-thiourea (ASU) in the analytical sample.

Comment 4 Recovery testing with triplicates measurement was conducted using compound fertilizer (2 samples), as a result, the mean recovery rates of 1-amidino-2-thiourea at the concentration level of 1.0 % (mass fraction), 0.5 % (mass fraction) and 0.25 % (mass fraction) were 99.0 % - 104.3 %, 97.7 % - 100.7 % and 99.7 % - 101.3 %.
Additionally, results from a collaborative study for test method validation and its analysis are in Table 1.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.005 % (mass fraction).

Table 1 Analysis resultsof the collaborative study
for the test method validation of 1-amidino-2-thiourea (ASU)

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Compound fertilizer 1	10	0.093	0.009	9.1	0.010	11.2
Compound fertilizer 2	10	0.246	0.021	8.6	0.021	8.6
Compound fertilizer 3	10	0.511	0.018	3.6	0.025	4.9
Compound fertilizer 4	10	0.759	0.039	5.1	0.040	5.3
Compound fertilizer 5	10	1.020	0.039	3.8	0.044	4.3

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Kazunori CHIBA : Analysis method of 1-amidino-2-thiourea (ASU) of Nitrification inhibitor in fertilizers with High-Performance Liquid Chromatograph, Validation Report of Fertilizers (in Japanese), **43** (**4**), p. 15 - 22 (1990)
2) Shigehiro KAI and Erina WATABE: Determination of 1-Amidino-2-thiourea as a Nitrification Inhibitor in Compound Fertilizer by High-Performance Liquid Chromatography: A Collaborative Study, Research Report of Fertilizers, **Vol. 6**, p. 36 - 42 (2013)

(5) **Flow sheet for 1-amidino-2-thiourea**: The flow sheet for 1-amidino-2-thiourea in fertilizers is shown below:

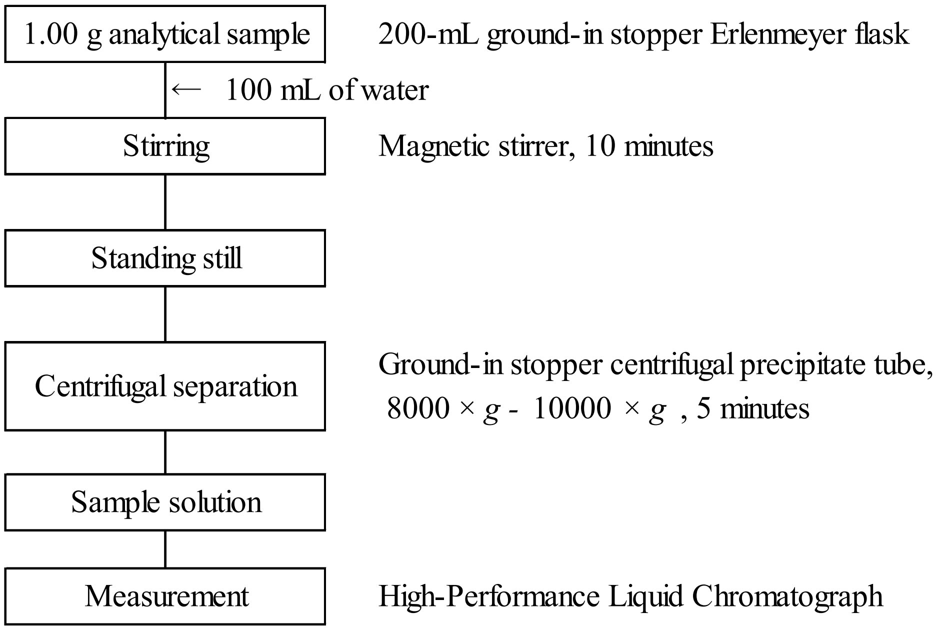
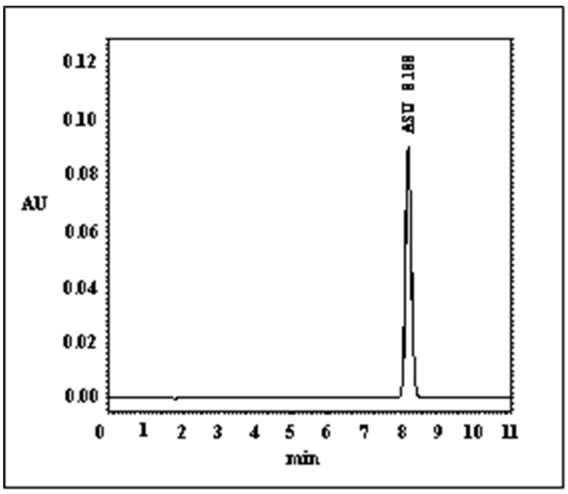


Figure Flow sheet for 1-amidino-2-thiourea (ASU) in fertilizers.

Reference: An example of the HPLC chromatogram of 1-amidino-2-thiourea (ASU) standard solution for the calibration curve preparation is shown below.



Reference diagram: HPLC chromatogram of 1-amidino-2-thiourea (ASU) standard solution

Measurement conditions for HPLC

Column: Mightysil RP-18 (4.6-mm inner diameter, 150-mm long, 5-µm particle diameter)

1-amidino-2-thiourea standard solution (the equivalents of 200 ng)

Other conditions are according to the example of HPLC measurement conditions in **(4.2) a)**

7.3 4-amino-1,2,4-triazole hydrochloride (ATC)

7.3.a High-Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing 4-amino-1,2,4-triazole hydrochloride (ATC) but not containing organic matters. This testing method is classified as Type C and its symbol is 7.3.a-2017 or ATC.a-1.

Add methanol to an analytical sample to extract 4-amino-1,2,4-triazole hydrochloride, introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with an aminopropyl silica gel column, and measure at a wavelength 220 nm to obtain 4-amino-1,2,4-triazole hydrochloride (ATC) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 4**.

(2) Reagent: Reagents are as shown below.

a) **Methanol**: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

b) **Methanol**: Methanol used in the eluent of a High-Performance Liquid Chromatograph is a regent of High-Performance Liquid Chromatograph analysis grade or equivalents.

c) **Acetonitrile**: Acetonitrile used in the eluent of a High-Performance Liquid Chromatograph is a regent of High-Performance Liquid Chromatograph analysis grade or equivalents.

d) **4-amino-1,2,4-triazole standard solution (1 mg/mL)** (1)(2): Put 0.1 g of 4-amino-1,2,4-triazole [$C_2H_4N_4$] (3) in a weighing dish and measure the mass to the order of 0.1 mg. Add methanol to dissolve, transfer to a 100-mL amber volumetric flask and add methanol to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

e) **4-amino-1,2,4-triazole standard solution (0.1 mg/mL)**: In the case of usage, put 10 mL of 4-amino-1,2,4-triazole standard solution (1 mg/mL) in a 100-mL volumetric flask and add methanol up to the marked line.

f) **4-amino-1,2,4-triazole standard solution (10 µg/mL - 50 µg/mL) for the calibration curve preparation**: In the case of usage, put 5 mL - 25 mL of 4-amino-1,2,4-triazole standard solution (0.1 mg/mL) in 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

g) **4-amino-1,2,4-triazole standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation**: In the case of usage, put 2.5 mL - 25 mL of 4-amino-1,2,4-triazole standard solution for the calibration curve preparation (20 µg/mL) in 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

Note (**1**) The solution contains 1.434 mg/mL as 4-amino-1,2,4-triazole hydrochloride.

(**2**) This is an example of preparation; prepare an amount as appropriate.

(**3**) A reagent of no less than 98 % (mass fraction) in purity is commercially sold as 4-amino-1,2,4-triazole.

Comment 1 4-amino-1,2,4-triazole is sold under production names such as 4-amino-1,2,4-triazole by FUJIFILM Wako Pure Chemical Co., Ltd., and Tokyo Chemical Industry Co., Ltd, and 4-amino-4H-1,2,4-triazole by Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below:

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A column of 4 mm - 6 mm inner diameter and 150 mm - 250 mm long stainless steel column tube filled with silica gel, to which amino or amino propyl chemically bonds.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 220 nm.

b) **Magnetic stirrer**
c) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 2 A column is sold under production names such as Hibar LiChrosorb NH_2, Inertsil NH_2, Unison UK-Amino, Mightysil NH_2, Shim-pack CLC-NH_2, Shodex NH-5A, Unisil Q NH_2, etc.

(4) **Test procedures**
(4.1) Extraction: Conduct extraction as shown below.
a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.
b) Add 100 mL of methanol and stir it with using a magnetic stirrer for about 10 minutes.
c) After allowing to stand still, put a supernatant solution in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).
d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (5) and use the supernatant as a sample solution.

Note **(4)** The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.
(5) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 3 Instead of the procedures in (**4.1**) **c**) - **d**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-μm) made of PTFE and the filtrate can be the sample solution.

(4.2) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.
a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions for the High-Performance Liquid Chromatograph is shown below. Set up the measurement conditions considering it:
1) **Column**: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-μm particle diameter) to which amino or amino propyl chemically bonds.
2) **Column bath temperature**: 30 °C - 40 °C
3) **Eluent**: Acetonitrile - methanol (9+1)
4) **Flow rate**: 1 mL/min
5) **Detection unit**: An absorptiometric detector, a measurement wavelength: 220 nm
b) **Calibration curve preparation**
1) Inject 10 μL of respective 4-amino-1,2,4-triazole standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 220 nm and obtain a peak area or height.
2) Prepare a curve for the relationship between the concentration and the peak area or height at a wavelength 220 nm of the 4-amino-1,2,4-triazole standard solutions for the respective calibration curve preparation.
c) **Sample measurement**
1) Subject 10 μL of the sample solution to the same procedure as in **b**) **1**)
2) Obtain the amount of 4-amino-1,2,4-triazole from the calibration curve to calculate 4-amino-1,2,4-triazole in the analytical sample.

3) Calculate the 4-amino-1,2,4-triazole hydrochloride (ATC) by the following formula.

4-amino-1,2,4-triazole hydrochloride in the analytical sample (% (mass fraction))
$= A \times 1.434$

A : 4-amino-1,2,4-triazole hydrochloride in the analytical sample (% (mass fraction))

Comment 4 Recovery testing was conducted using compound fertilizer (2 samples), as a result, the mean recovery rates of 4-amino-1,2,4-triazole hydrochloride at the concentration level of 0.5 % (mass fraction), 0.3 % (mass fraction) and 0.2 % (mass fraction) were 100.2 % - 104.9 %, 100.8 % - 103.0 % and 100.7 % - 104.2 %.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.005 % (mass fraction).

References

1) Koichi SAKAGAMI: Analysis methods of 4-amino-1,2,4-triazole hydrochloride with High-Performance Liquid Chromatography, Validation Report of Fertilizers (in Japanese), **40 (4)**, p.9 - 16 (1987)

(**5**) **Flow sheet for 4-amino-1,2,4-triazole hydrochloride (ATC)**: An example of the flow sheet for 4-amino-1,2,4-triazole hydrochloride in fertilizers is shown below:

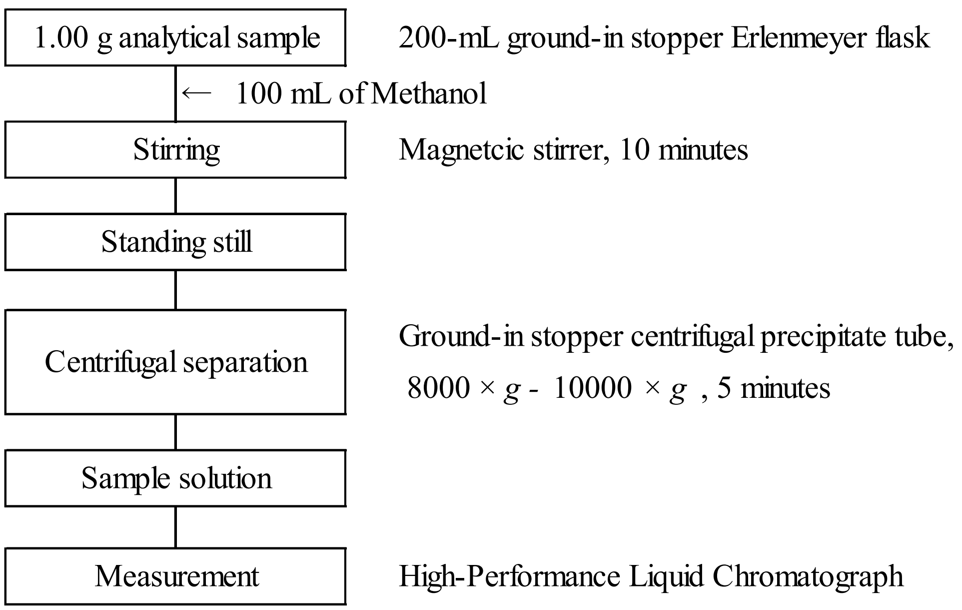


Figure Flow sheet for 4-amino-1,2,4-triazole hydrochloride (ATC) in fertilizers

7.4 N-2,5-dichlorophenyl succinamic acid (DCS)

7.4.a High-Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing N-2,5-dichlorophenyl succinamic acid (DCS) but not containing organic matters. This testing method is classified as Type C and its symbol is 7.4.a-2017 or DCS.a-1.

Add methanol - phosphoric acid (996+4) and water to an analytical sample to extract N-2,5-dichlorophenyl succinamic acid (DCS), introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with octadecyl silylation silica gel column, and measure at a wavelength 246 nm to obtain N-2,5-dichlorophenyl succinamic acid (DCS) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Methanol**: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

c) **Methanol**: Methanol used in the eluent of a High-Performance Liquid Chromatograph is a regent of High-Performance Liquid Chromatograph analysis grade or equivalents.

d) **Phosphoric acid**: A JIS Guaranteed Reagent specified in JIS K 9005 or a reagent of equivalent quality.

e) **N-2,5-dichlorophenyl succinamic acid standard solution (1 mg/mL)** (1): Put 0.1 g of N-2,5-dichlorophenyl succinamic acid [$C_{10}H_9C_{l2}NO_3$] in a weighing dish and measure the mass to the order of 0.1 mg. Add methanol to dissolve, transfer to a 100-mL volumetric flask and add methanol to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

f) **N-2,5-dichlorophenyl succinamic acid standard solution (0.1 mg/mL)**: In the case of usage, put 10 mL of N-2,5-dichlorophenyl succinamic acid standard solution (1 mg/mL) in a 100-mL volumetric flask and add methanol up to the marked line.

g) **N-2,5-dichlorophenyl succinamic acid standard solution (10 µg/mL - 50 µg/mL) for the calibration curve preparation**: In the case of usage, put 5 mL - 25 mL of N-2,5-dichlorophenyl succinamic acid standard solution (0.1 mg/mL) in 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

h) **N-2,5-dichlorophenyl succinamic acid standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation**: In the case of usage, put 2.5 mL - 25 mL of N-2,5-dichlorophenyl succinamic acid standard solution for the calibration curve preparation (20 µg/mL) in 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(3) Instruments: Instruments are as shown below:

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which octadecyl chemically bonds.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 246 nm.

b) **Magnetic stirrer**

c) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 1 A column is sold under production names such as Inertsil ODS, Mightysil RP-18, L-column ODS, Shim-pack VP-ODS, Silica C18M 4D, Puresil C_{18} and COSMOSIL 5C18-MS-II.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.

b) Add 100 mL of methanol - phosphate (996+4) and stir it with a magnetic stirrer for about 30 minutes.

c) After allowing to stand still, put a supernatant solution (2) in a 1.5-mL ground-in stopper centrifugal precipitate tube (3).

d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (4) and use the supernatant as a sample solution.

Note (**2**) If there is a possibility that the N-2,5-dichlorophenyl succinamic acid concentration in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of the outflow solution with methanol.

(**3**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement

(**4**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 2 Instead of the procedures in (**4.1**) **c**) - **d**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of PTFE and the filtrate can be the sample solution.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions for the High-Performance Liquid Chromatograph is shown below. Set up the measurement conditions considering it:

1) **Column**: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which octadecyl chemically bonds.

2) **Column bath temperature**: 30 °C - 40 °C

3) **Eluent**: Methanol - water (5) (55 + 45)

4) **Flow rate**: 0.8 mL/min

5) **Detection unit**: An absorptiometric detector, a measurement wavelength: 246 nm

Note (**5**) Adjust the water used to pH 3 with phosphoric acid in advance.

b) **Calibration curve preparation**

1) Inject 10 µL of respective N-2,5-dichlorophenyl succinamic acid standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 246 nm and obtain a peak area or height.

2) Prepare a curve for the relationship between the concentration and the peak area or height at a wavelength 246 nm of the respective N-2,5-dichlorophenyl succinamic acid standard solution for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain the N-2,5-dichlorophenyl succinamic acid content from the calibration curve to calculate N-2,5-dichlorophenyl succinamic acid (DCS) in the analytical sample.

Comment 3 Recovery testing was conducted using compound fertilizer (2 samples) and blended fertilizer (1 sample), as a result, the mean recovery rates of N-2,5-dichlorophenyl succinamic acid at the concentration level of 0.4 % (mass fraction), 0.2 % (mass fraction) and 0.1 % (mass fraction) were 100.9 % - 101.4 %, 100.8 % - 101.4 % and 101.2 % - 103.4 %.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.005 % (mass fraction).

References

1) Akira KUBO: Analysis methods of N-2,5-dichlorophenyl succinamic acid (DCS) of Nitrification suppression materials in fertilizers with High-Performance Liquid Chromatograph, Validation Report of Fertilizers (in Japanese), **44 (4)**, p.25 - 36 (1991).

(5) Flow sheet for N-2,5-dichlorophenyl succinamic acid (DCS): The flow sheet for N-2,5-dichlorophenyl succinamic acid (DCS) in fertilizers is shown below:

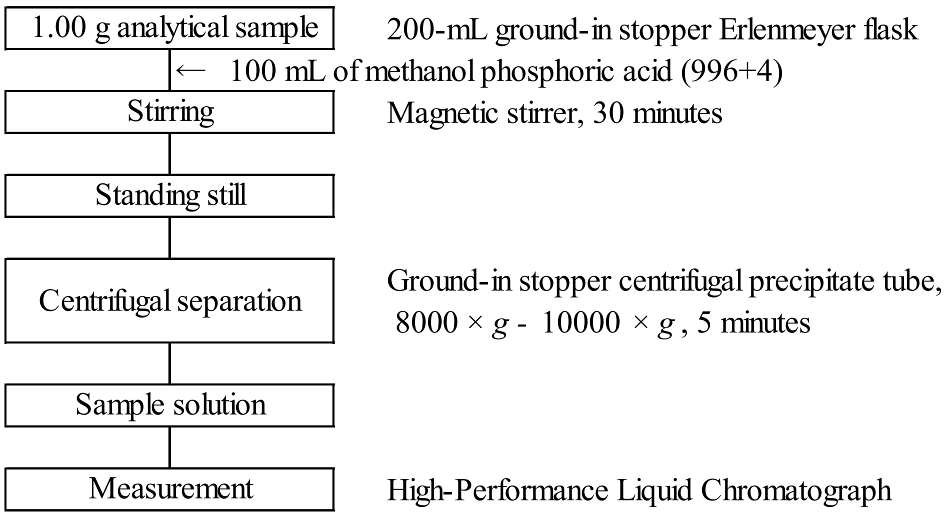
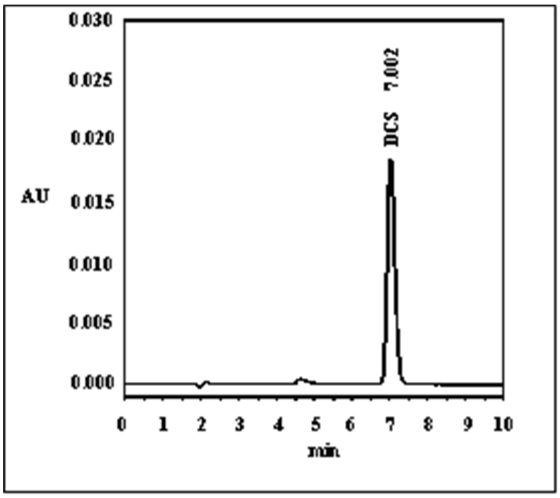


Figure Flow sheet for N-2,5-dichlorophenyl succinamic acid (DCS) in fertilizers

Reference: An example of the HPLC chromatogram of N-2,5-dichlorophenyl succinamic acid (DCS) standard solution for the calibration curve preparation is shown below.



Reference diagram: HPLC chromatogram of N-2,5-dichlorophenyl succinamic acid

Measurement conditions for HPLC

Column: Mightysil RP-18 (4.6-mm inner diameter, 150-mm long, 5-µm particle diameter)

N-2,5-dichlorophenyl succinamic acid standard solution (the equivalents of 100 ng)

Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**)

7.5 Dicyandiamide (Dd)
7.5.a High-Performance Liquid Chromatography
(1) Summary

This testing method is applicable to fertilizers containing dicyandiamide (Dd). This testing method is classified as Type B and its symbol is 7.5.a-2017 or Dd.a-1.

Add water to an analytical sample, leave at rest for a little while and add methanol to extract dicyandiamide. After removing interfering substances with a silica gel cartridge column, introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with an aminopropyl silica gel column, and measure at a wavelength 215 nm to obtain dicyandiamide (Dd) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 5**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Methanol**: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

c) **Methanol**: Methanol used in the eluent of a High Performance Liquid Chromatograph is a regent of High Performance Liquid Chromatograph analysis grade or equivalents.

d) **Acetonitrile**: A reagent of High Performance Liquid Chromatograph analysis grade or equivalents.

e) **Dicyandiamide standard solution (1 mg/mL)** (1): Put 0.1 g of dicyandiamide [$C_2H_4N_4$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of methanol to dissolve, transfer to a 100-mL volumetric flask and add the solvent up to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.

f) **Dicyandiamide standard solution (0.1 mg/mL)**: At the time of usage, put 10 mL of dicyandiamide standard solution (1 mg/ mL) in a 100-mL volumetric flask and add methanol up to the marked line.

g) **Dicyandiamide standard solution (10 µg/ mL - 50 µg/ mL) for the calibration curve preparation**: At the time of usage, put 5 mL - 25 mL of dicyandiamide standard solution (0.1 mg/ mL) in 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

h) **Dicyandiamide standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation**: In the case of usage, put 2.5 mL - 25 mL of dicyandiamide standard solution for the calibration curve preparation (20 µg/mL) in 50-mL volumetric flasks step-by-step and add methanol up to the marked line.

i) **Sodium sulfate**: A JIS Guaranteed Reagent specified in JIS K 8987 or a reagent of equivalent quality.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(2) A reagent of no less than 98 % in purity is commercially sold as dicyandiamide.

Comment 1 Dicyandiamide is commercially sold as dicyanodiamide by FUJIFILM Wako Pure Chemical Co., Ltd. and Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below:

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A column of 4 mm - 6 mm inner diameter and 150 mm - 250 mm long stainless steel column tube filled with silica gel, to which amino or amino propyl chemically bonds.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 40 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 215 nm.

b) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).
c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.
d) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.
e) **Silica gel cartridge column**: Link a 10-mL cylinder to the column (3) filled with 500 mg - 1 g of silica gel, add 3 mL of methanol to let it flow down.

Note (**3**) A cartridge with a 3-mL - 6-mL column filled with 500 mg – 1 g of silica gel can be used.

Comment 2 A column is sold under production names such as Hibar LiChrosorb NH_2, Inertsil NH_2, Unison UK-Amino, Mightysil NH_2, Shim-pack CLC-NH_2, Shodex NH-5A and Unisil Q NH_2.
Comment 3 A silica gel cartridge column is commercially sold under production names such as Sep-Pak Plus Silica, InertSep Si.

(4) Test procedures
(**4.1**) **Extraction**: Conduct extraction as shown below.
a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL ground-in stopper Erlenmeyer flask.
b) Add 1 mL of water (4) and leave at rest for 5 minutes.
c) Add about 100 mL of methanol, and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 10 minutes.
d) Add an appropriate amount of sodium sulfate (5).
e) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.
f) Centrifuge it at about 1700 × *g* centrifugal force for about five minutes (6) and use the supernatant (7) as an extract.

Note (**4**) Mix well until the whole sample comes in contact with water.
(**5**) About 5 g - 10 g.
(**6**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.
(**7**) If there is a possibility that the concentration of dicyandiamide in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of the extract with methanol.

(**4.2**) **Cleanup**: Conduct cleanup as shown below:
a) Put the extract in a silica gel cartridge column.
b) Dispose of about the first 3 mL of effluent and then put about the next 2 mL in a test tube.
c) Put the effluent in a 1.5-mL ground-in stopper centrifugal precipitate tube (8).
d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (9) and use the supernatant as a sample solution.

Note (**8**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.
(**9**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 4 Instead of the procedures in (**4.2**) **c**) - **d**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-μm) made of PTFE and the filtrate can be the sample solution.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions for the High-Performance Liquid Chromatograph is shown below. Set up the measurement conditions considering it:

1) **Column**: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-μm particle diameter) to which amino or amino propyl chemically bonds.

2) **Column bath temperature**: 30 °C - 40 °C

3) **Eluent:** Acetonitrile - methanol (6+1)

4) **Flow rate**: 0.5 mL/min - 1 mL/min

5) **Detection unit**: An absorptiometric detector, a measurement wavelength: 215 nm

b) **Calibration curve preparation**

1) Inject 10 μL of respective dicyandiamide standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 215 nm, and obtain a peak area or height.

2) Prepare a curve for the relationship between the concentration and the peak area or height at a wavelength 215 nm of the respective dicyandiamide standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 10 μL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain dicyandiamide content from the calibration curve to calculate dicyandiamide (Dd) in the analytical sample.

Comment 5 Recovery testing was conducted using inorganic compound fertilizer (2 samples) and organic compound fertilizer (3 samples), as a result, the mean recovery rates at the concentration level of 2 % (mass fraction) and 0.2% (mass fraction) were 101.2 % - 102.6 % and 98.4 % - 100.6 %.

Additionally, results from a collaborative study for test method validation and its analysis are in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.01 % (mass fraction).

Table 1 Analysis results of the collaborative study for the test method validation of dicyandiamide

Sample name	Number of laboratories[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	s_R[6)] (%)[3)]	RSD_R[7)] (%)
Compound fertilizer 1	11	0.263	0.009	3.2	0.019	7.4
Compound fertilizer 2	11	2.04	0.04	1.7	0.07	3.2
Compound fertilizer 3	13	0.548	0.011	2.0	0.033	6.0
Compound fertilizer 4	12	0.423	0.013	3.2	0.022	5.2
Compound fertilizer 5	12	1.02	0.01	1.4	0.04	4.3

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masakazu SAIKI: Development of High-Performance Liquid Chromatography for Determination of Dicyandiamide as a Nitrification Inhibitor in Fertilizer. Research Report of Fertilizer, **Vol. 3**, p. 43 - 50 (2010)

2) Masakazu SAIKI: Determination of Dicyandiamide as a Nitrification Inhibitor in Fertilizer by High-Performance Liquid Chromatography: Collaborative Study, Research Report of Fertilizer, **Vol. 4**, p. 16 - 22 (2011)

(5) **Flow sheet for dicyandiamide**: The flow sheet for dicyandiamide in fertilizers is shown.

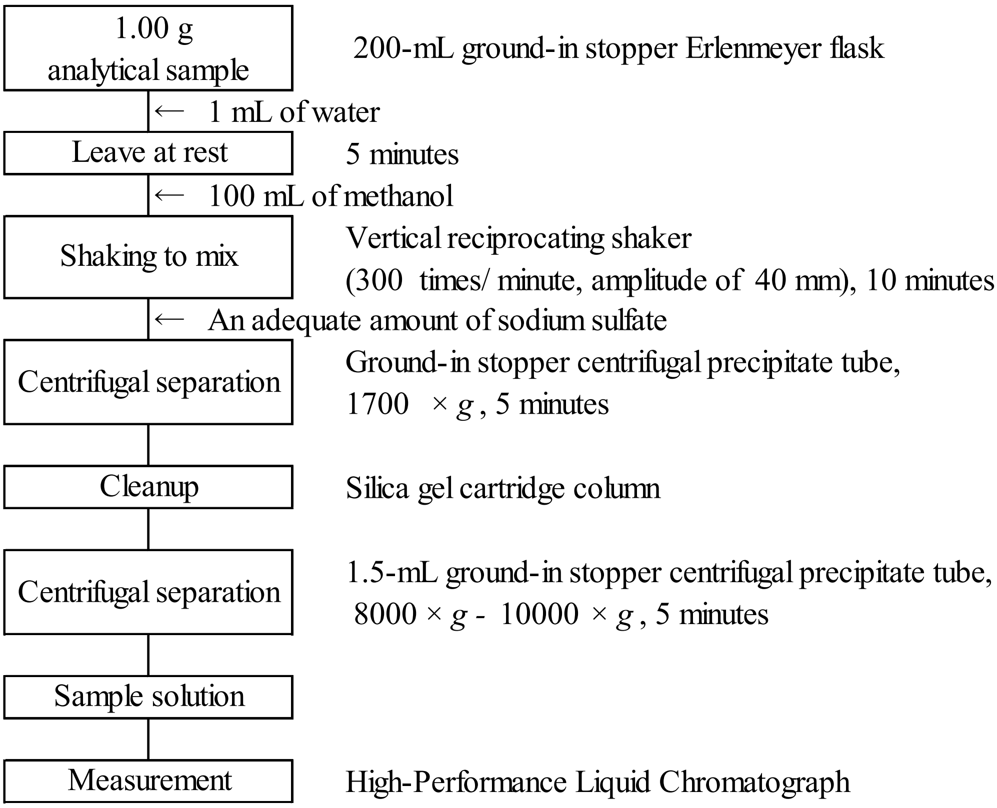
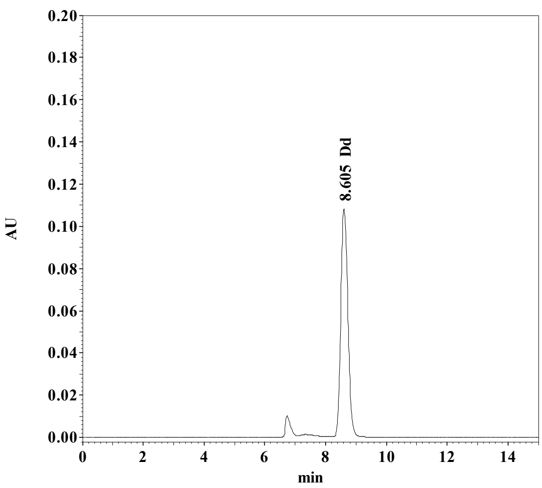
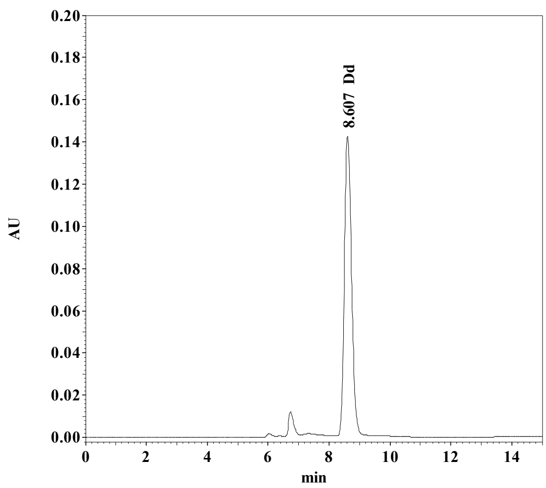


Figure Flow sheet for dicyandiamide (Dd) in fertilizers

Reference: Examples of the HPLC chromatogram of dicyandiamide standard solution for the calibration curve preparation and sample solution (compound fertilizer) are shown below.



1) Standard solution



2) Sample solution

Reference diagram HPLC chromatogram of dicyandiamide (Dd)

1) Dicyandiamide standard solution (the equivalents of 100 ng (10 µg/ mL, 10 µL) of dicyandiamide)
2) Sample solution (compound fertilizer)

Measurement conditions for HPLC

Column: Inertsil NH_2 (4.6-mm inner diameter, 250-mm long, 5-µm particle diameter)

Column oven temperature: 30 °C

Flow rate: 0.5 mL/min

Other conditions are according to the example of HPLC measurement conditions in **(4.3) a)**

7.6 2-sulfanilamide thiazole (ST)
7.6.a High-Performance Liquid Chromatography
(1) Summary
This testing method is applicable to fertilizers containing 2-sulfanilamide thiazole (ST). This testing method is classified as Type C and its symbol is 7.6.a-2017 or ST.a-1.
Add methanol−water (1+1) to an analytical sample to extract 2-sulfanilamide thiazole, introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with an octadecyl silylation silica gel column, and measure at a wavelength 285 nm to obtain 2-sulfanilamide thiazole (ST) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

(2) Reagent, etc.: Reagents and water are as shown below.
- **a) Water**: Water of A3 specified in JIS K 0557.
- **b) Methanol**: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.
- **c) Methanol**: Methanol used in the eluent of a High-Performance Liquid Chromatograph is a regent of High-Performance Liquid Chromatograph analysis grade or equivalents.
- **d) 2-sulfanilamide thiazole standard solution (1 mg/mL)** (1): Put 0.1 g of 2-sulfanilamide thiazole [$C_9H_9N_3O_2S_2$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add water to dissolve, transfer to a 1000-mL volumetric flask and add methanol−water (1+1) to the marked line. Store in a refrigerator, and do not use after 6 months after preparation.
- **e) 2-sulfanilamide thiazole standard solution (0.1 mg/mL)**: In the case of usage, put 10 mL of 2-sulfanilamide thiazole standard solution (100 µg/mL) in a 100-mL volumetric flask and add methanol−water (1+1) up to the marked line.
- **f) 2-sulfanilamide thiazole standard solution (10 µg/mL - 50 µg/mL) for the calibration curve preparation**: In the case of usage, put 5 mL - 25 mL of 2-sulfanilamide thiazole standard solution (0.1 mg/mL) in 50-mL volumetric flasks step-by-step and add methanol - water (1+1) up to the marked line.
- **g) 2-sulfanilamide thiazole standard solution (1 µg/mL - 10 µg/mL) for the calibration curve preparation**: In the case of usage, put 2.5 mL - 25 mL of 2-sulfanilamide thiazole standard solution for the calibration curve preparation (20 µg/mL) in 50-mL volumetric flasks step-by-step and add methanol−water (1+1) up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) A reagent of no less than 98 % (mass fraction) in purity is commercially sold as 2-sulfanilamide thiazole.

Comment 1 2-sulfanilamide thiazole is sold under the production name sulfathiazole by Tokyo Chemical Industry Co., Ltd, FUJIFILM Wako Pure Chemical Co., Ltd., and Kanto Chemical Co., Inc.

(3) Instruments: Instruments are as shown below:
- **a) High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.
  - **1) Column**: A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which octadecyl chemically bonds.
  - **2) Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
  - **3) Detection unit**: An absorptiometric detector that can measure at a wavelength around 285 nm.
- **b) Magnetic stirrer**
- **c) Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

d) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

e) **Acidic alumina cartridge column**: Link a 10-mL cylinder to a column (3) that is filled with 500 mg - 1 g of acidic alumina, put 3 mL of methanol and let it flow down.

Note (**3**) A cartridge with a 3-mL - 6-mL column filled with 500 mg – 1 g of silica gel can be used.

Comment 2 A column is sold under production names such as Inertsil ODS, Mightysil RP-18, L-column ODS, Shim-pack VP-ODS, Silica C18M 4D, Puresil C_{18} and COSMOSIL 5C18-MS-II.

Comment 3 An acidic alumina cartridge is commercially sold under production names such as Bond Elut AL-A, Sep-Pak Alumina-A and Supelclean LC-Alumina-A.

(4) Test procedures

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.

b) Add 100 mL of methanol - water (1+1) and stir it by using a magnetic stirrer for about 15 minutes.

c) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.

d) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (4) and use the supernatant (5) as an extract.

Note (**4**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**5**) If there is a possibility that the 2-sulfanilamide thiazole concentration in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of the extract with methanol.

(**4.2**) **Cleanup**: Conduct cleanup as shown below:

a) Put the extract in an acidic alumina cartridge column.

b) Dispose of about the first 3 mL of effluent and then put about the next 2 mL in a test tube.

c) Put the effluent in a 1.5-mL ground-in stopper centrifugal precipitate tube (6).

d) Centrifuge it at 8000 × g - 10000 × g centrifugal force for about five minutes (7) and use the supernatant as a sample solution.

Note (**6**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(**7**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 4 Instead of the procedures in (**4.2**) **c**) - **d**), it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of PTFE and the filtrate can be the sample solution.

Comment 5 The test is possible by the following procedures in the case of fertilizers not containing organic matters.

