INTERNATIONAL STANDARD

ISO 14674

IDF 190

First edition
2005-05-01

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

Reference numbers
ISO 14674:2005(E)
IDF 190:2005(E)

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Published in Switzerland

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Foreword

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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.

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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).

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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

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

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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.

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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.

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

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5.4 One-mark volumetric flask, of capacity 200 ml.

5.5 Measuring cylinder, of capacity 100 ml.

5.6 Disposable syringes, of capacities 10 ml and 100 ml.

5.7 Glass conical tube, of capacity 5 ml, to collect the extract after the clean-up step.

5.8 Evaporation system, rotary evaporator or by using a gentle stream of nitrogen.

5.9 Spectrometer, capable of measuring at wavelengths at between $\lambda = 200$ nm and $\lambda = 400$ nm, with quartz face cells of optical length 1 cm.

5.10 Water bath, capable of operating at between 35 °C and 37 °C.

5.11 Centrifuge, capable of producing a radial acceleration of at least 2 000g and, if possible, capable of cooling to +4 °C.

5.12 Filter paper (Whatman No. 4[1]) or equivalent).

5.13 Affinity columns

The immunoaffinity columns shall contain antibodies against AFM1. The columns shall have a maximum capacity of not less than 100 ng of AFM1 (which corresponds to 1 µg/l or 10 µg/kg when 100 ml of test portion is applied). They shall give a recovery of not less than 80 % for AFM1 when a calibrant solution containing 4 ng of toxin is applied (corresponding to 0,04 µg/l or 0,40 µg/kg applying a 100 ml of test portion). Any immunoaffinity column meeting the above-mentioned performance specifications may be used.

5.14 Analytical balance, capable of weighing to the nearest 0,1 g.

5.15 Magnetic stirrer.

5.16 Vortex type stirrer.

5.17 Vacuum system, for immunoaffinity cartridge.

5.18 Oven, capable of maintaining a temperature of approx. 50 °C.

5.19 Thin-layer chromatography (TLC) container.

5.20 Thin-layer chromatography (TLC) plates, of surface 10 cm × 10 cm or 20 cm × 20 cm, with silica gel 60, made of glass or aluminium.

5.21 UV lamp, capable of operating at 365 nm.

5.22 Densitometer (optional).

6 Sampling

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

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

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7 Preparation of test samples

7.1 Milk or liquid milk products

Warm the test sample in the water bath (5.10) to between 35 °C and 37 °C to dissolve the fat layer in order to obtain good homogenization of the test sample when mixing gently.

Centrifuge (5.11) the homogenized test sample at a radial acceleration of 2 000*g* for at least 15 min. If possible, cool the sample to obtain a better separation of the fat and discard the upper thin fat layer. Filter through one or more filter papers (5.12), if necessary. Collect at least 100 ml of the thus-prepared test portion.

7.2 Milk powders

Weigh, to the nearest 0,1 g, 20,0 g of test sample into a 250 ml glass beaker (5.3). Dissolve the test sample with about 150 ml of preheated water at 50 °C to 60 °C. Stir the obtained test solution with a magnetic stirrer (5.15) for about 10 min.

Allow the test solution to reach room temperature. Then, gently transfer the complete solution to a 200 ml volumetric flask (5.4). Dilute to the mark with water. Close the flask and shake its contents to obtain a good homogenized test solution.

Centrifuge (5.11) the test solution at a radial acceleration of 2 000*g* for 15 min. If possible, cool the solution to obtain a better separation of the fat and discard the upper thin fat layer. Filter through one or more filter papers (5.12), if necessary. Collect at least 100 ml of the thus-prepared test portion.

7.3 Immunoaffinity clean-up

Allow the immunoaffinity column (5.13) to reach room temperature. Attach a syringe barrel to the top of the immunoaffinity cartridge. Take 100 ml of prepared test portion (7.1 or 7.2) using a 100 ml measuring cylinder (5.5) or pipette.

Transfer the test portion to the syringe barrel and allow it to pass through the immunoaffinity column at a slow steady flow rate of about 2 ml/min to 3 ml/min. Use gravity or vacuum system (5.17) to control the flow rate. Do not allow the column to run dry. Wash the column with 40 ml of water at a steady flow rate. After washing, blow the column to dryness and discard the washing solution.

