

English Version

**Foodstuffs - Determination of aflatoxin B₁ in cereal based foods
for infants and young children - HPLC method with
immunoaffinity column cleanup and fluorescence detection**

Produits alimentaires - Dosage de l'aflatoxine B₁ dans les
produits pour nourrissons et jeunes enfants à base de
céréales - Méthode de chromatographie liquide haute
performance avec purification sur colonne d'immunoaffinité
et détection par fluorescence

Lebensmittel - Bestimmung von Aflatoxin B₁ in Säuglings-
und Kleinkindernahrung auf Getreidebasis - HPLC-
Verfahren mit Reinigung an einer Immunoaffinitätssäule
und Fluoreszenzdetektion

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Contents		Page
Foreword.....		3
1	Scope	4
2	Normative references	4
3	Principle	4
4	Reagents	4
5	Apparatus	7
6	Procedure	8
7	HPLC analysis	9
8	Calculation.....	11
9	Precision	11
10	Test report	12
Annex A (informative) Typical chromatogram		13
Annex B (informative) Precision data.....		14
Bibliography		15

Foreword

This document (EN 15851:2010) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by October 2010, and conflicting national standards shall be withdrawn at the latest by October 2010.

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1 Scope

This European Standard specifies a method for the determination of aflatoxin B₁ in baby food by high performance liquid chromatography (HPLC) with immunoaffinity cleanup and fluorescence detection. This method has been validated in an interlaboratory study via the analysis of both naturally contaminated and spiked samples ranging from 0,07 µg/kg to 0,18 µg/kg.

For further information on the validation, see Clause 9 and Annex B.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696:1995, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

A test portion is extracted with a mixture of methanol and water. The extract is filtered, diluted with phosphate buffered saline (PBS) to a specified solvent concentration, and applied to an immunoaffinity column containing antibodies specific to aflatoxin B₁. Aflatoxin B₁ is purified and concentrated on the column and removed from the antibodies using methanol as eluent. Aflatoxin B₁ is quantified by reverse-phase high performance liquid chromatography (RP-HPLC) with post column derivatization (PCD) involving bromination followed by fluorescence detection.

The post column derivatization is achieved with either electrochemically generated bromine or with pyridinium hydrobromide perbromide (PBPB).

4 Reagents

4.1 General

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. Solvents shall be of quality for HPLC analysis, unless otherwise specified. Commercially available solutions with equivalent properties to those listed may be used.

WARNING — Dispose of waste solvents according to applicable environmental rules and regulations. Decontamination procedures for laboratory wastes have been reported by the International Agency for Research on Cancer (IARC), see [4].

4.2 Helium purified compressed gas.

4.3 Nitrogen.

4.4 Disodium hydrogen phosphate, Na₂HPO₄ anhydrous or Na₂HPO₄·12 H₂O.

4.5 Potassium bromide.

4.6 Potassium chloride.

4.7 Potassium dihydrogen phosphate, KH₂PO₄.

4.8 Sodium chloride.

4.9 Sodium hydroxide.**4.10 Hydrochloric acid solution**, mass fraction $w(\text{HCl}) = 37\%$ in water.**4.11 Hydrochloric acid solution**, substance concentration $c(\text{HCl}) = 0,1 \text{ mol/l}$.

Dilute 8,28 ml of hydrochloric acid solution (4.10) to 1 l with water.

4.12 Sodium hydroxide solution, $c(\text{NaOH}) = 0,1 \text{ mol/l}$.

Dissolve 4 g of sodium hydroxide (4.9) in 1 l of water.

