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SWEDISH STANDARDS INSTITUTE

Livsmedel – Bestämning av deoxynivalenol i spannmål, spannmålsprodukter och spannmålsbaserade livsmedel för spädbarn och småbarn – HPLC-metod med upprening på immunoaffinitetskolonn och UV-detektion

Foodstuffs – Determination of deoxynivalenol in cereals, cereal products and cereal based foods for infants and young children – HPLC method with immunoaffinity column cleanup and UV detection

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Europastandarden EN 15891:2010 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN 15891:2010.

The European Standard EN 15891:2010 has the status of a Swedish Standard. This document contains the official version of EN 15891:2010.

Upplysningar om sakinnehållet i standarden lämnas av SIS, Swedish Standards Institute, telefon 08-555 520 00. Standarder kan beställas hos SIS Förlag AB som även lämnar allmänna upplysningar om svensk och utländsk standard.

Information about the content of the standard is available from the Swedish Standards Institute (SIS), telephone +46 8 555 520 00. Standards may be ordered from SIS Förlag AB, who can also provide general information about Swedish and foreign standards.

Denna standard är framtagen av kommittén för Livsmedel och foder, SIS/TK 435.

Har du synpunkter på innehållet i den här standarden, vill du delta i ett kommande revideringsarbete eller vara med och ta fram andra standarder inom området? Gå in på www.sis.se - där hittar du mer information.

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 15891

September 2010

ICS 67.060; 67.230

English Version

Foodstuffs - Determination of deoxynivalenol in cereals, cereal products and cereal based foods for infants and young children - HPLC method with immunoaffinity column cleanup and UV detection

Denrées alimentaires - Dosage du déoxynivalénol dans les céréales, les produits céréaliers, et céréales pour déjeuner en alimentation infantile - Méthode par CLHP avec purification sur colonne d'immunoaffinité et détection UV

Lebensmittel - Bestimmung von Deoxynivalenol in Getreide, Getreideerzeugnissen und Säuglings- und Kleinkindernahrung auf Getreidebasis - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule und UV-Detektion

This European Standard was approved by CEN on 28 August 2010.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 15891:2010) 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 March 2011, and conflicting national standards shall be withdrawn at the latest by March 2011.

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.

Annexes A and B are informative.

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

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Standard specifies a method for the determination of deoxynivalenol (DON) in cereals (grain and flour), cereal based foods and cereal based foods for infants and young children by high performance liquid chromatography (HPLC) with immunoaffinity cleanup and UV detection. This method has been validated in three interlaboratory studies. The first study was for the analysis of samples of wheat, rice flour, oat flour, maize, polenta, and wheat based breakfast cereal ranging from 85,4 µg/kg to 1 768 µg/kg, the second study was for wheat and maize ranging from 165 µg/kg to 4 700 µg/kg and the third study was for cereal based foods for infants and young children ranging from 58 µg/kg to 452 µg/kg.

For further information on the validation, see Clause 9 and Annex B.

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

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

Deoxynivalenol is extracted from the sample using water. The aqueous extract is cleaned up on an immunoaffinity column to remove impurities from the sample. Deoxynivalenol is then quantitatively determined by HPLC and UV detection.

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. Commercially available solutions with equivalent properties to the reagents listed may be used.

4.2 Disodium hydrogen phosphate, anhydrous or $Na_2HPO_4 \cdot 12\ H_2O$.

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(HCl) = 37 % in water.

4.8 Hydrochloric acid solution, substance concentration *c*(HCl) = 0,1 mol/l.

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

4.9 Sodium hydroxide solution, *c*(NaOH) = 0,1 mol/l.

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

4.10 Phosphate buffered saline (PBS) solution, c(NaCl) = 120 mmol/l, c(KCl) = 2,7 mmol/l, c(phosphate buffer) = 10 mmol/l, 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 $Na_2HPO_4 \cdot 12\ H_2O$ (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 Polyethylene glycol (PEG), with a molecular mass of approximately 8 000 g/mol.

4.13 Methanol.

4.14 Acetic acid, with a mass fraction of 96 % or glacial acetic acid, with a mass fraction of 100 %.

4.15 Diluent for HPLC analysis.

Mix 9,5 parts per volume of methanol (4.13) with 90,5 parts per volume of water.

4.16 HPLC mobile phase.

Mix 15 parts per volume of methanol (4.13) with 85 parts per volume of water. Add 0,1 parts per volume of acetic acid (4.14).The exact amount of methanol used and the use of acetic acid will depend on the HPLC column chosen for analysis and shall be adjusted if necessary. Degas this solution before use.

4.17 Wash solvent.

Mix 50 parts per volume of methanol (4.13) with 50 parts per volume of water.

