
**Animal feeding stuffs — Determination of
calcium, sodium, phosphorus,
magnesium, potassium, iron, zinc,
copper, manganese, cobalt, molybdenum,
arsenic, lead and cadmium by ICP-AES**

*Aliments des animaux — Détermination des teneurs en calcium,
sodium, phosphore, magnésium, potassium, fer, zinc, cuivre,
manganèse, cobalt, molybdène, arsenic, plomb et cadmium par
ICP-AES*



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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 document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 27085 was prepared by the European Committee for Standardization Technical Committee CEN/TC 327, *Animal feeding stuffs* (as EN 15510:2008), and was adopted under a special “fast-track procedure”, by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*, in parallel with its approval by the ISO member bodies.

Animal feeding stuffs — Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES

1 Scope

This International Standard specifies an inductively coupled plasma atomic emission spectroscopic (ICP-AES) method for the determination of:

- a) the minerals calcium, sodium, phosphorus, magnesium and potassium and the elements iron, zinc, copper, manganese, cobalt, and molybdenum in animal feeding stuffs;
- b) the elements arsenic, lead and cadmium in minerals on their own, in premixtures or mixtures for use in animal feeding stuffs.

The method detection limit for each element is dependent on the sample matrix as well as on the instrument. The method is not applicable for determination of low concentrations of elements. The limit of quantification is 3 mg/kg or lower.

NOTE This method can also be used for the determination of minerals in products with high mineral content (> 5 % mass fraction), yet for this purpose other more precise analytical techniques are available.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation*¹⁾

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

limit of detection

LOD

(animal feeding stuffs) smallest measured content, from which it is possible to deduce the presence of the analyte with reasonable statistical certainty

NOTE The limit of detection is numerically equal to three times the standard deviation of the mean of blank determinations ($n \geq 10$, where n is the number of measures) performed under reproducibility conditions.

1) To be published. (Revision of ISO 6498:1998)

3.2

limit of quantification

LOQ

⟨animal feeding stuffs⟩ lowest content of the analyte which can be measured with reasonable statistical certainty

NOTE If both trueness and precision are constant over a concentration range around the limit of detection, then the limit of quantification is numerically equal to 10 times the standard deviation of the mean of blank determinations ($n > 10$, where n is the number of measures) performed under reproducibility conditions.

3.3

feed additive

substance or preparation used in animal nutrition in order to: a) affect favourably the characteristics of feed materials or of compound feedingstuffs or of animal products; b) satisfy the nutritional needs of animals or improve animal production, in particular by affecting the gastro-intestinal flora or the digestibility of feedingstuffs; c) introduce nutrition elements conducive to attaining particular nutritional objectives or to meeting the specific nutritional needs of animals at a particular time; d) prevent or reduce the harmful effects caused by animal excretions or improve the animal environment

NOTE Adapted from Council Directive 96/51/EC^[3].

3.4

feedingstuff

animal feeding stuff

product of vegetable or animal origin, in its natural state, fresh or preserved, and products derived from the industrial processing thereof, and organic or inorganic substances, used singly or in mixtures, whether or not containing additives, for oral animal feeding

NOTE Adapted from Council Directive 2002/32/EC^[4].

4 Principle

For the determination of the minerals calcium, sodium, phosphorus, magnesium and potassium and the elements iron, zinc, copper, manganese, cobalt, and molybdenum, a test portion of the sample is ashed and dissolved in hydrochloric acid (in the case of organic feeding stuffs) or wet digested with hydrochloric acid (in the case of mineral compounds).

For the determination of the elements arsenic, cadmium and lead, a test portion of the sample is wet digested with nitric acid.

The concentration of minerals and the elements iron, zinc, copper, manganese, cobalt, and molybdenum is determined by ICP-AES using external calibration or standard addition technique.

WARNING — The use of this International Standard can involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate health and safety practices and determine the applicability of regulatory limitations prior to use.

5 Reagents

5.1 General

Use only reagents of recognized analytical grade, and water conforming to ISO 3696, grade 2.

5.2 Nitric acid, concentrated, not less than 65 % (mass fraction), having a density of approximately $\rho(\text{HNO}_3)$ 1,42 g/ml.

5.3 Dilute nitric acid, 50 % (volume fraction), prepared by mixing 1 volume of nitric acid (5.2) with 1 volume of water.

5.4 Nitric acid solution, 5 % (volume fraction), prepared by pipetting 160 ml dilute nitric acid (5.3) into a 1 000 ml volumetric flask (6.7) and making up to the mark with water.

5.5 Nitric acid solution, 2 % (volume fraction), prepared by pipetting 20 ml nitric acid (5.2) into a 1 000 ml volumetric flask (6.7) and making up to the mark with water.

5.6 Hydrochloric acid, concentrated, not less than 30 % (mass fraction), having a density of approximately $\rho(\text{HCl})$ 1,15 g/ml.

5.7 Dilute hydrochloric acid, 50 % (volume fraction), prepared by mixing 1 volume of hydrochloric acid (5.6) with 1 volume of water.

5.8 Hydrochloric acid solution, 1 % (volume fraction), prepared by pipetting 60 ml dilute hydrochloric acid (5.7) into a 1 000 ml volumetric flask (6.7) and making up to the mark with water.

5.9 Element stock solutions, Ca, Na, P, Mg, K, Fe, Zn, Cu, Mn, Co, Mo, Cd, Pb, As, $\rho = 1\,000\text{ mg/l}$.

The user should choose a suitable stock solution. Both single-element stock solutions and multi-element stock solutions with adequate specification stating the acid used and the preparation technique are commercially available. It is advisable to use certified stock solutions.

Stock solutions shall be replaced after one year, if nothing else is mentioned.

