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

**Part 1:
Molecular absorption spectrometric
method for cheese rind**

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

*Partie 1: Méthode par spectrométrie d'absorption moléculaire pour
croûte de fromage*



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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).

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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.

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

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

Part 1:

Molecular absorption spectrometric method for cheese rind

1 Scope

This document specifies a method for the determination in cheese rind of natamycin mass fraction of above 0,5 mg/kg and surface-area-related natamycin mass of above 0,03 mg/dm².

NOTE It is possible that the method is suitable for detecting migration of natamycin into the cheese.

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 molecular absorption spectrometry.

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 (CH_3OH).

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 natamycin standard stock solution (5.3) into 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.

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 1/2¹⁾).

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 C18¹⁾ or Waters No. 519101¹⁾).

6.14 Spectrometer, suitable for recording an ultraviolet (UV) spectrum between 300 nm and 340 nm, equipped with cells of optical path length 10 mm and a recorder.

6.15 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 the 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 dm² and 4 dm². 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 (6.15).

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.

8.2 Cheese interior

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 mm² and mix thoroughly. Immediately transfer a quantity of the sample thus prepared to a sample jar (6.15).

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.

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.

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

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 3 ml for direct measurement (9.3.3) and 25 ml or 50 ml for measurement at 5 or 10 times concentration (9.3.4), respectively.

9.2.2 Cheese interior

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).

Use a measuring cylinder to add 25 ml of water. Immediately place the conical flask in the freezer (6.12) for about 60 min.

9.2.2.2 Filtration

Filter the solution as described in 9.2.1.2.

9.3 Determination

9.3.1 Determination and detection limits

The laboratory applying the method shall establish the limits of detection and determination under its own instrumental conditions using recognized calculation methods to verify that natamycin can be determined down to levels of 0,5 mg/kg and 0,03 mg/dm².

9.3.2 UV absorbance of natamycin standard working solution

Record the spectrum of the natamycin standard working solution (5.4) in the range 300 nm to 340 nm. Measure the absorbance of natamycin at its maximum at about 317 nm, at its minimum at about 311 nm, and at 329 nm exactly. Use aqueous methanol (5.2) as blank.

An example of a natamycin standard working solution spectrum is shown in Figure A.3.

Because natamycin is unstable in aqueous methanol, carry out the measurement as rapidly as possible.

9.3.3 Test solution

Record the spectra of the test solutions (9.2.1.2 or 9.3.4.2 and 9.2.2.2) with aqueous methanol (5.2) as blank in the range 300 nm to 340 nm. Additionally, record in the same range the spectrum of the cheese rind test solution (9.2.1.2 or 9.3.4.2) with the cheese interior test solution (9.2.2.2) as blank.

Record the absorbance of the cheese rind test solution (9.2.1.2 or 9.3.4.2) with the cheese interior test solution (9.2.2.2) as blank at its maximum absorbance at about 317 nm, at its minimum at about 311 nm, and at 329 nm exactly. Examples of test solution spectra are shown in Figure A.4.

If the natamycin content of the test sample (8.1) is so low that no detection is possible or almost impossible (signal-to-noise ratio less than 3), but its determination is nevertheless required, proceed in accordance with 9.3.4.

NOTE The presence of spices, particularly pepper, in the cheese can interfere with the result, which might also be shown by an obvious distortion of the absorbance curve. Examples of spectra of various test solutions of cheeses are given in Figure A.4.

9.3.4 Low natamycin content

9.3.4.1 Concentration

Decide whether a concentration of about 5 times or about 10 times is desired. Base that decision on the result obtained in 9.3.3 and on the required limit of determination.

Then pipette 25 ml or 50 ml (for concentration times 5 or concentration times 10, respectively) of test solution (9.2.1.2) into a beaker. Add, depending on the concentration desired, 50 ml or 100 ml of water, respectively, and mix.

Activate an extraction cartridge (6.13) by using 3 ml to 5 ml of methanol (5.1). Then wash with 10 ml of water.

