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IDF 167

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Milk and dried milk — Determination of iodide content — Method using high-performance liquid chromatography

Lait et lait en poudre — Détermination de la teneur en iodure — Méthode par chromatographie en phase liquide à haute performance

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Foreword

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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 14378|IDF 167 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.

This second edition of ISO 14378|IDF 167 cancels and replaces the first edition (ISO 14378:2000), of which it constitutes a minor revision.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have 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 Standing 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.

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ISO 14378|IDF 167 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 former Joint ISO-IDF Group of Experts (E15 — *Heavy metals and other elements*) which is now part of the Joint ISO-IDF Action Team on *Minor compounds* of the Standing Committee on *Minor components and characterization of physical properties*.

This edition of ISO 14378|IDF 167 cancels and replaces IDF 167:1994, of which it constitutes a minor revision.

Milk and dried milk — Determination of iodide content — Method using high-performance liquid chromatography

WARNING — The use of this International Standard may involve hazardous materials, operations and equipment. Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish health and safety practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies a high-performance liquid chromatographic (HPLC) method for the determination of the iodide content of pasteurized whole milk and dried skimmed milk, when present at levels from 0,03 µg/g to 1 µg/g and 0,3 µg/g to 10,0 µg/g, respectively.

NOTE 1 The method has been collaboratively studied with samples of liquid whole milk and dried skimmed milk. There are no reasons to expect that the method does not apply to skimmed or partially skimmed milk as well as to dried whole milk.

NOTE 2 The method measures free (ionic) iodide. However, the total iodide content of fresh milk and good quality milk powder, in which no microbial growth has occurred, may contain a mass fraction of 5 % to 10 % of organically bound iodide. More iodide can be bound organically in milk where microbiological deterioration has occurred.

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*

3 Terms and definitions

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

3.1
iodide content of pasteurized whole milk
iodide content of dried skimmed milk
mass fraction of substances determined by the procedure specified in this International Standard

NOTE The iodide content is conventionally expressed in micrograms per gram.

4 Principle

A test portion is diluted with water. Insoluble and high-molecular-mass material is removed by filtration through a 25 000 D cut-off membrane. Iodide ions are separated by reverse-phase ion-pair HPLC with an electrochemical detector and a silver working electrode at 0 mV to 50 mV. The iodide content is calculated by means of a calibration graph.

5 Reagents

Use only reagents of recognized analytical grade or, if appropriate, of special HPLC grade.

5.1 Water, complying with ISO 3696, grade 2.

5.2 Iodide standard solutions.

WARNING — Protect aqueous solutions of iodides from light as they are unstable when exposed to visible frequencies.

5.2.1 Iodide stock solution, corresponding to 100 mg of iodide per litre.

Dissolve 130,8 mg of potassium iodide (KI) in water in a 1 000 ml one-mark volumetric flask (6.2). Dilute to the mark with water and mix.

The iodide stock solution may be kept for 1 month if stored in the dark at room temperature.

5.2.2 Iodide working standard solutions, corresponding to 20 µg, 50 µg, 150 µg and 250 µg of iodide per litre, respectively.

Pipette 20 µl, 50 µl, 150 µl and 250 µl of the iodide stock solution (5.2.1) into four separate 100 ml one-mark volumetric flasks (6.2). Dilute each solution to the mark with water and mix.

The iodide working standard solutions may be kept for 1 week if stored in the dark at room temperature.

5.3 Acetonitrile (CH_3CN), HPLC grade.

5.4 Hexadecyltrimethylammonium chloride solution [$CH_3(CH_2)_{15}N(CH_3)_3Cl$], 25 % (by mass) solution in water, ion-pair chromatography grade.

5.5 HPLC eluent: mixture of disodium hydrogenphosphate and hexadecyltrimethylammonium chloride in a mixture of acetonitrile and water (68 + 32 by volume), pH = 6,8.

Dissolve 1,42 g of disodium hydrogenphosphate (Na_2HPO_4) in about 600 ml of water in a 1 000 ml one-mark volumetric flask (6.2). Add 1,3 ml of hexadecyltrimethylammonium chloride solution (5.4) and mix well. Then add 320 ml of acetonitrile (5.3) and mix again. Adjust the pH to 6,8 with concentrated orthophosphoric acid (H_3PO_4). Dilute to the mark with water and mix well.

