Annexes for Testing Methods for Fertilizers

In revising the Testing Methods for Fertilizers, etc. (2024), contents that are difficult to insert into individual test methods due to the reasons such as a large amount of information were newly created as Annexes in accordance with the example of the Japanese Industrial Standards (JIS). The following descriptions were compiled accordingly.

In addition, when an annex provides standards, it is indicated as (Standards). When an annex provides reference information, it is indicated as (Reference).

Annex A (Standards) Procedure to validate testing methods

The procedures to validate test methods to be included in the Testing Methods for Fertilizers are shown in Annex A (Standards).

Annex B (Reference) List of extraction methods for main components, etc.

An extraction method for main components, etc., is identical with others in some cases. The sample solution extracted by a method may be applied to other components. For this reason, these same extraction methods are explicitly indicated in Annex B (Reference).

Annex C1 (Reference) List of concentration ranges of calibration curve in an ICP optical emission spectrometry
Annex C2 (Reference) List of concentration ranges of calibration curve in an ICP mass spectrometry

The preparation concentrations, etc. of respective components when preparing a mix standard solution in a simultaneous analysis using an ICP optical emission spectrometer or an ICP mass spectrometer are explicitly indicated in Annex C1 (Reference) or Annex C2 (Reference).

Annex D1 (Reference) Example IC column used for acid-soluble sulfur

Even if the functional group is the same, the bonding state of the functional group, degree of cross-linking, etc. varies depending on the model of the column. In order to separate the ion to be measured, measurement conditions suitable for the column to be used (e.g., composition of mobile phase (eluent), gradient) are applied. For that reason, a list of measurement conditions which were validated through collaborative studies, etc. are shown in Annex D1 (Reference).

Annex D2 (Reference) Examples of measurement conditions for the test method of organofluorine compounds

Even if a column has the same functional group with others, the bonding state of the functional group, degree of cross-linking, etc. varies depending on the model of a column. In order to separate the ion to be measured, measurement conditions suitable for the column to be used (e.g., composition of mobile phase (eluent), gradient) are applied. For this reason, the list of measurement conditions that have been validated through collaborative studies, etc. are shown in Annex D2 (Reference).

Annex A
(Standards)

The procedure to validate characteristics of testing methods

(1) Purposes

This article explains the procedure to validate characteristics of testing methods which will be listed in the Testing Methods of Fertilizers. In addition, when testing institutes conduct a test which is not included in the Testing Methods of Fertilizers, a procedure to evaluate the validity of the test method should conform to a method stipulated in this article.

Additionally, this article targets chemical testing methods.

Comment 1 The contents of effective figures (acid-, citric acid- and water-soluble) are stipulated in a notification of the Ministry of Agriculture, Forestry and Fisheries. In addition, the change of extraction conditions such as extraction temperatures may affect observed values even in total content. Therefore, the change of extraction methods (including refining of an extract, etc.) is limited to the case that the change can be compared with the original extraction conditions.

(2) Definition of terminology [1, 2, 3, 4, 5, 6, 7)]

The definition of terminology in this article is as shown below.

a) **Selectivity**: Capability to accurately measure components subjected to analysis under the existence of materials which seem to exist in a sample.

b) **Trueness**: The degree of agreement between the mean obtained from multiple measurement results and the true value [(1)].

c) **Precision**: The degree of agreement (or the degree of variation) among the independent measurement results which are repeatedly measured under the determined conditions.

d) **Repeatability**: The precision of the measurement results of analytical samples, which are regarded to be all identical, obtained under condition (repeatability conditions) that independent measurement results are measured in a short time, using the same method, in the same laboratory, by the same operator and with the same instrument.

e) **Intermediate precision**: The precision of a measurement result of analytical samples, which are regarded to be all identical, obtained under condition (intermediate conditions) that independent measurement results are measured, using the same method, in the same laboratory and in different factors (such as different time and a different operator).

f) **Reproducibility**: The precision of a measurement result of analytical samples, which are regarded to be all identical, obtained under condition (reproducibility conditions) that independent measurement results are measured, using the same method, in different laboratories, by different operators and with different instruments.

g) **Minimum Limit of Quantification (LOQ)**: The quantifiable lowest volume or minimum concentration of a component subjected to analysis which is contained in an analytical sample.

h) **Minimum Limit of Detection (LOD)**: The detectable lowest volume or minimum concentration of a component subjected to analysis which is contained in an analytical sample.

i) **Reference material**: A material which is uniform and stable enough for one or more prescribed properties, and is made suitable for the purpose of use in a measurement process.

j) **Certified reference material**: A reference material, whose values of one or more prescribed properties are characterized by a reasonable metrological procedure, having a certificate of attestation on which the characteristics of prescribed properties and their uncertainty and metrological traceability are stated.

k) **Blank sample**: An analytical sample not containing components subjected to analysis [(2)].

l) **Addition sample**: An analytical sample the content of whose components subjected to analysis is known, or an analytical sample to which reference materials are added [(3) (4)] or compounded [(3)].

m) **Natural contamination sample**: A test sample prepared from fertilizers which naturally contain the components subjected to analysis such as harmful components.

n) **Distribution sample**: An analytical sample prepared from fertilizers [(5)] which are manufactured in a fertilizer production factory, etc.

o) **Surrogate**: A material which is added to an analytical sample in order to conduct a pre-process operation, correct yields in respective steps of measurement procedure and confirm recovery rates, whose chemical structure is identical or similar to a target component.

p) **SN ratio**: Intensity ratio of a signal (response value) S originating from the analysis target and a signal (usually noise) N based on the other factors.

Note (**1**) In reality, the certified value of a certified reference material, the chemical composition of a compound, the added content of a reference material, etc. and others.

(**2**) Reagents, etc. containing a target matrix can be used in the case that there is no distribution fertilizer used as a blank sample for a recovery test and the confirmation of the minimum limit of quantification, etc.

(**3**) Mix a component subjected to analysis with a mortar, etc. to sufficient uniformity

(**4**) In the case of adding a standard solution, vaporize the solvent sufficiently conducting measures such as letting it stand for one night.

(**5**) A fertilizer containing components subjected to analysis whose formation or form changed due to a chemical or physical process (a granulation process, etc.).

(**3**) Validation method

Test necessary items of (3.1) to (3.8) in a planned manner and estimate performance parameters from the obtained results.

Confirm whether the estimated values of performance parameters are suitable to target values (performance norm) respectively, and evaluate that the test method is validated if they are all suitable.

(**3.1**) Scope of application

As a result of a validation test in a single laboratory and a collaborative study, if the result is suitable up to reproducibility, the test method is evaluated as a validated test method as far as the kind of a fertilizer used in the test and the range of concentration are concerned. Therefore, a laboratory where the said test is conducted can use the performance (reproducibility, etc.) as a validated method through implementing internal quality control, etc.

As a result of a validation test in a single laboratory, if the result is suitable to trueness, repeatability and intermediate precision, etc., the test method is evaluated as a validated test method as far as the laboratory where the test was conducted and as far as the kind of a fertilizer used in the test and the range of concentration are concerned. Therefore, another laboratory which wants to introduce the test method is required to carry out the validation anew in an individual laboratory with the above test method.

(**3.2**) Selectivity [8, 9, 10, 11)]

(**3.2.1**)Case of Chromatography

Conduct a procedure for a blank sample and confirm that there is no peak (interference peak) which affects the measurement of components subjected to analysis [(6)]. In addition, in the case of the simultaneous measurement of multi components, confirm that adjacent peaks are sufficiently separated [(6)].

Note (**6**) Resolution (R) should be 1.0 or more at minimum though 1.5 or more is preferable.

Comment 2 Resolution (R) is used as a separation indicator of peaks. If Resolution (R) is 1.5 or more, the adjacent two peaks are sufficiently separated and they do not affect a measurement, whether a peak height or peak area is used. If Resolution (R) is 1.0 or more, there is no problem if peak height is used for a measurement even if the adjacent two peaks overlap to some extent.

Resolution (R) can be obtained using a peak width by the formula (1a). In addition, if the peak is a normal distribution, it can be obtained using a peak width at half height by the formula (1b). With the data processing device of a chromatograph, the formula (1b) is often used to obtain Resolution (R).

$$\text{Resolution } (R)=\frac{t_2-t_1}{\frac{1}{2}\times(W_1+W_2)} \quad \cdots(1a)$$

$$\text{Resolution } (R)=\frac{1.18\times(t_2-t_1)}{\left(W_{\frac{1}{2},1}+W_{\frac{1}{2},2}\right)} \quad \cdots(1b)$$

t_1 : Retention time of Peak 1 t_2 : Retention time of Peak 2

W_1 : Peak width of Peak 1 W_2 : Peak width of Peak 1

$W_{\frac{1}{2},1}$: Peak width at half height of Peak 1

$W_{\frac{1}{2},2}$: Peak width at half height of Peak 2

(3.2.2)Case of a method other than Chromatography [7]

Conduct a procedure for a blank sample and confirm that there is no response which originates from other components than a component subjected to analysis and can be a factor of positive error of a quantification value [8].

Note (**7**) A test method such as Molecular absorption spectrometry, Atomic absorption spectrometry or Titration analysis which does not isolate with a measurement instrument.
(**8**) Absorbance, titer, etc.

(3.3) Calibration curve [8, 12, 13]

Measure respective standard solutions for the calibration curve preparation of the concentration or the content [9] of level 6 to 8 a few times [10] to make a figure plotting the obtained signals [11] as a function of the concentration or the content of a component subjected to analysis and evaluate its linearity visually using the figure.

If linearity is recognized, calculate the inclination (b) and the intercept (a) of a calibration curve, its confidence interval and the coefficient of determination (r^2) using a statistical method such as the calculation of a regression equation by the least square method. Moreover make the plot of residuals [12] in respective levels.