Put another dry clean barrel on the cartridge. Slowly elute AFM1 from the column by passing 2,5 ml of acetonitrile/methanol solution (4.2) and then 2,5 ml of pure methanol (4.1.4). Allow these solvents to be in contact with the column for at least 1 min while keeping a steady flow rate. Then blow the column to dryness.

Collect the eluate in a conical tube and evaporate nearly to dryness (i.e. the last drop) using the rotary evaporator (5.8) at a temperature of $\leqslant$ 40 °C or by using a gentle stream of nitrogen. Do not evaporate it to dryness to allow a good resuspension of the AFM1 present in the test portion residue.

Add 200 µl of pure acetonitrile (4.1.2) to the test portion residue. Cap the tube and mix, preferably by using a Vortex type stirrer (5.16), vigorously for about 1 min. Evaporate again nearly to dryness (i.e. the last drop) using the rotary evaporator (5.8) at a temperature of $\leqslant$ 40 °C or by using a gentle stream of nitrogen.

NOTE The former step is necessary to avoid emulsion and to obtain a good resuspension when adding the toluene/acetonitrile solution.

Open the tube and add 100 µl of toluene/acetonitrile solution (4.3) to the test portion residue. Cap the tube and mix, preferably by using a Vortex type stirrer (5.16), vigorously again for about 1 min. Store the thus-purified test portion until the start of the procedure.

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8 Procedure

8.1 Unidirectional TLC

Add onto a TLC plate (5.20), a spot of 10 µl to 20 µl of the purified test portion (7.3). Add also, for example, 5 µl, 10 µl and 20 µl aliquots of the appropriate AFM1 standard working solution prepared in 4.5.2 (solution A or B).

Place the plate in the TLC container (5.19) containing the unidirectional TLC solution (4.4.1). Develop the plate until the solvent front reaches a height of about 10 cm.

Remove the plate from the container and let it dry in air. Read the obtained spots by using the UV-lamp (5.21) at 365 nm or by using a densitometer (5.22) at 365 nm. Compare the fluorescent intensity of the spot of the test portion to those obtained with the AFM1 standard working solutions. The spots may also be compared approaching extinction.

Calculate the AFM1 content in the test portion as described in Clause 9.

If the fluorescence of the test portion spot is more intense than that of the highest AFM1 standard working solution, estimate the contamination level. Based on that level, dilute the purified test portion (7.3) with an appropriate volume of toluene/acetonitrile solution (4.3).

Repeat the procedure with the diluted purified test portion restarting as in 8.1.

8.2 Bidirectional TLC

If any interference or streak appears in the developed plate close to fluorescent spots, carry out bidirectional TLC. Add onto a TLC plate (5.20), a spot of 20 µl of the purified test portion (7.3). Add spots of 5 µl, 10 µl and 20 µl of AFM1 standard working solution (4.5.2) to run into direction 1, and spots of 5 µl and 10 µl of same AFM1 standard working solution (4.5.2) to run in direction 2 (see Figure 1).

Place the plate in the TLC container (5.19) containing the unidirectional TLC solution (4.4.1). Develop the plate in the first direction until the solvent front reaches a height of about 7 cm. Remove the plate from the container and allow it to dry in air.

Heat the dry plate in the oven (5.18) set at 50 °C for 5 min. Cool the plate and place it with the second direction downwards in another TLC container (5.19) containing the bidirectional TLC solution (4.4.2). Develop the plate in the second direction until the solvent front also reaches a height of about 7 cm.

Remove the plate from the container and allow it to dry in air. Read the obtained spots using the UV-lamp (5.21) at 365 nm or by using the densitometer (5.22) at 365 nm. Compare the fluorescent intensity of the spot of the test portion to those obtained with the AFM1 standard working solutions. The spots may also be compared approaching extinction.

Calculate the AFM1 content in the test portion as described in Clause 9.

If the fluorescence of the test portion spot is more intense than that of the highest AFM1 standard working solution, repeat the procedure with a smaller purified test portion (7.4) restarting as in 8.2.

After the TLC analysis, store the remaining purified test portion in a closed vial in a freezer for further quantification or confirmation of identity, if needed.

