BS EN 15891:2010

BSI Standards Publication

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

raising standards worldwide™

National foreword

This British Standard is the UK implementation of EN 15891:2010.

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.

ISBN 978 0 580 63013 2

ICS 67.060; 67.230

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 October 2010.

Amendments issued since publication

Date	Text affected

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.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN Management Centre or to any CEN member.

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.

CEN members are the national standards bodies of 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 United Kingdom.

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

Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN 15891:2010: E

Contents

Page

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:

5.15.1 Injection system, capable of injecting e.g. 100 µl to 300 µl.

5.15.2 Mobile phase pump, pulse free, capable of maintaining a volume flow rate of 1 ml/min.

5.15.3 Analytical reverse-phase HPLC separating column, for example C18 octadecylsilane (ODS), length of 15 cm to 25 cm, inner diameter of 4,6 mm and a particle size of 5 µm, which ensures resolution of deoxynivalenol from all other peaks.

The maximum overlapping of peaks shall be less than 10 %. It can be necessary to adjust the mobile phase for a sufficient baseline resolution. A suitable corresponding reverse-phase guard column should be used.

5.15.4 UV detector, set at 220 nm.

5.15.5 Recorder, integrator or computer based data processing system.

5.15.6 Mobile phase switching unit, or second HPLC pump, if necessary.

5.16 UV spectrometer.

6 Procedure

6.1 General

This method has been validated in three interlaboratory studies. These studies were performed by different laboratories at different times. This is the reason that slightly different procedures were used for extraction and immunoaffinity column cleanup for wheat, rice flour, oat flour, maize, polenta and wheat based breakfast cereal (described in 6.2 and 6.4) and for cereal based food for infants and young children (described in 6.3 and 6.5). The procedures described here are similar to the ones described in the original interlaboratory studies.

6.2 Extraction for wheat, rice flour, oat flour, maize, polenta and wheat based breakfast cereal

Weigh, to the nearest 0,1 g, a 25 g (m_s) test portion and 5 g of PEG (4.12) into a centrifuge tube (5.8). Add 200 ml (V_1) of water (or another volume as specified by the IA column manufacturer) and homogenize at high speed for 3 min using a homogenizer (5.4). Alternative extraction procedures have been shown to give equivalent results. Either shake the sample and the extraction solvent on a wrist action shaker for 2 h or add a magnetic stirrer bar to the flask, cap it and place it on a magnetic stirrer (5.5) to mix at medium-high speed for 30 min. In both cases, shake the sample and reagents together thoroughly by hand to ensure they are well mixed before placing on shaker. Centrifuge the homogenized sample for 15 min at 2 500 *g*. After centrifugation filter the sample with a glass fibre filter (5.10).

NOTE It has been shown during the validation study that for some matrices (maize), samples that have been extracted on a magnetic stirrer do not require centrifugation.

6.3 Extraction for cereal based food for infants and young children

Weigh, to the nearest 0,1 g, a 25 g (m_s) test portion into a 250 ml or 500 ml conical flask. Add 200 ml (V_1) of water, cap and shake for 1 h on a laboratory shaker (5.5). Allow the sample to settle after shaking and transfer 50 ml of supernatant to a centrifuge tube (5.8) and centrifuge for 15 min at 2 500 *g*.

Prepare a funnel and filter paper (5.9). Pour the extracted sample after centrifugation into a 250 ml or 500 ml conical flask through the prepared funnel and filter paper.

6.4 Immunoaffinity column cleanup for wheat, rice flour, oat flour, maize, polenta and wheat based breakfast cereal

Prepare the IA column and proceed with the cleanup procedure in accordance with the manufacturer's instructions. Transfer 2,0 ml (V_3) of the filtered aqueous extract (6.2) onto the immunoaffinity column (4.18). Pass the extract completely through the column at a rate of about one drop per second. Wash the column with 5 ml of water or PBS (4.10). Dry the column by pushing air through it. Using a pipette, transfer 2 ml of acetonitrile (4.11) or methanol (4.13), depending on manufacturer's instructions, to the column reservoir. Allow elution solvent to pass slowly into the column. Stop the flow then wait 1 min before eluting deoxynivalenol from the column at a rate of one drop per second and collect in a 4 ml vial or assay tube (5.13). Carefully pass air through the column in order to collect any final drops.

