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**Cheese, cheese rind and processed  
cheese — Determination of natamycin  
content —**

Part 2:  
**High-performance liquid  
chromatographic method for cheese,  
cheese rind and processed cheese**

*Fromage, croûte de fromage et fromages fondus — Détermination de  
la teneur en natamycine —*

*Partie 2: Méthode par chromatographie liquide à haute performance  
pour fromage, croûte de fromage et fromages fondus*

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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.

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](http://www.iso.org/directives)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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](http://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.

This second edition cancels and replaces the first edition (ISO 9233-2 | IDF 140-2:2007), of which it constitutes a minor revision to incorporate the amendment ISO 9233-2:2007/Amd.1:2012.

A list of all parts in the ISO 9233 | IDF 140 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.

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

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This document was prepared by the IDF Standing Committee on *Analytical methods for additives and contaminants* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

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# Cheese, cheese rind and processed cheese — Determination of natamycin content —

## Part 2:

## High-performance liquid chromatographic method for cheese, cheese rind and processed cheese

### 1 Scope

This document specifies a method for the determination of natamycin mass fraction in cheese, cheese rind and processed cheese of above 0,5 mg/kg and of the surface-area-related natamycin mass in cheese rind of above 0,03 mg/dm<sup>2</sup>.

### 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 <https://www.iso.org/obp>

#### 3.1

##### **natamycin content**

mass fraction of substances determined by the procedure specified in this document

Note 1 to entry: The natamycin content is expressed in milligrams per kilogram.

#### 3.2

##### **surface-area-related natamycin mass in cheese rind**

surface-area-related mass of substances determined by the procedure specified in this document

Note 1 to entry: The surface-area-related natamycin mass is expressed in milligrams of natamycin per square decimetre of cheese rind.

#### 3.3

##### **cheese rind**

outer layer of the cheese, excluding the coating layer, if present

### 4 Principle

A known quantity of sample is extracted with methanol. The extract is diluted with water followed by cooling to between -15 °C and -20 °C to precipitate most of the fat, followed by filtration. The natamycin content or surface-area-related natamycin mass is determined in the filtrate (after concentration, if necessary) by high-performance liquid chromatography (HPLC).

## 5 Reagents

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

### 5.1 Methanol ( $\text{CH}_3\text{OH}$ ).

### 5.2 Methanol, aqueous solution.

Mix two volumes of methanol (5.1) with one volume of water.

### 5.3 Natamycin standard stock solution, of concentration 500 mg/l.

Immediately before use, dissolve in methanol (5.1) a quantity of a natamycin preparation of known natamycin content, corresponding to 50 mg of pure natamycin ( $\text{C}_{33}\text{H}_{47}\text{NO}_{13}$ ), in a 100 ml one-mark volumetric flask (6.1). Make up to the mark with water and mix.

### 5.4 Natamycin standard working solution, of concentration 5 mg/l.

Pipette 5,0 ml of the natamycin standard stock solution (5.3) in a 50 ml one-mark volumetric flask (6.1). Dilute to the mark with aqueous methanol (5.2) and mix.

Pipette 5,0 ml of the thus diluted solution into another 50 ml one-mark volumetric flask (6.1). Dilute to the mark with aqueous methanol (5.2) and mix. The concentration of this natamycin standard working solution is 5  $\mu\text{g}/\text{ml}$ .

This concentration shall be close to that of the test solution measured in 9.3.3. Adjust the standard working dilution by pipetting and diluting another quantity, if required.

### 5.5 Acetic acid ( $\text{CH}_3\text{CO}_2\text{H}$ ), glacial.

## 6 Apparatus

Usual laboratory equipment and, in particular, the following.

### 6.1 One-mark volumetric flasks, of capacities 50 ml and 100 ml.

### 6.2 Slicer or similar apparatus, capable of cutting cheese portions of thickness 5 mm and of width about 30 mm (Figure A.1 shows an example).

### 6.3 Fine slicer, capable of cutting thin cheese slices of maximum thickness 1 mm (Figure A.2 shows an example).

### 6.4 Grinder or blender.

### 6.5 Sharp knife, capable of cutting cheese slices into small pieces.

### 6.6 Magnetic stirrer or shaking machine.

### 6.7 Conical flasks, of capacities 100 ml and 200 ml, made of coloured glass and fitted with ground-glass stoppers.

### 6.8 Syringes, disposable, of capacity 10 ml.

**6.9 Membrane microfilters**, of pore size 0,20 µm and 0,45 µm, resistant to attack by alcoholic solutions.

**6.10 Folded paper filters, fast speed**, of diameter 150 mm. (e.g. S and S, No. 595 1/2<sup>1</sup>).

**6.11 Funnel**, of diameter approximately 70 mm.

**6.12 Freezer**, capable of freezing at a temperature of between –15 °C and –20 °C.

**6.13 Extraction cartridges**, to concentrate the filtered extract, if necessary (e.g. Sep-pack C18<sup>1</sup>) or Waters No. 51910<sup>1</sup>).

