SLOVENSKI STANDARD

SIST EN ISO 14501:1999

01-maj-1999

Mleko in mleko v prahu ï Doloļ evanje vsebnosti aflatoksina M1 - Ļiğļ enje z imunoafinitetno kromatografijo in doloļ evanje s tekoļ insko kromatografijo visoke loļ ljivosti (ISO 14501:1998)

Milk and milk powder - Determination of aflatoxin M1 content - Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography (ISO 14501:1998)

Milch und Milchpulver - Bestimmung des Gehalts an Aflatoxin M1 - Reinigung durch Immunaffinitäts-Chromatographie und Bestimmung mit Hochleistungs-Flüssigchromatographie (ISO 14501:1998)

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Lait et lait en poudre - Détermination de la teneur en aflatoxine M1 - Purification par chromatographie d'immunoaffinité et détermination par chromatographie en phase liquide a haute performance (ISO 14501:1998)

Ta slovenski standard je istoveten z: **EN ISO 14501:1998**

ICS:

67.100.10	Mleko in predelani mleļ ni proizvodi	Milk and processed milk products

SIST EN ISO 14501:1999 **en**

2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 14501

November 1998

ICS 67.100.10

Descriptors: see ISO document

English version

Milk and milk powder - Determination of aflatoxin M1 content - Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography (ISO 14501:1998)

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This European Standard was approved by CEN on 15 November 1998.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

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Foreword

The text of the International Standard ISO 14501:1998 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 302 "Milk and milk products - Methods of sampling and analysis", the secretariat of which is held by NNI.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 1999, and conflicting national standards shall be withdrawn at the latest by May 1999.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 14501:1999 was approved by CEN as a European Standard without any modification.

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INTERNATIONAL STANDARD

ISO 14501

First edition
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Milk and milk powder — Determination of aflatoxin M_1 content — Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography

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Lait et lait en poudre — Détermination de la teneur en aflatoxine M_1 — Purification par chromatographie d'immunoaffinité et détermination par chromatographie en phase liquide à haute performance

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Reference number
ISO 14501:1998(E)

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 3.

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.

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International Standard ISO 14501 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with International Dairy Federation (IDF) and the Association of Official Analytical Chemists (AOAC International), and will also be published by these organizations.

Annex A of this International Standard is for information only.

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Milk and milk powder — Determination of aflatoxin M_1 content — Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography

WARNINGS

1 The method described in this International Standard requires the use of chloroform and aflatoxin M_1 solutions. Chloroform is an ozone-depleting substance. Aflatoxins are carcinogenic to human subjects. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO) [4, 5].

2 Adequately protect from daylight the laboratory where the analyses are performed, and keep aflatoxin standard solutions protected from light, for example by using aluminium foil.

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3 The use of non-acid-washed glassware (e.g. tubes, vials, flasks, beakers, syringes) for aqueous aflatoxin solutions may cause loss of aflatoxin. Moreover, brand new laboratory glassware coming into contact with aqueous solutions of aflatoxin should be soaked in dilute acid (e.g. sulfuric acid, 2 mol/l) for several hours, then rinsed well with distilled water to remove all traces of acid (check to ensure pH is in the range 6 to 8).

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4 Use a decontamination procedure for laboratory wastes such as solid compounds, solutions in organic solvents, glassware, aqueous solutions and spills. The procedure for decontamination was developed and validated in a programme of the International Agency for Research on Cancer (WHO) [4, 5]

1 Scope

This International Standard specifies a method for the determination of aflatoxin M_1 content of milk and milk powder. The lowest level of validation is 0,08 µg/kg for whole milk powder i.e. 0,008 µg/l for reconstituted liquid milk. The method is also applicable to low fat milk, skimmed milk, low fat milk powder and skimmed milk powder.

2 Term and definition

For the purposes of this International Standard, the following term and definition apply.

2.1
aflatoxin M_1 content
mass fraction of substances determined by the procedure specified in this International Standard

NOTE The aflatoxin M_1 content is expressed as micrograms per litre or micrograms per kilogram.

3 Principle

Aflatoxin M_1 is extracted by passing the test portion through an immunoaffinity column. The column contains specific antibodies bound onto a solid support material. As the sample passes through the column, the antibodies selectively bind with any aflatoxin M_1 (antigen) present and form an antibody-antigen complex. All other components of the sample matrix are washed off the column with water. Then aflatoxin M_1 is eluted from the column and the eluate is collected. The amount of aflatoxin M_1 present in this eluate is determined by means of high-performance liquid chromatography (HPLC) coupled with fluorimetric detection.