Note (**9**) The blank test solution for the calibration curve preparation can be included.
(**10**) In order to avoid nonlinear confusion due to the variation of sensitivity, etc., conduct measurements randomly for each replicate determination.
(**11**) Absorbance, fluorescence intensity, peak height, peak area, etc.
(**12**) The difference between a signal obtained by measurement and a signal estimated using a regression equation.

Comment 3 It is recommended that the 95% confidence interval of an intercept (a) includes the origin (0).
Comment 4 Though it is usable if the coefficient of determination (r^2) is 0.99 or more, it is recommended that the coefficient of determination (r^2) is 0.999 or more for a precise analysis. If it is less than 0.99, use the equation of a higher order or consider changing the measuring area.
Comment 5 The mean of residuals is 0 and the residuals indicate a random pattern.

(3.4) Trueness [7, 8, 12, 14, 15]

As the estimation method of trueness, the methods are recommended in the following order. (1) Use of a certified

reference material (**3.4.1**), (2) Comparison with an observed value by a validated method (**3.4.2**) and (3) Recovery test (**3.4.3**).

In addition, if a surrogate is used, it is recommended that a recovery rate is about 40% or more.

(3.4.1)Use of a certified reference material

With regard to a component which has matrixes similar to a fertilizer subjected to test and can use a certified reference material containing components subjected to measurement of the concentration in a measurement level, conduct repeatability testing using 3 or more analytical samples (n) according to the test method of the certified reference material. As a result, the absolute value of the difference between the mean observed values and the certified value (characteristic value) must not exceed twice the standard uncertainty combined respective standard uncertainties of the mean observed values and the certified value [(13)].

Note (**13**) The evaluation procedure of the difference between a measurement result and a certified value (characteristic value) is shown in **Reference 1 Procedure to compare an observed value and a certified value**.

(3.4.2)Use of another validated test method

For a component for which a certified reference material is not usable but another validated test method (hereinafter referred to as "a standard test method") is applicable, confirm that the condition **a**) or **b**) is satisfied.

a) **In case 12 or more samples are available**: Conduct respective tests of 12 or more test samples composed of addition samples, natural contamination samples or distribution samples according to a new test method and a standard test method, create the correlation chart of observed values with two methods for each sample and calculate the inclination (b) and the intercept (a) of a regression line, and a correlation coefficient (r). Further confirm a prediction interval.

However, in case that the width between the minimum and the maximum observed value is small, conduct the paired samples t-test to confirm that a significant difference is not observed.

Comment 6 It is recommended that the 95% confidence interval of an inclination (b) includes 1, the 95% confidence interval of an intercept (a) includes the origin (0) and the correlation coefficient (r) is no less than 0.99.

b) **In case fewer samples are available**: With regard to 3 or more test samples of different concentration, conduct respective repeatability addition tests using 4 analytical samples according to the new test method and a standard test method, confirm the homoscedasticity of the results of 2 groups and conduct a t-test for each concentration to confirm that significant difference is not observed under the two-sided significant level of 5%.

(3.4.3) In case neither certified reference material nor other validated test methods are usable

For 3 or more test samples of different concentration, conduct respective repeatability tests using 3 analytical samples and evaluate by obtaining the recovery using the mean of the observed values. The criteria of the trueness are shown in **Separate sheet: The target of trueness and the criteria of precision in respective concentration levels**.

(3.5) Precision [8, 12, 16, 17)]

Evaluate reproducibility and repeatability by a collaborative study (**3.5.1**). Or evaluate an intermediate precision and repeatability by a repeatability test (**3.5.2**).

(3.5.1)Reproducibility and repeatability by a collaborative study

The number of laboratories to obtain effective data should be 8 or more [(15)]. Conduct undisclosed duplicate collaborative studies for 5 or more kinds of samples with different concentration. Obtain reproducibility and repeatability from the observed values [(16)] to evaluate.

Criteria to evaluate these precisions are shown in **Separate sheet: The target of trueness and the criteria of precision in respective concentration levels**.

Note (**14**) In case the number of laboratories which have required facility/instruments is limited, this should be 5 or more.
(**15**) The calculation method is shown in **Reference 2: Calculation of reproducibility or intermediate precision and repeatability**

(3.5.2)Intermediate precision and repeatability by a repeatability test in a single laboratory on different days

Conduct a duplicate test [(17)] per test day for 5 to 7 days using two analytical samples of different concentration which is included in a normal range [(18)]. Obtain intermediate precision and repeatability from the observed values [(19)] to evaluate.

Criteria to evaluate these precisions are shown in **Separate sheet: The target of trueness and the criteria of precision in respective concentration levels**.

Note (**16**) The data of internal quality control can be used.
(**17**) It is not necessary for the same tester to conduct a test through 5 to 7 days.
(**18**) The calculation method is shown in **Reference 2: Calculation of reproducibility or intermediate precision and repeatability**

(3.6) Minimum Limit of Quantification (LOQ) [7, 11)]

Estimate Minimum Limit of Quantification according to (**3.6.1**) to (**3.6.3**). Prepare test samples which include the concentration estimated to be near the Minimum Limit of Quantification step by step as necessary. And conduct respective repeatability tests using 3 analytical samples and define the concentration of a prepared test sample as the Minimum Limit of Quantification, where the mean of the obtained values using the prepared test sample is suitable to the target value of trueness.

Comment 7 In case permissible content and equivalent level is 1.0 mg/kg or more, the Minimum Limit of Quantification (LOQ) of harmful components and restricted components, etc. should be no more than 1/5 of the permissible content and equivalent level. In case permissible content and equivalent level is no more than 1.0 mg/kg, the Minimum Limit of Quantification (LOQ) should be no more than 2/5 of the permissible content. Moreover, it is recommended that the Minimum Limit of Quantification of main components/major components and material components should be no more than 1/5 of minimum volume to be contained and the minimum content of a distribution fertilizer. In addition, in case the Minimum Limit of Quantification exceeds 1/5 of these minimum volumes, conduct the above-mentioned repeatability test, confirm the Minimum Limit of Quantification and state clearly the fact in the applicable range of a test method.

Comment 8 There are some methods to estimate Minimum Limit of Quantification. The methods differ depending on whether they are based on an instrument analysis or not and depending on instruments used. A method different from the methods shown in (**3.6.1**) to (**3.6.3**) is allowed. However, the definition of a method and Minimum Limit of Quantification by the method should be clearly stated.

(3.6.1)Estimation method by a repeatability test

With regard to a test sample with concentration near Minimum Limit of Quantification, conduct a repeatability test using 7 to 10 analytical samples, obtain repeatability standard deviation and estimate Minimum Limit of Quantification (LOQ) in an analytical sample by the formula (3).

$$\text{Estimated value of Minimum Limit of Quantification (LOQ)} = 10 \times s_r \quad \cdots (3)$$

s_r: Repeatability standard deviation

(3.6.2) Estimation method using a calibration curve

In case a calibration curve is linear, estimate Minimum Limit of Quantification (LOQ) in an analytical sample by

the formula (4) using the standard deviation of the residuals of a calibration curve or estimated signals in concentration 0 and the inclination of a calibration curve.

$$\text{Estimated value of Minimum Limit of Quantification (LOQ)} = \frac{10 \times s}{b} \quad \cdots(4)$$

s: The standard deviation of residuals. Or the standard deviation of signasls in concentration 0, which are estimated from a regression line

b: The inclination of a calibration curve

(3.6.3)Estimation method using an SN ratio

In a test method such as Chromatography, etc. which has a baseline noise, calculate from a concentration in an analytical solution whose SN ratio is 10 to 1 at the peak and estimate Minimum Limit of Quantification (LOQ) in an analytical sample.

(3.7) Minimum Limit of Detection (LOD) [7, 11)]

Estimate Minimum Limit of Detection according to (**3.7.1**) to (**3.7.3**).

Comment 9 There are some methods to estimate Minimum Limit of Detection. The methods differ depending on whether they are based on an instrument analysis or not and depending on instruments used. A method different from the methods shown in (**3.7.1**) to (**3.7.3**) is allowed. However, the definition of a method and Minimum Limit of Detection by the method should be clearly stated.

(3.7.1)Estimation method by a repeatability test

With regard to a test sample or a blank sample with concentration near Minimum Limit of Quantification, conduct repeatability tests using 7 to 10 analytical samples, obtain repeatability standard deviation and estimate Minimum Limit of Detection (LOD) in an analytical sample by the formula (5).

$$\text{Estimated value of Minimum Limit of Detection (LOD) in an analytical sample} = 2 \times t(n\text{-}1, 0.05) \times s_r \quad \cdots(5)$$

s_r: Repeatability standard deviation

$t\,(n\text{-}1,0.05)$: The Student value of Significance Level 5% (one side) [(20)]

n: The number of analytical samples in a repeatability test

Note (**19**) In case of a repeatability test using 7 analytical samples, the value is 1.94. In case of using 10 analytical samples, the value is 1.83.

(3.7.2)Estimation method using a calibration curve

In case a calibration curve is linear, estimate Minimum Limit of Detection (LOD) in an analytical sample by the formula (6) using the standard deviation of the residuals of a calibration curve or estimated signals in concentration 0 and the inclination (b) of a calibration curve.

$$\text{Estimated value of Minimum Limit of Detection (LOD)} = \frac{2 \times t(n\text{-}2, 0.05) \times s}{b} \quad \cdots(6)$$

s: The standard deviation of residuals. Or the standard deviation of signals in concentration 0, which are

estimated from a regression line

b: The inclination of a calibration curve

$t(n\text{-}2, 0.05)$: The Student value of Significance Level 5% (one side)

n: The number of a measurement point on a calibration curve

(3.7.3)Estimation method using an SN ratio

In a test method such as Chromatography, etc. which has a baseline noise, calculate from a concentration in an analytical solution whose SN ratio is 3 to 1 at the peak and estimate Minimum Limit of Detection (LOD) in an analytical sample.

