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

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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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Contents

Page

Foreword 3
1 **Scope** 4
2 **Normative references** 4
3 **Principle** 4
4 **Reagents** 4
5 **Apparatus** 7
6 **Procedure** 9
7 **HPLC analysis** 10
8 **Calculation** 12
9 **Precision** 12
10 **Test report** 13
Annex A (informative) **Typical chromatograms** 15
Annex B (informative) **Precision data** 16
Bibliography 18

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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of an identical text or by endorsement, at the latest by October 2010, and conflicting national standards shall be withdrawn at the latest by October 2010.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 $Na_2HPO_4 \cdot 12\ H_2O$.

4.3 Potassium chloride (KCl).

4.4 Potassium dihydrogen phosphate, KH_2PO4.

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.

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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 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.

4.21 Immunoaffinity column.

The immunoaffinity column shall contain antibodies raised against zearalenone. The column shall have a capacity of not less than 1 500 ng of zearalenone and shall give a recovery of not less than 80 % when 75 ng of zearalenone is applied in 10 ml of a mixture of 15 parts per volume of methanol and 85 parts per volume of PBS.

4.22 Zearalenone, in crystal form or as a film in ampoules, purity not less than 98 % mass fraction or in form of commercially available Zearalenone solution.

WARNING — Zearalenone is an oestrogenic compound and 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.

4.23 Zearalenone stock solution, $c \approx 200$ µg/ml.

Add 4,0 ml of acetonitrile (4.11) to 5 mg of zearalenone (4.22) to form a solution with a mass concentration of approximately 1,25 mg/ml. Dilute 800 µl of this solution to 5 ml with acetonitrile (4.11) to form a stock solution with a concentration of approximately 200 µg/ml.

Store this solution in a freezer at - 18 °C to - 20 °C. Allow to reach room temperature before opening. A solution stored in this way is usually stable for 12 months. Confirm the mass concentration of the solution if it is older than six months.

4.24 Zearalenone spiking solution, $c \approx 10$ µg/ml.

Dilute 250 µl of stock solution (4.23) with 4,75 ml of acetonitrile (4.11) to form a solution with a mass concentration of approximately 10 µg/ml.

To determine the exact concentration, record the absorption curve of this solution between 200 nm to 300 nm in a 1 cm quartz cell in the spectrometer (5.25) with acetonitrile (4.11) as reference. Identify the wavelength for maximum absorption (λ is approximately 274 nm). Calculate the mass concentration of zearalenone, ρ_{zon}, in micrograms per millilitre using Equation (1):

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

where

A_{max} is the absorption determined at the maximum of the absorption curve (274 nm);

M is the molar mass, in grams per mole, of zearalenone (M = 318,4 g/mol);

ε is the molar absorption coefficient, in square metres per mole of zearalenone in acetonitrile (4.11) (1 262 m^2/mol, see [1]);

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

Store this solution in a freezer at - 18 °C to - 20 °C. Allow to reach room temperature before opening. A solution stored in this way is usually stable for 12 months. Confirm the mass concentration of the solution if it is older than six months.

4.25 Zearalenone standard solution A, $\rho = 2$ µg/ml, for maize based baby food, barley flour, maize flour, polenta and wheat flour.

Transfer an aliquot of the spiking solution (4.24) equivalent to 10 µg of zearalenone into either a vial (5.9) or a calibrated volumetric flask (5.10). Add acetonitrile (4.11) to make the total volume up to 5 ml.

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Store this solution in a freezer at - 18 °C to - 20 °C. Allow to reach room temperature before opening. A solution stored in this way is usually stable for 12 months. Confirm the mass concentration of the solution if it is older than six months.

4.26 Zearalenone standard solution B, ρ = 0,4 µg/ml, for cereal based foods for infants and young children.

Transfer an aliquot of the spiking solution (4.24) equivalent to 2 µg of zearalenone into either a vial (5.9) or a calibrated volumetric flask (5.10). Add acetonitrile (4.11) to make the total volume up to 5 ml.

Store this solution in a freezer at - 18 °C to - 20 °C. Allow to reach room temperature before opening. A solution stored in this way is usually stable for 12 months. Confirm the mass 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 High speed blender or homogenizer.