4.13 Phosphate buffered saline (PBS) solution, $c(\text{NaCl}) = 120 \text{ mmol/l}$, $c(\text{KCl}) = 2,7 \text{ mmol/l}$, $c(\text{phosphate buffer}) = 10 \text{ mmol/l}$, $\text{pH} = 7,4$.Dissolve 8,0 g of sodium chloride (4.8), 1,2 g of anhydrous disodium hydrogen phosphate or 2,9 g of $\text{Na}_2\text{HPO}_4 \cdot 12 \text{ H}_2\text{O}$ (4.2), 0,2 g of potassium dihydrogen phosphate (4.7) and 0,2 g of potassium chloride (4.6) in 900 ml of water. After dissolution, adjust the pH to 7,4 with hydrochloric acid solution (4.11) or sodium hydroxide solution (4.12) as appropriate, then dilute to 1 l with water.

Alternatively, a PBS solution with equivalent properties can be prepared from commercially available PBS material.

4.14 Pyridinium hydrobromide perbromide (PBPB), [CAS: 39416-48-3].**4.15 Acetonitrile.****WARNING — Acetonitrile is hazardous and samples shall be blended using an explosion proof blender which is housed within a fume cupboard. After blending, samples shall be filtered inside a fume cupboard.****4.16 Methanol**, HPLC grade.**4.17 Methanol**, technical grade.**4.18 Toluene.****4.19 Extraction solvent.**

Mix eight parts per volume of methanol (4.17) with two parts per volume of water.

4.20 Nitric acid, $c(\text{HNO}_3) = 4 \text{ mol/l}$.**4.21 HPLC mobile phase A**, for use with PBPB.

Mix six parts per volume of water with two parts per volume of acetonitrile (4.15) and three parts per volume of methanol (4.16). Degas mobile phase A with for example helium (4.2).

4.22 HPLC mobile phase B, for use with electrochemically generated bromine.Mix six parts per volume of water with two parts per volume of acetonitrile (4.15) and three parts per volume of methanol (4.16). Add 120 mg of potassium bromide (4.5) and 350 μl of nitric acid (4.20) per litre of mobile phase. Degas mobile phase B with for example helium (4.2).**4.23 Post-column reagent.**

Dissolve 50 mg of PBPB (4.14) in 1 l of water. To be used with mobile phase solvent A (4.21). The solution may be used up to four days if stored in a dark place at room temperature.

4.24 Mixture of toluene and acetonitrile.

Mix nine parts per volume of toluene (4.18) with one part per volume of acetonitrile (4.15).

4.25 Immunoaffinity column.

The immunoaffinity column shall contain antibodies raised against aflatoxin B₁. The column shall have a capacity of not less than 100 ng of aflatoxin B₁ and shall give a recovery of not less than 80 % when 5 ng of aflatoxin B₁ are applied as a standard solution in a mixture of ten parts per volume of methanol and 90 parts per volume of water.

4.26 Aflatoxin B₁, in crystal form or as a film in ampoules or in form of commercially available aflatoxin B₁ solution.

WARNING — Aflatoxins are subject to light degradation. Protect the laboratory, where the analyses are done, adequately from daylight. This can be achieved effectively by using ultraviolet (UV) absorbing foil on the windows in combination with subdued light (no direct sunlight) or curtains or blinds in combination with artificial light (fluorescent tubes are acceptable).

Protect aflatoxin containing solutions from light as much as possible (keep in the dark, use aluminium foil or amber-coloured glassware).

4.27 Aflatoxin B₁ stock solution, $c \approx 10 \mu\text{g/ml}$.

Prepare a solution of aflatoxin B₁ in the mixture of toluene and acetonitrile (4.24) to give a solution with a mass concentration of approximately 10 $\mu\text{g/ml}$.

To determine the exact mass concentration, record the absorption curve between 330 nm and 370 nm in 1 cm quartz cells in a spectrometer (5.14) with the mixture of toluene and acetonitrile (4.24) as reference. Identify the wavelength for maximum absorption (between 330 nm and 370 nm). Calculate the mass concentration of aflatoxin B₁, ρ_{afl} , in $\mu\text{g/ml}$, using Equation (1):

$$\rho_{\text{afl}} = \frac{A_{\text{max}} \times M \times 100}{\varepsilon \times b} \tag{1}$$

where

- A_{max} is the absorption determined at the maximum of the absorption curve (between 330 nm and 370 nm);
- M is the molar mass, in g/mol, of aflatoxin B₁ ($M = 312 \text{ g/mol}$);
- ε is the molar absorption coefficient, in square metres per mole, of aflatoxin B₁ in the mixture of toluene and acetonitrile (4.24) ($1\,930 \text{ m}^2/\text{mol}$, see [5]);
- b is the optical path length, in centimetres, of the quartz cell.