(3.8) Robustness [7, 11, 12]

Robustness should be studied when an analysis method is developed, and the estimation method depends on the type of analysis method to be developed. Robustness expresses the reliability of an analysis method when its analysis conditions are intentionally changed. If an observed value tends to be easily affected by the variation of an analysis condition, it is necessary to consider a method to control an analysis condition appropriately or to state the fact as a precaution in a testing method. The evaluation of robustness enables the establishment of a series of parameters such as Resolution related to system conformance. Similarly, the confirmation of these parameters ensures that the validation of an analysis method is maintained in a daily analysis.

Typical variation factors are as follows.

(3.8.1)Common variation factors: Typical variation factors common to various kinds of test methods are as follows.

a) Extraction time, extraction temperature
b) Stability of a test solution in respective steps
c) Reagent's grade

(3.8.2)Variation factors in Chromatography, etc.: Typical variation factors of measurements by Chromatography or refining by solid phase extraction are as follows.

a) Change of a column or a cartridge (A different lot or a different brand)
b) Influence by the variation of pH and composition of an eluent or a wash
c) Temperature
d) Flow rate
e) Influence of a matrix and effect of dilution

References

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Reference 1: Procedure to compare an observed value and a certified value

Obtain the total mean (m) of the replication test results and the certified value (μ), and the absolute value (Δ_m) of the difference of the two values by the formula (R1.1). Next, obtain the standard uncertainty (u_{CRM}) of the certified value of a certified reference material by the formula (R1.2), and obtain the standard uncertainty (u_m) of the total mean by the formula (R1.3). Calculate the combined standard uncertainty ($u_{C(\Delta_m)}$) of Δ_m by the formula (R1.4) using the obtained u_m and u_{CRM}. Further, calculate an expanded uncertainty (U_{Δ_m}) by the formula (R1.5) using the coverage factor ($k = 2$).

Compare Δ_m and U_{Δ_m} to confirm that the criterion (the formula (R1.6)) is satisfied, that is, Δ_m is no more than U_{Δ_m}.

The absolute value (Δ_m) of the difference of the total mean of repeatability test results and a certified value=$|m-\mu|$ ･･･(R1.1)

The standard uncertainty (u_{CRM}) of the certified value=$\frac{U_{95\,\%}}{k_{CRM}}$ ･･･(R1.2)

The standard uncertainty of the measurement of a total mean (u_m)=$\frac{s_r}{\sqrt{n}}$ ･･･(R1.3)

The combined standard uncertainty ($u_{C(\Delta_m)}$) of Δ_m=$\sqrt{u_m{}^2+u_{CRM}{}^2}$ ･･･(R1.4)

The expanded uncertainty (U_{Δ_m}) of Δ_m =$k_{C(\Delta_m)}\times u_{C(\Delta_m)}$=$2\times u_{C(\Delta_m)}$ ･･･(R1.5)

Criterion $\Delta_m \leqq U_{\Delta_m}$ ･･･(R1.6)

m: The total mean of observed values

μ: A certified value

$U_{95\,\%}$: The expanded uncertainty of a certified value

k_{CRM}: The coverage factor of an expanded uncertainty of a standard reference material

s_r: Repeatability standard deviation

n: The number of repeatability test samples

$k_{C(\Delta_m)}$: The coverage factor of an expanded uncertainty of Δ_m ($k_{C(\Delta_m)}$=2)

Reference 2: Calculation of reproducibility or intermediate precision and repeatability

(1) Structure of an observed value

An observed value (x_{ij}) in Table 1, as is shown in the formula (R2.1), consists of a true value (μ), a variation (β) by a factor and a variation (e) by an accidental error under repeatability conditions (hereinafter referred to as "an accidental error"). When p laboratories conduct a collaborative study in which respective laboratories conduct repeatability tests using n samples, the formula (R2.2) is introduced on the assumption that the distribution of β is equivalent to $N(0, \sigma_L^2)$ which depends on a pure between-laboratory variation and the distribution of e is equivalent to $N(0, \sigma_r^2)$ which depends on an accidental error. In addition, when the same laboratory conducts replicate tests for p days using n samples on respective test days, the formula (R2.3) is introduced on the assumption that the distribution of β is equivalent to $N(0, \sigma_{(T)}^2)$ which depends on test days variation (factor T) and the distribution of e is equivalent to $(0, \sigma_r^2)$ which depends on an accidental error.

$$\text{Observed value } (x_{ij}) = \mu + \beta_i + e_{ij} \quad \cdots \quad \text{(R2.1)}$$

$$\text{Observed value } (x_{ij}) = \mu + N(0,\sigma_L^2) + N(0,\sigma_r^2) \quad \cdots \quad \text{(R2.2)}$$

$$\text{Observed value } (x_{ij}) = \mu + N(0,\sigma_{(T)}^2) + N(0,\sigma_r^2) \quad \cdots \quad \text{(R2.3)}$$

μ: True value

β_i: Variation of a factor $\quad$ e_{ij}: Accidental error

$N(0,\sigma_L^2)$: Normal distribution of β_i with the mean 0 and standard deviation σ_{L_i}

$N(0,\sigma_r^2)$: Normal distribution of e_{ij} with the mean 0 and standard deviation σ_r

σ_L^2: Pure between - laboratory variance $\quad$ σ_r^2: Repeatability variance

$N(0, \sigma_{(T)}^2)$: Normal distribution of β_i with the mean 0 and standard deviation $\sigma_{(T)}$

$\sigma_{(T)}^2$: Test days variance

Table 1 Test results of collaborative studies or repeatability tests on different days

Laboratory or test day	Analytical sample number						
(Factor)	1	2	3	•••	j	•••	n
1	x_{11}	x_{12}	x_{13}	•••	x_{1j}	•••	x_{1n}
2	x_{21}	X_{22}	x_{23}	•••	x_{2j}	•••	x_{2n}
3	x_{31}	X_{32}	x_{33}	•••	x_{3j}	•••	x_{3n}
•••	•••	•••	•••	•••	•••	•••	•••
i	x_{i1}	X_{i2}	x_{i3}	•••	x_{ij}	•••	x_{in}
•••	•••	•••	•••	•••	•••	•••	•••
p	x_{p1}	X_{p2}	x_{p3}	•••	x_{pj}	•••	x_{pn}

(2) Calculation procedure of reproducibility and repeatability of the results of a collaborative study

(2.1) Estimation of true value and variance

In an actual statistical analysis, a true value (μ), a true and pure between-laboratory variance (σ_L^2) and a true repeatability variance (σ_r^2) are unknown. Therefore, they are replaced with estimated values obtained from the results of a collaborative study and are expressed as a mean (m), a pure between-laboratory variance (s_L^2) and a repeatability variance (s_r^2) respectively.

(2.2) One-way analysis of variance

Exclude ineffective observed values which have clearly objective reasons such as deviation from a protocol and

malfunction of instruments from the report values by laboratories which participated in a collaborative study. Further exclude outliers by conducting Cochran's test and Grubb's test. And conduct one-way analysis of variance for the remaining results to obtain the unbiased variance (V) of respective variation factors in Table 2.

Table 2 Table of one-way analysis of variance

Variation factor	Sum of squares (S)	Dgree of freedom (φ)	Unbiased variance (V)	Expectation of variance ($E(V)$)
Between-laboratory (L)	SS_L	$p-1$	V_L	$\sigma_r^2+n\times\sigma_L^2$
Accidental error (e)	SS_r	$p\times(n-1)$	V_r	σ_r^2

Comment 1 It is possible to conduct one-way analysis of variance easily using a statistical program or a tool of a spreadsheet program. In this case, it should be noted that different terminologies may be used (Between-laboratory (L)→ Between-group, Accidental error (e) → Within-group, Unbiased variance→ Mean square, etc.).

Comment 2 Unbiased variance (V) is calculated by (Sum of squares)/ (Degree of freedom).

(2.3) The calculation of reproducibility and repeatability

The relation of the expectation of variance E (V) of respective factors in Table 2 holds true. Therefore, calculate repeatability variance (s_r^2) and pure between-laboratory variance (s_L^2) by the formula (R2.4) and (R2.5), and further calculate reproducibility variance (s_R^2) by the formula (R2.6) [(1) (2)].

$$\text{Repeatability variance } (s_r^2) = V_r \quad \cdots \text{ (R2.4)}$$

$$\text{Pure between - laboratory variance } (s_L^2) = \frac{V_L - V_r}{n} \quad \cdots \text{ (R2.5)}$$

$$\text{Reproducibility variance } (s_R^2) = s_L^2 + s_r^2 \quad \cdots \text{ (R2.6)}$$

V_r: The unbiased variance of a variation factor (accidental error (e)) in the table of one − way analysis of variance (Table 2)

V_L: The unbiased variance of a variation factor (between − laboratories (L)) in the table of one − way analysis of variance (Table 2)

Calculate a repeatability standard deviation (s_r) and a reproducibility standard deviation (s_R) by the formula (R2.7) and (R2.8) using the obtained repeatability variance and reproducibility variance, and further calculate a repeatability relative standard deviation (RSD_r) and a reproducibility relative standard deviation (RSD_R) by the formula (R2.9) and (R2.10) [(2) (3)].

$$\text{Repeatability standard deviation } (s_r) = \sqrt{s_r^2} \quad \cdots \text{ (R2.7)}$$

$$\text{Reproducibility standard deviation } (s_R) = \sqrt{s_R^2} \quad \cdots \text{ (R2.8)}$$

$$\text{Repeatability relative standard deviation } (RSD_r, \%) = \frac{s_r}{m}\times 100 \quad \cdots \text{ (R2.9)}$$

$$\text{Reproducibility relative standard deviation } (RSD_R, \%) = \frac{s_R}{m}\times 100 \quad \cdots \text{ (R2.10)}$$

m: the gross mean of the effective data of collaborative study results

Note **(1)** In case $V_L<V_r$, assume $V_L=V_r$ (that is, the pure between-laboratory variance (s_L^2)= 0 in the formula (R2.5)) and let the formula (R2.6) form $s_R^2=s_r^2$.