5.3 Analytical balance, capable of weighing to 0,000 1 g.

5.4 Laboratory balance, capable of weighing to 0,1 g.

5.5 Adjustable vertical or horizontal shaker.

5.6 Vortex mixer, or equivalent.

5.7 Mills, various screens.

5.8 Tumble mixer.

5.9 Glass vials, of various sizes

5.10 Volumetric flasks, of 3 ml or 5 ml and 10 ml capacity.

5.11 Beaker, of 250 ml capacity.

5.12 Conical flask, with screw cap or glass stopper of 100 ml, 250 ml and 500 ml capacity.

5.13 Filter paper, e.g., qualitative, strong, fast flow, 24 cm diameter, 30 µm pore size, prefolded or equivalent.

5.14 Glass microfibre filter, e.g. 1,6 µm retention size or equivalent.

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

5.16 Displacement micropipettes or syringes, gas tight of e.g. 100 µl, 500 µl, 1 000 µl capacity.

5.17 Vacuum manifold or automated system, to accommodate immunoaffinity columns.

5.18 Reservoirs, of 50 ml to 75 ml capacity, and attachments for immunoaffinity columns.

5.19 Plastic syringes, 5 ml.

5.20 Vacuum pump, capable of for example pulling a vacuum of 1 kPa and or pumping 18 l/min.

5.21 Solvent vacuum filtration system, fitted with 47 mm glass microfibre filter.

5.22 Disposable syringe filter unit, nylon with a pore size of 0,45 µm.

Prior to usage, verify that no zearalenone losses occur during filtration (recovery testing).

NOTE There is a possibility that various filter materials retain zearalenone.

5.23 Ultrasonic bath.

5.24 HPLC apparatus, comprising the following:

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

5.24.2 Mobile phase pump, pulse free, capable of maintaining a volume flow rate of 0,5 ml/min to 1,5 ml/min.

5.24.3 Analytical reverse-phase HPLC separating column, that allows a sufficient baseline separation of zearalenone from other interfering components. The maximum overlap shall be less than 10 % peak height.

Phenomenex Prodigy® ODS 3 [1)] (150 mm × 4,6 mm internal diameter, 5 µm particle size, 25 nm pore size) or Spherisorb® [1)] ODS-2 Excel (250 mm × 4,6 mm internal diameter, 5 µm particle size, 25 nm pore size) have been found to be suitable when used with mobile phase A (4.16).

Supelcosil® [1)] (C_{18}), fully end capped (250 mm × 4,6 mm internal diameter, 5 µm particle size, 18 nm pore size), carbon loading of 12 %, or similar, has been found to be suitable when used with mobile phase B (4.20).

5.24.4 Pre-column, with preferably the same stationary phase material as the analytical column, an internal diameter of 4 mm, 5 µm particle size.

5.24.5 Fluorescence detector, fitted with a flow cell and suitable for measurements with excitation wavelength of 274 nm or 275 nm and emission at 446 nm or 450 nm.

5.24.6 Recorder, integrator or computer based data processing system.

5.24.7 Degasser (optional).

5.25 UV spectrometer, with suitable quartz cells.

1) Phenomenex Prodigy®, Spherisorb®, Supelcosil® are examples of a suitable products available commercially. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can show to lead to the same results.

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6 Procedure

6.1 Extraction

6.1.1 Extraction for barley flour, maize flour, wheat flour, polenta and maize based baby food

Weigh, to the nearest 0,1 g, a 25 g test portion of the ground sample into a beaker (5.11). Add 100 ml of extraction solvent A (4.14). Homogenize for 3 min with a homogenizer (5.2) set at high speed. Filter the extract through a folded filter paper (5.13). Transfer 88 ml of PBS (4.10) to a conical flask (5.12). Pipette (5.15), add 12 ml of the filtrate and mix well by shaking by hand. If on addition of the filtrate to PBS the sample becomes cloudy filter through a glass microfibre filter paper (5.14) prior to cleanup.

6.1.2 Extraction for cereal based foods for infants and young children

Weigh, to the nearest 0,1 g, a 20 g test portion into a screw capped conical flask (5.12). Add 150 ml of extraction solvent B (4.17). Mix shortly by hand for a few seconds to obtain a homogeneous suspension, then either shake for 1 h in a shaker (5.5) or sonicate for 15 min in an ultrasonic bath (5.23) and shake on a shaker (5.5) for another 15 min.

