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**Milk and milk products — Determination  
of lactose content by high-performance  
liquid chromatography (Reference  
method)**

*Lait et produits laitiers — Détermination de la teneur en lactose par  
chromatographie liquide haute performance (Méthode de référence)*

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## Contents

Page

Foreword.....	iv
Foreword.....	v
1 Scope .....	1
2 Normative references .....	1
3 Terms and definitions.....	1
4 Principle .....	1
5 Reagents .....	1
6 Apparatus .....	2
7 Sampling.....	3
8 Preparation of test sample.....	3
9 Procedure .....	4
9.1 Preparation of the standard solution.....	4
9.2 Preparation of test portion.....	4
9.3 Preparation of filtrate.....	4
9.4 HPLC determination .....	5
10 Calculation and expression of results.....	6
10.1 Calculation.....	6
10.2 Expression of results .....	6
11 Precision.....	7
11.1 Interlaboratory test .....	7
11.2 Repeatability.....	7
11.3 Reproducibility.....	7
12 Test report .....	7
Annex A (informative) Results of interlaboratory test.....	8
Bibliography .....	10

## 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 22662|IDF 198 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.

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## 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.

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.

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 22662|IDF 198 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 *Lactose & lactate determination* of the Standing Committee on *Main components in milk* under the aegis of its project leader, Mr. R. Kouaouci (CA).

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# Milk and milk products — Determination of lactose content by high-performance liquid chromatography (Reference method)

## 1 Scope

This International Standard specifies the reference method for the determination of lactose content of raw milk, heat-treated milks, dried milk and raw and pasteurized cream.

The method is not applicable to fermented milks and milks to which oligosaccharides have been added.

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

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## 3 Terms and definitions

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

### 3.1

#### **lactose content**

mass fraction of substances determined by the procedure specified in this International Standard

NOTE The mass fraction can be expressed as a percentage.

## 4 Principle

An internal standard [D(+)-melezitose] is added to a weighed volume of milk and to lactose standards. A chemical reagent (Biggs-Szijarto solution) is added to precipitate out the fat and the protein component fractions of milk. The sample is filtered twice prior to injection, first through paper filter and then through a 0,45 µm nylon filter. The lactose and the internal standard are separated by a cation exchange column in the lead form and detected by a differential refractometer detector or other suitable detector. As mobile phase, HPLC grade water is used.

## 5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

### 5.1 Degassed HPLC grade water.

Filter the water, conforming to the requirements of ISO 3696, Grade 1, obtained from the water purification unit (6.9) using the solvent filtration unit (6.10). To improve the pump performance and to obtain a stable baseline, degas the mobile phase daily by selecting one of the available techniques such as sparging with helium, sonication, vacuum or in-line degassing system.

### 5.2 D(+)-Melezitose hydrate solution, $c(\text{C}_{18}\text{H}_{32}\text{O}_{16}\cdot\text{H}_2\text{O}) = 50 \text{ mg/ml}$ .

Dissolve an amount of D(+)-melezitose hydrate in water (5.1) to give a final concentration equivalent to 50 mg/ml of the anhydrous form.

The D(+)-melezitose solution can be stored at 4 °C for no longer than 1 week.

### 5.3 $\alpha$ -Lactose monohydrate, $\text{C}_{12}\text{H}_{22}\text{O}_{11}\cdot\text{H}_2\text{O}$ .

Before use, dry the  $\alpha$ -lactose monohydrate at 70 °C for 4 h. Cool it to room temperature in a desiccator.

NOTE After drying, the lactose remains in the monohydrate form.

### 5.4 Biggs-Szijarto solution.

Dissolve 25 g of zinc acetate dihydrate,  $\text{Zn}(\text{CH}_3\text{COO})_2\cdot 2\text{H}_2\text{O}$  and 12,5 g of phosphotungstic acid monohydrate ( $\text{W}_{12}\text{O}_{36}\cdot\text{H}_3\text{PO}_4\cdot\text{H}_2\text{O}$ ) in about 100 ml of HPLC grade water (5.1) in a 200 ml one-mark volumetric flask.

Add 20 ml of glacial acetic acid ( $\text{CH}_3\text{COOH}$ ). Dilute to the 200 ml mark with HPLC grade water (5.1) and mix. After use, the solution may be stored at 4 °C for no longer than 1 week.

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## 6 Apparatus

Usual laboratory equipment and, in particular, the following.

**6.1 HPLC ion exchange resin column**, of length 300 mm, of internal diameter 7,8 mm, with 8 % cross-linked copolymer, based on polystyrene-divinylbenzene cation exchange resin, packed in the lead form.

**6.2 Guard column.**

In order to prolong ion exchange resin column life, replace the guard column after about 200 injections.

**6.3 Micro-guard holder.**

**6.4 Column heater**, capable of maintaining a constant temperature of 85 °C  $\pm$  1 °C.

**6.5 HPLC pump**, capable of maintaining a flow rate of between 0 ml/min and 10 ml/min.

**6.6 HPLC autosampler.**

NOTE Manual injection can also be used.

**6.7 Differential refractometer detector**, highly sensitive.

NOTE Other detectors, e.g. an evaporative light scattering detector, can also be used.



**6.8 Software**, capable of: automating injections, performing data acquisition, processing, and managing chromatographic information.

**6.9 Water purification unit**, capable of providing water complying with grade 1 requirements of ISO 3696, with a resistivity of between 10 M $\Omega$ ·cm and 18 M $\Omega$ ·cm.

**6.10 Solvent filtration unit**, including a vacuum source, with a membrane filter of 0,45  $\mu$ m pore size and of diameter 47 mm.

**6.11 Analytical balance**, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

**6.12 Water bath**, capable of maintaining a temperature of between 38 °C and 40 °C.

**6.13 Accurate dispenser, accurate automatic pipette, or one-mark pipettes** conforming to the requirements of ISO 648, Class A, of capacity 2 ml.

**6.14 Filter funnel**, of diameter 75 mm.

**6.15 Filter paper**, of diameter 110 mm, Whatman<sup>1)</sup> No 1 or equivalent.

**6.16 Nylon syringe filter**, of porosity 0,45  $\mu$ m.

NOTE An in-line filter of the same porosity may also be used.

**6.17 Syringe**, with Luer-lock, of capacity 5 ml.

**6.18 HPLC vials**, with caps.

**6.19 One-mark volumetric flasks**, of capacity 10 ml  $\pm$  0,02 ml.

NOTE Flasks with a capacity of more than 10 ml can also be used by taking into account the concentration factor.

## 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

For fluid milk and cream, warm the test sample in the water bath (6.12) to between 38 °C and 40 °C. Gently mix the test sample by repeatedly inverting the bottle. Cool the sample quickly to 20 °C  $\pm$  1 °C while gently mixing the sample immediately prior to weighing the test portion (9.2).

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1) Whatman 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 constitute an endorsement by ISO or by IDF of this product.