4 Reagents

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

4.1 Immunoaffinity column

The immunoaffinity column shall contain antibodies against aflatoxin M_1. The column shall have a maximum capacity of not less than 100 ng of aflatoxin M_1 (which corresponds to 2 µg/l when a volume of 50 ml of test portion is applied), and shall give a recovery of not less than 80 % for aflatoxin M_1 when a standard solution containing 4 ng of toxin is applied (which corresponds to 80 ng/l when a volume of 50 ml of sample is applied). Any immunoaffinity column meeting the performance specifications mentioned above can be used. The performance of the columns shall be checked regularly and at least once for every batch of columns (see 4.1.1 and 4.1.2).

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4.1.1 Capacity check

By means of a pipette (5.4) transfer 1,0 ml of the stock aflatoxin M_1 solution (4.5.2) to a 20 ml conical tube (5.9). Evaporate the solution slowly to dryness using a constant stream of nitrogen (4.3) and dissolve the residue obtained in 10 ml of the 10 % acetonitrile solution (4.2.2). Shake vigorously.

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Add this solution to 40 ml of water. Mix well and apply the whole volume to the immunoaffinity column. Be careful to follow the recommendations given by the manufacturer for the use of the columns. Wash the column, elute the toxin and determine the amount bound to the column by HPLC after suitable dilution of the final eluate.

Calculate the recovery for the aflatoxin. Compare the result with the specification under 4.1.

4.1.2 Recovery check

By means of a pipette (5.4) dilute 0,8 ml of the 0,005 µg/ml aflatoxin M_1 working solution (4.5.3) to 10 ml with water. Mix well and apply the whole volume to the immunoaffinity column. Be careful to follow the recommendations given by the manufacturer for the use of the columns. Wash the column, elute the toxin and determine the amount bound to the column by HPLC after suitable dilution of the final eluate. Calculate the recovery for the aflatoxin. Compare the result with the specification under 4.1.

4.2 Acetonitrile, pure, HPLC grade.

4.2.1 Acetonitrile, in water, 25 % solution by volume.

Add 250 ml of acetonitrile (4.2) to 750 ml of water (degas before use).

4.2.2 Acetonitrile, in water, 10 % solution by volume.

Add 100 ml of acetonitrile (4.2) to 900 ml of water (degas before use).

4.3 Nitrogen gas

4.4 Chloroform, stabilized with 0,5 % to 1,0 % ethanol (by mass).

4.5 Aflatoxin M_1 standard solutions

4.5.1 Calibrant solution

Standard solution of aflatoxin M_1 in chloroform with a nominal concentration of 10 µg/ml.

Determine the concentration by measurement of its absorbance at the wavelength for maximum absorption as follows.

By using the spectrometer (5.11), record the absorbance of the calibrant solution against chloroform as blank between 340 nm and 370 nm. Measure the absorbance, A, at the wavelength of maximum absorption, λ_{max}, close to 360 nm. Calculate the concentration, c_i in micrograms per millilitre, using the following equation:

$$c_i = A \times M \times 100/\varepsilon$$

where

A is the numerical value of the absorbance at λ_{max};

M is the numerical value of the molar mass of the aflatoxin M_1 in grams per mole (M = 328 g/mol);

ε is the numerical value of the absorption coefficient of the toxin in chloroform, in square metres per mole (ε = 1 995 m^2/mol).

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4.5.2 Stock solution

After checking the concentration of the calibrant solution (4.5.1), dilute the calibrant solution in chloroform to give an aflatoxin M_1 stock solution of 0,1 µg/ml. The stock solution shall be well-stoppered and wrapped in aluminium foil to exclude light.

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Store the stock solution in a refrigerator at a temperature below 5 °C in the dark. Under these conditions the stock solution is stable for about 2 months. After 2 months, the stability should be checked.

4.5.3 Working solutions of aflatoxin M_1

Before preparing working dilutions of the aflatoxin M_1 standard solution, allow the stock solution (4.5.2) to attain ambient temperature before removing aliquots of the solution for subsequent dilution. Prepare working solutions on the day of use.

Prepare a solution with a concentration of 0,005 µg/ml as follows. By means of a pipette (5.4) transfer 1,0 ml of the stock solution (4.5.2) to a 20 ml conical tube (5.9). Evaporate the solution to dryness using a gentle stream of nitrogen (4.3) and dissolve the residue obtained in 20,0 ml of the diluted acetonitrile (4.2.2). Shake occasionally over a period of 30 min.

Care should be taken when evaporating the solution to dryness to ensure the temperature does not drop so low that condensation occurs.

Use this diluted solution for the preparation of a series of appropriate dilutions of aflatoxin M_1 standard solution to provide, depending on the injection loop volume, for injection of 0,05 ng, 0,1 ng, 0,2 ng and 0,4 ng of aflatoxin M_1. Dilute by using diluted acetonitrile solution (4.2.2).

5 Apparatus

Usual laboratory equipment and, in particular, the following.