Guidelines for the validation of analytical methods for testing agricultural chemical residues in food

1 Scope

These guidelines describe the procedure for validating analytical methods used in a laboratory to decide whether the concentrations of pesticide, veterinary drug, or feed additive (hereinafter referred to as agricultural chemicals) residues in food comply with the specifications of food.

These guidelines are applicable to analytical methods in which instruments are used.

Note: The procedures described in these guidelines are examples of procedures for validating analytical methods. Other internationally approved procedures can be used. These guidelines are in accordance with ISO 5724-1994 and JIS 8402 1999 and are notified as analytical methods for testing agricultural chemical residues in food.

2 Subject of these guidelines

The subject of these guidelines is the analytical methods used to decide compliance with the specifications of food and are not validated. These analytical methods include analytical methods shown in notification[*1] concerning residue standards for agricultural chemicals, testing methods shown in notification[*2] concerning testing methods, and/or other methods.

*1 Specifications and Standards for Food, Food Additives, Etc.(Ministry of Health and Welfare Notification No.370, 1959), Part I “Food,” Section A “General Compositional Standards for Food,” Nos. 5, 6, and 7

*2 Analytical Method for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food (Shoku-An No.0124001, January 24, 2005)

3 Definitions of terms

(1) Selectivity is the ability to quantify analyte(s) accurately in the presence of materials in a sample.

(2) Trueness is the degree of agreement between the average of sufficient test results and the accepted reference value (spiked concentration etc.).

(3) Precision is the degree of closeness between independent test results obtained under stipulated conditions.

(4) Repeatability is precision in repeatability conditions, where samples regarded as identical are quantified by an identical analyst with use of an identical instrument, in an identical laboratory using an identical method within a short period of time.

(5) Intralaboratoty precision is precision in intralaboratory conditions, where samples regarded as identical are quantified independently in an identical laboratory using an identical

method.

(6) The limit of quantification is the lowest quantity or concentration of an analyte that can be quantified with appropriate trueness and precision. These guidelines, as a general rule, adopt the limit of quantification shown in the notification concerning the testing method. When the agricultural chemical is specified as "should not be detected" in the notification concerning residue standards for agricultural chemicals, the limit of detection shown in the director notice (Syoku-An No.1129001 November 29, 2005) is treated as the limit of quantification in these guidelines.

(7) Nested experimental design is a design where all levels of a factor appear in only one level of other factors.

4 Validation procedure

Add the agricultural chemical(s) that is the analyte(s) of the method to be validated to the blank sample (the sample that does not contain the analyte(s)) and prepare a spiked sample. Analyze the spiked sample with the method and estimate the performance parameters (shown below) based on the analytical results. Confirm that the estimate of each performance parameter meets the specified target value.

The concentration of the agricultural chemical in the spiked sample is, as a general rule, the maximum residue level (MRL) of the agricultural chemical in the target food. When the agricultural chemical is specified as "should not be detected" in the notification concerning residue standards for agricultural chemicals, the concentration in the spiked sample should be the limit of detection shown in the notification (that is defined as the limit of quantification in the guidelines).

When a multi-analyte method is validated, the MRLs of target agricultural chemicals are different and the addition of agricultural chemicals following their MRLs may be difficult. In such a case, the concentration of agricultural chemicals in spiked samples can be two fixed levels, where one is a fixed concentration close to their MRLs and the other is the uniform limit.

Table 1 MRL and spiking concentration

MRL	Spiking Concentration
Should not be detected	Quantification limit
Other than “should not be detected”	MRL When a multi-analyte method is validated, the spiking concentration can be 2 levels such as “concentration similar to MRLs of analytes” and the uniform limit.

(1) Selectivity

Analyze the blank samples following the method and confirm that there are no peaks interfering with the quantification (interfering peak).

When an interfering peak is recognized, compare the area (or height) of the interfering peak with that of the peak obtained from a standard solution corresponding to the MRL or the limit of quantification, and confirm that the ratio meets the following conditions (See Table 2).

i) When the limit of quantification is not more than 1/3 of the MRL, the area (or height) of interfering peak should be less than 1/10 of that of the peak corresponding to the MRL.

ii) When the limit of quantification is more than 1/3 of the MRL, the area (or height) of the interfering peak should be less than 1/3 of that of the peak corresponding to the MRL.

iii) When an agricultural chemical is specified as "should not be detected" in a notification concerning residue standards for agricultural chemicals, the area (or height) of the interfering peak should be less than 1/3 of that of the peak corresponding to the limit of detection (limit of quantification in the guidelines) shown in Director Notice (Syoku-An No.1129001 November 29, 2005).