NOTE Care should be taken not to exceed the capacity of the immunoaffinity column.

6.5 Immunoaffinity column cleanup for cereal based food for infants and young children

Prepare the IA column and proceed with the cleanup procedure in accordance with the manufacturer's instructions. Transfer 10 ml (V_3) of the aqueous layer (6.3) into the reservoir (5.12) of an immunoaffinity column (4.18). Allow this solution to pass slowly through the column at a rate of approximately one drop per second. When the extract has passed completely through the immunoaffinity column, pass 5 ml of water or PBS (4.10) through the column. Dry the column by passing nitrogen through the column for about 5 s. Then discard all the eluent from this stage of the cleanup procedure. Finally, place a 2,0 ml to 2,5 ml vial (5.13) under the column and pass 0,5 ml of methanol (4.13) through the column, collecting the eluate. Allow the methanol to remain on the column for approximately 1 min before allowing to pass through. Then add a further 1,0 ml of methanol and continue to collect the eluate. Carefully pass air through the column in order to collect any final drops.

NOTE Methods for loading onto IA columns, washing and elution vary slightly between column manufacturers and the specific instructions supplied with the columns should be followed precisely. In general for deoxynivalenol, procedures involve sample extraction with water, centrifugation, filtration, loading under pressure onto a possibly pre-washed column, washing the column with distilled water or (phosphate buffered saline PBS) and elution of deoxynivalenol with methanol or acetonitrile. Take care not to exceed the maximum loading volume or capacity of the column.

6.6 Preparation of sample test solution

Place the vial in a heating block or thermostatic waterbath (5.14) and evaporate under nitrogen at no more than 50 °C. Re-dissolve the residue at ambient temperature in a final volume of 0,5 ml of HPLC diluent (4.15) or mobile phase (4.16). Mix well to ensure the residue is completely re-dissolved by shaking for at least 30 s, for example with a vortex mixer (5.6).

NOTE 1 If necessary the sample can be filtered before analysis by HPLC. A check should be made with a standard solution to assess any loss of deoxynivalenol due to this filtration step.

NOTE 2 After evaporation, the residue can be stored one week at (4 ± 2) °C protected from light.

6.7 Spiking procedure

To determine the recovery carry out the spiking procedure using the spiking solution (4.23). The spiking level should be within the calibration range (preferably mid-range). Add the spiking solution to a test portion of material previously shown not to contain deoxynivalenol and leave to stand for 30 min before adding the extraction solvent.

7 HPLC analysis

7.1 HPLC operating conditions

Using the equipment specified in 5.15, and the column specified in 5.15.3 the following conditions have proved to produce adequate separation, see Figure A.1. The exact amount of methanol used in the mobile phase and the use of acetic acid will depend on the HPLC column chosen for analysis and shall be adjusted if necessary to achieve separation of deoxynivalenol.

— Flow rate mobile phase (column): 1,0 ml/min;

— UV detection wavelength: 220 nm;

— Injection volume: 100 µl to 300 µl.

Using a mobile phase switching unit (5.15.6), the mobile phase profile is set as in Table 1:

Table 1 — Mobile phase profile

Time min	**Flowrate** ml/min	**Mobile phase (4.16)** %	**Wash solvent (4.17)** %
0 to 15	1	100	0
15 to 25	1	0	100
25 to 35	1	100	0

Using the HPLC conditions shown above, deoxynivalenol usually elutes with a retention time of approximately 11 min, see Figure A.1.

NOTE Mobile phases prepared with acetonitrile and water have also been shown to be suitable alternatives. Such mobile phases can be used provided sufficient separation is achieved.

