INTERNATIONAL STANDARD

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Animal feeding stuffs — Determination of potassium and sodium contents — Methods using flame-emission spectrometry

Aliments des animaux — Détermination des teneurs en potassium et sodium — Méthodes par spectrométrie d'émission de flamme

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 7485 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Annex A of this International Standard is for information only.

Animal feeding stuffs — Determination of potassium and sodium contents — Methods using flame-emission spectrometry

1 Scope

This International Standard specifies a calibration method and a standard addition method for the determination of potassium and sodium contents of animal feeding stuffs by flame-emission spectrometry.

The standard addition method applies if it is not known whether the emissions measured for potassium or sodium are subject to matrix effects. The limit of determination for potassium and sodium is 0,04 g/kg.

The calibration method applies if such matrix effects have been shown to be absent.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 6498:1998, *Animal feeding stuffs — Preparation of test samples.*

3 Principle

The test portion is incinerated to destroy any organic matter. The potassium and sodium are dissolved by treatment with hydrochloric acid. The potassium and sodium contents are determined by flame-emission spectrometry at wavelengths of 766 nm and 589 nm respectively, by means of a calibration curve (calibration method) or a standard addition graph (standard addition method).

4 Reagents

Use only reagents of recognized analytical quality.

4.1 **Water**, double distilled, or double deionized.

4.2 **Potassium chloride** (KCl), dried for 2 h at (110 ± 2) °C.

4.3 **Sodium chloride** (NaCl), dried for 2 h at (110 ± 2) °C.

4.4 **Concentrated hydrochloric acid**, c(HCl) = 12 mol/l (ρ_{20}(HCl) = 1,19 g/ml).

4.5 **Hydrochloric acid**, c(HCl) = 6 mol/l.

4.6 **Dilute hydrochloric acid**, c(HCl) = 0,1 mol/l.

4.7 Ionization and release buffer solution.

Dissolve 50 g of caesium chloride and 250 g of aluminium nitrate nonahydrate [$Al(NO_3)_3 \cdot 9H_2O$] in water. Dilute to 1 litre with water and mix. Store in a plastic bottle, e.g. polyethene or polypropylene.

4.8 Potassium standard solutions.

4.8.1 Potassium stock solution, ρ(K) = 1 mg/ml.

Weigh 1,906 g of potassium chloride (4.2) and transfer to a 1 000 ml volumetric flask with approximately 250 ml of dilute hydrochloric acid (4.6). Dissolve and dilute to the mark with the hydrochloric acid (4.6).

Store in a plastic bottle, e.g. polyethene or polypropylene. At room temperature the solution is stable for up to 6 months.

4.8.2 Potassium intermediate solution, ρ(K) = 100 μg/ml.

Transfer 10 ml of the potassium stock solution (4.8.1) to a 100 ml volumetric flask. Dilute to the mark with dilute hydrochloric acid (4.6) and mix.

Store in a plastic bottle, e.g. polyethene or polypropylene. At room temperature the solution is stable for 1 month.

4.8.3 Potassium calibration solution, ρ(K) = 10 μg/ml.

Transfer 10 ml of the potassium intermediate solution (4.8.2) to a 100 ml volumetric flask. Add 10 ml of the ionization and release buffer solution (4.7). Dilute to the mark with dilute hydrochloric acid (4.6) and mix.

Prepare fresh on the day of use.

4.9 Sodium standard solutions.

4.9.1 Sodium stock solution, ρ(Na) = 1 mg/ml.

Weigh 2,542 g of sodium chloride (4.3) and transfer to a 1 000 ml volumetric flask with approximately 250 ml of dilute hydrochloric acid (4.6). Dissolve and dilute to the mark with the hydrochloric acid (4.6). Mix well.

Store in a plastics bottle. At room temperature the solution is stable for up to 6 months.

4.9.2 Sodium intermediate solution, ρ(Na) = 100 μg/ml.

Transfer 10 ml of the sodium stock solution (4.9.1) to a 100 ml volumetric flask. Dilute to the mark with dilute hydrochloric acid (4.6) and mix.

Store in a plastics bottle. At room temperature the solution is stable for 1 month.

4.9.3 Sodium calibration solution, ρ(Na) = 10 μg/ml.

Transfer 10 ml of the sodium intermediate solution (4.9.2) to a 100 ml volumetric flask. Add 10 ml of the ionization and release buffer solution (4.7). Dilute to the mark with the hydrochloric acid (4.6) and mix.

Prepare fresh on the day of use.

4.10 Biological Certified Reference Materials.

For example: V8-Rye flour or V10-Hay (powdered), from the International Atomic Energy Agency (IAEA), Austria; SRM 157-Wheat flour, SRM 1568a-Rice flour or SRM 1548-Total diet, from the National Institute of Standards and Technology (NIST), USA.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 **Analytical balance**, capable of weighing to the nearest 1 mg.

5.2 **Muffle furnace**, electrically heated, capable of being maintained at (550 ± 20) °C.

5.3 **Ashing dishes**, of platinum or silica.