Filtrate the extract through a folded filter paper (5.13) and collect the extract in a conical flask of 100 ml (5.12). Transfer exactly 30 ml of the filtrate extract into a 150 ml volumetric cylinder with stopper. Dilute the extract in the cylinder with PBS (4.10) to achieve a final volume of 150 ml. Shake and filter approximately 20 ml of this diluted extract through a glass microfibre filter (5.14) into a glass beaker by applying a slight vacuum (5.21). Discard this 20 ml and filter again a further approximately 70 ml for analysis.

NOTE Do not apply a strong vacuum in the beginning of the filtration process, as this can lead to turbid filtered extracts after filtration.

Proceed immediately with the immunoaffinity column cleanup procedure (6.2).

6.2 Immunoaffinity column cleanup

Allow the immunoaffinity column to reach room temperature prior to conditioning. Connect the immunoaffinity column (4.21) to the vacuum manifold (5.17), and attach the reservoir (5.18) on top of the immunoaffinity column. Precondition the immunoaffinity column with 20 ml of PBS (4.10) using a flow rate of 3 ml/min to 5 ml/min. Transfer 50 ml of diluted (and possibly filtered) sample extract (see 6.1.1 or 6.1.2) into the reservoir. Draw the extract through the column by gravity at a steady flow rate until all the extract has passed through the column and the last solvent portion reaches the frit of the column. The flow rate should result in a dropping speed of one drop per second to two drops per second.

After the extract has passed through the column, wash the column with 20 ml of water or for baby food 5 ml of washing solvent (4.18) followed by 15 ml of water at a rate of one drop per second to two drops per second.

Remove residual water from the immunoaffinity column for example by passing 3 ml of air or nitrogen through the column. Discard all the eluent from this stage of the cleanup procedure.

NOTE 1 The conditions above for preconditioning and washing elution can be altered to comply with individual column manufacturer's instructions for use.

NOTE 2 Care should be taken not to exceed the capacity of the immunoaffinity column.

6.3 Preparation of the sample test solution

6.3.1 Preparation for barley flour, maize flour, wheat flour, polenta and maize based baby food

Place a vial (5.9) under each immunoaffinity column. Pipette 1,5 ml of acetonitrile (4.11) to the column reservoir (5.18). Allow the solvent to remain in contact with the immunoaffinity column for 1 min then elute the

zearalenone from the immunoaffinity column by allowing the solvent to pass through the column at a speed of one drop per second to two drops per second. Ensure that all the solvent has been collected for example by passing 5 ml of air through the column.

Evaporate the immunoaffinity column eluate to dryness under nitrogen. Redissolve in 1 ml of HPLC injection solvent A (4.15). Mix well on a vortex mixer (5.6). Transfer to for example a glass vial (5.9).

Alternatively, the column eluate may be diluted to a known volume (for example 3 ml) by the addition of water and an aliquot analyzed by HPLC.

6.3.2 Preparation for cereal based foods for infants and young children

Place a 3,0 ml volumetric flask (5.10) under the column and pass 0,75 ml of methanol (4.12) through the column, collecting the eluate. After the last drops of methanol have passed through the column allow the methanol to remain on the column for approximately 1 min. Then add a further 0,75 ml of methanol and continue to collect the eluate. Carefully pass air through the column in order to collect any final drops.

Fill the volumetric flask up to the mark with water and shake. In case of turbid samples, filter the test solution through a HPLC syringe filter (5.22) with a plastic syringe (5.19).

NOTE 1 Methanol and water undergo volume contraction when mixed. Adjust volume if necessary after shaking.

NOTE 2 Alternatively to a manual procedure (see 6.3.1 and 6.3.2) the immunoaffinity cleanup and elution can be performed with an automatic sample preparation unit, provided that volumes and flow rates remain unchanged.

6.4 Spiking procedure

To determine the recovery carry out the spiking procedure using the standard solution (4.25). The spiking level should be within the calibration range (preferably mid-range). Leave the spiked sample to stand for a minimum of 30 min to ensure evaporation of the solvent.

7 HPLC analysis

7.1 HPLC operating conditions

When the column specified in 5.24.3 and the mobile phase specified in 4.16 or 4.20 were used, the following settings were found to be appropriate, see also Figures A.1 and A.2:

— flow rate mobile phase (column): 0,7 ml/min to 1,0 ml/min;

— fluorescence detection, emission wavelength: 446 nm to 450 nm;

— fluorescence detection, excitation wavelength: 274 nm to 275 nm;

— injection volume: 100 µl to 300 µl.