7.2 Preparation of calibration solutions for HPLC

Prepare calibration solutions from standard solution B (4.24). For normal practice evaporate the following amount of standard solution B in 10 ml volumetric flasks in accordance with Table 2 and Table 3. Fill the flasks up to the mark with HPLC diluent (4.15) or mobile phase (4.16).

Table 2 — Preparation of HPLC calibration solutions for wheat, rice flour, oat flour, maize, polenta and wheat based breakfast cereal

HPLC calibration solution	Deoxynivalenol standard solution B (4.24)	Final deoxynivalenol concentration in calibration solution	Equivalent sample concentration
	µl	ng/ml	µg/kg
1	1 000	1 000	2 000
2	750	750	1 500
3	500	500	1 000
4	250	250	500
5	50	50	100

Table 3 — Preparation of HPLC calibration solutions for cereal based food for infants and young children

HPLC calibration solution	Deoxynivalenol standard solution B (4.24)	Final deoxynivalenol concentration in calibration solution	Equivalent sample concentration
	µl	ng/ml	µg/kg
1	1 250	1 250	500
2	1 000	1 000	400
3	750	750	300
4	500	500	200
5	250	250	100
6	125	125	50

7.3 Calibration curve

Prepare a calibration curve by injecting a volume of 100 µl to 300 µl of the calibration solutions (7.2) at different suitable concentrations into the chromatograph at the beginning of every day of the analysis. Establish the calibration curve prior to analysis of test samples by plotting the concentration of deoxynivalenol in nanograms per millilitre on the x-axis against peak signal as area or height on the y-axis and check the plot for linearity using linear regression ($r^2 \geq 0{,}998$).

7.4 Determination of deoxynivalenol in test solutions

Inject 100 µl to 300 µl aliquots of the sample test solutions (6.6) into the chromatograph using the same conditions used for the preparation of the calibration curve.

7.5 Peak identification

Identify the deoxynivalenol peak in the test solution by comparing the retention time with that of the nearest HPLC standard injected in the HPLC run. The concentration of deoxynivalenol in the test solution shall fall within the calibration range. If the deoxynivalenol level in the sample test solution exceeds the mass concentration of the highest standard solution, dilute the sample extract with HPLC diluent or mobile phase to bring it within calibration range, and reanalyse. The dilution factor shall be incorporated into all subsequent calculations.

8 Calculation

Determine the concentration of deoxynivalenol in the sample test solution (6.6), in nanograms per millilitre, directly from the calibration curve (7.3). Calculate the mass fraction, w_{DON}, of deoxynivalenol in micrograms per kilogram, using Equation (3):

$$w_{DON} = \frac{\rho_{DON} \times V_1 \times V_2}{V_3 \times m_s} \quad (3)$$

where

ρ_{DON} is the concentration of deoxynivalenol, in nanograms per millilitre, in the aliquot of test solution derived from the calibration curve;

V_1 is the volume, in millilitres, of the solvent used for extraction in 6.2 or 6.3 (here: 200 ml);

V_2 is the final volume, in millilitres, of the test solution in 6.6 (here: 0,5 ml);

V_3 is the volume, in millilitres, of the extract aliquot used for immunoaffinity clean up (here: 2,0 ml in 6.4 and 10 ml in 6.5);

m_s is the mass, in grams, of sample material taken for analysis (here: 25 g).

Report the result to three significant figures.

EXAMPLE

P_{DON} 545 ng/ml,

V_1 200 ml,

V_2 0,5 ml,

V_3 2,0 ml,

m_s 25,0 g,

$$w_{DON} = \frac{545 \times 200 \times 0{,}5}{2{,}0 \times 25{,}0} = 1\,090 \ \mu g/kg \ .$$

9 Precision

9.1 General

Details of the interlaboratory tests on the precision of the method for wheat, rice flour, oat flour, maize, polenta, and wheat based breakfast cereal are given in [2] and [3]. Details of an interlaboratory test on the precision of the method for cereal based foods for infants and young children are given in [4]. Precision data are given in Annexes A and B. The values derived from these interlaboratory tests may not be applicable to concentration ranges and/or matrices other than those given.