Store this solution in a freezer at approximately - 18 °C. Allow to reach room temperature before opening. A solution stored in this way is usually stable for 12 months. Confirm the concentration of the solution if it is older than 12 months.

4.28 Aflatoxin B₁ standard solution, $\rho = 5,00 \text{ ng/ml}$.

Pipette a volume of aflatoxin B₁ stock solution (4.27) containing exactly 1,00 μg aflatoxin B₁ into a 200 ml calibrated volumetric flask and dilute to the mark with the mixture of toluene and acetonitrile (4.24). This solution contains 5,00 ng/ml aflatoxin B₁.

Wrap the flask tightly in aluminium foil and store it at less than 4 °C. Before use, do not remove the aluminium foil until the contents have reached room temperature to avoid incorporation of water by condensation. A solution stored in this way is stable for at least four weeks.

4.29 Aflatoxin B₁ spiking solution, $\rho = 2 \mu\text{g/ml}$.

Pipette a volume of aflatoxin B₁ stock solution (4.27) containing exactly 20 μg aflatoxin B₁ into a 10 ml calibrated volumetric flask. Evaporate the mixture of toluene and acetonitrile solution just to dryness under a stream of nitrogen at room temperature. Make up to volume with methanol (4.16) and shake well. The concentration of this spiking solution is 2 $\mu\text{g/ml}$ for aflatoxin B₁.

Wrap the flask tightly in aluminium foil and store it at less than 4 °C. Before use, do not remove the aluminium foil until the contents have reached room temperature to avoid incorporation of water by condensation. A solution stored in this way is stable for at least three months.

5 Apparatus

WARNING — All glassware coming into contact with aqueous solutions of aflatoxins shall be washed with acid solution before use. Many laboratory washing machines do this as part of the washing programme. Otherwise soak laboratory glassware coming into contact with aqueous solutions of aflatoxins in sulfuric acid ($c = 2 \text{ mol/l}$) for several hours (e.g. 15 h overnight), then rinse well (e.g. at least three times) with water to remove all traces of acid. Check the absence of acid with pH paper.

This treatment is necessary, because the use of non-acid washed glassware can cause losses of aflatoxins. In practice, the treatment is necessary for round bottomed flasks, volumetric flasks, measuring cylinders, vials or tubes used for calibration solutions and final extracts (particularly autosampler vials), and Pasteur pipettes, if these are used to transfer calibration solutions or extracts.

Usual laboratory glassware and equipment and, in particular, the following.

- 5.1 **Analytical balance**, capable of weighing to 0,000 1 g.
- 5.2 **Laboratory balance**, capable of weighing to 0,01 g.
- 5.3 **Adjustable vertical or horizontal shaker**.
- 5.4 **Filter paper**, e.g. 24 cm diameter, prefolded.
- 5.5 **Conical flask**, with screw top or glass stopper of 500 ml capacity.
- 5.6 **Glass microfibre filter**, retention size 1,6 μm or smaller.
- 5.7 **Reservoir**, of 75 ml capacity with luer tip connector and attachments for immunoaffinity column (IAC).
- 5.8 **Hand pump**, 20 ml syringe with luer lock or rubber stopper for IAC.
- 5.9 **Volumetric flasks**, of 5 ml, 10 ml, 20 ml, 150 ml, and 200 ml capacity with an accuracy of at least 0,5 %.
- 5.10 **Disposable syringe filter unit**, with pore size of 0,45 μm .