The procedures in (**4.1**) **c**) - **d**) and (**4.2**) **a**) - **b**) are omitted and “Put the effluent” in

(**4.2**) **c**) is replaced with the "After allowing to stand still, put the supernatant" to operate.

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions for the High-Performance Liquid Chromatograph is shown below. Set up the measurement conditions considering it:

1) **Column**: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter) to which octadecyl chemically bonds.

2) **Column bath temperature**: 30 °C - 40 °C

3) **Eluent**: Methanol−water (2+8)

4) **Flow rate**: 1 mL/min

5) **Detection unit**: An absorptiometric detector, a measurement wavelength: 285 nm

b) **Calibration curve preparation**

1) Inject 10 µL of respective 2-sulfanilamide thiazole standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 285 nm and obtain a peak area or height.

2) Prepare a curve for the relationship between the concentration and the peak area or height at a wavelength 285 nm of the respective 2-sulfanilamide thiazole standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 10 µL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain the 2-sulfanilamide thiazole content from the calibration curve to calculate 2-sulfanilamide thiazole (ST) in the analytical sample.

Comment 6 Recovery testing was conducted using compound fertilizer (1 sample) and blended fertilizer (2 sample), as a result, the mean recovery rates of 2-sulfanilamide thiazole at the concentration level of 1.0 % (mass fraction), 0.4 % (mass fraction) and 0.1 % (mass fraction) were 101.2 % - 102.1 %, 99.6 % - 101.7 % and 99.4 % - 101.0 %.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.005 % (mass fraction).

References

1) Yuji SHIRAI: The volumetric analysis of 2-sulfanilamide thiazole in fertilizers with High-Performance Liquid Chromatograph, Validation Report of Fertilizers (in Japanese), **44 (1)**, p. 10 - 20 (1991)

(5) Flow sheet for 2-sulfanilamide thiazole (ST): The flow sheet for 2-sulfanilamide thiazole in fertilizers is shown below:

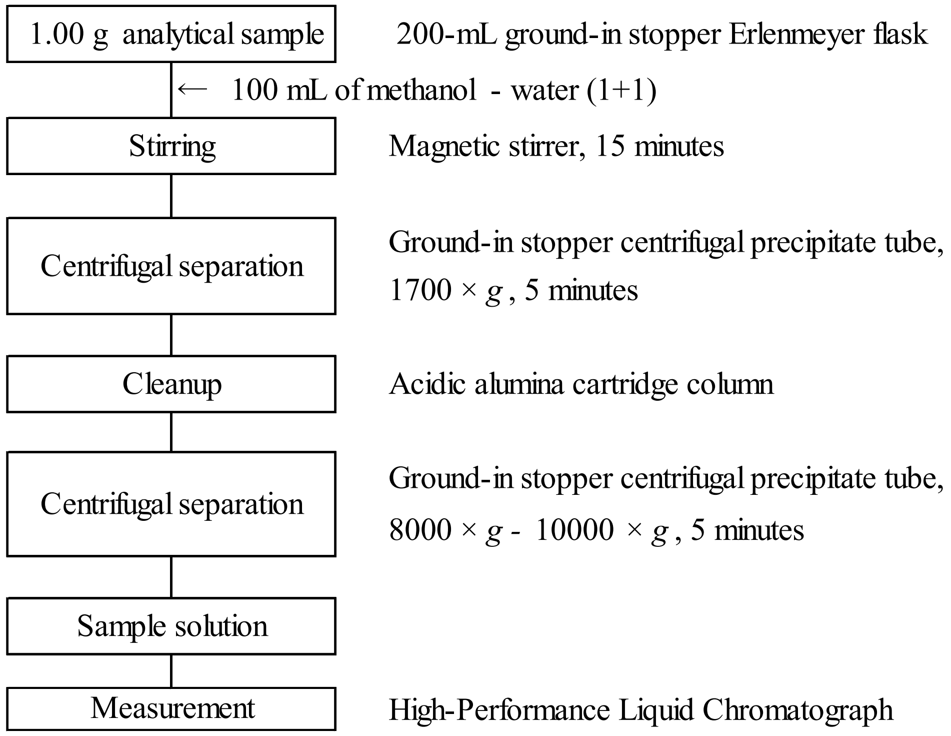
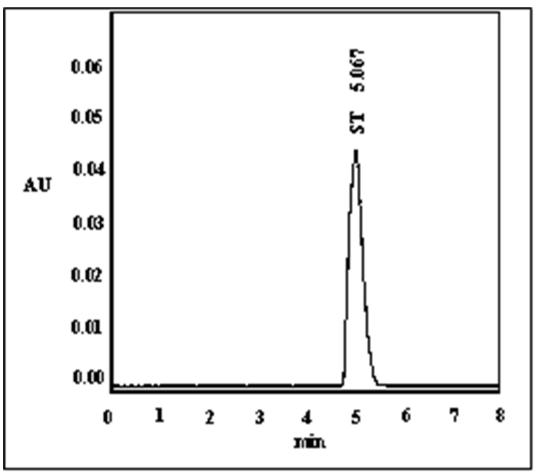


Figure Flow sheet for 2-sulfanilamide thiazole (ST) in fertilizers.

Reference: An example of the HPLC chromatogram of 2-sulfanilamide thiazole (ST) standard solution for the calibration curve preparation is shown below.



Reference diagram: HPLC chromatogram of 2-sulfanilamide thiazole (ST)

Measurement conditions for HPLC

Column: Mightysil RP-18 (4.6-mm inner diameter, 150-mm long, 5-µm particle diameter)

2-sulfanilamide thiazole standard solution (the equivalents of 200 ng)

Other conditions are according to the example of HPLC measurement conditions in **(4.3) a)**

7.7 3,4-Dimethylpyrazole phosphate (DMPP)

7.7.a High-Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers containing 3.4-dimethylpyrazole phosphate (DMPP). However, fertilizers containing organic matters and formaldehyde processed urea fertilizers are excluded from the scope of application. This testing method is classified as Type D and its symbol is 7.7.a-2021 or DMPP.a-1.

Add water to an analytical sample, agitate to extract, introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with an octadecyl silylation silica gel column, and measure at a wavelength of 224 nm to obtain 3,4-dimethylpyrazole phosphate (DMPP) in the analytical sample. Note that the performance of this testing method is shown in **Comment 5**.

(2) Reagent, etc.: Reagents and water are as shown below.

a) Water: Water of A3 specified in JIS K 0557.

b) Acetonitrile: A regent of High-Performance Liquid Chromatograph analysis grade or equivalents.

c) 3,4-Dimethylpyrazole phosphate (DMPP) standard solution (2 mg/mL) (1): Put 0.200 g of 3,4-dimethylpyrazole phosphate (DMPP) [$C_5H_{11}N_2O_4P$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to transfer to a 100-mL volumetric flask, sonicate using an ultrasonic generator to dissolve, and add water up to the marked line.

d) 3,4-Dimethylpyrazole phosphate (DMPP) standard solution (200 µg/mL) for the calibration curve preparation (1): Put 20 mL of 3,4-dimethylpyrazole phosphate standard solution (DMPP 2 mg/mL) in a 200-mL volumetric flask and add water up to the marked line.

e) 3,4-Dimethylpyrazole phosphate (DMPP) standard solutions (50 µg/mL - 100 µg/mL) for the calibration curve preparation (1): Put 25 mL - 50 mL of 3,4-dimethylpyrazole phosphate (DMPP) standard solution (200 µg/mL) in a 100-mL volumetric flask and add water up to the marked line.

f) 3,4-Dimethylpyrazole phosphate (DMPP) standard solutions (5 µg/mL - 25 µg/mL) for the calibration curve preparation (1): Put 5 mL - 25 mL of 3,4-dimethylpyrazole phosphate standard solution (DMPP 100 µg/mL) in 100-mL volumetric flasks step by step and add water up to the marked line.

g) 3,4-Dimethylpyrazole phosphate (DMPP) standard solutions (0.5 µg/mL - 2.5 µg/mL) for the calibration curve preparation (1): Put 5 mL - 25 mL of 3,4-dimethylpyrazole phosphate standard solution (DMPP 10 µg/mL) in 100-mL volumetric flasks step by step and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate. A DMPP standard solution is unstable: prepare at least the standard solutions in **d**) - **g**) at time of use. The standard solution prepared in **c**) is usable for about 1 month when stored refrigerated.

(**2**) A reagent of no less than 98 % (mass fraction) in purity as 3,4-dimethylpyrazole phosphate (DMPP) is commercially sold.

Comment 1 3,4-Dimethylpyrazole phosphate is sold by Tokyo Chemical Industry Co., Ltd. It is also commercially sold as 3,4-dimethylpyrazole phosphate by FUJIFILM Wako Pure Chemical Co., Ltd.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) High Performance Liquid Chromatograph: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) Column: A 4.6-mm inner diameter 150-mm long stainless steel column tube filled with 5-µm particle diameter silica gel, to which octadecyl chemically bonds.

2) **Column bath:** A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
3) **Detection unit:** An absorptiometric detector that can measure at a wavelength around 224 nm.

b) **Magnetic stirrer**

c) **High speed centrifugal separator:** A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 2 A column is sold under the production name Discovery C18, etc.

(**4**) **Test procedure**

(**4.1**) **Extraction**: Conduct extraction as shown below.

(**4.1.1**) **Solid test sample**

a) Weigh 1.0 g of an analytical sample (3), and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, put the supernatant solution (4) in a 1.5-mL ground-in stopper centrifugal precipitate tube (5).

d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (6) and use the supernatant as a sample solution.

Note (**3**) Crush the sample immediately after obtaining it.

(**4**) If there is a possibility that the 3,4-dimethylpyrazole phosphate (DMPP) concentration in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of supernatant with water.

(**5**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(**6**) 7.2-cm – 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8000 × *g* - 10000 × *g* centrifugal force.

Comment 3 Instead of the procedures in (**4.1**) **c**) - **d**), it is allowed to filter with a membrane filter (pore size: no more than 0.5 µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(**4.2**) **Measurement**: Conduct measurement according to JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph (HPLC) used in measurement.

a) **Measurement conditions for High Performance Liquid Chromatograph:** An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) **Column**: Octadecyl silylation silica gel column (4.6-mm inner diameter, 150-mm long, 5-µm particle diameter)
2) **Column bath temperature**: 40 °C
3) **Eluent** (1): Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL, and mix in 175 mL of acetonitrile. Filter with a membrane filter (pore size: no more than 0.5-µm) made of hydrophilic PTFE.
4) **Flow rate**: 0.7 mL/min
5) **Injection volume**: 10 µL
6) **Detection unit**: An absorptiometric detector, measurement wavelength: 224 nm

Comment 4 Eluent can be prepared as follows. Dissolve 15.6 g of sodium dihydrogen phosphate with water to make 1000 mL and store in a refrigerator. At the time of use, dilute a predetermined volume of the solution by a factor of 10, mix in acetonitrile 0.175 folds of the volume, and filter with a membrane filter (pore size: no more than 0.5 µm) made of hydrophilic PTFE.

b) Calibration curve preparation

1) Inject 10 µL of standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 224 nm and obtain a peak area.

2) Prepare a calibration curve for the relationship between the concentration and the ratio of peak area at a wavelength 224 nm of 3,4-dimethylpyrazole phosphate (DMPP).

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1)**, and obtain a peak area.

2) Obtain the 3,4-dimethylpyrazole phosphate (DMPP) content from the calibration curve, and calculate 3,4-dimethylpyrazole phosphate (DMPP) in the analytical sample.

Comment 5 Triplicates additive recovery testing was conducted to evaluate trueness using urea, compound fertilizer, and blended fertilizer (1 brand each) not containing 3,4-dimethylpyrazole phosphate (DMPP). As a result, the mean recovery rates at the additive level of 0.01 % (mass fraction) - 0.5 % (mass fraction) were 96.9 % - 101.3 %.
The results of repeated analyses on different days using urea, mixed nitrogen fertilizer, compound fertilizer (4 samples), and blended fertilizer were analyzed by the one-way analysis of variance. Table 1 shows the estimation results of intermediate precision and repeatability.
Note that the estimated minimum limit of quantification of this testing method was estimated to be about 0.003 % (mass fraction).

Table 1 Estimation results of repeatability and intermediate precision

Name of sample	Test days of repeatability (T)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Urea	5	0.258	0.0011	0.4	0.0036	1.4
Mixed nitrogen fertilizer	5	0.145	0.0011	0.8	0.0039	2.7
Compound fertilizer 1	5	0.105	0.0004	0.4	0.0044	4.1
Compound fertilizer 2	5	0.0533	0.0004	0.7	0.0019	3.5
Compound fertilizer 3	5	0.0372	0.0003	0.8	0.0012	3.3
Compound fertilizer 4	5	0.0325	0.0004	1.4	0.0006	1.9
Blended fertilizer	5	0.0184	0.0004	2.4	0.0005	2.8

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) × the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

(**5**) **Flow sheet for testing method**: The flow sheet for 3,4-dimethylpyrazole phosphate (DMPP) in fertilizers is shown below:

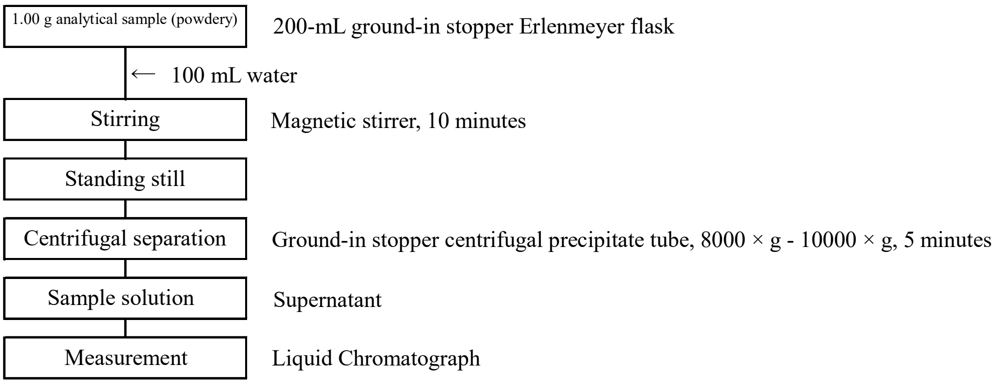
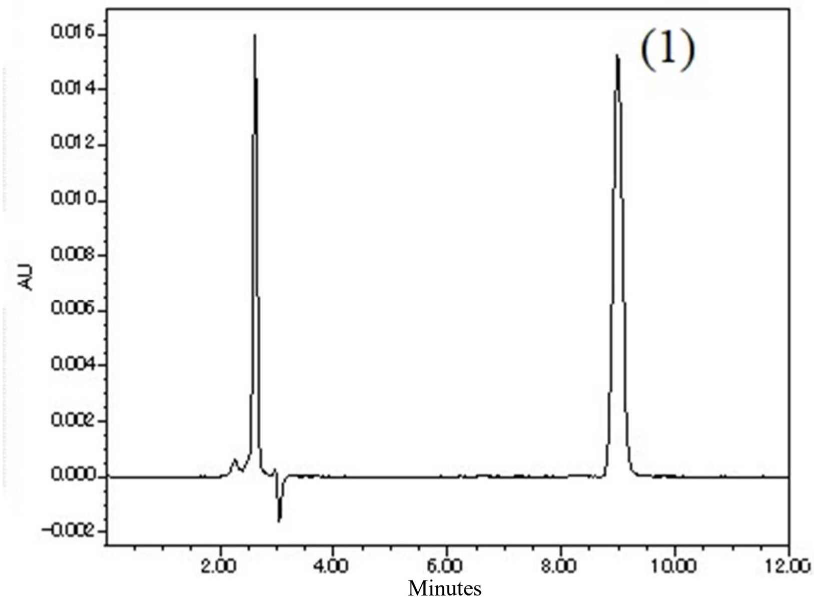


Figure Flow sheet for 3,4-dimethylpyrazole phosphate (DMPP) in fertilizers (Extraction procedure (4.1.1) and measurement)

Reference: An example of the chromatogram of the standard solution for calibration curve preparation of 3,4-dimethylpyrazole phosphate (DMPP) is shown below.



Reference diagram: HPLC chromatogram of the standard solutions for calibration curb preparation (10 mg/L)

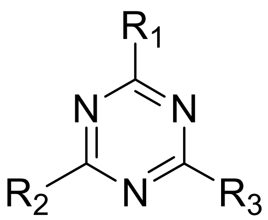
Peak name
(1) 3,4-Dimethylpyrazole phosphate (DMPP)
Measurement conditions for HPLC
Column: Discovery C18 (4.6-mm inner diameter, 150-mm long, 5-µm particle diameter)
Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**).

8. Others
8.1 Melamine and its degradation products
8.1.a Gas Chromatography/Mass Spectrometry
(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 8.1.a-2017 or Mel.a-1.

Extract melamine and its derivative substances (hereinafter referred to as “melamine derivations”) in organic matters and fertilizers containing organic matters with diethylamine−water−acetonitrile (1+4+5) and derivatize with BSTFA−TMCS (99+1) and then measure with a Gas Chromatography/ Mass Spectrometer to obtain melamine deviations in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

Comment 1 The structural formula of melamine and its degradation products is shown in the figure 1. During the production process of melamine, a by-product that replaces “-NH_2” of R1 - R3 with “-OH” is formed in some cases.



	R_1	R_2	R_3	MW
Melamine	NH_2	NH_2	NH_2	126.12
Ammeline	OH	NH_2	NH_2	127.10
Ammelide	OH	OH	NH_2	128.09
Cyanuric acid	OH	OH	OH	129.07

Figure 1 Structural formula of melamine and its degradation products

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Acetonitrile**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8039 or a reagent of equivalent quality.

c) **Diethylamine**: A guaranteed reagent or a reagent of equivalent quality.

d) **Pyridine (dehydration)** (1): A reagent of organic synthesis grade of no less than 99.5 % (mass fraction) in purity and no more than 0.05 mg/mL in moisture or a reagent of equivalent quality.

e) **Derivatization reagent** (2): Bis (trimethylsilyl) trifluoroacetamide−trimethylchlorosilane (99+1).

f) **Melamine derivations standard solution (0.5 mg/mL)**: Put about 0.05 g of melamine [$C_3H_6N_6$] (3), ammeline [$C_3H_5N_5O$] (3), ammelide [$C_3H_4N_4O_2$] (3) and cyanuric acid [$C_3H_3N_3O_3$] (3) into a weighing dish and measure the mass to the order of 0.1 mg. Dissolve in a small amount of diethylamine−water (1+4), transfer to a 100 mL volumetric flask respectively, and add the solvent up to the marked line.

g) **Mixture standard solution (50 µg/mL)** (3): Put 5 mL of respective melamine derivations standard solutions (0.5 mg/mL) in 50-mL volumetric flasks and add diethylamine−water−acetonitrile (1+4+5) up to the marked line.

Note **(1)** After it is opened once, add a proper amount of sodium sulfate (anhydrous) and seal tightly to store.

(2) A mixed derivatization reagent is commercially sold under the name BSTFA−TMCS (99+1).

(3) The respective standard reagents of melamine, ammeline, ammelide and cyanuric acid are commercially sold.

Comment 2 BSTFA−TMCS (99+1) is sold as 1-mL ampoule by SUPELCO. After it is opened once, use it on the same day.

Comment 3 The standard reagent of melamine, ammeline, ammelide and cyanuric acid are sold by FUJIFILM Wako Pure Chemical Co., Ltd. and Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(**3**) **Instruments**: Instruments are as shown below:

a) **Gas Chromatograph/Mass Spectrometer (GC/MS)**: GC/MS specified in JIS K 0123 that satisfies the following requirements.

1) **Gas Chromatograph**

(i) Sample injector: An injector that enables split less system.

(ii) Capillary column: A capillary column (0.25-mm - 0.32-mm inner diameter and 30-m long) made of fused silica. 5 % phenyl 95 % methyl polysiloxane chemically bonds to the inner surface of a capillary column with 0.25 µm thickness. The column is according to the specification of mass spectrometer.

(ii) Carrier gas: High purity helium of no less than 99.999 % (volume fraction) in purity.

2) **Mass Spectrometer**

(i) Ionization method: Electron-Impact ionization (EI) method

(ii) Ion detection method: Selected Ion Monitoring (SIM) method

b) **Ultrasonic generator**: An ultrasonic washer can be used.

c) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

d) **Concentrator**: A Centrifugal evaporator that can be adjusted to 70 ºC ± 2 ºC.

e) **Water bath**: Water bath that can be adjusted to 70 ºC ± 2 ºC.

Comment 4 A capillary column is commercially sold under the names such as DB-5ms, Rtx-5ms, HP-5ms, SLB-5ms, BPX-5, CP-Sil 8CB low Bleed/MS and TC -5HT for GC/MS.

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 0.50 g of an analytical sample, and put it into a 200 mL - 200-mL ground-in stopper Erlenmeyer flask.

b) Add 160 mL - 200 mL of diethylamine−water−acetonitrile (1+4+5), and subject to ultra-sonication for about 30 minutes using an ultrasonic generator.

c) Put about 1.5 mL in a 1.5-mL ground-in stopper centrifugal precipitate tube (4), and centrifuge at 8000 × *g* - 10000 × *g* for about 5 minutes (5).

d) Put 1 mL of the supernatant solution in 5-mL - 50-mL volumetric flasks, add diethylamine−water−acetonitrile (1+4+5) up to the marked line to make an extract.

Note (**4**) Confirm that the tube is made of polypropylene, etc. to not affect testing results.

(**5**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 5 Grind until it completely passes through a sieve of 500 µm aperture to prepare the test sample.

Comment 6 Weigh 0.5 g of an analytical sample, extract with 200 mL of diethylamine – water − acetonitrile (1+4+5). If it is diluted 50 times in the procedure in **d**), the quantitative range of melamine derivations in the analytical sample is 0.2 % (mass fraction) - 10 % (mass fraction). In the case of measuring melamine derivations which do not reach the quantitative range, make the dilution factor in the procedure in **d**) decrease. In addition, if the contents of melamine derivations exceed 10 % (mass fraction) respectively, the sampling volume of an analytical sample should be reduced.

(**4.2**) **Derivatization**: Conduct derivatization as shown below.

a) Put 0.2 mL of the extract in a 5-mL - 10-mL test tube with a screw stopper.

b) Place a test tube in a concentrator, concentrate under reduced pressure at 70 ºC ± 2 ºC and vaporize the solvent completely (6).

c) Add 0.3 mL of pyridine (dehydration) (1) and 0.2 mL of derivatization reagent (2) to the residue to mix, and then stopple to seal tightly.

d) After heating in a water bath at 70 ºC ± 2 °C for about 45 minutes (7), let it stand to cool to make a sample solution (8).

Note (**6**) A spraying type concentrator can be used.

(**7**) If moisture remains after the procedure in **b**) or the reagent used in the procedure in **c**) contains moisture, the reaction of the derivatization in **d**) does not advance enough in some cases.

(**8**) If necessary, put the sample solution into a 1.5-mL ground-in stopper centrifugal precipitate tube (4) to centrifuge at 8000 × g - 10000 × g for about 5 minutes (5).

(**4.3**) **Measurement**: Conduct the measurement as indicated in JIS K 0123 and as shown below. Specific measurement procedures are according to the operation method of the Gas Chromatograph/Mass Spectrometer used in measurement.

a) **Measurement conditions for the Gas Chromatograph/Mass Spectrometer**: An example of measurement conditions for the Gas Chromatograph/Mass Spectrometer is shown below. Set up the measurement conditions considering it:

1) **Gas Chromatograph**

(i) Sample injection method: split less injection method (1min)

(ii) Temperature of sample injector: 280 °C

(ii) Capillary column: A capillary column (0.25-mm - 0.32-mm inner diameter, 30-m long, 0.25 µm layer thickness) made of fused silica. 5 % phenyl 95 % methyl polysiloxane chemically bonds to the inner surface of the capillary column.

(iv) Temperature of column bath: 100 °C (1 min) → (15 °C /min) → 320 °C (3 min)

(v) Temperature of GC/MS coupling portion: 250 °C

(vi) Carrier gas: helium, flow rate: 1.5 mL/min

2) **Mass Spectrometer**

(i) Ionization method: Electron-Impact ionization (EI) method

(ii) Ionization voltage: 70 V

(iii) Temperature of ion source: 230 °C

(iv) Ion detection method: Selected Ion Monitoring (SIM) method

(v) Measurement of ion: Shown in table 1

Table 1 Fragment ion of materials subjected to measurement

Materials to be measured	Measured fragment ion (*m/z*)				
	Determination	Validation	Validation	Validation	Validation
Melamine	342	344	327	285	213
Ammeline	328	345	343	285	214
Ammelide	344	346	329	214	198
Cyanuric acid	345	347	330	215	188
DACP(I.S.)	288	289	290	273	275

b) **Calibration curve preparation**

1) Put 5 mL of mixture standard solution (50 µg/mL) in a 50-mL volumetric flask and add diethylamine−water−acetonitrile (1+4+5) up to the marked line to make a mixture standard solution (5 µg/mL).

2) Put 1 mL - 20 mL of the mixture standard solution (5 µg/ mL) in 50 mL volumetric flasks step-by-step and add diethylamine−water−acetonitrile (1+4+5) up to the marked line to make a mixture standard solution (0.1 µg/mL - 2 µg/mL).

3) Conduct the procedures of (**4.2**) **b**) - **d**) for the mixture standard solution (0.1 µg/mL - 2 µg/mL) to make a mixture standard solution, the equivalents of 0.04 µg/mL - 0.8 µg/mL, for calibration curve.

4) Inject 1 µL of respective mixture standard solutions for calibration curve to the GC/MS and record the chromatogram of ion (*m/z*) for determination and ion (*m/z*) for validation of materials subjected to measurement to obtain a peak area or height.

5) Calculate the peak area ratio or height ratio of ion (*m/z*) for determination and ion (*m/z*) for validation of respective materials subjected to measurement.

6) Prepare a curve for the relationship between the concentration of material subjected to measurement and the peak area or height of ion (*m/z*) for determination of respective mixture solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 1 µL of the sample solution to the same procedure as in **b**) **4**) - **5**) (9).

2) Obtain the content of respective materials subjected to measurement from the calibration curve to calculate respective materials subjected to measurement in the analytical sample.

Note (**9**) Confirm that the peak area ratio or height ratio of ion (*m*/*z*) for determination to ion (*m*/*z*) for validation is within the range of about ± 30 % of the peak area ratio or height ratio for the standard solution. In addition, the peak area ratio or height ratio depends on the concentration.

Comment 7 If the variation of sensitivity of melamine derivations is observed, conduct measurement by the following method of **a**) or **b**).

a) In the procedure in (**4.3**) **c**) **1**), inject the sample solution into the GC/MS predetermined times, and then correct the calibration curve according to (**4.3**) **b**) **4**) - **6**).

b) Add 2,6-diamino-4-chloropyrimidine (the equivalents of 0.5 µg) as an internal reference material to the standard solution and the sample solution, conduct the same procedures as (**4.2**) **c**) - **d**), (**4.3**) **b**) **4**) - **6**) and **c**) **1**). However, prepare the calibration curve from the peak area ratio or height ratio of ion (*m/z*) for determination of respective materials subjected to measurement and internal reference material, and calculate the concentration of respective materials subjected to measurement in the analytical sample.

Comment 8 Recovery testing of melamine derivations was conducted using soybean meal, fish meal, fish waste processed fertilizer, mixed organic fertilizer, blended fertilizer and compound fertilizer, as a result, the mean recovery rates at the additive level of 10 % (mass fraction) and 0.2 % (mass fraction) were 92.1 % - 102.9 % and 90.3 % - 102.2 %. Note that the minimum limit of quantification of melamine derivations of this test method was estimated to be about 0.01 % (mass fraction).

References

1) Yuji SHIRAI, Jun OKI: Validation of Gas Chromatography/Mass Spectrometry for Determination of Melamine and Its Degradation Products in Fertilizers, Research Report of Fertilizer, **Vol. 1**, p. 114 - 121 (2008)

(5) **Flow sheet for melamine derivations**: The flow sheet for melamine derivations in fertilizers is shown below.

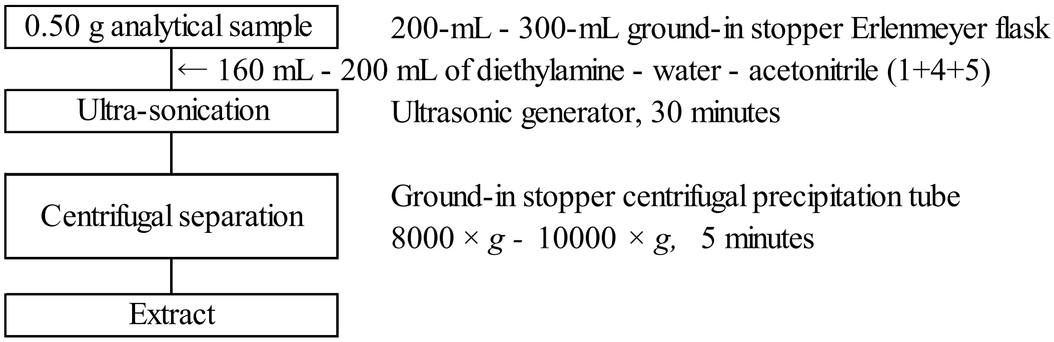


Figure 1 Flow sheet for melamine derivations in fertilizers (Extraction procedure)

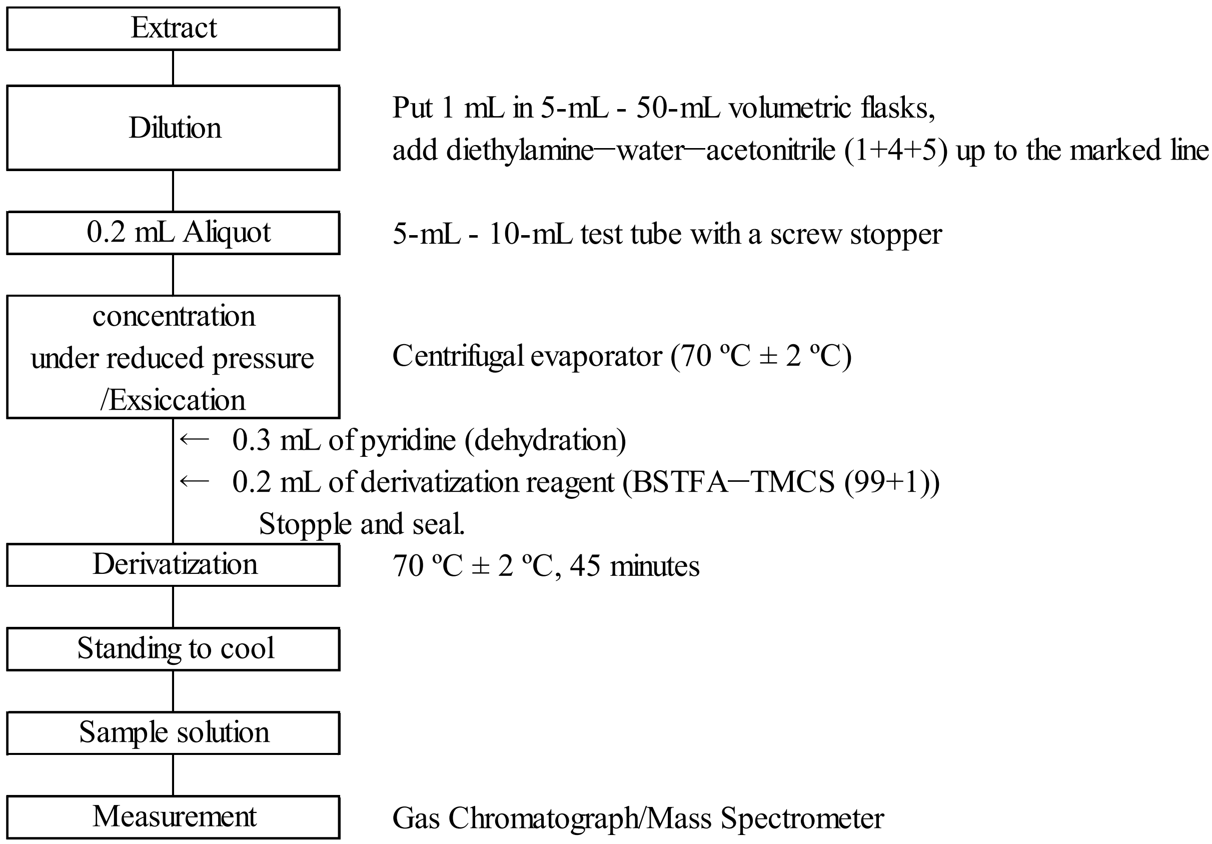


Figure 2 Flow sheet for melamine derivations in fertilizers
(Derivatization and measurement procedure)

Reference: An example of the Total Ion Chromatogram (TIC) of GC/MS of the mixture standard solution for calibration curve preparation of melamine derivations is shown below.

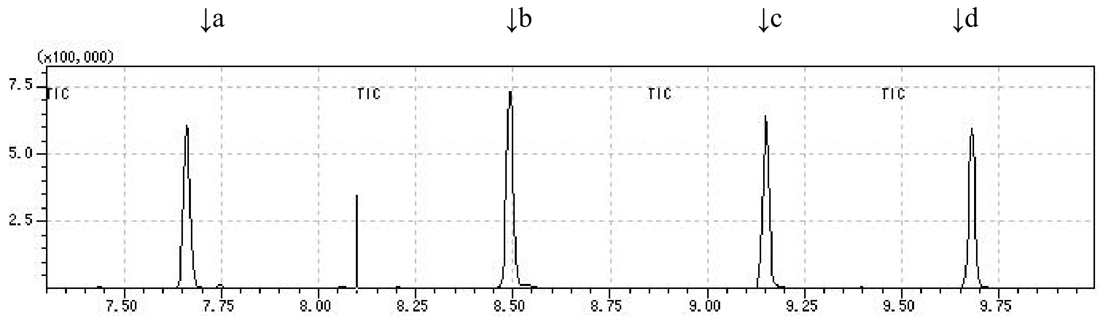


Figure 3 Total Ion Chromatogram (TIC) of GC/MS for melamine derivations

Measurement conditions of GC/MS

Capillary column: Rtx-5ms (0.25-mm inner diameter, 30-m long, 0.25 µm layer thickness)

Other conditions are according to the example of HPLC measurement conditions in **(4.3) a)**

Peak name of respective total ion chromatograms

a) Cyanuric acid b) Ammelide

c) Ammeline d) Melamine

Sample and amount injected into GC/MS

Injected sample: Mixture standard solutions (the equivalents of 2 µg/mL) for the calibration curve preparation of respective melamine derivations.

Injected amount: 1 µL (the equivalents of 2 ng of respective melamine derivations)

8.1.b

8.1.c High-Performance Liquid Chromatography (Fertilizers not containing organic matters)

(1) Summary

This testing method is applicable to fertilizers not containing organic matters. This testing method is classified as Type B and its symbol is 8.1.c-2017 or Mel.c-1.

Add hydrochloric acid (1+15) to an analytical sample, extract melamine and its degradation products (hereinafter referred to as “melamine derivations”), introduce them into a High-Performance Liquid Chromatograph (HPLC), isolate with a silica gel column to which carbamoyl chemically bonds, and measure at a wavelength 214 nm to obtain melamine derivations in an analytical sample. In addition, the performance of this testing method is shown in **Comment 4**.

(2) **Reagent, etc**.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Acetonitrile**: A JIS Guaranteed Reagent specified in JIS K 8032 or a reagent of equivalent quality. In addition, acetonitrile used in the eluent of a High-Performance Liquid Chromatograph is a reagent of High-Performance Liquid Chromatograph analysis grade.

c) **Hydrochloric acid**: A guaranteed reagent or a reagent of equivalent quality.

d) **Phosphate buffer solution** (1): Dissolve 0.237 g of disodium hydrogen-phosphate specified in JIS K 9020 and 0.520 g of sodium dihydrogen-phosphate dihydrate specified in JIS K 9009 in water to make 1000 mL (2). If it is used for the eluent of a High-Performance Liquid Chromatograph, filter with a membrane filter (pore size: no more than 0.5-µm) made of hydrophilic PTFE.

e) **Melamine derivations standard solution (0.5 mg/mL)**: Put about 0.05 g of melamine [$C_3H_6N_6$] (3), ammeline [$C_3H_5N_5O$] (3), ammelide [$C_3H_4N_4O_2$] (3) and cyanuric acid [$C_3H_3N_3O_3$] (3) into a weighing dish and measure the mass to the order of 0.1 mg. Dissolve them with a small amount of hydrochloric acid (1+15), transfer to 100 mL volumetric flasks respectively, and add the solutions up to the marked line.

f) **Mixture standard solution (50 µg/mL)** (1): Put 5 mL of respective melamine derivations standard solutions (0.5 mg/mL) in 50-mL volumetric flasks and add acetonitrile - phosphate buffer solutions (4+1) up to the marked line.

g) **Mixture standard solution for calibration curve preparation (1 µg/mL - 5 µg/mL)**: In the case of usage, put 1 mL - 5 mL of mixture standard solution (50 µg /mL) in 50-mL volumetric flasks step-by-step and add acetonitrile‒phosphate buffer solutions (4+1) up to the marked line.

h) **Mixture standard solution for calibration curve preparation (0.05 µg/mL - 0.5 µg/mL)**: In the case of usage, put 2.5 mL - 25 mL of mixture standard solution (1 µg /mL) in 50-mL volumetric flasks step-by-step and add acetonitrile‒phosphate buffer solutions (4+1) up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) The phosphate buffer solution becomes pH 6.7 ± pH 0.2.