(**2**) The rounding of a numerical value is not executed in the middle of the calculation.
(**3**) The mean and the standard deviation are expressed rounding to the digit of the observed value. The relative standard deviation is expressed rounding to the first decimal place.

(3) Calculation procedure of intermediate precision and repeatability by the replicate test results on different days

(3.1) Estimation of a true value and a variance

In an actual statistical analysis, a true value (μ), a true test day variance ($\sigma_{(T)}^2$) and a true repeatability variance (σ_r^2) are unknown. Therefore, they are replaced with estimated values obtained from the repeatability test results on different days and are expressed as a mean (m), test days variance ($s_{(T)}^2$) and a repeatability variance (s_r^2) respectively.

(3.2) One-way analysis of variance

Conduct one-way analysis of variance for the replicate test results on different days to obtain the unbiased variance (V) of respective variation factors in Table 3.

Table 3 Table of one-way analysis of variance

Variation factor	Sum of squares (S)	Dgree of freedom (φ)	Unbiased variance (V)	Expectation of variance ($E(V)$)
Test days (T)	SS_T	$p-1$	V_T	$\sigma_e^2+n\times\sigma_{(T)}^2$
Accidental error (e)	SS_e	$p\times(n-1)$	V_e	σ_e^2

Comment 3 It is possible to conduct one-way analysis of variance easily using a statistical program or a tool of a spreadsheet program. In this case, it should be noted that different terminologies may be used (Test days (T) → between-group, Accidental error (e) → within-group, Unbiased variance → Mean square, etc.).

Comment 4 Unbiased variance (V) is calculated by (Sum of squares)/ (Degree of freedom).

(3.3) The calculation of intermediate precision and repeatability

The relation of the expectation of variance E (V) of respective factors in Table 3 holds true. Therefore, calculate repeatability variance (s_r^2) and test days variance ($s_{(T)}^2$) by the formula (R2.11) and (R2.12), and further calculate intermediate variance ($s_{I(T)}^2$) by the formula (R2.13) [2] [4].

$$\text{Repeatability variance } (s_r^2) = V_r \quad \cdots \quad \text{(R2.11)}$$

$$\text{Test days variance } \left(s_{(T)}^2\right) = \frac{V_T - V_r}{n} \quad \cdots \quad \text{(R2.12)}$$

$$\text{Intermediate Variance } \left(s_{I(T)}^2\right) = s_{(T)}^2 + s_r^2 \quad \cdots \quad \text{(R2.13)}$$

V_r: The unbiased variance of a variation factor (accidental error (e)) in the table of one − way analysis of variance (Table 3)

V_T: The unbiased variance of a variation factor (test days (T)) in the table of one − way analysis of variance (Table 3)

Calculate a repeatability standard deviation (s_r) and an intermediate standard deviation ($s_{I(T)}$) by the formula (R2.14) and (R2.15) using the obtained estimated values of repeatability variance and intermediate variance, and further calculate a repeatability relative standard deviation (RSD_r) and an intermediate relative standard deviation ($RSD_{I(T)}$) by the formula (R2.16) and (R2.17) [2] [3].

Repeatability standard deviation $(s_r) = \sqrt{s_r^2}$ ··· (R2.14)

Intermediate standard deviation $\left(s_{I_{(T)}}\right) = \sqrt{s_{I_{(T)}}^2}$ ··· (R2.15)

Repeatability relative standard deviation $(RSD_r, \%) = \frac{s_r}{m} \times 100$ ··· (R2.16)

Intermediate relative standard deviation $\left(RSD_{I_{(T)}}, \%\right) = \frac{s_I}{m} \times 100$ ··· (R2.17)

m: Gross mean of the replicate test results on different days

Note (**4**) In case $V_T < V_r$ assume $V_T = V_r$ (that is, the test days variance $(s_{(T)}^2)$ in the formula (R2.12) = 0) and let the formula (R2.13) form $s_{I(T)}^2 = s_r^2$.

(4) Examples of the calculation of intermediate precision and repeatability by the replicate test results on different days.

An example of repeatability test results on different days of citric acid-soluble phosphoric acid using sample 1 and sample 2 containing phosphite is shown in Table 4. Conduct one-way analysis of variance for the test results of respective samples to obtain the unbiased variance (V) of respective variation factors (Table 5).

Examples of the calculation of intermediate precision and repeatability for the sample 1 and sample 2 using the formula (R2.11) to the formula (R2.17) are shown in Table 6-1 and 6-2. In addition, the results of respective standard deviation are expressed rounding to the digit of the observed value and the results of the respective relative standard deviations are expressed rounding to the first decimal place.

Table 4 Example of repeatability test results on different days (mass fraction (%))

Sample No	Test day (factor) 1	2	3	4	5	6	7	Total mean (m) 1)
Sample 1	51.20	52.15	51.00	51.35	51.35	51.38	51.28	51.38
	51.45	51.85	51.09	51.28	51.10	51.38	51.43	
Sample 2	5.18	4.90	5.01	5.15	5.14	5.13	5.21	5.10
	5.00	5.12	5.06	5.14	5.07	5.11	5.18	

1) The mean is expressed rounding to the digit of the observed value.

Table5 Table of one-way analysis of variance

Sample No	Variation factor	Sum of squares (S)	Dgree of freedom (φ)	Unbiased variance (V)	Expectation of variance $(E(V))$
Sample 1	Test days (T)	1.0570	6	0.17616	$\sigma_e^2 + n \times \sigma_{I(T)}^2$
	Accidental error (e)	0.1253	7	0.01789	σ_e^2
Sample 2	Test days (T)	0.0478	6	0.00797	$\sigma_e^2 + n \times \sigma_{I(T)}^2$
	Accidental error (e)	0.0448	7	0.00640	σ_e^2

Table 6-1 Calculation of intermediate precision and repeatability using the sample 1 replicate test results on different days[1)]

Variation factor	Formula	Calculation	Result
Repeatability variance (s_r^2)	$= V_r$	= 0.01789	0.01789
Repeatability standard deviation (s_r)[2)]	$= \sqrt{s_r^2}$	$= \sqrt{0.01789}$	0.13 (%)[4)]
Repeatability relative standard deviation (RSD_r)[3)]	$= (s_r/m)\times 100$	= (0.1338/51.38)×100	0.3 (%)
Teat days variance ($s_{(T)}^2$)	$= (V_T - V_r)/n$	= (0.17616−0.01789)/2	0.07914
Intermediate variance ($s_{I(T)}^2$)	$= s_T^2 + s_r^2$	= 0.07914+0.01789	0.09703
Intermediate standard deviation ($s_{I(T)}$)[2)]	$= \sqrt{s_{I(T)}^2}$	$= \sqrt{0.09703}$	0.31 (%)[4)]
Intermediate relative standard deviation ($RSD_{I(T)}$)[3)]	$= (s_{I(T)}/m)\times 100$	= (0.3115/51.38)×100	0.6 (%)

1) The rounding of a numerical value is not executed in the middle of the calculation.
2) The standard deviation is expressed rounding to the digit of the observed value.
3) The relative standard deviation is expressed rounding to the first decimal place.
4) Mass fraction

Table 6-2 Calculation of intermediate precision and repeatability using the sample 2 replicate test results on different days[1)]

Variation factor	Formula	Calculation	Result
Repeatability variance (s_r^2)	$= V_r$	= 0.00640	0.00640
Repeatability standard deviation (s_r)[2)]	$= \sqrt{s_r^2}$	$= \sqrt{0.00640}$	0.08 (%)[4)]
Repeatability relative standard deviation (RSD_r)[3)]	$= (s_r/m)\times 100$	= (0.0800/5.10)×100	1.6 (%)
Teat days variance ($s_{(T)}^2$)	$= (V_T - V_r)/n$	= (0.00797−0.00640)/2	0.00078
Intermediate variance ($s_{I(T)}^2$)	$= s_T^2 + s_r^2$	= 0.00078+0.00640	0.00718
Intermediate standard deviation ($s_{I(T)}$)[2)]	$= \sqrt{s_{I(T)}^2}$	$= \sqrt{0.00718}$	0.08 (%)[4)]
Intermediate relative standard deviation ($RSD_{I(T)}$)[3)]	$= (s_{I(T)}/m)\times 100$	= (0.0848/5.10)×100	1.7 (%)

Footnote: Refer to Table 6-1

Separate sheet: The target of trueness and the criteria of precision in respective concentration levels

The target of trueness (recovery rate) and the criteria of precision in respective concentration levels to evaluate test methods of Chromatography (1) as well as other than Chromatography used for the analysis of fertilizers are shown in Table 1 and Table 2. The target of trueness is generally within the recovery rate of Table 1. As for precision, the permissible level may exceed respective relative standard deviations in Table 2 by a factor of 2.0.

Note that the target of trueness and criteria of precision were compiled from the performance evaluation results of analysis methods for fertilizers and discussed and approved by the Technical Committee for Fertilizers, etc. The concentration level setting, target values and permissible ranges are established with reference to the guideline of Codex Alimentarius Commission (CAC), IUPAC protocol and the guideline of AOAC INTERNATIONAL, etc.