7.2 Preparation of calibration solutions for HPLC

Use a pipette (5.15) or syringe (5.16) to prepare five HPLC calibration solutions in separate 10 ml volumetric flasks by transferring the volumes given in Table 1 and Table 2 appropriate for the sample to be tested. Dilute each solution to the mark with the appropriate injection solvent for HPLC (4.15 for Table 1 or 4.19 for Table 2).

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Table 1 — Preparation of HPLC calibration solutions for maize based baby food, barley flour, maize flour, polenta and wheat flour

HPLC calibration solution	Aliquot taken from zearalenone standard solution A (see 4.25) µl	Final zearalenone mass concentration in calibration solution ng/ml
1	25	5
2	175	35
3	375	75
4	550	110
5	750	150

Table 2 — Preparation of HPLC calibration solutions for cereal based foods for infants and young children

HPLC calibration solution	Aliquot taken from zearalenone standard solution B (see 4.26) µl	Final zearalenone mass concentration in calibration solution ng/ml
1	50	2
2	200	8
3	350	14
4	500	20
5	650	26

The preparation of the calibration solutions can be performed either by the use of pipettes or calibrated glassware as available.

7.3 Calibration curve

Prepare a calibration curve at the beginning of every day of the analysis by injecting the calibration solutions appropriate for the samples under test (see Table 1 or Table 2) into the HPLC system. Establish the calibration curve prior to analysis of test samples by plotting the mass concentration of zearalenone, in nanograms per millilitre, on the x-axis against the 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 zearalenone in sample test solutions

Inject aliquots of the sample test solutions (6.3.1 or 6.3.2) into the HPLC system using the same conditions used for the preparation of the calibration curve.

7.5 Peak identification

Identify the zearalenone peak in the sample test solution by comparing the retention time with that of the nearest HPLC standard injected in the HPLC run. The mass concentration of zearalenone in the sample test solution shall fall within the calibration range. If the zearalenone level in the sample test solution exceeds the mass concentration of the highest standard dilute the sample extract with HPLC mobile phase, to bring it

within calibration range, and reanalyse. The dilution factor shall be incorporated into all subsequent calculations.

8 Calculation

Determine the mass concentration of zearalenone in the sample test solution (6.3.1 or 6.3.2), in nanograms per millilitre, directly from the calibration curve (7.3). Calculate the mass fraction, w_{zon}, of zearalenone in nanograms per gram, using Equation (2):

$$w_{zon} = \frac{\rho_a \times V_1 \times V_2}{V_3 \times m_s} \quad (2)$$

where

ρ_a is the mass concentration of zearalenone, in nanograms per millilitre, in the aliquot of test solution injected and determined from the calibration curve;

V_1 is the volume, in millilitres, of the solvent taken for extraction (100 ml for 6.1.1 or 150 ml for 6.1.2);

V_2 is the volume, in millilitres, obtained after elution from the immunoaffinity column after re-dissolving with mobile phase (1,0 ml or 3,0 ml);

V_3 is the volume, in millilitres, of the extract aliquot used for immunoaffinity cleanup (6 ml for 6.1.1 and 10 ml for 6.1.2);

m_s is the mass, in grams, of sample material taken for analysis (25 g for 6.1.1 and 20 g for 6.1.2).

This results in the following simplified equations:

$w_{zon} = \rho_a \times 0{,}667$ (for samples prepared following 6.1.1, final volume of 1 ml);

$w_{zon} = \rho_a \times 2{,}0$ (for samples prepared following 6.1.1, final volume of 3 ml);

$w_{zon} = \rho_a \times 2{,}25$ (for samples prepared following 6.1.2, final volume of 3 ml).

9 Precision

9.1 General

Details of an interlaboratory test on the precision of the method for maize based baby food, barley flour, maize flour, polenta and wheat flour are given in Table B.1. Details of an interlaboratory test on the precision of the method for cereal based foods for infants and young children are given in Table B.2. The values derived from these interlaboratory tests may not be applicable to concentration ranges and/or matrices other than those given in Annex B.

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.

