
**Milk and milk products — Determination
of lead content — Graphite furnace
atomic absorption spectrometric method**

*Lait et produits laitiers — Détermination de la teneur en plomb —
Méthode spectrométrique d'absorption atomique avec four de graphite*

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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 6733|IDF/RM 133 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a Standing Committee may decide to publish an other type of normative document which is called by IDF: *Reviewed method*. Such a method represents an agreement between the members of a Standing Committee and is accepted for publication if it is approved by at least 50 % of the committee members casting a vote. A *Reviewed method* is equal to an ISO/PAS or ISO/TS and will, therefore, also be published jointly under ISO conditions.

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

ISO/TS 6733|IDF/RM 133 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

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All work was carried out by the Joint ISO-IDF Action Team on *Minor compounds*, of the Standing Committee on *Minor components and characterization of physical properties*, under the aegis of its project leader, Dr. G. Ellen (NL).

This edition of ISO/TS 6733|IDF/RM 133 cancels and replaces IDF 133A:1992, which has been technically revised.

Introduction

The method described in the first edition of IDF 133A:1992 was not up-to-date, was based on another technique and also did not fulfil the requirements for a fully validated International Standard. A new interlaboratory test on the up-dated method as described in this Technical Specification (Reviewed Method) was organized in 2004 in accordance with ISO 5725-1 and ISO 5725-2. However, the requirements for a reliable study were not fulfilled due to outliers, lack of participants and misinterpretations on the use of the method.

To maintain the method because several laboratories are using it, experts decided to publish the method as an ISO Technical Specification and IDF Reviewed Method rather than an International Standard.

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Milk and milk products — Determination of lead content — Graphite furnace atomic absorption spectrometric method

1 Scope

This Technical Specification describes a method for the quantitative determination of the total lead content in milk and milk products.

The detection limit of the method, defined as three times the standard deviation of the blank determination, is 0,001 mg/kg for liquid products and 0,01 mg/kg for solid products if dry ashing is applied. For pressurized wet mineralization, these figures are the same for test portions containing 0,2 g of dry matter. Microwave mineralization allows for larger test portions, and detection limits are accordingly lower.

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 648, *Laboratory glassware — One-mark pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

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

ISO 6732:1985, *Milk and milk products — Determination of the iron content — Spectrometric method (Reference method)*¹⁾

3 Terms and definitions

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

3.1

lead content

mass fraction of substance determined by the procedure specified in this Technical Specification

NOTE The lead content is expressed as milligrams per kilogram of sample.

4 Principle

A test portion is mineralized by means of programmed dry ashing with sulfuric acid as the ashing aid. Alternatively, it is mineralized by pressurized wet mineralization with nitric acid in a teflon or quartz vessel, or by microwave wet mineralization. The ash is dissolved in dilute nitric acid, or the wet mineralisate is evaporated to dryness and the residue is dissolved in dilute nitric acid. The atomic absorption of the resulting

1) Equivalent to IDF 103A.

solution is measured at a wavelength of 283,3 nm with a graphite furnace atomic absorption spectrometer with Zeeman or deuterium-arc background correction. Quantification is carried out by means of a calibration graph or by the method of standard additions.

5 Reagents

Unless otherwise stated, all reagents and solvents shall be of analytical reagent (AR) grade.

5.1 Water, double-distilled or of similar purity, complying with the requirements of ISO 3696, grade 2.

5.2 Concentrated nitric acid (HNO_3), $\rho_{20}(\text{HNO}_3) = 1,40$ g/ml, for cleaning purposes.

5.3 Concentrated nitric acid (HNO_3), $\rho_{20}(\text{HNO}_3) = 1,40$ g/ml, Merck "Suprapur" ²⁾ or equivalent.

5.4 Concentrated sulfuric acid (H_2SO_4), $\rho_{20}(\text{H}_2\text{SO}_4) = 1,84$ g/ml, Merck "Suprapur" ²⁾ or equivalent.

5.5 Sulfuric acid solution, 20 % (mass/volume fraction).

Dilute 130 ml of concentrated sulfuric acid (5.4) with water to 1 000 ml.

5.6 Matrix modifiers.

5.6.1 Dissolve 2,0 g of ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) (Baker "Ultrex") ²⁾ and 0,17 g of magnesium nitrate [$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] (Merck "Suprapur") in water. Dilute to 100 ml.

Suitable solutions are also commercially available [e.g. from Perkin-Elmer No. B019-0634 ²⁾ (1 % Mg solution) and No. N930-3445 (10 % $\text{NH}_4\text{H}_2\text{PO}_4$ solution)].