Prior to usage, verify that no aflatoxin losses occur during filtration (recovery testing).

NOTE There is a possibility that various filter materials retain aflatoxins.

- 5.11 **Volumetric pipettes**, with 2 ml and 10 ml capacity.
- 5.12 **Calibrated microlitre syringe(s) or microlitre pipette(s)**, with 25 μl to 500 μl capacity.

5.13 HPLC apparatus comprising the following:

5.13.1 Injection system, capable of injecting e.g. 1 000 µl. Total loop injection is recommended.

In the case that a different volume is used, it shall be ensured that the limit of detection (LOD) for the system is $\leq 0,05 \mu\text{g/kg}$ (signal-to-noise-ratio = 3) and the limit of quantification (LOQ) is $\leq 0,1 \mu\text{g/kg}$ for aflatoxin B₁. The measurement of the LOQ shall be performed by multiple injection ($n = 10$) of a standard solution of aflatoxin B₁ (concentration equivalent to a contamination level of $0,1 \mu\text{g/kg}$). The relative standard deviation (RSD) of the obtained signals shall not exceed a value of 10 %. This data shall be reported.

5.13.2 Mobile phase pump, capable of maintaining a volume flow rate of 1,0 ml/min.

5.13.3 Fluorescence detector, with a wavelength of $\lambda = 360 \text{ nm}$ excitation filter and a wavelength of $\lambda > 420 \text{ nm}$ cut-off emission filter, or equivalent (e.g. a detector with an adjustable monochromator).

5.13.4 Recorder, integrator or computer based data processing system.

5.13.5 Analytical reverse-phase HPLC separating column, e.g. C₁₈ or ODS-2, and a suitable corresponding reverse phase guard column.

This column shall ensure a baseline resolved resolution of the aflatoxin B₁ peak from all other peaks. The maximum overlapping of peaks shall be less than 10 % of the maximum peak height. It could be necessary to adjust the mobile phase for a sufficient baseline resolution. A suitable pre-column should be used.

5.13.6 Post-column derivatization system, with PBPB (only to be used with mobile phase A, see 4.21).

Consisting of a second HPLC pulseless pump, zero-dead volume T-piece, reaction tubing of polytetrafluoroethylene (PTFE) with minimum 45 cm length and 0,5 mm internal diameter.

5.13.7 System for derivatization with electrochemically generated bromine, e.g. KOBRA cell^{® 1)} (only to be used with mobile phase B, see 4.22).

Recommended settings for adjustable detectors are 365 nm (excitation wavelength), 435 nm (emission wavelength) and a bandwidth of 18 nm.

5.13.8 Degasser (optional).

5.14 UV spectrometer, with suitable quartz cells.

6 Procedure

6.1 Extraction

Mix the sample thoroughly before removing an analytical test portion.

Weigh, to the nearest 0,1 g, 50 g of the test portion of baby food (infant formula) into a 500 ml conical flask (5.5). Add 5 g of sodium chloride (4.8) and 250 ml of extraction solvent (4.19). Shake intensively by hand for the first 15 s to 30 s and then for 30 min with a shaker (5.3). Filter the extract using a prefolded filter paper (5.4) and, using a pipette (5.11), pipette 10 ml of the clear filtrate into a 100 ml calibrated volumetric flask (5.9) and fill up with PBS (4.13) to the mark. Re-filter through a glass microfibre filter (5.6) and transfer a volume of

1) KOBRA cell[®] is a trade name of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

50 ml to 100 ml of the clear filtrate on a reservoir (5.7) that is placed on a conditioned immunoaffinity column (see 4.25). Transfer the solution on column as described in 6.2.

6.2 Immunoaffinity column cleanup

The cleanup may be carried out by using a vacuum, by positive pressure or by allowing the specified volumes to pass through the column under gravity. Do not exceed the maximum specified flow rates. Extra care is needed to ensure this when a vacuum manifold is used.