Note (**1**) Gas chromatography, Gas Chromatography/Mass Spectrometry, High-Performance Liquid Chromatography, High-Performance Liquid Chromatography/Tandem Mass Spectrometry, Ion Chromatography, etc.

Table 1 The target of trueness in respective concentration levels

Concentration level	Chromatography Recovery rate (%)	Test methods other than Chromatography Recovery rate (%)
≧25 % (mass fraction)	90～108	98～102
≧10 % (mass fraction)	90～108	97～103
≧1 % (mass fraction)	85～110	96～104
≧0.1 % (mass fraction)	85～110	94～106
≧100 mg/kg	80～115	92～108
≧10 mg/kg	70～120	90～110
≧1 mg/kg	70～120	85～115
≧100 µg/kg	70～120	85～115
≧10 µg/kg	70～120	80～120
<10 µg/kg	60～125	75～125

Table 2 Criteria of precision [1)] in respective concentration levels

Concentration level	Chromatography			Test methods other than Chromatography		
	Reproducibility relative standard deviation (%)	Intermediate relative standard deviation (%)	Repeatability relative standard deviation (%)	Reproducibility relative standard deviation (%)	Intermediate relative standard deviation (%)	Repeatability relative standard deviation (%)
≧25 % (mass fraction)	8	6.5	4	2.5	2	1
≧10 % (mass fraction)	8	6.5	4	3	2.5	1.5
≧1 % (mass fraction)	8	6.5	4	4	3.5	2
≧0.1 % (mass fraction)	8	6.5	4	6	4.5	3
≧100 mg/kg	8	6.5	4	8	6.5	4
≧10 mg/kg	11	9	6	11	9	6
≧1 mg/kg	16	13	8	16	13	8
≧100 µg/kg	22	18	11	22	18	11
≧10 µg/kg	22	18	11	22	18	11
<10 µg/kg	22	18	11	22	18	11

1) As for precision, the permissible level may exceed respective relative standard deviations by a factor of 2.0.

Reference

1) ISO/IEC 17025 (2017): “General requirements for the competence of testing and calibration laboratories” (JIS Q 17025: 2018, “General requirements for the competence of testing and calibration laboratories”)
2) Codex Alimentarius Commission: “PROCEDURAL MANUAL, Twentieth edition, (2011) <ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_20e.pdf>
3) AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals, AOAC INTERNATIONAL (2002) <http://www.aoac.org/Official_Methods/slv_guidelines.pdf>
4) Codex: “Guideline on Analytical Terminology”, CAC/GL 72-2009 (2009) <www.codexalimentarius.net/download/standards/11357/cxg_072e.pdf>
5) ISO 13528 (2015): “Statistical methods for use in proficiency testing by interlaboratory comparisons” (JIS Z 8405: 2021, “Statistical methods for use in proficiency testing by interlaboratory comparisons”)
6) ISO Guide 35 (2006): “Reference materials - General and statistical principles for certification” (JIS Q 0035 : 2008, “Reference materials - General and statistical principles for certification”)
7) AOAC OFFICIAL METHODS OF ANALYSIS Appendix E: Laboratory Quality Assurance, AOAC INTERNATIONAL, Gaithersburg (2005)

Annex B
(Reference)

List of extraction methods for main components, etc.

(1) List of extraction methods for main components, etc.

Lists of extraction methods for main components, etc. used in the Testing Methods for Fertilizers are shown in Table 1 to Table 4.

Table 1 List of extraction methods for main components, harmful components (e.g., heavy metals), etc.

Component name	Test method		Extraction method [a]					
			Kjeldahl digestion	Incineration-hydrochloric acid boiling	Incineration-aqua regia digestion	Nitric acid, sulfuric acid, perchloric acid digestion	Microwave headed acid digestion	Other
Total nitrogen	4.1.1.a	Kjaeldahl method	○[b]					
	4.1.1.b	Combustion method						A
	4.1.1.c	Devarda's alloy – Kjeldahl method						B
	4.1.1.d	Reduced iron - Kjeldahl method						C
Total phosphoric acid	4.2.1.a	Ammonium vanadomolybdate absorptiometric analysis	○	○	○			
	4.2.1.b	Quinoline gravimetric analysis	○					
	4.2.1.c	ICP Optical Emission Spectrometry			○			
Total potassium	4.3.1.a	Flame atomic absorption spectrometry or flame photometry		○	○			
	4.3.1.b	Sodium tetraphenylborate gravimetric analysis		○				
	4.3.1.c	ICP Optical Emission Spectrometry			○			
Total lime	4.5.1.a	Flame atomic absorption spectrometry		○	○			
	4.5.1.b	ICP Optical Emission Spectrometry (Internal standard method)			○			
Total magnesia	4.6.1.a	Flame atomic absorption spectrometry		○	○			
	4.6.1.b	ICP Optical Emission Spectrometry (Internal standard method)			○			

a) Respective extraction method
A: Introduction of analytical sample into measuring instrument
B: Reduction by Devarda's alloy – Kjeldahl digestion
C: Reduction by reduced iron – Kjeldahl digestion
D: Dichromate oxidation
E: Hydrochloric acid treatment
F: Extraction with water and sulfuric acid (1+5)
G: Boiling with potassium hydroxide/ethanol solution
H: Nitric acid, perchloric acid digestion
I: Decomposition at microwave decomposition temperature of 240 °C
J: Incinerate, phosphoric acid (1+1) processing with magnesium nitrate added
K: Nitric acid, sulfuric acid, perchloric acid digestion with ammonium sulfate added
L: Ammonium hydrogensulfate melting
b) Filtration is not necessary

Table 1 (cont.)

Component name	Test method		Extraction method [a)]					
			Kjeldahl digestion	Incineration-hydrochloric acid boiling	Incineration-aqua regia digestion	Nitric acid, sulfuric acid, perchloric acid digestion	Microwave headed acid digestion	Other
Total zinc	4.9.1.a	Flame atomic absorption spectrometry		○	○			
	4.9.1.b	ICP Optical Emission Spectrometry			○			
	4.9.1.c	ICP Optical Emission Spectrometry (Internal standard method)			○			
Total copper	4.10.1.a	Flame atomic absorption spectrometry		○	○			
	4.10.1.b	ICP Optical Emission Spectrometry			○			
	4.10.1.c	ICP Optical Emission Spectrometry (Internal standard method)			○			
Organic carbon	4.11.1.a	Dichromate oxidation						D
	4.11.1.b	Combustion method						E
Total sulfur content	4.12.1.a	Potassium permanganate analysis						F
	4.12.1.b	Barium chloride gravimetric analysis						G
	4.12.1.c	Transmitted light analysis						G
Total iron	4.13.1.a	Flame atomic absorption spectrometry			○			
Mercury	5.1.a	Cold vapor atomic absorption spectrometry						H
	5.1.b	Cold vapor atomic absorption spectrometry (Fluid sludge fertilizers)					I	
Arsenic	5.2.a	Hydride generation atomic absorption spectrometry				○		
	5.2.b	Silver diethyl dithiocarbamate absorptiometric analysis				○		
	5.2.c	ICP Mass Spectrometry					○	
	5.2.d	Hydride generation atomic absorption spectrometry (sulfur)						J
Cadmium	5.3.a	Flame atomic absorption spectrometry			○			
	5.3.b	ICP Optical Emission Spectrometry			○			
	5.3.c	ICP Mass Spectrometry					○	
	5.3.e	ICP Optical Emission Spectrometry (Internal standard method)			○			
Nickel	5.4.a	Flame atomic absorption spectrometry			○			
	5.4.b	ICP Optical Emission Spectrometry			○			
	5.4.c	ICP Mass Spectrometry					○	
	5.4.e	ICP Optical Emission Spectrometry (Internal standard method)			○			

Table 1 (cont.)

Component name	Test method		Extraction method a)					
			Kjeldahl digestion	Incineration-hydrochloric acid boiling	Incineration-aqua regia digestion	Nitric acid, sulfuric acid, perchloric acid digestion	Microwave headed acid digestion	Other
Chromium	5.5.a	Flame atomic absorption spectrometry (Fertilizers containing organic matters)			○			
	5.5.b	Flame atomic absorption spectrometry (Fertilizers mainly containing fused matters, slag, etc.)						K
	5.5.c	Flame atomic absorption spectrometry (Fertilizers not containing organic matters)				○		
	5.5.d	ICP Optical Emission Spectrometry			○			
	5.5.e	ICP Mass Spectrometry (Fertilizers containing organic matters)					○	
	5.5.g	ICP Optical Emission Spectrometry (Internal standard method)			○			
Lead	5.6.a	Flame atomic absorption spectrometry			○			
	5.6.b	ICP Optical Emission Spectrometry			○			
	5.6.c	ICP Mass Spectrometry					○	
	5.6.e	ICP Optical Emission Spectrometry (Internal standard method)			○			
Titanium	5.11.a	ICP Optical Emission Spectrometry (1)				○		
	5.11.b	ICP Optical Emission Spectrometry (2)						L
Sodium	8.4.a	Flame atomic absorption spectrometry		○				

Table 2 List of extraction methods for soluble main components, etc.