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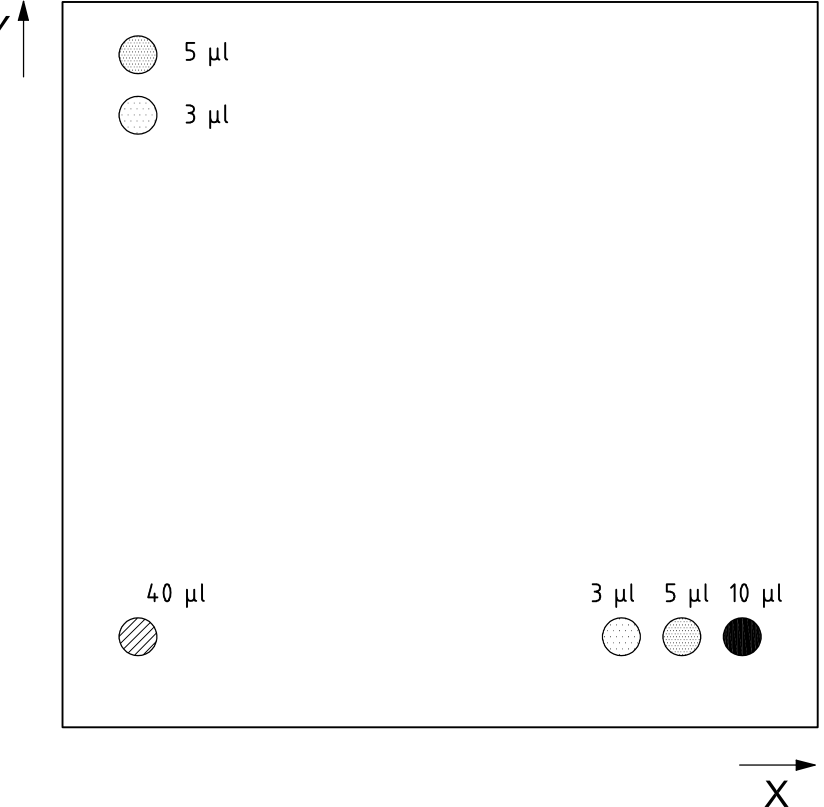

Key

X Direction 2

Y Direction 1

Figure 1 — Bidirectional TLC

9 Calculation and expression of results

9.1 Calculation

Calculate the mass fraction of AFM1 in the test sample (7.1 or 7.2), w_A, expressed either in µg/l or µg/kg, by using the following equation:

$$w_A = \frac{v_s \times c_s \times v_1}{v_2 \times m_t}$$

where

v_s is the numerical value of the volume of the spot of the AFM1 standard working solution A or B (4.5.2), comparable to the spot of the test portion (8.1 or 8.2) and used in the calculation, expressed in microlitres;

c_s is the AFM1 content of the used standard working solution A or B (4.5.2) comparable to the spot of the test portion (8.1 or 8.2) and used in the calculation, expressed in nanograms per microlitre;

v_1 is the volume of dissolved residue, expressed in microlitres (v_1 = 100 µl);

v_2 is the volume of the spot (8.1 or 8.2) of the purified test portion comparable to the spot of the standard working solution A or B (4.5.2) and used in the calculation, expressed in microlitres;

m_t is the volume (7.1) or the mass (7.2) of the test sample, expressed either in millilitres or grams.

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9.2 Expression of results

Express the test results to two decimal places.

10 Precision

10.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

10.2 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 following.

a) For liquid samples:

- with low contamination level (± 0,10 µg/l), uncorrected results: 0,17 µg/l;
- with low contamination level (± 0,10 µg/l), corrected for recovery: 0,10 µg/l;
- with high contamination level (± 0,50 µg/l), uncorrected: 0,63 µg/l;
- with high contamination level (± 0,50 µg/l), corrected for recovery: 0,96 µg/l.

b) For powder samples:

- with low contamination level (± 1,00 µg/kg), uncorrected: 0,73 µg/kg;
- with low contamination level (± 1,00 µg/kg), corrected for recovery: 1,04 µg/kg;
- with high contamination level (± 5,00 µg/kg), uncorrected: 3,04 µg/kg;
- with high contamination level (± 5,00 µg/kg), corrected for recovery: 5,79 µg/kg.

