



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 precolumn derivatization

National foreword

This Draft for Development is the UK implementation of CEN/TS 16187:2011.

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It is being issued in the Draft for Development series of publications and is of a provisional nature. It should be applied on this provisional basis, so that information and experience of its practical application can be obtained.

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According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into an international Standard, to extend the life of the Technical Specification or to withdraw it. Comments should be sent to the Secretary of the responsible BSI Technical Committee at British Standards House, 389 Chiswick High Road, London W4 4AL.

The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Food analysis - Horizontal methods.

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

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Amendments issued since publication

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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 precolumn derivatization**

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 Technical Specification (CEN/TS) was approved by CEN on 22 February 2011 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

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Foreword

This document (CEN/TS 16187:2011) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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Annexes A, B and C are informative.

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1 Scope

This Technical Specification 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 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

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 derivatization 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 [3].

4.1 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.

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

4.8 Extraction solvent.

Mix two parts per volume of citrate buffer solution (4.7) with one part per volume of methanol (4.2) and one 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 one part per volume of acetonitrile (4.1) with one part per volume of water. Use this solvent to prepare spiking solutions.

4.12 Mixture of acetonitrile and water B.

Mix three parts per volume of acetonitrile (4.1) with seven 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 one 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 one part per volume of glacial acetic acid (4.9).