BS EN 16187:2015

BSI Standards Publication

Foodstuffs — Determination of fumonisin B1 and fumonisin B2 in processed maize containing foods for infants and young children — HPLC method with immunoaffinity column cleanup and fluorescence detection after pre-column derivatisation

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National foreword

This British Standard is the UK implementation of EN 16187:2015. It supersedes DD CEN/TS 16187:2011 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Foodstuffs - Determination of fumonisin B1 and fumonisin B2 in processed maize containing foods for infants and young children - HPLC method with immunoaffinity column cleanup and fluorescence detection after pre-column derivatisation

Denrées alimentaires - Dosage de la fumonisine B1 et de la fumonisine B2 dans les aliments pour nourrissons et jeunes enfants contenant du maïs transformé - Méthode par CLHP avec purification sur colonne d'immunoaffinité et détection de fluorescence après dérivation précolonne

Lebensmittel - Bestimmung von Fumonisin B1 und Fumonisin B2 in Säuglings- und Kleinkindernahrung auf Maisbasis - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule und Fluoreszenzdetektion nach Vorsäulenderivatisierung

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Foreword

This document (EN 16187:2015) 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of an identical text or by endorsement, at the latest by December 2015, and conflicting national standards shall be withdrawn at the latest by December 2015.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes CEN/TS 16187:2011.

No technical change has been introduced during the conversion of CEN/TS 16187:2011 into this final draft European Standard.

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1 Scope

This European Standard specifies a method for the determination of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) in processed maize-containing foods for infants and young children by high performance liquid chromatography (HPLC) with immunoaffinity cleanup and fluorescence detection (FLD). This method has been validated in an interlaboratory study via the analysis of both naturally contaminated and spiked samples ranging from 112 µg/kg to 458 µg/kg for FB_1+FB_2, 89 µg/kg to 384 µg/kg for FB_1 and 22 µg/kg to 74 µg/kg for FB_2.

For further information on the validation, see Clause 8 and Annex B.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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

Fumonisins are extracted from the sample with a mixture of citrate-phosphate buffer with methanol and acetonitrile. The filtered extract is diluted with water and applied to an immunoaffinity column containing antibodies specific to fumonisins. Fumonisins are eluted from the column with methanol and water and quantified by HPLC-FLD with pre-column derivatisation with *o*-phthaldialdehyde (OPA) reagent.

4 Reagents

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 [1].

4.1 Acetonitrile

WARNING — Acetonitrile is hazardous and samples shall be shaken using an orbital shaker which is housed within a fume cupboard. After shaking, samples shall be filtered inside a fume cupboard.

4.2 Methanol

4.3 O-phthaldialdehyde (OPA)

4.4 Citric acid solution, substance concentration $c(C_6H_8O_7 \cdot H_2O)$ = 0,1 mol/l

Dissolve 21,0 g of $C_6H_8O_7 \cdot H_2O$ in water and dilute to 1 l.

4.5 Disodium hydrogen phosphate solution, $c(Na_2HPO_4)$ = 0,2 mol/l

Dissolve 28,4 g of Na_2HPO_4 in water and dilute to 1 l.

4.6 2-mercaptoethanol

4.7 Citrate-phosphate buffer solution

Mix 1 part per volume of citric acid solution (4.4) with 1 part per volume of disodium hydrogen phosphate solution (4.5).

4.8 Extraction solvent

Mix 2 parts per volume of citrate-phosphate buffer solution (4.7) with 1 part per volume of methanol (4.2) and 1 part per volume of acetonitrile (4.1).

4.9 Glacial acetic acid

4.10 Phosphate buffered saline (PBS) solution, *c*(NaCl) = 137 mmol/l, *c*(KCl) = 2,7 mmol/l, *c*(phosphate buffer) = 10 mmol/l, pH = 7,4 at T = 25 °C

Dissolve one tablet of commercially available PBS material in 200 ml of water.

4.11 Mixture of acetonitrile and water A

Mix 1 part per volume of acetonitrile (4.1) with 1 part per volume of water. Use this solvent to prepare spiking solutions.

4.12 Mixture of acetonitrile and water B

Mix 3 parts per volume of acetonitrile (4.1) with 7 parts per volume of water. Use this solvent to prepare calibration solutions and to redissolve dried extracts from immunoaffinity cleanup.

4.13 Sodium tetraborate solution, *c*($Na_2B_4O_7 \cdot 10H_2O$) = 0,1 mol/l

Dissolve 3,8 g of $Na_2B_4O_7 \cdot 10H_2O$ in 100 ml of water.