4.18 Immunoaffinity (IA) column.

The immunoaffinity column shall contain antibodies raised against DON. The column shall have a capacity of not less than 1 000 ng of DON and shall have a recovery of not less than 80 % when 500 ng of DON are applied in 1 ml to 2 ml of water.

4.19 Deoxynivalenol, purity not less than 97 % mass fraction**.**

WARNING — Deoxynivalenol is highly toxic. 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 Deoxynivalenol stock solution 1, mass concentration $\rho \approx 1{,}25$ mg/ml**.**

Add 4,0 ml of acetonitrile (4.11) to approximately 5 mg of deoxynivalenol (4.19) to form a solution with a concentration of approximately 1,25 mg/ml. Alternatively, available commercial solutions with equivalent properties can be used.

Store this solution in a freezer at approximately - 18 °C. A solution stored in this way is stable for 12 months. Confirm the concentration of the solution if it is older than six months.

4.21 Deoxynivalenol stock solution 2, $\rho \approx 250$ µg/ml.

Dilute 800 µl of stock solution 1 (4.20) to 4 ml with acetonitrile (4.11) to form a solution with a concentration of approximately 250 µg/ml.

Store this solution in a freezer at approximately - 18 °C. A solution stored in this way is stable for 12 months. Confirm the concentration of the solution if it is older than six months.

4.22 Deoxynivalenol standard solution A.

Dilute 200 µl of stock solution 2 (4.21) to 2,0 ml with acetonitrile (4.11) to form a solution with a concentration of approximately 25 µg/ml.

To determine the exact mass concentration, record the absorption curve between a wavelength of 200 nm to 270 nm, e.g. in 5 nm steps; in the spectrometer (5.16) against acetonitrile as reference. Identify the wavelength for maximum absorption and calculate the mass concentration of deoxynivalenol, ρ_{DON}, in micrograms per millilitre using Equation (1):

$$\rho_{DON} = \frac{A_{max} \times M \times 100}{\varepsilon \times b} \quad (1)$$

where

A_{max} is the absorption determined at the maximum of the absorption curve (here: at 220 nm);

M is the molar mass, in grams per mole, of deoxynivalenol (M = 296,3 g/mol);

ε is the molar absorption coefficient, in square metres per mole, of deoxynivalenol in acetonitrile (4.11) (here: 681 m^2/mol, see [1]);

b is the optical path length, in centimetres, of the quartz cell.

Calculate the mass concentration of the stock solution 2 (4.21), ρ_{DON2}, in micrograms per millilitre using Equation (2):

$$\rho_{DON2} = \rho_{DON} \times 10 \quad (2)$$

Store this solution in a freezer at approximately - 18 °C. A solution stored in this way is stable for 12 months. Confirm the concentration of the solution if it is older than six months.

NOTE Preparation of standard solutions can be carried out gravimetrically by accurately weighing the deoxynivalenol standard material and the solvent used to dissolve it.

4.23 Deoxynivalenol spiking solution, ρ = 100 µg/ml.

Pipette an aliquot of the stock solution 2 (4.21) equivalent to 500 µg of deoxynivalenol in a 5 ml volumetric flask. Dilute to the mark with acetonitrile (4.11).

Store this solution in a freezer at approximately - 18 °C. A solution stored in this way is stable for 12 months. Confirm the concentration of the solution if it is older than six months.

4.24 Deoxynivalenol standard solution B, ρ = 10 µg/ml.

Pipette 500 µl of the spiking solution (4.23) in a 5 ml volumetric flask. Dilute to the mark with acetonitrile (4.11).

Store this solution in a freezer at approximately - 18 °C. A solution stored in this way is stable for 12 months. Confirm the concentration of the solution if it is older than six months.

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 High speed blender or homogenizer.

5.5 Laboratory shaker or magnetic stirrer, speed adjustable to approximately 500 min^{-1}.

5.6 Vortex mixer, or equivalent.

5.7 Centrifuge, capable of a centrifugal force of 2 500 *g*.

5.8 Centrifuge tube, of 250 ml capacity.

5.9 Filter paper, qualitative, strong, fast flow, pre-folded and with a diameter of 18,5 cm**.**

5.10 Glass fibre filter, fast flow, fine porosity, retention size 1,6 µm or smaller.

5.11 Pipettes, e.g. of 10 ml, 5 ml, 1 ml, and 25 µl to 250 µl capacity.

5.12 Reservoirs for immunoaffinity columns, of for example 20 ml capacity, with appropriate adaptors.

5.13 Glass vials or assay tubes, of various size.

5.14 Heating block or thermostatic waterbath, capable of maintaining approximately 50 °C.

5.15 HPLC apparatus, comprising the following: