International Standard 6490/2

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Animal feeding stuffs — Determination of calcium content —
Part 2: Atomic absorption spectrometric method

Aliments des animaux — Détermination de la teneur en calcium — Partie 2: Méthode par spectrométrie d'absorption atomique

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

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It has been approved by the member bodies of the following countries:

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No member body expressed disapproval of the document.

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Animal feeding stuffs — Determination of calcium content — Part 2: Atomic absorption spectrometric method

1 Scope and field of application

This part of ISO 6490 specifies an atomic absorption spectrometric method for the determination of the calcium content of animal feeding stuffs.

The limit of detection is in the region of 10 mg/kg.

2 References

ISO 6497, *Animal feeding stuffs — Sampling.*[1]

ISO 6498, *Animal feeding stuffs — Preparation of test samples.*[1]

3 Principle

Following any necessary destruction of organic matter by incineration of a test portion, dissolution of the calcium by treatment with hydrochloric acid and dilution of the solution obtained, in the presence of lanthanum which is used as a spectral buffer. Determination of the calcium content by atomic absorption spectrometry.

4 Reagents

All reagents shall be of recognized analytical quality and the water used shall be double-distilled, deionized and distilled or double deionized water.

4.1 Hydrochloric acid, concentrated (ϱ_{20} = 1,18 to 1,19 g/ml).

4.2 Hydrochloric acid, 6 mol/l solution.

4.3 Lanthanum chloride, solution prepared as follows:

In a 1 000 ml one-mark volumetric flask, dissolve 25 g of lanthanum oxide of low calcium content in 75 ml of the hydrochloric acid (4.1). Following reaction, allow to cool, add a little water, agitate, dilute to the mark with water and mix.

4.4 Calcium, standard solution corresponding to 40 mg/l.

4.4.1 Stock solution corresponding to 1 g/l.

Weigh 2,497 g of calcium carbonate which has been previously dried at 105 °C for 1 h. Transfer quantitatively to a 1 000 ml one-mark volumetric flask, rinsing with about 100 ml of water. Add 50 ml of the hydrochloric acid (4.1) to dissolve the carbonate, dilute to the mark with water and mix.

4.4.2 Standard solution corresponding to 40 mg/l.

By means of a pipette, transfer 10 ml of the stock solution (4.4.1) into a 250 ml one-mark volumetric flask. Add several drops of the hydrochloric acid (4.1), dilute to the mark with water and mix.

1 ml of this standard solution corresponds to 40 µg of calcium.

5 Apparatus

Usual laboratory equipment, and in particular

5.1 Electrically heated muffle furnace, preferably of silica, capable of being controlled at 550 ± 10°C.

5.2 Incineration dishes, of platinum, or, failing this, silica or porcelain.

5.3 Atomic absorption spectrometer, equipped to determine calcium, using an air/acetylene flame.

5.4 Ashless filter paper.

5.5 Beakers, of capacity 250 ml.

5.6 Volumetric flasks, of capacities 100 and 250 ml.

5.7 Pipettes, to deliver 5, 10, 15, 20 and 25 ml.

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

5.9 Analytical balance.

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[1] At present at the stage of draft.

6 Sampling

Take the laboratory sample in accordance with ISO 6497.

7 Procedure

7.1 Preparation of the test sample

Prepare the test sample in accordance with ISO 6498.

7.2 Test portion

7.2.1 Samples containing organic matter

Weigh, to the nearest 1 mg, 1 to 5 g of the test sample, according to the expected calcium content, into an incineration dish (5.2).

7.2.2 Samples not containing organic matter

Weigh, to the nearest 1 mg, 1 to 5 g of the test sample, according to the expected calcium content, into a 250 ml beaker (5.5).

7.3 Preparation of the test solution

7.3.1 Incineration and rendering the silica insoluble (only in the case of samples containing organic matter)

Place the incineration dish in the muffle furnace (5.1) while cold, close the furnace and gradually increase the temperature so as to reach 550 ± 10 °C in approximately 1 h 30 min. Maintain this temperature until the ash is free of carbon particles (if necessary, maintain this temperature for 16 h, i.e. overnight), then remove the incineration dish and leave to cool. Transfer the ash to a 250 ml beaker (5.5), moisten the ash with water, then rinse the incineration dish with a total of approximately 5 ml of the hydrochloric acid (4.1), collecting the rinsings in the beaker; take care during the addition as a violent reaction may occur.

Evaporate the contents of the beaker to dryness on the sand bath or hot plate (5.8) controlled at approximately 150 °C.

7.3.2 Dissolution of calcium

7.3.2.1 Add to the beaker containing the test portion (see 7.2.2), or the ash after incineration and rendering the silica insoluble (see 7.3.1), 15 ml of the hydrochloric acid solution (4.2) and 120 ml of water, and bring to the boil.

Filter through the filter paper (5.4) and collect the filtrate in a 250 ml one-mark volumetric flask (5.6).

7.3.2.2 If the residue on the filter shows only traces of carbon, ignore this, and wash the filter with 5 ml of the hydrochloric acid solution (4.2) and a little hot water, collecting the washings in the volumetric flask. Dilute to the mark with water and mix.