9.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit *r* in not more than 5 % of the cases.

Using the extraction procedure given in 6.2 and cleanup procedure given in 6.4:

The values for oat flour are:	$\bar{x}$ = 1 768 µg/kg	*r* = 153,8 µg/kg	(naturally contaminated)
The values for rice flour are:	$\bar{x}$ = 458 µg/kg	*r* = 83,6 µg/kg	(fortified)
	$\bar{x}$ = 85 µg/kg	*r* = 33,7 µg/kg	(fortified)
The values for wheat flour are:	$\bar{x}$ = 678 µg/kg	*r* = 113,7 µg/kg	(fortified)
The values for polenta are:	$\bar{x}$ = 123 µg/kg	*r* = 22,1 µg/kg	(fortified)
The values for breakfast cereal are:	$\bar{x}$ = 217 µg/kg	*r* = 80,6 µg/kg	(fortified)
The values for wheat are:	$\bar{x}$ = 165 µg/kg	*r* = 95 µg/kg	(naturally contaminated)
	$\bar{x}$ = 1 466 µg/kg	*r* = 197,1 µg/kg	(naturally contaminated)
	$\bar{x}$ = 4 612 µg/kg	*r* = 579,2 µg/kg	(naturally contaminated)
The values for maize are:	$\bar{x}$ = 501 µg/kg	*r* = 145,6 µg/kg	(naturally contaminated)
	$\bar{x}$ = 2 763 µg/kg	*r* = 274,5 µg/kg	(naturally contaminated)
	$\bar{x}$ = 4 712 µg/kg	*r* = 480,8 µg/kg	(naturally contaminated)

Using the extraction procedure given in 6.3 and cleanup procedure given in 6.5:

The values for cereal based foods for infants and young children are:

$\bar{x}$ = 115 µg/kg	*r* = 24,2 µg/kg	(fortified)
$\bar{x}$ = 212 µg/kg	*r* = 83,0 µg/kg	(fortified)
$\bar{x}$ = 58 µg/kg	*r* = 22,8 µg/kg	(naturally contaminated)
$\bar{x}$ = 115 µg/kg	*r* = 20,5 µg/kg	(naturally contaminated)
$\bar{x}$ = 452 µg/kg	*r* = 103,0 µg/kg	(naturally contaminated)

9.3 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit *R* in not more than 5 % of the cases.

Using the extraction procedure given in 6.2 and cleanup procedure given in 6.4:

The values for oat flour are:	$\bar{x}$ = 1 768 µg/kg	*R* = 685,3 µg/kg	(naturally contaminated)
The values for rice flour are:	$\bar{x}$ = 458 µg/kg	*R* = 146,9 µg/kg	(fortified)
	$\bar{x}$ = 85 µg/kg	*R* = 35,5 µg/kg	(fortified)
The values for wheat flour are:	$\bar{x}$ = 678 µg/kg	*R* = 309,3 µg/kg	(fortified)
The values for polenta are:	$\bar{x}$ = 123 µg/kg	*R* = 79,6 µg/kg	(fortified)
The values for breakfast cereal are:	$\bar{x}$ = 217 µg/kg	*R* = 160 µg/kg	(fortified)
The values for wheat are:	$\bar{x}$ = 165 µg/kg	*R* = 177,4 µg/kg	(naturally contaminated)
	$\bar{x}$ = 1 466 µg/kg	*R* = 844,7 µg/kg	(naturally contaminated)
	$\bar{x}$ = 4 612 µg/kg	*R* = 3 881,3 µg/kg	(naturally contaminated)
The values for maize are:	$\bar{x}$ = 501 µg/kg	*R* = 315,8 µg/kg	(naturally contaminated)
	$\bar{x}$ = 2 763 µg/kg	*R* = 1 834,6 µg/kg	(naturally contaminated)
	$\bar{x}$ = 4 712 µg/kg	*R* = 2 700,9 µg/kg	(naturally contaminated)

