SLOVENSKI STANDARD
kSIST-TS FprCEN/TS 16187:2010

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Živila - Določevanje fumonizina B1 in fumonizina B2 v predelanih koruznih kašicah za dojenčke in majhne otroke - HPLC metoda s čiščenjem z imunoafinitetno kolono in fluorescenčno detekcijo po predkolonski derivatizaciji

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

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

Produits alimentaires - Fumonisine B1 et B2 dans les aliments à base de maïs pour bébés et jeunes enfants

Ta slovenski standard je istoveten z: **FprCEN/TS 16187**

ICS:

67.060	Žita, stročnice in proizvodi iz njih	Cereals, pulses and derived products
67.230	Predpakirana in pripravljena hrana	Prepackaged and prepared foods

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TECHNICAL SPECIFICATION

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FINAL DRAFT FprCEN/TS 16187

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ICS

English Version

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COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (FprCEN/TS 16187:2010) 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 Formal Vote.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

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.

Annexes A, B and C are informative.

1 Scope

This Technical Specification 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. This method has been validated in an interlaboratory study via the analysis of both naturally contaminated and spiked samples ranging from 111,6 µg/kg to 458,0 µg/kg for FB_1+FB_2, 89,1 µg/kg to 384,4 µg/kg for FB_1 and 22,5 µg/kg to 73,6 µg/kg for FB_2.

For further information on the validation see Clause 8 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

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

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 [1].

4.2 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.

4.3 Methanol.

4.4 O-phthaldialdehyde (OPA).

4.5 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.

4.6 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.

4.7 2-mercaptoethanol.

4.8 Citrate buffer solution.

Mix one part per volume of citric acid solution (4.5) with one part per volume of disodium hydrogen phosphate solution (4.6).

4.9 Extraction solvent.

Mix two parts per volume of citrate buffer solution (4.8) with one part per volume of methanol (4.3) and one part per volume of acetonitrile (4.2).

4.10 Glacial acetic acid.

4.11 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.

To obtain this solution, dissolve one tablet of commercially available PBS material in 200 ml of water.

4.12 Mixture of acetonitrile and water A.

Mix one part per volume of acetonitrile (4.2) with one part per volume of water. Use this solvent to prepare spiking solutions.

4.13 Mixture of acetonitrile and water B.

Mix three parts per volume of acetonitrile (4.2) with seven parts per volume of water. Use this solvent to prepare calibration solutions and to redissolve dried extracts from immunoaffinity cleanup.

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

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

4.15 OPA reagent solution.

Dissolve 40 mg of OPA (4.4) in 1 ml of methanol (4.3) and dilute with 5 ml of sodium tetraborate solution (4.14). Add 50 µl of 2-mercaptoethanol (4.7) 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.

4.16 HPLC mobile phase.

4.16.1 HPLC mobile phase A.

Mix thirty parts per volume of acetonitrile (4.2) with sixty-nine parts per volume of water and one part per volume of glacial acetic acid (4.10).

4.16.2 HPLC mobile phase B.

Mix sixty parts per volume of acetonitrile (4.2) with thirty-nine parts per volume of water and one part per volume of glacial acetic acid (4.10).

4.16.3 Linear gradient settings.

Table 1 — 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 C_{18} SymmetryShield™ [1)] column. The use of a different column may necessitate adjustment of these conditions until acceptable resolution is achieved.

4.17 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.

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

4.19 Certified standard solution of fumonisin B_2 (FB_2), $\rho(FB_2)$ = 50 µg/ml ml in a mixture of one part per volume of acetonitrile and one 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.

4.20 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 (0) and 500 µl of the FB_2 certified standard solution (0) 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.

1) Waters C_{18} SymmetryShield™, Biopure RK 002003 and RK 002004 are examples of suitable products available commercially. This information is given for the convenience of users of this Technical Specification 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.

4.21 Diluted mixed FB_1 and FB_2 stock solution.

Pipette 500 µl of the mixed stock solution (4.20) into a 10 ml calibrated volumetric flask. Fill up to the mark with the mixture of acetonitrile water B (4.13) 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.

4.22 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 (4.21) according to Table 2. Make up each calibration solution to volume (5 ml) with the mixture of acetonitrile and water B (4.13) and mix well.

Table 2 — Preparation of mixed FBs calibration solutions for HPLC

HPLC calibration solution	Diluted mixed FBs stock solution (4.21)	Final concentration of mixed FBs calibration solutions (4.22)		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 (4.13).

5 Apparatus

5.1 General

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

5.2 Analytical balance, capable of weighing to 0,000 1 g.

5.3 Laboratory balance, capable of weighing to 0,1 g.

5.4 Thermostated water bath.

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

5.6 Orbital shaker.

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

5.8 Centrifuge bottles, of 250 ml capacity with screw caps.

5.9 Calibrated microlitre pipettes or microlitre syringes, of 100 µl, 200 µl or 1 000 µl capacity.

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

5.11 Vacuum manifold, to accommodate immunoaffinity columns (4.17).

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

5.13 Vacuum pump.

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

5.15 Glass microfibre binder free filter paper, e.g. 1,6 µm pore size or equivalent.

5.16 Heating block with nitrogen or air gas supply.

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

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

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

5.20 Vortex mixer, or equivalent.

5.21 HPLC apparatus, comprising the following:

5.21.1 Injection system.

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

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

5.21.4 Analytical reverse-phase HPLC separating column, e.g. C_{18} reverse-phase column, 150 mm × 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 C_{18} SymmetryShield™[2)] , Agilent Zorbax SB-C_{18}[2)] or similar have been found to be suitable.

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

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

5.21.7 Recorder, integrator or computer based data processing system.

6 Procedure

6.1 Extraction

The sample should be finely ground to pass through 1 mm sieve and thoroughly mixed to ensure complete homogenization.

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2) Waters C_{18} SymmetryShield™ and Agilent Zorbax SB-C_{18} are examples of suitable products available commercially. This information is given for the convenience of users of this Technical Specification 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.