5.6.2 Mix 200 μl of a solution containing 1 % Pd (as nitrate, e.g. Perkin-Elmer No. B019-0635) ²⁾ and 20 μl of a solution containing 1 % Mg (as nitrate, e.g. Perkin-Elmer No. B019-0634) ²⁾ with 1,67 ml of water and 2 drops of nitric acid (5.3)

5.7 Concentrated lead standard solution, $c(\text{Pb}) = 100$ mg/l.

Dissolve 159,9 mg of lead nitrate [$\text{Pb}(\text{NO}_3)_2$] in water in a 1 000 ml one-mark volumetric flask (6.4). Add 10 ml of concentrated nitric acid (5.3). Dilute to the mark with water and mix.

Store the solution in a borosilicate glass bottle with a ground glass stopper.

NOTE As an alternative, a commercially available standard solution may be used, e.g. Merck Titrisol 9969 ²⁾.

5.8 Standard lead solution, $c(\text{Pb}) = 1$ mg/l.

Pipette 1,0 ml of concentrated lead standard solution (5.7) into a 100 ml volumetric flask (6.4). Add 1 ml of concentrated nitric acid (5.3). Dilute to the mark with water and mix.

Store the solution in a polyethylene flask (6.6) with a screw cap.

5.9 Working lead standard solutions.

Pipette 0 ml, 0,50 ml, 1,0 ml, 2,0 ml, and 3,0 ml, respectively, of standard lead solution (5.8) into 100 ml one-mark volumetric flasks (6.4). Add 1 ml of nitric acid (5.3). Dilute to the mark with water and mix. Prepare the working lead standard solutions every week.

²⁾ Baker "Ultrex", Merck "Suprapur", Titrisol and Perkin-Elmer solutions are examples of products available commercially. This information is given for the convenience of the users of this Technical Specification and does not constitute an endorsement by ISO or IDF of these products.

NOTE These working lead standard solutions have concentrations of 0,0 µg/l, 5,0 µg/l, 10,0 µg/l, 20,0 µg/l and 30,0 µg/l of lead, respectively.

Store the working lead standard solutions for not more than one week in polyethylene flasks (6.6) with screw caps.

5.10 Ultrapure nitrogen or argon.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Crucibles and glassware, cleaned as follows.

Wash and rinse with tap water, then rinse with distilled water. Soak in 10 % (mass fraction) concentrated nitric acid (5.2) for at least 12 h. Rinse three times with double-distilled water before use. Allow to dry and store the thus-cleaned crucibles and glassware in a dust-free environment.

6.2 Analytical balance, capable of weighing to the nearest 0,1 mg.

6.3 Quartz crucibles, flat bottomed, with covers (lids), of capacity approx. 25 ml to 50 ml.

6.4 One-mark volumetric flasks, of capacities 10 ml, 100 ml and 1 000 ml, with ground glass stoppers.

6.5 One-mark pipettes, of capacities 1 ml, 2 ml and 10 ml.

6.6 Polyethylene flasks, of capacity 100 ml, with screw caps.

6.7 Dosing pipettes, with adjustable volumes of between 50 µl and 250 µl, between 250 µl and 1 000 µl and between 1 000 µl and 5 000 µl, with plastic tips.

6.8 Muffle furnace, temperature-programmable to approx. 800 °C, with a lining of material with a low lead content (e.g. quartz).

Heat the muffle furnace regularly (e.g. once a fortnight) to a temperature of between 700 °C and 800 °C.

6.9 Wet-pressurized mineralization equipment, such as conventional or microwave thermal energy source, with mineralization vessels of contamination-free materials.

6.10 Stove, capable of operating at 150 °C ± 5 °C.

6.11 Hotplate, with adjustable thermostat.

6.12 Atomic absorption spectrometer, equipped with an electrothermal atomizer (graphite furnace), a Zeeman or deuterium-arc background correction system, autosampler with sample cups, printer or recorder, hollow cathode or electrodeless discharge lamp for lead, graphite tubes, L'vov platforms, if appropriate, etc.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707 | IDF 50.

8 Preparation of test sample

Prepare the test sample from the various types of product in accordance with ISO 6732:1985, 8.1. Avoid contamination with lead.

9 Procedure

9.1 Dry ashing

9.1.1 Test portion

Weigh, to the nearest 1 mg, approx. 1 g of test sample of dry products, or 5 g to 10 g of test sample of wet products, into a quartz crucible (6.3). Add to the sample of dry products, 2 ml to 3 ml of water to obtain a thick paste. Add to either test portion 2 ml of sulfuric acid solution (5.5). Mix carefully with a glass rod. Rinse the rod with a little water.