Using the extraction procedure given in 6.3 and cleanup procedure given in 6.5:

The values for cereal based foods for infants and young children are:

$\bar{x}$ = 115 µg/kg	R = 40,9 µg/kg	(fortified)
$\bar{x}$ = 212 µg/kg	R = 119,0 µg/kg	(fortified)
$\bar{x}$ = 58 µg/kg	R = 24,7 µg/kg	(naturally contaminated)
$\bar{x}$ = 115 µg/kg	R = 30,2 µg/kg	(naturally contaminated)
$\bar{x}$ = 452 µg/kg	R = 133,9 µg/kg	(naturally contaminated)

10 Test report

The test report shall contain at least the following data:

a) all information necessary for the identification of the sample (kind of sample, origin of sample, designation);

b) a reference to this European Standard;

c) the date and type of sampling procedure (if known);

d) the date of receipt;

e) the date of test;

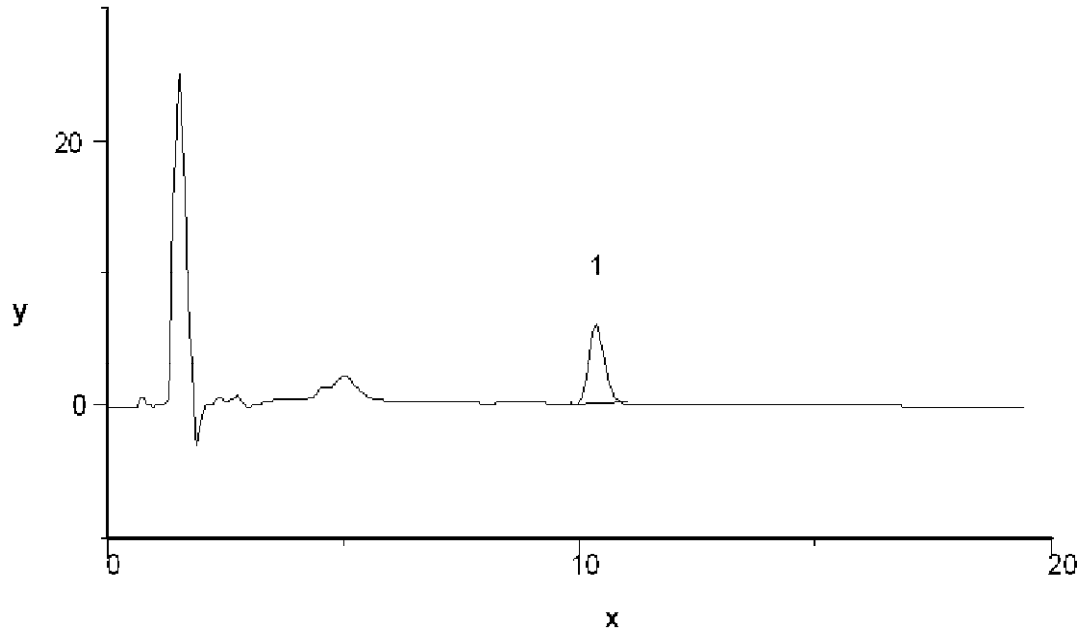
f) the test results and the units in which they have been expressed;

g) any particular points observed in the course of the test;

h) any operations not specified in the method or regarded as optional, which might have effected the results.

Annex A
(informative)

Typical chromatogram



Key

x time, in minutes
y UV signal, in millivolts
1 deoxynivalenol

Figure A.1 — Typical chromatogram naturally contaminated wheat flour sample at a concentration of approximately 900 µg/kg

Annex B
(informative)

Precision data

The following data in Table B.1 for oat flour, rice flour, wheat flour, polenta and wheat based breakfast cereal were obtained in an interlaboratory test [2] according to AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis [5]. The data for wheat and corn were obtained in an interlaboratory test organized by BIPEA (Interprofessional Bureau of Analytical Studies – France) in May 2004 [3].

The data in Table B.2 were obtained in an interlaboratory test [4] according to AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis [5].