(**3**) The respective standard reagents of melamine, ammeline, ammelide and cyanuric acid are commercially sold.

Comment 1 The standard reagent of melamine, ammeline, ammelide and cyanuric acid are sold by FUJIFILM Wako Pure Chemical Co., Ltd. and Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(3) **Instruments**: Instruments are as shown below:

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube

filled with silica gel, to which carbamoyl chemically bonds.

2) **Column bath**: A column bath whose temperature can be adjusted to 40 ºC ± 1 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 214 nm.

b) **Ultrasonic generator**: An ultrasonic washer can be used.

c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

d) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 2 A column is sold under the production name TSKgel Amide-80, etc. A column that has been confirmed to be able to completely separate melamine, ammeline, ammelide and cyanuric acid should be used.

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 0.50 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.

b) Add 100 mL of hydrochloric acid (1+15) and conduct ultra-sonication for about 30 minutes using an ultrasonic generator.

c) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.

d) Centrifuge it at 1700 × *g* centrifugal force for about five minutes (4) and use the supernatant as an extract.

e) Put 5 mL of the extract (5) into a 50-mL volumetric flask, and add an acetonitrile‒phosphate buffer solution (4+1) up to the marked line to dilute.

f) Put dilution liquid in a 1.5-mL ground-in stopper centrifugal precipitate tube (6).

g) Centrifuge at 8000 × *g* - 10000 × *g* for about 5 minutes (7) to make supernatant as a sample solution

Note (**4**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**5**) If there is a possibility that the concentration of melamine derivations in the sample solution exceeds the maximum limit of the calibration curb, the amount of a supernatant solution to be transferred should be1 mL - 2.5 mL.

(**6**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement

(**7**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 3 Instead of the procedures in (**4.1**) **f**) - **g**), it is allowed to filter with a membrane filter (pore size: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions for the High-Performance Liquid Chromatograph is shown below. Set up the measurement conditions considering it:

1) **Column**: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm

particle diameter column) to which carbamoyl chemically bonds.

2) **Column bath temperature**: 40 °C ± 1 °C

3) **Eluent**: Acetonitrile - phosphate buffer solution (4+1)

4) **Flow rate**: 1 mL/min

5) **Detection unit**: An absorptiometric detector, a measurement wavelength: 214 nm

b) **Calibration curve preparation**

1) Inject 10 µL of respective mixture standard solutions for calibration curve preparation into the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 214 nm and obtain a peak area or height.

2) Prepare a curve for the relationship between the concentration and the peak area or height at a wavelength 214 nm of respective mixture solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 10 µL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain the melamine derivations content from the calibration curve to calculate respective melamine derivations in the analytical sample.

Comment 4 Recovery testing was conducted using 3 brands of nitrolime, 1 brand of compound fertilizer containing nitrolime, 2 brands of compound fertilizers not containing nitrolime, 1 brand of ammonium sulfate and 1 brand of urea, as a result, the recovery rates of melamine derivations at the concentration level of 4 % (mass fraction) and 0.1 % (mass fraction) were 90.5 % - 106.3 % and 92.2 % - 107.0 %.

Additionally, results from a collaborative study for test method validation and its analysis are in Table 1.

Note that the minimum limit of quantification of the test method was estimated to be about 0.02 % (mass fraction) for melamine and cyanuric acid and about 0.01 % (mass fraction) for ammeline and ammelide. In the case of ammelide and cyanuric acid, the sufficient reproducibility was observed in the range of 0.188 % (mass fraction) - 1.10 % (mass fraction) and 0.105 % (mass fraction) - 1.15 % (mass fraction) respectively.

Table 1 Analysis resultsof the collaborative study
for the test method validation of melamine deivations

Agrichemical name	Sample name	Number of laboratories[1)]	Mean[2)] (%)[7)]	s_r[3)] (%)[7)]	RSD_r[4)] (%)	s_R[5)] (%)[7)]	RSD_R[6)] (%)
Melamine	Nitrolime 1	9	2.83	0.04	1.4	0.12	4.3
	Nitrolime 2	10	0.391	0.003	0.8	0.023	5.8
	Compound fertilizer containing nitrolime	9	0.845	0.019	2.2	0.036	4.2
	Compound ferttilizer	11	0.198	0.005	2.6	0.012	6.2
	Ammonium sulfate	10	0.0343	0.0015	4.5	0.0040	11.6
Ammeline	Nitrolime 1	9	1.60	0.02	1.3	0.06	3.8
	Nitrolime 2	10	0.105	0.001	1.3	0.002	2.3
	Compound fertilizer containing nitrolime	9	0.629	0.027	4.3	0.023	3.7
	Compound ferttilizer	11	0.195	0.004	2.1	0.009	4.5
	Ammonium sulfate	10	0.0346	0.0013	3.7	0.0024	6.9
Ammelide	Nitrolime 1	9	1.10	0.02	2.1	0.08	7.6
	Nitrolime 2	11	0.361	0.008	2.2	0.023	6.5
	Compound fertilizer containing nitrolime	9	0.188	0.004	2.2	0.014	7.5
	Compound ferttilizer	11	0.718	0.028	3.9	0.052	7.2
	Ammonium sulfate	11	0.0345	0.0031	8.9	0.0056	16.1
Cyanuric acid	Nitrolime 1	9	1.15	0.06	4.8	0.09	7.7
	Nitrolime 2	10	0.390	0.018	4.5	0.029	7.4
	Compound fertilizer containing nitrolime	9	0.105	0.003	2.9	0.014	13.2
	Compound ferttilizer	9	0.788	0.026	3.2	0.054	6.8
	Ammonium sulfate	10	0.0365	0.0015	4.2	0.0067	18.3

1) Number of laboratories used in analysis
2) Gross mean (n = number of laboratories × number of repeated tests (2))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Reproducibility standard deviation
6) Reproducibility relative standard deviation
7) Mass fraction

References

1) Etsuko BANDO and Yuji SHIRAI: Validation of High-Performance Liquid Chromatography (HPLC) for Determination of Melamine and Its Related Substances in Fertilizer, Research Report of Fertilizer, **Vol. 6**, p. 27 - 35 (2013)

2) Etsuko BANDO and Sigehiro KAI: Determination of Melamine and Its Related Substances in Fertilizer by High-Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer, **Vol. 7**, p. 10 - 21 (2014)

(5) Flow sheet for melamine derivations: The flow sheet for melamine derivations in fertilizers is shown below.

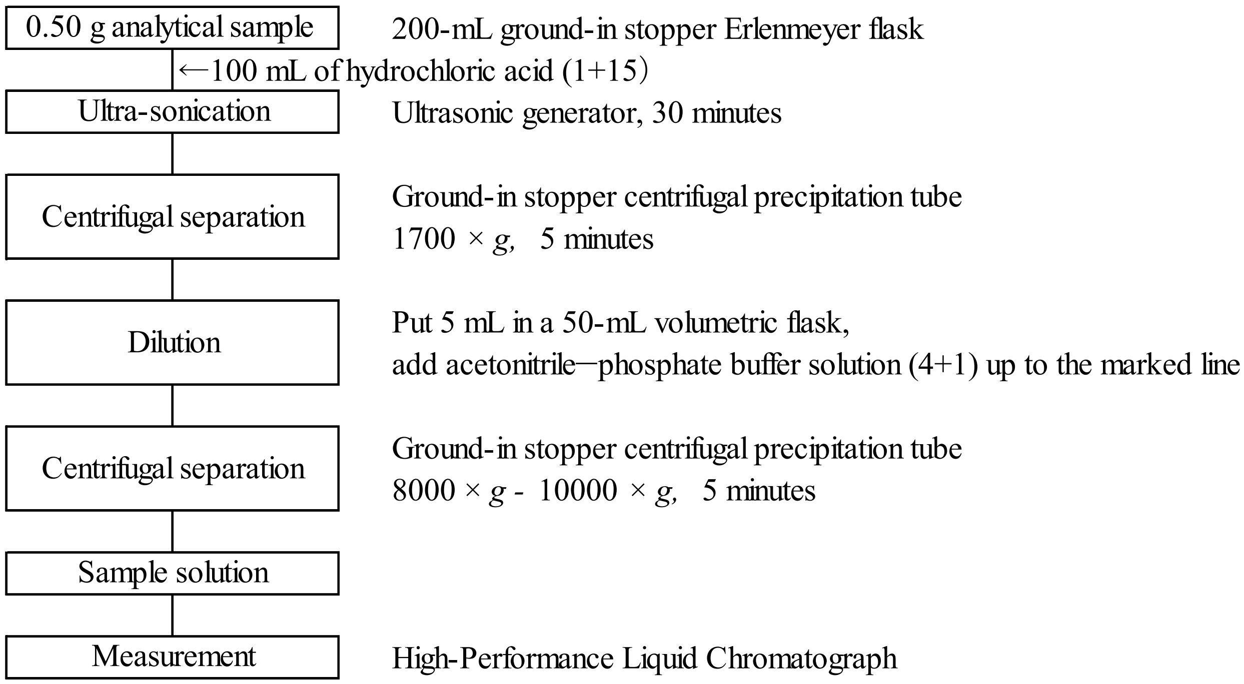
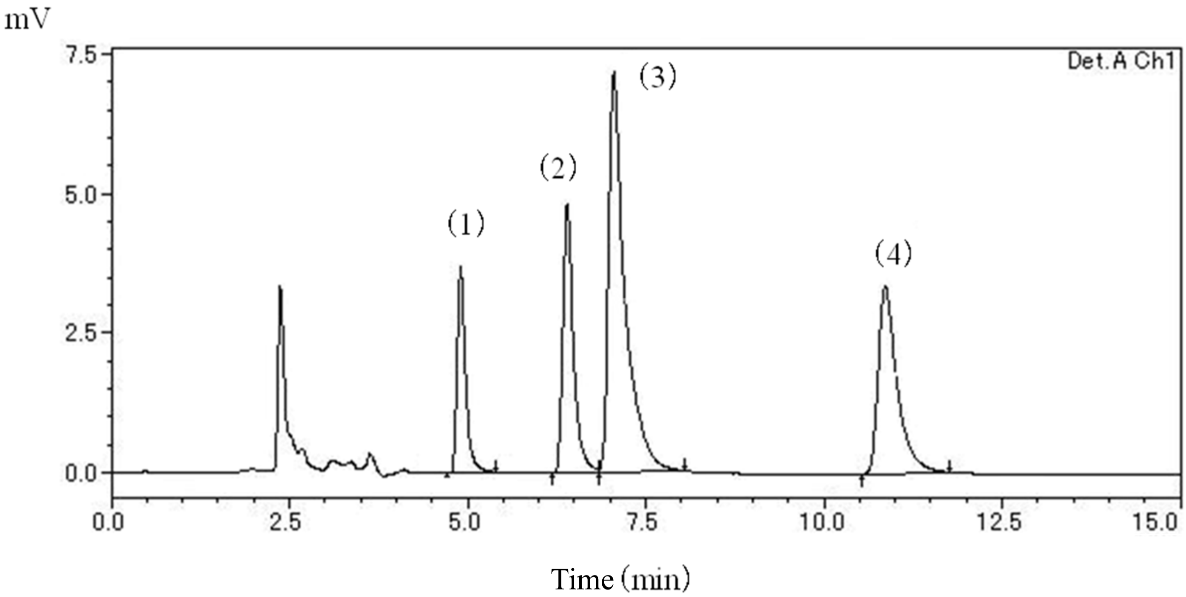


Figure Flow sheet for melamine derivations in fertilizers

Reference: An example of the HPLC chromatogram of the mixture standard solution for calibration curve preparation of melamine derivations is shown below.



Reference diagram: HPLC chromatogram of melamine derivations

The names of materials for respective peaks
(1) cyanuric acid (2) ammelide (3) melamine (4) ammeline
Measurement conditions for HPLC
Column: TSKgel Amide-80 (4.6-mm inner diameter, 250-mm long, 5-µm particle diameter)
Mixture standard solutions (respective equivalents of 10 ng (1 µg/mL, 10 µL)) for calibration curve preparation of respective melamine derivations
Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**)

8.1.d High-Performance Liquid Chromatography (Fertilizers containing organic matters)

(1) Summary

This testing method is applicable to organic fertilizers and fertilizers containing organic matters. This testing method is classified as Type D and its symbol is 8.1.d-2017 or Mel.d-1.

Add water to an analytical sample to extract melamine, introduce it into a High-Performance Liquid Chromatograph (HPLC), isolate with a silica gel column to which carbamoyl chemically bonds, and measure at a wavelength 214 nm to obtain melamine in an analytical sample. However, cyanuric acid, ammelide and ammeline which are melamine derivations are excluded from components subjected to measurement. In addition, the performance of this testing method is shown in **Comment 4**.

(**2**) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Acetonitrile**: A JIS Guaranteed Reagent specified in JIS K 8032 or a reagent of equivalent quality. In addition, acetonitrile used in the eluent of a High-Performance Liquid Chromatograph is a reagent of High-Performance Liquid Chromatograph analysis grade.

c) **Phosphate buffer solution** (1): Dissolve 0.237 g of disodium hydrogen-phosphate specified in JIS K 9020 and 0.520 g of sodium dihydrogen-phosphate dihydrate specified in JIS K 9009 in water to make 1000 mL (2). If it is used for the eluent of a High-Performance Liquid Chromatograph, filter with a membrane filter (pore size: no more than 0.5-µm) made of hydrophilic PTFE.

d) **Melamine standard solution (0.5 mg/mL)** (1): Put 0.05 g of melamine [$C_3H_6N_6$] (3) in a weighing dish and measure the mass to the order of 0.1 mg. Dissolve it with a small amount of water, transfer to a 100 mL volumetric flask and add the solution up to the marked line.

e) **Melamine standard solution (50 µg/mL)** (1): Put 5 mL of melamine solution (0.5 mg/mL) in a 50-mL volumetric flask and add acetonitrile - phosphate buffer solutions (82+18) up to the marked line.

f) **Melamine standard solution for calibration curve preparation (1 µg/mL - 5 µg/mL)**: In the case of usage, put 1 mL - 5 mL of melamine standard solution (50 µg /mL) in 50-mL volumetric flasks step-by-step and add acetonitrile−phosphate buffer solutions (82+18) up to the marked line.

g) **Melamine standard solution for calibration curve preparation (0.05 µg/mL - 0.5 µg/mL)**: In the case of usage, put 2.5 mL - 25 mL of melamine standard solution (1 µg/ mL) in 50-mL volumetric flasks step-by-step, add acetonitrile−phosphate buffer solutions (82+18) up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) The phosphate buffer solution becomes pH 6.7 ± 0.2.
(**3**) A standard reagent as melamine is commercially sold.

Comment 1 A standard reagent of melamine is sold by FUJIFILM Wako Pure Chemical Co., Ltd., Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(**3**) **Instruments**: Instruments are as shown below:

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 4-mm - 6-mm inner diameter 150-mm - 250-mm long stainless steel column tube filled with silica gel, to which carbamoyl chemically bonds.

2) **Column bath**: A column bath whose temperature can be adjusted to 40 ºC ± 1 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 214 nm.

b) **Ultrasonic generator**: An ultrasonic washer can be used.

c) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

d) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 2 Column is sold under the production name TSKgel Amide-80, etc. A column which has actually isolated melamine, ammeline, ammelide and cyanuric acid should be used.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 0.50 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and conduct ultra-sonication for about 10 minutes using an ultrasonic generator.

c) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.

d) Centrifuge it at 1700 × *g* centrifugal force for about 10 minutes (4) and use the supernatant as an extract.

e) Put 5 mL of the extract (5) into a 50-mL volumetric flask, and add an acetonitrile−phosphate buffer solution (82+18) up to the marked line to dilute.

f) Put dilution liquid in a 1.5-mL ground-in stopper centrifugal precipitate tube (6).

g) Centrifuge at 8000 × *g* - 10000 × *g* for about 5 minutes (7) to make supernatant as a sample solution

Note (**4**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**5**) If there is a possibility that the concentration of melamine derivations in the sample solution exceeds the maximum limit of the calibration curb, the aliquot volume to be taken of a supernatant solution should be 1 mL - 2.5 mL.

(**6**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement

(**7**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 3 Instead of the procedures in (**4.1**) **f**) - **g**), it is allowed to filter with a membrane filter (pore size: no more than 0.5 µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(4.2) Measurement: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions for the High-Performance Liquid Chromatograph is shown below. Set up the measurement conditions considering it:

1) **Column**: A silica gel column (4-mm - 6-mm inner diameter, 150-mm - 250-mm long, 5-µm particle diameter column) to which carbamoyl chemically bonds.

2) **Column bath temperature**: 40 °C ± 1 °C

3) **Eluent**: Acetonitrile - phosphate buffer solution (82+18)

4) **Flow rate**: 1 mL/min

5) **Detection unit**: An absorptiometric detector, measurement a wavelength: 214 nm

b) Calibration curve preparation

1) Inject 10 µL of respective melamine standard solutions for calibration curve preparation into the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 214 nm and obtain a peak area or height.
2) Prepare a curve for the relationship between the concentration and the peak area or height at a wavelength 214 nm of respective melamine solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b) 1**)
2) Obtain the melamine content from the calibration curve to calculate melamine in the analytical sample.

Comment 4 Additive recovery testing was conducted to evaluate trueness using rape seed meal, soybean meal, compound fertilizer containing lime nitrogen and organic matter, compound fertilizer containing organic matter and blended fertilizer containing organic matter (1 brand for each). As a result, the mean recovery rates at the additive level of 2 % (mass fraction), 0.4 % (mass fraction) and 0.1 % (mass fraction) were 94.6 % - 99.8 %, 92.4 % - 98.5 % and 93.1 % - 98.4 % respectively.

The results of the repeatability tests on different days using soybean meal and compound fertilizer containing organic matter, to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.02 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of melamine

Name of sample	Test days of repeatability (T)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Soybean meal	5	1.91	0.03	1.7	0.04	2.2
Compound fertilizer containing organic matter	5	0.100	0.001	1.4	0.002	2.5

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Etsuko FUNAMIZU: Determination Method for Melamine in Organic Fertilizer and Fertilizer Containing Organic Matter using High-Performance Liquid Chromatograph (HPLC), Research Report of Fertilizers, **Vol. 9**, p. 33 - 42 (2016)

(5) Flow sheet for melamine derivations: The flow sheet for melamine derivations in fertilizers is shown below.

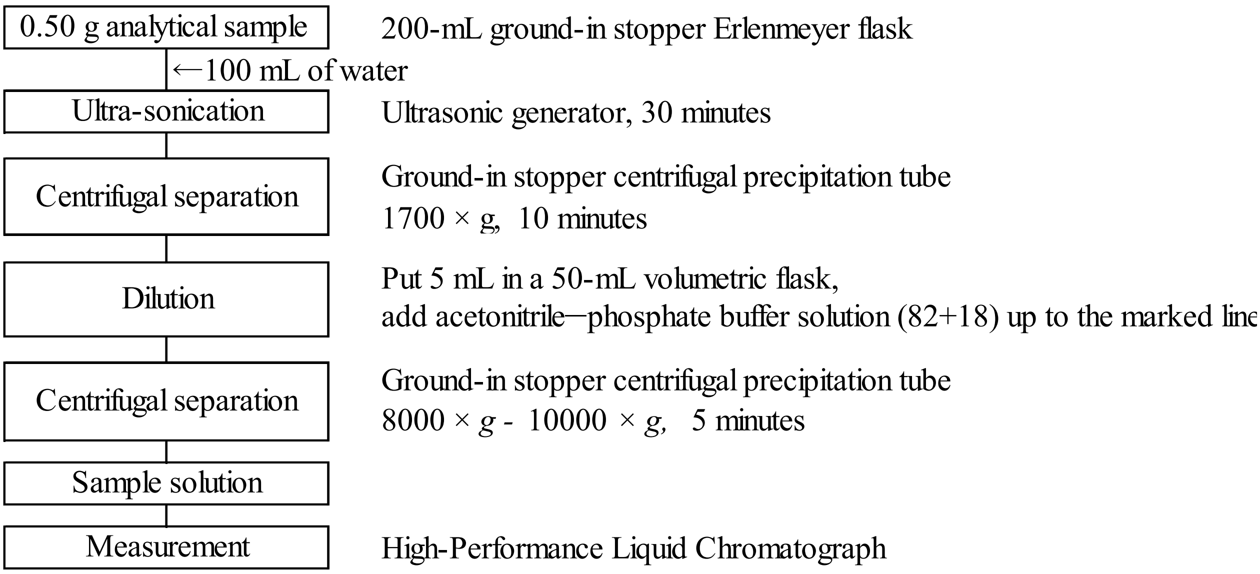
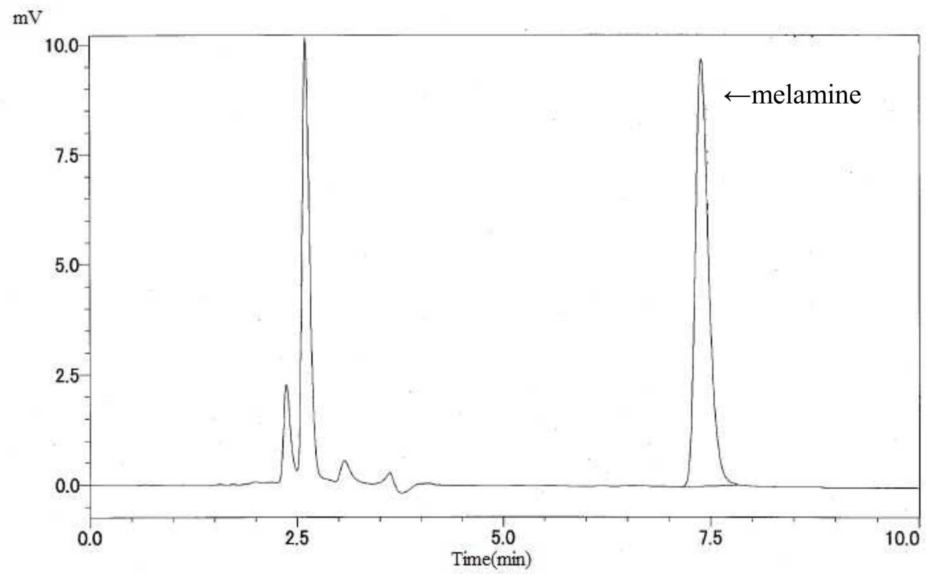


Figure Flow sheet for melamine in fertilizers containing organic matter

Reference: An example of the HPLC chromatogram of the standard solution for calibration curve preparation of melamine is shown below.



Reference diagram: HPLC chromatogram of melamine

Measurement conditions for HPLC

Column: TSKgel Amide-80 (4.6-mm inner diameter, 250-mm long, 5-µm particle diameter)

Melamine standard solution (the equivalents of 10 ng (1 µg/mL, 10 µL) for each)

Other conditions are according to the example of HPLC measurement conditions in **(4.2) a)**

8.2 Clopyralid and its degradation products

8.2.a High-Performance Liquid Chromatography/Tandem Mass Spectrometry (Simultaneous analysis of three components for clopyralid, etc.)

(1) Summary

This testing method is applicable to compost and composted sludge fertilizers. This testing method is classified as Type B and its symbol is 8.2.a-2017 or CLP.a-1.

Extract clopyralid, aminopyralid and picloram with methanol under alkaline condition, refine with a cleanup cartridge by taking advantage of characteristics that the behavior of elution varies between acidity and alkaline, and then measure with a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer (LC-MS/MS) to obtain clopyralid, aminopyralid and picloram in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

Comment 1 Structural formulas of clopyralid, aminopyralid and picloram are as shown in Figure 1.

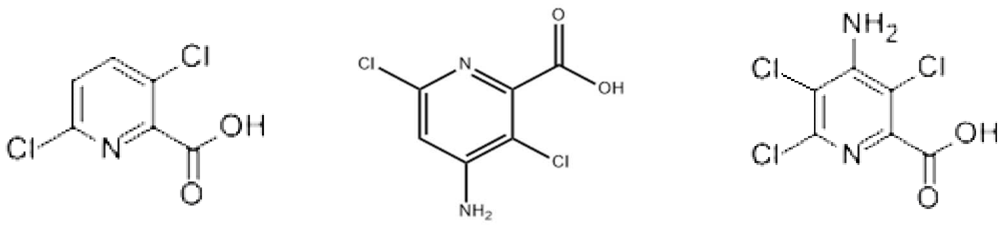


Figure 1 Structural formula of clopyralid, aminopyralid and picloram

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557. Note that water of A4 should be used as the eluent which is introduced to a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer.

b) **Acetonitrile**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8039 or a reagent of equivalent quality.

c) **Methanol**: A JIS Guaranteed Reagent specified in a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer or a reagent of equivalent quality.

d) **Methanol**: Methanol used in the eluent of a High-Performance Liquid Chromatograph/ Tandem Mass Spectrometer is a reagent of LC-MS analysis grade or equivalents.

e) **Sodium hydroxide**: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

f) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

g) **Ammonia solution**: A JIS Guaranteed Reagent of 28 % (mass fraction) specified in JIS K 8085 or a reagent of equivalent quality.

h) f**ormic acid**: A JIS Guaranteed Reagent specified in JIS K 8264 or a reagent of equivalent quality.

i) **Ammonia solution (0.0028 % (mass fraction))** [(1)]: Add 0.1 mL of ammonia solution to 1000 mL of water.

j) **Respective agrichemical standard solutions (0.1 mg/mL)** [(1)]: Put about 0.01 g of clopyralid [$C_6H_3Cl_2NO_2$] [(2)], aminopyralid [$C_6H_4Cl_2N_2O_2$] [(2)] and picloram [$C_6H_3Cl_3N_2O_2$] [(2)] in weighing dishes and measure the mass to the order of 0.1 mg. Dissolve with a small amount of acetonitrile, transfer to 100-mL volumetric flasks and add the solvent up to the marked line.

k) **Mixture standard solution (100 ng/mL)** (1): Dilute a predetermined amount of respective agrichemical standard solutions (0.1 mg/mL) with formic acid (1+1000) to prepare mixture standard solution (100 ng/mL).

l) **Mixture standard solution for calibration curve preparation (5 ng/mL - 50 ng/mL)** (1): In the case of usage, put 2.5 mL - 25 mL of mixture standard solution (100 ng/mL) in 50 mL volumetric flasks step-by-step, and add formic acid (1+1000) up to the marked line.

m) **Mixture standard solution for calibration curve preparation (0.5 ng/mL - 5 ng/mL)** (1): In the case of usage, put 2.5 mL - 25 mL of mixture standard solution (10 ng/mL) in 50 mL volumetric flasks step-by-step, and add formic acid (1+1000) up to the marked line.

Note **(1)** This is an example of preparation; prepare an amount as appropriate.
(2) A standard reagent is commercially sold.

Comment 2 Standard reagents of clopyralid, aminopyralid and picloram are sold by FUJIFILM Wako Pure Chemical Co., Ltd., Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(3) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **High-Performance Liquid Chromatograph/Mass Spectrometer** : A high-performance liquid chromatograph/tandem mass spectrometer specified in JIS K 0136 that satisfies the following requirements.

1) **High-Performance Liquid Chromatograph**
(i) Column bath: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
(ii) Column: A 2-mm - 3-mm inner diameter 50-mm - 150-mm long 1.6-µm - 2.2-µm particle diameter stainless steel column tube filled with silica gel to which octadecyl chemically bonds. The specification is according to the mass spectrometer specification.

2) **Mass Spectrometer**
(i) Ionization method: Electro-Spray Ionization (ESI) method
(ii) Ion detection method: Selected Reaction Monitoring

b) **Vertical reciprocating shaker**: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

c) **Manifold**

d) **Centrifugal separator**: A centrifugal separator that can work at about 1700 × *g*.

e) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

f) **Concentrator**: An evaporator that can adjust to 40 ºC ± 2 ºC.

g) **Copolymer cartridge column**: A divinylbenzene-N-vinylpyrrolidone copolymer mini column (200 mg)

Comment 3 Column is sold under the production name ACQUITY UPLC HSS C18, etc.
Comment 4 A copolymer cartridge is sold under the production names such as Oasis HLB 6cc (200 mg) and Oasis PRiME HLB Plus Short Cartridge (225 mg).

(4) **Test procedures**

(4.1) **Extraction**: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample, and put it into a 200-mL - 300-mL ground-in stopper Erlenmeyer flask.

b) Add 1 mL of sodium hydroxide solution (40 g/L) and 99 mL of methanol (3), and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 30 minutes.
c) After allowing to stand still, put a supernatant solution in a 50-mL ground-in stopper centrifugal precipitate tube.
d) Centrifuge it at about 1700 × *g* centrifugal force for about five minutes (4) and use the supernatant as an extract.

Note (**3**) It is also allowed to add 100 mL of sodium hydroxide solution (40 g/L) − methanol [1+99]
(**4**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

Comment 5 Grind until it completely passes through a sieve of 500 µm aperture to prepare the test sample.

(**4.2**) **Cleanup (1)** (5): Conduct cleanup (1) as shown below.
a) Wash a copolymer cartridge column with about 5 mL of methanol and 5 mL of water in advance.
b) Place a 100-mL round-bottom flask (6) under the cartridge column, put 5 mL or 7 mL (7) of the extract in the cartridge column and allow the extract to overflow until the surface of the liquid reaches the top of the packing materials.
c) Add about 5 mL of sodium hydroxide solution (0.4 g/L) − methanol (1+1) to the cartridge column 2 times and allow the liquid to overflow in the same manner in b).
d) Add 5 mL of methanol.

Note (**5**) Use a pressure reducing device in the procedure in (**4.2**) and (**4.3**) as appropriate.
(**6**) When pretreating many analytical samples, a free-standing type vessel that can contain a solution with a liquid volume of 20 mL may be used. In this case, instead of procedure **d**), put an effluent into a round-bottle flask, wash the vessel 2 times with 2.5 mL of methanol and add washing to the previous effluent.
(**7**) When using Oasis HLB 6cc (200 mg), add 5 mL of the extract 2 times.

(**4.3**) **Cleanup (2)** (5): Conduct cleanup (2) as shown below.
a) Wash a new copolymer cartridge column with about 5 mL of acetonitrile and 5 mL of hydrochloric acid (1+120) in advance.
b) Concentrate the effluent in (**4.2**) **d**) under reduced pressure to 5 mL or less on a water bath at no more than 40 °C.
c) Add 3 mL of hydrochloric acid (1 +11), put the concentrated effluent into the cartridge column and allow the effluent to overflow until the surface of liquid reaches the top of packing materials.
d) Wash a round-bottom flask with about 5 mL of hydrochloric acid (1+120) 2 times and add washing into the cartridge column successively.
e) Then, add about 5 mL of hydrochloric acid (1+120) − acetonitrile (9+1) and about 5 mL of water into the cartridge column successively and allow the liquid to overflow.
f) Place a 5-mL volumetric flask under the cartridge column, add 4 mL of ammonia solution (0.0028 % (mass fraction)) − acetonitrile (9+1) to the cartridge column and allow clopyralid, aminopyralid and picloram to elute quickly.
g) Add formic acid (1+1000) up to the marked line (8) and put it in 1.5-mL ground-in stopper centrifugal precipitate tube (9).
h) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (10) and use the supernatant as a sample solution

Note (**8**) If there is a possibility that the clopyralid, aminopyralid and picloram concentration in the sample solution exceed the maximum limit of the calibration curve, dilute a predetermined amount of effluent with formic acid (1 +1000).

(**9**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement

(**10**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

(**4.4**) **Measurement**: Conduct the measurement as indicated in JIS K 0136 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph/Tandem Mass Spectrometer used in measurement.

a) **The measurement conditions of the High-Performance Liquid Chromatograph/Tandem Mass spectrometer**: An example of measurement conditions for the High-Performance Liquid Chromatograph/Tandem Mass Spectrometer is shown below. Set up the measurement conditions considering it:

1) **High-Performance Liquid Chromatograph**

(i) Column: A silica gel column (2-mm - 3-mm inner diameter, 50-mm - 150-mm long, 1.6-µm - 2.2-µm particle diameter column) to which octadecyl chemically bonds.

(ii) Flow rate: 0.2 mL/min - 0.5 mL/min

(iii) Eluent: A: Formic acid (1+1000) B: Methanol:

(iv) Gradient: 0 min (5 %B) → 5 min (60 %B) → 6 min (95 %B) → 7 min (5 %B)

(v) Temperature of column bath: 40 °C

(vi) Injection volume: 5 µL

2) **Mass Spectrometer**

(i) Ionization method: Electro-Spray Ionization (ESI) method

(ii) Mode: Positive

(iii) Monitor ion: Shown in table 1

Table 1Monitor ion of respective agrichemicals

Substance name	Mass-to-charge ratio (*m*/*z*)		
	Precursor ion	Product ion (Determination)	Product ion (Validation)
Clopyralid	192	146	110
Aminopyralid	207	161	189
Picloram	241	195	223

b) **Calibration curve preparation**

1) Inject 5 µL of respective mixture standard solutions for calibration curve into the LC-MS/MS, record the chromatograms of ion (*m/z*) for determination and ion (*m/z*) for validation of clopyralid, aminopyralid and picloram and obtain respective peak areas.

2) Calculate the peak area ratio or height ratio of ion (*m/z*) for determination and ion (*m/z*) for validation of clopyralid, aminopyralid and picloram.

3) Prepare a curve for the relationship between the agrichemical concentration and the peak area of ion (*m/z)* for determination of respective mixture standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 5 µL of the sample solution to the same procedure as in **b**) **2**) - **3**) (11).

2) Obtain the content of material subjected to measurement from the calibration curve to calculate the concentration of the material subjected to measurement in the analytical sample.

Note (11) Confirm that the peak area ratio or height ratio of ion (*m*/*z*) for determination to ion (*m*/*z*) for validation is within the range of about ± 30 % of the peak area ratio or height ratio for the standard solution. In addition, the peak area ratio or height ratio depends on the concentration.

Comment 6 Additive recovery testing of clopyralid, aminopyralid and picloram was conducted using cow dung compost (2 kinds), composted sludge fertilizers containing cow dung (2 kinds) and composted sludge fertilizers containing swine manure (1 kind), as a result, the mean recovery rates at the additive level of 1000 µg/kg, 400 µg/kg and 40 µg/kg were 78.1 % - 90.0 %, 81.0 % - 117.6 % and 71.2 % - 101.3 % respectively.

Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 10 µg/kg for clopyralid, aminopyralid, and picloram.