10.3 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 following.

a) For liquid products:

- with low contamination level (± 0,10 µg/l), uncorrected: 0,18 µg/l;
- with low contamination level (± 0,10 µg/l), corrected for recovery: 0,14 µg/l;
- with high contamination level (± 0,50 µg/l), uncorrected: 0,84 µg/l;
- with high contamination level (± 0,50 µg/l), corrected for recovery: 0,96 µg/l.

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b) For milk powder:

— with low contamination level (± 1,00 µg/kg), uncorrected: 1,01 µg/kg;

— with low contamination level (± 1,00 µg/kg), corrected for recovery: 1,25 µg/kg;

— with high contamination level (± 5,00 µg/kg), uncorrected: 5,06 µg/kg;

— with high contamination level (± 5,00 µg/kg), corrected for recovery: 8,63 µg/kg.

11 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, together with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may influence the final test result(s);

e) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.

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Annex A
(informative)

Results of interlaboratory test

The interlaboratory test involved nine laboratories for liquid milk samples and eleven laboratories for milk powder, and was carried out on duplicate samples. The liquid test samples were contaminated at two levels: at 0,13 µg/l (L1) and at 0,68 µg/l (L2). The powder test samples were also contaminated at two levels: at 1,17 µg/kg (P1) and at 5,49 µg/kg (P2). Statistical analysis was carried out on uncorrected results and on results corrected for recovery.

AFSSA-LERHQA, France, organized the trial. The AFM1 content of the distributed contaminated milk was determined using the HPLC method described in ISO 14501.

The results obtained were subjected to statistical analysis in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Table A.1.

Table A.1 — Results of the interlaboratory test (uncorrected data)

	Batches							
	L1		L2		P1		P2	
	A [a]	B [b]	A [a]	B [b]	A [a]	B [b]	A [a]	B [b]
Expected values (µg/l or µg/kg)	0,13	0.13	0,68	0,68	1,17	1,17	5,49	5,49
Number of laboratories	9	8	11	11	9 [c]	10	11	10 [d]
Overall mean (µg/l or µg/kg)	0,13	0,14	0,46	0,63	0,65	0,93	3,36	5,74
Standard deviation for repeatability, s_r (µg/l or µg/kg)	0,06	0,04	0,22	0,34	0,26	0,37	1,08	2,04
Relative standard deviation for repeatability (%)	45,10	25,90	48,70	54,10	40,10	39,30	32,00	35,60
Repeatability limit at 95 %, r (µg/l or µg/kg)	0,17	0,10	0,63	0,96	0,73	1,04	3,04	5,79
Standard deviation for reproducibility, s_R (µg/l or µg/kg)	0,06	0,05	0,25	0,30	0,36	0,44	1,79	3,05
Relative standard deviation for reproducibility (%)	49,00	33,70	55,10	46,90	55,40	47,50	53,30	53,10
Reproducibility limit at 95 %, R (µg/l or µg/kg)	0,18	0,14	0,72	0,84	1,01	1,25	5,06	8,63

[a] A is uncorrected data.

[b] B is data corrected for recovery.

[c] Data from laboratory 7 were found to be outliers for the batch P1 by the Cochran test.

[d] Data from laboratory 4 were found to be outliers for the batch P2 by the Cochran test; data from laboratory 6 were found to be stragglers for the batch P2 by the Cochran test but were included in the calculation.

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Bibliography

[1] ISO 707 | IDF 50, *Milk and milk products — Guidance on sampling*

[2] ISO 14501 | IDF 171, *Milk and milk powder — Determination of aflatoxin M_1 content — Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography*

[3] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[4] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[5] CASTEGNARO, M., HUNT, D.C., SANSONE, E.B., SCHULLER, P.L., SIRIWARDANA, M.G., TELLING, G.M., VAN EGMOND, H.P., WALKER, E.A. *Laboratory decontamination and destruction of aflatoxins B_1, B_2, G_1 and G_2 in laboratory wastes.* IARC Scientific Publication N° 37, Int. Agency for Research on Cancer (WHO), Lyon (France), 1980, p. 59

[6] AOAC 1995 49.3.02 (980.21 Method)

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