Prepare the immunoaffinity column (4.25) according to the manufacturers' instructions, with special attention for avoiding column drying, if specified. Columns should be allowed to reach room temperature prior to conditioning.

Pass the filtrate through the column at a flow rate of approximately 3 ml/min (approximately 1 drop/s). Do not exceed a flow rate of 5 ml/min. Wash the column with approximately 15 ml of PBS (4.13), applied in little portions of approximately 5 ml at a flow rate of maximum 5 ml/min and dry by applying little vacuum for 5 s to 10 s or by passing air through the immunoaffinity column by means of a syringe for 10 s.

6.3 Preparation of the sample test solution

Elute the aflatoxin B₁ by transferring 0,50 ml of methanol (4.16) on the column and let it pass through by gravity. Collect the elute in a calibrated volumetric flask of 5 ml (5.9).

Wait for 1 min and transfer a second portion of 0,75 ml methanol (4.16) on the column. Collect as much of the extract as possible by pressing air through. Fill the flask to the mark with water and shake well. If the solution is clear it can be used directly for HPLC analysis. If the solution is not clear, pass it through a disposable filter unit (5.10) prior to HPLC injection.

NOTE Methods for loading onto immunoaffinity columns, washing the column and elution vary slightly between column manufacturers and the specific instructions supplied with the columns should be followed.

6.4 Spiking procedure

For the determination of the recovery, carry out a spiking procedure using a methanol spiking solution (4.29). The spiking level shall be within the calibration range (preferably at the value corresponding at the mid range of the calibration curve). Take care that not more than 2 ml (5.11) of the spiking solvent is added and that the subsequent evaporation takes place in the dark and should last about 0,5 h to 2 h.

7 HPLC analysis

7.1 HPLC operating conditions

Aflatoxin B₁ is separated by isocratic reverse-phase high performance liquid chromatography (RP-HPLC) at ambient temperature with a reverse-phase column (5.13.5) and appropriate mobile phase (see 4.21 or 4.22). The injection volume is 1 000 µl (to ensure a maximum precision total loop mode (see 5.13.1) according to the instructions of the HPLC injection valve or injection port manufacturer is advisable). The recommended flow rate is 1 ml/min for a column with an inner diameter of 4,6 mm. Other column dimensions may be used, provided that the required limit of quantification is achieved. This shall be demonstrated. Thus the flow rate may be adjusted according to the column dimension.

Aflatoxin B₁ elutes with retention time of approximately 11 min and should be baseline resolved from any interfering substances, if present. The mobile phase may be adjusted by addition of water, methanol (4.16) or acetonitrile (4.15) for maximum peak resolution and performance. A typical chromatogram is enclosed in Annex A.

7.2 Post-column derivatization

When using PBPB, mount the post-column derivatization system (see 5.13.6), and then operate using the following parameters:

- flow rate of 1,0 ml/min for the mobile phase;
- flow rate of 0,30 ml/min for the derivatization reagent (flow rate can be adjusted to the flow rate of the mobile phase).

When using electrochemically generated bromine (KOBRA cell^{®2)}) follow the instructions for the installation of the cell as supplied by the manufacturers and operate using the following parameters:

- flow rate of 1,0 ml/min for the mobile phase;
- current of 100 µA.

7.3 Preparation of calibration solutions for HPLC

Pipette from the aflatoxin B₁ standard solution (4.28) the volumes as listed in Table 1 into a set of 10 ml calibrated volumetric flasks, then evaporate the mixture of toluene and acetonitrile solution just to dryness under a stream of nitrogen at room temperature. To each flask, add 3,5 ml of methanol (4.16), let the aflatoxin B₁ dissolve, dilute to 10 ml with water, and shake well. These solutions cover the range of 0,05 µg/kg to 0,35 µg/kg for aflatoxin B₁ under the conditions of this protocol.

NOTE Methanol and water are subject to volume contraction when mixed.