4.14 OPA reagent solution

Dissolve 40 mg of OPA (4.3) in 1 ml of methanol (4.2) and dilute with 5 ml of sodium tetraborate solution (4.13). Add 50 µl of 2-mercaptoethanol (4.6) and mix for 1 min. This reagent solution is stable for up to one week at room temperature in the dark in a capped amber vial.

4.15 HPLC mobile phase

4.15.1 HPLC mobile phase A

Mix 30 parts per volume of acetonitrile (4.1) with 69 parts per volume of water and 1 part per volume of glacial acetic acid (4.9).

4.15.2 HPLC mobile phase B

Mix 60 parts per volume of acetonitrile (4.1) with 39 parts per volume of water and 1 part per volume of glacial acetic acid (4.9).

4.16 Immunoaffinity column

The immunoaffinity column shall contain antibodies raised against FB_1 and FB_2. The column shall have a capacity of not less than 5 µg of fumonisins and shall give a recovery of not less than 80 % for the sum of FB_1 and FB_2 when applied as a standard solution in PBS containing 5 µg of fumonisins. The columns shall be warmed up to room temperature before use. With these columns the working range of the method reaches

5 000 µg/kg of total fumonisins. The use of columns with a capacity less than 5 µg of fumonisins will reduce the working range of the method.

4.17 Certified standard solution of fumonisin B_1 (FB_1), mass concentration $\rho(FB_1)$ = 50 µg/ml in a mixture of 1 part per volume of acetonitrile and 1 part per volume of water (e.g. Biopure RK 002003 [1)] or equivalent)

4.18 Certified standard solution of fumonisin B_2 (FB_2), $\rho(FB_2)$ = 50 µg/ml in a mixture of 1 part per volume of acetonitrile and 1 part per volume of water (e.g. Biopure RK 002004 [1)] or equivalent)

WARNING — Fumonisins are nephrotoxic, hepatotoxic and carcinogenic to rats and mice and classified as possible human carcinogen by IARC. These compounds should be treated with extreme caution. Gloves and safety glasses shall be worn at all times and all standard and sample preparation stages shall be carried out in a fume cupboard.

4.19 Mixed FB_1 and FB_2 stock solution

Prepare a mixed FB_1 and FB_2 stock solution by pipetting 2 000 µl of the FB_1 certified standard solution (4.17) and 500 µl of the FB_2 certified standard solution (4.18) into a vial. Cap the vial and shake well to obtain a stock solution containing 40,0 µg/ml of FB_1 and 10,0 µg/ml of FB_2.

4.20 Diluted mixed FB_1 and FB_2 stock solution

Pipette 500 µl of the mixed stock solution (4.19) into a 10 ml calibrated volumetric flask. Fill up to the mark with the mixture of acetonitrile water B (4.12) and shake well to obtain a diluted mixed stock solution containing 2,0 µg/ml of FB_1 and 0,5 µg/ml of FB_2. Store the solution at less than 4 °C. This solution is stable for at least 6 months at these conditions.

4.21 Mixed FBs calibration solutions for HPLC

Prepare five HPLC mixed calibration solutions in 5 ml calibrated volumetric flasks by further diluting the diluted mixed FBs stock solution (4.20) according to Table 1. Make up each calibration solution to volume (5 ml) with the mixture of acetonitrile and water B (4.12) and mix well. Store the solution at less than 4 °C. This solution is stable for at least 6 months at these conditions.

Table 1 — Preparation of mixed FBs calibration solutions for HPLC

HPLC calibration solution	Diluted mixed FBs stock solution (4.20)	Final concentration of mixed FBs calibration solutions (4.21)		Sample equivalent levels of FB_1 and FB_2[a]	
	µl	FB_1 µg/ml	FB_2 µg/ml	FB_1 µg/kg	FB_2 µg/kg
1	100	0,04	0,01	20,0	5,0
2	200	0,08	0,02	40,0	10,0
3	400	0,16	0,04	80,0	20,0
4	1 000	0,40	0,10	200,0	50,0
5	2 400	0,96	0,24	480,0	120,0

[a] For a sample extract reconstituted in 500 µl of the mixture of acetonitrile and water B (4.12).

1) Biopure RK 002003 and RK 002004 are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products. Equivalent products may be used if they can be shown to lead to the same results.

5 Apparatus

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 0,1 g.

