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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 precolumn derivatization

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Normcommissie 370275 "Voedingsmiddelenanalyse - Horizontale methoden"

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EUROPEAN STANDARD

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DRAFT
prEN 16187

December 2012

ICS 67.230

Will supersede CEN/TS 16187:2011

English Version

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 precolumn 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

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 275.

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Foreword

This document (prEN 16187:2012) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

This document will supersede CEN/TS 16187:2011.

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

WARNING — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This document 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 recognised 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 $c(C_6H_8O_7 \cdot H_20) = 0{,}1$ mol/l.

Dissolve 21,0 g of $C_6H_8O_7 \cdot H_20$ 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.

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 2. Make up each calibration solution to volume (5 ml) with the mixture of acetonitrile and water B (4.12) and mix well.

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.

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