**6.14 Liquid chromatograph**, with UV detector, capable of measuring at 303 nm and equipped with a recorder and/or integrator.

**6.15 Analytical column**, of length 150 mm, of internal diameter 4,6 mm, type C8, having a particle size of 5 µm (e.g. Lichrosorb RP8<sup>1</sup>).

**6.16 Guard column**, of length 100 mm, of internal diameter 2,1 mm, type C8, having a particle size of 30 µm to 40 µm (e.g. Perisorb RP8<sup>1</sup>).

**6.17 Sample jar**, of suitable capacity.

## 7 Sampling

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

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

The laboratory sample shall be a whole cheese, or a segment of a cheese representative of the whole.

## 8 Preparation of test sample

### 8.1 Cheese rind

If necessary, cut the test sample into sectors or smaller portions so that the width of the cheese rind is not more than about 30 mm. Using the slicer (6.2), remove the whole rind from all obtained sectors or portions by slicing off a maximum thickness of 5 mm excluding coating layer if present.

NOTE This document can also be used for analysis of cheese rind plus coating layer.

From the rind obtained, cut, using a sharp knife (6.5), a rectangular piece of area between 2 dm<sup>2</sup> and 4 dm<sup>2</sup>. Determine its surface area, in square decimetres, and its mass, in kilograms.

Grind (6.4) carefully the whole rind, including the weighed and measured piece, and mix thoroughly. Immediately transfer a quantity of the sample thus prepared to a sample jar (6.17).

After preparing each test sample, clean all tools that have been in contact with the sample with hot water and then with methanol (5.1). Dry all tools thoroughly, e.g. by using a stream of compressed air.

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1) 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 by IDF of this product.

## 8.2 Cheese interior and processed cheese

After removing the rind (8.1), use the fine slicer (6.3) to remove a slice of maximum thickness 1 mm from the whole of the outer section of the test sample.

Cut all cheese slices into small pieces of about 50 mm<sup>2</sup> and mix thoroughly. Immediately transfer a quantity of the sample thus prepared to a sample jar (6.17).

After preparing each test sample, clean all tools that have been in contact with the test sample with hot water and then with methanol (5.1). Dry all tools thoroughly, e.g. by using a stream of compressed air.

## 9 Procedure

### 9.1 Test portion

#### 9.1.1 Cheese rind

Weigh, to the nearest 10 mg, approximately 10,00 g of test sample (8.1) into a 200 ml conical flask (6.7).

#### 9.1.2 Cheese interior and processed cheese

Weigh, to the nearest 10 mg, approximately 5,00 g of test sample (8.2) into a 100 ml conical flask (6.7).

### 9.2 Preparation of test solution

#### 9.2.1 Cheese rind

##### 9.2.1.1 Initial steps

Add 100 ml of methanol (5.1) to the test portion in the conical flask (9.1.1). Stir the contents of the conical flask for 90 min with a magnetic stirrer (6.6) or shake for 90 min in a shaking machine (6.6).

Add 50 ml water. Immediately place the conical flask in the freezer (6.12) for about 60 min.

##### 9.2.1.2 Filtration

Filter the cold extract through a folded filter paper (6.10) while discarding the first 5 ml of filtrate. The filtration should be carried out while the suspension is still cold to avoid dissolution of the fat and consequently turbid filtrates.

Bring the filtrate to room temperature. Take a portion of the filtrate in a syringe (6.8). Filter through a membrane microfilter of pore size 0,45 µm (6.9) and then through a membrane microfilter of pore size 0,20 µm (6.9).

The minimum amount of test solution (filtrate) required is 20 µl per injection for direct chromatographic measurement (9.3.4), and 25 ml or 50 ml for measurement at 5 or 10 times concentration (9.3.5), respectively.

#### 9.2.2 Cheese interior and processed cheese

##### 9.2.2.1 Initial steps

Use a measuring cylinder to add 50 ml of methanol (5.1) to the test portion in the conical flask (9.1.2). Stir the contents of the conical flask for 90 min with a magnetic stirrer (6.6) or shake for 90 min in a shaking machine (6.6).