5.3 Thermostated water bath.

5.4 Conical flasks, of 250 ml capacity with screw caps.

5.5 Orbital shaker.

5.6 Centrifuge, capable of a centrifugal force up to 3 000 *g*.

5.7 Centrifuge bottles, of 250 ml capacity with screw cap*s*.

5.8 Calibrated microliter pipettes or microliter syringe*s*, of 100 µl, 200 µl or 1 000 µl capacity.

5.9 Displacement pipettes, of 5 ml, 10 ml or 25 ml capacity.

5.10 Vacuum manifold, to accommodate immunoaffinity columns (4.16).

5.11 Reservoirs (of 25 ml capacity) **and attachments to fit to columns.**

5.12 Vacuum pump.

5.13 Filter paper, e.g. qualitative, strong, fast flow, 24 cm diameter, 30 µm pore size, prefolded or equivalent.

5.14 Glass microfibre filter paper, e.g. 1,6 µm pore size or equivalent.

5.15 Heating block with nitrogen or air gas supply.

5.16 Vials, of 4 ml to 12 ml capacity with screw caps.

5.17 HPLC autosampler vials, of 1,8 ml capacity with caps.

5.18 Glass flat bottom vial insert, of 250 µl volume capacity.

5.19 Vortex mixer, or equivalent.

5.20 HPLC apparatus, comprising the following:

5.20.1 Injection system.

5.20.2 Mobile phase pump, (binary, ternary or quaternary pump), capable of generating a binary gradient at 1 ml/min.

5.20.3 Autosampler, capable of performing automated pre-column derivatisation with OPA reagent according to 6.4.1.

5.20.4 Analytical reverse-phase HPLC separating column, e.g. C18 reverse-phase column, 150 mm x 4,6 mm, 5 µm preceded by a suitable pre-column or guard filter, which provides acceptable retention and resolution for FB_1 and FB_2.

Waters C18 SymmetryShield™ [2)], Agilent Zorbax SB-C18 [2)] or similar have been found to be suitable.

5.20.5 Column oven, capable to operate at 20 °C.

NOTE C18 reverse-phase columns can also operate at ambient temperatures.

5.20.6 Fluorescence detector, fitted with a flow cell and suitable for measurements with excitation wavelength of 335 nm and emission wavelength of 440 nm.

5.20.7 Recorder, integrator or computer based data processing system.

5.21 Linear gradient settings

The gradient conditions are given in Table 2.

Table 2 — Gradient conditions

Time min	Flow rate ml/min	Mobile phase A %	Mobile phase B %
0,00	1,00	60	40
5,00	1,00	60	40
26,00	1,00	12	88
29,00	1,00	12	88
29,10	1,00	-	100
30,00	1,00	-	100
30,10	1,00	60	40
38,00	1,00	60	40

The gradient programme above has proven to give acceptable resolution for FB_1 and FB_2 by using a Waters C18 SymmetryShield™ [2)] column. The use of a different column may necessitate adjustment of these conditions until acceptable resolution is achieved.

6 Procedure

6.1 Extraction

The sample should be finely ground to pass through a 0,5 mm sieve and thoroughly mixed to ensure complete homogenization.

Weigh, to the nearest 0,1 g, a 20 g test portion of the ground sample into a conical flask (5.4). Add 100 ml of extraction solvent (4.8) and close the flask with screw cap. Heat at 55 °C (static condition) in the thermostated water bath (5.3) for 60 min. Put the flask on the orbital shaker (5.5) and shake for 30 min at room temperature. Transfer the sample into a centrifuge bottle (5.7) and centrifuge (5.6) at 3 000 *g* for 15 min. Filter through a filter paper (5.13) and collect 10 ml filtrate. Dilute the 10 ml filtrate with 40 ml of water and mix. Filter through a

2) Waters C18 SymmetryShield™ and Agilent Zorbax SB-C18 are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products. Equivalent products may be used if they can be shown to lead to the same results.

microfibre filter (5.14), collect 25 ml filtrate (equivalent to 1 g of test sample) and proceed rapidly to immunoaffinity cleanup (i.e. within the next hour).

6.2 Immunoaffinity column cleanup

Accommodate the immunoaffinity column (4.16) to the vacuum manifold (5.10) and attach the reservoir. Do not empty storage solution from column. Add the 25 ml of the diluted filtered extract to the reservoir. Elute the extract at a flow rate of about one drop per second. Wash the column with 10 ml of PBS solution (4.10) followed by 2 ml of water at a flow rate of about one drop per second. Pass air or nitrogen for 5 s through the column to expel water. Place a vial or tube under the column.

Elute fumonisins with two times 1 ml of methanol (4.2) and 2 ml water in the following way.