NOTE The addition of sulfuric acid is to prevent losses of lead. Experience, however, has shown that such an addition is not needed provided that the ashing is carried out with temperature programming and the maximum temperature does not exceed (500 to 550) °C.

9.1.2 Ashing

Place the quartz crucible on a hotplate (6.11) and evaporate the contents to dryness. If the reaction is too intense, interrupt the heating process. Continue to heat until no more white sulfur trioxide fumes are formed.

Transfer the crucible to the muffle furnace (6.8). Increase the furnace temperature, starting at room temperature, by approximately 50 °C per hour until 300 °C is reached then maintain this temperature for 2 h. Then increase the furnace temperature again by approximately 50 °C per hour to about 550 °C. Keep the crucible and its contents at this temperature for approx. 6 h.

Remove the crucible from the furnace and leave to cool. Moisten the ash with 1 ml of water and 1 ml of nitric acid (5.3). Use the hotplate (6.11) to evaporate the contents of the quartz crucible to dryness.

Then return the crucible to the muffle furnace (6.8) which, in the meantime, has cooled down from 550 °C to approx. 350 °C. Increase the furnace temperature again to 550 °C. Keep the furnace at this temperature for 30 min. Repeat this process until the ash in the crucible is free of carbon (white).

CAUTION — Place the hotplate and the muffle furnace in a hood, since sulfuric acid and nitrogen oxide vapours are released during decomposition. Always place the lids on the crucibles when they are transferred to another place or are stored between handling.

9.1.3 Test solution

Add 1,0 ml of water and 0,5 ml of nitric acid (5.3) to the contents of the quartz crucible. Heat the crucible carefully on the hotplate (6.11) to dissolve the ash. Transfer the contents of the crucible quantitatively to a 10 ml one-mark volumetric flask (6.4). Dilute to the mark with water. Mix well and carry out the measurements.

9.1.4 Blank

Carry out all the steps described in 9.1.1 to 9.1.3, inclusive, but omit the test portion.

9.2 Mineralization in a closed vessel (bomb digestion)

9.2.1 Test portion

Weigh, to the nearest 0,1 mg, 1 g of test sample of a liquid product, or 0,2 g of test sample of a solid product, into a teflon mineralization vessel (6.9). If applicable, adjust the amount of test portion of a liquid product so that no more than 0,2 g of organic material is involved.

These amounts apply for a mineralization vessel of 23 ml capacity. Adapt these amounts for vessels with other volumes.

9.2.2 Mineralization

Add 3 ml of nitric acid (5.3). Close the teflon mineralization vessel. Transfer it to a steel vessel and close that tightly with the screw cap. Place the whole in the stove (6.10) at room temperature. Increase the stove temperature to 150 °C and maintain this temperature for at least 3 h.

9.2.3 Test solution

After cooling to room temperature, open the teflon mineralization vessel. Evaporate the contents of the vessel to nearly dryness on a hotplate (6.11). Add 250 µl of nitric acid (5.3) and transfer the contents of the vessel quantitatively with water to a 10 ml one-mark volumetric flask (6.4). Dilute to the mark with water.

Mix well and carry out the measurements.

9.2.4 Blank

Carry out all the steps described in 9.2.1 to 9.2.3, inclusive, but omit the test portion.

Several laboratories use microwave mineralization with nitric acid rather than mineralization by heating. This technique may also be used, provided that the laboratory has proven that microwave mineralization yields measuring solutions that are comparable with those from pressurized mineralization when analysed by graphite furnace AAS.

9.3 Measurements

9.3.1 Instrument settings

NOTE A large variety of brands and types of AAS spectrometers are in use in analytical laboratories. Provided that an instrument is equipped with a proper light source, optical system and appropriate background correction system (e.g. based upon the Zeeman effect or a deuterium-arc lamp), it is suitable. Several types of graphite tubes are available; the best results can be expected with tubes of pyrolytic carbon, pyrolytically coated or coated with zirconium, and fitted with L'vov platforms.

Adjust all instrument parameters in accordance with the manufacturer's instructions for the measuring of lead and optimize to obtain an acceptable peak shape and background correction.

9.3.2 Calibration and measuring of sample solutions

9.3.2.1 General

Calibration may be carried out by means of a calibration graph or by the method of standard additions. If the calibration graph procedure is chosen, it shall be confirmed by the method of standard additions that the slopes of the calibration graphs are the same for standard solutions and test solutions.

Injection volumes depend on the type of instrument and may vary between 5 µl and 40 µl. For standard solutions and test solutions, the same amount of matrix modifier (5.6.1 or 5.6.2) shall be added to the graphite tube.