Component name	Test method		Extraction method [a]			
			Boiling	Constant-temperature vertical rotating shaker	Horizontal reciprocating water bath shaker	Other
Citrate-soluble phosphoric acid	4.2.2.a	Ammonium vanadomolybdate absorptiometric analysis				A
	4.2.2.b	Quinoline gravimetric analysis				A
	4.2.2.c	ICP Optical Emission Spectrometry				A
Acid and base-soluble silicic acid	4.4.1.a	Potassium fluoride method		○	○	
	4.4.1.b	Potassium fluoride method (Silica gel fertilizers, etc.)				B
	4.4.1.c	Potassium fluoride method (Fertilizers containing silica gel fertilizers)				C
	4.4.1.d	Perchloric acid method		○		
Acid-soluble lime	4.5.2.a	Flame atomic absorption spectrometry	○			
Alkalinity	4.5.5.a	Ethylenediamine tetraacetate method	○			
Acid-soluble magnesia	4.6.2.a	Flame atomic absorption spectrometry	○			
Acid-soluble manganese	4.7.1.a	Flame atomic absorption spectrometry	○			
Acid-soluble sulfur	4.12.2.a	Ion Chromatography				D

a) Respective extraction method

A: Heating in Petermans citrate solution after separating water-soluble components with water

B: Heating in sodium hydroxide solution (20 g/L)

C: Heating in sodium hydroxide solution (20 g/L) after separating acid-soluble components by heating in hydrochloric acid (1+23)

D: Extraction with hydrochloric acid (1+23)

Table 3 List of extraction methods for citric acid-soluble main components

Component name	Test method		Extraction method	
			Constant-temperature vertical rotating mixer	Horizontal reciprocating water bath shaker
Citric acid-soluble phosphoric acid	4.2.3.a	Ammonium vanadomolybdate absorptiometric analysis	○	○
	4.2.3.b	Ammonium vanadomolybdate absorptiometric analysis (Fertilizers containing phosphorous acid or phosphite)	○	○
	4.2.3.c	Quinoline gravimetric analysis	○	
	4.2.3.d	ICP Optical Emission Spectrometry	○	○
Citric acid-soluble potassium	4.3.2.a	Flame atomic absorption spectrometry or flame photometry	○	○
	4.3.2.b	Sodium tetraphenylborate gravimetric analysis	○	
	4.3.2.c	Sodium tetraphenylborate volumetric analysis	○	
	4.3.2.d	ICP Optical Emission Spectrometry	○	○
Citric acid-soluble lime	4.5.3.a	Flame atomic absorption spectrometry	○	○
	4.5.3.b	ICP Optical Emission Spectrometry	○	○
Citric acid-soluble magnesia	4.6.3.a	Flame atomic absorption spectrometry	○	○
	4.6.3.b	ICP Optical Emission Spectrometry	○	○
Citric acid-soluble manganese	4.7.2.a	Flame atomic absorption spectrometry	○	○
	4.7.2.b	ICP Optical Emission Spectrometry	○	○
Citric acid-soluble boron	4.8.1.a	Azomethine-H method	○	○
	4.8.1.b	ICP Optical Emission Spectrometry	○	○

Table 4 List of extraction methods for water-soluble main components, etc.

Component name	Test method		Extraction method [a]				
			Vertical rotating shaker	Vertical reciprocating shaker	Boiling	Shaking to mix [b]	Other
Ammoniacal nitrogen	4.1.2.a	Distillation method	○, A	○, A			B
	4.1.2.b	Formaldehyde method	○,C, D				
Nitrate nitrogen	4.1.3.a	Devarda's alloy - distillation method					B
	4.1.3.b	Reduced iron- distillation method					B
	4.1.3.c	Phenol sulfuric acid method	E	E		E	
Water-soluble phosphoric acid	4.2.4.a	Ammonium vanadomolybdate absorptiometric analysis	○	○		○	
	4.2.4.b	Ammonium vanadomolybdate absorptiometric analysis (Fertilizers containing phosphorous acid or phosphite)	○	○		○	
	4.2.4.c	Quinoline gravimetric analysis	○				
	4.2.4.d	ICP Optical Emission Spectrometry	○	○		○	
Water-soluble potassium	4.3.3.a	Flame atomic absorption spectrometry or flame photometry	○	○	○	○	
	4.3.3.b	Sodium tetraphenylborate gravimetric analysis	○		○		
	4.3.3.c	Sodium tetraphenylborate volumetric analysis	○		○		
	4.3.3.d	ICP Optical Emission Spectrometry	○	○	○	○	
Water-soluble silicic acid	4.4.2.a	Potassium fluoride method	○			F	
Water-soluble calcium	4.5.4.a	Flame atomic absorption spectrometry	G	H		○	
	4.5.3.b	ICP Optical Emission Spectrometry				○	
Water-soluble magnesia	4.6.4.a	Flame atomic absorption spectrometry			I	○	
	4.6.4.b	ICP Optical Emission Spectrometry			I	○	
Water-soluble manganese	4.7.3.a	Flame atomic absorption spectrometry	○	○		○	
	4.7.3.b	ICP Optical Emission Spectrometry	○	○		○	
Water-soluble boron	4.8.2.a	Azomethine-H method			○	○	
	4.8.2.b	ICP Optical Emission Spectrometry			○	○	

a) Respective extraction method
A: Extraction with hydrochloric acid (1+23)
B: Introduction of analytical sample into distillation apparatus
C: Extraction with hydrochloric acid (1+20)
D: Extraction with potassium chloride solution (1 mol/L)
E: Extraction with copper sulfate - silver sulfate solution
F: 5 g of analytical sample - 400 mL of water
G: 1 g of analytical sample - 400 mL of water
H: 0.5 g of analytical sample - 200 mL of water
I: 1 g of analytical sample - 400 mL of water, boiling
b) Extraction method for liquid fertilizers, 1 g of analytical sample - Add about 50 mL of water and shake to mix

Table 4(cont.)

Component name	Test method		Extraction method [a)]				
			Vertical rotating mixer	Vertical reciprocating shaker	Boiling	Shaking to mix [b)]	Other
Water-soluble zinc	4.9.2.a	Flame atomic absorption spectrometry	○			○	
	4.9.2.b	ICP Optical Emission Spectrometry				○	
Water-soluble copper	4.10.1.a	Flame atomic absorption spectrometry	○			○	
	4.10.2.b	ICP Optical Emission Spectrometry				○	
Water-soluble iron	4.13.1.a	Flame atomic absorption spectrometry	○			○	
	4.13.1.b	ICP Optical Emission Spectrometry				○	
Water-soluble molybdenum	4.14.1.a	Sodium thiocyanate absorptiometric analysis	○			○	
	4.14.1.b	ICP Optical Emission Spectrometry				○	
Water-soluble cobalt	4.15.1.a	Flame atomic absorption spectrometry				○	
	4.15.1.b	ICP Optical Emission Spectrometry				○	

Annex C1
(Reference)

List of concentration ranges of calibration curve in ICP optical emission spectrometry

(1) List for ICP optical emission spectrometry

A list of concentration ranges of calibration curve in ICP optical emission spectrometry used in the Testing Methods for Fertilizers is shown in Table 1.

Note that when preparing mixed standard solutions, avoid using standard solutions made of compounds (potassium dihydrogenphosphate etc.) which contain anything other than a target component (phosphoric acid, etc.) or prepare separately the standard solution for calibration curve of a component (potassium, etc.) which affects measurement. When preserving mixed standard solutions for calibration curve preparation, use a container, which can be sealed tightly, made of material such as PTFE that does not elute boron easily.

Table 1 Concentration range of calibration curve and measurement wavelength for each element in ICP Optical Emission Spectrometry

Component name	Test method	Concentration range of calibration curve				Measurement wavelength (nm)
		Element concentration (mg/L)	Conversion factor	Oxide concentration (mg/L)		
Phosphoric acid	4.2.3.d, 4.2.4.d	P 1~200	2.2914	P_2O_5	2.291~458.2	178.287
Potassium	4.3.2.d, 4.3.3.d	K 1~200	1.2046	K_2O	1.205~241.0	766.491, 769.896
Lime (calcium)	4.5.3.b, 4.5.4.b	Ca 0.1~20	1.3992	CaO	0.1399~27.98	393.366, 317.933
Magnesia	4.6.3.b, 4.6.4.b	Mg 0.1~20	1.6583	MgO	0.1658~33.16	279.553, 280.270
Manganese	4.7.2.b, 4.7.3.b	Mn 0.05~10	1.2912	MnO	0.06455~12.91	257.610, 260.569
Boron	4.8.1.b, 4.8.2.b	B 0.05~10	3.2199	B_2O_3	0.1610~32.20	249.773, 249.678
Zinc	4.9.2.b	Zn 0.1~20	—	—		213.856, 206.200
Copper	4.10.2.b	Cu 0.1~20	—	—		327.396, 224.700, 324.754
Iron	4.13.2.b	Fe 0.1~20	—	—		259.940, 238.204
Molybdenum	4.14.1.b	Mo 0.1~20	—	—		202.030, 277.540
Cobalt	4.15.1.b	Co 0.1~20	—	—		228.616

Annex C2

(Reference)

List of concentration ranges of calibration curve in ICP mass spectrometry

(1) List for ICP mass spectrometry

A list of concentration ranges of calibration curve in ICP mass spectrometry used in the Testing Methods for Fertilizers is shown in Table 1.