Element stock solutions with concentrations different from 1 000 mg/l may also be used.

5.10 Standard solutions

5.10.1 General

Depending on the scope, different multi-element standard solutions may be necessary. In general, when combining multi-element standard solutions, their chemical compatibility and the possible hydrolysis of the components shall be taken into account. The examples given in 5.10.2 to 5.10.4 also take into account the measuring range of various ICP-AES instruments and the expected concentration of the element in animal feeding stuffs.

The multi-element standard solutions are considered to be stable for several months, if stored in the dark (observe the expiry date specified by the manufacturer).

Other combinations of elements at different concentrations can be used, provided that the element stock solutions (5.9) are diluted with the same acid at a similar concentration to the acid in the test solution to prepare a range of standards that covers the concentrations of the elements to be determined.

5.10.2 Multi-element standard solution — Minerals in HCl (5.8), $\rho(\text{Ca, Na, P, Mg, K}) = 40\text{ mg/l}$.

Pipette 40,0 ml of each element stock solution (Ca, Na, P, Mg, K) (5.9) into a 1 000 ml volumetric flask (6.7). Add 60 ml dilute hydrochloric acid (5.7). Make up to the mark with water and transfer to a suitable storage bottle.

5.10.3 Multi-element standard solution — Fe, Zn, Cu, Mn, Co, Mo in HCl (5.8), $\rho(\text{Fe, Zn, Cu, Mn, Co, Mo}) = 50\text{ mg/l}$.

Pipette 50,0 ml of each element stock solution (Fe, Zn, Cu, Mn, Co, Mo) (5.9) into a 1 000 ml volumetric flask (6.7). Add 60 ml dilute hydrochloric acid (5.7). Make up to the mark with water and transfer to a suitable storage bottle.

5.10.4 Multi-element standard solution — Cd, Pb, As in HNO₃ (5.4), $\rho(\text{Cd, Pb, As}) = 100 \text{ mg/l}$.

Pipette 100,0 ml of each element stock solution (Cd, Pb, As) (5.9) into a 1 000 ml volumetric flask (6.7). Add 160 ml dilute nitric acid (5.3). Make up to the mark with water and transfer to a suitable storage bottle.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Laboratory grinders

6.1.1 Laboratory grinder capable of grinding to a particle size of less than or equal to 1 mm, e.g. a knife mill or equivalent.

6.1.2 Laboratory grinder capable of grinding to a particle size of less than or equal to 0,1 mm, e.g. a ball mill or equivalent.

6.1.3 Mortar with pestle, free of contamination.

6.2 Analytical balance, capable of weighing to an accuracy of 1 mg.

6.3 Electric hot plate, with temperature control.

6.4 Ashing crucibles, of platinum, quartz or porcelain.

6.5 Electric muffle-furnace, capable of being maintained at a temperature of $450 \text{ }^{\circ}\text{C} \pm 20 \text{ }^{\circ}\text{C}$.

The real temperature in the furnace has to be checked, because this temperature may be substantially different from the adjust temperature.

6.6 Beakers, of capacities 100 ml, 250 ml.

6.7 One-mark volumetric flasks, of capacities 100 ml, 500 ml, 1 000 ml.

6.8 ICP-AES instrument

The instrument shall be equipped with a radial plasma as a minimum requirement; an axial plasma is equally acceptable. Background correction shall also be performed when necessary. Settings of the working conditions (e.g. viewing height, gas flows, RF or plasma power, sample uptake rate, integration time, number of replicates) shall be optimized according to the manufacturer's instructions.

6.9 Freeze drying equipment, capable of freeze-drying liquid animal feeding stuffs.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497^[2].

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

8 Preparation of the test sample

8.1 General

Prepare the test sample in accordance with ISO 6498:

- the grinding shall be done in conditions such that the substance is not appreciably heated;
- the operation is to be repeated as many times as is necessary and it shall be effected as quickly as possible in order to prevent any gain or loss of constituents (water);
- the whole ground product is placed in a flask made of e.g. polypropylene, which can be stoppered and stored in such way to prevent any change in composition;
- before any weighing is carried out for the analysis, the whole test sample shall be thoroughly mixed for reasons of homogeneity.

8.2 Animal feeding stuffs which can be ground as such

Grind the laboratory sample (usually 500 g), using a grinder (6.1.1) or mortar, until a particle size of 1 mm or less has been reached.

8.3 Liquid animal feeding stuffs

Liquid feeding stuffs shall be pre-dried according to the procedure described in 8.3.1 or freeze-dried according to the procedure described in 8.3.2.

8.3.1 Pre-drying

Pre-dry the laboratory sample at a temperature of $70\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ during at least 16 h to reduce the moisture content. The mass of the sample before and after the pre-drying is to be determined using an analytical balance (6.2). Grind the pre-dried sample in accordance with 8.2.

8.3.2 Freeze-drying

Freeze-dry the laboratory sample following the instructions of the freeze-drying equipment (6.9). The mass of the sample before and after the freeze-drying is to be determined using an analytical balance (6.2). Grind the freeze-dried sample in accordance with 8.2.

8.4 Mineral animal feeding stuffs

Mineral compounds, except mineral products containing crystalline water, e.g. $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, shall be ground using a grinder (6.1.2) or mortar until a particle size of 0,1 mm or less has been reached. Mineral products containing crystalline water should not be ground.

9 Procedure

9.1 Digestion

9.1.1 Selection of the procedure

9.1.1.1 Determination of Ca, Na, P, Mg, K, Fe, Zn, Cu, Mn, Co, Mo

If the test sample concerns a mineral compound or a product potentially containing phosphates, proceed in accordance with 9.1.2.