Add 1 ml of methanol on the column. Press the liquid through the column with slight over pressure of air (e.g. with Pasteur balloon or syringe) until the first drop comes out. Close the stopcock immediately. Wait for 1 min. Open the stopcock. Allow elution to take place by gravity force, until the liquid reaches the top layer of the column. Close the stopcock immediately and wait for 1 min. Add the second 1 ml of methanol. Open the stopcock and let half of the liquid (approx. 500 µl) flow through the column by gravity force. Close the stopcock and wait 1 min. Open the stopcock and elute the remaining liquid by gravity force. Pass air or nitrogen through the column to remove the last methanol from the column.

Add 2 ml of water and elute the liquid through the column by gravity force. Pass air or nitrogen through the column to remove the last liquid from the column. Evaporate the eluates to dryness under a stream of air or nitrogen at ca 55 °C (5.15). Redissolve the residue in 500 µl of the mixture of acetonitrile and water B (4.12). Cap the vial and shake on a vortex mixer (5.19) for 30 s, making sure the lower part of the vial is thoroughly rinsed by the solvent and store at 4 °C until HPLC analysis that should be performed within one week.

6.3 Spiking procedure

To determine recovery spike a fumonisin-free representative ground baby food material with a spiking solution prepared by appropriately diluting the mixed FB1 and FB2 stock solution (4.19) with the mixture of acetonitrile and water A (4.11). No more than 1 ml of spiking solution should be added to each test portion. The fumonisin mass concentration of the spiking solution should be prepared according to the spiking level chosen. Leave the spiked sample to stand overnight to ensure evaporation of the solvent.

6.4 Derivatisation and HPLC determination

6.4.1 Automated pre-column derivatisation programme

Transfer 110 µl of extract to an HPLC autosampler vial containing a glass flat bottom vial insert (5.18). Put the vial containing the sample extract in the autosampler.

The following conditions have proven to produce satisfying results.

Aspirate an air segment of 5 µl to separate the wash solvent in the buffer tubing from the OPA reagent (4.14). Aspirate 50 µl of OPA reagent to flush the tubing and needle. Unload the syringe to the syringe-waste position. Aspirate 110 µl of OPA reagent and dispense it to the vial insert containing 110 µl of sample extract or calibration solution. Rinse the buffer tubing and needle with the mixture of acetonitrile and water A (4.11). Aspirate 55 µl of derivatised solution and dispense it back into the vial insert; repeat this four times. Rinse the buffer tubing and needle with wash solvent (4.11). Wait for 20 s. Inject 50 µl in full loop mode. Rinse the buffer tubing and needle with wash solvent (4.11) five times. This automated derivatisation procedure takes 2,5 min.

NOTE Different pre-column derivatisation conditions (volumes, derivatisation programme, etc.) could be used according to your autosampler, bearing in mind that the signals (peak area or height) of FB_1 or FB_2 in the sample extract should fall within the calibration range.

6.4.2 HPLC injections

Once the instrument settings are acceptable, inject 50 µl of derivatised calibration and sample extracts solutions. Re-runs of the calibration solutions should be interspersed in regular intervals when running the derivatised sample extract solutions. The frequency of these calibration re-runs depend on the stability of the chromatographic system. Typical chromatograms of spiked and naturally contaminated baby food samples are shown in Figure A.1 and Figure A.2 (Annex A).

6.4.3 Peak identification

Identify FB_1 and FB_2 peaks in the sample extract by comparing the retention times with those of the calibration solutions.

6.4.4 Determination

To carry out the determination by the external standard method, integrate the peak area or determine the peak height and compare with the calibration curve. The signals (peak area or height) of FB_1 or FB_2 in the sample extract shall fall within the calibration range. If the FB_1 and/or FB_2 signal in the sample extract exceeds the signals of the highest calibration solution the sample extract should be diluted with the mixture of acetonitrile and water solution B (4.12) to bring it within the calibration range and be reanalysed. The dilution factor shall be incorporated into Formula (1).

7 Calculation

Read off the mass of each fumonisin (m_a), in nanograms, corresponding to the peak area or height of sample extract from the calibration curve.

Calculate the mass fraction of each fumonisin, w_i, in micrograms per kilogram, in the sample using Formula (1):

$$w_i = \frac{m_a \times V_t \times D}{W \times V_i} \quad (1)$$

where

m_a is the mass of each fumonisin, in nanograms, in the aliquot of derivatised sample extract solution injected in the HPLC column;

V_t is the volume of the derivatised solution, in microliters (V_t = 220 µl);

D is the dilution factor that may have been used;

W is the sample equivalent mass derivatised, in grams (W = 0,22 g in 110 µl of sample extract solution);

V_i is the injected volume, in microliters (V_i = 50 µl).

