
**Cheese — Determination of propionic
acid level by chromatography —**

**Part 2:
Method by ion exchange
chromatography**

*Fromages — Détermination de la teneur en acide propionique par
chromatographie —*

Partie 2: Méthode par chromatographie par échange d'ions

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Forewords

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document 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.

A list of all parts in the ISO/TS 19046 | IDF/RM 233 series can be found on the ISO website.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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ISO/TS 19046-2 | IDF/RM 233-2 was prepared by the IDF Standing Committee on *Analytical Methods for Composition* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*.

The IDF Reviewed method is equal to an ISO Publicly Available Specification (ISO/PAS) or an ISO Technical Specification (ISO/TS) and is therefore published jointly under ISO conditions.

The work was carried out by the IDF/ISO Project Group on Propionic acid (C25) of the Standing Committee on *Analytical Methods for Composition* under the aegis of its project leader P. Trossat (FR).

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Cheese — Determination of propionic acid level by chromatography —

Part 2:

Method by ion exchange chromatography

WARNING — This document can involve the use of products and implementation of procedures and equipment of a hazardous nature. This document does not aim to address all the risks related to its use. It is the responsibility of the user of this document to establish appropriate hygiene and safety practices before using it, and to determine the applicability of any other restrictions.

1 Scope

This document specifies a method for the determination of propionic acid level in cheese, using ion exchange chromatography.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

3.1

level of propionic acid

mass fraction of propionic acid determined following the procedure described in this document

Note 1 to entry: The level of propionic acid is expressed in mg/100 g of cheese.

4 Principle

Aqueous extraction of propionic acid by dilution of samples in the mobile phase, centrifugation of the solution obtained, and filtration and assay of the propionic acid in the filtrate by ion exchange chromatography.

5 Reagents

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

5.1 Propionic acid [CH₃ CH₂COOH] of purity ≥ 99,5 % mass fraction.

5.2 Sulfuric acid [H₂SO₄] containing a mass/volume fraction of 98 %.

5.3 Water, high performance liquid chromatography (HPLC) grade.

5.4 Mobile phase

Weigh exactly 10,1 g of sulfuric acid (5.2) and transfer into a 5 000 ml flask (6.3) already containing 2 l of water (5.3), make up to the mark with water (5.3) and filter using a vacuum filtration system (6.7).

5.5 Standard solutions

5.5.1 Standard stock solution of propionic acid

Weigh exactly 200 mg of propionic acid (5.1) in a 100 ml one mark volumetric flask (6.3). Dilute to the mark with the mobile phase (5.4) and filter standard stock solution using a vacuum filtration system (6.7).

5.5.2 Standard solutions of propionic acid

Prepare two additional standard solutions by diluting standard stock solution (5.5.1) in mobile phase (5.4).

- 25 ml of standard stock solution in a 50 ml one mark volumetric flask (6.3). Dilute to the mark with the mobile phase (5.4).
- 25 ml of standard stock solution in a 100 ml one mark volumetric flask (6.3). Dilute to the mark with the mobile phase (5.4).

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Grinding or grating device.

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

6.3 Volumetric flasks, of capacity 50 ml, 100 ml and 5 000 ml.

6.4 Single-use syringes, 1 ml capacity equipped with a 0,2 µm polyvinylidene fluoride (PVDF) syringe filter.

6.5 Hot plate stirrer.

6.6 Laboratory centrifuge, capable to produce a centrifugal acceleration of approximately 13 000*g*.

6.7 Vacuum filtration system, equipped with a 0,2 µm filtration membrane.

6.8 HPLC system, with the following equipment.

6.8.1 Column oven, set at 50 °C.

6.8.2 Ion exchange column, Aminex HPX 87 H BIO RAD¹⁾ (column size: 300 mm × 7,8 mm, particle size: 9 µm) equipped with a Carbo H BIO RAD precolumn or equivalent.

1) Aminex HPX 87 H is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

6.8.3 Chromatographic conditions

- Mobile phase: H₂SO₄ 0,02 mol/l (5.4) kept under helium at 20 ml/min;
- flow rate: 0,6 ml/min;
- quantity injected: 10 µl;
- rinse under H₂SO₄ 0,02 mol/l, inverted column, for 4 h (needed after each analytical series).

An example of the HPLC chromatogram profile obtained with these conditions is shown in [Annex A](#).

6.8.4 UV detector, set at 210 nm.

6.8.5 Injection syringe or injection system, capable to inject 10 µl.

6.8.6 Integration system, preferably being computerized.

7 Sampling

Sampling is not part of the method specified in this document. A recommended sampling method is given in ISO 707 | IDF 50.

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

8 Preparation of test sample

Prior to analysis, grind or grate the test sample by using an appropriate grinding or grating device (6.1), if necessary, after removing the rind, the smear or the mouldy surface layer of the cheese.

Weigh, to the nearest 0,1 mg, about 5 g to 10 g of cheese in a glass flask and record the mass m_1 . Add around 35 ml of mobile phase (5.4) to the test sample.

- Mix with a magnetic stirrer (6.5) for 1 h at 70 °C.
- Cool down the solution and transfer into a 50 ml one mark volumetric flask. Dilute to the mark with the mobile phase (5.4).

Filter the solution using a 0,2 µm syringe filter (6.4).

NOTE A centrifugation at 13 000g for 10 min can be applied if difficulties are met with the direct filtration.

9 Quantitative determination by HPLC

9.1 Calibration

Inject 10 µl of the three standard solutions (5.5) into the HPLC system (6.8) applying chromatographic conditions described in 6.8.3 and calibrate according the manufacturer requirements.