Table 2 Permissible range of interfering peak

Relation between MRL and Limit of Quantification (LOQ)	Permissible Range of Interfering Peak
LOQ ≦ MRL/3	< peak corresponding to MRL/10
LOQ > MRL/3	< peak corresponding to LOQ/3
should not be detected	< peak corresponding to LOQ/3

(2) Trueness

Measure not less than 5 spiked samples and estimate the trueness by calculating the ratio of the average of results to spiked concentration. [Note]

Note: When a surrogate (a reference standard containing stable isotope spiked to a sample to correct fluctuations in trueness) is used, recovery of the surrogate should not be less than 40%.

(3) Precision

Repeat the measurement of the spiked sample, calculate the standard deviation and the relative standard deviation. Evaluate the repeatability and intralaboratory precision derived from different analysts and/or days with the calculated value. The degree of freedom should not be less than 4.

Target values of trueness, repeatability and intralaboratory precision are shown in Table 3.

Table 3 Target values of trueness, repeatability and intralaboratory precision

Concentration (ppm)	Trueness (%)	Repeatability (RSD%)	Intralaboratory precision (RSD%)
< 0.001	70 - 120	30 >	35 >
0.001< - ≦0.01	70 - 120	25 >	30 >
0.01< - ≦0.1	70 - 120	15 >	20 >
0.1 <	70 - 120	10 >	15 >

(4) Limit of quantification

When the MRL is equivalent to the limit of quantification or the agricultural chemical is specified as "should not be detected" in the notification concerning residue standards for agricultural chemicals, confirm that the following two conditions are satisfied.

i) The trueness, repeatability and intralaboratory precision estimated based on the result of the spiked sample satisfy the target values shown in Table 3.

ii) In measurement with chromatography, the S/N ratio of the peak corresponding to the limit of quantification (peak obtained in i) or peak obtained from the analyte dissolved in the sample solution prepared with a blank sample) is not less than 10.

When a method validated following these guidelines is introduced to a laboratory or is modified partially, it is not necessary to evaluate all performance parameters described above. In such cases, the performance parameters shown in Appendix 1 should be evaluated.

The nested experimental design makes it possible to estimate the trueness, repeatability and intralaboratory precision simultaneously (see Appendix 2). Existing data can also be used, if they are obtained from the appropriate spiked samples in concentration and other characteristics, to estimate the trueness, repeatability and intralaboratory precision (see Appendix 3).

5 Type of food used for preparation of a spiked sample and spiking concentration

(1) Type of food used for preparation of a spiked sample

Food for spiking is selected, as a general rule, from foods that are intended to be tested by the method. Although all foods are potential subjects of a method, if the uniform limit is concerned, it is not practical to validate a method with all foods. Therefore it is advisable to select representative food and validate a method using this food first, and extend the range of foods gradually. Representative foods are selected from the list shown below, as a general rule, taking the characteristics of the food component and the difference of the extraction methods into consideration.

i) Agricultural products

Grains (brown rice, for example)

Legumes (soybeans, for example)

Nuts and seeds

Vegetables (spinach, which contains chlorophyll, cabbage, which contains sulfur compounds, and potato, which contains starch)

Fruit (oranges, apples, and the like)

Tealeaves

Hops

Spices

ii) Animal and fishery products

Muscle of cattle, pigs, chicken and the like

Fat of cattle, pigs, chicken and the like

Liver of cattle, pigs, chicken and the like

Kidney of cattle, pigs, chicken and the like

Chicken eggs

Cow milk

Apiculture products such as honey

Fish and shellfish (eel, which contains fat)

(2) Notices for preparing spiked samples

i) Use fresh food for preparation of spiked samples, as a general rule, and homogenize and weigh before spiking agricultural chemicals.

Avoid the use of frozen food or homogenized frozen food, if possible, because the composition of food changes as a result of freezing and the change may affect the performance of the method. Vegetables and fruits that are difficult to store for a long time without freezing may be stored in a frozen state without homogenization, but repeated freezing and thawing of food should be avoided.

