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Milk and milk powder — Determination of aflatoxin M_1 content — Clean-up by immunoaffinity chromatography and determination by thin-layer chromatography

Lait et lait en poudre — Détermination de la teneur en aflatoxin M_1 — Purification par chromatographie d'immunoaffinité et détermination par chromatographie sur couche mince

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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 14674 | IDF 190 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.

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 the National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Organic contaminants*, of the Standing Committee on *Analytical methods for additives and contaminants*, under the aegis of its project leader, Mrs S Dragacci (FR).

Milk and milk powder — Determination of aflatoxin M_1 content — Clean-up by immunoaffinity chromatography and determination by thin-layer chromatography

WARNING — The method described in this International Standard requires the use of aflatoxin M_1 solutions. Aflatoxins are carcinogenic to human subjects. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO). [5]

Aflatoxins are subject to light degradation. Adequately protect analytical work from daylight and keep aflatoxin standard solutions protected from light, for example by using amber vials or aluminium foil.

The use of non-acid-washed glassware (e.g. vials, tubes, flasks) for aqueous aflatoxin solutions can cause a loss of aflatoxin. Take special care with new glassware. Before use, soak the new glassware in diluted acid (e.g. 2 mol/l sulfuric acid) for several hours, then rinse extensively with distilled water to remove all traces of acid. Check to ensure that the pH is in the range of 6 to 8 by using a pH-paper.

Use the decontamination procedure for laboratory wastes developed and validated by the International Agency for Research on Cancer (WHO). [5]

1 Scope

This International Standard specifies a method for the determination of the aflatoxin M_1 (AFM1) content of milk and milk powder by a method including a clean-up step using immunoaffinity chromatography followed by a thin-layer chromatography (IAC-TLC).

The method is applicable to raw milk, low fat or skimmed liquid milk and milk powder.

The lowest quantity of AFM1 that can commonly be determined is 2 ng, which corresponds to a limit of quantification close to 0,10 µg/l for liquid milk or dissolved milk powder (for a spot of 20 µl).

2 Terms and definitions

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

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

NOTE The aflatoxin M_1 content is expressed as micrograms per litre for liquid milk products, and as micrograms per kilogram for milk powder.

3 Principle

Aflatoxin M_1 (AFM1) 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 AFM1 (antigen) present and form an antibody-antigen complex. All

other components of the sample matrix are washed off the column with water. Then the AFM1 is eluted from the column with methanol and acetonitrile. After concentration of the eluate, the amount of AFM1 is determined by one-dimensional thin-layer chromatography. In the case of interference, two-dimensional thin-layer chromatography is carried out to separate the AFM1 from its impurities.

4 Reagents

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

4.1 Pure solvents.

WARNING — Some of the pure solvents (e.g. chloroform, acetonitrile, toluene and methanol) are toxic. Take all necessary precautions where needed.

4.1.1 Chloroform ($CHCl_3$).

4.1.2 Acetonitrile (CH_3CN).

4.1.3 Diethyl ether $(C_2H_5)_2O$.

4.1.4 Methanol (CH_3OH).

4.1.5 Toluene ($C_6H_5CH_3$).

4.1.6 Acetone ($CH_3.COCH_3$), optional.

4.1.7 Isopropanol ($CH_3CHOHCH_3$), optional.

4.2 Acetonitrile/methanol solution, of volume ratio 3:2.

Add 30 ml of acetonitrile (4.1.2) to 20 ml of methanol (4.1.4) and mix.

4.3 Toluene/acetonitrile solution, of volume ratio 9:1.

Add 9 ml of toluene (4.1.5) to 1 ml of acetonitrile (4.1.2) and mix. Use this solution to resuspend the AFM1 standard solutions (4.5) and the evaporated eluate before the TLC analysis.

4.4 TLC development solvents.

4.4.1 Unidirectional TLC solution.

Prepare a 100 ml unidirectional TLC solution by adding 4 ml of methanol (4.1.4) and 1 ml of water to 95 ml of diethyl ether (4.1.3) and mix well (volume ratio 95:4:1).

4.4.2 Bidirectional TLC solution, optional.

Prepare a 100 ml bidirectional TLC solution by adding 10 ml of acetone (4.1.6) and 3 ml of isopropanol (4.1.7) to 87 ml of chloroform (4.1.1) and mix well (volume ratio 87:10:3).

4.5 Aflatoxin M_1 standard solution.

4.5.1 AFM1 standard stock solution.

Prepare an AFM1 standard stock solution with a nominal concentration of 10 µg/ml chloroform (4.1.1); i.e. by resuspending a lyophilized film of 10 µg of AFM1 to 1 ml of chloroform.

In accordance with the AOAC protocol [6], determine the concentration of the AFM1 standard stock solution by measuring its absorbance at the wavelength of maximum absorption and use a calibrated spectrometer to record the absorbance of the standard stock solution against chloroform (4.1.1), used as blank, at between $\lambda = 200$ nm and $\lambda = 400$ nm.

Check the purity of the AFM1 by recording the spectrum between 200 nm and 400 nm. Measure the absorbance (A) at the wavelength for maximum absorption (λ_{max}), i.e. close to 365 nm.

Calculate the mass concentration, c, expressed in micrograms per millilitre, by using the following equation:

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

where

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

M is the numerical value of the molar mass of the AFM1, in grams per mole ($M = 328$ g/mol);

ε is the numerical value of the absorption coefficient of AFM1 in chloroform, in square metres per mole ($\varepsilon = 1\ 995\ m^2 \cdot mol^{-1}$).

Keep the AFM1 standard stock solution in a well-stoppered amber-coloured vial protected from light. Store the standard solution at below 0 °C. Under these conditions, the AFM1 standard stock solution is stable for about one year.

4.5.2 AFM1 standard working solution.

4.5.2.1 Working solution A.

Use a volumetric pipette or a Hamilton-like microsyringe (5.2) to transfer 50 µl of AFM1 standard stock solution (4.5.1) into a vial. Evaporate the solution to dryness. Resuspend the dried solution with 500 µl of toluene/acetonitrile solution (4.3) to obtain an AFM1 standard working solution with concentration of 1 µg/ml (working solution A). Use solution A to spot onto TLC plates for test samples with a high contamination level or when the determination level is close to 0,50 µg/l.

4.5.2.2 Working solution B.

Transfer 100 µl of solution A to a vial. Add 900 µl of toluene/acetonitrile solution (4.3) to obtain an AFM1 standard working solution with concentration of 0,1 µg/ml (working solution B). Use solution B to spot onto TLC plates for test samples with a low contamination level or when the determination level is close to 0,10 µg/l.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Volumetric pipettes, of required capacities.

5.2 Hamilton-like microsyringes.[1)]

5.3 Laboratory glassware, such as glass beakers and funnels, of appropriate capacities.

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1) Hamilton-like syringes and Whatman No. 4 are examples of suitable products available commercially.

This information is given for the convenience of users of this document and does not constitute an endorsement by either ISO or IDF of these products.