Clarify the solution by filtering first through a 1,2 µm membrane filter and then through a 0,5 µm membrane filter. Swirl the solution to mix it and simultaneously degas by means of a vacuum or sonification for 2 min before initial use. The eluent can be modified by the addition of small amounts of water or acetonitrile to achieve minor adjustments in the retention time of iodide. The eluent may be kept for 1 year, if stored in a tightly closed container.

5.6 *n*-Pentanol.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 0,01 g, with a readability of three decimal places.

6.2 One-mark volumetric flasks, of capacities 100 ml and 1 000 ml, ISO 1042[3] class A.

6.3 Micropipettes, capable of delivering 20 µl, 50 µl, 150 µl and 250 µl respectively, ISO 8655-2[6].

6.4 Graduated pipette, of capacity 2 ml, with 0,1 ml graduations, ISO 835[2] class A or class AS.

6.5 Measuring cylinder, of capacity 500 ml, ISO 4788[4] class A.

6.6 pH-meter, with combined glass electrode.

6.7 Membrane filters, 1,2 µm and 0,5 µm, nylon-6-6, or equivalent, with filter equipment to clarify HPLC eluent.

6.8 Centrifuge, capable of holding 50 ml centrifuge tubes and capable of producing a radial acceleration of 1 000*g*.

6.9 Centrifuge tubes, of capacity 50 ml, internal diameter 27 mm, conical, made of disposable plastic, with screw caps.

6.10 Conical membrane supports, to support membrane filter cone (6.11) in centrifuge tubes (6.9) [Amicon CS1A[1)], or equivalent].

6.11 Membrane filter cones, 25 000 D to 30 000 D cut-off [Amicon Centreflo CF-25[1)], or equivalent].

Prepare new membrane filter cones before use as follows. Soak in a mixture of ethanol and water (2 + 8 parts by volume) for 1 h. Remove the cone and drain. Mount it in a conical membrane support (6.10) and place it in a 50 ml centrifuge tube. Centrifuge the cone at a radial acceleration of 900*g* to 1 000*g* for 5 min to 10 min.

Invert the new membrane filter cone to drain any remaining solvent. Place the prepared cones into supports in clean, labelled centrifuge tubes (6.9) for sample analysis. After each use, soak the cones immediately in hot water, flush well with hot water and store them in a mixture of ethanol and water (1 + 5 parts by volume). Remove the solvent before the next use of the cones as described above for new cones.

Alternatively, Millipore Ultrafre-PF [1)] (UFP1, 10 000 D cut-off) filtration units may be used. These disposable filters do not need any pretreatment, and filtering can be carried out by slight pressure or vacuum; no centrifuge is needed.

6.12 HPLC equipment, consisting of the following.

6.12.1 Pump, capable of delivering a volume flow rate of 2 ml/min.

6.12.2 Injector, manual or automatic, with injection capacities of 50 µl to 200 µl.

6.12.3 Analytical column, Partisphere C-18[1)], 5 µm, internal diameter 4,7 mm, length 110 mm, or equivalent.

1) Example of a suitable product available commercially. This information is given for the convenience of users of this International Standard, and does not constitute an endorsement by ISO or IDF of this product. Equivalent products may be used if they can be shown to produce comparable results.

6.12.4 Guard column (optional), Spheri-5 C-18[1)] cartridge, internal diameter 3,2 mm, length 15 mm, or equivalent.

6.12.5 Electrochemical detector, to be used in the d.c. mode or pulsed amperometric mode, with a silver working electrode at 0 mV to + 50 mV potential.

6.12.6 Strip chart recorder or **integrator**, capable of peak area measurement, preferably using an electronic integrator having so-called "negative peak" function [Spectra Physics[1)] is suitable].

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707 | IDF 50[1].

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

8 Preparation of test sample

8.1 General

Avoid any bacterial contamination during preparation of the sample.

8.2 Milk

Bring the test sample to 20 °C ± 2 °C and mix carefully. If a homogeneous dispersion of the fat is not obtained, heat the sample slowly to 40 °C and mix gently by inversion. Then cool the sample to 20 °C ± 2 °C.

8.3 Dried milk

Transfer the sample to a container of capacity about twice the volume of the sample, provided with an airtight lid. Close the container immediately and mix the sample thoroughly by repeatedly shaking and inverting the container.

