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**Milk and milk products — Determination  
of furosine content — Ion-pair reverse-  
phase high-performance liquid  
chromatography method**

*Lait et produits laitiers — Détermination de la teneur en furosine —  
Méthode par chromatographie liquide à haute performance en phase  
inverse par paire d'ions*

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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.

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 18329|IDF 193 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

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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 and AOAC International 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 IDF National Committees casting a vote.

International Standard ISO 18329|IDF 193 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Characterization of heat treatment*, of the Standing Committee on *Minor components and characterization of physical properties*, under the aegis of its project leader, Prof. P. Resmini (IT).

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# Milk and milk products — Determination of furosine content — Ion-pair reverse-phase high-performance liquid chromatography method

## 1 Scope

This International Standard specifies a method for the quantitative determination of furosine ( $\epsilon$ -furoylmethyl-lysine) in milk and milk products. The method is particularly applicable to raw or heat-treated milk and to cheese.

## 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 8968-1 | IDF 20-1, *Milk — Determination of nitrogen content — Part 1 — Kjeldahl method*

[ISO 18329:2004](https://standards.iteh.ai/catalog/standards/sist/e17f78a6-2b12-4f5d-b1df-57bab0e8ca44/iso-18329-2004)

## 3 Terms and definitions

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

### 3.1

#### **furosine content**

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

NOTE Furosine content is expressed in milligrams per 100 g of protein.

## 4 Principle

The first stable “Maillard Reaction” (MR) product formed in milk and in cheese,  $\epsilon$ -lactulosyl-lysine, is partially converted by warm acid-hydrolysis into furosine, the determination of which allows the extent of the early stage of MR to be evaluated. The MR extent is related to the type and intensity of heat treatments applied both to raw material and in processing. The determination of furosine is performed by ion-pair reverse-phase (IP-RP) HPLC with UV detection at 280 nm. Quantification of furosine is obtained by reference to a standard sample of furosine.

## 5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

### 5.1 Hydrochloric acid (HCl), fuming, with a mass fraction 37 %.

**5.2 Hydrochloric acid solution I**,  $c(\text{HCl}) = 10,6 \text{ mol/l}$ .

Mix 8 volumes of hydrochloric acid (5.1) with 1 volume of water to obtain hydrochloric acid solution I.

**5.3 Hydrochloric acid solution II**,  $c(\text{HCl}) = 8,0 \text{ mol/l}$ .

Mix 2 volumes of hydrochloric acid (5.1) with 1 volume of water to obtain hydrochloric acid solution II.

**5.4 Hydrochloric acid solution III**,  $c(\text{HCl}) = 3,0 \text{ mol/l}$ .

Mix 1 volume of hydrochloric acid (5.1) with 3 volumes of water to obtain hydrochloric acid solution III.

**5.5 Methanol** ( $\text{CH}_3\text{OH}$ ).

**5.6 HPLC elution solvents**

Prepare HPLC elution solvents by using HPLC-grade reagents.

**5.6.1 Water**, of HPLC-grade.

**5.6.2 Dilute acetic acid** ( $\text{CH}_3\text{CO}_2\text{H}$ )

Dilute 4 ml of glacial acetic acid with water to 1 000 ml.

**5.6.3 Potassium chloride solution**,  $c(\text{KCl}) = 3 \text{ g/l}$ .

Dissolve 3 g of potassium chloride in 1 000 ml of dilute acetic acid (5.6.2).

**5.7 Furosine**, (e.g. Neosystem<sup>1)</sup> or equivalent). [ISO 18329:2004](https://standards.iteh.ai/catalog/standards/sist/e17f78a6-2b12-4f5d-b1df-67b10e8e415a/iso-18329-2004)  
<https://standards.iteh.ai/catalog/standards/sist/e17f78a6-2b12-4f5d-b1df-67b10e8e415a/iso-18329-2004>

**5.8 Furosine standard solution**,  $c[\epsilon\text{-N-(2-furoylmethyl)-L-lysine}] = 1 \text{ nmol/ml}$  (approx.).

Dissolve 15 mg of furosine (5.7) in 25 ml of hydrochloric acid solution III (5.4) and mix. Dilute 5 ml of this solution with the hydrochloric acid solution III (5.4) to 100 ml. Mix again to obtain dilution 1. Dilute 1 ml of dilution 1 with hydrochloric acid solution III (5.4) to 100 ml to obtain a furosine standard solution of about 1 nmol/ml furosine.

Calculate the exact concentration of furosine in the final furosine standard solution on the basis of the net content declared for the commercial product.

The furosine standard solution remains stable for 24 months, if stored at  $-20^\circ\text{C}$ .

**5.9 Nitrogen**, gas chromatography purity.

## 6 Apparatus

Usual laboratory equipment and, in particular, the following.

**6.1 C 18 cartridge**, or minicolumn (500 mg), used in the solid-phase extraction.

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1) Furosine is supplied by Neosystem, Rue de Bologne 7, 67100 Strasbourg, France. This 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 IDF of this product.



**6.2 HPLC equipment**, provided with gradient pumping system, metal-free injector with injection loop between 20 µl and 50 µl, and a thermostatic column oven.

**6.3 UV-detector**, capable of operating at 280 nm wavelength with minimum AUFS of 0,010 or lower.

Under these chromatographic conditions and with an injection of 10 pmol of furosine, the signal-to-noise ratio shall be higher than 10.

**6.4 “Furosine dedicated” column**, of diameter 4,6 mm and of length 250 mm, 5 µm particle size (e.g. Alltech<sup>2)</sup>), or an equivalent column capable of separating the furosine peak on the baseline without interfering with other peaks.

**6.5 Integrator**, or data-reprocessing software, capable of measuring the peak areas.

**6.6 Analytical balance**, capable of weighing to the nearest 1 mg.

**6.7 Oven**, capable of being maintained at 110 °C ± 2 °C.

**6.8 Screw-cap Pyrex<sup>3)</sup> vials**, or other heat-resistant sealing vials.

**6.9 Paper filters**, of medium porosity.

**6.10 Glass syringe**, of capacity of 10 ml.

**6.11 Kjeldahl apparatus**, conforming to ISO 8968-1/IDF 20-1.

## 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.

## 8 Preparation of test portion

### 8.1 Milk

Pipette 2 ml of test sample into a screw-cap Pyrex-vial (6.8). Add 6 ml of hydrochloric solution I (5.2) and mix.

### 8.2 Cheese

Weigh an aliquot of test sample corresponding to an amount of 40 mg to 50 mg of protein into a screw-cap vial (6.8). Add 8 ml of hydrochloric solution II (5.3) and mix.

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2) The “Furosine dedicated” column is supplied by Alltech-Europe, Laarne, Belgium.

3) Pyrex 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 IDF of these products.