8 Precision

8.1 General

Details of an interlaboratory test on the precision of the method are given in Tables B.1, B.2 and B.3. The values derived from this interlaboratory test may not be applicable to concentration ranges and/or matrices other than those given in Annex B.

8.2 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 FB_1+FB_2 in processed maize-containing foods for infants and young children are:

$\bar{x}$ = 112 µg/kg	*r* = 51,0 µg/kg	(fortified)
$\bar{x}$ = 293 µg/kg	*r* = 56,6 µg/kg	(fortified)
$\bar{x}$ = 211 µg/kg	*r* = 135,5 µg/kg	(naturally contaminated)
$\bar{x}$ = 322 µg/kg	*r* = 126,3 µg/kg	(naturally contaminated)
$\bar{x}$ = 458 µg/kg	*r* = 107,5 µg/kg	(naturally contaminated)

The values for FB_1 in processed maize-containing foods for infants and young children are:

$\bar{x}$ = 89 µg/kg	*r* = 40,3 µg/kg	(fortified)
$\bar{x}$ = 240 µg/kg	*r* = 45,4 µg/kg	(fortified)
$\bar{x}$ = 177 µg/kg	*r* = 16,2 µg/kg	(naturally contaminated)
$\bar{x}$ = 258 µg/kg	*r* = 51,2 µg/kg	(naturally contaminated)
$\bar{x}$ = 384 µg/kg	*r* = 91,3 µg/kg	(naturally contaminated)

The values for FB_2 in processed maize-containing foods for infants and young children are:

$\bar{x}$ = 22 µg/kg	*r* = 13,2 µg/kg	(fortified)
$\bar{x}$ = 53 µg/kg	*r* = 11,5 µg/kg	(fortified)
$\bar{x}$ = 34 µg/kg	*r* = 22,1 µg/kg	(naturally contaminated)
$\bar{x}$ = 51 µg/kg	*r* = 18,2 µg/kg	(naturally contaminated)
$\bar{x}$ = 74 µg/kg	*r* = 17,6 µg/kg	(naturally contaminated)

8.3 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 FB_1+FB_2 in processed maize-containing foods for infants and young children are:

$\bar{x}$ = 112 µg/kg	*R* = 83,2 µg/kg	(fortified)
$\bar{x}$ = 293 µg/kg	*R* = 136,9 µg/kg	(fortified)
$\bar{x}$ = 211 µg/kg	*R* = 157,1 µg/kg	(naturally contaminated)
$\bar{x}$ = 322 µg/kg	*R* = 218,1 µg/kg	(naturally contaminated)
$\bar{x}$ = 458 µg/kg	*R* = 213,4 µg/kg	(naturally contaminated)

The values for FB_1 in processed maize-containing foods for infants and young children are:

$\bar{x}$ = 89 µg/kg	*R* = 65,2 µg/kg	(fortified)
$\bar{x}$ = 240 µg/kg	*R* = 107,2 µg/kg	(fortified)
$\bar{x}$ = 177 µg/kg	*R* = 127,1 µg/kg	(naturally contaminated)
$\bar{x}$ = 258 µg/kg	*R* = 132,2 µg/kg	(naturally contaminated)
$\bar{x}$ = 384 µg/kg	*R* = 165,5 µg/kg	(naturally contaminated)

The values for FB_2 in processed maize-containing foods for infants and young children are:

$\bar{x}$ = 22 µg/kg	R = 23,0 µg/kg	(fortified)
$\bar{x}$ = 53 µg/kg	R = 32,2 µg/kg	(fortified)
$\bar{x}$ = 34 µg/kg	R = 34,7 µg/kg	(naturally contaminated)
$\bar{x}$ = 51 µg/kg	R = 44,0 µg/kg	(naturally contaminated)
$\bar{x}$ = 74 µg/kg	R = 58,5 µg/kg	(naturally contaminated)

9 Test report

The test report shall contain at least the following data:

a) all information necessary for the identification of the sample (kind of sample, origin of sample, designation);

b) a reference to this European Standard;

c) the date and type of sampling procedure (if known);

d) the date of receipt;

e) the date of test;

