

Foodstuffs — Determination of zearalenone in maize based baby food, barley flour, maize flour, polenta, wheat flour and cereal based foods for infants and young children — HPLC method with immunoaffinity column cleanup and fluorescence detection

ICS 67.060

National foreword

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Foodstuffs - Determination of zearalenone in maize based baby food, barley flour, maize flour, polenta, wheat flour and cereal based foods for infants and young children - HPLC method with immunoaffinity column cleanup and fluorescence detection

Produits alimentaires - Dosage de la zéaralénone dans la farine d'orge, de maïs et de blé, la polenta et les produits pour nourrissons et jeunes enfants à base de céréales - Méthode par chromatographie liquide haute performance avec purification sur colonne d'immunoaffinité et détection par fluorescence

Lebensmittel - Bestimmung von Zearalenon in Säuglingsnahrung auf Maisbasis, Gerstenmehl, Maismehl, Maisgrieß, Weizenmehl und Lebensmittel auf Getreidebasis für Säuglinge und Kleinkinder - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule und Fluoreszenzdetektion

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Foreword

This document (EN 15850:2010) 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of an identical text or by endorsement, at the latest by October 2010, and conflicting national standards shall be withdrawn at the latest by October 2010.

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1 Scope

This European Standard specifies a method for the determination of zearalenone in maize based baby food, barley flour, maize flour, polenta, wheat flour and cereal based foods for infants and young children by high performance liquid chromatography (HPLC) with immunoaffinity cleanup and fluorescence detection. This method has been validated in two interlaboratory studies. The first study was for the analysis of samples of maize based baby food, barley flour, maize flour, polenta and wheat flour ranging from 10 µg/kg to 335 µg/kg, and the second study was for samples of cereal based foods for infants and young children ranging from 9 µg/kg to 44 µg/kg.

Further information on validation, see Clause 9 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

A test portion is extracted with aqueous acetonitrile or methanol according to the products analyzed. The extract is then diluted with phosphate buffered saline (PBS) to give an aqueous extract that is applied to an immunoaffinity column containing antibodies specific for zearalenone. Zearalenone is purified and concentrated on the column and removed from the antibodies using acetonitrile or methanol as eluent. Zearalenone is quantified by reverse-phase high performance liquid chromatography (RP-HPLC) with fluorescence detection.

4 Reagents

4.1 General

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.

4.2 Disodium hydrogen phosphate, Na_2HPO_4 anhydrous or $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$.

4.3 Potassium chloride (KCl).

4.4 Potassium dihydrogen phosphate, KH_2PO_4 .

4.5 Sodium chloride (NaCl).

4.6 Sodium hydroxide (NaOH).

4.7 Hydrochloric acid solution, mass fraction $w(\text{HCl}) = 37 \%$ in water.

4.8 Hydrochloric acid solution, substance concentration $c(\text{HCl}) = 0,1 \text{ mol/l}$.

Dilute 8,28 ml of hydrochloric acid solution (4.7) to 1 l with water.

4.9 Sodium hydroxide solution, $c(\text{NaOH}) = 0,1 \text{ mol/l}$.

Dissolve 4 g of sodium hydroxide (4.6) in 1 l of water.

4.10 Phosphate buffered saline (PBS) solution, $c(\text{NaCl}) = 120 \text{ mmol/l}$, $c(\text{KCl}) = 2,7 \text{ mmol/l}$, $c(\text{phosphate buffer}) = 10 \text{ mmol/l}$, $\text{pH} = 7,4$.

Dissolve 8,0 g of sodium chloride (4.5), 1,2 g of anhydrous disodium hydrogen phosphate or 2,9 g of $\text{Na}_2\text{HPO}_4 \cdot 12 \text{ H}_2\text{O}$ (4.2), 0,2 g of potassium dihydrogen phosphate (4.4) and 0,2 g of potassium chloride (4.3) in 900 ml of water. After dissolution, adjust the pH to 7,4 with hydrochloric acid solution (4.8) or sodium hydroxide solution (4.9) as appropriate, then dilute to 1 l with water.

Alternatively, a PBS solution with equivalent properties can be prepared from commercially available PBS material.

4.11 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.

4.12 Methanol, HPLC grade.

4.13 Methanol, technical grade.

4.14 Extraction solvent A.

Mix 75 parts per volume of acetonitrile (4.11) with 25 parts per volume of water.

4.15 Injection solvent A for HPLC analysis.

Mix four parts per volume of acetonitrile (4.11) with six parts per volume of water.

4.16 HPLC mobile phase A.

Mix 53 parts per volume of acetonitrile (4.11) with 47 parts per volume of water. Filter and degas the HPLC mobile phase before use.

4.17 Extraction solvent B.

Mix 75 parts per volume of methanol (4.13) with 25 parts per volume of water.

4.18 Washing solvent.

Mix 15 parts per volume of methanol (4.12) with 85 parts per volume of PBS (4.10).

4.19 Injection solvent B for HPLC analysis.

Mix five parts per volume of methanol (4.12) with five parts per volume of water.

4.20 HPLC mobile phase B.

Mix 75 parts per volume of methanol (4.12) with 25 parts per volume of water. Filter and degas the HPLC mobile phase before use.