Pass the diluted test solution through the cartridge at a speed of 3 ml/min to 5 ml/min with the aid of a syringe (6.8). Rinse the cartridge with 10 ml of water with the aid of a syringe (6.8). Elute the natamycin with 3 ml of methanol (5.1) with the aid of a syringe (6.8).

9.3.4.2 Spectrometric measurement

Add 1,5 ml of water to the eluate (9.3.4.1) and mix. Aspirate the solution into a syringe (6.8). Filter the solution through a microfilter of pore size 0,45 µm (6.9), and then through a microfilter of pore size 0,20 µm (6.9), into a cell.

Proceed as in [9.3.3](#).

10 Calculation and expression of results

10.1 Calculation of natamycin mass fraction

Calculate the natamycin content as a mass fraction, w , in milligrams per kilogram, of the test sample by using [Formula \(1\)](#):

$$w = \frac{A_t \times c_n \times V}{A_s \times m} \quad (1)$$

where

- A_t is the numerical value of the net absorbance difference of the cheese rind test solution measured against the test solution of the interior of the cheese at about 317 nm ([9.3.3](#));
- c_n is the concentration, in micrograms per millilitre, of natamycin in the natamycin standard working solution ([5.4](#));
- V is the total volume, in millilitres, of the test solution ([9.2.1.2](#));
- A_s is the numerical value of the net absorbance of the natamycin standard working solution at about 317 nm ([9.3.2](#));
- m is the mass, in grams, of the test portion ([9.1](#)).

10.2 Calculation of absorbance

A_s can be taken from the UV spectrum of the natamycin standard working solution (see, for example, [Figure A.3](#)), using the straight line between the absorbance at about 311 nm and that at 329 nm as a baseline, or can be calculated by using [Formula \(2\)](#):

$$A_s = A_{s1} - \frac{2}{3}A_{s2} - \frac{1}{3}A_{s3} \quad (2)$$

where

- A_{s1} is the numerical value of the maximum absorbance of the natamycin standard working solution at about 317 nm ([9.3.2](#));
- A_{s2} is the numerical value of the minimum absorbance of the natamycin standard working solution at about 311 nm ([9.3.2](#));
- A_{s3} is the numerical value of the absorbance of the natamycin standard working solution at 329 nm ([9.3.2](#)).

A_t can be taken from the UV spectrum of the cheese rind test solution measured against the cheese interior test solution, using the straight line between the absorbance at about 311 nm and that at 329 nm as a baseline, or can be calculated by using [Formula \(3\)](#):

$$A_t = A_{t1} - \frac{2}{3}A_{t2} - \frac{1}{3}A_{t3} \quad (3)$$

where

- A_{t1} is the numerical value of the maximum absorbance of the cheese rind test solution measured against the cheese interior test solution at about 317 nm (9.3.3);
- A_{t2} is the numerical value of the minimum absorbance of the cheese rind test solution measured against the cheese interior test solution at about 311 nm (9.3.3);
- A_{t3} is the numerical value of the absorbance of the cheese rind test solution measured against the cheese interior test solution at 329 nm (9.3.3).

10.3 Calculation of surface-area-related natamycin mass

The surface-area-related natamycin mass, $m_{A,n}$, in milligrams per square decimetre, is calculated by using Formula (4):

$$m_{A,n} = w \times \frac{m}{A} \quad (4)$$

where

- w is the natamycin mass fraction, in milligrams per kilogram, of the test sample (10.1);
- m is the mass, in kilograms, of the weighed piece of the test sample (8.1);
- A is the area, in square decimetres, of the weighed piece of the test sample (8.1).

10.4 Correction of results

If the filtered extract has been concentrated as in 9.3.4, correct the test results obtained for w (10.1) and $m_{A,n}$ (10.3) as follows:

- a) for approximately 5 times concentration, divide the result obtained by 5,6;
- b) for approximately 10 times concentration, divide the result obtained by 11,1.

If determination in duplicate is required, and provided that the requirements for repeatability are satisfied, take as the final natamycin content of the test sample the arithmetic mean of two determinations obtained as specified in Clause 11, rounded to the first decimal place.