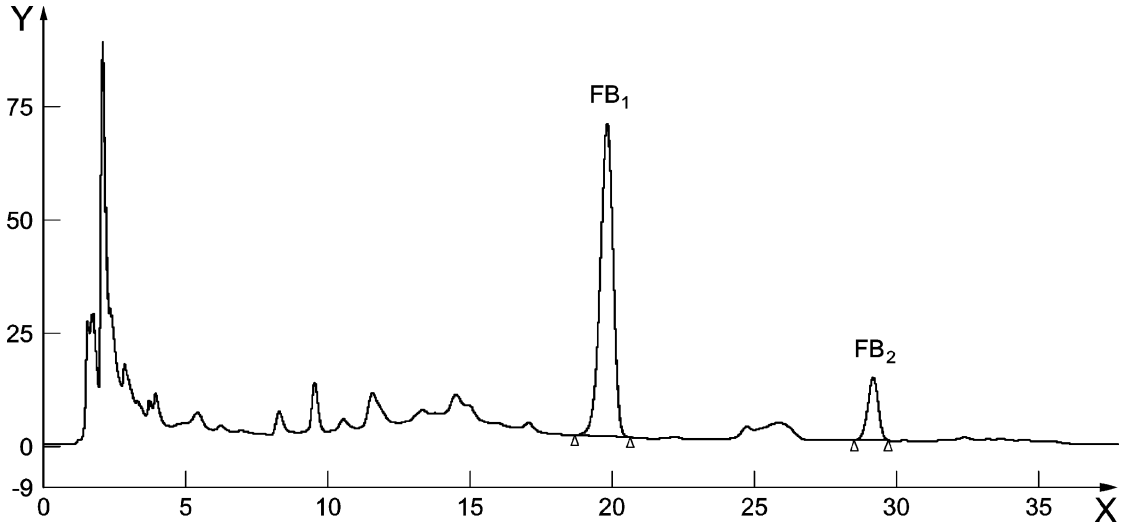
f) the test results and the units in which they have been expressed;

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 affected the results.

Annex A
(informative)

Typical chromatograms

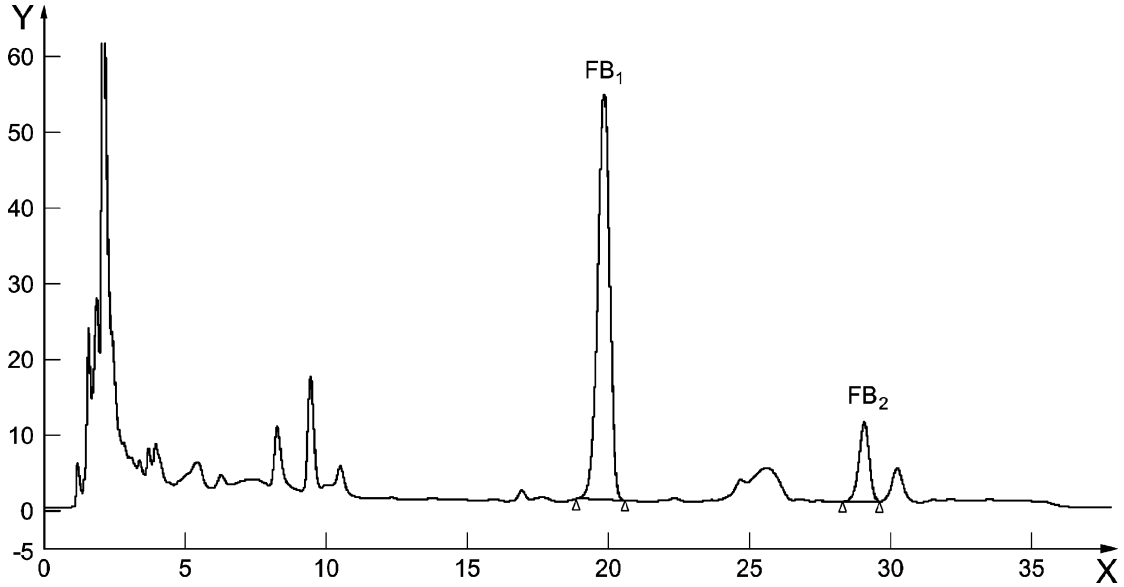


Key

x = t in min

y = mVolts

Figure A.1 — Typical chromatogram of baby food (pre-cooked flours of diastased maize and tapioca) spiked with 250,0 µg/kg fumonisin B_1 (FB_1) and 62,5 µg/kg fumonisin B_2 (FB_2)



Key

x = t in min

y = mVolts

Figure A.2 — Typical chromatogram of baby food (biscuits containing wheat, barley, rye, rice and maize) naturally contaminated with 164,8 µg/kg FB_1 and 35,6 µg/kg FB_2

Annex B
(informative)

Precision data

The data given in Tables B.1, B.2 and B.3 were obtained in an interlaboratory study [2] conducted in accordance with the IUPAC/ISO/AOAC protocol [3] for collaborative study procedures to validate characteristics of a method of analysis.