Table 1 Concentration range of calibration curve and mass number for each element in ICP Mass Spectrometry

Component name	Test method	Concentration range of calibration curve		Internal standard element	
		Element and concentration (μg/L)	Mass number	Element and concentration (μg/L)	Mass number
Arsenic	5.2.c	As 0.2~20	75	Te 10	125
Cadmium	5.3.c	Cd 0.05~5	111	Rh 5	103
Nickel	5.4.c	Ni 0.5~50	60	Rh 5	103
Chromium	5.5.e	Cr 1~100	52	Sc 50	45
Lead	5.6.c	Pb 0.1~10	208	Tl 5	205

Annex D1
(Reference)

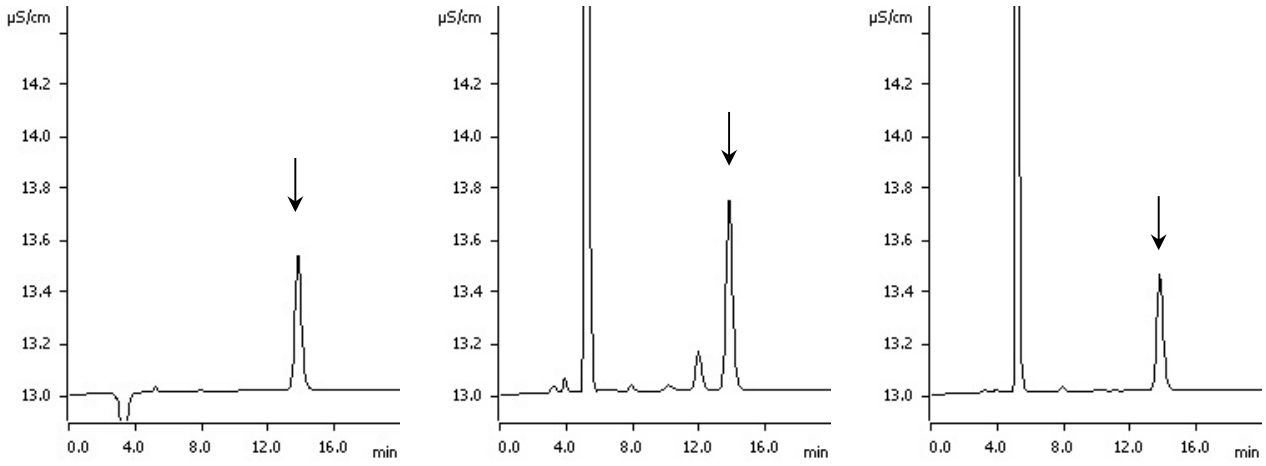
Example IC column used for acid-soluble sulfur (4.12.2.a Ion Chromatography)

This Annex shows the ion exchange column and measurement conditions used for acid-soluble sulfur (4.12.2.a Ion Chromatography), as summarized in Table 1.

Example IC chromatograms of standard solution, compound fertilizer sample solution and gypsum sample solution produced by the measurement conditions provided in Table 1 are shown in Figure 1 to Figure 4. In the figures, the peak of sulfate iron (SO_4^{2-}) is indicated by an arrow (↓).

Table 1 Example ion exchange column and measurement conditions in ion chromatography for acid-soluble sulfur

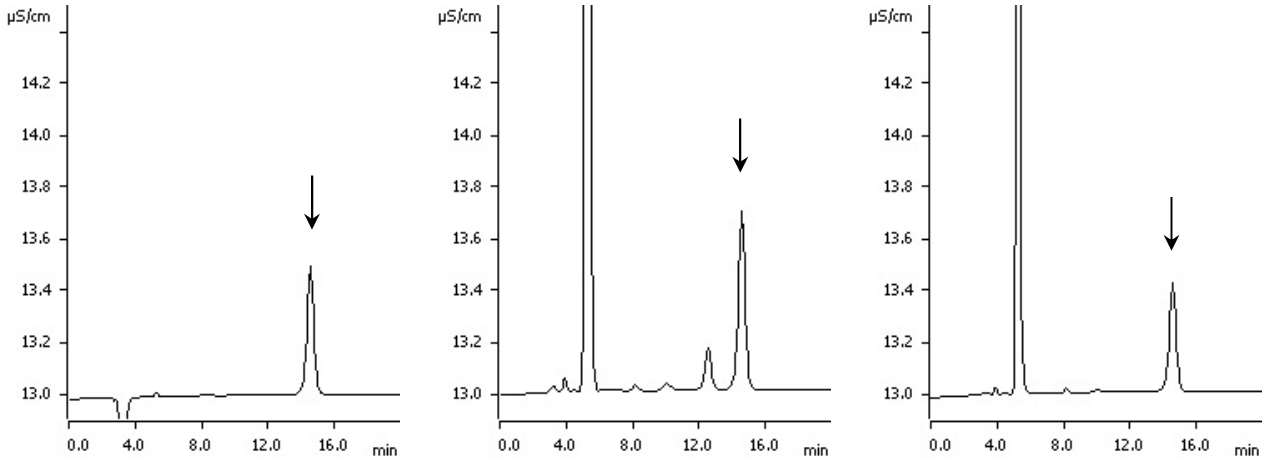
Ion exchange column			Measurement conditions				Note
Functional group	IC column name	Inner diameter × Length Particle diameter	Eluent and eluting conditions	Flow rate	Sample injection volume	Column temperature	
Quaternary ammonium group	Shodex IC SI-90 4E	4.0 mm×250 mm, 9 μm	1.8-mM sodium carbonate - 1.7-mM sodium hydrogencarbonate solution	1.0 mL/min	20 μL	25℃	A
Quaternary ammonium group	Metrosep A Supp 4-250/4.0	4.0 mm×250 mm, 9 μm	1.8-mM sodium carbonate - 1.7-mM sodium hydrogencarbonate solution	1.0 mL/min	20 μL	Room temperature	–
						25℃	B
Quaternary ammonium group	Shim-pack IC-SA2	4.0 mm×250 mm, 9 μm	1.8-mM sodium carbonate - 1.7-mM sodium hydrogencarbonate solution	1.0 mL/min	20 μL	25℃	C
Quaternary ammonium group	PCI-205	4.0 mm×250 mm, 9 μm	1.8-mM sodium carbonate - 1.7-mM sodium hydrogencarbonate solution	1.0 mL/min	20 μL	37℃	–
Quaternary ammonium group	TSKgel SuperIC-Anion HS	4.6 mm×100 mm, 3.5 μm	0.8-mM sodium carbonate - 7.5-mM sodium hydrogencarbonate solution	1.5 mL/min	30 μL	40℃	–
			1.1-mM sodium carbonate - 7.5-mM sodium hydrogencarbonate solution				–
Quaternary ammonium group	TSKgel SuperIC-AZ	4.6 mm×150 mm, 4 μm	1.7-mM sodium carbonate - 6.3-mM sodium hydrogencarbonate solution	0.8 mL/min	20 μL	40℃	D
Quaternary alkylamines	IonPac AS12A	4.0 mm×200 mm, 9 μm	2.7-mM sodium carbonate - 0.3-mM sodium hydrogencarbonate solution	1.2 mL/min	20 μL	32℃	–
Quaternary alkanolamines	IonPac AS22	4.0 mm×250 mm, 6 μm	4.5-mM sodium carbonate - 1.4-mM sodium hydrogencarbonate solution	1.0 mL/min	50 μL	35℃	–
				1.2 mL/min	25 μL		–
Quaternary alkanolamines	IonPac AS19	4.0 mm ×250 mm, 7.5 μm	10-mM potassium hydroxide solution	1.0 mL/min	20 μL	30℃	–
Quaternary alkanolamines	IonPac AS20	4.0 mm ×250 mm, 7.5 μm	Gradient elution by 5.0 mM-47.0 mM potassium hydroxide solutions 5.0 mM (-7.0 min) → 5.0 mM (0.0 min) → 5.0 mM (6.0 min) → 47.0 mM (25.0 min) → 47.0 mM (30.0 min)	1.5 mL/min	25 μL	35℃	–
Quaternary alkanolamines	IonPac AS11-HC	4.0 mm×250 mm, 9 μm	Gradient elution by 1.0 mM-70 mM potassium hydroxide solutions 70.0 mM (-10.5 min) → 70.0 mM (-10.1 min) → 15 mM (-10.0 min) → 15 mM (0.0 min) → 22 mM (10.0 min) → 22 mM (14.0 min) → 42 mM (17.5 min) → 70 mM (20.0 min) → 70 mM (25.0 min) → 1 mM (25.1 min)	1.2 mL/min	25 μL	40℃	–
			Gradient elution by 15 mM-50 mM potassium hydroxide solutions 15 mM (0.0 min) → 15 mM (18.0 min) → 50 mM (18.0 min) → 50 mM (20.0 min)			35℃	–



Sulfate ion standard solution (3 μg/mL) | Compound fertilizer | Gypsum

Figure 1 Chromatograms of sulfate ion (Part 1)

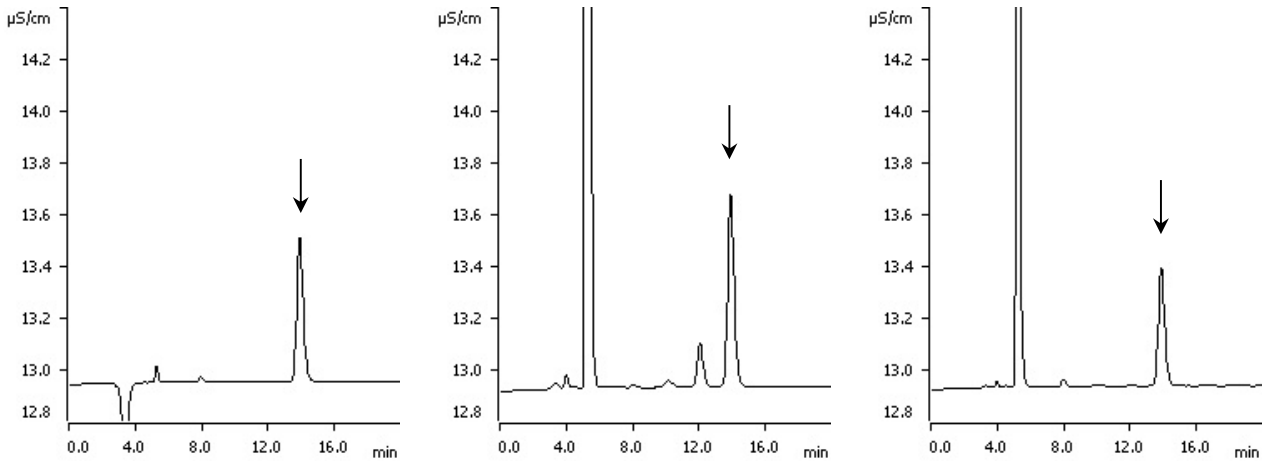
Column and measurement conditions: "A" in the Note column in Table 1.