For preparation of spiked samples, the volume of a standard solution spiked to a blank sample should be 1/10-1/20 of the sample amount. Use solvent mixable with the sample. Mix the sample thoroughly after spiking of agricultural chemicals, let stand for about 30 minutes and start the extraction procedure.

ii) When analyses in the validation procedure are carried out over several days such as in the use of the nested experimental design, spiked samples should be prepared on the day of each analysis avoiding repeated freezing and thawing.

Appendix 1

Performance parameters that should be evaluated when a method validated following the guidelines is introduced to a laboratory or is modified partially.

1 Introduction of a validated method to a laboratory

When a validated method is introduced to a laboratory, the performance parameters described in the guidelines except intralaboratory precision should be evaluated for verification of the method.

2 Application of a validated method to food that was not evaluated in the original validation procedure

When a validated method or a method introduced after verification is applied to food similar to the foods on which the method has been validated, selectivity and trueness should be evaluated, and if necessary repeatability should be evaluated. When the MRL is equivalent to the limit of quantification or the agricultural chemical is specified as "should not be detected" in a notification concerning residue standards for agricultural chemicals, the limit of quantification should also be evaluated.

3 Partial modification of a validated method

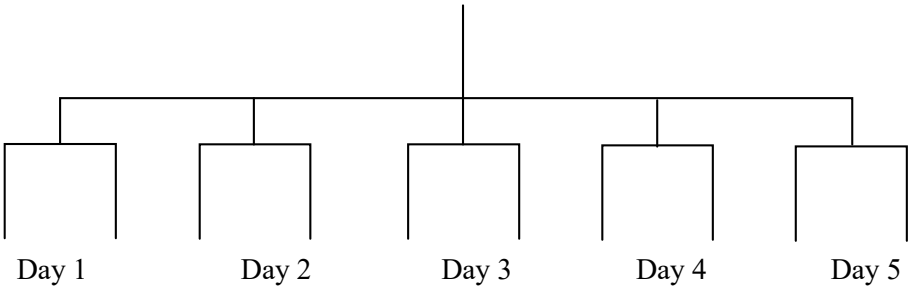
When a validated method or an introduced method after verification is modified partially, the evaluation of some performance parameters described in the guidelines may be omitted. Selectivity and trueness, however, must be evaluated after partial modification.

Selectivity, trueness and, if necessary, repeatability should be evaluated, after modification of the final volume of the testing solution or measurement conditions (injection volume, type and the size of the analytical column, carrier gas, temperature gradient, composition of the mobile phase, flow rate and gradient of the mobile phase, column temperature, mode of MS measurement and measurement ion). When the MRL is equivalent to the limit of quantification or the agricultural chemical is specified as "should not be detected" in a notification concerning residue standards for agricultural chemicals, the limit of quantification should be evaluated.

When the procedures of a method are modified except described above, the performance of the method may change considerably. Then the modified method is regarded as a new method as a general rule, and should be revalidated following the guidelines. The amount of sample used for analysis, sampling procedure, type and amount of solvent for extraction should not be changed.

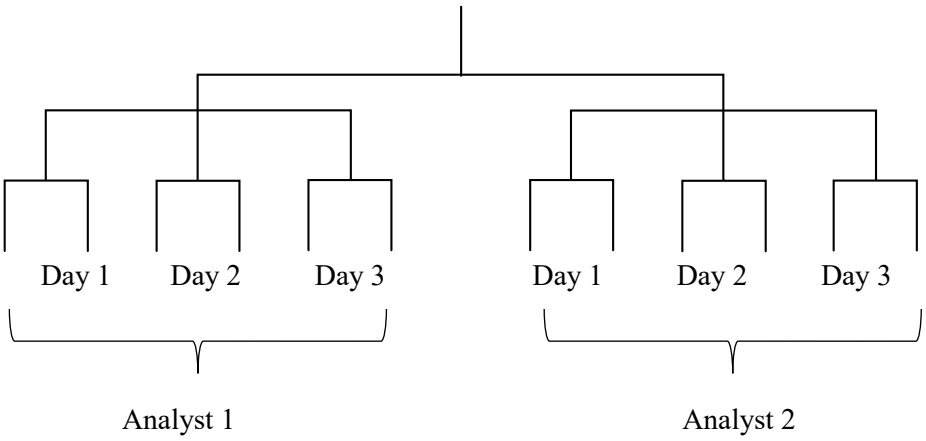
Appendix 2
Examples of experimental design for evaluation of intralaboratory precision

Example 1　Experimental design where an analyst analyzes an identical spiked sample twice a day in repeatability condition for 5 days.