For samples prepared using procedure 6.1.1:

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The values for maize based baby food are: $\bar{x}$ = 10,9 µg/kg r = 11,0 µg/kg (fortified)
The values for barley flour are: $\bar{x}$ = 143 µg/kg r = 27,5 µg/kg (fortified)
The values for maize flour are: $\bar{x}$ = 87,2 µg/kg r = 34,8 µg/kg (naturally contaminated)
The values for maize flour are: $\bar{x}$ = 335 µg/kg r = 82,9 µg/kg (naturally contaminated)
The values for polenta are: $\bar{x}$ = 66,5 µg/kg r = 16,6 µg/kg (fortified)
The values for wheat flour are: $\bar{x}$ = 227 µg/kg r = 52,9 µg/kg (fortified)

For samples prepared using procedure 6.1.2:

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

$\bar{x}$ = 9,1 µg/kg r = 1,5 µg/kg (naturally contaminated)
$\bar{x}$ = 17,1 µg/kg r = 2,5 µg/kg (naturally contaminated)
$\bar{x}$ = 44,0 µg/kg r = 3,4 µg/kg (naturally contaminated)
$\bar{x}$ = 18,4 µg/kg r = 4,5 µg/kg (fortified)
$\bar{x}$ = 26,6 µg/kg r = 4,2 µg/kg (fortified)

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.

For samples prepared using procedure 6.1.1:

The values for maize based baby food are: $\bar{x}$ = 10,9 µg/kg R = 11,7 µg/kg (fortified)
The values for barley flour are: $\bar{x}$ = 143 µg/kg R = 71,8 µg/kg (fortified)
The values for maize flour are: $\bar{x}$ = 87,2 µg/kg R = 50,4 µg/kg (naturally contaminated)
The values for maize flour are: $\bar{x}$ = 335 µg/kg R = 343,7 µg/kg (naturally contaminated)
The values for polenta are: $\bar{x}$ = 66,5 µg/kg R = 30,6 µg/kg (fortified)
The values for wheat flour are: $\bar{x}$ = 227 µg/kg R = 107,9 µg/kg (fortified)

For samples prepared using procedure 6.1.2:

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

$\bar{x}$ = 9,1 µg/kg R = 3,3 µg/kg (naturally contaminated)
$\bar{x}$ = 17,1 µg/kg R = 6,2 µg/kg (naturally contaminated)
$\bar{x}$ = 44,0 µg/kg R = 12,5 µg/kg (naturally contaminated)
$\bar{x}$ = 18,4 µg/kg R = 6,6 µg/kg (fortified)
$\bar{x}$ = 26,6 µg/kg R = 6,1 µg/kg (fortified)

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) reference to this European Standard;

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

d) date of receipt;

e) date of test;

f) 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.

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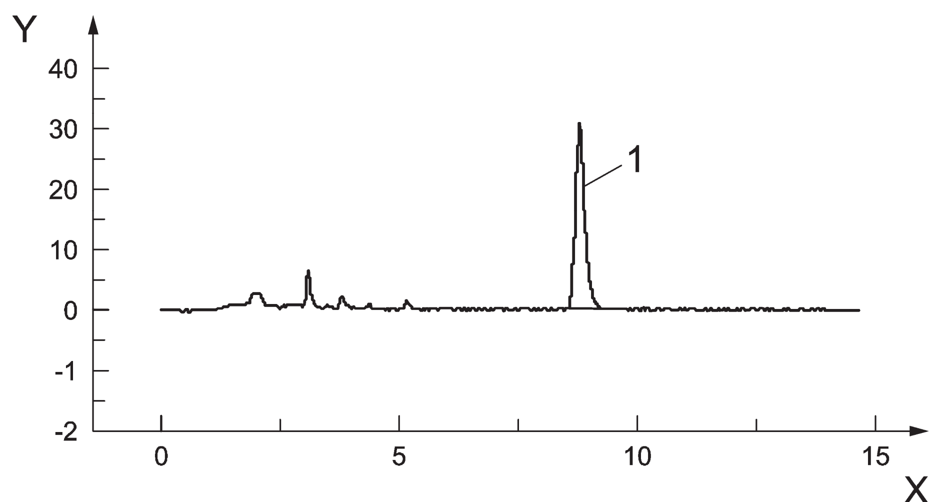
Annex A
(informative)

