



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

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 It supersedes DD CEN/TS 16187:2011 which is withdrawn.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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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 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of an identical text or by endorsement, at the latest by December 2015, and conflicting national standards shall be withdrawn at the latest by December 2015.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 B₁ (FB₁) and fumonisin B₂ (FB₂) 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 FB₁+FB₂, 89 µg/kg to 384 µg/kg for FB₁ and 22 µg/kg to 74 µg/kg for FB₂.

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

Fumonisin is 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. Fumonisin is eluted from the column with methanol and water and quantified by HPLC-FLD with pre-column derivatisation with *o*-phthalaldehyde (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*-phthalaldehyde (OPA)

4.4 Citric acid solution, substance concentration $c(\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}) = 0,1 \text{ mol/l}$

Dissolve 21,0 g of $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ in water and dilute to 1 l.

4.5 Disodium hydrogen phosphate solution, $c(\text{Na}_2\text{HPO}_4) = 0,2 \text{ mol/l}$

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, $c(\text{NaCl}) = 137 \text{ mmol/l}$, $c(\text{KCl}) = 2,7 \text{ mmol/l}$, $c(\text{phosphate buffer}) = 10 \text{ mmol/l}$, $\text{pH} = 7,4$ at $T = 25 \text{ }^{\circ}\text{C}$

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, $c(\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}) = 0,1 \text{ mol/l}$

Dissolve 3,8 g of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ 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 B₁ (FB₁), mass concentration $\rho(\text{FB}_1) = 50 \text{ µg/ml}$ 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 B₂ (FB₂), $\rho(\text{FB}_2) = 50 \text{ µg/ml}$ 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 FB₁ and FB₂ stock solution

Prepare a mixed FB₁ and FB₂ stock solution by pipetting 2 000 µl of the FB₁ certified standard solution (4.17) and 500 µl of the FB₂ 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 FB₁ and 10,0 µg/ml of FB₂.

4.20 Diluted mixed FB₁ and FB₂ 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 FB₁ and 0,5 µg/ml of FB₂. 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) µl	Final concentration of mixed FBs calibration solutions (4.21)		Sample equivalent levels of FB ₁ and FB ₂ ^a	
		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.