9 Procedure

9.1 Test portion

9.1.1 Milk

Weigh, to the nearest 0,1 g, 45 g ± 5 g of the test sample into a 100 ml one-mark volumetric flask (6.2). Make up to the mark with water and mix well.

9.1.2 Dried milk

Weigh, to the nearest 0,01 g, 4,2 g ± 0,2 g of the test sample into a 100 ml one-mark volumetric flask (6.2). Add 70 ml to 80 ml of water and shake briskly for 5 min to 10 min to obtain a complete solution of the sample. Add 1 drop of *n*-pentanol (5.6) to reduce foaming, and mix. Make up to the mark with water and mix well.

9.2 Clean-up

From the diluted test portion (9.1.1 or 9.1.2), fill two membrane cones to within 5 mm of the top and centrifuge at a radial acceleration of 900g to 1 000g for 15 min to 20 min. The clear filtrates obtained (i.e. two test solutions for each sample) may be injected directly into the HPLC system.

NOTE For an alternative clean-up procedure, see 6.11, last paragraph.

9.3 Determination by HPLC

9.3.1 Optimization of HPLC conditions

Wash a new HPLC column by pumping through it a mixture of acetonitrile (5.3) and water (5.1) (1 + 1 parts by volume), followed by 30 ml of HPLC eluent (5.5). Then recycle the eluent at 2 ml/min for at least 1 h.

Switch on the electrochemical detector (6.12.5) (potential 0 mV to + 50 mV; output 10 nA to 20 nA full scale). Recycle continuously the HPLC eluent (5.5) until a stable baseline is obtained.

Inject repeatedly 50 µl of the iodide standard working solution with an iodide concentration of 250 µg/l (5.2.2) until the retention time and peak height are constant; i.e. the absolute difference between the peak heights of two successive injections is not greater than 3 %. The retention time for iodides shall lie between 4 min and 8 min; if not, adjust the composition of the eluent (5.5). Adjust the applied electrode potential within 0 mV to + 50 mV to optimize the peak shape and peak height (see Figure 1).

Determine the injection volume for the 250 µg/l iodide standard working solution (5.2.2) that gives a peak height of about 80 % full scale. Use that injection volume afterwards for all the test and standard solutions.

The HPLC eluent (5.5) may be recycled between sample analyses or when standard solutions alone are being injected. However, do not recycle eluent when test solutions are being injected. In routine use, recycle eluent at 0,2 ml/min to maintain system readiness. During extended intervals between use, flush the HPLC system with a mixture of acetonitrile (5.3) and water (5.1) (1 + 1 parts by volume) and re-equilibrate with HPLC eluent (5.5) before the next use.

9.3.2 Measurement

Inject the four iodide working standard solutions (5.2.2). Wait for 5 min after the elution of the iodide before the next injection. Measure the iodide peak heights or peak areas for the iodide working standard solutions.

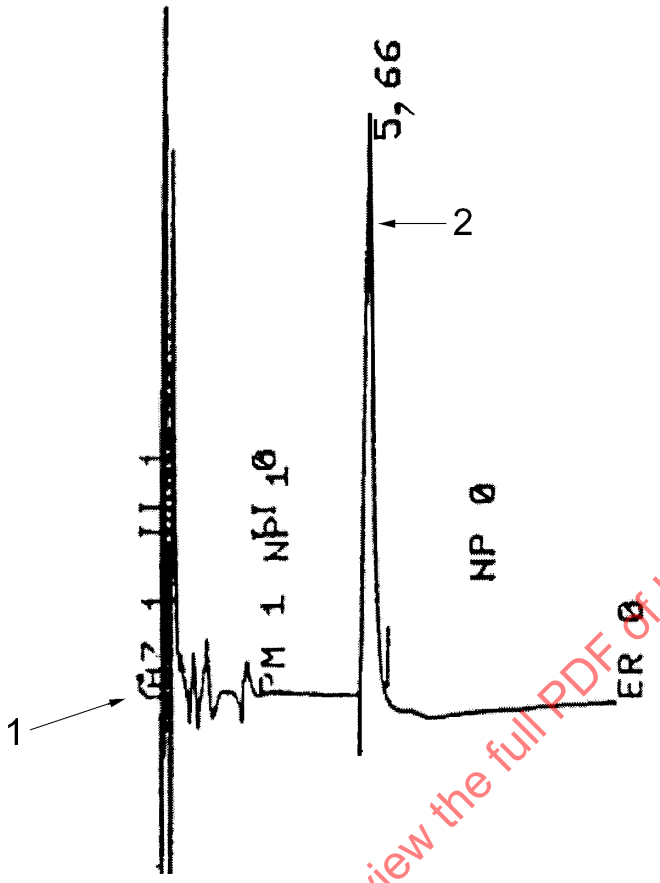
Inject the test portions (duplicates as obtained in 9.2). Wait again for 5 min after the elution of the iodide before the next injection. Measure the iodide peak heights or peak areas.