Table 1 — Preparation of HPLC calibration solutions

HPLC calibration solution	Aliquot taken from aflatoxin B ₁ standard solution, ρ = 5,00 ng/ml (see 4.28) µl	Final aflatoxin B ₁ mass concentration in calibration solution ng/ml
1	20	0,01
2	40	0,02
3	60	0,03
4	80	0,04
5	100	0,05
6	120	0,06
7	140	0,07

The preparation of the calibration solutions can be performed either by the use of pipettes or calibrated glassware as available.

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7.4 Calibration curve

Prepare a calibration curve by injecting 1 000 µl of the calibration solutions listed in Table 1 into the HPLC system at the beginning of every day of the analysis. Plot the peak area against the mass of the injected aflatoxin B₁ and check the curve for linearity.

Prepare appropriate calibration curves in case that the content of aflatoxin B₁ in the sample is outside of the calibration range. Alternatively the injection solution for HPLC analysis can be diluted to an aflatoxin B₁ content appropriate for the established calibration curve.

7.5 Determination of aflatoxin B₁ in sample test solutions

Inject aliquots of the sample test solution (6.3) into the HPLC system using the same conditions used for the preparation of the calibration curve.

7.6 Confirmation of aflatoxin B₁

In order to confirm aflatoxin B₁ disconnect the HPLC column from the bromination device and connect it directly to the fluorescence detector. The aflatoxin B₁ signal is significantly lower (factor 10 or more) when the derivatization system is removed. Do not switch off the electrical current with the bromination device still in line due to the possibility of remaining bromine in the cell membrane of the device.

Alternatively, mass spectrometric identification could be applied by performing LC-MS or LC-MS/MS analysis.

8 Calculation

Determine from the calibration curve the mass concentration of aflatoxin B₁, in nanograms per millilitre (ng/ml), in the injection solution. Calculate the mass fraction, w_{afla} , of aflatoxin B₁ in nanograms per gram (ng/g), using Equation (2):

$$w_{\text{afla}} = \frac{\rho_{\text{afla}} \times V_1 \times V_3}{V_2 \times m_s} \quad (2)$$

where

- ρ_{afla} is the concentration of aflatoxin B₁, in nanograms per millilitre, in the test solution injected and derived from the calibration curve;
- V_1 is the volume, in millilitres, of the solvent taken for extraction (here: 250 ml);
- V_2 is the volume, in millilitres, of the undiluted aliquot of sample extract taken for immunoaffinity cleanup (here: 5 ml to 10 ml);
- V_3 is the volume, in millilitres, obtained after elution from the immunoaffinity column (here: 5 ml);
- m_s is the mass, in grams, of sample material taken for analysis (here: 50 g).

9 Precision

9.1 General

Details of the interlaboratory test on the precision of the method are given in Table B.1. The values derived from this interlaboratory test may not be applicable to analyte concentration ranges and matrices other than given in Annex B.

9.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit *r* in not more than 5 % of the cases.

The values for baby food (infant formula) are:

$\bar{x} = 0,10 \text{ }\mu\text{g/kg}$	$r = 0,011 \text{ }\mu\text{g/kg}$	(fortified)
$\bar{x} = 0,18 \text{ }\mu\text{g/kg}$	$r = 0,067 \text{ }\mu\text{g/kg}$	(fortified)
$\bar{x} = 0,07 \text{ }\mu\text{g/kg}$	$r = 0,028 \text{ }\mu\text{g/kg}$	
$\bar{x} = 0,09 \text{ }\mu\text{g/kg}$	$r = 0,020 \text{ }\mu\text{g/kg}$	
$\bar{x} = 0,17 \text{ }\mu\text{g/kg}$	$r = 0,059 \text{ }\mu\text{g/kg}$	

9.3 Reproducibility

The absolute difference between two single test results found on identical test material reported by two laboratories will exceed the reproducibility limit *R* in not more than 5 % of the cases.