Table B.1 — Precision data for oat flour, rice flour, wheat, wheat flour, maize, polenta and wheat based breakfast cereal

Sample	Oat flour n.c.[a]	Rice flour 1 f.[a]	Rice flour 2 f.[a]	Wheat flour f.[a]	Polenta f.[a]	Breakfast cereal f.[a]	Wheat 1 n.c.[a]	Wheat 2 n.c.[a]	Wheat 3 n.c.[a]	Maize 1 n.c.[a]	Maize 2 n.c.[a]	Maize 3 n.c.[a]
Year of interlaboratory test	2003	2003	2003	2003	2003	2003	2004	2004	2004	2004	2004	2004
Number of laboratories	13	13	13	13	13	13						
Number of laboratories retained after eliminating outliers	10	11	10	12	11	12	16	16	17	16	16	17
Number of outliers (laboratories)	2	1	2	2	1	1						
Number of accepted results	10	11	10	12	11	12	16	16	17	17	16	17
Mean value, $\bar{x}$, µg/kg	1 768	458	85	678	123	217	165	1 466	4 612	501	2 763	4 712
Repeatability standard deviation s_r, µg/kg	54,92	29,87	12,04	40,62	7,88	28,77	34,3	71,1	209	52,5	99	173,4
Repeatability relative standard deviation, RSD_r, %	3,1	6,5	14,1	6,0	6,4	13,2	21	5	5	10	4	4
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	153,8	83,6	33,7	113,7	22,1	80,6	95	197,1	579,2	145,6	274,5	480,8
Reproducibility standard deviation s_R, µg/kg	244,8	52,5	12,7	110,5	28,4	57,1	64	304,7	1 400,2	113,9	661,9	974,4
Reproducibility relative standard deviation, RSD_R, %	13,8	11,5	14,8	16,3	23,1	26,3	39	21	30	23	24	21
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	685,3	146,9	35,5	309,3	79,6	160,0	177,4	844,7	3 881,3	315,8	1 834,6	2 700,9
Recovery, %[b]	81	86	n.a.[a]	81	86	86	n.a.[a]	n.a.[a]	n.a.[a]	n.a.[a]	n.a.[a]	n.a.[a]
HorRat value, according to [6]	0,9	0,6	0,6	1,0	1,1	1,3	1,9	1,4	2,4	1,3	1,8	1,7
HorRat value, according to [7]	0,9	0,6	0,7	1,0	1,1	1,3	1,9	1,4	2,4	1,3	1,8	1,7

[a] n.c. is naturally contaminated, f. is fortified and n.a. is not applicable.

[b] Recovery values were derived independently from the analysis of single spiked samples of each matrix (1 000 µg/kg) by each laboratory that participated in the interlaboratory study.

Table B.2 — Precision data for cereal based food for infants and young children

Sample	**Cereal based food for infants and young children**					
	f. [a]	f. [a]	n.c. [a]	n.c. [a]	n.c. [a]	n.c. [a]
Year of interlaboratory test	2005	2005	2005	2005	2005	2005
Number of laboratories	12	14	14	11	12	14
Number of laboratories retained after eliminating outliers	11	13	14	11	10	13
Number of outliers (laboratories)	1	1	0	0	2	1
Number of accepted results	11	13	14	11	10	13
Mean value, $\bar{x}$, µg/kg	115	212	< 6	58	115	452
Repeatability standard deviation s_r, µg/kg	8,6	29,6	n.a. [a]	8,2	7,3	36,7
Repeatability relative standard deviation, RSD_r, %	7,5	14,0	n.a. [a]	14,0	6,4	8,1
Repeatability limit r [$r = 2{,}8 \times s_r$], µg/kg	24,2	83,0	n.a. [a]	22,8	20,5	103,0
Reproducibility standard deviation s_R, µg/kg	14,6	42,5	n.a. [a]	8,8	10,8	47,8
Reproducibility relative standard deviation, RSD_R, %	12,7	20,1	n.a. [a]	15,2	9,4	10,6
Reproducibility limit R [$R = 2{,}8 \times s_R$], µg/kg	40,9	119,0	n.a. [a]	24,7	30,2	133,9
Recovery, %	89	85	n.a. [a]	n.a. [a]	n.a. [a]	n.a. [a]
HorRat value, according to [6]	0,6	1,0	n.a. [a]	0,6	0,4	0,6
HorRat value, according to [7]	0,7	1,0	n.a. [a]	0,7	0,4	0,6

[a] f. is fortified, n.c. is naturally contaminated and n.a. is not applicable.