5.4 **Flame photometer** with an air-butane, air-propane or air-acetylene flame in a ratio allowing complete combustion; or an **atomic absorption spectrometer** operating in emission mode and equipped to determine potassium and sodium with an air-acetylene flame.

5.5 **Sand bath** or **hot plate**, capable of being maintained at approximately 150 °C.

5.6 **TD (to deliver) precision pipettes** or **microburette** of 5 ml capacity.

5.7 **TC (to contain) precision pipettes,** of various capacities.

5.8 **Beakers,** of 250 ml capacity.

5.9 **Volumetric flasks,** of various capacities.

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [1].

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

7 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

8 Procedure

8.1 Test portion

8.1.1 Samples containing organic matter

Depending on the expected content, weigh 1 g to 5 g of the prepared test sample (clause 7) to the nearest 1 mg into an ashing dish (5.3). Proceed in accordance with 8.2.1.

8.1.2 Samples not containing organic matter

Depending on the expected content of potassium and sodium, weigh 1 g to 5 g of the prepared test sample to the nearest 1 mg into a beaker (5.8). Proceed in accordance with 8.2.2.

8.2 Preparation of test solution

8.2.1 Incineration and rendering silica insoluble

Place the incineration dish containing the test portion (8.1.1) in the cold muffle furnace (5.2). Close the furnace and gradually increase the temperature to 550 °C and ash for 3 h at this temperature.

If many carbon particles are present, allow to cool down, moisten the contents of the dish with 2 ml of water and dry the dish on the sand bath or hot plate (5.5). Ash for another 2 h at 550 °C in the muffle furnace.

Allow to cool down, moisten the ash with a few drops of water and transfer it to a 250 ml beaker (5.8). Rinse the incineration dish with a total of approximately 5 ml of concentrated hydrochloric acid (4.4) and then with a few millilitres of water, collecting the rinsings in the beaker.

CAUTION — Take care during the addition of hydrochloric acid, as an effervescent reaction may occur with some samples.

Put a watch glass on the beaker to reduce spattering and slowly evaporate the contents of the beaker to near dryness on the sand bath or hot plate (5.5). Allow to cool to room temperature.

Proceed in accordance with 8.2.3.

8.2.2 Dissolution of potassium and sodium

Add to the beaker containing the test portion (8.1.2), slowly and carefully, a sufficient quantity (15 ml to 30 ml) of concentrated hydrochloric acid (4.4). Put a watch glass on the beaker to reduce spattering and slowly evaporate the contents of the beaker to dryness on the sand bath or hot plate (5.5). Allow to cool to room temperature.

8.2.3 Re-dissolution of potassium and sodium

Treat the residue (8.2.1 or 8.2.2) with 5 ml of hydrochloric acid (4.5) and 45 ml of water. Bring to the boil and allow to cool to room temperature. Transfer quantitatively into a 100 ml volumetric flask. Dilute to the mark with water and mix. Allow particles to settle for at least 4 h. Filter if the solution is not clear.

CAUTION — Perform the above operations in an effective fume-removal device.

8.3 Blank test solution

Prepare a solution in the same way as for the test solution, carrying out all the operations specified in 8.2 but omitting the test portion.

8.4 Selection of method

If it is not known whether the emissions measured for potassium or sodium are subject to matrix effects, proceed in accordance with 8.6.

If such matrix effects have been shown to be absent, proceed in accordance with 8.5.

8.5 Calibration method

8.5.1 Test solutions

Transfer to a 100 ml volumetric flask an aliquot portion of the test solution (8.2.3) containing a maximum of 1 mg of potassium or sodium. Add 10 ml of the ionization and release buffer solution (4.7), dilute to the mark with water and mix.

Prepare the same dilution of the blank test solution (8.3) in the same way.

8.5.2 Calibration solutions

Transfer to six 100 ml volumetric flasks 0 ml, 2,0 ml, 4,0 ml, 6,0 ml, 8,0 ml and 10,0 ml of the potassium intermediate solution (4.8.2) or sodium intermediate solution (4.9.2). Add 10 ml of the ionization and release buffer solution (4.7) to each flask. Dilute to the mark with hydrochloric acid (4.6) and mix. The potassium or sodium contents of these solutions are 0 μg/ml, 2,0 μg/ml, 4,0 μg/ml, 6,0 μg/ml, 8,0 μg/ml and 10,0 μg/ml respectively.

Prepare fresh on the day of use.

8.5.3 Spectrometric measurements

8.5.3.1 Adjustment of the instrument

Adjust the instrument in accordance with the manufacturer's instructions.

Light the flame and allow some minutes for flame stabilization. Adjust the burner position, the gas flows and the wavelengths (theoretically 766 nm for potassium and 589 nm for sodium) or select the filters so that maximum reading is obtained when the calibration solution of potassium (4.8.3) or sodium (4.9.3) is atomized in the flame.

8.5.3.2 Test solution measurements

Measure the emissions of the calibration solutions (8.5.2) and then the emission of the test solution (8.5.1).

