
**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*



Reference numbers
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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;
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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order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn.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again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.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.

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.

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.

9.3.2.2 Procedure with calibration graph

Measure the five working lead standard solutions (5.9) in triplicate. Then measure, also in triplicate, the test solution (9.1.3 or 9.2.3) and the blank (9.1.4 or 9.2.4). Individual readings corrected for background (peak areas or peak heights) for the same standard or test solution shall differ no more than 10 %.

Check the performance of the instrument after every five test portion solutions by measuring the 10 µg/l of working lead standard solution (5.9).

9.3.2.3 Procedure with standard additions

Measure all blanks (9.1.4 or 9.2.4) and test solutions (9.1.3 or 9.2.3) as follows. Inject x µl of test solution plus x µl of matrix modifier (5.6.1 or 5.6.2) and measure the atomic absorption corrected for background absorption. Repeat this procedure.

Then do the same, but additionally inject x µl of a suitable working lead standard solution (5.9), and measure the atomic absorption corrected for background absorption. Repeat this procedure too.

Do the same again, but inject additionally $2x$ µl of the same working lead standard solution. Carry out this measurement twice. The injection sequence is given schematically in Table 1.

The difference between each one of a pair of replicate results shall not exceed 10 %. As a check for linearity, the increase in signal resulting from the second addition shall be between 90 % and 110 % of that resulting from the first addition.

NOTE Depending on the type of instrument, x will vary from 5 to 20.

Table 1 — Injection sequence

Source	Injection number	Injection sample
Blank	1	blank
	2	blank
	3	blank + addition 1
	4	blank + addition 1
	5	blank + addition 2
	6	blank + addition 2
Test solution 1	1	sample 1
	2	sample 1
	3	sample 1 + addition 1
	4	sample 1 + addition 1
	5	sample 1 + addition 2
	6	sample 1 + addition 2
Test solution 2	1	sample 2
	2	sample 2
	3	sample 2 + addition 1
	4	sample 2 + addition 1
	5	sample 2 + addition 2
	6	sample 2 + addition 2
Restart with blank, etc.		etc.

EXAMPLE The following settings for a Perkin-Elmer AAS 4100 ZL³⁾ atomic absorption spectrometer, equipped with a hollow cathode lamp have been used:

Graphite tubes:	pyrolytically coated, fitted with L'vov platforms of pyrolytic carbon (Perkin-Elmer ³⁾ No. B050-4033, for AAS 4100 ZL)
Lamp current:	10 mA
Wavelength:	283,3 nm
Slit width:	0,7 nm (low)
Heath gas:	nitrogen
Pipette speed:	100 %
Injection volume:	10 µl
Injection temperature:	20 °C
Matrix modifier:	10 µl [0,05 % Pd/0,03 % Mg(NO ₃) ₂] (5.6.2)
Signal measurement:	peak area
Calibration:	standard addition method

Table 2 — Temperature programme

Step	Furnace temperature °C	Time s		Internal gas flow ml/min
		Ramp	Hold	
1	120	10	30	250
2	130	25	40	250
3	1 200	20	30	250
4 ^a	1 850	0	5	0
5	2 500	1	5	250

^a The signal is only registered during the atomization step (4).

10 Calculation and expression of results

10.1 Automated calculation

Modern instruments are supplied with software programs capable of calculating means and standard deviations of replicate measurements. These programs can also make calculations of calibration graphs for external standards, as well as for the standard additions method. In addition, the mass of the test portion and dilution factors can usually be introduced, so that the instrument calculates directly the result expressed in mg/kg of test sample.

If the instrument is not equipped with such a program, proceed as in 10.2 or 10.3

10.2 Calculation using a calibration graph

10.2.1 Calculate the mean values of the signals obtained for the standard solutions, the blank solution and the test solutions (see 9.3.2.1).

3) Perkin-Elmer AAS 4100 ZL is an example of a product 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 this product.

Plot the absorbance values of the working lead standard solutions (5.9) against the corresponding concentrations and construct the best fitting linear plot or, alternatively, calculate the regression line by the method of least squares. Read from the graph, or calculate, the concentration in the test solution and in the blank solution in micrograms per litre.