Table B.1 — Precision and recovery data for fumonisins B_1+B_2 in baby food

Sample	1 [a]	2 [b]	3 [c]	4 [d]	5 [e]
Year of interlaboratory test	2009	2009	2009	2009	2009
Number of laboratories	10 [f]	10 [f]	10 [f]	10 [f]	10 [f]
Number of laboratories retained after eliminating outliers	8	10	10	10	9
Number of outliers (laboratories)	2	0	0	0	1
Number of accepted results	16	20	20	20	18
Mean value, $\bar{x}$, µg/kg	111,6	293,4	211,2	322,5	458,0
Repeatability standard deviation s_r, µg/kg	18,2	20,2	48,4	45,1	38,4
Repeatability relative standard deviation, RSD_r, %	16,3	6,9	22,9	14,0	8,4
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	51,0	56,6	135,5	126,3	107,5
Reproducibility standard deviation s_R, µg/kg	29,7	48,8	56,1	77,9	76,2
Reproducibility relative standard deviation, RSD_R, %	26,6	16,6	26,6	24,1	16,6
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	83,2	136,9	157,1	218,1	213,4
Recovery, %	89	94	-	-	-
HorRat value, according to [4]	1,2	0,9	1,3	1,3	0,9
HorRat value, according to [5]	1,2	0,9	1,3	1,3	0,9

[a] Spiked sample ('Bambix fijne granen' [3)] containing wheat, oat, rice, millet, barley, maize and rye).

[b] Spiked sample (flour of diastased maize and tapioca, spices and vitamin B_1).

[c] Naturally contaminated sample (biscuits containing wheat, barley, rye, rice and maize).

[d] Naturally contaminated sample (milk powder, vegetal oil, rice, maize, sugars, minerals and vitamins).

[e] Naturally contaminated sample (pre-cooked flours of wheat, oat, rye, barley, rice, millet and maize).

[f] One laboratory out of original eleven participants was removed because judged invalid.

3) 'Bambix fijne granen' is an example 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 this product.

Table B.2 — Precision and recovery data for fumonisin B_1 in baby food

Sample	1 [a]	2 [b]	3 [c]	4 [d]	5 [e]
Year of interlaboratory test	2009	2009	2009	2009	2009
Number of laboratories	10 [f]	10 [f]	10 [f]	10 [f]	10 [f]
Number of laboratories retained after eliminating outliers	8	10	10	9	9
Number of outliers (laboratories)	2	0	0	1	1
Number of accepted results	16	20	20	18	18
Mean value, $\bar{x}$, µg/kg	89,1	240,1	176,7	257,6	384,4
Repeatability standard deviation s_r, µg/kg	14,4	16,2	41,5	18,3	32,6
Repeatability relative standard deviation, RSD_r, %	16,2	6,8	23,5	7,1	8,5
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	40,3	45,4	116,2	51,2	91,3
Reproducibility standard deviation s_R, µg/kg	23,3	38,3	45,4	47,2	59,1
Reproducibility relative standard deviation, RSD_R, %	26,2	16,0	25,7	18,3	15,4
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	65,2	107,2	127,1	132,2	165,5
Recovery, %	89	96	-	-	-
HorRat value, according to [4]	1,1	0,8	1,2	0,9	0,8
HorRat value, according to [5]	1,2	0,8	1,2	0,9	0,8

[a] Spiked sample ('Bambix fijne granen' [4)] containing wheat, oat, rice, millet, barley, maize and rye).

[b] Spiked sample (flour of diastased maize and tapioca, spices and vitamin B_1).

[c] Naturally contaminated sample (biscuits containing wheat, barley, rye, rice and maize).

[d] Naturally contaminated sample (milk powder, vegetal oil, rice, maize, sugars, minerals and vitamins).

[e] Naturally contaminated sample (pre-cooked flours of wheat, oat, rye, barley, rice, millet and maize).

[f] One laboratory out of original eleven participants was removed because judged invalid.

4) 'Bambix fijne granen' is an example 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 this product.