Table 2 Analysis results of the collaborative study
for the test method validation of clopyralid and its degradation products

Agrichemical name	Sample name	Number of laboratories[1)]	Mean[2)] (μg/kg)	s_r[3)] (μg/kg)	RSD_r[4)] (%)	s_R[5)] (μg/kg)	RSD_R[6)] (%)
Clopyralid	Compost 1	10	128	6	4.5	21	16.4
	Compost 2	10	835	41	4.9	100	11.9
	Composted sludge fertilizer 1	9	16.2	1.7	10.6	5.2	31.8
	Composted sludge fertilizer 2	10	89.6	11.3	12.6	11.3	12.6
	Composted sludge fertilizer 3	10	339	28	8.3	28	8.3
Aminopyralid	Compost 1	8	324	15	4.5	39	12.0
	Compost 2	8	21.2	5.2	24.7	6.4	30.3
	Composted sludge fertilizer 1	7	5.39	1.41	26.2	2.22	41.2
	Composted sludge fertilizer 2	10	701	146	20.8	263	37.6
	Composted sludge fertilizer 3	9	59.5	8.9	15.0	16.6	28.0
Picloram	Compost 1	10	840	50	5.9	175	20.8
	Compost 2	9	37.7	3.5	9.4	10.3	27.3
	Composted sludge fertilizer 1	9	90.2	11.1	12.3	30.3	33.5
	Composted sludge fertilizer 2	8	341	19	5.6	67	19.8
	Composted sludge fertilizer 3	8	182	16	8.6	56	31.0

1) Number of laboratories used in analysis
2) Grossl mean (n = number of laboratories × number of repeated tests (2))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Reproducibility standard deviation
6) Reproducibility relative standard deviation

References

1) Toshiharu YAGI, Yuko SEKINE, Yuji SHIRAI: Determination of Clopyralid in Fertilizer by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), Research Report of Fertilizer **Vol. 3**, p. 51 - 59 (2010)

2) Hisanori ARAYA, Toshiharu YAGI, Yoshimi HASHIMOTO and Yuji SHIRAI: Determination of Clopyralid, Aminopyralid and Picloram in Compost and Composted Sludge Fertilizer by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS), Research Report of Fertilizer **Vol. 7**, p. 1 - 9 (2014)

3) Kenji KOZUKA, Mayu OSHIMA, Yoshimi HASHIMOTO, Naoko TAMARU, and Yuji SHIRAI: Determination of Clopyralid, Aminopyralid and Picloram in Compost and Composted Sludge Fertilizer by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS): A Collaborative Study, Research Report of Fertilizer **Vol. 10**, p. 61 - 71 (2017)

(5) **Flow sheet for clopyralid and its derivative substances**: The flow sheet for clopyralid and its derivative substances in compost and composted sludge fertilizer is shown below:

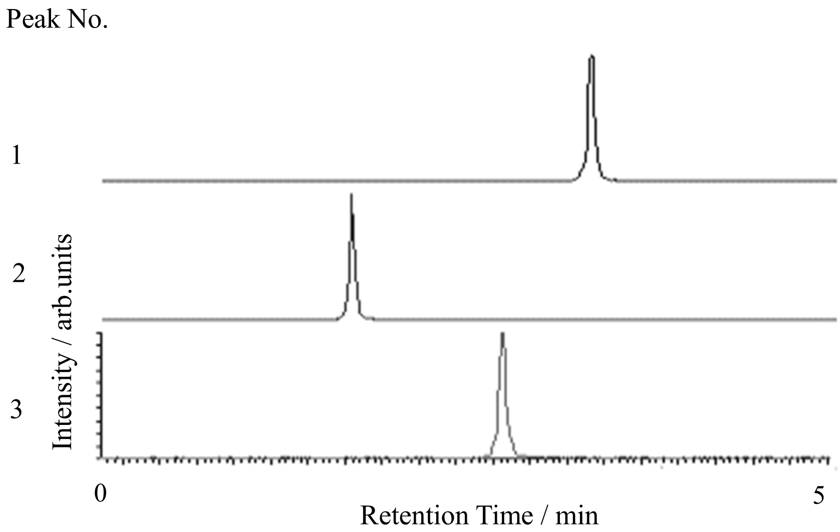
5.00 g analytical sample — 200-mL - 300-mL ground-in stopper Erlenmeyer flask
← 1 mL of sodium hydroxide solution (4 g/L)
← 99 mL of methanol
shaking to mix — Vertical reciprocating shaker (300 times/ minute, amplitude of 40 mm), 30 minutes
Centrifugal separation — 50 mL centrifugal precipitate tube, 1700 × *g*, 5 minutes
Extract

Figure 1 Flow sheet for clopyralid and its derivative substances in compost and composted sludge fertilizers (Extraction procedure)

Extract
Aliquot (5 mL or 10 mL)
Cleanup (1) — copolymer cartridge column (1) (Wash with about 5 mL of methanol, then 5 mL of water in advance)
[Collection, 100-mL round-bottom flask]
← About 5 mL of sodium hydroxide solution (0.4 g/L) - methanol [1+1] × 2 times
[Collection, same as above vessel]
← About 5 mL of methanol
Concentration under reduced pressure — 40 °C, until the volume reaches about 5 mL
← 3 mL of hydrochloric acid (1+11)
Cleanup (2) — copolymer cartridge column (1) (Wash with about 5 mL of acetonitrile, then 5 mL of hydrochloric acid (1+120) in advance)
← Wash the vessel 2 times with about 5mL of hydrochloric acid (1+120)
← Wash with about 5mL of hydrochloric acid (1+120) -acetonitrile [9+1]
← Wash with about 5mL of water
← 4 mL of ammonia solution (0.0028 w/v %)- acetonitrile [9+1]
[Elute, 5-mL volumetric flask]
Precise adjustment
← Formic acid (1+1000) (up to the marked line)
Centrifugal separation — 1.5-mL ground-in stopper centrifugal precipitate tube, 8000 × *g* - 10000 × *g*, 5 minutes
Measurement — High-Performance Liquid Chromatograph/Tandem Mass Spectrometer

Figure 2 Flow sheet for clopyralid and its derivative substances in compost and composted sludge fertilizers (Cleanup (1), cleanup (2) and measurement procedure)

References: An example of the Selected Reaction Monitoring chromatograms of the mixture standard solution for calibration curve preparation and the sample solution (cow dung compost) is shown below



Peak No.1: Picloram
No.2: Aminopyralid
No.3: Clopyralid

Reference diagram: SRM chromatograms of respective agrichemicals
Mixture standard solution (the equivalents of 200 pg as respective agrichemicals)

LC-MS/MS measurement conditions

Column: ACQUITY UPLC HSS C18 (2.1-mm inner diameter, 100-mm long, 1.8-µm particle diameter)

Flow rate: 0.4 mL/min

Capillary voltage: 1.0 kV

Temperature of ion source: 120 °C

Desolvation temperature: 400 °C

Cone voltage: Shown in reference table

Collision energy: Shown in reference table

Other conditions are according to the examples of the measurement conditions in (**4.4**) **a**) LC-MS/MS.

Reference table Parameters of mass spectrometer

Substance name		Mass-to-charge ratio (m/z)		Cone voltage (V)	Collision energy (eV)
		Precursor ion	Product ion		
Clopyralid	For measurement	192	146	20	20
	For validation	192	110	20	30
Aminopyralid	For measurement	207	161	22	22
	For validation	207	189	22	16
Picloram	For measurement	241	195	28	22
	For validation	241	223	28	16

8.2.b High-Performance Liquid Chromatography/Tandem Mass Spectrometry (Microanalysis for clopyralid (1))

(1) Summary

This testing method is applicable to compost and composted sludge fertilizers. This testing method is classified as Type B and its symbol is 8.2.b-2018 or CLP.b-1.

Extract clopyralid in compost and composted sludge fertilizer with methanol under alkaline condition, refine with a cleanup cartridge and dichloromethane by taking advantage of characteristics that the behavior of elution varies between acidity and alkaline then measure with a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer (LC-MS/MS) to obtain clopyralid in an analytical sample. In addition, the performance of this testing method is shown in **Comment 8**.

Comment 1 Structural formula of clopyralid is as shown in Figure 1.

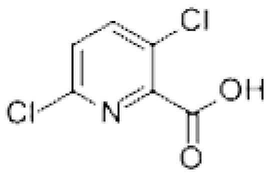


Figure 1 Structural formula of clopyralid

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557. Note that water of A4 should be used as the eluent which is introduced to a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer.
b) **Acetonitrile**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8039 or a reagent of equivalent quality.
c) **Methanol**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) or a reagent of equivalent quality.
d) **Methanol**: Methanol used in the eluent of a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer is a reagent of LC-MS analysis grade or equivalents.
e) **Sodium hydroxide**: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.
f) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.
g) **Ammonia solution**: A JIS Guaranteed Reagent of 28 % (mass fraction) specified in JIS K 8085 or a reagent of equivalent quality.
h) **Formic acid**: A JIS Guaranteed Reagent specified in JIS K 8264 or a reagent of equivalent quality.
i) **Formic acid**: Formic acid used in the eluent of a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer is a reagent of LC-MS analysis grade or equivalents.
j) **Dichloromethane**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8117 or a reagent of equivalent quality.
k) **Sulfuric acid**: A JIS Guaranteed Reagent specified in JIS K 8951 or a reagent of equivalent quality.
l) **Acetone**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8040 or a reagent of equivalent quality.

m) Ammonia solution (0.0028 % (mass fraction)) (1): Add 0.1 mL of ammonia solution to 1000 mL of water.

n) Clopyralid standard solution (0.1 mg/mL) (1): Put about 0.01 g of clopyralid [$C_6H_3C_{12}NO_2$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Dissolve with a small amount of acetonitrile, transfer to 100-mL volumetric flasks and add the solvent up to the marked line.

o) Clopyralid standard solution (100 ng/mL) (1): Dilute a predetermined amount of clopyralid standard solution (0.1 mg/mL) with formic acid (1+1000) to prepare a clopyralid standard solution (100 ng/mL).

p) Clopyralid standard solution for calibration curve preparation (5 ng/mL - 50 ng/mL) (1): In the case of usage, put 2.5 mL - 25 mL of clopyralid standard solution (100 ng/mL) in 50 mL volumetric flasks step-by-step, and add formic acid (1+1000) up to the marked line.

q) Clopyralid standard solution for calibration curve preparation (0.5 ng/mL - 5 ng/mL) (1): In the case of usage, put 2.5 mL - 25 mL of clopyralid standard solution (10 ng/mL) in 50 mL volumetric flasks step-by-step, and add formic acid (1+1000) up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) A standard reagent is commercially sold.

Comment 2 A standard reagent of clopyralid is sold by FUJIFILM Wako Pure Chemical Co., Ltd., Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) High-Performance Liquid Chromatograph/ Mass Spectrometer : A high-performance liquid chromatograph/tandem mass spectrometer specified in JIS K 0136 that satisfies the following requirements.

1) **High-Performance Liquid Chromatograph**
   - (i) Column bath: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
   - (ii) Column: A 2-mm - 3-mm inner diameter 50-mm - 150-mm long 1.6-µm - 2.2-µm particle diameter stainless steel column tube filled with silica gel to which octadecyl chemically bonds. The specification is according to the mass spectrometer specification.

2) **Mass Spectrometer**
   - (i) Ionization method: Electro-Spray Ionization (ESI) method
   - (ii) Ion detection method: Selected Reaction Monitoring

b) Vertical reciprocating shaker: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

c) Manifold

d) Centrifugal separator: A centrifugal separator that can work at 700 × *g* - 2000 × *g*.

e) High speed centrifugal separator: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

f) Concentrator: An evaporator that can adjust to 40 ºC ± 2 ºC.

g) Copolymer cartridge column: A divinylbenzene-N-vinylpyrrolidone copolymer mini column (200 mg or 335 mg)

h) Filter: A funnel for filtering under reduced pressure (compatible filter diameter: 60 mm)

i) Glass fiber filter paper: A filter paper made of glass fiber (filter diameter: 60 mm) that can keep particle diameter 0.8 µm.

j) Test tube mixer: Vortex mixer

Comment 3 Column is sold under the production name ACQUITY UPLC HSS C18, etc.
Comment 4 A copolymer cartridge is sold under the production names such as Oasis HLB 6cc (225

mg) and Oasis PRiME HLB Plus Short Cartridge (225 mg).

Comment 5 A funnel for filtering under reduced pressure is sold under the production name KIRIYAMA Funnel SB-60, KIRIYAMA Funnel SU-60, etc.

Comment 6 A glass fiber filter is sold under the production name Glass filter paper GFP-60, etc.

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample to the order of 1 mg and put it in a 100-mL centrifugal precipitate tube with a screw cap (3) (4).

b) Add 50 mL of sodium hydroxide solution (40 g/L) − methanol [1+99] and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 30 minutes.

c) Centrifuge at about 1700 × *g* for about 3 minutes (5) and put the supernatant in a 100-mL Erlenmeyer flask.

d) Add 40 mL of sodium hydroxide solution (40 g/L) − methanol [1+99] to residue and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 30 minutes.

e) Centrifuge at about 1700 × *g* for about five minutes (5).

f) Filter supernatant in **c**) and **e**) under reduced pressure with a filter that places a glass fiber filter paper to a 100-mL short-neck volumetric flask as an acceptor (6).

g) Wash the vessel and residue with a small amount of sodium hydroxide (40 g/L)−methanol [1+99] several times and put the washing in the previous filter to filter under pressure.

h) Add sodium hydroxide solution (40 g/L) - methanol [1+99] to the marked line to make an extract.

Note (**3**) A vessel used for an extract procedure should be made of glass or polypropylene and it can be used with a shaker and a centrifugal separator.

(**4**) A 100-mL - 200-mL ground-in stopper or screw cap Erlenmeyer flask can also be used. In this case, however, suspension should be transferred to a ground-in stopper or screw cap centrifugal precipitate tube before the procedure **c**) and **e**).

(**5**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**6**) An Erlenmeyer flask can also be used. In this case, however, filtrate should be transferred to a 100-mL volumetric flask before the procedure **h**).

Comment 5 Grind until it completely passes through a sieve of 500 μm aperture to prepare the test sample.

(**4.2**) **Cleanup (1)** (7): Conduct cleanup (1) as shown below.

a) Wash a cartridge column with about 5 mL of methanol and 5 mL of water in advance.

b) Place a 100-mL round-bottom flask (8) under the cartridge column, put 9 mL (9) of the extract in the cartridge column and allow the extract to overflow until the surface of the liquid reaches the top of the packing materials.

c) Add about 5 mL of sodium hydroxide solution (0.4 g/L) − methanol [1+1] to the cartridge column 2 times and allow the liquid to overflow in the same manner in **b**).

d) Add 5 mL of methanol.

Note (**7**) Use a pressure reducing device in the procedure in (**4.2**) and (**4.3**) as appropriate.

(**8**) When making a pretreatment of many analytical samples, a free-standing type vessel that can contain a solution with a liquid volume of 20 mL may be used. In this case, instead of procedure **d**), put an effluent into a round-bottle flask, wash the vessel 2 times with 2.5 mL of methanol and add washing to the previous effluent.

(**9**) When using Oasis HLB 6cc (200 mg), add 5 mL of the extract 2 times.

(**4.3**) **Cleanup (2)** (7): Conduct cleanup (2) as shown below.

a) Wash a new cartridge column with about 5 mL of acetonitrile and 5 mL of hydrochloric acid (1+120) in advance.

b) Concentrate the effluent in (**4.2**) **d**) under reduced pressure to 5 mL or less on a water bath at no more than 40 °C.

c) Add 3 mL of hydrochloric acid (1 +11), put the concentrated effluent into the cartridge column and allow the effluent to overflow until the surface of liquid reaches the top of packing materials.

d) Wash a round-bottom flask with about 5 mL of hydrochloric acid (1+120) 2 times and add washing into the cartridge column successively to allow the liquid overflow.

e) Then, add about 5 mL of hydrochloric acid (1+120) − acetonitrile (9+1) and about 5 mL of water into the cartridge column successively and allow the liquid to overflow.

f) Place a 10-mL cone shaped centrifugal precipitate tube with a screw cap (10) under the cartridge column, add 4 mL of ammonia solution (0.0028 % (mass fraction)) − acetonitrile [9+1] to the cartridge column and allow clopyralid to elute.

Note (**10**) The part under 2 mL from the bottom forms a cone shape.

(**4.4**) **Cleanup (3)**: Conduct cleanup (3) as shown below.

a) Add 0.1 mL of sodium hydroxide (40 g/L) to the elute in (**4.3**) **f**) and shake to mix by using a test tube mixer.

b) Add about 2 mL of dichloromethane, and shake to mix using a test tube mixer for about 30 minutes.

c) Centrifuge at about 740 × *g* for about 3 minutes (11) and remove a low layer by using a Pasteur pipet (12) or a syringe.

d) Repeat the procedure in **b**) - **c**) 1 time.

e) Add about 0.15 mL of sulfuric acid (1+2), and shake to mix using a test tube mixer.

f) Add about 2 mL of dichloromethane, and shake to mix using a test tube mixer for about 30 minutes (13).

g) Centrifuge at about 740 × *g* for about five minutes (11) and put a low layer in a 50mL round-bottle flask by using a Pasteur pipet (14) or a syringe.

h) Repeat the procedure in **f**) - **g**) 2 times. Note that lower layers are added to the same round-bottle flask.

i) Add 5 mL of acetone.

j) Concentrate under reduced pressure in a water bath of no more than 40 ºC until most of the elute exsiccates and send a nitrogen gas to exsiccate.

k) Add 1 mL of formic acid (1+1000) and transfer it to a 1.5-mL ground-in stopper centrifugal precipitate tube (15).

l) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (16) and use the supernatant as a sample solution

Note (**11**) 16.5-cm of rotor radius and 2000 rpm of revolutions makes about 740 × *g* centrifugal force. Confirm the permissible range of centrifugal force of a 10-mL cone shaped bottle centrifugal precipitate tube with a screw cap used.

(**12**) When using a Pasteur pipet, use the same Pasteur pipet through a series of procedures in **c**) - **d**).

(**13**) Disperse dichloromethane sufficiently. If a shaking to mix process is conducted while dichloromethane layer remains caked, the extraction efficiency of clopyralid deteriorates and measurements are affected.

(**14**) When using a Pasteur pipet, use the same Pasteur pipet through a series of procedures in **g**) - **h**). Do not use the Pasteur pipet used in Note (**12**).

(**15**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement

(**16**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

(**17**) If there is a possibility that the clopyralid concentration in the sample solution exceed the maximum limit of the calibration curve, dilute a predetermined amount of effluent with formic acid (1 +1000).

Comment 6 As an alternative to the procedures in (**4.4**) **k**) - **l**), filtration is allowed with a membrane filter (pore size: no more than 0.5 µm) made of hydrophilic PTFE or conduct centrifugal filtration with a centrifugal type filter unit (Ultrafree-MC PVDF membrane (0.22 µm), etc.) and the filtrate can be the sample solution.

Comment 7 When further concentration is required to ensure minimum quantitation, dissolve concentrated matters in the procedure in **j**) by adding acetone, transfer to a nitrogen concentration tube with the same solvent and send a nitrogen gas to exsiccate. Then add 0.2 mL of formic acid (1+1000) and conduct centrifugal filtration with a centrifugal type filter unit (Ultrafree-MC PVDF membrane (0.22 µm), etc.) and the filtrate can be the sample solution. In this case, don not conduct the procedure in **i**).

(**4.5**) **Measurement**: Conduct the measurement as indicated in JIS K 0136 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph/Mass Spectrometer used in measurement.

a) **The measurement conditions of the High-Performance Liquid Chromatograph/Mass spectrometer**: An example of measurement conditions for the High-Performance Liquid Chromatograph/Mass Spectrometer is shown below. Set up the measurement conditions considering it:

1) **High-Performance Liquid Chromatograph**

(i) Column: A silica gel column (2-mm - 3-mm inner diameter, 50-mm - 150-mm long, 1.6-µm - 2.2-µm particle diameter column) to which octadecyl chemically bonds.

(ii) Flow rate: 0.2 mL/min - 0.5 mL/min

(iii) Eluent: A: Formic acid (1+1000) B: Methanol:

(iv) Gradient: 0 min (5 %B) → 5 min (60 %B) → 6 min (95 %B) → 7 min (5 %B)

(v) Temperature of column bath: 40 °C

(vi) Injection volume: 5 µL

2) **Mass Spectrometer**

(i) Ionization method: Electro-Spray Ionization (ESI) method

(ii) Mode: Positive

(iii) Monitor ion: Precursor ion m/z 192
Product ion *m/z* 146 for determination, *m/z* 110 for validation

b) **Calibration curve preparation**

1) Inject 5 µL of respective clopyralid standard solutions for calibration curve into the High-Performance Liquid Chromatograph/Tandem Mass Spectrometer, record the chromatograms of ion (*m/z*) for determination and ion (*m/z*) for validation of clopyralid and obtain respective peak areas.

2) Calculate the peak area ratio or height ratio of ion (*m/z*) for determination and ion (*m/z*) for validation of clopyralid.

3) Prepare a curve for the relationship between the agrichemical concentration and the peak area of ion (*m/z*) for determination of respective mixture standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 5 µL of the sample solution to the same procedure as in **b) 2) - 3)** [(18)].

2) Obtain the clopyralid content from the calibration curve to calculate clopyralid in the analytical sample.

Note (**18**) Confirm that the ratio against the peak area ratio or height ratio of the standard solution is within the range of about ± 30 %. In addition, the peak area ratio or height ratio may depend on the concentration.

Comment 8 Additive recovery testing of clopyralid was conducted using cow dung compost (1 kinds), as a result, the mean recovery rates at the additive level of 50 µg/kg, 10 µg/kg and 2 µg/kg were 78.9 %, 78.3 % and 71.5 % respectively. In addition, additive recovery testing of clopyralid was conducted using swine manure compost, poultry manure compost and composted sludge fertilizer (1 sample for each), as a result, the mean recovery rates at the additive level of 200µg/kg, 2 µg/kg and 80µg/kg were 88.6 %, 81.2 % and 94.2 % respectively.

Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 1.

Note that the minimum limit of quantification of this testing method was estimated to be about 2 µg/kg for clopyralid.

Table 1 Analysis results of the collaborative study for the test method validation of clopyralid

Sample name	Number of laboratories[1)]	Mean[2)] (µg/kg)	s_r[3)] (µg/kg)	RSD_r[4)] (%)	s_R[5)] (µg/kg)	RSD_R[6)] (%)
Cow dung compost 1	10	128	10	7.9	15	11.4
Cow dung compost 2	10	2.28	0.35	15.3	0.40	17.6
Swine manure compost	9	22.5	2.3	10.3	3.4	15.3
Poultry manure compost	9	1.20	0.06	5.0	0.14	12.0
Composted sludge fertilizer	9	48.1	1.2	2.5	5.6	11.6

1) Number of laboratories used in analysis
2) Grossl mean (*n* = number of laboratories × number of repeated tests (2))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Reproducibility standard deviation
6) Reproducibility relative standard deviation

References

1) National Research and Development Agency: Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization: High-sensitive analysis for clopyralid in cow dung compost (Reference method)
< http://www.naro.affrc.go.jp/publicity_report/pub2016_or_later/pamphlet/tech-pamph/078229.html>

2) Kohei ITO, Kenji KOZUKA, Keisuke AOYAMA, Yuji SHIRAI: Validation of Microanalysis Determination of Clopyralid in Compost and Composted Sludge Fertilizer by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS), Research Report of Fertilizer **Vol. 11**, p. 63 – 74 (2018)

3) Kohei ITO, Kenji KOZUKA, Satono AKIMOTO, Satoko SAKAIDA, Mayu OSHIMA, Nobuhito NAKAMURA, Yuji SHIRAI: Microanalysis Determination of Clopyralid in Compost and Composted Sludge Fertilizer by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS): A Collaborative Study, Research Report of Fertilizer **Vol. 11**, p. 75 – 85 (2018)

(5) Flow sheet for clopyralid: The flow sheet for clopyralid in compost is shown below:

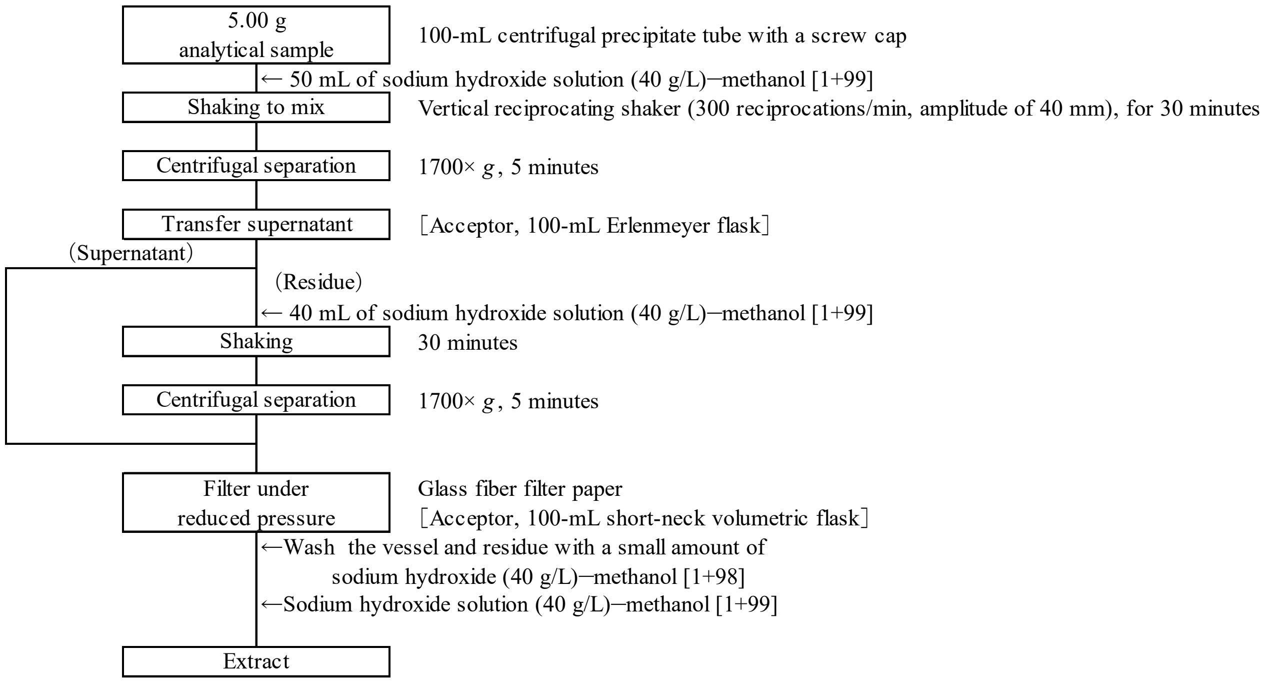


Figure 1 Flow sheet for clopyralid in compost and composted sludge fertilizers (Extraction procedure)

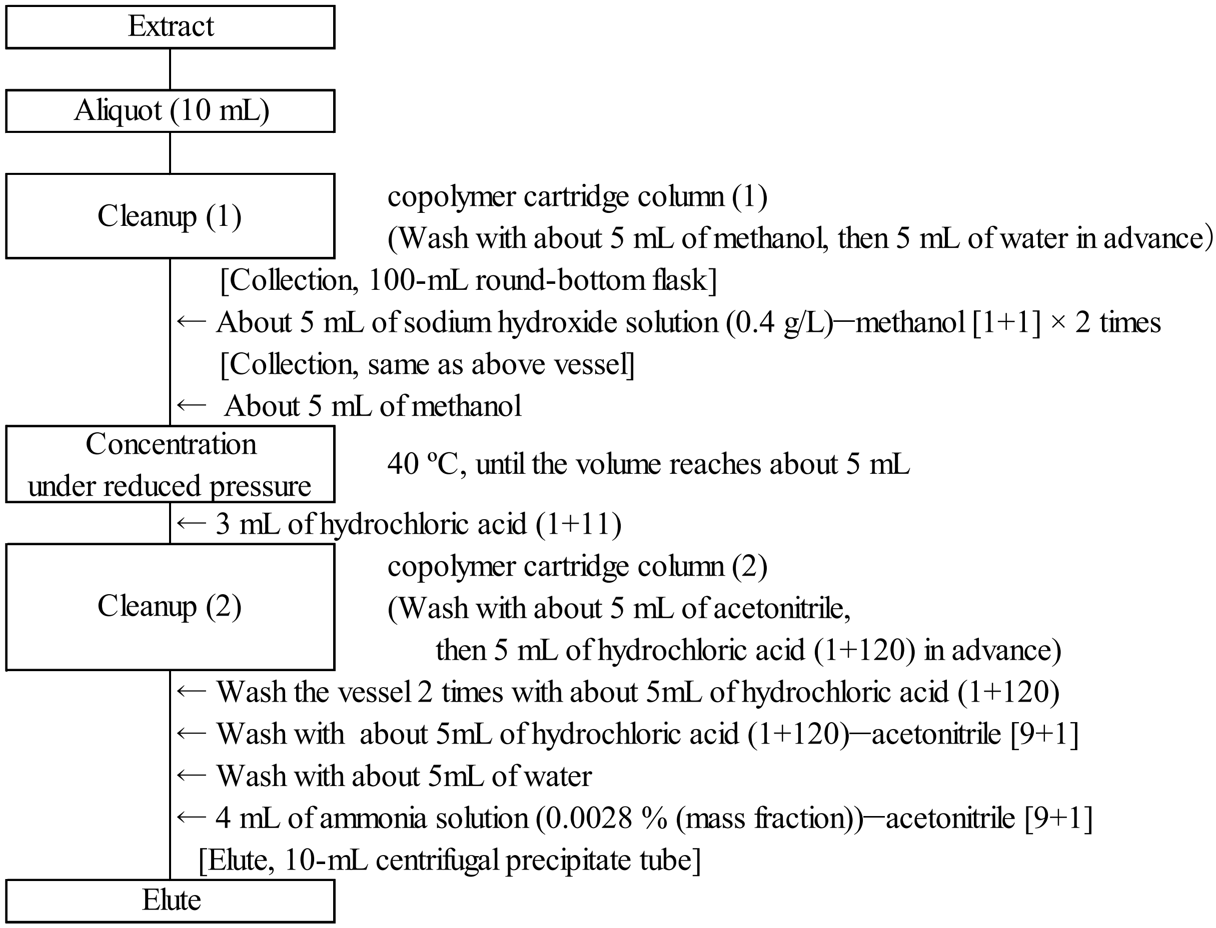


Figure 2 Flow sheet for clopyralid in compost and composted sludge fertilizers
(Cleanup (1) and cleanup (2) procedure)

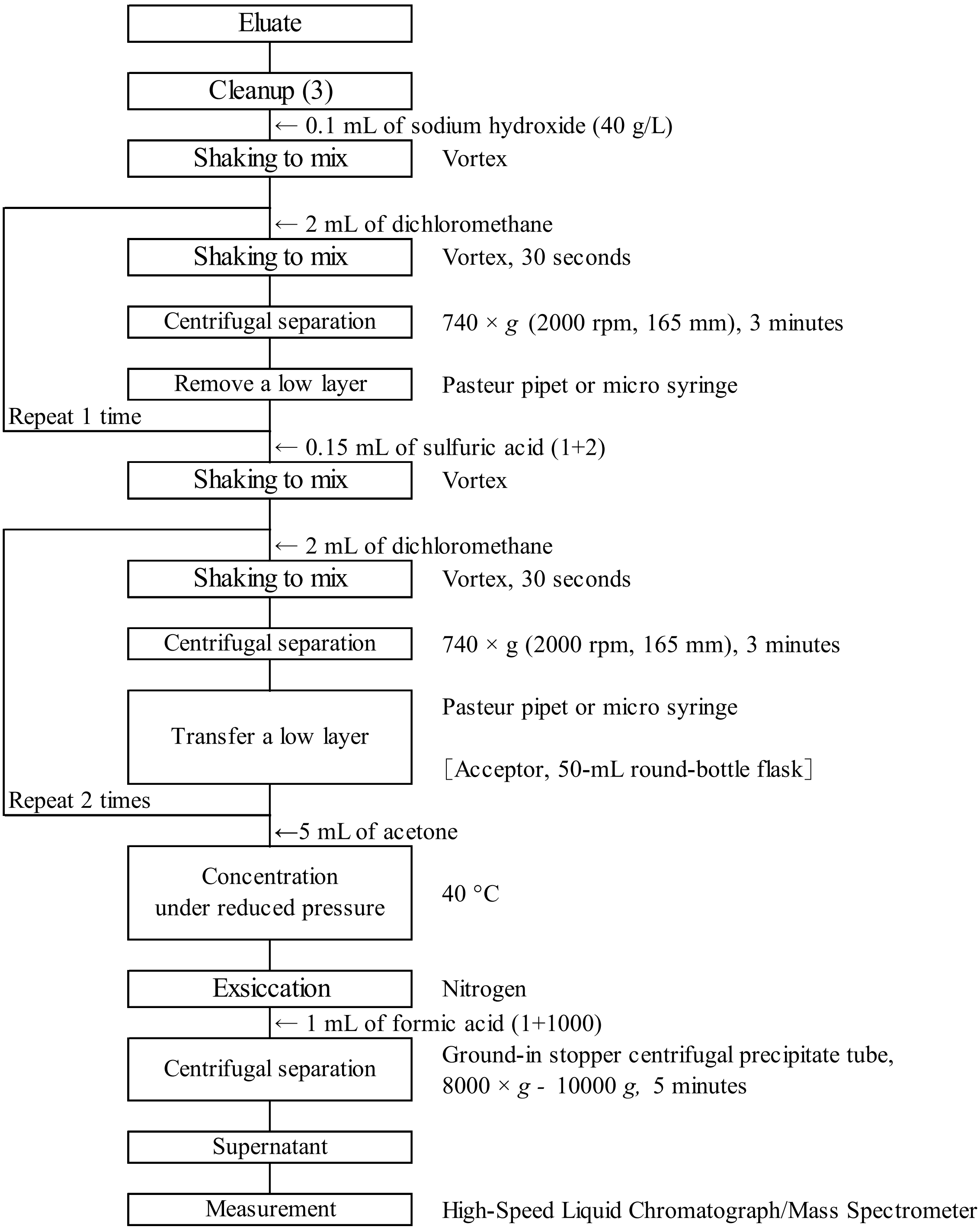
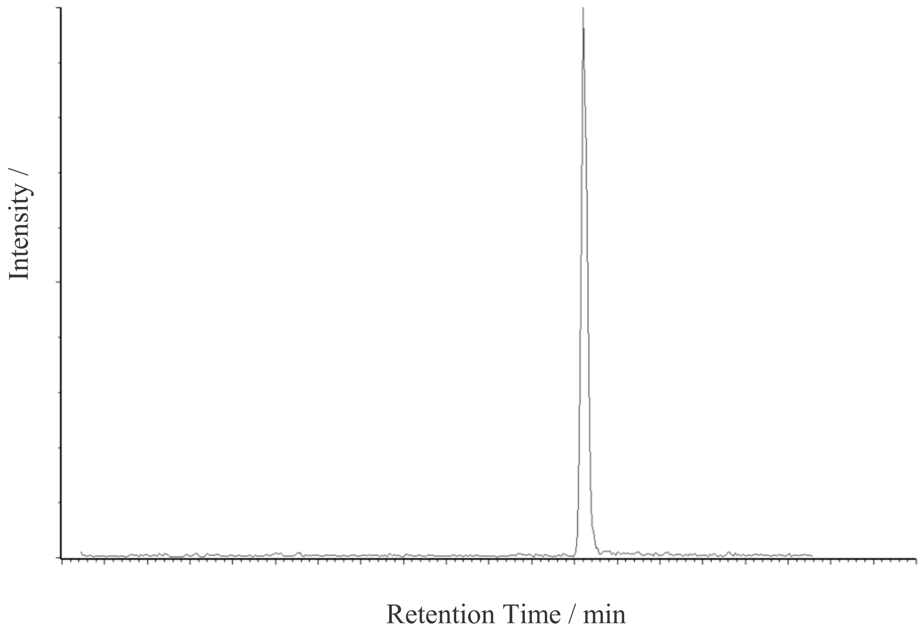


Figure 3 Flow sheet for clopyralid in compost and composted sludge fertilizers (Cleanup (3) and measurement procedure)

References: An example of the Selected Reaction Monitoring chromatogram of clopyralid standard solution for calibration curve preparation is shown below



Reference diagram: SRM chromatograms of clopyralid
Clopyralid standard solution (the equivalents of 100 pg as clopyralid)

LC-MS/MS measurement conditions

1) Column: ACQUITY UPLC HSS C18 (2.1-mm inner diameter, 100-mm long, 1.8-µm particle diameter)
Flow rate: 0.4 mL/min
Capillary voltage: 1.0 kV
Temperature of ion source: 120 °C
Desolvation temperature: 400 °C
Cone voltage: 20 V
Collision energy: 20 eV for determination, 30 eV for validation
Other conditions are according to the examples of the measurement conditions in (**4.4**) **a**) LC-MS/MS.

8.2.c High-Performance Liquid Chromatography/Tandem Mass Spectrometry (Microanalysis for clopyralid (2))

(1) Summary

This testing method is applicable to compost and composted sludge fertilizers. This testing method is classified as Type B and its symbol is 8.2.c-2021or CLP.c-2.

Extract clopyralid in compost and composted sludge fertilizer with methanol under alkaline condition, refine with two kinds of cleanup cartridge by taking advantage of characteristics that the behavior of elution varies between acidity and alkaline then measure with a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer (LC-MS/MS) to obtain clopyralid in an analytical sample. In addition, the performance of this testing method is shown in **Comment 9**.

Comment 1 Structural formula of clopyralid is as shown in Figure 1.