10.2.2 Calculate the lead content of the test sample, w , in milligrams per kilogram, using the following equation:

$$w = \frac{(w_s - w_0) V}{1000 m}$$

where

w_s is the numerical value of the lead content of the test solution (9.1.3 or 9.2.3), in micrograms per litre;

w_0 is the numerical value of the lead content of the blank solution (9.1.4 or 9.2.4), in micrograms per litre;

V is the numerical value of the volume of the test solution (9.1.3 or 9.2.3), in millilitres;

m is the numerical value of the mass of the test portion (9.1.1 or 9.2.1), in grams.

10.3 Calculation using standard additions

10.3.1 Calculate the lead content of the test solution, w_s , and that of the blank, w_0 , both expressed in micrograms per litre, using standard additions and by using the following equation:

$$w_s(w_0) = A_0 \sqrt{\left[\frac{(A_2 - A_1) + (A_1 - A_0)}{2} \right]} \times c_s$$

where

w_s is the lead content of the test solution, expressed in micrograms per litre;

w_0 is the lead content of the blank solution, expressed in micrograms per litre;

A_0 is the numerical value of the signal (area or height) for the test solution or the blank (9.3.2.2);

A_1 is the numerical value of the signal for the test solution or the blank for one addition (9.3.2.2);

A_2 is the numerical value of the signal for the test solution or the blank for two additions (9.3.2.2);

c_s is the numerical value of the concentration in the added lead standard solution (5.9), in micrograms per litre;

10.3.2 Calculate the lead content in the test sample, w , expressed in milligrams per kilogram, by using the same equation as in 10.2.2 but replacing the lead content of the blank, w_0 , and that of the test solution, w_s , by the numerical value obtained in 10.3.1.

10.4 Expression of results

Express the test results obtained in 10.2.2 or those in 10.3.2 to two decimal places.

11 Precision

11.1 Interlaboratory test

The values for the repeatability and reproducibility were derived from the results of an interlaboratory test carried out in accordance with ISO 5725-1 and ISO 5725-2. Details of the interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this 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 be greater than the values given in Table A.1.

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 be greater than the values given in Table A.1.

12 Test report

The test report shall specify:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, with reference to this Technical Specification;
- d) all operational details not specified in this Technical Specification, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- e) the test result(s) obtained and, if the repeatability has been checked, the final quoted result obtained.

Annex A (informative)

Interlaboratory trials

A.1 Study report

An international collaborative test involving nine laboratories has been carried out on eight samples of various dried milk products. However, the reports of five participating laboratories showed difficulties in using the method or that its procedure was not followed exactly.

The results of the remaining four laboratories, all using pressurized mineralization in a stove or in a microwave oven, were subjected to statistical analysis in accordance with ISO 5725-1 and ISO 5725-2 to obtain the precision data shown in Table A.1. The Grubbs and Cochran tests revealed that there were no more outliers.

Table A.1 — Interlaboratory test results

	Type of product	Mean µg/kg	CVr %	<i>r</i> µg/kg	CVR %	<i>R</i> µg/kg	CVR % ^a
A (1/13)	Freeze-dried evaporated milk 1	38,7	16,8	18,40	20,9	22,89	26,1
B (2/7)	Whole milk powder	23,6	58,9	39,34	80,9	54,05	28,1
C (3/10)	Processed cheese	9,5	72,7	19,55	80,1	21,53	32,2
D (4/11)	Whey powder	16,3	69,9	32,24	127	58,58	29,7
E (5/15)	Caseinate	25,3	49,0	35,08	75,5	54,06	27,8
F (6/12)	Freeze-dried evaporated milk 2	19,4	103	56,55	77,3	42,44	29,0
G (8/14)	Freeze-dried cheese	22,0	36,4	22,66	49,5	30,82	28,4
H (9/16)	Skim milk powder	19,6	37,8	20,97	29,6	16,42	28,9

^a Coefficient of variation of reproducibility predicted by the Horwitz equation.

A.2 Conclusions and recommendations

The results of the study show that the precision data do not meet expectations. Only the reproducibility of sample pair A with by far the highest lead content of all samples is in agreement with the predicted value of the Horwitz equation, while that of sample H may be considered as 'acceptable'. For the remaining sample pairs, the reproducibility is roughly two to three times higher than predicted.