10.5 Expression of results

Express the test results to one decimal place.

11 Precision

11.1 Interlaboratory tests

The values for repeatability and reproducibility have been derived from the results of an interlaboratory test in accordance with ISO 5725:1986 (for results, see Reference [4]).

11.2 Repeatability

The absolute difference between two independent single test results, obtained using 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, will in not more than 5 % of cases be greater than the values indicated in Table B.1.

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the values indicated in [Table B.1](#).

12 Test report

The test report shall specify:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, with reference to this document, i.e. ISO 9233-1 | IDF 140-1;
- d) all operational details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- e) the test result(s) obtained, and, if the repeatability has been checked, the final quoted result obtained.

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Annex A (informative)

Examples

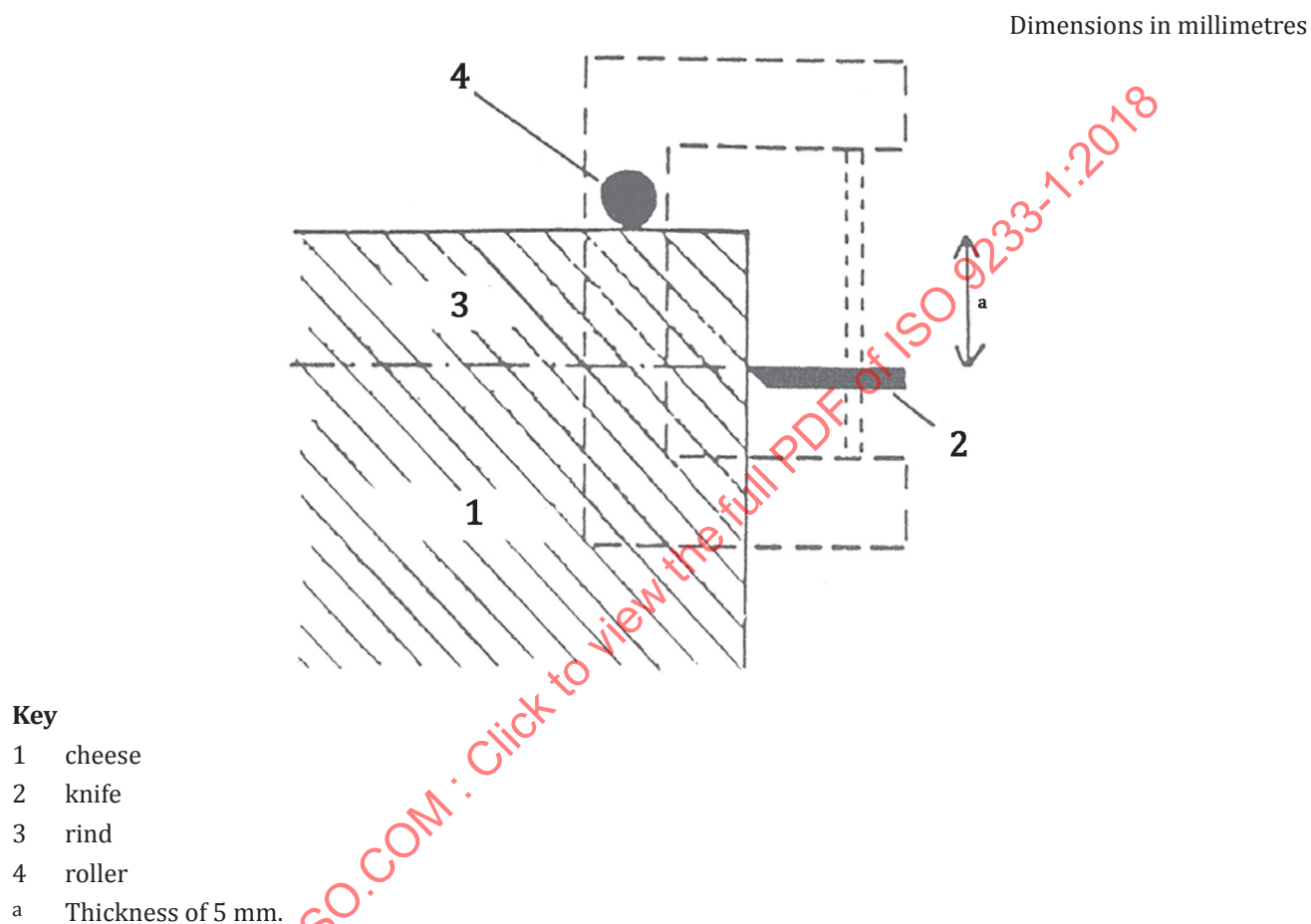


Figure A.1 — Example of a slicer for cutting portions of cheese rind 5 mm thick (6.2)

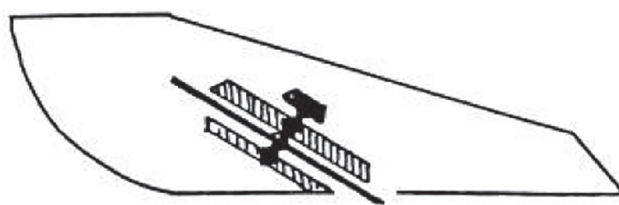
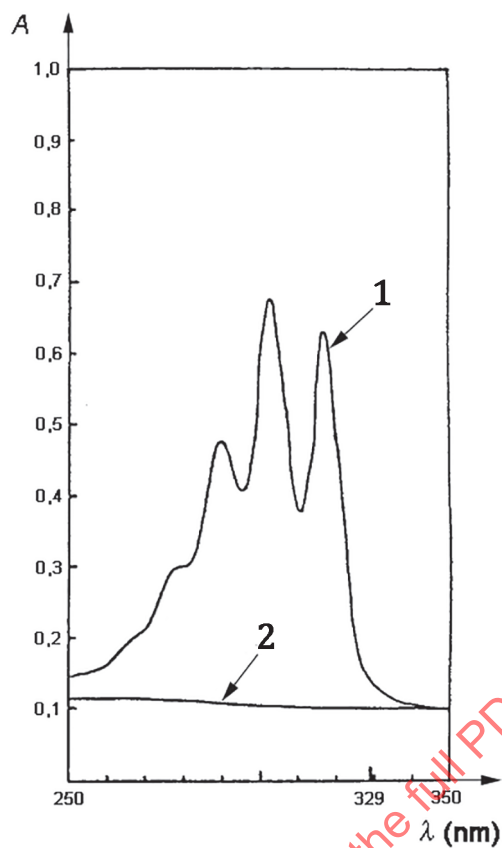


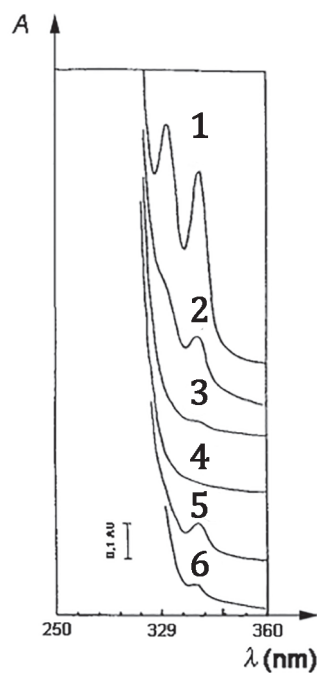
Figure A.2 — Example of a fine slicer for cutting slices of cheese of maximum thickness 1 mm (6.3)



Key

- A absorbance
- λ wavelength
- 1 sample
- 2 blank

Figure A.3 — Example of a spectrum of a natamycin standard solution and a blank



Key

A	absorbance	4	cheese, natamycin mass fraction 0,3 mg/kg
t	time	5	as 3, after concentration times 5
AU	absorbance unit	6	as 4, after concentration times 10
1	cheese rind, natamycin mass fraction 61 mg/kg		
2	cheese rind, natamycin mass fraction 15 mg/kg		
3	cheese, natamycin mass fraction 1,7 mg/kg		

Figure A.4 — Examples of spectra of various test solutions