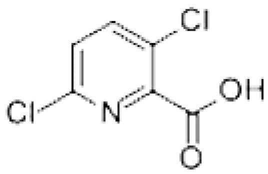


Figure 1 Structural formula of clopyralid

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557. Note that water of A4 should be used as the eluent which is introduced to a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer.

b) **Acetonitrile**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8039 or a reagent of equivalent quality.

c) **Methanol**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) or a reagent of equivalent quality. Note that a regent of LC-MS analysis grade or equivalents should be used as the eluent which is introduced to a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer.

d) **Sodium hydroxide**: A JIS Guaranteed Reagent specified in JIS K 8576 or a reagent of equivalent quality.

e) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

f) **Ammonia solution**: A JIS Guaranteed Reagent of 28 % (mass fraction) specified in JIS K 8085 or a reagent of equivalent quality.

g) **Formic acid**: A JIS Guaranteed Reagent specified in JIS K 8264 or a reagent of equivalent quality. Note that a regent of LC-MS analysis grade or equivalents should be used as the eluent which is introduced to a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer.

h) **Ammonia solution (0.0028 % (mass fraction))** [(1)]: Add 0.1 mL of ammonia solution to 1000 mL of water.

i) **Clopyralid standard solution (0.1 mg/mL)** [(1)]: Put about 0.01 g of clopyralid [$C_6H_3C_{12}NO_2$] [(2)] in a weighing dish and measure the mass to the order of 0.1 mg. Dissolve with a small amount of acetonitrile, transfer to 100-mL volumetric flasks and add the solvent up to the marked line.

j) **Clopyralid standard solution (100 ng/mL)** [(1)]: Dilute a predetermined amount of clopyralid standard solution (0.1 mg/mL) with formic acid (1+1000) to prepare clopyralid standard solution (100 ng/mL).

k) Clopyralid standard solution for calibration curve preparation (5 ng/mL - 50 ng/mL) (1): In the case of usage, put 2.5 mL - 25 mL of clopyralid standard solution (100 ng/mL) in 50 mL volumetric flasks step-by-step, and add formic acid (1+1000) up to the marked line.

l) Clopyralid standard solution for calibration curve preparation (0.5 ng/mL - 5 ng/mL) (1): In the case of usage, put 2.5 mL - 25 mL of clopyralid standard solution (10 ng/mL) in 50 mL volumetric flasks step-by-step, and add formic acid (1+1000) up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) A standard reagent is commercially sold.

Comment 2 A standard reagent of clopyralid is sold by FUJIFILM Wako Pure Chemical Co., Ltd., Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(**3**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) High-Performance Liquid Chromatograph/Mass Spectrometer : A high-performance liquid chromatograph/tandem mass spectrometer specified in JIS K 0136 that satisfies the following requirements.

1) High-Performance Liquid Chromatograph

(i) Column bath: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

(ii) Column: A 2-mm - 3-mm inner diameter 50-mm - 150-mm long 1.6-µm - 2.2-µm particle diameter stainless steel column tube filled with silica gel to which octadecyl chemically bonds. The specification is according to the mass spectrometer specification.

2) Mass Spectrometer

(i) Ionization method: Electro-Spray Ionization (ESI) method

(ii) Ion detection method: Selected Reaction Monitoring

b) Vertical reciprocating shaker: A vertical reciprocating shaker that can shake a 250-mL volumetric flask using an adapter to reciprocate vertically at a rate of 300 reciprocations/min (amplitude of 40 mm).

c) Manifold

d) Centrifugal separator: A centrifugal separator that can work at 700 × *g* - 2000 × *g*.

e) High speed centrifugal separator: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

f) Concentrator: An evaporator that can adjust to 40 ºC ± 2 ºC.

g) Copolymer cartridge column: A cartridge column whose syringe barrel (12 mL) is filled with a divinylbenzene-*N*-vinylpyrrolidone copolymer (500 mg) or cartridge column whose syringe barrel (20 mL) is filled with an N-containing vinyl polymer-containing styrenedivinylbenzene composite polymer (500 mg).

h) Zirconia coated silica gel cartridge column: A cartridge column whose syringe barrel (6 mL) is filled with silica gel (500 mg) coated by zirconia group.

Comment 3 A column is sold under production names such as CQUITY UPLC HSS C18, ACQUITY UPLC HSS C18, ACQUITY UPLC HSS T3, InertSustain AQ-C18, Shim-pack Scepter C18-120, C18U 2B, and ZORBAX Eclipse Plus C18.

Comment 4 A copolymer cartridge column is sold under the production names Oasis HLB 12cc (500 mg), InertSep HLB FF 500 mg/20 mL, etc.

Comment 5 A zirconia coated silica gel cartridge column is commercially sold under production names HybrideSPE®Phospholipid (500 mg), etc.

(**4**) **Test procedures**

(**4.1**) **Extraction**: Conduct extraction as shown below.

a) Weigh 2.00 g of an analytical sample and put it in a 50-mL centrifugal precipitate tube with a screw cap (3).

b) Add 50 mL of sodium hydroxide solution (40 g/L) − methanol [1+99] and shake to mix by reciprocating vertically at 300 times/min (amplitude of 40 mm) for about 30 minutes.

c) Centrifuge at about 1700 × *g* for about 5 minutes (4) and put the supernatant in a 200-mL round-bottle flask(5).

d) Add 10 mL of sodium hydroxide solution (40 g/L) − methanol [1+99] to residue and shake to mix.

e) Centrifuge it at about 1700 × *g* centrifugal force for about 5 minutes (4), and add the supernatant to the supernatant of **c**) (5).

f) Conduct the procedures in **d**) – **e**) two times, wash the solution attached at the connecting part of the round-bottom flask with methanol to add, and use it as an extract.

Note (**3**) A vessel used for an extract procedure should be made of glass or polypropylene and it can be used with a shaker and a centrifugal separator.

(**4**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**5**) Transfer by decantation.

Comment 6 Grind until it completely passes through a sieve of 500 µm aperture to prepare the test sample.

Comment 7 In the procedure in (**4.2**), to reduce the factors of the copolymer cartridge column being clogged, rotate the centrifugal precipitate tube with a screw cap to remove the sample attached to the wall prior to the centrifugal separation operation in **c**) and **e**), and pay attention not to include solid materials to the extent possible when transferring or adding the supernatant.

(**4.2**) **Cleanup (1)** (6): Conduct cleanup (1) as shown below.

a) Wash a copolymer cartridge column with about 5 mL of acetonitrile and 5 mL of hydrochloric acid (1+120) in advance.

b) Concentrate the extract under reduced pressure to 3 mL or less on a water bath at no more than 40 °C.

c) Add 3 mL of hydrochloric acid (1+11) and transfer it to a 10-mL ground-in stopper centrifugal precipitate tube (7) (hereinafter in this section simply “centrifugal precipitate tube”) (8).

d) Centrifuge at about 740 × *g* for about five minutes (9).

e) Put the supernatant into the cartridge column (8) and allow the effluent to overflow until the surface of liquid reaches the top of packing materials.

f) Wash the round-bottom flask with about 5 mL of hydrochloric acid (1+120), and add the washing into the centrifugal precipitate tube (8) (10).

g) Centrifuge at about 740 × *g* for about five minutes.

h) Wash the round-bottom flask with about 5 mL of hydrochloric acid (1+120), and add the washing into the centrifugal precipitate tube (8) (10).

i) Centrifuge at about 740 × *g* for about five minutes (9).

j) Add the supernatant and the washings into the cartridge column successively and allow the liquid overflow.

k) Repeat the procedure in **h**) - **j**) 2 times.

l) Then, add about 10 mL of hydrochloric acid (1+120) - acetonitrile (9+1) and about 5 mL of water into the cartridge column successively and allow the liquid to overflow.

m) Place a 10-mL ground-in stopper tube under the cartridge column, add 8 mL of ammonia solution (0.0028 % (mass fraction)) - acetonitrile [9+1] to the cartridge column and allow clopyralid to elute.

n) Add 2 mL of formic acid (1+1000) to the effluent and mix well.

Note (**6**) Use a pressure reducing device or pressurize in the procedure in (**4.2**) and (**4.3**) as appropriate.

(**7**) A centrifugal precipitate tube with a screw cap may be used.

(**8**) The same Pasteur pipet, etc. should be used in a series of procedures.

(**9**) 16.5-cm of rotor radius and 2000 rpm of revolutions makes about 740 × g centrifugal force. The permissible range of centrifugal force of a 10-mL ground-in stopper centrifugal precipitate tube used to be confirmed.

(**10**) To reduce the factors of the copolymer cartridge column being clogged, pay attention not to disturb the centrifugal precipitate to the extent possible.

(**4.3**) **Cleanup (2)** (5): Conduct cleanup (2) as shown below.

a) Wash a zirconia coated silica gel cartridge column with about 5 mL of acetonitrile and 5 mL of formic acid (1+1000) in advance.

b) Put the solution in (**4.2**) **f**) into the cartridge column (6) and allow the effluent to overflow until the surface of liquid reaches the top of packing materials.

c) Wash a ground-in stopper tube with about 5 mL of acetonitrile, put the washing to the same cartridge and allow the effluent to overflow until the surface of liquid reaches the top of packing materials.

d) Place a 50-mL round-bottom flask (8) under the cartridge column, add 10 mL of formic acid-acetonitrile [2+98] (9) to the cartridge column and allow the solution to elute.

e) Concentrate the elute under reduced pressure in a water bath of no more than 40 ºC until most of the elute exsiccates and send a nitrogen gas to exsiccate.

f) Add 4 mL of formic acid (1+1000) and put a predetermined amount of the solution in a ground-in stopper centrifugal precipitate tube (10).

g) Centrifuge it at 8000 × g - 10000 × g centrifugal force for about five minutes (11) to make the sample solution (12).

Note (**8**) When making a pretreatment of the multicomponent of an analytical sample, a 10-mL test tube may be used. In this case, before the procedure in **e**), put the eluate into a 50-mL round-bottle flask, wash the previous test tube 2 times with 2.5 mL of acetonitrile and add the washing to the previous eluate.

(**9**) Prepare at the time of usage. Using the solution that has passed one day after the preparation affects the measurement results.

(**10**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement

(**11**) 7.2-cm – 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × g - 10000 × g centrifugal force.

(**12**) If there is a possibility that the clopyralid concentration in the sample solution exceed the maximum limit of the calibration curve, dilute a predetermined amount of the sample solution with formic acid (1 +1000).

Comment 8 Instead of the procedures in (**4.3**) **f**) - **g**), it is allowed to filter with a membrane filter (pore size: no more than 0.5 μm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(**4.4**) **Measurement**: Conduct the measurement as indicated in JIS K 0136 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph/ Mass Spectrometer used in measurement.

a) **The measurement conditions of the High-Performance Liquid Chromatograph/Mass spectrometer**: An example of measurement conditions for the High-Performance Liquid Chromatograph/Mass Spectrometer is shown below. Set up the measurement conditions considering it:

1) **High-Performance Liquid Chromatograph**

(i) Column: A silica gel column (2-mm - 3-mm inner diameter, 50-mm - 150-mm long, 1.6-µm – 3.0-µm particle diameter column) to which octadecyl chemically bonds.

(ii) Flow rate: 0.2 mL/min - 0.5 mL/min

(iii) Eluent: A: Formic acid (1+1000) B: Methanol:

(iv) Gradient: 0 min (5 %B) → 5 min (60 %B) → 6 min (95 %B) → 7 min (5 %B)

(v) Temperature of column bath: 40 °C

(vi) Injection volume: 5 µL

2) **Mass Spectrometer**

(i) Ionization method: Electro-Spray Ionization (ESI) method

(ii) Mode: Positive

(iii) Monitor ion: Precursor ion m/z 192
Product ion *m/z* 146 for determination, *m/z* 110 for validation

b) **Calibration curve preparation**

1) Inject 5 µL of respective clopyralid standard solutions for calibration curve preparation into the High-Performance Liquid Chromatograph/Tandem Mass Spectrometer, record the chromatograms of ion (*m/z*) for determination and ion (*m/z*) for validation of clopyralid and obtain respective peak areas [(13)].

2) Calculate the peak area ratio or height ratio of ion (*m/z*) for determination and ion (*m/z*) for validation of clopyralid.

3) Prepare a curve for the relationship between the clopyralid concentration and the peak area of ion (*m/z*) for determination of respective clopyralid standard solutions for the calibration curve preparation.

c) **Sample measurement**

1) Subject 5 µL of the sample solution to the same procedure as in **b**) **1**) - **2**) [(14)].

2) Obtain the content of material subjected to measurement from the calibration curve to calculate the concentration of the material subjected to measurement in the analytical sample.

Note (**13**) According to the sensitivity of an instrument, ion for determination may be regarded as ion for validation and vice versa.

Note (**14**) Confirm that the peak area ratio or height ratio of ion (*m*/*z*) for determination to ion (*m*/*z*) for validation is within the range of about ± 30 % of the peak area ratio or height ratio for the standard solution. In addition, the peak area ratio or height ratio may vary depending on the concentration.

Comment 9 The comparison of the measurement value (y_i : 1.5 µg/kg – 88.5 µg/kg) of microanalysis for clopyralid (2) and the measurement value (x_i) of microanalysis for clopyralid (1) was conducted to evaluate trueness using cow dung compost (5 samples), horse dung compost (2 samples), swine manure compost (4 samples), poultry manure compost (4 samples) and composted sludge fertilizer (5 samples). As a result, a regression equation was y=−0.43+1.005x and its correlation coefficient (r) was 0.996. The results of the repeatability tests on different days using compost and composted sludge fertilizers were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 2 µg/kg for clopyralid.

Table 1 Analysis results of the collaborative study for the test method validation of clopyralid

Sample name	Number of laboratories[1)]	Mean[2)] (mg/kg)	s_r [3)] (mg/kg)	RSD_r [4)] (%)	s_R [5)] (mg/kg)	RSD_R [6)] (%)
Cow dung compost	5	87.2	3.6	4.1	3.6	4.1
Swine manure compost	5	2.79	0.29	10.3	0.29	10.3
Poultry manure compost	5	20.5	0.8	3.8	3.2	15.8
Composted sludge fertilizer	5	6.27	0.36	5.8	0.46	7.3

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of repeated tests (2))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Reproducibility standard deviation
6) Reproducibility relative standard deviation

Table 2 Statistical analysis results of collaborative study results

Measurement method[1)]	Sample name	Number of laboratories[2)]	Mean[3)] (µg/kg)	s_r [4)] (µg/kg)	RSD_r [5)] (%)	s_R [6)] (µg/kg)	RSD_R [7)] (%)
m/z 146 Area	Poultry manure compost	11	5.30	0.73	13.8	1.50	28.4
	Swine manure compost	12	50.3	2.8	5.6	9.1	18.0
	Cow dung compost 1	11	115	14	12.6	22	19.1
	Cow dung compost 2	12	6.67	0.44	6.5	1.48	22.1
	Horse manure compost	12	22.6	3.2	14.1	3.4	15.0
	Composted sludge fertilizer	12	15.3	1.0	6.4	4.2	27.5

1) Top: measured product ion, bottom: peak area or height used to calculate clopyralid content
2) Number of laboratories used in analysis
3) Gross mean (n = [Number of laboratories] × [Number of samples (2)])
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Nobuhito NAKAMURA, Kenji KOZUKA and Yuji SHIRAI: Improvement of Microanalysis Determination of Clopyralid in Compost and Composted Sludge Fertilizer by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS), Research Report of Fertilizer Vol. 12, p. 69 – 83 (2019)

(5) **Flow sheet for clopyralid**: The flow sheet for clopyralid in compost and composted sludge fertilizer is shown below:

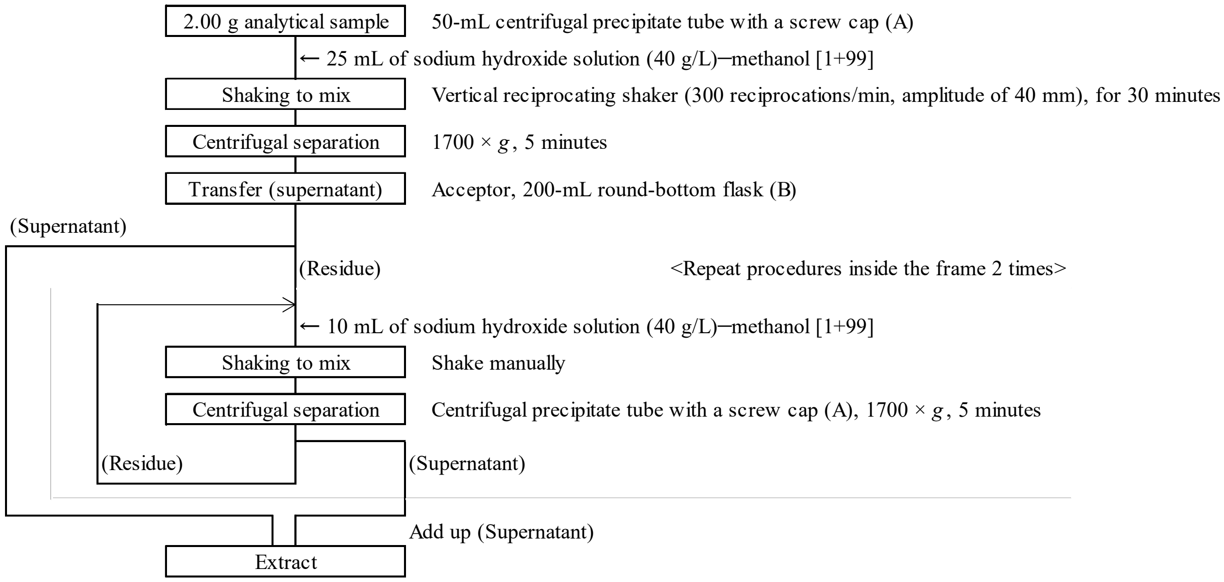


Figure 2-1 Flow sheet for clopyralid in compost and composted sludge fertilizers (Extraction procedure)

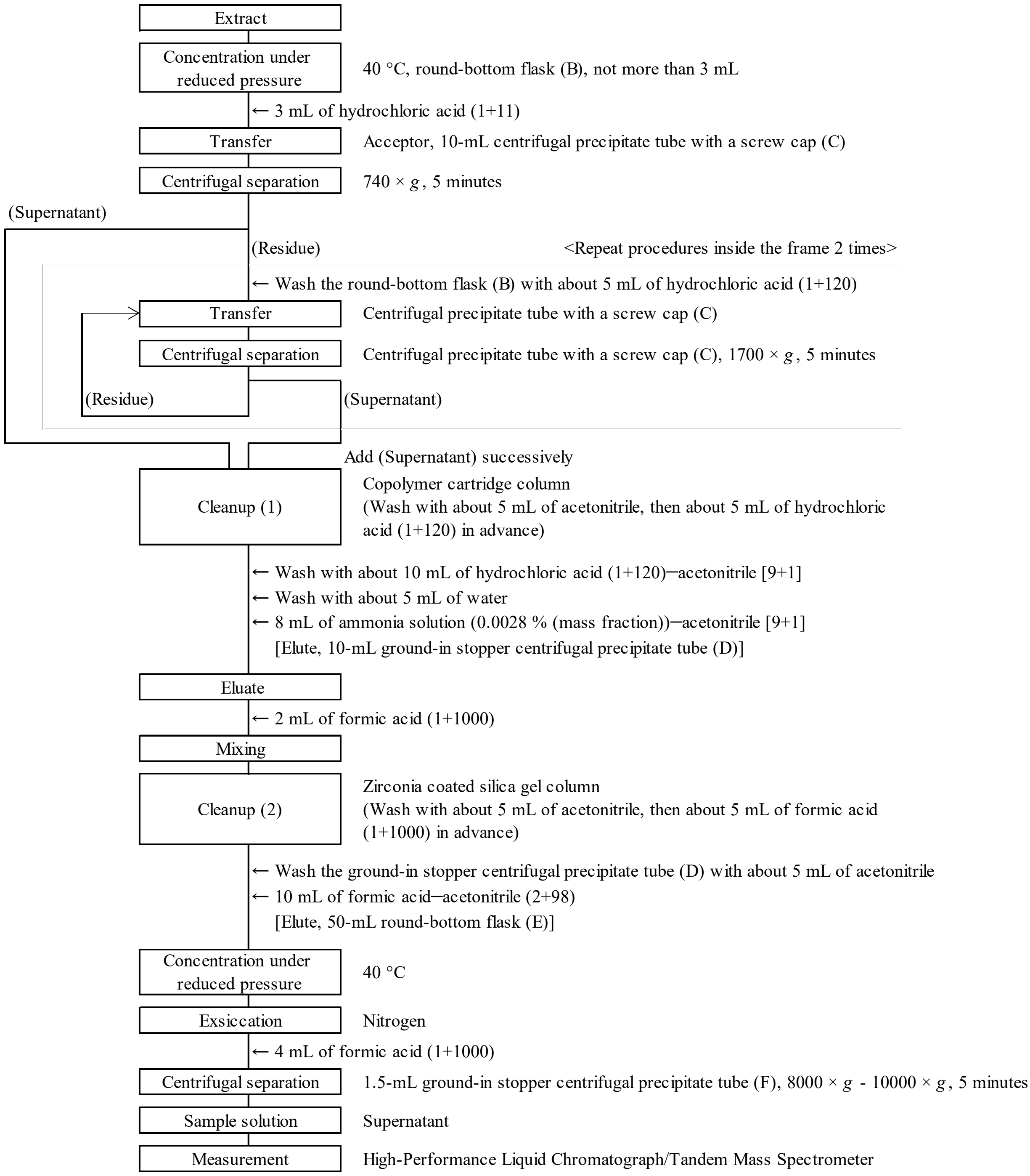
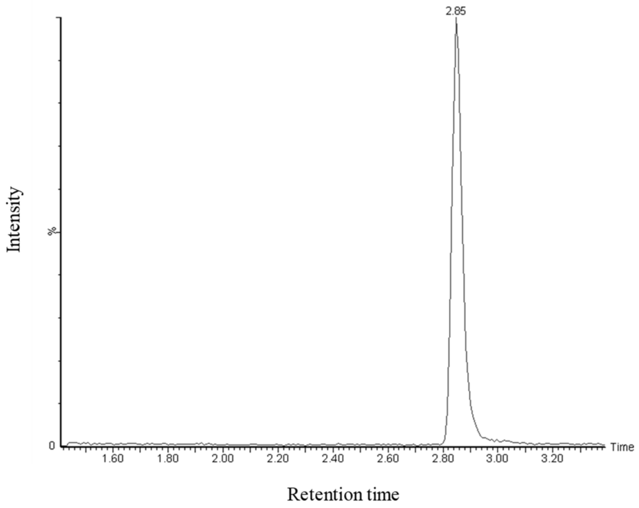


Figure 2-2 Flow sheet for clopyralid in compost and composted sludge fertilizers (Cleanup (1), cleanup (2) and measurement procedures)

References: An example of the Selected Reaction Monitoring chromatogram of a clopyralid standard solution for calibration curve preparation is shown below.



Reference diagram: SRM chromatograms of clopyralid
Clopyralid standard solution (the equivalents of 50 pg as clopyralid)

LC-MS/MS measurement conditions

Column: ACQUITY UPLC HSS C18 (2.1-mm inner diameter, 100-mm long, 1.8-μm particle diameter)

Flow rate: 0.4 mL/min

Capillary voltage: 1.0 kV

Temperature of ion source: 120 °C

Desolvation temperature: 400 °C

Cone voltage: 20 V

Collision energy: 20 eV for determination, 30 eV for validation

Other conditions are according to the examples of the measurement conditions in (**4.4**) **a)** LC-MS/MS.

8.3 Residue agrichemicals (multicomponent)
8.3.1 Residue agrichemicals multicomponent analysis (1)
8.3.1.a High-Performance Liquid Chromatography/Tandem Mass Spectrometry

(1) **Compounds subjected to analysis** Abamectin: abamectin B1a, ivermectin: 22, 23-dihydro avermectin B1a (Another name: ivermectin B1a), eprinomectin: eprinomectin B1a, rotenone: rotenone, piperonylbutoxide: piperonylbutoxide, pyrethrin: pyrethrin I and pyrethrin II

(2) **Summary**

This testing method is applicable to fluid home garden-use compound fertilizer and fluid compound fertilizer. This testing method is classified as Type B and its symbol is 8.3.1.a-2017 or AG-C-1.a-1.

Dissolve respective agricultural chemicals in fertilizers with acetonitrile and water, and extract. Refine by using 2 kinds of cleanup cartridge, and then measure with a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer (LC-MS/MS) to obtain compounds subjected to analysis in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(3) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Acetonitrile**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8039 or a reagent of equivalent quality.

c) **Methanol**: A JIS Guaranteed Reagent specified in JIS K 8891 or a reagent of equivalent quality.

d) **Methanol**: Methanol used in the eluent of a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer is a reagent of LC/MS analysis grade or equivalents.

e) **Ethyl acetate**: A JIS Guaranteed Reagent specified in JIS K 8361 or a reagent of equivalent quality.

f) **Toluene**: A JIS Guaranteed Reagent specified in JIS K 8680 or a reagent of equivalent quality.

g) **Ammonium formate**: A JIS Guaranteed Reagent (no less than 95 % (mass fraction) in purity) or a reagent of equivalent quality.

h) **Ammonium formate solution (0.1 mol/L)** (1): Add 6.306 g of ammonium formate into 1000 mL of water.

i) **Ammonium formate solution (0.1 mmol/L)** (1): Add 1mL of ammonium formate solution (0.1 mol/L) into 1000 mL water.

j) **Formic acid**: A JIS Guaranteed Reagent specified in JIS K 8264 or a reagent of equivalent quality.

k) **Formic acid solution (0.1 v/ v %)** (1): Add 1 mL of formic acid to 1,000 mL of water.

l) **Acetonitrile formate solution (0.1 v/v %)** (1): Add 1 mL of formic acid to 1,000 mL of acetonitrile.

m) **Respective agricultural chemicals standard solutions (0.1 mg/mL)** (1): Put about 0.01 g of abamectin [$C_{48}H_{72}O_{14}$] (2), ivermectin [$C_{48}H_{74}O_{14}$] (2), eprinomectin [$C_{50}H_{75}NO_{14}$] (2), rotenone [$C_{23}H_{22}O_6$] (2), piperonylbutoxide [$C_{19}H_{30}O_5$] (2) and pyrethrin [pyrethrin I: $C_{21}H_{28}O_3$ and pyrethrin II: $C_{22}H_{28}O_5$] (2) in a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve with a small amount of methanol, transfer to a 100-mL volumetric flask and add the solvent up to the marked line. (However, 0.1 mg/mL of pyrethrin contains total of pyrethrin I/II)

n) **Mixture standard solution (10 µg/mL)**: Transfer 10 mL of respective agricultural chemical standard solutions to a 100-mL volumetric flask and add methanol up to the marked line.

o) **Mixture standard solution (1000 ng/mL)**: Transfer 10 mL of mixture standard solution (10 µg/mL) to a 100-mL volumetric flask and add methanol up to the marked line.

p) **Mixture standard solution for calibration curve preparation (50 ng/mL - 500 ng/mL)**: In the case of usage, put 2.5 mL - 25 mL of mixture standard solution (1000 ng/ mL) in 50-mL volumetric flasks step-by-step, and add methanol up to the marked line.

q) **Mixture standard solution for calibration curve preparation (5 ng/mL - 50 ng/mL)**: In the case of usage, put 2.5 mL - 25 mL of mixture standard solution (100 ng/ mL) in 50-mL volumetric flasks step-by-step, and add methanol up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) A standard reagent is commercially sold.

Comment 1 A standard reagent of respective agricultural chemicals is sold by FUJIFILM Wako Pure Chemical Co., Ltd., Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(**4**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **High-Performance Liquid Chromatograph/Mass Spectrometer** : A high-performance liquid chromatograph/tandem mass spectrometer specified in JIS K 0136 that satisfies the following requirements.

1) **High-Performance Liquid Chromatograph**
   (i) Column bath: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.
   (ii) Column: A 2-mm - 3-mm inner diameter 50-mm - 150-mm long 1.6-µm - 3.0-µm particle diameter stainless steel column tube filled with silica gel to which octadecyl chemically bonds. The specification is according to the mass spectrometer specification (3).

2) **Mass Spectrometer**
   (i) Ionization method: Electro-Spray Ionization (ESI) method
   (ii) Ion detection method: Selected Reaction Monitoring

b) **Ultrasonic generator**: An ultrasonic washer can be used.

c) **Concentrator**: An evaporator whose temperature can be adjusted up to 40 ºC.

d) **Porous diatomaceous earth cartridge column**: A column that is filled with the porous diatomaceous earth (capacity: 5 mL) (4)

e) **Graphite carbon-NH_2 laminate cartridge column**: A 6-mL cylinder on which 500 mg of graphite carbon and 500 mg of aminopropyl silylation silica gel is laminated (5).

Note (**3**) The column is sold under the names ACQUITY UPLC HSS C18, etc.
(**4**) The column is sold under the names Chem Elut (5 mL), etc.
(**5**) The column is sold under the names Envi-carb/LC-NH_2 (500 mg/500 mg, 6 mL), etc.

(**5**) **Test procedures**

(**5.1**) **Extraction**: Conduct extraction as shown below.

a) Put about 5.00 mL (6) of an analytical sample into a 10-mL volumetric flask.

b) Add 3 mL of acetonitrile to the same volumetric flask, and add water up to the marked line to shake to mix well.

c) Conduct ultra-sonication for about 30 minutes using an ultrasonic generator (7) to make an extract.

Note (**6**) After measuring the specific gravity of sample, calculate the concentration of materials subjected to measurement in the analytical sample.
(**7**) Note that the volume of the solution may expand as a result of ultra-sonication. It is recommended to leave it at room temperature for a while when it expands.

Comment 2 The specific gravity (density) can be calculated by placing a 10-mL volumetric flask on an electric balance, aligning the scale to zero, putting 5.00 mL of the analytical sample in the volumetric flask and reading the weighing value.

(**5.2**) **Cleanup (1)**: Conduct cleanup (1) as shown below.

a) Put 5 mL of extract into a porous diatomaceous earth cartridge column and keep it in the column for about 5 minutes.

b) Place a 100-mL round-bottom flask under the same cartridge column, add about 5 mL of ethyl acetate into the same cartridge column 4 times successively and allow the solution to elute until the surface of the solution reaches the top of packing materials (8).

c) After conducting vacuum concentration of elute in a water bath of no more than 40 ºC until most of the elute exsiccate, send a nitrogen gas to exsiccate the elute (9), and add 2 mL of acetonitrile−toluene (3+1) to dissolve the residue.

Note (**8**) Confirm the solution to elute before conducting the testing.
(**9**) There is a possibility for agricultural chemicals to vaporize if it is exsiccated excessively.

(**5.3**) **Cleanup (2)**: Conduct cleanup (2) as shown below.

a) Wash the graphite carbon-NH_2 laminate cartridge column with about 10 mL of acetonitrile−toluene (3+1) in advance

b) Place a 100-mL round-bottom flask under the same cartridge column, put the solution in (**5.2**) **c**) in the same cartridge column, and allow the solution to overflow until the surface of the solution reaches the top of packing materials.

c) Wash the vessel with about 5 mL of acetonitrile−toluene (3+1) 5 times and add washing to the same cartridge successively to allow it to overflow.

d) After conducting vacuum concentration of elute in a water bath of no more than 40 ºC until most of the elute exsiccates, send a nitrogen gas to exsiccate the elute (10), and add 2 mL of methanol (11) to dissolve the residue. Put a predetermined amount of the solution precisely and dilute with methanol exactly by a factor of 5 to make the solution as a sample solution.

Note (**10**) There is a possibility for agricultural chemicals to vaporize if it is exsiccated excessively.
(**11**) If there is a possibility that the concentration of agricultural chemicals in the sample solution exceeds the maximum limit of the calibration curve, dilute a predetermined amount of the sample solution with methanol.

(**5.4**) **Measurement**: Conduct the measurement as indicated in JIS K 0136 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph/Mass Spectrometer used in measurement.

a) **The measurement conditions of the High-Performance Liquid Chromatograph/Mass spectrometer**: An example of measurement conditions for the High-Performance Liquid Chromatograph/Mass Spectrometer is shown below. Set up the measurement conditions considering it:

1) **High-Performance Liquid Chromatograph**

(i) Column: A silica gel column (2-mm - 3-mm inner diameter, 50-mm - 150-mm long, 1.6-µm - 3.0-µm particle diameter column) to which octadecyl chemically bonds.

(ii) Flow rate: 0.2 mL/min - 0.5 mL/min

(iii) Eluent: A: Ammonium formate solution (0.1 mmol/L) − formic acid solution (0.1 v/v%) [1+1]
B: Acetonitrile formate solution (0.1 v/v%)

(iv) Gradient: 0 min (50 %B) → 15 min (95 %B) → 20 min (98 %B) → 30 min (50 %B)

(v) Temperature of column bath: 40 °C
(vi) Injection volume: 5 µL

2) **Mass Spectrometer**
(i) Ionization method: Electro-Spray Ionization (ESI) method
(ii) Mode: Positive
(iii) Monitor ion: Shown in Table 1

Table 1 Monitor ion of respective agrichemicals

Agrichemicals	Mass-to-charge ratio (*m/z*)		
	Precursorion	Production (determination)	Production (validation)
Abamectin B1a	891	305	567
Ivermectin B1a	893	307	551
Eprinomectin B1a	915	186	298
Rotenone	395	213	192
Piperonylbutoxide	356	177	147
PyrethrinI	329	161	133
PyrethrinII	373	161	133

b) **Calibration curve preparation**

1) Inject 5 µL of respective mixture standard solutions for calibration curve preparation into the High-Performance Liquid Chromatograph/Tandem Mass Spectrometer, record the chromatograms of ion (*m/z*) for determination and ion (*m/z*) for validation of materials subjected to measurement.

2) Calculate the peak area ratio or height ratio of ion (*m/z*) for determination and ion (*m/z*) for validation of respective materials subjected to measurement.

3) Prepare a curve for the relationship between the concentration of material subjected to measurement and the peak area or height of ion (*m/z*) for determination of respective mixture solutions for the calibration curve preparation. Prepare a calibration curve when the sample is measured.

c) **Sample measurement**

1) Subject 5 µL of the sample solution to the same procedure as in **b**) **2**) - **3**) (12).

2) Obtain the content of materials subjected to measurement from the calibration curve of the peak area or height to calculate materials subjected to measurement in the analytical sample.

Note (12) Confirm that the peak area ratio or height ratio of ion (*m/z*) for determination to ion (*m/z*) for validation is within the range of about ± 30 % of the peak area ratio or height ratio for the standard solution. In addition, the peak area ratio or height ratio depends on the concentration.

(5.5) Calculation

Calculate the respective concentration of agricultural chemicals in the analytical sample by the following formula. in the analytical sample by the following formula:

Respective concentration of agricultural chemicals in the analytical sample (µg/kg)

$= (A \times B \times 10) / C$

A : Concentration (ng/mL) of respective materials subjected to measurement in the final sample solution obtained from the calibration curve

B : Dilution factor in the case that the final sample solution is further diluted because it exceeds the upper limit of the calibration curve.

C : Specific gravity of the analytical sample (density) (g/mL)

Comment 3 Recovery testing was conducted using fluid home garden-use compound fertilizer (3 kinds) and fluid compound fertilizer (2 kinds), as a result, the mean recovery rates at the additive level of 4000 µg/kg and 400 µg/kg (However, 4000 µg/kg and 400 µg/kg of pyrethrin contain total of pyrethrin I/II) were 77.0 % - 104.5 % and 85.6 % - 107.9 % respectively.

Additionally, results from a collaborative study for test method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 10 µg/kg for each of the agrichemicals.