Bibliography

[1] Krska R., Schubert-Ullrich P., Josephs R.D., Emteborg H., Buttinger G., Petterson H., Van Egmond H.P., Schothorst R.C., MacDonald S. and Chan D., 2007, Determination of molar absorptivity coefficients for major type-B trichothecenes and certification of calibrators for deoxynivalenol and nivalenol, Anal. Bioanal. Chem, **388**, 1215-1226

[2] MacDonald S.J., Chan D., Brereton P., Damant A. and Wood R., 2005, Determination of Deoxynivalenol in Cereals and Cereal Products by Immunoaffinity Column Cleanup with Liquid Chromatography: Interlaboratory Study, *Journal of AOAC International*, **88**, 1197-1204

[3] IRTAC – BIPEA - Rapport de comparaisons inter laboratoires – Mars 2004 N° 002

[4] Stroka J., Derbyshire M., Mischke C., Ambrosio M., Kroeger K., Arranz I., Sizoo E. and Van Egmond H.P., 2006, Liquid Chromatographic Determination of Deoxynivalenol in Baby Food and Animal Feed: Interlaboratory Study, *Journal of AOAC International*, **89**, 1012-1020

[5] AOAC International 1995, AOAC Official Methods Program, Associate Referee's Manual on development, Study, Review, and Approval Process. Part IV AOAC Guidelines for Collaborative Studies, 23-51

[6] Horwitz, W. and Albert, R., 2006, The Horwitz Ratio (HorRat): A Useful Index of Method Performance with Respect to Precision, *Journal of AOAC International*, **89**, 1095-1109

[7] Thompson, M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, *Analyst*, **125**, 385-386

This page deliberately left blank

This page deliberately left blank

British Standards Institution (BSI)

BSI is the independent national body responsible for preparing British Standards and other standards-related publications, information and services.
It presents the UK view on standards in Europe and at the international level.

It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover.

Tel: +44 (0)20 8996 9001 Fax: +44 (0)20 8996 7001

BSI offers Members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Tel: +44 (0)20 8996 7669 Fax: +44 (0)20 8996 7001
Email: plus@bsigroup.com

Buying standards

You may buy PDF and hard copy versions of standards directly using a credit card from the BSI Shop on the website **www.bsigroup.com/shop.** In addition all orders for BSI, international and foreign standards publications can be addressed to BSI Customer Services.

Tel: +44 (0)20 8996 9001 Fax: +44 (0)20 8996 7001
Email: orders@bsigroup.com

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Knowledge Centre.

Tel: +44 (0)20 8996 7004 Fax: +44 (0)20 8996 7005
Email: knowledgecentre@bsigroup.com

Various BSI electronic information services are also available which give details on all its products and services.

Tel: +44 (0)20 8996 7111 Fax: +44 (0)20 8996 7048
Email: info@bsigroup.com

BSI Subscribing Members are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration.

Tel: +44 (0)20 8996 7002 Fax: +44 (0)20 8996 7001
Email: membership@bsigroup.com

Information regarding online access to British Standards via British Standards Online can be found at **www.bsigroup.com/BSOL**

Further information about BSI is available on the BSI website at **www.bsigroup.com/standards**

Copyright

BSI Group Headquarters

389 Chiswick High Road London W4 4AL UK

Tel +44 (0)20 8996 9001
Fax +44 (0)20 8996 7001
www.bsigroup.com/standards

raising standards worldwide™