Sulfate ion standard solution (3 μg/mL) | Compound fertilizer | Gypsum

Figure 2 Chromatograms of sulfate ion (Part 2)

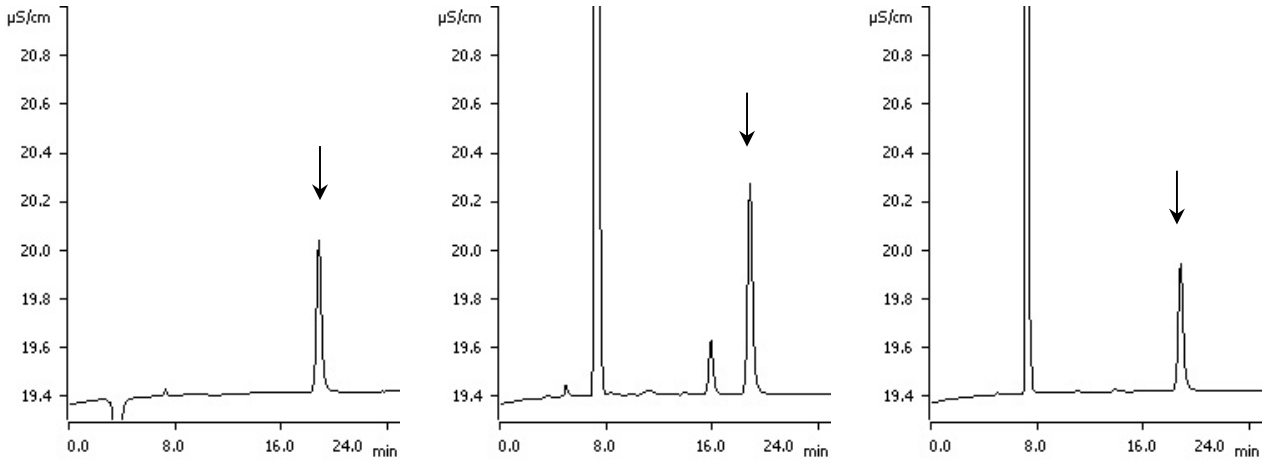
Column and measurement conditions: "B" in the Note column in Table 1.



Sulfate ion standard solution (3 μg/mL) | Compound fertilizer | Gypsum

Figure 3 Chromatograms of sulfate ion (Part 3)

Column and measurement conditions: "C" in the Note column in Table 1.



Sulfate ion standard solution (3 μg/mL) | Compound fertilizer | Gypsum

Figure 4 Chromatograms of sulfate ion (Part 4)

Column and measurement conditions: "D" in the Note column in Table 1.

Annex D2 (Reference)

Example of Measurement conditions for the test method of Organofluorine compounds (8.7.a High-Performance Liquid Chromatography/Tandem Mass Spectrometry)

This Annex shows the columns and the measurement conditions used for organofluorine compounds (8.7.a High-Performance Liquid Chromatography/Tandem Mass Spectrometry), as summarized in Table 1.

Table 1 Columns and measurement conditions used for High-Performance Liquid Chromatography/Tandem Mass Spectrometry of organofluorine compounds

Separation column	Eluent	Flow rate	Temperature of column bath	Gradient		
(Inner diameter × length, particle dimeter)	Top: A Bottom: B	(mL/min)	(℃)	Time(min)	A:(%)	B:(%)
InertSustain C18, GL Sciences (2.1 mm×150 mm, 3.0 μm)	10 mmol/L ammonium acetate	0.2	40	0.0 ~ 1.5 min	A: 60	B: 40
	acetonitrile			1.5 min～10.0 min	A: 60→0	B: 40→100
				10.0 min～12.0 min	A: 0	B: 100
				12.0 min～12.2 min	A: 0→60	B: 100→40
				12.2 min～20.0 min	A: 60	B: 40
InertSustain C18 HP, GL Sciences (2.1 mm×150 mm, 3.0 μm)	10 mmol/L ammonium acetate	0.2	40	0.0 ~ 1.5 min	A: 60	B: 40
	acetonitrile			1.5 min～10.0 min	A: 60→0	B: 40→100
				10.0 min～12.0 min	A: 0	B: 100
				12.0 min～12.2 min	A: 0→60	B: 100→40
				12.2 min～20.0 min	A: 60	B: 40
InertSustain AQ-C18, GL Sciences (2.1 mm×150 mm, 3.0 μm)	10 mmol/L ammonium acetate	0.2	40	0.0 ~ 1.5 min	A: 60	B: 40
	acetonitrile			1.5 min～10.0 min	A: 60→0	B: 40→100
				10.0 min～12.0 min	A: 0	B: 100
				12.0 min～12.2 min	A: 0→60	B: 100→40
				12.2 min～18.0 min	A: 60	B: 40
Shim-pack Velox SP-C18, Shimadzu (2.1 mm×100 mm, 1.8 μm)	10 mmol/L ammonium acetate	0.2~0.35	40	0.0 ~ 7.0 min	A: 70→35	B: 30→65
	acetonitrile			7.0 min～7.1 min	A: 35→5	B: 65→95
				7.1 min～7.5 min	A: 5	B: 95
				7.5 min～10.5 min	A: 5	B: 95
				10.5 min～10.6 min	A: 5→70	B: 95→30
				10.6 min～15.0 min	A: 70	B: 30
InertSustain C18, GL Sciences (2.1 mm×150 mm, 3.0 μm)	0.5 mmol/L ammonium acetate (containing 0.1 % formic acid)	0.2	40	0.0 ~ 13.0 min	A: 60→5	B: 40→95
	acetonitrile			13.0 min～18.0 min	A: 5	B: 95
				18.0 min～21.0 min	A: 5→60	B: 95→40
				21.0 min～25.0 min	A: 60	B: 40
ACQUITY UPLC BEH C18, Waters (2.1 mm×50 mm, 1.7 μm)	5 mmol/L ammonium acetate	0.25	45	0.0 ~ 4.8 min	A: 70→5	B: 30→95
	acetonitrile			4.8 min～5.4 min	A: 5	B: 95
				5.4 min～5.5 min	A: 5→70	B: 95→30
ZORBAX Eclipse Plus C18, Agilent (2.1 mm×100 mm, 1.8 μm)	10 mmol/L ammonium acetate	0.2	40	0.0 ~ 20.0 min	A: 70→10	B: 30→90
	acetonitrile			20.0 min～25.0 min	A: 10	B: 90
				25.0 min～25.1 min	A: 10→70	B: 90→30
InertSustain C18, GL Science (2.1 mm×150 mm, 3.0 μm)	10 mmol/L ammonium acetate	0.35	40	0.0 ~ 1.0 min	A: 75	B: 25
	acetonitrile			1.0 min～16.0 min	A: 75→2	B: 25→98
				16.0 min～20.0 min	A: 2	B: 98
				20.0 min～20.1 min	A: 2→75	B: 98→25
				20.1 min～22.5 min	A: 75	B: 25
InertSustain C18, GL Sciences (2.1 mm×150 mm, 3.0 μm)	10 mmol/L ammonium acetate	0.2	40	0.0 ~ 20.0 min	A: 75→0	B: 25→100
	acetonitrile			20.0 min～23.0 min	A: 0	B: 100
				23.0 min～23.1 min	A: 0→75	B: 100→25
				23.1 min～30.0 min	A: 75	B: 25

Table 1 cont.

Atlantis T3, Waters (2.1 mm×150 mm, 3.0 μm)	10 mmol/L ammonium acetate	0.2	40	0.0 ~ 20.0 min	A: 70→5	B: 30→95
	acetonitrile			20.0 min～23.0 min	A: 5	B: 95
				23.0 min～23.1 min	A: 5→70	B: 95→30
				23.1 min～25.0 min	A: 70	B: 30
InertSustainSwift C18 HP, GL Science (2.1 mm×100 mm, 3.0 μm)	10 mmol/L ammonium acetate (containing 0.1 % formic acid)	0.3	40	0.0 ~ 3.0 min	A: 70	B: 30
	acetonitrile			3.0 min～10.0 min	A: 70→0	B: 30→100
				10.0 min～15.0 min	A: 0	B: 100
				15.0 min	A: 0→70	B: 100→30
				15.0 min～20.1 min	A: 70	B: 30
ACQUITY UPLC C18, Waters (2.1 mm×50 mm, 1.7 μm)	2 mmol/L ammonium acetate	0.3	40	0.0 ~ 8.0 min	A: 99→5	B: 1→95
	ammonium acetate			8.0 min～9.0 min	A: 5	B: 95
				9.0 min～9.1 min	A: 5→99	B: 95→1
InertSustain C18 HP, GL Sciences (2.1 mm×150 mm, 3.0 μm)	10 mmol/L ammonium acetate	0.2	40	0.0 ~ 1.5 min	A: 60	B: 40
	acetonitrile			1.5 min～10.0 min	A: 60→0	B: 40→100
				10.0 min～12.0 min	A: 0	B: 100
				12.0 min～12.2 min	A: 0→60	B: 100→40
				12.2 min～20.0 min	A: 60	B: 40