Table 2 Analysis results of the collaborative study for the testing method validation of multicomponent analysis of pesticide

Agrichemicals	Sample name	Number of laboratories[1]	Mean[2] (µg/kg)	Additive amount (µg/kg)	Recovery (%)	RSD_r[3] (%)	RSD_R[4] (%)
Abamectin B1a	Home garden-use compound fertilizer1	8	286.8	333.3	86.1	13.3	14.4
	Home garden-use compound fertilizer2	8	358.9	416.7	86.1	13.4	14.8
	Home garden-use compound fertilizer3	8	425.8	500.0	85.2	8.6	11.6
	Fluid compound fertilizer1	8	288.6	333.3	86.6	7.1	8.5
	Fluid compound fertilizer2	8	405.5	500.0	81.1	7.1	7.2
Ivermectin B1a	Home garden-use compound fertilizer1	8	298.9	333.3	89.7	14.9	15.0
	Home garden-use compound fertilizer2	8	382.5	416.7	91.8	14.1	19.3
	Home garden-use compound fertilizer3	8	431.1	500.0	86.2	9.8	10.9
	Fluid compound fertilizer1	8	298.8	333.3	89.6	10.1	12.8
	Fluid compound fertilizer2	8	405.2	500.0	81.0	3.8	5.8
Eprinomectin B1a	Home garden-use compound fertilizer1	8	293.5	333.3	88.1	7.0	10.4
	Home garden-use compound fertilizer2	8	361.9	416.7	86.9	9.2	14.3
	Home garden-use compound fertilizer3	8	425.3	500.0	85.1	7.0	10.0
	Fluid compound fertilizer1	8	277.3	333.3	83.2	9.0	12.0
	Fluid compound fertilizer2	8	398.2	500.0	79.6	7.5	11.6
Rotenone	Home garden-use compound fertilizer1	8	276.8	333.3	83.1	5.7	7.8
	Home garden-use compound fertilizer2	8	353.5	416.7	84.8	9.8	12.5
	Home garden-use compound fertilizer3	8	426.6	500.0	85.3	6.6	8.5
	Fluid compound fertilizer1	8	263.5	333.3	79.1	11.0	12.3
	Fluid compound fertilizer2	8	385.2	500.0	77.0	5.7	12.1

1) Number of laboratories used in analysis
2) Gross mean (n = number of laboratories × number of repeated tests (2))
3) Repeatability (relative standard deviation)
4) Reproducibility (relative standard deviation)

Table 2 (Continued)

Agrichemicals	Sample name	Number of laboratories[1)]	Mean[2)] (μg/kg)	Additive amount (μg/kg)	Recovery (%)	RSD_r[3)] (%)	RSD_R[4)] (%)
Piperonyl butoxide	Home garden-use compound fertilizer1	8	318.2	333.3	95.5	8.1	13.2
	Home garden-use compound fertilizer2	8	395.6	416.7	94.9	8.4	13.6
	Home garden-use compound fertilizer3	8	450.3	500.0	90.1	4.6	9.3
	Fluid compound fertilizer1	8	299.7	333.3	89.9	7.4	11.0
	Fluid compound fertilizer2	8	435.8	500.0	87.2	5.8	7.4
Pyrethrin I	Home garden-use compound fertilizer1	8	160.7	186.0	86.4	9.3	11.9
	Home garden-use compound fertilizer2	8	202.2	232.5	87.0	12.6	12.8
	Home garden-use compound fertilizer3	8	228.6	279.0	81.9	5.4	8.8
	Fluid compound fertilizer1	8	158.2	186.0	85.1	6.8	10.4
	Fluid compound fertilizer2	8	223.1	279.0	80.0	8.5	9.1
Pyrethrin II	Home garden-use compound fertilizer1	8	131.1	147.3	89.0	6.5	9.7
	Home garden-use compound fertilizer2	8	163.2	184.2	88.6	10.8	13.6
	Home garden-use compound fertilizer3	8	182.0	221.0	82.4	5.4	8.9
	Fluid compound fertilizer1	8	126.2	147.3	85.7	7.8	11.4
	Fluid compound fertilizer2	8	180.2	221.0	81.5	6.3	8.3

References

1) Toshiharu YAGI, Masayuki YAMANISHI, Yuji SHIRAI: Simultaneous Determination of Agricultural chemicals in Fluid Fertilizer by Liquid Chromatography/Tandem Mass Spectrometry, Research Report of Fertilizer, **Vol. 4**, p. 36 - 48 (2011)

2) Toshiharu YAGI, Masayuki YAMANISHI, Yuji SHIRAI and Masato SHIBATA: Simultaneous Determination of Six Kind of Agricultural Chemicals in Fluid Fertilizer by Liquid Chromatograph-Tandem Mass Spectrometer (LC-MS/MS): A Collaborative Study, Research Report of Fertilizer, **Vol. 5**, p. 48 - 59 (2012)

(6) **Flow sheet for simultaneous analysis of 6 kinds of agrichemicals**: The flow sheet for simultaneous analysis of 6 kinds of agrichemicals in fertilizer is shown below.

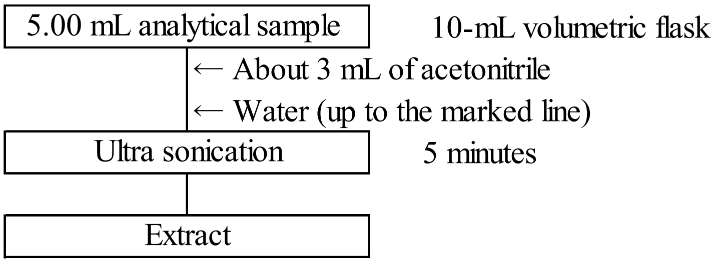


Figure 1 Flow sheet for residue agrichemicals multicomponent analysis
((1) : simultaneous analysis of 6 kinds of agrichemicals) in fertilizers
(Extraction procedure)

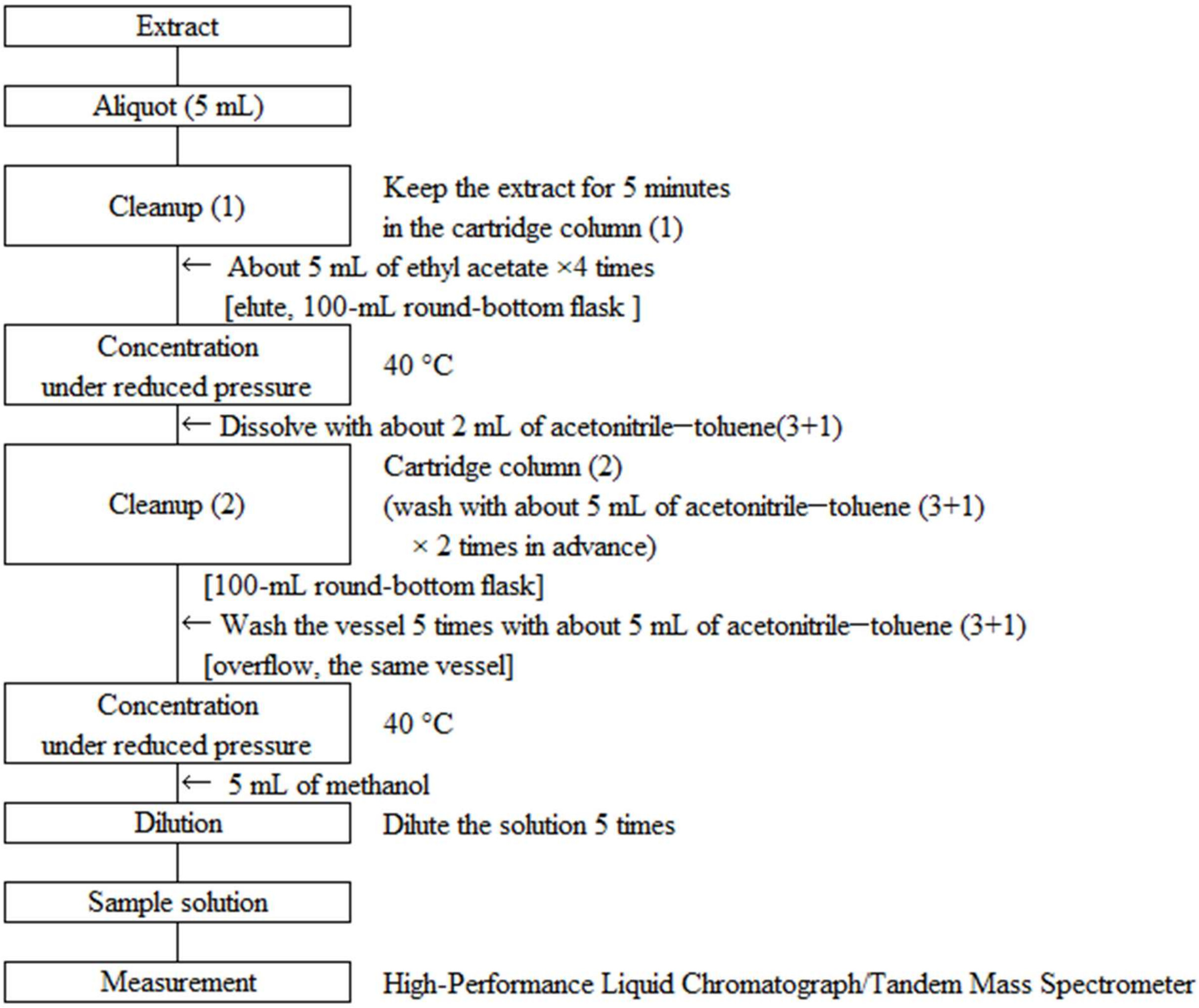
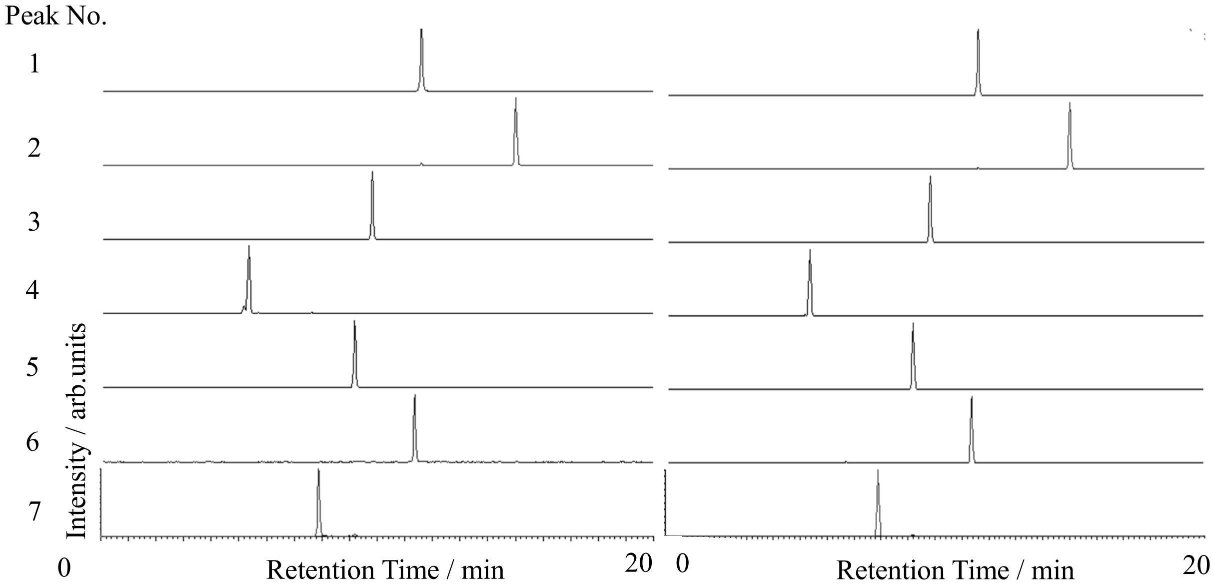


Figure 2 Flow sheet for residue agrichemicals multicomponent analysis
((1) : simultaneous analysis of 6 kinds of agrichemicals) in fertilizers
(Cleanup (1) and cleanup (2) and measurement procedure)

Reference: An example of the Selected Reaction Monitoring chromatograms of a mixture standard solution for calibration curve preparation and a sample solution (fluid home garden-use compound fertilizer) are shown below.



Peak No.1: AbamectinB1a
No.2: IvermectinB1a
No.3: Eprinomectin B1a
No.4: Rotenone
No.5: Piperonylbutoxide
No.6: Pyrethrin I
No.7: Pyrethrin II

1) Mixed standard solution   2) Sample solution

Reference diagram: Selected Reaction Monitoring chromatograms of respective agricultural chemicals

1) Mixture standard solution (the equivalents of 2,500 pg as respective agrichemicals)
(For pyrethrin, the equivalents of 2,500 pg of the total of pyrethrin I/II)
2) Sample solution (fluid home garden-use compound fertilizer, additive of the equivalents of 400 µg/ kg in the sample)
(For pyrethrin, the equivalents of 400 µg/kg of the total of pyrethrin I/II)

LC-MS/MS measurement conditions
Column: ACQUITY UPLC HSS C18 (2.1-mm inner diameter, 100-mm long, 1.8-µm particle diameter)
Flow rate: 0.2 mL/min
Capillary voltage: 3.0 kV
Temperature of ion source: 120 °C
Desolvation temperature: 400 °C
Cone voltage: Shown in Table 1

Collision energy: Shown in reference table
Other conditions are according to the examples of the measurement conditions in (**5.4**) **a**) LC-MS/MS and the reference table.

Reference table Parameters of mass spectrometer

Agrichemicals	Mass-to-charge ratio (*m*/*z*)			Cone voltage (V)	Collision energy (eV)
	Precursorion	Production (determination)	Production (validation)		
Abamectin B1a	891	305	567	20	25
Ivermectin B1a	893	307	551	25	25
Eprinomectin B1a	915	186	298	20	20
Rotenone	395	213	192	35	25
Piperonylbutoxide	356	177	147	20	15
PyrethrinI	329	161	133	20	10
PyrethrinII	373	161	133	20	10

8.3.2 Residue agrichemicals multicomponent analysis (2)

8.3.2.a Gas Chromatography

(1) **Compounds subjected to analysis** *β*-HCH (*β*-BHC), *γ*-HCH (*γ*-BHC), *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT,aldrin,endrin, dieldrin, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor,heptachlor, heptachlor epoxide and hexachlorobenzene

(2) **Summary**

This testing method is applicable to compost and straw, raw materials of compost. This testing method is classified as Type D and its symbol is 8.3.2.a-2017 or AG-C-2.a-1.

Extract respective agricultural chemicals in fertilizers or raw materials with acetonitrile and water, refine by using a porous diatomaceous earth column, a gel permeation chromatograph and a synthetic magnesium silicate cartridge column, and then measure with an electron capture detector equipped gas chromatograph to obtain compounds subjected to analysis in an analytical sample. In addition, the performance of this testing method is shown in **Comment 7**.

(3) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Acetonitrile**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8039 or a reagent of equivalent quality.

c) **Hexane**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8825 or a reagent of equivalent quality.

d) **Sodium chloride**: A reagent of agricultural chemicals residue/PCB testing grade or a reagent of equivalent quality.

e) **Cyclohexane**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) or a reagent of equivalent quality.

f) **Acetone**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8040 or a reagent of equivalent quality.

g) **Diethyl ether**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) specified in JIS K 8357 or a reagent of equivalent quality.

h) **2,2,4-trimethylpentane**: A reagent of High-Performance Liquid Chromatograph analysis grade or a reagent of equivalent quality.

i) **Respective agricultural chemicals standard solutions (0.2 mg/mL)** (1): Put about 0.02 g of *β*-HCH (*β*-BHC) [$C_6H_6Cl_6$] (2), *γ*-HCH (*γ*-BHC) [$C_6H_6Cl_6$] (2), *o,p*'-DDD [$C_{14}H_{10}Cl_4$] (2), *p,p*'-DDD [$C_{14}H_{10}Cl_4$] (2), *o,p*'-DDE [$C_{14}H_8Cl_4$] (2), *p,p*'-DDE [$C_{14}H_8Cl_4$] , *o,p*'-DDT [$C_{14}H_9Cl_5$] (2), *p,p*'-DDT [$C_{14}H_9Cl_5$] ,aldrin [$C_{12}H_8Cl_6$] ,endrin [$C_{12}H_8Cl_6O$] , dieldrin [$C_{12}H_8Cl_6O$] (2), *trans*-chlordane [$C_{10}H_8Cl_6$] (2), *cis*-chlordane [$C_{10}H_8Cl_6$] (2), *trans*-nonachlor [$C_{10}H_5Cl_9$] (2), *cis*-nonachlor [$C_{10}H_5Cl_9$] (2), heptachlor [$C_{10}H_5Cl_7$] (2), heptachlor epoxide [$C_{10}H_5Cl_7O$] (2) and hexachlorobenzene [C_6Cl_6] (2) to a weighing dish, and measure the mass to the order of 0.1 mg. Dissolve them with 20 mL of acetone, transfer to 100 mL volumetric flasks respectively, and add 2,2,4-trimethylpentane up to the marked line.

j) **Mixture standard solution (1 µg/mL)** (1): Transfer 1 mL of respective agricultural chemicals standard solutions to a 100-mL volumetric flask and add 2,2,4-trimethylpentane-acetone (4+1) up to the marked line.

k) **Mixture standard solution for calibration curve preparation (0.02 µg/mL - 0.2 µg/mL)** (1): In the case of usage, put 1 mL - 10 mL of mixture standard solution (1 µg/mL) in 50 mL volumetric flasks step-by-step, and add 2,2,4-trimethylpentane–acetone (4+1) up to the marked line.

l) **Mixture standard solution for calibration curve preparation (0.005 µg/mL - 0.02 µg/mL)** (1): In the case of usage, put 2.5 mL - 10 mL of mixture standard solution (0.1 µg/mL) in 50 mL volumetric flasks step-by-step, and add 2,2,4-trimethylpentane−acetone (4+1) up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) A standard reagent is commercially sold.

Comment 1 A standard reagent of respective agricultural chemicals is sold by FUJIFILM Wako Pure Chemical Co., Ltd., Kanto Chemical Co., Inc. and Hayashi Pure Chemical Industries., Ltd.

(**4**) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **Gas Chromatograph (GC)**: GC specified in JIS K 0114 that satisfies the following requirements.

1) **Sample injector**: An injector that enables split less system.

2) **Capillary column**: A capillary column (0.25-mm inner diameter and 30-m long) made of fused silica. 14 % cyanopropylphenyl -86 % dimethyl polysiloxane chemically bonds to the inner surface of a capillary column with 0.25 µm thickness.

3) **Detection unit**: Electron capture detector (ECD)

b) **Gel Permeation Chromatograph (GPC)**: Preparative liquid chromatograph specified in JIS K 0135 that satisfies the following requirements. No detector is required.

1) **Sample injector**: A sample injector that can inject 5 mL of sample solution.

2) **Column**: A 20-mm inner diameter 300-mm long stainless-steel column tube filled with styrendivynylbenzene copolymer system hard gel

3) **Guard column**: A 20-mm inner diameter 100-mm long stainless steel column tube filled with styrendivynylbenzene copolymer system hard gel

4) **Fraction collecto**r: A fraction collector that can set up a fraction to which agrichemical components elute.

c) **Shaking apparatus**

d) **Concentrator**: An evaporator whose temperature can be adjusted up to 40 ºC.

e) **Filter**: A funnel for filtering under reduced pressure (compatible filter diameter: 60 mm)

f) **Porous diatomaceous earth cartridge column**: A column that is filled with the porous diatomaceous earth (capacity: 20 mL).

g) **Synthetic magnesium silicate cartridge column**: A cartridge column that is filled with 910 mg of synthetic magnesium silicate.

h) **Membrane filters**: Made of PTFE (pore size is no more than 0.5 µm)

Comment 2 Column for GC is sold under the production name DB-1701, Rtx-1701, SPB-1701, etc. A column which has actually isolated compounds subjected to analysis should be used.

Comment 3 GPC is an aliquot liquid chromatograph that collects a fraction of a material subjected to measurement sieved and isolated by packing materials of the column for GPC according to the size of the molecule of the material. A column for GPC is sold under the production name Shodex CLNpak EV-2000 AC, etc. In addition, a guard column for GPC is sold under the production name Shodex CLNpak EV-G AC, etc.

Comment 4 A funnel for filtering under educed pressure is sold under the production name KIRIYAMA Funnel SB-60, KIRIYAMA Funnel SU-60, etc.

Comment 5 A porous diatomaceous earth cartridge is commercially sold under production name Chem Elut (20 mL), etc.

Comment 6 Synthetic magnesium silicate is commercially sold under the production names such as Sep-Pak Florisil Plus Long Cartridge (910 mg).

Comment 7 A membrane filter is sold under the production name HLC-DISK 25 Solvent system (pore size: 0.45 µm), DISMIC 25JP050, Millex FH (diameter: 25 mm, pore size: 0.45 µm), etc.

(5) Test procedures

(5.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample, and put it into a 200-mL ground-in stopper Erlenmeyer flask.

b) Add 20 mL of acetonitrile−water (3 +1) to moisten.

c) After leaving at rest for ten minutes, add 100 mL of acetonitrile and shake to mix for about 30 minutes.

d) Place a 300-mL round-bottom flask under the filter and filtrate the extract under reduced pressure with a filter paper (type 5 B)

e) Conduct cleanup of the previous Erlenmeyer flask and residue with 50 mL of acetonitrile successively, similarly filtrate under reduced pressure and pool with the filtrate in **d**) to make an extract.

(5.2) Cleanup (1): Conduct cleanup (1) as shown below.

a) Concentrate under reduced pressure in a water bath of no more than 40 ºC until most of the extract exsiccates.

b) Add 20 mL of sodium chloride saturated solution, put it into a porous diatomaceous earth cartridge column and leave it at rest for about 5 minutes.

c) Place a 300-mL round-bottom flask under the same cartridge column, wash the vessel 3 times with about 20 mL of hexane to add the washing to the same cartridge column successively and allow the solution to elute until the surface of the solution reaches the top of packing materials.

d) Further add about 60 mL of hexane into the same cartridge and allow the solution to elute until the surface of the solution reaches the top of packing materials.

e) After concentrating the eluate under reduced pressure in a water bath of no more than 40 ºC until most of the eluate exsiccates, send a nitrogen gas to exsiccate the eluate (3), and add 10 mL of cyclohexane−acetone (4+1) to dissolve the residue.

f) Filter with a membrane filter (pore size: no more than 0.5 µm).

Note (**3**) There is a possibility for agricultural chemicals to vaporize if it is exsiccated excessively.

(5.3) Cleanup (2): Conduct cleanup (2) as shown below.

a) Inject 5 mL of the filtrate in (**5.2**) **e**) into a gel permeation chromatograph and take a fraction eluted from respective agrichemicals which are determined according to the procedure condition in **b**) into a 100-mL round-bottom flask.

b) **Procedure conditions for the Gel Permeation Chromatograph**: An example of procedure conditions for the Gel Permeation Chromatograph is shown below. Set up the measurement conditions considering it:

1) **Column**: A styrendivynylbenzene copolymer column (20-mm inner diameter, 300-mm long, 15-µm particle diameter)
2) **Guard column**: A styrendivynylbenzene copolymer column (20-mm inner diameter, 100-mm long, 15-µm particle diameter)
3) **Eluent**: Cyclohexane-acetone (4 +1)
4) **Flow rate**: 5 mL/min
5) **Aliquot fraction**: 70 mL -120 mL

c) After concentrating the eluate under reduced pressure in a water bath of no more than 40 ºC until most of the eluate exsiccates, send a nitrogen gas to exsiccate the eluate (3), and add 2 mL of hexane to dissolve the residue.

(**5.4**) **Cleanup (3)**: Conduct cleanup (3) as shown below.

a) Conduct cleanup of a synthetic magnesium silicate cartridge column (910 mg) with about 5 mL of hexane.

b) Place a 50-mL round-bottom flask under the same cartridge column, put the solution in (**5.3**) **c**) to the same cartridge column, and allow the solution to overflow until the surface of the solution reaches the top of packing materials.

c) Wash the vessel with about 2 mL of hexane 2 times and add washing to the same cartridge successively to allow it to overflow.

d) Further add about 15 mL of hexane−diethyl ethel (9+1) into the same cartridge and allow respective materials subjected to measurement to elute.

e) After concentrating the eluate under reduced pressure in a water bath of no more than 40 ºC until most of the eluate exsiccates, send a nitrogen gas to exsiccate the eluate (3). And add 1 mL of 2,2,4-trimethylpentane−acetone (4+1) (4) to dissolve the residue, making a sample solution.

Note (**4**) If there is a possibility that the concentration of respective agricultural chemicals in the sample solution exceed the maximum limit of the calibration curve, dilute a predetermined amount of the sample solution with 2,2,4-trimethylpentane−acetone (4+1).

(**5.4**) **Measurement**: Conduct the measurement as indicated in JIS K 0114 and as shown below. Specific measurement procedures are according to the operation method of the Gas Chromatograph used in measurement.

a) **The measurement conditions of the Gas Chromatograph**: An example of measurement conditions for the Gas Chromatograph is shown below. Set up the measurement conditions considering it:

1) **Sample injection method**: split less injection method (1min)

2) **Temperature of sample injector**: 250 °C

3) **Capillary column**: A capillary column (0.25-mm inner diameter, 30-m long, 0.25 μm layer thickness) made of fused silica. 14 % cyanopropylphenyl−86 % dimethyl polysiloxane chemically bonds to the inner surface of the capillary column with 0.25 μm thickness.

4) **Column bath temperature**: 60 °C (1 min) → (20 °C/min) → 180 °C→ (2 °C/min) → 260 °C→ (5 °C/min) → 275 °C (1 min)

5) **Carrier gas**: helium**, Flow rate**: 1.5 mL/min

6) **Addition gas**: Nitrogen, **Flow rate**: 60 mL/min

7) **Detection unit**: Electron capture detector (ECD)

8) **Detector temperature**: 280 °C

b) **Calibration curve preparation**

1) Inject 1 μL of respective mixture standard solutions for calibration curve preparation into the GC, record a chromatogram and obtain a peak area or height.

2) Prepare a curve for the relationship between the concentration and the peak area or height of respective mixture solutions for the calibration curve preparation. Prepare a calibration curve when the sample is measured.

c) **Sample measurement**

1) Subject 1 μL of the sample solution to the same procedure as in **b**) **1**)

2) Obtain the content of materials subjected to measurement from the calibration curve of the peak area or height to calculate materials subjected to measurement in the analytical sample.

Comment 8 Recovery testing of compounds subjected to analysis in compost was conducted, as a result, the mean recovery rates at the additive level of 20 µg/kg and 50 µg/kg were 82.1 % - 118.1 % and 62.5 % - 120.2 % respectively. The results of the repeatability tests on different days using compost to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Note that *α*-HCH (*α*-BHC), *δ*-HCH (*δ*-BHC), and oxychlordane, which were subjected to simultaneous analysis, were excluded from the compounds subjected to analysis because sufficient recovery rates were not obtained.

Note that the minimum limit of quantification for respective agrichemicals of the test method is no more than 20 µg/kg.

Table 1 Analysis results of the repeatability tests on different days of residue agrichemicals multicomponent

Name of sample	Test days of repeatability (T) 1)	Mean 2) (µg/kg)	s_r 3) (µg/kg)	RSD_r 4) (%)	$s_{I(T)}$ 5) (µg/kg)	$RSD_{I(T)}$ 6) (%)
β-BHC	5	15.7	1.3	8.3	2.0	12.8
γ-BHC	5	14.7	1.3	8.6	1.6	10.9
δ-BHC	5	50	11.8	23.6	14.5	29.1
hexachlorobenzene	5	15.3	1.4	9.3	2.5	16.0
heptachlor	5	16.9	1.1	6.7	2.4	14.3
aldrin	5	12.8	1.0	7.8	3.4	26.4
oxychlordane	5	32.4	2.8	8.7	2.8	8.6
heptachlor epoxide (1)	5	17.8	2.0	11.1	1.6	9.0
heptachlor epoxide (2)	5	17.9	1.8	10.1	1.8	10.0
trans-chlordane	5	17.6	1.7	9.9	1.9	10.8
cis-chlordane	5	17.8	1.3	7.2	2.0	11.3
trans-nonachlor	5	15.9	1.3	8.3	1.5	9.6
cis-nonachlor	5	16.7	1.5	8.8	2.1	12.4
dieldrin	5	16.6	1.4	8.5	2.0	11.9
endrin	5	17.8	1.4	7.9	1.7	9.5
o,p'-DDE	5	18.7	2.7	14.4	2.7	14.6
p,p'-DDE	5	16.8	1.6	9.8	1.8	10.9
o,p'-DDD	5	16.9	1.2	7.3	1.3	7.8
p,p'-DDD	5	16.3	1.7	10.7	1.8	10.9
o,p'-DDT	5	17.9	1.4	7.8	2.2	12.0
p,p'-DDT	5	16.6	1.4	8.1	2.2	13.0

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Intermediate standard deviation
6) Intermediate relative standard deviation

References

1) Tomoharu NOZAKI: Determination of Organic Chloride Pesticides in Composts using Gas Chromatography-Electron Capture Detector (GC-ECD), Research Report of Fertilizers, **Vol. 10**, p. 41 - 60 (2017)

(6) **Flow sheet for simultaneous analysis of chloride pesticides**: The flow sheet for simultaneous analysis of chloride pesticides in fertilizer is shown below.

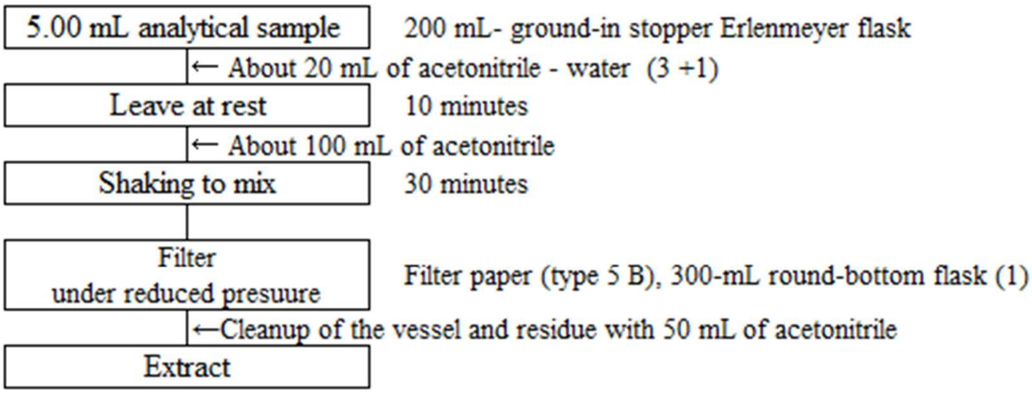


Figure 1 Flow sheet for residue agrichemicals multicomponent analysis
((2) : simultaneous analysis of chloride agrichemicals) in fertilizers
(Extraction procedure)

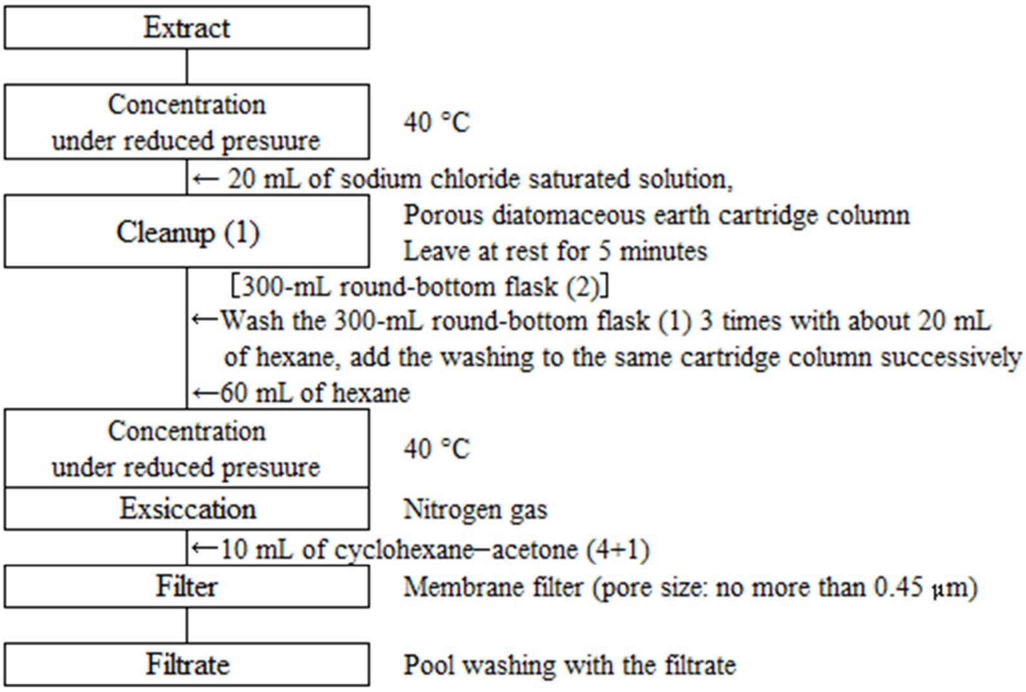


Figure 2 Flow sheet for residue agrichemicals multicomponent analysis
((2) : simultaneous analysis of chloride agrichemicals) in fertilizers
(Cleanup (1) procedure)

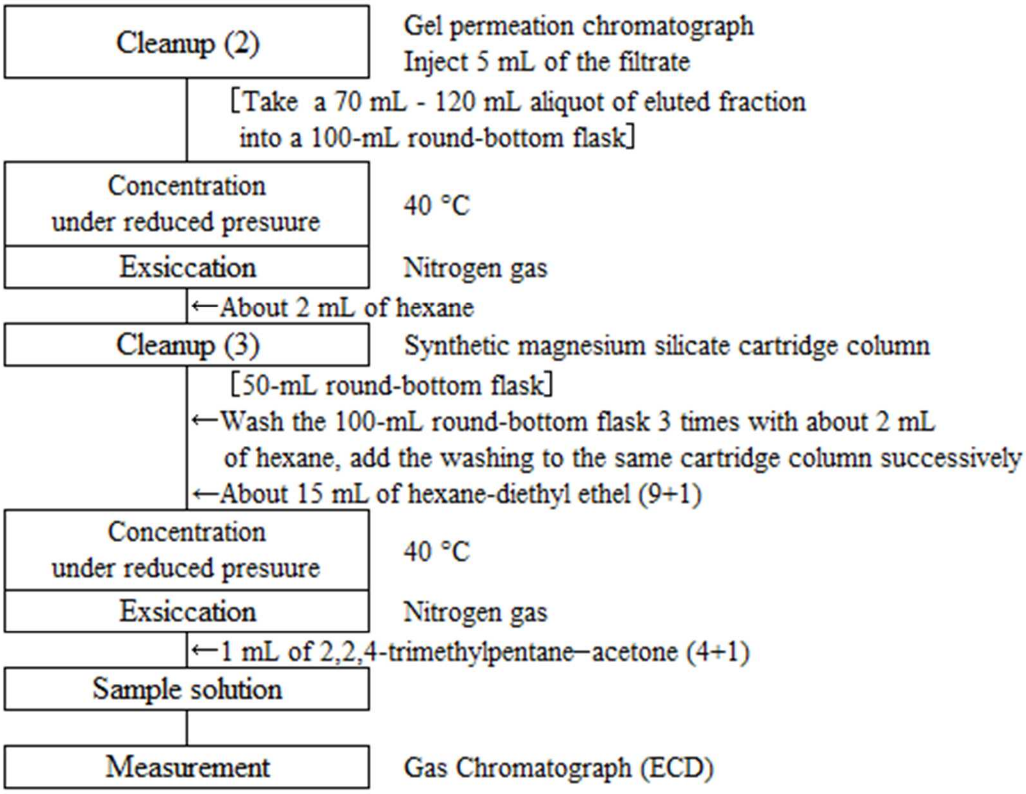


Figure 3 Flow sheet for residue agrichemicals multicomponent analysis
((2) : simultaneous analysis of chloride agrichemicals) in fertilizers
(Cleanup (2) and cleanup (3) and measurementprocedure)

8.4 Sodium

8.4.a Flame atomic absorption spectrometry

(1) Summary

This testing method is applicable to fertilizers containing organic matters. This testing method is classified as Type D and its symbol is 8.4.a-2017 or Na.a-1.

Pretreat an analytical sample with incineration and hydrochloric acid, spray into an acetylene−air flame, and measure the atomic absorption with sodium at a wavelength of 589.0 nm or 589.6 nm to obtain sodium (Na) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 3**.

(**2**) **Reagent**: Reagents are as shown below.

a) **Hydrochloric acid**: A JIS Guaranteed Reagent specified in JIS K 8180 or a reagent of equivalent quality.

b) **Sodium standard solution (Na 1 mg/mL)** (1): Heat sodium chloride specified in JIS K 8150 at 600 ºC ± 10 ºC for about 1 hour, let it stand to cool in a desiccator, and weigh 2.542 g to a weighing dish. Dissolve in a small amount of water, transfer to a 1000-mL volumetric flask, and add water up to the marked line.

c) **Sodium standard solution (Na 0.1 mg/mL)** (1): Put 20 mL of sodium standard solution (Na 1 mg/mL) in a 200-mL volumetric flask, and add hydrochloric acid (1+23) up to the marked line.

d) **Sodium standard solutions (Na 1 µg/mL - 10 µg/mL) for the calibration curve preparation** (2): Put 2.5 mL - 25 mL of sodium standard solution (Na 0.1 mg/mL) in 250-mL volumetric flasks step-by-step, and add hydrochloric acid (1+23) up to the marked line (2).

e) **Blank test solution for the calibration curve preparation**: Hydrochloric acid (1+23) used in the procedures in **d**) (3).

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) When using a device model which cannot degrade the sensitivity of the device by tilting the burner head, conduct the model appropriate dilution. (For example, 0.1 µg/mL - 4 µg/mL)

(**3**) When preserving, use a container, which can be sealed tightly, made of material such as polypropylene, PTFE that sodium hardly elutes.

Comment 1 Instead of the sodium standard solution in (**2**) **b**), a sodium standard solution (Na 0.1 mg/mL 1 mg/mL or 10 mg/mL for Atomic Absorption Spectrometry traceable to National Metrology can also be used.