After 6 to 8 injections of test solutions, inject the 150 µg/l iodide standard working solution (5.2.2) again. The iodide peak height or area shall differ by no more than 5 % from the value obtained earlier.

Depending on the clean-up conditions, the quality of the HPLC solvents and the electrode behaviour, a negative dip below the baseline on the descending side of the iodide peak in the chromatogram may be observed. The integrator should be adjusted so that the negative part of the peak (below the original baseline) is not included in the peak height or peak area measurement.

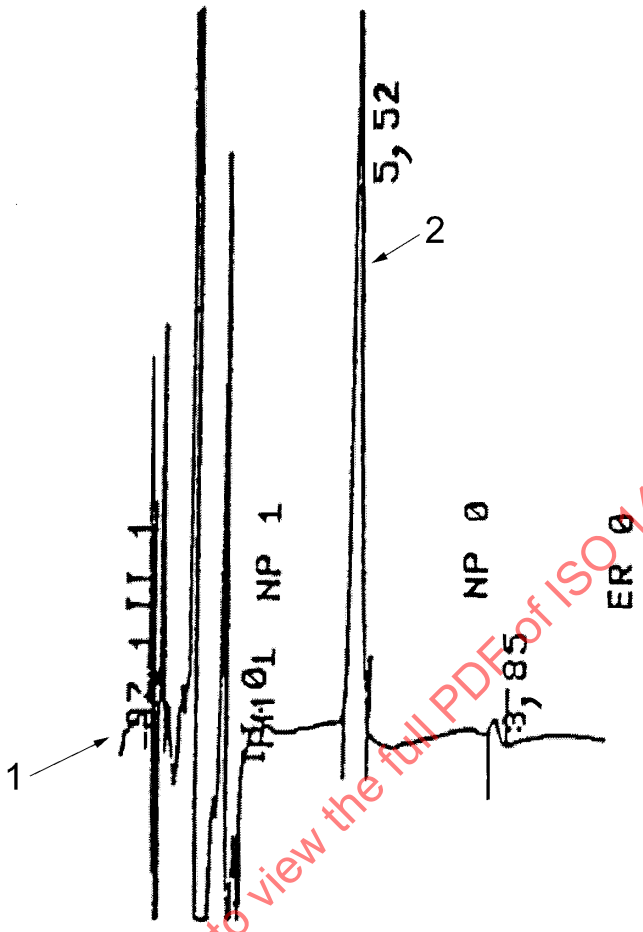
9.4 Preparation of calibration graph

Perform linear least-squares analysis on the relationship between the concentrations and signals obtained (peak heights or areas) for the four iodide standard working solutions. Do not include the zero point (0,0) in the calculation. The correlation coefficient should be $\geqslant$ 0,99.



a) Iodide standard (102 µg/l)

Figure 1 (*continued*)



b) Dried skimmed milk (2,3 µg/l)

Key

1 injection

2 iodide

NOTE Typical LC chromatograms of iodides in an iodide working standard solution and in a dried milk sample analysed on a Partisphere C-18 5 µm column, of internal diameter 4,7 mm, length 110 mm.

Figure 1 — Typical chromatograms of iodide solutions

10 Calculation and expression of results

10.1 Calculation

The iodide content, w_I, in micrograms per gram, is calculated by the equation:

$$w_I = \frac{\rho_t}{m} \times 0{,}1$$

where

ρ_t is the iodide content, in micrograms per litre, of the test solution calculated from the regression line or read from the calibration graph;

m is the mass, in grams, of the test portion (9.1).