The values for baby food (infant formula) are:

$\bar{x} = 0,10 \text{ }\mu\text{g/kg}$	$R = 0,034 \text{ }\mu\text{g/kg}$	(fortified)
$\bar{x} = 0,18 \text{ }\mu\text{g/kg}$	$R = 0,118 \text{ }\mu\text{g/kg}$	(fortified)
$\bar{x} = 0,07 \text{ }\mu\text{g/kg}$	$R = 0,048 \text{ }\mu\text{g/kg}$	
$\bar{x} = 0,09 \text{ }\mu\text{g/kg}$	$R = 0,022 \text{ }\mu\text{g/kg}$	
$\bar{x} = 0,17 \text{ }\mu\text{g/kg}$	$R = 0,109 \text{ }\mu\text{g/kg}$	

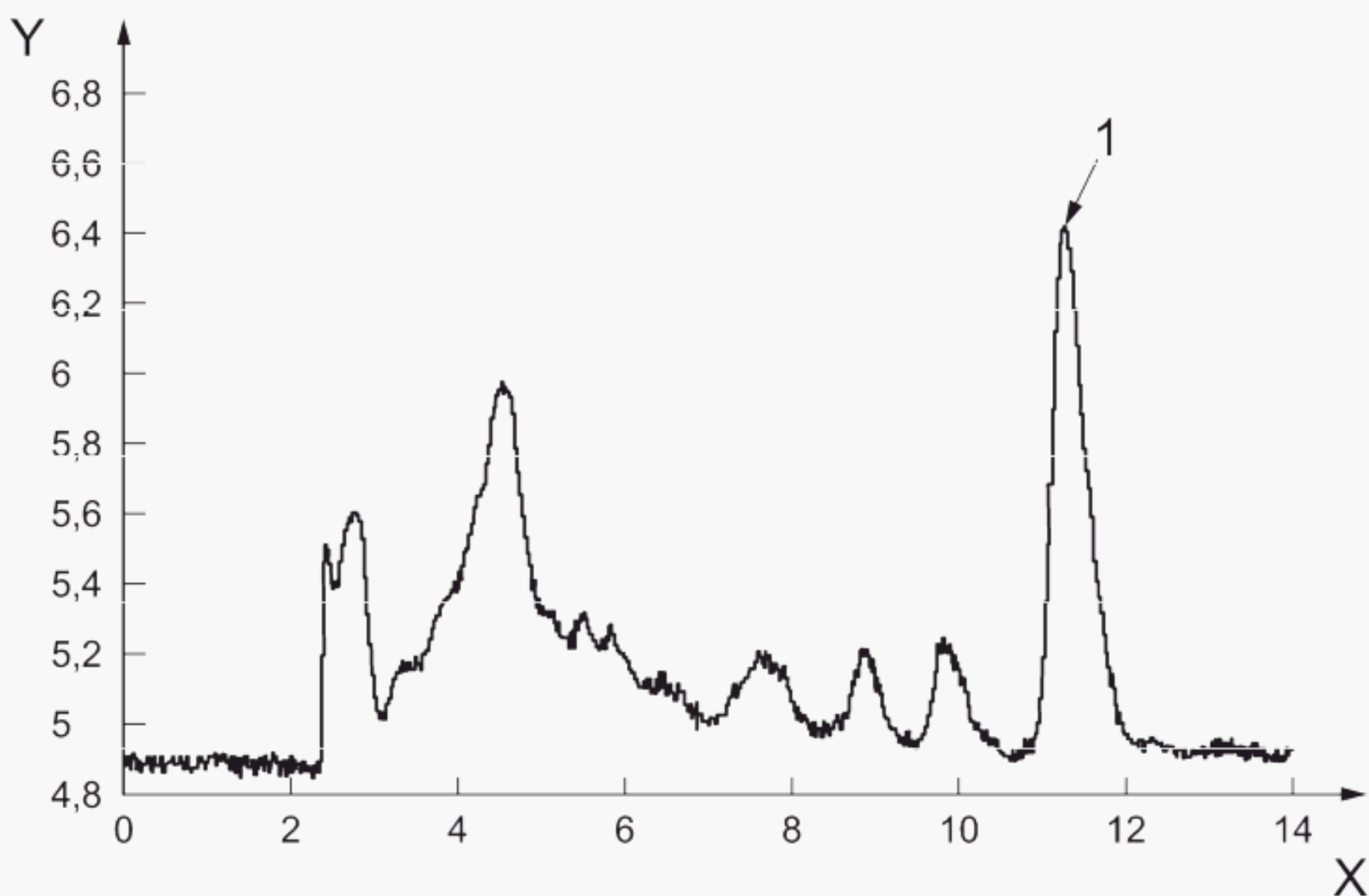
10 Test report

The test report shall contain the following data:

- a) all information necessary for the identification of the sample (kind of sample, origin of sample, designation);
- b) reference to this European Standard;
- c) date and type of sampling procedure (if known);
- d) date of receipt;
- e) date of test;
- f) test results and the units in which they have been expressed;
- g) any particular points observed in the course of the test;
- h) any operations not specified in the method or regarded as optional, which might have effected the results.

Annex A
(informative)

Typical chromatogram



Key

- X time (min)
- Y fluorescence (mV)
- 1 aflatoxin B₁

Operating conditions:

Column:	LC-18 Supelcosil [®] 3), inner diameter 4,6 mm, length 25 cm
Flow rate:	1 ml/min
Mobile phase:	HPLC mobile phase B, see 4.22
Column temperature:	Room temperature
Injection volume:	1 000 µl
Derivatization:	Electrochemically generated bromine
Detection:	Fluorescence, 365 nm excitation, 435 nm emission, bandwidth 18 nm

Figure A.1 — Typical chromatogram of aflatoxin B₁ in infant formula after immunoaffinity cleanup (contamination level 0,1 µg/kg aflatoxin B₁)