(**3**) **Instruments**: Instruments are as shown below:

a) **Flame atomic absorption spectrometer**: An atomic absorption spectrometer specified in JIS K 0121.

1) **Light source**: A sodium hollow cathode lamp

2) **Gas**: Gas for heating by flame

(i) Fuel gas: acetylene

(ii) Auxiliary gas: Air sufficiently free of dust and moisture.

b) **Electric furnace**: An electric furnace that can be adjusted to 550 ºC ± 5 ºC.

c) **Hot plate or sand bath**: A hot plate whose surface temperature can be adjusted up to 250 ºC. Adjust the amounts of gas and silica sand of a sand bath so that the sand bath temperature can be set to 250 ºC.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 5.00 g of an analytical sample and put it in a 200-mL - 300-mL tall beaker.
b) Put the tall beaker in an electric furnace, and heat gently to char (4).
c) Ignite at 550 ºC ± 5 ºC for no less than 4 hours to incinerate (4).
d) After standing to cool, moisten the residue with a small amount of water, gradually add about 10 mL of hydrochloric acid, and further add water to make 20 mL.
e) Cover the tall beaker with a watch glass, and heat on a hot plate or a sand bath to boil for about 5 minutes.
f) After standing to cool, transfer the solution to a 250-mL - 500-mL volumetric flask with water
g) Add water up to the marked line.
h) Filter with Type 3 filter paper to make a sample solution.

Note (**4**) Example of charring and incineration procedure: After raising the temperature from room temperature to about 250 °C in 30 minutes to 1 hour, continue heating for about 1 hour and further raise to 550 °C in 1 to 2 hours.

Comment 2 A sample solution obtained in the procedure in (**4.1**) is also applicable to the components shown in Annex B.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0121 and as shown below. Specific measurement procedures are according to the operation method of the Atomic absorption spectrometer used in measurement.

a) **Measurement conditions for the Atomic absorption spectrometer**: Set up the measurement conditions for the Atomic absorption spectrometer considering the following:
Analytical line wavelength: 589.0 nm or 589.6 nm

b) **Calibration curve preparation**
1) Spray the sodium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation into a flame, and read the indicated value at a wavelength of 589.0 nm or 589.6 nm.
2) Prepare a curve for the relationship between the sodium concentration and the indicated value of the sodium standard solutions for the calibration curve preparation and the blank test solution for the calibration curve preparation.

c) **Sample measurement**
1) Put a predetermined amount of the sample solution (the equivalents of 0.1 mg - 1 mg as Na) (5) in a 100-mL volumetric flask.
2) Add hydrochloric acid (1+23) to the marked line.
3) Subject to the same procedure as in **b) 1**) to read the indicated value.
4) Obtain the sodium content from the calibration curve, and calculate the sodium (Na) in the analytical sample.

Note (**5**) Sample a predetermined amount of sample solution according to the device model in **Note (2**).

Comment 3 Additive recovery testing with triplicates measurement was conducted using fish caked powder, fish waste processed fertilizers, rape seed meal and its powder, composted sludge fertilizers and compost, as a result, the mean recovery rate at the additive concentration of sodium in the range of 1 % (mass fraction) - 10 % (mass fraction) was 97.0 % - 103 %.
The results of the repeatability tests on different days using fish caked powder (sample to which sodium chloride is added) and compost to evaluate precision were analyzed

by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.
Note that the minimum limit of quantification of this testing method was estimated to be about 0.02 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of sodium

Name of sample	Test days of repeatability (T)[1)]	Mean[2)] (%)[3)]	s_r[4)] (%)[3)]	RSD_r[5)] (%)	$s_{I(T)}$[6)] (%)[3)]	$RSD_{I(T)}$[7)] (%)
Fish cakes powder	5	9.08	0.06	0.6	0.09	1.0
Compost	5	0.0973	0.0019	2.0	0.0037	3.8

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviatio

References

1) Kimie KATO, Masaki CHIDA and Toshifumi FUJITA: Method Validation for Determination of Sodium in Fertilizer by Atomic Absorption Spectrometry , Research Report of Fertilizers, **Vol. 8**, p. 61 - 69 (2015)

(5) **Flow sheet for sodium testing method**: The flow sheet for sodium testing method in fertilizers is shown below:

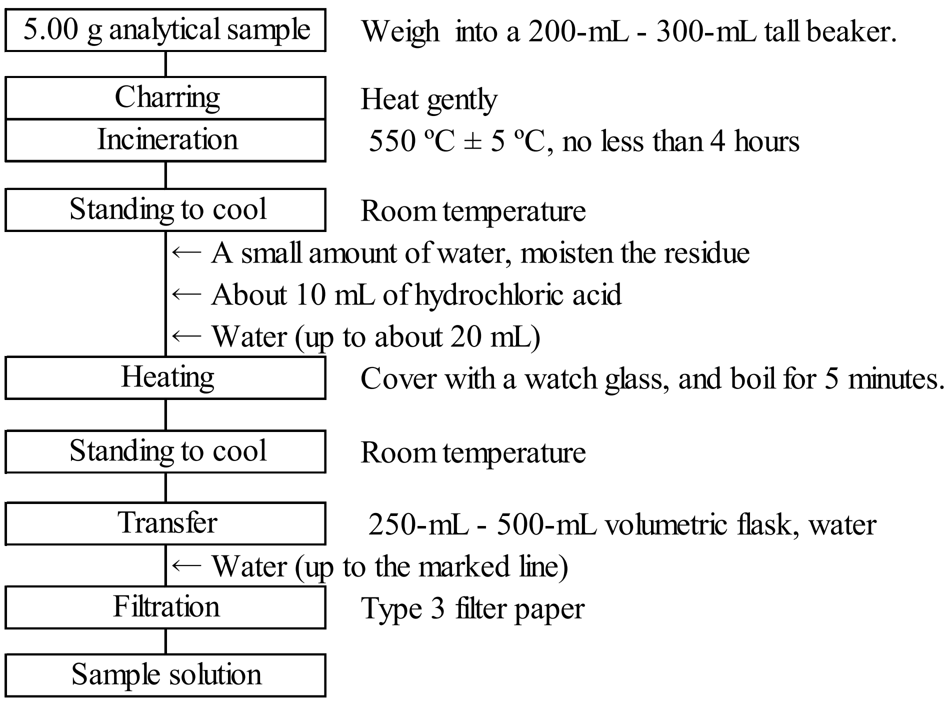


Figure 1 Flow sheet for sodium in fertilizers (Extraction procedure)

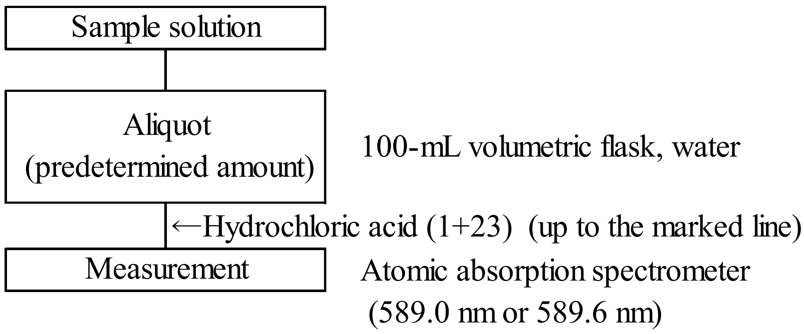


Figure 2 Flow sheet for sodium in fertilizers (Measurement procedure)

8.5 Guanylurea nitrogen
8.5.a High-Performance Liquid Chromatography
(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type B and its symbol is 8.5.a-2017 or GU-N.a-1.

Add water to an analytical sample to extract guanylurea, introduce it to a High-Performance Liquid Chromatograph (HPLC) to isolate it with a weak acid ion-exchange column, and then measure at a wavelength 190 nm to obtain guanylurea nitrogen (GU-N) in an analytical sample. In addition, the performance of this testing method is shown in **Comment 6**.

Biuret nitrogen (B-N), dicyandiamide nitrogen (Dd-N), urea nitrogen(U-N) and guanidine nitrogen (Gd-N) can be simultaneously quantified by using this method. (Refer to **Comment 5**).

(2) **Reagent, etc.**: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557.

b) **Potassium dihydrogen phosphate**: A JIS Guaranteed Reagent specified in JIS K 9007 or a reagent of equivalent quality.

c) **Phosphoric acid**: A JIS Guaranteed Reagent specified in JIS K 9005 or a reagent of equivalent quality.

d) **Guanylurea nitrogen standard solution (GU-N 2 mg/mL)** (1): Put 0.540 g of guanylurea sulfate [$C_4H_{12}N_8O_2 \cdot H_2SO_4$] (2) in a weighing dish and measure the mass to the order of 0.1 mg. Add a small amount of water to dissolve, then transfer to a 100-mL volumetric flask and add water up to the marked line.

e) **Guanylurea nitrogen standard solution (GU-N 200 µg/mL)**: Put 10 mL of guanylurea nitrogen standard solution (GU-N 2 mg/mL) in a 100-mL volumetric flask and add water up to the marked line.

f) **Guanylurea nitrogen standard solution (GU-N 50 µg/mL - 100 µg/mL)**: Put 25 mL - 50 mL of guanylurea nitrogen standard solution (GU-N 200 µg/mL) in 100-mL volumetric flasks and add water up to the marked line.

g) **Guanylurea nitrogen standard solution for the calibration curve preparation (GU-N 1 µg/mL - 50 µg/mL)**: At the time of usage, put 1 mL - 50 mL of guanylurea nitrogen standard solution (GU-N 100 µg/mL) in 100-mL volumetric flasks step-by-step and add water up to the marked line.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.

(**2**) A reagent of no less than 98 % (mass fraction) in purity as guanylurea sulfate is commercially sold.

Comment 1 Guanylurea sulfate is commercially sold by Kanto Chemical Co., Inc. and Tokyo Chemical Industry Co., Ltd.

(3) **Apparatus and instruments**: Apparatus and instruments are shown below.

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 7.5-mm inner diameter 100-mm long stainless steel column tube filled with weak acid ion-exchange resin.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 190 nm.

b) **Magnetic stirrer**

c) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000

× *g*.

Comment 2 A column is sold under the production name Asahipak ES-502C 7C, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

(4.1.1) Powdery test sample

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.

b) Add 100 mL of water and stir it by using a magnetic stirrer for about 10 minutes.

c) After allowing to stand still, transfer a supernatant solution (3) to a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (5) and use the supernatant as a sample solution.

Note (**3**) If there is a possibility that the guanylurea nitrogen (GU-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of supernatant with water.

(**4**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement.

(**5**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

(4.1.2) Fluid test sample

a) Weigh 1.00 g of an analytical sample, and put it in a 100-mL volumetric flask.

b) Add about 50 mL of water, and shake to mix.

c) Add water up to the marked line (6) and pit it in a 1.5-mL ground-in stopper centrifugal precipitate tube (4).

d) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes (5) and use the supernatant as a sample solution.

Note (**6**) If there is a possibility that the guanylurea nitrogen (GU-N) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of precisely adjusted solution with water.

Comment 3 Instead of procedures in (**4.1.1**) **c)** - **d)** or (**4.1.2**) **c)** - **d)**, it is allowed to filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of a High-Performance Liquid Chromatograph (HPLC) used in measurement.

a) Measurement conditions for a High-Performance Liquid Chromatograph (HPLC): An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) **Column**: A weak acid ion-exchange resin column (4.0-mm - 7.5-mm inner diameter, 100-mm - 150-mm long, 5-µm – 10-µm particle diameter)

2) **Column bath temperature**: 40 °C

3) **Eluent** (1): Dissolve 3.92 g of potassium dihydrogen phosphate and 0.12 g of phosphoric acid in water to make 1000 mL. Filter with a membrane filter (aperture diameter: no more than 0.5-

μm) made of hydrophilic PTFE.

4) **Flow rate**: 0.6 mL/min
5) **Injection volume**: 10 μL
6) **Detection unit**: An absorptiometric detector, a measurement wavelength: 190 nm

Comment 4 Eluent can be prepared as follows. Dissolve 19.6 g of potassium dihydrogen phosphate and 0.584 g of phosphoric acid with water to make 500 mL and store in a refrigerator. At the time of usage, dilute a predetermined volume of the solution by a factor of 10 and filter with a membrane filter (aperture diameter: no more than 0.5-μm) made of hydrophilic PTFE.

b) Calibration curve preparation

1) Inject 10 μL of respective standard solutions for the calibration curve preparation to the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 190 nm and obtain a peak height.
2) Prepare a curve for the relationship between the guanylurea nitrogen (GU-N) concentration and the peak height at a wavelength 190 nm of the respective standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 μL of the sample solution to the same procedure as in **b**) **1**)
2) Obtain the guanylurea nitrogen (GU-N) content from the peak height using the calibration curve to calculate guanylurea nitrogen (GU-N) in the analytical sample.

Comment 5 This testing method enables the simultaneous measurement of biuret nitrogen (B-N), urea nitrogen (U-N), dicyandiamide nitrogen (Dd-N), guanidine urea (Gd-N) and guanylurea nitrogen (GU-N). In that case, see **5.10.a Comment 5**.

Comment 6 Additive recovery testing was conducted using a preparation sample for a guanylurea fertilizer (one brand). As a result, the mean recovery rates at the additive level of 36.7 % (mass fraction), 35.2 % (mass fraction) and 33.4 % (mass fraction) were 103.8 %, 104.6 % and 105.6 % respectively.

The results of the repeatability tests on different days using a guanylurea fertilizer to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability. Additionally, results from a collaborative study for testing method validation and its analysis are shown in Table 2.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.006 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of guanylurea nitrogen

Name of sample	Test days of repeatability (T) 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	$s_{I(T)}$ 6) (%) 3)	$RSD_{I(T)}$ 7) (%)
Guanylurea nitrogen fertilzier	5	37.0	0.3	0.7	0.3	0.8

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

Table 2 Analysis results of the collaborative study for the test method validation of guanylurea nitrogen

Sample name	Number of laboratories 1)	Mean 2) (%) 3)	s_r 4) (%) 3)	RSD_r 5) (%)	s_R 6) (%) 3)	RSD_R 7) (%)
Compound fertilizer 1	12	2.20	0.09	4.2	0.17	7.7
Compound fertilizer 2	11	4.38	0.07	1.5	0.19	4.3
Compound fertilizer 3	11	5.83	0.08	1.4	0.52	8.9
Compound fertilizer 4	12	7.43	0.43	5.7	0.78	10.5
Guanylurea fertilizer	12	30.3	0.4	1.5	1.1	3.6

1) Number of laboratories used in analysis
2) Mean (n = number of laboratories × number of samples (2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Reproducibility standard deviation
7) Reproducibility relative standard deviation

References

1) Masahiro ECHI, Yasuharu KIMURA and Yuji SHIRAI: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography: A Single-Laboratory Validation, Research Report of Fertilizer, **Vol. 10**, p. 72 - 85 (2017)

2) Norio FUNAKI and Yasuharu KIMURA: Determination of Urea Nitrogen, Biuret Nitrogen, Dicyandiamide Nitrogen, Guanidine Nitrogen and Guanyl urea Nitrogen in Fertilizer by High-Performance Liquid Chromatography (HPLC): A Collaborative Study, Research Report of Fertilizer, **Vol. 10**, p. 86 - 100 (2017)

(5) **Flow sheet for testing method**: The flow sheet for guanylurea nitrogen in fertilizers is shown below:

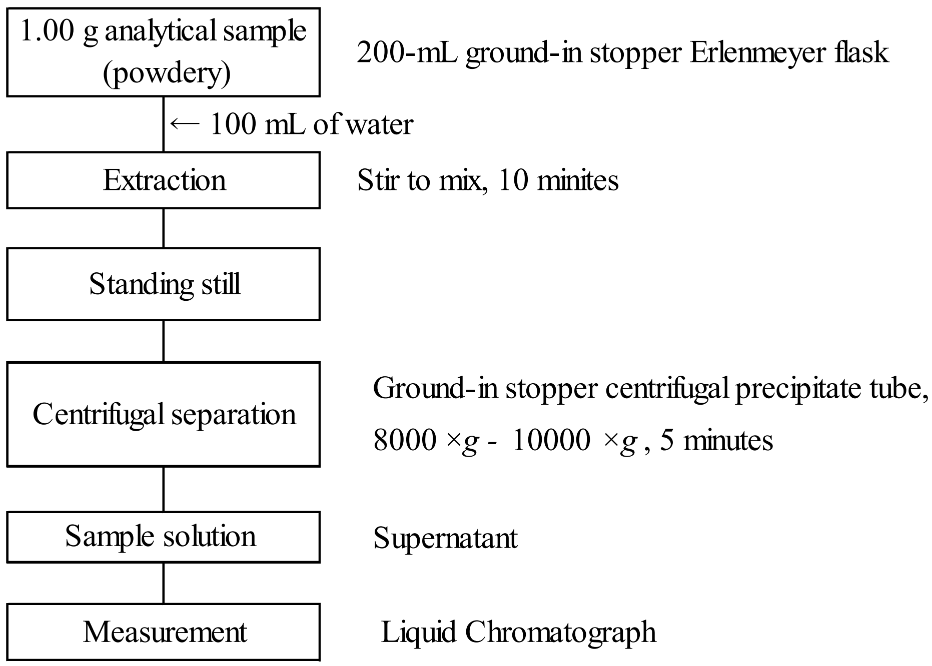


Figure 1 Flow sheet for guanylurea nitrogen in fertilizers
(Extraction procedure (4.1.1) and mesurement)

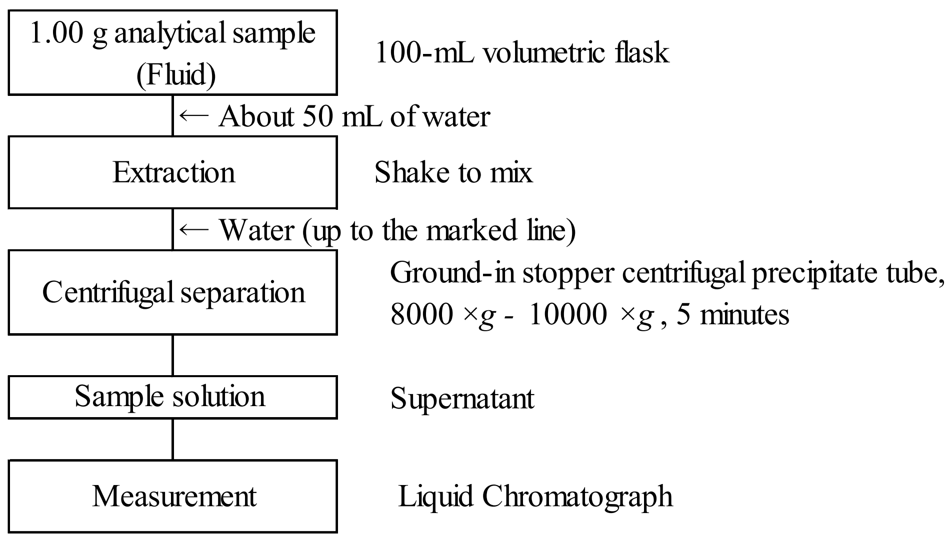
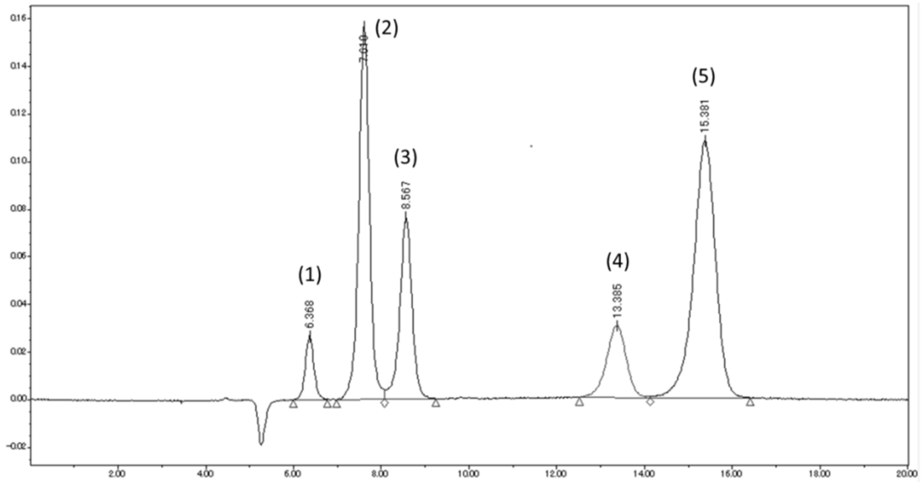


Figure 2 Flow sheet for guanylurea nitrogen in fertilizers
(Extraction procedure (4.1.2) and mesurement)

Reference: An example of the chromatogram of the standard solution for calibration curve preparation of guanylurea nitrogen is shown below.



Reference diagram: HPLC chromatogram of the mixture standard solutions for calibration curb preparation (10 mg/L for each)

Peak name

(1) Urea nitrogen (2) Biuret nitrogen (3) Dicyandiamide nitrogen

(4) Guanidine nitrogen (5) Guanylurea nitrogen

Measurement conditions for HPLC

Column: Asahipak ES-502C 7C (7.5-mm inner diameter, 100-mm long, 9-μm particle diameter)

Other conditions are according to the example of HPLC measurement conditions in (**4.2**) **a**)

8.6 Uric acid

8.6.a High-Performance Liquid Chromatography

(1) Summary

This testing method is applicable to fertilizers. This testing method is classified as Type D and its symbol is 8.6.a-2018 or U-acid.a-1.

Add phosphate solution (pH 8) to an analytical sample to extract uric acid, introduce it to a High-Performance Liquid Chromatograph (HPLC) to isolate it with a multi-mode ODS (reverse phase + strong anion-exchange + strong cation-exchange + normal phase) column, and then measure at a wavelength 290 nm to obtain uric acid (U-acid) in an analytical sample. The performance of this testing method is shown in **Comment 5.**

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A3 specified in JIS K 0557. Note that water of A4 should be used as the eluent which is introduced to a High-Performance Liquid Chromatograph.

b) **Potassium dihydrogenphosphate**: A reagent specified in JIS K 9007 or a reagent of equivalent quality.

c) **Disodium hydrogenphosphate:** A reagent specified in JIS K 9020 or a reagent of equivalent quality.

d) **Phosphate solution**: Dissolve 9.073 g of potassium dihydrogenphosphate in water to make 1000 mL and dissolve 9.464 g of disodium hydrogenphosphate in water to make 1000 mL. Mix these solutions so that pH 8.0 ± 0.1 is reached.

e) **Lithium carbonate solution**: Dissolve 0.739 g of lithium carbonate (Li_2CO_3) of no less than 99 % (mass fraction) in purity in water to make 1000 mL.

f) **Uric acid standard solution (U-acid 1 mg/mL)** (1): Put 0.100 g of uric acid in a weighing dish and measure the mass to the order of 0.1 mg. Dissolve it with a small amount of lithium carbonate solution, transfer to a 100 mL volumetric flask and add the solution up to the marked line.

g) **Uric acid standard solution for the calibration curve preparation (U-acid 100 µg/mL)**: Put 10 mL of uric acid standard solution (U-acid 1 mg/mL) in a 100-mL volumetric flask and add the phosphate solution up to the marked line.

h) **Uric acid standard solution for the calibration curve preparation (U-acid 10 µg/mL - 50 µg/mL)**: Put 10 mL - 50 mL of uric acid standard solution (U-acid 100 µg/mL) in a 100-mL volumetric flask and add the phosphate solution up to the marked line.

i) **Uric acid standard solution for the calibration curve preparation (U-acid 0.1 µg/mL - 5 µg/mL)**: At the time of usage, put 1 mL - 50 mL of uric acid standard solution (U-acid 10 µg/mL) in 100-mL volumetric flasks step-by-step and add the phosphate solution up to the marked line.

j) **Ammonium acetate**: A reagent specified in JIS K 8359 or a reagent of equivalent quality.

k) **Methanol**: A reagent of High-Performance Liquid Chromatograph analysis grade or a reagent of equivalent quality.

Note (1) This is an example of preparation; prepare an amount as appropriate.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) **High-Performance Liquid Chromatograph**: A high-performance liquid chromatograph specified in JIS K 0124 that satisfies the following requirements.

1) **Column**: A 4.6-mm inner diameter 250-mm long stainless steel column tube filled with silica gel, to which octadecyl, ion-exchange group for strong acidity and strong basic anion-exchange group of a 3-µm particle diameter chemically bond.

2) **Column bath**: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

3) **Detection unit**: An absorptiometric detector that can measure at a wavelength around 290 nm.

b) **Water bath**: Water bath that can be adjusted to 60 ºC ± 2 ºC.

c) **Magnetic stirrer**

d) **Centrifugal separator**: A centrifugal separator that can work at 1700 × *g*.

e) **High speed centrifugal separator**: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

Comment 1 A column is sold under the production name Scherzo SS-C18, etc.

(4) Test procedures

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 1.00 g of an analytical sample, and put it into a 200 mL- ground-in stopper Erlenmeyer flask.

b) Add 100 mL of phosphate solution (2) and heat for 30 minutes while shaking to mix (3) at every 10 minutes in a water bath at 60 ºC ± 2 ºC.

c) Immediately stir it by using a magnetic stirrer for about 10 minutes.

d) After allowing to stand still, put supernatant in a 15-mL or 50-mL ground-in stopper centrifugal precipitate tube (4), and centrifuge at 1700 × *g* for about 5 minutes (5).

e) Put the supernatant (6) in a 1.5-mL ground-in stopper centrifugal precipitate tube (4), and centrifuge at 8000 × *g* - 10000 × *g* for about 5 minutes (7) to make supernatant as a sample solution.

Note (**2**) Use a silicone stopper instead of a glass stopper as the solution is heated.

(**3**) Steam easily expels silicon stoppers, so while lightly holding down the stopper from the top with your finger, shake it so that the water drops on the inside of the flask come down as much as possible. In addition, conduct this procedure before and after the heating procedure.

(**4**) The ground-in stopper centrifugal precipitate tube should be made of polypropylene, etc. to not affect the measurement

(**5**) 7.2 cm - 18.9-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**6**) If there is a possibility that the uric acid (U-acid) concentration in the sample solution will exceed the maximum limit of the calibration curve, dilute a predetermined amount of supernatant with phosphate solution.

(**7**) 7.2-cm - 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

Comment 2 Instead of the procedures in (**4.1**) **e**), it is allowed to filter with a membrane filter (pore size: no more than 0.5-µm) made of hydrophilic PTFE and the filtrate can be the sample solution.

(**4.2**) **Measurement**: Conduct the measurement as indicated in JIS K 0124 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph used in measurement.

a) **Measurement conditions for the High-Performance Liquid Chromatograph**: An example of measurement conditions is shown below. Set up the measurement conditions considering it:

1) **Column**: A silica gel column (4.6-mm inner diameter, 250-mm long, 3-µm particle diameter), to which octadecyl, ion-exchange group for strong acidity and strong basic anion-exchange group chemically bond

2) **Column bath temperature**: 40 °C
3) **Eluent** (1): Dissolve 1.54 g of ammonium acetate with water to make 1000 mL. Get 900 mL of the solution to mix with 100 mL of methanol. Filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.
4) **Flow rate**: 0.4 mL/min
5) **Injection volume**: 10 µL
6) **Detection unit**: An absorptiometric detector, a measurement wavelength: 290 nm

Comment 3 Eluent can be prepared as follows. Dissolve 15.4 g of ammonium acetate with water to make 1000 mL and store in a refrigerator. At the time of usage, dilute a predetermined volume of the solution by a factor of 10 to mix with methanol of volume ratio 1/9 and filter with a membrane filter (aperture diameter: no more than 0.5-µm) made of hydrophilic PTFE.

b) Calibration curve preparation

1) Inject 10 µL of respective standard solutions for calibration curve preparation into the High-Performance Liquid Chromatograph, record a chromatogram at a wavelength 290 nm and obtain a peak area or height.
2) Prepare a curve for the relationship between the uric acid (U-acid) concentration and the peak area or height at a wavelength 290 nm of respective standard solutions for the calibration curve preparation.

c) Sample measurement

1) Subject 10 µL of the sample solution to the same procedure as in **b**) **1**)
2) Obtain the uric acid (U-A) content from the peak area or height using the calibration curve to calculate the uric acid (U-A) in the analytical sample.

Comment 4 In addition to uric acid, allantoin and allantoic acid can be measured simultaneously by this measurement method (when using a Scherzo SS-C18 column). Note that the detection wavelength of allantoin and allantoic acid is 210 nm.

Comment 5 Additive recovery testing was conducted using one brand of a compound fertilizer, a composted sludge fertilizer, a mixed compost compound fertilizer and compost. As a result, the mean recovery rates at the additive level of 0.1 % (mass fraction), 0.01 % (mass fraction) and 0.005 % (mass fraction) were 92.4 % - 101.8 %, 85.3 % - 105.0 % and 92.5 % - 114.1 % respectively.

The results of the repeatability tests on different days using one brand of a compound fertilizer, a composted sludge fertilizer, a mixed compost compound fertilizer and compost to evaluate precision were analyzed by one-way analysis of variance. Table 1 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to be about 0.0008 % (mass fraction).

Table 1 Analysis results of the repeatability tests on different days of uric acid

Name of sample	Test days of repeatability (T)[1]	Mean[2] (%)[3]	s_r[4] (%)[3]	RSD_r[5] (%)	$s_{I(T)}$[6] (%)[3]	$RSD_{I(T)}$[7] (%)
Compound fertilzier	7	0.0989	0.0006	0.6	0.0015	1.6
	7	0.0102	0.0001	0.7	0.00042	4.2
Composted sludge fertilizer	7	0.0932	0.0004	0.5	0.0016	1.7
	7	0.00938	0.00009	0.9	0.00031	3.3
Mixed compost compound fertilizer	7	0.0924	0.0004	0.4	0.0015	1.7
	7	0.00921	0.00005	0.6	0.00032	3.5
Compost	7	0.101	0.001	1.3	0.0029	2.8
	7	0.00966	0.00018	1.8	0.00049	5.0

1) The number of test days conducting a duplicate test
2) Mean (the number of test days(T) ×the number of duplicate testing(2))
3) Mass fraction
4) Repeatability standard deviation
5) Repeatability relative standard deviation
6) Intermediate standard deviation
7) Intermediate relative standard deviation

References

1) Norio FUNAKI: Determination of Uric Acid in Fertilizer by High Performance Liquid Chromatography, Research Report of Fertilizer, **Vol. 11**, p. 86 – 105 (2018)

(5) Flow sheet for testing method: The flow sheet for uric acid in fertilizers is shown below:

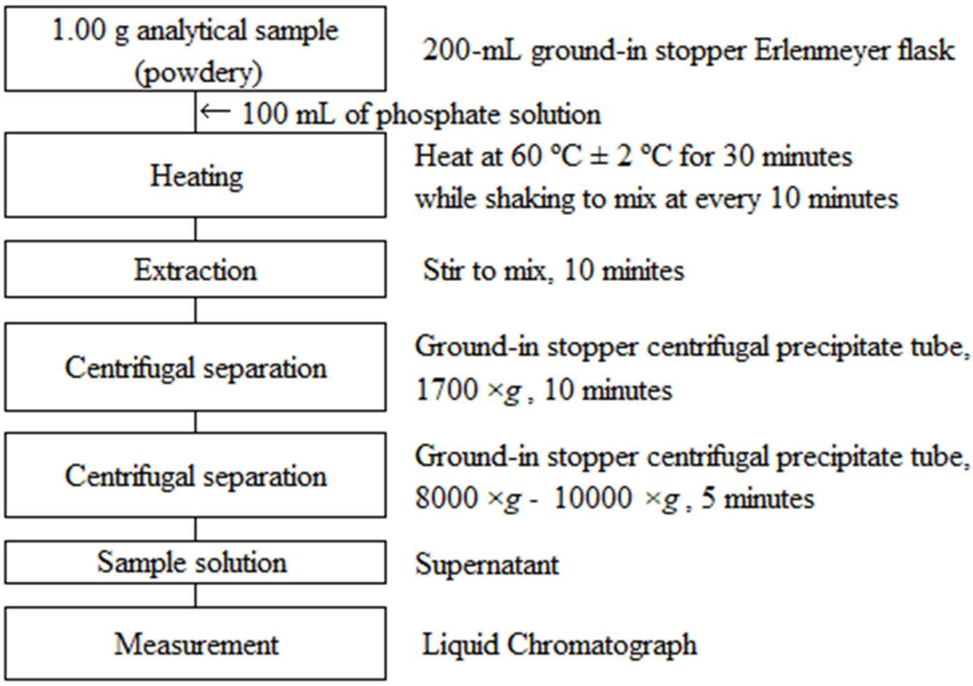
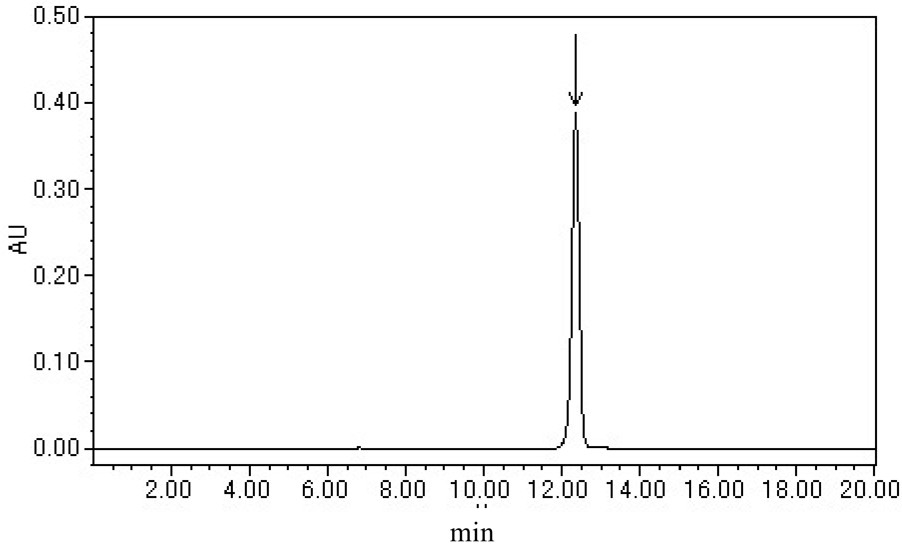


Figure Flow sheet for uric acid in fertilizers

Reference: An example of the chromatogram of the standard solution for calibration curve preparation of uric acidis shown below.



Reference diagram: HPLC chromatogram of the uric acid standard solution for calibration curve preparation (50 µg/mL)

Peak name (↓) Uric acid

Measurement conditions for HPLC

Column: Scherzo SS-C18 (4.6-mm inner diameter, 250-mm long, 3-µm particle diameter)

Other conditions are according to the example of HPLC measurement conditions in **(4.2) a**)

8.7 Organofluorine compounds
8.7.a High-Performance Liquid Chromatography/Tandem Mass Spectrometry
(1) Summary

This testing method is applicable to compost and composted sludge fertilizers. This testing method is classified as Type D and its symbol is 8.7.a-2021 or PFC.a-1.

Extract organofluorine compounds (perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA)) in sludge fertilizers with methanol under acidic condition, refine using two kinds of cleanup cartridges, and then measure using a High-Performance Liquid Chromatograph/Tandem Mass Spectrometer (LC-MS/MS) to obtain PFOS and PFOA in the analytical sample. Note that the performance of this testing method is shown in **Comment 14**.

Comment 1 Structural formulas of PFOS and PFOA are as shown in Figure 1-1 and Figure 1-2.

