INTERNATIONAL STANDARD

ISO 6491

Second edition
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Animal feeding stuffs — Determination of phosphorus content — Spectrometric method

Aliments des animaux — Détermination de la teneur en phosphore — Méthode spectrométrique

Foreword

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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.

International Standard ISO 6491 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 10, *Animal feeding stuffs*.

This second edition replaces the first edition (ISO 6491:1980), which has been technically revised.

Annexes A and B of this International Standard are for information only.

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Animal feeding stuffs — Determination of phosphorus content — Spectrometric method

1 Scope

This International Standard specifies a spectrometric method for the determination of the phosphorus content of animal feeding stuffs.

The method is applicable to animal feeding stuffs with a phosphorus content less than 50 g/kg. It is particularly appropriate for the analysis of products with low phosphorus content. For products with higher phosphorus content, application of a gravimetric method is advised, using for instance quinoline phosphomolybdate.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

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

3 Principle

A test portion of the sample is either ashed with lime and heated with acid (in the case of organic feeding stuffs) or wet oxidized with a mixture of sulfuric and nitric acids (in the case of mineral compounds and liquid feeding stuffs).

An aliquot portion of the acid solution is mixed with molybdovanadate reagent and the absorbance of the yellow solution obtained is measured at a wavelength of 430 nm.

4 Reagents

Use only reagents of recognized analytical grade.

4.1 Water, complying to at least grade 3 in accordance with ISO 3696.

4.2 Calcium carbonate.

4.3 Hydrochloric acid, $c(HCl) \approx 6$ mol/l.

4.4 Nitric acid, $c(HNO_3) \approx 1$ mol/l.

4.5 Nitric acid, $c(HNO_3) = 14$ mol/l, $\rho(HNO_3) \approx 1{,}40$ g/ml.

4.6 Sulfuric acid, $c(H_2SO_4)$ = 18 mol/l, $\rho(H_2SO_4)$ = 1,84 g/ml.

4.7 Ammonium heptamolybdate solution.

Dissolve in hot water 100 g of ammonium heptamolybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$. Add 10 ml of ammonia [$c(NH_4OH)$ = 14 mol/l, $\rho(NH_4OH)$ = 0,91 g/ml] and dilute to 1 l with water.

4.8 Ammonium monovanadate solution.

Dissolve 2,35 g of ammonium monovanadate (NH_4VO_3) in 400 ml of hot water. Stirring constantly, slowly add 7 ml of nitric acid (4.5) and dilute to 1 l with water.

4.9 Molybdovanadate reagent.

In a 1 l volumetric flask, mix 200 ml of the ammonium heptamolybdate solution (4.7), 200 ml of the ammonium monovanadate solution (4.8) and 135 ml of nitric acid (4.5). Dilute to the mark with water. Filter if insoluble particles are present.

4.10 Reference solution.

Dilute 10 ml of molybdovanadate reagent (4.9) with 10 ml of water.

4.11 Phosphorus standard solution, $\rho(P)$ = 1 mg/ml.

In a 1 l volumetric flask, dissolve in water 4,394 g of potassium dihydrogen phosphate (KH_2PO_4), previously dried at 103 °C for 1 h. Dilute to the mark with water.

5 Apparatus

Usual laboratory apparatus and, in particular, the following:

5.1 Ashing crucibles, of silica or porcelain.

5.2 Electric muffle-furnace, capable of being maintained at a temperature of 550 °C ± 20 °C.

5.3 Kjeldahl flask, of capacity 250 ml.

5.4 One-mark volumetric flasks, of capacities 500 ml and 1 000 ml.

5.5 Spectrometer, fitted with 10 mm cells, suitable for measurements at a wavelength of 430 nm.

5.6 Glass test tubes, of capacity 25 ml to 30 ml, fitted with ground glass stoppers.

5.7 Sand bath.

5.8 Beaker, of capacity 250 ml.

5.9 Graduated pipettes.

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [4].

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.

If solid, grind the laboratory sample (usually 500 g) so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

8 Procedure

8.1 Selection of procedure

If the test sample contains organic substances and if it is free from phosphates rendering insoluble products on ashing, proceed in accordance with 8.2.

If the test sample concerns a mineral compound or a liquid feeding stuff, proceed in accordance with 8.3.

8.2 Dry ashing

Weigh about 2,5 g of the prepared test sample to the nearest 1 mg in an ashing crucible (5.1).

Mix the test portion thoroughly with 1 g of the calcium carbonate (4.2). Ash in the furnace (5.2), set at a temperature of 550 °C, until white or grey ash is obtained (a small quantity of carbon does not interfere).

Transfer the ash to a 250 ml beaker (5.8) with 20 ml to 50 ml of water. Add hydrochloric acid (4.3) until effervescence ceases. Add a further 10 ml of the hydrochloric acid (4.3).

Place the beaker on the sand bath (5.7) and evaporate to dryness to render the silica insoluble. Allow to cool.

Add 10 ml of nitric acid (4.4) to the residue and boil on the sand bath for 5 min, without evaporating to dryness.

Decant the liquid into a 500 ml volumetric flask (5.4), rinsing the beaker several times with hot water.

Leave to cool, dilute to the mark with water, mix and filter.

Proceed in accordance with 8.4.

8.3 Wet destruction

Weigh 1 g or more of the prepared test sample to the nearest 1 mg.