The results of the study are not satisfying and, as a whole, the number of laboratories presenting data that could be included in the validation is far too low, and thus the precision data are not acceptable. Probably, the explanation of the unsatisfying precision is related to the low lead content levels of the samples. Only two samples were spiked with lead, while the others had a 'natural' lead contamination level. From the viewpoint of product quality, the very low lead contents are very positive. On the other hand, that implies a serious challenge for analytical laboratories.

For liquid milk, the EU limit for lead has been set at 20 µg/kg. For milk powder and other dried milk products, this corresponds to a limit of 150 µg/kg to 200 µg/kg. The limit for cheese is about 75 µg/kg.

The lead content levels in the test samples used in the study were much lower. Considering that precision and accuracy were acceptable for sample A with a lead content of around 35 µg/kg, it may be estimated that for test samples having a lead content of between 50 µg/kg and 100 µg/kg, the method might give more satisfying results.

Keeping in mind that determination of lead content is not a defining method, but the estimation of the concentration of a well-defined chemical substance, the future criteria approach might be most appropriate for this topic.

Based on experience, precise and accurate results can be produced with the described method for lead content levels of more than 40 µg/kg on a dry matter basis.

Annex B (informative)

Applicability of dry ashing as a mineralization procedure

Only one laboratory participating in the interlaboratory test made use of dry ashing. Unfortunately, this laboratory did not follow precisely the protocol specified, as ashing was performed at $700\text{ °C} \pm 50\text{ °C}$ instead of at a maximum of 550 °C . The obtained digest was also extracted with APDC into MIBK, which is not in the protocol. Nevertheless, the results of this laboratory were not statistically different from those of four laboratories using a bomb digestion technique, and were quite in line with those of the three laboratories using the ICP-MS and solid sampling GFAAS technique, see Table B.1.

Table B.1 — Comparison of the mean results obtained using this method (with bomb digestion) with those of laboratories using solid sampling GFAAS and ICP-MS (B) technique and dry ashing at 700 °C (C)

Sample	Type of product	A mean µg/kg	B mean µg/kg	C mean µg/kg
A (1/13)	Freeze-dried evaporated milk 1	38,7	33,6	29,8
B (2/7)	Whole milk powder	23,6	10,1	6,0
C (3/10)	Processed cheese	9,5	5,8	7,2
D (4/11)	Whey powder	16,3	6,1	0
E (5/15)	Caseinate	25,3	19,3	21,2
F (6/12)	Freeze-dried evaporated milk 2	19,4	13,5	11,6
G (8/14)	Freeze-dried cheese	22,0	15,2	15,7
H (9/16)	Skim milk powder	19,6	8,8	6,3

One laboratory reported being unable to obtain good test results with digests obtained from dry ashing due to very high background absorption, which the instrument could not compensate for. Probably, the fact that sulfuric acid was used as an aid during dry ashing may have caused these high background signals. Experience has shown that older types of graphite furnaces with relatively large graphite tubes have no problems with background compensation with this type of digests [6], but newer instruments with much smaller graphite tubes can have these compensation problems.

Based on this, it is questionable as to whether the use of sulfuric acid is strictly necessary, as its main purpose is to prevent loss of lead by volatilization. Following experience and additional information from laboratories, the addition of sulfuric acid is found to be unnecessary to prevent such losses if ashing is carried out with temperature programming with a maximum ashing temperature of between 500 °C and 550 °C [5].

Severe loss of lead does occur when the sample contains almost no inorganic material, which results in a very small amount of residual ash. In addition, when only a small amount of a standard solution is ashed, the recovery is very low. This suggests that the inorganic matrix encloses the lead and thus prevents its volatilization. Most milk and dairy products, however, yield a fair amount of ash from their content of inorganic material.

Dry ashing is a relatively simple and not laborious technique that allows the mineralization of several samples simultaneously. Depending on the size of the muffle furnace, the amount may vary between 10 and 50 samples. Thus, experts recommended not to delete this alternative digestion method from this Technical Specification. Therefore, this option is included, but with a note (see 9.1.1) to omit the addition of sulfuric acid.

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