3) Supelcosil[®] is a trade name of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Annex B
(informative)

Precision data

The following data (see Table B.1) were obtained in an interlaboratory study organized by the European Communities, Standards Measurement and Testing Programme [6] in accordance with ISO 5725-2 [1], ISO 5725-4 [2] and ISO 5725-6 [3]. The test samples were baby food (infant formula) both naturally contaminated and spiked with aflatoxin B₁. The limit of quantification has been demonstrated to be 0,05 µg/kg, depending on the equipment used.

Table B.1 — Precision data interlaboratory study for baby food (infant formula)

Sample	1	2	3 ^a	4 ^a	5 ^a
Year of interlaboratory test	1999	1999	1999	1999	1999
Number of laboratories	14	14	14	14	14
Number of sample duplicates	1	1	1	1	1
Number of laboratories retained after eliminating outliers	11	14	14	11	13
Number of outliers (laboratories)	3	0	0	3	1
Number of accepted results	11	14	14	11	13
Mean value, \bar{x} , µg/kg	0,10	0,18	0,07	0,09	0,17
Repeatability standard deviation s_r , µg/kg	0,004	0,024	0,010	0,007	0,021
Repeatability relative standard deviation, RSD_r , %	3,5	13	14	8	12
Repeatability limit r [$r = 2,8 \times s_r$], µg/kg	0,011	0,067	0,028	0,020	0,059
Reproducibility standard deviation s_R , µg/kg	0,012	0,042	0,017	0,008	0,039
Reproducibility relative standard deviation, RSD_R , %	12	23	23	9	23
Reproducibility limit R [$R = 2,8 \times s_R$], µg/kg	0,034	0,118	0,048	0,022	0,109
Recovery, %	101	92	-	-	-
HorRat value, according to [7]	0,19	0,39	0,34	0,14	0,39
HorRat _R value, according to [8]	0,55	1,05	1,01	0,41	1,05
^a Naturally contaminated.					

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