9.2 Sample test

Inject 10 µl of the test sample prepared as specified in [Clause 8](#) into the HPLC system (6.8) applying the chromatographic conditions described in 6.8.3.

10 Calculation and expression of results

10.1 Calculation of propionic acid in cheese

Calculate the mass fraction of propionic acid, w_e , expressed in mg/100 g of cheese using [Formula \(1\)](#):

$$w_e = P_e \times \frac{50}{m_1} \quad (1)$$

where

w_e is the mass fraction of propionic acid, in mg/100 g, of cheese;

P_e is the concentration of propionic acid, in g/100 ml, of the cheese-mobile phase mixture calculated by the integrator after calibration;

50 is the final volume of cheese-mobile phase mixture, in ml;

m_1 is the mass of the test sample, in g.

10.2 Expression of results

The results are expressed in mg/100 g of cheese, without decimals.

11 Precision

11.1 Interlaboratory test

The values for the repeatability and reproducibility are derived from the results of an interlaboratory test carried out in accordance with ISO 5725-1 and ISO 5725-2 by three laboratories (see [Annex B](#)) and therefore shall be considered as indicative only.

11.2 Repeatability

An indicative value of absolute difference between two single test results obtained with 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 of 48 mg of propionic acid/100 g of cheese should be considered.

12 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this document, i.e. ISO/TS 19046-2 | IDF/RM 233-2;
- all operating details not specified in this document, or regarded as optional, together with details of any incident which might have affected the result(s);
- the test result(s) obtained and, if the repeatability has been checked, the final quoted result obtained.

Annex A (informative)

Example of HPLC chromatogram

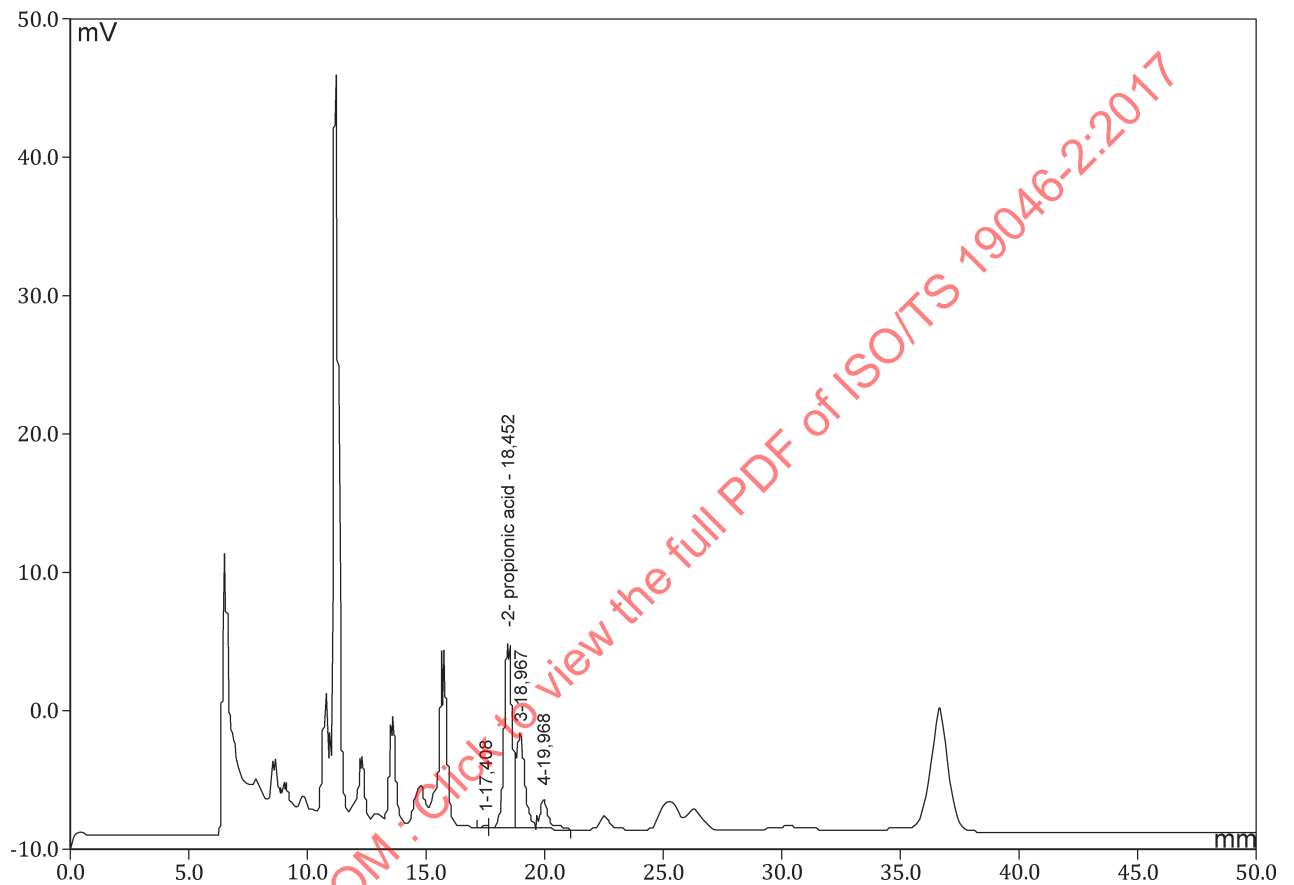


Figure A.1 — Example of HPLC chromatogram