Correct the emission of the test solution for the emission of the diluted blank test solution (8.5.1), if the latter differs from the emission of the zero concentration calibration solution.

8.5.4 Calibration graph

Draw the calibration graph by plotting the emissions of the calibration solutions against their respective potassium or sodium contents in micrograms per millilitre.

8.6 Standard addition method

8.6.1 Test solutions

Carry out the dilutions according to the expected potassium or sodium content.

Transfer to each of three volumetric flasks of suitable capacity (flasks A, B and C) the same aliquot portion of the test solution (8.2.3). Add to flasks B and C aliquot portions of the potassium intermediate solution (4.8.2) or sodium intermediate solution (4.9.2) corresponding to a content of 2,5 μg/ml for flask B and 5 μg/ml for flask C. Add to each flask an aliquot portion of the ionization and release buffer solution (4.7) corresponding to 10 ml per 100 ml of the final solution.

Dilute the contents of the three flasks (A, B and C) to the mark with water and mix.

The potassium or sodium content of the solution in flask A shall be smaller than 5 μg/ml.

Prepare the same dilution of the blank test solution (8.3) in the same way as for the solution in flask A.

8.6.2 Spectrometric measurements

8.6.2.1 Adjustment of the instrument

Adjust the instrument in accordance with 8.5.3.1.

8.6.2.2 Test solution measurements

Set the instrument to zero with the diluted blank test solution (8.6.1).

Reset the maximum reading with the calibration solution (4.8.3 for potassium and 4.9.3 for sodium). Atomize the solutions from flasks A, B and C (8.6.1) and measure the corresponding emissions (E_1, E_2 and E_3 respectively).

8.6.3 Graphic presentation

Draw a graph by plotting the measured emissions against the respective added potassium or sodium contents in micrograms per millilitre. The graph shall be a straight line intersecting the ordinate axis at E_2. The intersection of the extension of this line with the concentration line at ρ_x represents the unknown content of the test solution (in flask A).

9 Expression of results

Calculate the numerical value of the potassium content or sodium content of the test sample by the equation:

$$w = \frac{\rho_x \cdot f}{m}$$

where

w is the potassium or sodium content, in grams per kilogram, of the test sample;

ρ_x is the potassium or sodium content, in micrograms per millilitre, of the test solution determined from the calibration curve (8.5.4) or from the graph (8.6.3);

f is the reciprocal dilution factor;

m is the mass, in grams, of the test portion (8.1).

Report the result rounded in accordance with Table 1.

Table 1 — Rounding of the calculated content

Values in grams per kilogram

Content		Round to
from	to	
—	1,0	0,01
1,0	10,0	0,1
10,0	—	1,0

10 Control test

Analyse a Biological Certified Reference Material (4.10) with known contents of potassium and sodium in order to check the reagents and apparatus.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases exceed the repeatability limit (r) derived from Table 2.

Table 2 — Repeatability limit (r) and reproducibility limit (R)

Element	r g/kg	R g/kg
Potassium	0,168 g/kg + 0,073 $\overline{w}$	0,555 g/kg + 0,161 $\overline{w}$
Sodium	0,0054 g/kg + 0,105 $\overline{w}$	0,03 g/kg + 0,164 $\overline{w}$
$\overline{w}$ is the mean of the two single test results, in grams per kilogram.		

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit (R) derived from Table 2.

12 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— the test result obtained, or the two test results obtained if the repeatability has been checked.

Annex A
(informative)

Results of the interlaboratory tests

The precision of the method was established by interlaboratory tests carried out in accordance with ISO 5725[1)]. In this test 13 laboratories from 5 countries participated, each performing determinations in duplicate on 5 different samples (maize, soybean meal, alfalfa meal, finished mixed feed and mixed feed concentrate). The statistical results are summarized in Tables A.1 and A.2.

Table A.1 — Statistical results for the determination of potassium content

Parameter	Sample[a]				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	13	13	13	13	13
Number of outliers (laboratories)	-	-	-	-	-
Number of accepted results	26	26	26	26	26
Mean potassium content, g/kg	3,30	23,00	28,30	9,34	12,05
Repeatability standard deviation (s_r), g/kg	0,148	0,70	0,78	0,28	0,40
Repeatability coefficient of variation, %	4,5	3,0	2,8	3,0	3,3
Repeatability limit (r) [$r = 2{,}8 \times s_r$], g/kg	0,414	1,96	2,18	0,78	1,12
Reproducibility standard deviation (s_R), g/kg	0,40	1,43	2,13	0,69	0,92
Reproducibility coefficient of variation, %	12,2	6,2	7,5	7,4	7,6
Reproducibility limit (R) [$R = 2{,}8 \times s_R$], g/kg	1,12	4,00	5,96	1,93	2,58

[a] Sample 1: Maize;
Sample 2: Soybean meal;
Sample 3: Alfalfa meal;
Sample 4: Finished mixed feed;
Sample 5: Mixed feed concentrate.

1) ISO 5725:1986 (now withdrawn) was used to obtain the precision data.