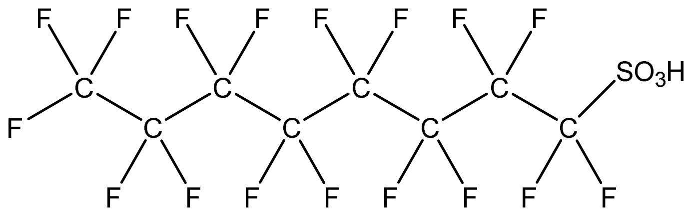


Figure 1-1 Structural formula of PFOS

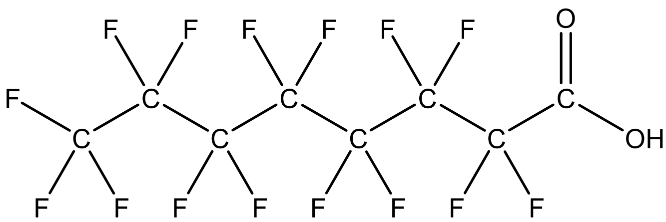


Figure 1-2 Structural formula of PFOA

(2) Reagent, etc.: Reagents and water are as shown below.

a) **Water**: Water of A4 specified in JIS K 0557.

b) **Acetonitrile**: A reagent of LC-MS analysis grade or equivalents.

c) **Methanol**: A reagent of agricultural chemicals residue/PCB testing grade (concentration: no less than 300) or a reagent of equivalent quality.

f) **Ammonia solution**: A JIS Guaranteed Reagent of 28 % (mass fraction) specified in JIS K 8085 or a reagent of equivalent quality.

e) **Formic acid**: A JIS Guaranteed Reagent of no less than 98 % (mass fraction) specified in JIS K 8264 or a reagent of equivalent quality.

f) **Ammonium acetate solution (1 mol/L)**: A regent of High-Performance Liquid Chromatograph analysis grade or equivalents.

g) **Ammonium acetate solution (10 mmol/L)** (1): Add water to 10 mL of ammonium acetate solution (1 mol/L) to make 1000 mL.

h) **PFOS standard stock solution**: A standard solution of PFOS [$C_8HF_{17}SO_3$] with known concentration and uncertainty (2).

i) **PFOA standard stock solution**: A standard solution of PFOA [$C_8HF_{15}O_2$] with known concentration and uncertainty (2).

j) **PFOS standard solution (1 µg/mL)** (1): Dilute a predetermined amount of PFOS standard stock solution with methanol to prepare a PFOS standard solution (1 µg/mL).

k) **PFOA standard solution (1 μg/mL)** (1): Dilute a predetermined amount of PFOA standard stock solution with methanol to prepare a PFOA standard solution (1 μg/mL).

l) **Mixed standard solution (100 ng/mL)** (1): Dilute a predetermined amount of PFOS standard solution (1 μg/mL) and PFOA standard solution (1 μg/mL) with methanol - water (1+1) to prepare a mixed standard solution (100 ng/mL).

m) **Mixed standard solution (10 ng/mL)** (1): Dilute a predetermined amount of mixed standard solution (100 ng/mL) with methanol - water (1+1) to prepare a mixed standard solution (10 ng/mL).

n) **Mixed standard solution (1 ng/mL)** (1): Dilute a predetermined amount of mixed standard solution (10 ng/mL) with methanol - water (1+1) to prepare a mixed standard solution (1 ng/mL).

o) **^{13}C-labeled PFOS internal standard stock solution**: A standard solution of $^{13}C_4$-PFOS [$C_8HF_{17}SO_3$] or $^{13}C_8$-PFOS [$C_8HF_{17}SO_3$] with known concentration and uncertainty (2).

p) **^{13}C-labeled PFOA internal standard stock solution**: A standard solution of $^{13}C_4$-PFOA [$C_8HF_{15}O_2$] or $^{13}C_8$-PFOA [$C_8HF_{15}O_2$] with known concentration and uncertainty (2).

q) **^{13}C-labeled PFOS internal standard solution (1 μg/mL)** (1): Dilute a predetermined amount of ^{13}C-labeled PFOS internal standard stock solution with methanol to prepare a PFOS internal standard solution (1 μg/mL).

r) **^{13}C-labeled PFOA internal standard solution (1 μg/mL)** (1): Dilute a predetermined amount of ^{13}C-labeled PFOA internal standard stock solution with methanol to prepare a PFOA internal standard solution (1 μg/mL).

s) **^{13}C-labeled mixed internal standard solution (200 ng/mL)** (1): Dilute a predetermined amount of ^{13}C-labeled PFOS internal standard solution (1 μg/mL) and ^{13}C-labeled PFOA internal standard solution (1 μg/mL) with methanol to prepare a ^{13}C-labeled mixed internal standard solution (200 ng/mL).

t) **^{13}C-labeled mixed internal standard solution (20 ng/mL)** (1): Dilute a predetermined amount of ^{13}C-labeled mixed internal standard solution (200 ng/mL) with methanol - water (1+1) to prepare a ^{13}C-labeled mixed internal standard solution (20 ng/mL).

u) **Mixed standard solutions for calibration curve preparation (0.1 ng/mL - 50 ng/mL)** (1): Put 1 mL - 5 mL of mixed standard solution (100 ng/ mL) in 20-mL volumetric flasks step-by-step, add 1 mL of ^{13}C-labeled mixed internal standard solution (20 ng/mL) to each of them, and add methanol - water (1+1) up to the marked line.
Put 1 mL - 5 mL of mixed standard solution (10 ng/ mL) in 20-mL volumetric flasks step-by-step, add 1 mL of ^{13}C-labeled mixed internal standard solution (20 ng/mL), and add methanol - water (1+1) up to the marked line for each of them.
Put 1 mL - 5 mL of mixed standard solution (1 ng/ mL) in 20-mL volumetric flasks step-by-step, add 1 mL of ^{13}C-labeled mixed internal standard solution (20 ng/mL) to each of them, and add methanol - water (1+1) up to the marked line.

v) **^{13}C-labeled mixed internal standard solution (20 ng/mL) for adding analytical sample** (1): Dilute a predetermined amount of ^{13}C-labeled mixed internal standard solution (200 ng/mL) with methanol to prepare a ^{13}C-labeled mixed internal standard solution (20 ng/mL) for adding analytical sample.

Note (**1**) This is an example of preparation; prepare an amount as appropriate.
(**2**) A standard reagent is commercially sold.

Comment 2 Molecules in a PFOS standard stock solution, PFOA standard stock solution, ^{13}C-labeled PFOS internal standard stock solution and ^{13}C-labeled PFOA internal standard stock solution shall be primarily linear.

Comment 3 A PFOS standard stock solution, PFOA standard stock solution, ^{13}C-labeled PFOS internal standard stock solution and ^{13}C-labeled PFOA internal standard stock solution are sold by Wellington Laboratories Inc., Cambridge Isotope Laboratories, Inc., FUJIFILM Wako Pure Chemical Co., Ltd., and GL Sciences Inc.

Comment 4 A HPLC grade ammonium acetate solution (1 mol/L) is sold by FUJIFILM Wako Pure Chemical Co., Ltd. and Kanto Chemical Co., Inc.

(3) Apparatus and instruments: Apparatus and instruments are shown below.

a) High-Performance Liquid Chromatograph/Mass Spectrometer: A high-performance liquid chromatograph/tandem mass spectrometer specified in JIS K 0136 that satisfies the following requirements.

1) High-Performance Liquid Chromatograph:

(i) Column bath: A column bath whose temperature can be adjusted to 30 ºC - 45 ºC.

(ii) Column: A 2-mm - 3-mm inner diameter 50-mm - 150-mm long 1.6-µm - 3.0-µm particle diameter stainless steel column tube filled with silica gel to which octadecyl chemically bonds. The specification is according to the mass spectrometer specification.

2) Mass Spectrometer:

(i) Ionization method: Electro-Spray Ionization (ESI) method

(ii) Ion detection method: Selected Reaction Monitoring

b) Ultrasonic generator: An ultrasonic washer can be used.

c) Manifold

d) Centrifugal separator: A centrifugal separator that can work at 700 × *g* - 2000 × *g*.

e) High speed centrifugal separator: A centrifugal separator that can work at 8000 × *g* - 10000 × *g*.

f) Test tube mixer: Vortex mixer

g) Weak anion exchange polymer cartridge column: A cartridge column whose syringe barrel (6 mL) is filled with polymer (500 mg) to which weak anion exchange groups bond.

h) Graphite carbon cartridge column: A cartridge column filled with 400 mg of graphite carbon.

i) 50-mL centrifugal precipitate tube with a screw cap: A 50-mL test tube with a screw cap made of polypropylene that can be used in a centrifugal separator.

j) 50-mL test tube with a screw cap: A 50-mL test tube with a screw cap made of polypropylene that has a line marked at 50 mL.

k) 15-mL test tube with a screw cap: A 15-mL test tube with a screw cap made of polypropylene.

l) Graduated test tube: A 7-mL - 10-mLtest tube that has lines marked at 0.5 mL and 1 mL.

m) Vial for standard solution and sample solution: A 0.3-mL - 1-mL vial with a screw cap made of polypropylene.

Comment 5 A column is sold under the production name InertSustain C18, etc.

Comment 6 A weak anion exchange polymer cartridge column is sold under the production names InertSep MA-2 500 mg/6 mL, Oasis WAX 6 cc (500 mg), etc.

Comment 7 A graphite carbon cartridge column is sold under the production names InertSep Slim GC 400 mg, etc.

Comment 8 A test tube with a 50 mL line (ASTM Standard E1272 Class A (± 0.25 mL)) is sold under the production name DigiTUBEs, etc. Since an internal standard solution is added, the solution does not have to be accurately 50 mL in the procedure in (**4.1**) **f**).

Comment 9 The containers, whole pipettes, Pasteur pipettes, and piston pipette tips used in **i**) - **l**) shall be washed with methanol specified in JIS K 8891 and allow the methanol to evaporate.

Comment 10 For containers used in **i**) - **k**) and **m**), screw caps made of polyethylene or polypropyrene shall be used. To prevent contamination, do not use packings of polytetrafluoroethylene resin, etc.

(4) Test procedure

(4.1) Extraction: Conduct extraction as shown below.

a) Weigh 2.00 g of an analytical sample and put it in a 50-mL centrifugal precipitate tube with a screw cap.

b) Put 1 mL of ^{13}C-labeled mixed internal standard solution (20 ng/mL) for adding analytical sample.

c) Add 15 mL of methanol and 0.1 mL of formic acid and conduct ultra-sonication for 20 minutes using an ultrasonic generator.

d) Centrifuge it at about 1700 × *g* centrifugal force for about 5 minutes (3), and add the supernatant to a 50-mL test tube with a screw cap (4).

e) Repeat the procedure in **c**) - **d**) 2 times and add up the supernatants.

f) Add methanol to the 50 mL line to make an extract.

g) As a blank test, conduct the procedures in **b**) - **f**) using another centrifugal precipitate tube with a screw cap to prepare an extract for blank test.

Note (**3**) 16.5-cm of rotor radius and 3000 rpm of revolutions makes about 1700 × *g* centrifugal force.

(**4**) Transfer by decantation.

Comment 11 Grind until it completely passes through a sieve of 500 µm aperture to prepare the test sample.

(4.2) Cleanup (5): Conduct cleanup as shown below:

a) Successively wash the weak anion exchange polymer cartridge column with about 5 mL of ammonia solution (6) - methanol (1+100), about 5 mL of methanol, and then about 5 mL of methanol - water (1+1) in advance.

b) Wash the graphite carbon cartridge column with about 5 mL of methanol in advance.

c) Put 5 mL of the extract in (**4.1**) **f**) in a 15-mL test tube with a screw cap, add 5 mL of water, shake to mix, put it into the weak anion exchange polymer cartridge column and allow the effluent to overflow until the surface of liquid reaches the top of packing materials.

d) Wash the test tube with a screw cap with about 5 mL of methanol - water (1+1), put the washing to the same column and allow the effluent to overflow until the surface of liquid reaches the top of packing materials.

e) Add about 5 mL of methanol twice, and allow the effluent to overflow until the surface of liquid reaches the top of packing materials.

f) Connect (8) the graphite carbon cartridge column washed in b) under the weak anion exchange polymer cartridge column.

g) Add 2 mL of ammonia solution (6) - methanol (1+100), and allow the effluent to overflow until the surface of liquid reaches the top of packing materials.

h) Place a graduated test tube under the cartridge column, add 4 mL of ammonia solution (6) - methanol (1+100) to the cartridge column and allow PFOS and PFOA to elute.

i) Gently spray nitrogen gas onto the eluate to concentrate it to the 0.5 mL line.

j) Add a small amount of water (9), and shake to mix using a test tube mixer (10).

k) Further add water to the 1 mL line, shaking to mix using a test tube mixer, and put it in a 1.5-mL ground-in stopper centrifugal precipitate tube.

l) Centrifuge it at 8000 × *g* - 10000 × *g* centrifugal force for about five minutes [(11)] and use the supernatant as a sample solution.

m) Conduct the procedures in **a**) - **l**) using the extract for blank test in (**4.1**) **g**) in place of the extract in (**4.1**) **f**) in the procedure in **c**) to prepare a blank test solution.

Note (**5**) Use a pressure reducing device or pressurize in the procedure in (**5**) **c**) and **d**) as appropriate.
(**6**) Use an ammonia solution with an ammonia concentration of 28 % (mass fraction).
(**7**) Put methanol into the graphite carbon cartridge column using a reservoir.
(**8**) Remove the reservoir of the graphite carbon cartridge column and directly connect it to the weak anion exchange polymer cartridge column.
(**9**) About 0.4 mL.
(**10**) Dissolve precipitates.
(**11**) 7.2-cm – 8.9-cm of rotor radius and 10000 rpm of revolutions makes about 8100 × *g* - 10000 × *g* centrifugal force.

(**4.3**) **Measurement**: Conduct measurement according to JIS K 0136 and as shown below. Specific measurement procedures are according to the operation method of the High-Performance Liquid Chromatograph/ Mass Spectrometer used in measurement.

a) **The measurement conditions of the High-Performance Liquid Chromatograph/Mass spectrometer**: An example of measurement conditions for the High-Performance Liquid Chromatograph/Mass Spectrometer is shown below. Set up the measurement conditions considering it:

1) **High-Performance Liquid Chromatograph**:
(i) Column: A silica gel column (2-mm - 3-mm inner diameter, 50-mm - 150-mm long, 1.6-µm - 3.0-µm particle diameter column) to which octadecyl chemically bonds.
(ii) Flow rate: 0.2 mL/min～0.5 mL/min
(iii) Eluent: A: Ammonium acetate solution (10 mmol/L) B: Acetonitrile
(iv) Gradient: 0 min (40 %B)→1.5 min (40 %B)→10 min (100 %B)→12 min (100 %B)→12.1 min (40 %B)
(v) Temperature of column bath: 40 °C
(vi) Injection volume: 5 µL

2) **Mass Spectrometer**:
(i) Ionization method: Electro-Spray Ionization (ESI) method
(ii) Mode: Negative
(iii) Monitor ion: Shown in Table 1

Table 1Example monitor ions of materials subjecetd to measurement and internal standard materials

Compound name	Mass-to-charge ratio (*m*/*z*) Precursor ion	Product ion (Determination)	Product ion (Validation)
PFOS	499	80	99
$^{13}C_4$-PFOS	503	80	99
$^{13}C_8$-PFOS	507	80	99
PFOA	413	169	369
$^{13}C_4$-PFOA	417	169	372
$^{13}C_8$-PFOA	421	172	376

Comment 12 If peaks of impurities originating from the mobile phase or the High-Performance Liquid Chromatograph/Mass Spectrometer appear at the retention time of PFOS or PFOA, it is desirable to take a measure to eliminate their influence on the measurement by, for example, attaching a delay column (2-mm - 4.6-mm inner diameter 10-mm - 50-mm long stainless steel column tube filled with silica gel to which octadecyl chemically bonds or with highly pure activated carbon) between the eluent supply pump and the inlet of the solution to be measured to separate them from the peaks of PFOS and PFOA in the test solution. A delay column is sold under the production name Delay Column for PFAS, etc.

b) Calibration curve preparation

1) Inject 5 µL of respective mixed standard solutions for calibration curve preparation into the High-Performance Liquid Chromatograph/Tandem Mass Spectrometer, record the chromatograms of ion (*m*/*z*) for determination and ion (*m*/*z*) for validation of PFOS, PFOA, ^{13}C-labeled PFOS, and ^{13}C-labeled PFOA, and obtain respective peak areas.

2) Calculate the ratio of peak area of ion (*m*/*z*) for determination for PDOS and PFOA and peak area of ion (*m*/*z*) for validation for ^{13}C-labeled PFOS and ^{13}C-labeled PFOA.

3) Calculate the ratio of peak areas of ion (*m*/*z*) and ion (*m*/*z*) for validation for PFOS, PFOA, ^{13}C-labeled PFOS, and ^{13}C-labeled PFOA.

4) Prepare a calibration curve for the relationship between the concentrations of PFOS and PFOA and the peak area ratio derived in **2**).

c) Sample measurement

1) Subject 5 µL of the sample solution in (**4.2**) **l**) to the same procedure as in **b**) **1**) - **3**) (12).

2) Obtain the PFOS and PFOA concentrations in the sample solution from the calibration curve, and calculate the PFOS and PFOA concentrations in the analytical sample.

d) Blank test sample measurement

1) Subject 5 µL of the blank test solution in (**4.2**) **m**) to the same procedure as in **b**) **1**) - **3**) (13).

2) Obtain the PFOS and PFOA concentrations in the blank test solution from the calibration curve, and confirm that they are below the lower limit of the calibration curve concentration range (0.5 ng/mL).

Note (**12**) Confirm that the peak area ratio of ion (*m*/*z*) for determination to ion (*m*/*z*) for validation is within the range of about ± 30 % of the peak area ratio for the standard solution. In addition, the peak area ratio may vary depending on the concentration.

Comment 13 If the PFOS and PFOA concentrations in the blank test solution obtained in **d**) **2**) are not lower than the lower limit of the calibration curve concentration range, investigate the cause, reduce the blank value to the level that does not affect the measurement, prepare a blank test solution again and redo the test.

Comment 14 Additive recovery testing was conducted to evaluate trueness using sewage sludge fertilizer, industrial sludge fertilizer, and composted sludge fertilizer (1 brand each). As a result, the mean recovery rates at the additive levels of 200 µg/kg, 20 µg/kg and 2 µg/kg were 96.5 % - 101.1 %, 93.8 % - 96.5 % and 83.3 % - 102.1 % for PFOS and 100.4 % - 107.3 %, 92.3 % - 96.0 % and 99.7 % - 102.7 % for PFOA, respectively.

The results of the repeatability tests on different days using human waste sludge fertilizer and composted sludge fertilizer were analyzed by one-way analysis of variance. Table 2 shows the calculation results of intermediate precision and repeatability.

Note that the minimum limit of quantification of this testing method was estimated to

be about 0.5 μg/kg for PFOS and PFOA.

Table 2 Analysis results of the repeatability tests on different days of organofluorine compounds

Compound name	Sample name	Test days of repeatability (T)1)	Mean2) (μg/kg)	s_r 3) (μg/kg)	RSD_r 4) (%)	$s_{I(T)}$ 5) (μg/kg)	$RSD_{I(T)}$ 6) (%)
PFOS	Composted sludge fertilizer	5	66.8	2.0	3.0	2.2	3.3
	Human waste sludge fertilizer	5	4.46	0.16	3.6	0.21	4.6
PFOA	Composted sludge fertilizer	5	140	2	1.4	4	2.6
	Human waste sludge fer	5	2.08	0.13	6.3	0.19	9.1

1) The number of test days conducting a duplicate test
2) Mean ([Number of test days (T)] × [Number of duplicate testing (2)])
3) Repeatability standard deviation
4) Repeatability relative standard deviation
5) Intermediate standard deviation
6) Intermediate relative standard deviation

(5) Flow sheet for organofluorine compounds: The flow sheet for organofluorine compounds in sludge fertilizers is shown below:

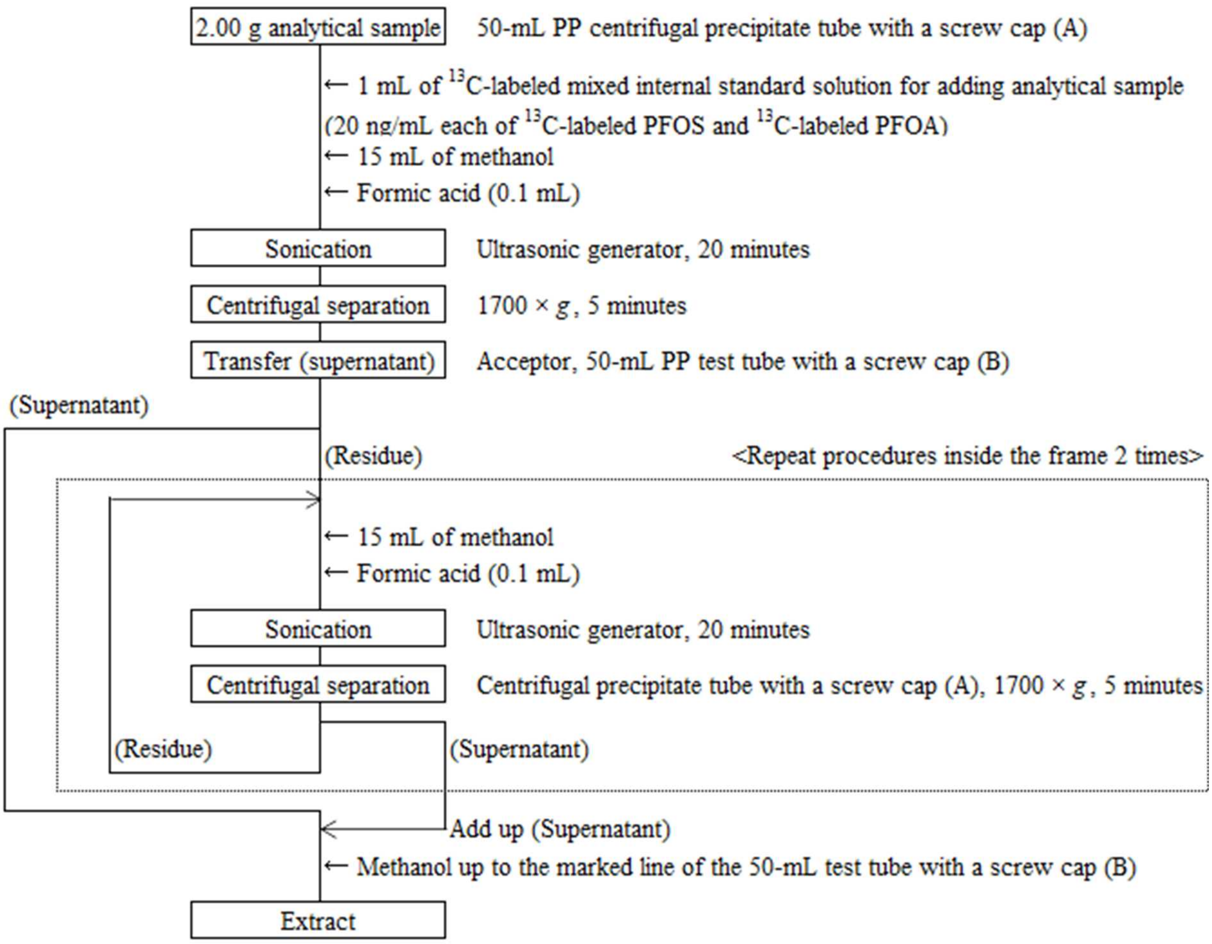


Figure 2-1 Flow sheet for organofluorine compounds in sludge fertilizers (Extraction procedure)

Extract

Aliquot (5 mL) — Acceptor, 15-mL PP test tube with a screw cap (C)

← 5 mL of water

Mixing

Cleanup — Weak anion exchange polymer cartridge column
(Wash with about 5 mL of ammonia solution (28 % (mass fraction))-methanol (1+100), about 5 mL of methanol, and then about 5 mL of water in advance)

← Wash the test tube with a screw cap (C) with 5 mL of methanol-water (1+1)
← Wash with 5 mL of methanol

Connection of cartridge columns — (Connect the graphite carbon cartridge column (wash with about 5 mL of methanol in advance) under the weak anion exchange polymer cartridge column)

← 2 mL of ammonia solution (28 % (mass fraction))─methanol [1+100] [Discard]
← 4 mL of ammonia solution (28 % (mass fraction))─methanol [1+100] [Elute, graduated test tube (D)]

Concentration — Gently spray nitrogen gas, down to the 0.5 mL line

← A small amount (about 0.4 mL) of water

Mixing — Test tube mixer

← Water, up to the 1 mL line

Mixing — Test tube mixer

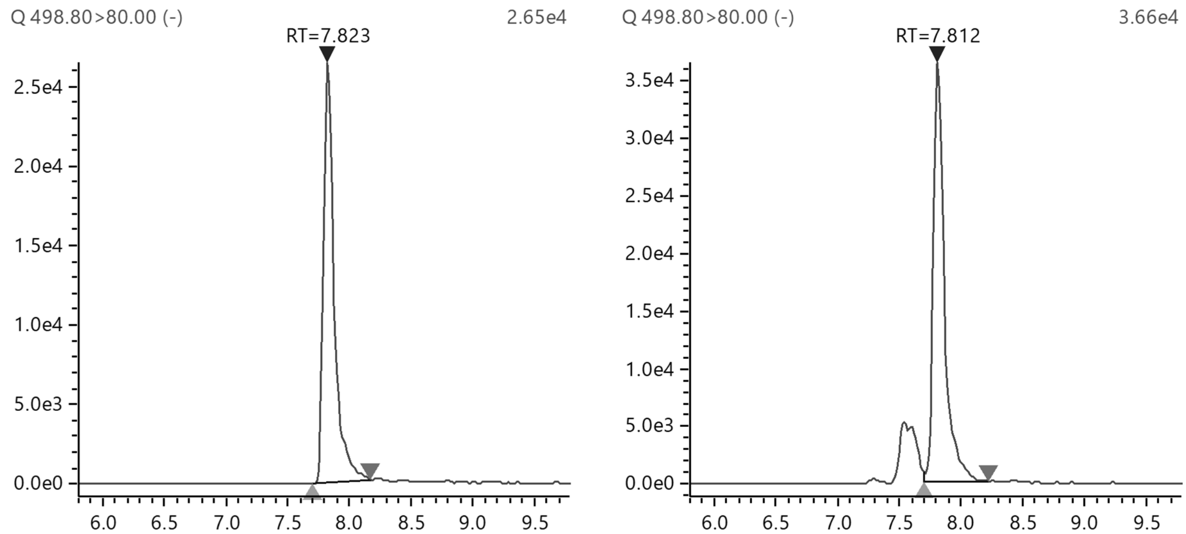
Centrifugal separation — 1.5-mL ground-in stopper centrifugal precipitate tube (E), 8000 × *g* - 10000 × *g*, 5 minutes

Sample solution — Supernatant

Measurement — High-Performance Liquid Chromatograph/Tandem Mass Spectrometer

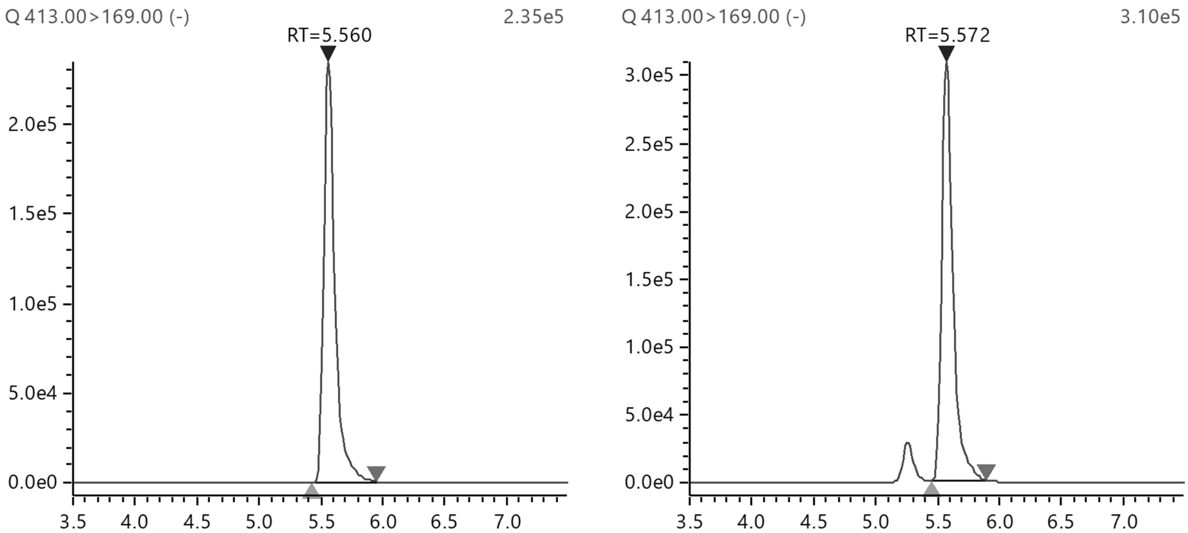
Figure 2-2 Flow sheet for organofluorine compounds in sludge fertilizers (Cleanup and measurement procedures)

References: An example of the Selected Reaction Monitoring chromatograms of product ion for determination of mixed standard solution for calibration curve preparation and sample solution is shown below .



Standard solution (20 ng/mL as PFOS) Sample solution (composted sludge fertilizer)

Reference diagram 1: MRM chromatograms of PFOS



Standard solution (20 ng/mL as PFOA) Sample solution (composted sludge fertilizer)

Reference diagram 2: MRM chromatograms of PFOA

LC-MS/MS measurement conditions

Column for separation: InertSustain C18 (2.1-mm inner diameter, 150-mm long, 3-μm particle diameter)

Delay column: Delay column for PFAS (3-mm inner diameter, 30-mm long)

Flow rate: 0.2 mL/min

Probe voltage: -1 kV

DL temperature: 200 °C

Heat block temperature: 300 °C

Interface temperature: 300 °C

Nebulizing gas rate: 3 L/min

Drying gas rate: 5 L/min
Heating gas rate: 15 L/min
Other conditions are according to the examples of the measurement conditions in (**4.3**) **a**) LC-MS/MS and the reference table.

Reference table Parameters of mass spectrometer after optimization

		Mass-to-charge ratio (m/z)		Collision energy (eV)
		Precursor ion	Product ion	
PFOS	For measurement	498.8	80.0	54.0
	For validation	498.8	98.9	44.0
$^{13}C_4$-PFOS	For measurement	502.8	80.0	52.0
	For validation	502.8	98.9	45.0
$^{13}C_8$-PFOS	For measurement	506.8	80.0	54.0
	For validation	506.8	99.0	46.0
PFOA	For measurement	412.8	169.0	18.0
	For validation	412.8	369.0	10.0
$^{13}C_4$-PFOA	For measurement	416.8	162.0	18.0
	For validation	416.8	372.0	9.0
$^{13}C_8$-PFOA	For measurement	421.1	172.0	19.0
	For validation	421.1	376.0	9.0

Explanation of Testing Methods for Fertilizers (2021)

"The Official Methods of the Analysis of Fertilizers" stipulated by the National Institute for Agro-Environmental Sciences (currently the National Agriculture and Food Research Organization) of the Ministry of Agriculture, Forestry and Fisheries (herein after referred to as MAFF) is adopted for the MAFF Public Notices (Prescribing for the official specifications for normal fertilizers pursuant to the Fertilizer Regulation Act, etc.) which prescribes the quality or the labeling method of fertilizers, and contributes to maintaining the quality of and securing the safety of fertilizers. On February 28, 2020, according to the amendment of these MAFF Public Notices, "Testing Methods for fertilizers" were adopted as the official methods of analysis for effective components, harmful components, etc., as stipulated by the Incorporated Administrative Agency: Food and Agricultural Materials Inspection Center (herein after referred to as "FAMIC")

"Testing Methods for Fertilizers" collected simple testing methods using new analytical instruments and the performance results of testing methods for new ingredients and fertilizers. The contents were edited and made public by FAMIC based on the views of experts and MAFF officers in the technical committee for fertilizers, etc. established at FAMIC.

In the course of editing the "Testing Methods for Fertilizes", attention was paid to the following amendment points to make the description easier to understand: Reagents and instruments used should be clarified by specifying JIS standards, etc., certified standard solutions and titrant have been revised for ease of use and operation flow sheets for respective testing methods have been appended in order for analysts to work easily. In addition, a testing method type specified by the validation level (Citrate-soluble phosphoric acid 4.2.3.a: Ex. Type B) and its symbol indicating the amendment year or revision history (Citrate-soluble phosphoric acid 4.2.3.a: Ex. 4.2.3.a-2018 or C-P.a-2) were described in the "Summary". Also, as for the testing methods whose validation was conducted, the results of trueness and precision were listed in the "Comments".

In the "Testing Methods for Fertilizers (2021)", as a result of the investigation and research issues by FAMIC, (1) testing methods which were newly studied in FY2020 were added, (2) extraction procedures and measurement procedures (including expansion of scope) were added to existing testing methods, (3) the classification of the testing methods carried out in collaborative studies and whose validation was confirmed by multiple laboratories was revised to Type B, and (4) some descriptions were revised according to indications by the technical committee for fertilizers, etc.

In addition, upon revising to the "Testing Methods for Fertilizers (2021)", following suit the example set by the Japanese Industrial Standards (JIS), (5) content that is difficult to be included in descriptions of a testing method due to, for example, a large amount of information was newly arranged as annexes. When an annex provides standards, it is indicated as (Standards). When an annex provides reference information, it is indicated as (Reference).

(1) Newly added testing methods

7.7 3,4-Dimethylpyrazole phosphate (DMPP) 7.7.a High-Performance Liquid Chromatography

8.7 Organofluorine compounds 8.7.a High-Performance Liquid Chromatography/Tandem Mass Spectrometry

(2) Testing methods to which procedures, etc. were added

4.1.2 Ammoniacal nitrogen 4.1.2.a Distillation method

4.1.3 Nitrate nitrogen 4.1.3.c Phenol sulfuric acid method

4.3.1 Total potassium 4.3.1.a Flame atomic absorption spectrometry or flame photometry

4.3.2 Citric acid-soluble potassium 4.3.2.a Flame atomic absorption spectrometry or flame photometry

4.3.3 Water-soluble potassium 4.3.2.a Flame atomic absorption spectrometry or flame photometry

4.6.1 Total magnesia 4.6.1.a Flame atomic absorption spectrometry

4.6.2 Acid-soluble magnesia 4.6.2.a Flame atomic absorption spectrometry

4.6.3 Citric acid-soluble magnesia 4.6.3.a Flame atomic absorption spectrometry

4.6.4 Water-soluble magnesia 4.6.4.a Flame atomic absorption spectrometry

4.7.2 Citric acid-soluble manganese 4.7.2.a Flame atomic absorption spectrometry

4.7.3 Water-soluble manganese 4.7.3.a Flame atomic absorption spectrometry

5.2 Arsenic 5.2.c ICP Mass Spectrometry

5.3 Cadmium 5.3.c ICP Mass Spectrometry

5.4 Nickel 5.4.c ICP Mass Spectrometry

5.5 Chromium 5.5.c ICP Mass Spectrometry

5.6 Lead 5.6.c ICP Mass Spectrometry

(3) Testing methods whose classification was revised to Type B

4.5.3 Citric acid-soluble lime 4.5.3.a Flame atomic absorption spectrometry

4.12.2 Acid-soluble sulfur 4.12.2.a Ion Chromatography

8.2 Clopyralid and its degradation products 8.2.c High-Performance Liquid Chromatography/Tandem Mass Spectrometry (Microanalysis for clopyralid (2))

(4) Revised descriptions

- For extractor, “rotary shaker” was changed to “vertical rotating mixer,” and “constant-temperature rotary shaker” was changed to “constant-temperature vertical rotating mixer.” For the extraction procedure, only “shake to mix” was used as a phrase about shaking to mix (in the previous Japanese versions, multiple expressions meaning “shake to mix” were used).
- Among the measurement conditions of high-performance liquid chromatograph/mass spectrometer used in high-performance liquid chromatograph mass spectrometry and high-performance liquid chromatograph/tandem mass spectrometry, those that depend on the model of mass spectrometer, namely, capillary voltage, temperature of ion source, desolvation temperature, cone temperature, and collision energy, were removed from the

descriptions in the body, and described in the measurement conditions for example chromatograph shown as an example instead.

(5) New establishment of annexes

- New establishment of annexes for Testing Methods for Fertilizers
- Annex A (Standards)   Procedure to validate characteristics of testing methods
- Annex B (Reference)   List of extraction methods for main components, etc.
- Annex C1 (Reference)   Standard solution, etc. preparation methods in simultaneous analysis using ICP optical emission spectrometer
- Annex C2 (Reference)   Standard solution, etc. preparation methods in simultaneous analysis using ICP mass spectrometer
- Annex D (Reference)   Example IC column used for acid-soluble sulfur

Further, information, such as consistency and revised contents between “Testing Methods for Fertilizers (2021)” and “Official Methods of Analysis of Fertilizers (1992)”, was put together in the “Changes in the Testing Methods for Fertilizers (2021) (latest information is shown in red) and performance evaluation of the Testing Methods for Fertilizers (2020)” (Reference material).

These testing methods are utilized for inspection and investigation by FAMIC. FAMIC hopes that the testing methods will help people engaged in the production/quality control and products inspection of fertilizers to ensure the quality of their activities.

We are grateful to the members of the Testing Methods for Fertilizers Technical Committee and the Testing Methods for Fertilizers and Sampling Methods Study Group for their helpful advice and timely guidance regarding technical issues on the occasion of making the “Testing Methods for Fertilizers (2021)”.

Testing Methods for Fertilizers Technical Committee in FY2020

(Without honorifics, as of March 3, 2021)

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Testing Methods for Fertilizers Technical Committee Testing Methods for Fertilizers and Sampling Methods Study Group in FY2021

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