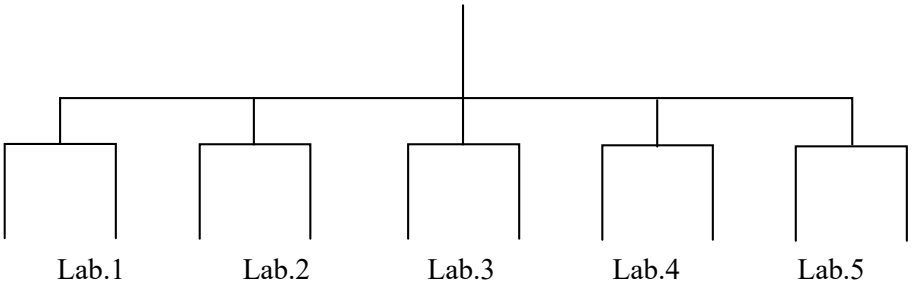


If the internal quality control has been put into practice and a spiked sample is analyzed twice in repeatability condition each day, or a spiked sample is analyzed twice in repeatability condition simultaneously with testing the sample, more than 4 data sets of 2 results can be used as a data set as shown in Example 1.

Example 2　Experimental design where 2 analysts analyze an identical spiked sample twice a day in repeatability condition for 3 days.



Example 3　Experimental design where an identical spiked sample is analyzed twice a day in repeatability condition in 5 laboratories.



Appendix 3
Example of method validation using existing data

1 Use of internal quality control data

Laboratories testing agricultural chemical residues implement the internal quality control following the general guidelines of internal quality control described in the appendix of the notification “Practice of Inspection Task Control in Food Sanitation Inspection Facilities” (Ei-Shoku No.117, April 1, 1997). When the method under the internal quality control is identical to the method intended to be validated, the existing data obtained from the internal quality control can be used to validate the method.

In the internal quality control following the general guidelines, blank samples and a sample that is spiked with an analyte of known concentration are analyzed periodically and furthermore the sample that is spiked with the analyte of known concentration is measured 5 times in repeatability condition.

Based on such data

(1) Selectivity is evaluated with the analytical results of blank samples.

(2) Trueness is estimated as the average of more than 4 results obtained by periodical analysis of the sample spiked with an analyte of known concentration. Intralaboratory precision is estimated as the standard deviation of the same analytical data. When a sample spiked with an analyte of known concentration is analyzed twice in repeatability condition for more than 4 days, the data may be regarded as data from the nested experimental design shown in Appendix 2 and trueness, repeatability and intralaboratory precision can be estimated with them.

(3) When a sample spiked with an analyte of known concentration is measured 5 times in repeatability condition periodically for more than 4 days, trueness, repeatability and intralaboratory precision can be estimated.

2 Use of data that is obtained from a spiked sample analyzed simultaneously with the testing

When a sample spiked with an analyte of known concentration is analyzed simultaneously with the testing with use of the testing method intended to be validated, the analytical results of the spiked sample can be used to validate the testing method.

A blank sample and 1-3 samples spiked with an analyte of known concentration are, in general, analyzed simultaneously with the testing.

(1) Selectivity is evaluated with the analytical results of the blank sample.

(2) When a sample spiked with an analyte of known concentration is analyzed 2-3 times in repeatability condition, the data sets from more than 4 testings may be regarded as the data

from the nested experimental design shown in Appendix 2 and trueness, repeatability and intralaboratory precision can be estimated.

(3) When one sample spiked with an analyte of known concentration is analyzed, trueness is estimated by the average of the data from more than 4 testings and intralaboratory precision is estimated by their standard deviation. Repeatability should be evaluated from the analysis of the sample more than 4 times in repeatability condition. However, when intralaboratory precision is lower than the target value of repeatability, the repeatability is also considered to be lower than the target value.