Table B.3 — Precision and recovery data for fumonisin B_2 in baby food

Sample	1 [a]	2 [b]	3 [c]	4 [d]	5 [e]
Year of interlaboratory test	2009	2009	2009	2009	2009
Number of laboratories	10 [f]	10 [f]	10 [f]	10 [f]	10 [f]
Number of laboratories retained after eliminating outliers	8	10	10	9	9
Number of outliers (laboratories)	2	0	0	1	1
Number of accepted results	16	20	20	18	18
Mean value, $\bar{x}$, µg/kg	22,5	53,3	34,5	50,8	73,6
Repeatability standard deviation s_r, µg/kg	4,7	4,1	7,9	6,5	6,3
Repeatability relative standard deviation, RSD_r, %	21,0	7,6	22,9	12,9	8,6
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	13,2	11,5	22,1	18,2	17,6
Reproducibility standard deviation s_R, µg/kg	8,2	11,5	12,4	15,7	20,9
Reproducibility relative standard deviation, RSD_R, %	36,3	21,6	36,0	30,8	28,4
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	23,0	32,2	34,7	44,0	58,5
Recovery, %	90	85	-	-	-
HorRat value, according to [4]	1,3	0,9	1,4	1,2	1,2
HorRat value, according to [5]	1,7	1,0	1,6	1,4	1,3

[a] Spiked sample ('Bambix fijne granen' [5)] containing wheat, oat, rice, millet, barley, maize and rye).

[b] Spiked sample (flour of diastased maize and tapioca, spices and vitamin B_1).

[c] Naturally contaminated sample (biscuits containing wheat, barley, rye, rice and maize).

[d] Naturally contaminated sample (milk powder, vegetal oil, rice, maize, sugars, minerals and vitamins).

[e] Naturally contaminated sample (pre-cooked flours of wheat, oat, rye, barley, rice, millet and maize).

[f] One laboratory out of original eleven participants was removed because judged invalid.

5) 'Bambix fijne granen' is an example 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 this product.

Annex C
(informative)

Comparison between the method in this document and EN 14352:2004 and EN 13585:2001 on fumonisins in maize

The data in Table C.1 were obtained in one laboratory where four maize samples naturally contaminated with fumonisin B_1 and B_2 were analysed with the method given in this document and EN 14352:2004 [6] and EN 13585:2001 [7] on the analysis of fumonisins. The results are the mean of four replicated analyses. The results reported in Table C.1 demonstrate the applicability of the present method also to maize flour samples having different particle size.

Table C.1 — Comparison between the method in this document and EN 14352:2004 and EN 13585:2001

		Present method		EN 14352:2004 [d]		EN 13585:2001 [e]	
		Mean µg/kg	RSD_r %	Mean µg/kg	RSD_r %	Mean µg/kg	RSD_r %
Ground whole maize (particle size < 500 µm)	FB_1+FB_2	580,6 [a]	9	612,5 [a]	12	648,9 [a]	7
	FB_1	485,0 [a]	10	498,3 [a]	11	509,9 [a]	7
	FB_2	95,6 [a]	9	114,2 [c]	15	139,0 [b]	8
Ground whole maize (particle size > 500 µm)	FB_1+FB_2	804,3 [a]	8	791,5 [c]	12	635,9 [b]	7
	FB_1	640,3 [a]	8	619,1 [c]	14	500,9 [b]	7
	FB_2	164,0 [a]	9	172,4 [a]	6	135,0 [b]	8
Maize flour (particle size 350 µm to 500 µm)	FB_1+FB_2	210,6 [a]	5	204,5 [a]	3	161,1 [b]	10
	FB_1	175,2 [a]	5	166,9 [a]	3	135,1 [b]	12
	FB_2	35,4 [a]	6	37,6 [a]	3	26,0 [b]	5
Maize flour (particle size < 350 µm)	FB_1+FB_2	3 148,1 [a]	8	3 264,3 [a]	4	2 276,9 [b]	5
	FB_1	2 561,3 [a]	9	2 642,6 [a]	4	1 868,8 [b]	5
	FB_2	586,8 [a]	7	621,7 [a]	6	408,1 [b]	8

[a] Mean fumonisin levels are statistically significantly different ($P < 0,05$, Tukey one-way ANOVA with post-test, parametric test) from those with footnote b in the same line.

[b] Mean fumonisin levels are statistically significantly different ($P < 0,05$, Tukey one-way ANOVA with post-test, parametric test) from those with footnote a in the same line.

[c] Mean fumonisin levels are not statistically significantly different ($P < 0,05$, Tukey one-way ANOVA with post-test, parametric test) from those with footnote a or b in the same line.

[d] EN 14352:2004 Fumonisins are extracted from maize with a mixture of water-acetonitrile-methanol and the filtered extract is purified on immunoaffinity column.

[e] EN 13585:2001 Fumonisins are extracted from maize with a mixture of water-methanol and the filtered extract is purified on strong anion exchange column.

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[6] EN 14352:2004, *Foodstuffs - Determination of fumonisin B1 and B2 in maize based foods - HPLC method with immunoaffinity column clean up*

[7] EN 13585:2001, *Foodstuffs - Determination of fumonisins B1 and B2 in maize - HPLC method with solid phase extraction clean-up*

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