Place the test portion in a Kjeldahl flask (5.3). Add 20 ml of sulfuric acid (4.6). Shake to impregnate the substance completely with acid and to prevent it from sticking to the wall of the flask. Heat and keep at boiling point for 10 min.

Leave to cool slightly. Add 2 ml of nitric acid (4.5), heat gently then leave to cool slightly. Add a little more nitric acid (4.5) and bring back to the boiling point.

Repeat this procedure until a colourless solution is obtained.

Cool, add a little water and decant the liquid into a 500 ml volumetric flask (5.5), rinsing the Kjeldahl flask with hot water.

Leave to cool, dilute to the mark with water, mix and filter.

8.4 Development of the colour and measurement of absorbance

Dilute an aliquot portion of the filtrate obtained (8.2 or 8.3) with water, to obtain a phosphorus content not exceeding 40 µg/ml.

Transfer, by means of a pipette (5.9), 10 ml of this solution to a test tube (5.6). Add, by means of another pipette, 10 ml of the molybdovanadate reagent (4.9).

Mix and leave to stand for at least 10 min at 20 °C.

Transfer a portion of the obtained solution to a measuring cell and measure the absorbance in the spectrometer (5.5) at a wavelength of 430 nm against the reference solution (4.10).

8.5 Preparation of the calibration curve

8.5.1 Using the phosphorus standard solution (4.11), and by means of graduated pipettes (5.9), prepare solutions with a phosphorus content of 5 µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml respectively.

8.5.2 Transfer, by means of pipettes (5.9), 10 ml of each of these solutions to a series of five test tubes (5.6). Add, to each by means of another pipette, 10 ml of the molybdovanadate reagent (4.9).

Mix and leave to stand for at least 10 min at 20 °C.

Measure the absorbance of each solution as specified in 8.4.

8.5.3 Draw the calibration curve by plotting the absorbances against the corresponding phosporus contents, in micrograms per millilitre, of the phosphorus standard solutions (8.5.1).

For phosphorus contents between 0 µg/ml and 40 µg/ml, the curve shall be linear.

8.6 Blank test

Carry out a blank test in parallel with the determination, using the same procedure and the same quantities of the reagents, but omitting the test portion.

9 Expression of results

Calculate the phosphorus content of the test sample, w_P, in grams per kilogram, using the following equation:

$$w_P = \frac{50 \cdot f_1 \cdot f_2 \cdot w_{Pc} \cdot V}{m}$$

where

f_1 is the reciprocal dilution factor for the aliquot portion (see 8.4);

f_2 is the units correction factor, in grams per milligram ($f_2 = 10^{-3}$ g/mg);

w_{Pc} is the phosphorus content, in micrograms per millilitre, of the diluted aliquot portion of the test solution, read from the calibration curve (8.5.3);

V is the volume, in millilitres, of each of the calibration solutions taken in 8.5.2 (V = 10 ml);

m is the mass, in grams, of the test portion (8.2 or 8.3).

Report the result to the nearest 0,1 g/kg.

10 Precision

10.1 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.

10.2 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 1 g/kg.

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases exceed 7 g/kg.

11 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 that occurred which may have influenced the test result(s);

— the test result obtained; or

— if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Results of interlaboratory test

An interlaboratory test was organized by ISO/TC 34/SC 10, *Animal feeding stuffs*, in 1987 and carried out in accordance with ISO 5725 [1][1)]. The final statistical analysis was carried out in accordance with ISO 5725-2 [3]. In this test 24 laboratories participated. Samples of corn gluten feed, finished mixed feed stuff, fish meal, mixed feed stuff concentrate (two types), premixed feed stuff and yeast were investigated.

Table A.1 — Statistical results of interlaboratory test

Parameter	Sample[1)]						
	1	2	3	4 [2)]	5	6	7
Number of laboratories retained after eliminating outliers	24	24	24	24	24	24	24
Mean phosphorus content, g/kg[3)]	28	5,4	9,2	80,1	27,4	22,5	11,4
Repeatability standard deviation (s_r), g/kg	0,40	0,32	0,11	1,48	0,75	0,30	0,24
Repeatability relative standard deviation, %	1,4	5,9	1,2	1,9	2,7	1,3	2,1
Repeatability limit (r) [$r = 2{,}8 \times s_r$], g/kg	1,12	0,90	0,31	4,14	2,10	0,84	0,67
Reproducibility standard deviation (s_R), g/kg	2,6	3,0	1,9	14,5	4,1	2,0	1,4
Reproducibility relative standard deviation, %	9,4	55,3	21,1	18,1	14,9	8,7	12,3
Reproducibility limit (R) [$R = 2{,}8 \times s_R$], g/kg	7,28	8,40	5,32	40,60	11,48	5,60	3,92

1) Sample 1: fish meal;
Sample 2: corn gluten feed;
Sample 3: yeast;
Sample 4: premixed feed stuff;
Sample 5: mixed feed stuff concentrate;
Sample 6: mixed feed stuff concentrate;
Sample 7: finished mixed feed stuff.

2) Since the phosphorus content was outside the limits of the method, the results were not included in precision data in clause 10.

3) Based on dry matter.

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

Annex B
(informative)

Bibliography

[1] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*.

[2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*.

[3] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*.

[4] ISO 6497:—[2)], *Animal feeding stuffs — Sampling*.

2) To be published.