7.3.2.3 If the residue on the filter appears black (presence of carbon), place the filter containing the residue in a dish (5.2) and incinerate again in the muffle furnace, controlled at 550 ± 10 °C, until all the carbonaceous matter has completely disappeared (this operation usually requires 3 to 5 h). Allow to cool, add 2 ml of the hydrochloric acid (4.1) and evaporate to dryness on the sand bath or hot plate (5.8) controlled at approximately 150 °C, then add 5 ml of the hydrochloric acid solution (4.2). Heat, filter through a filter paper, collecting the solution in the 250 ml volumetric flask containing the filtrate previously collected (see 7.3.2.1), wash the filter paper with water, and dilute to the mark with water. Mix.

NOTE — For the analysis of products such as alumino-calcium phosphates which are not readily soluble in hydrochloric acid, it is recommended that an alkaline fusion be carried out as follows:

Mix the sample for analysis in a platinum crucible with 5 times its mass of a mixture of equal parts of potassium carbonate and sodium carbonate. Heat carefully until the mixture has completely melted. Cool and carefully dissolve the residue in the hydrochloric acid solution as described in 7.3.2.

7.4 Blank test

Prepare a solution in the same way as for the test solution, carrying out all the operations specified in 7.3 and using all the reagents, but omitting the test portion.

7.5 Preparation of the calibration graph

7.5.1 Preparation of calibration solutions

Prepare a series of six 100 ml one-mark volumetric flasks (5.6), and by means of a pipette (5.7), introduce, respectively, 0 — 5 — 10 — 15 — 20 and 25 ml of the standard calcium solution (4.4).

Add to each flask 20 ml of the lanthanum chloride solution (4.3), dilute to the mark with water and mix.

These solutions correspond respectively to 0 — 2 — 4 — 6 — 8 and 10 µg of calcium per millilitre.

This series of calibration solutions is given as a guide, with the understanding that it may be displaced slightly depending on the sensitivity of the instrument. If this is the case, the operator should determine the final dilution of the test portion to obtain the optimum conditions.

NOTE — For the analysis of mineral compounds which are rich in alkali metals, it is recommended that the calibration solutions be prepared using a solution containing sodium and potassium ions in the same proportions as those of the product for analysis.

7.5.2 Spectrometric measurements

Measure the absorbances of the calibration solutions (7.5.1), using the atomic absorption spectrometer (5.3), set at a wavelength of 422,7 nm, with an air/acetylene flame.

7.5.3 Plotting the graph

Plot a graph having, for example, the calcium contents, in micrograms per millilitre, as abscissae, and the corresponding absorbances of the calibration solutions as ordinates.

7.6 Determination

7.6.1 Preparation of dilutions

Carry out the dilutions according to the expected calcium content.

Using an aliquot part of the solution obtained, carry out an initial dilution using a volumetric flask of suitable capacity, diluting to the mark with water.

Carry out a second dilution by adding 20 ml of the lanthanum chloride solution (4.3) to an aliquot portion of the initial dilution in a 100 ml one-mark volumetric flask, then dilute to the mark with water.

7.6.2 Spectrometric measurements

Measure the absorbances of the test solution [second dilution (7.6.1)] and of the blank test solution (7.4) proceeding as described in 7.5.2.

Correct the absorbance of the test solution if that of the blank test solution differs from that of the zero term calibration solution.

7.7 Number of determinations

Carry out two determinations on test portions taken from the same test sample.

8 Expression of results

The calcium content, expressed as a percentage by mass of the product as received, is equal to

$$\frac{c \times F}{40 \times m}$$

where

c is the calcium content, in micrograms per millilitre, of the test solution, determined from the calibration graph (7.5.3);

F is the reciprocal dilution factor of the test solution (see 7.6.1);

m is the mass, in grams, of the test portion (7.2).

9 Precision

Two inter-laboratory tests carried out at the national level, in which 13 and 12 laboratories, respectively, each performing three determinations, participated, gave the statistical results (evaluated in accordance with ISO 5725[1]) summarized in the following table.

Table

Results expressed as percentages by mass

Sample	Granulated feed for pigs	Powdered feed for chickens	Meat meal	Monodiphos mixture
Number of laboratories retained after eliminating outliers	11	11	10	10
Mean	0,83	3,41	5,54	18,5
Standard deviation of repeatability (s_r)	0,03	0,05	0,07	0,20
Coefficient of variation of repeatability	3,4 %	1,4 %	1,3 %	1,1 %
Repeatability (2,83 × s_r)	0,08	0,14	0,20	0,57
Standard deviation of reproducibility (s_R)	0,04	0,11	0,17	0,54
Coefficient of variation of reproducibility	4,9 %	3,2 %	3,1 %	2,9 %
Reproducibility (2,83 × s_R)	0,11	0,31	0,48	1,53

10 Test report

The test report shall show the method used and the result obtained. It shall also mention any operations not specified in this International Standard, or regarded as optional, as well as any incidents likely to have affected the result.

The test report shall give all the information necessary for the complete identification of the sample.

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[1] ISO 5725, *Precision of test methods — Determination of repeatability and reproducibility by inter-laboratory tests.*

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