INTERNATIONAL STANDARD

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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

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 3.

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 International Standard may be the subject of patent rights other than those identified above. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14378 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC International, and will also be published by these organizations.

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. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish safety and health 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 skim 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 skim milk. There are no reasons to expect that the method would not be applicable 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 might be bound organically in milk where microbiological deterioration has been occurred.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, this publication do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

3 Term and definition

For the purposes of this International Standard, the following term and definition applies.

3.1
iodide content of pasteurized whole milk or dried skim 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 grade 2 of ISO 3696.

5.2 Iodide standard solutions

WARNING: Aqueous solutions of iodides are unstable when exposed to light and shall be protected from light.

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 hydrogen phosphate and hexadecyltrimethylammonium chloride in a mixture of acetonitrile and water (68:32 by volume), pH = 6,8.

Dissolve 1,42 g of disodium hydrogen phosphate (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.

6.3 Micropipettes, capable of delivering 20 μl, 50 μl, 150 μl and 250 μl, respectively.

6.4 Graduated pipette, of capacity 2 ml, with 0,1 ml graduations.

6.5 Graduated cylinder, of capacity 500 ml.

6.6 pH-meter, with combined glass electrode.

6.7 Membrane filters, 1,2 μm and 0,5 μm, Nylon-6-6[1)], 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[1)] CF-25, or equivalent).

Prepare new membrane filter cones before use as follows. Soak in a mixture of ethanol and water (2:8 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 by volume). Remove the solvent before the next use of the cones as described above for new cones.

NOTE 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[2)], 5 μm, internal diameter 4,7 mm, length 110 mm, or equivalent.

6.12.4 Guard column (optional), SPHERI-5 C-18 cartridge,[3)] 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 (for example, Spectra Physics[4)] is suitable).

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1) Nylon 6-6, Amicon CS1A, Amicon Centreflo and Millipore Ultrafre-PF are examples of suitable products available commercially.

2) PARTISPHERE C-18 is an example of a suitable product available from Whatman Inc.

3) SPHERI-5 C-18 cartridge is an example of a suitable product available from Brownlee.

4) Spectra Physics is an example of a suitable product available commercially.

This information is given for the convenience of users of this International Standard and does not consitute an endorsement by ISO of these products.

7 Sampling

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

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

Store the sample in such a way that deterioration and change in its composition are prevented.

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 repeated 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). Dilute the sample 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. Dilute 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 900 *g* to 1000 *g* 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 note to 6.11.

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 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 (see 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 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 after the elution of the iodide for 5 min 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 0,99.

10 Calculation and expression of results

10.1 Calculation

The iodide content is calculated by the following equation:

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

where

w_I is the numerical value of the iodide content of the test sample, in micrograms per gram;

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

m is the numerical value of the mass of the test portion, in grams.