Typical chromatograms



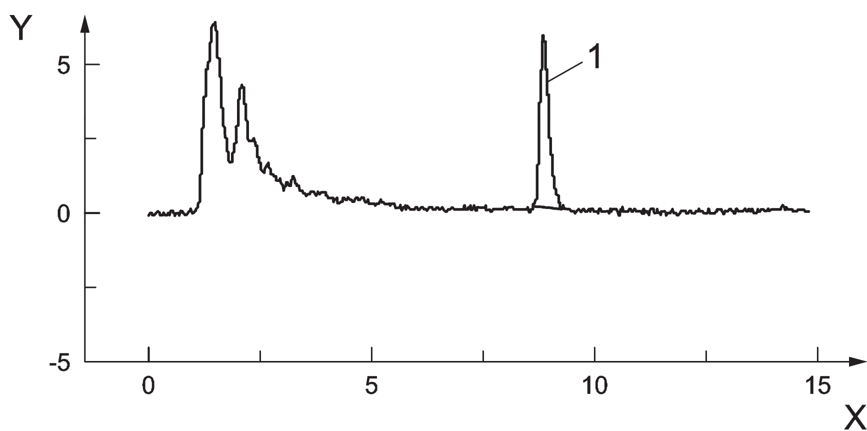
Key

X time (min)

Y signal (mV)

1 zearalenone

Figure A.1 — Typical chromatogram, wheat sample naturally contaminated with 190 µg/kg zearalenone, analyzed using HPLC conditions described in 7.1



Key

X time (min)

Y signal (mV)

1 zearalenone

Figure A.2 — Typical chromatogram, wheat sample naturally contaminated with 36 µg/kg zearalenone, analyzed using HPLC conditions described in 7.1

Annex B
(informative)

Precision data

The data given in Table B.1 were obtained in interlaboratory tests [2], [3] according to AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis [4].

Table B.1 — Precision data for samples prepared using procedure 6.1.1

Sample	Maize based baby food (fortified)	Barley flour (fortified)	Maize flour (naturally contam.)	Polenta (fortified)	Wheat flour (fortified)	Maize flour (naturally contam.)
Year of interlaboratory test	2002	2002	2002	2002	2002	2002
Number of laboratories	29	29	29	29	29	28
Number of laboratories retained after eliminating outliers	28	28	29	29	29	27
Number of outliers (laboratories)	1	1	0	0	0	1
Number of accepted results	23	25	27	27	27	27
Mean value, $\bar{x}$, µg/kg	10,9	143	87,2	66,5	227	335
Repeatability standard deviation s_r, µg/kg	3,9	9,8	12,4	5,9	18,9	29,6
Repeatability relative standard deviation, RSD_r, %	35,8	6,9	14,2	8,9	8,3	8,8
Repeatability limit r [$r = 2,8 \times s_r$], µg/kg	11,0	27,5	34,8	16,6	52,9	82,9
Reproducibility standard deviation s_R, µg/kg	4,2	25,6	18,0	10,9	38,6	123
Reproducibility relative standard deviation, RSD_R, %	38,2	17,9	20,6	16,4	17	36,6
Reproducibility limit R [$R = 2,8 \times s_R$], µg/kg	11,7	71,8	50,4	30,6	108	344
Recovery, % [a]	100	92	91	91	95	98
HorRat value, calculated using Predicted Standard Deviation ($PSRD_R$) from Thompson, see [5] and [6]	1,7	0,8	0,9	0,7	0,9	1,9

[a] Recovery values were derived independently from the analysis of single spiked samples of each matrix (100 µg/kg) by each laboratory that participated in the collaborative study.

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Table B.2 — Precision data for samples prepared using procedure 6.1.2

Sample	Cereal based foods for infants and young children					
	(fortified)		(naturally contaminated)			
Year of interlaboratory test	2005	2005	2005	2005	2005	2005
Number of laboratories	17	17	19	19	19	19
Number of laboratories retained after eliminating outliers	17	17	19	17	18	17
Number of outliers (laboratories)	0	0	0	2	1	2
Number of accepted results	17	17	19	17	18	17
Mean value, $\bar{x}$, µg/kg	18,4	26,6	< 2	9,1	17,1	44
Repeatability standard deviation s_r, µg/kg	1,6	1,5	n.a.	0,5	0,9	1,2
Repeatability relative standard deviation, RSD_r, %	8,7	5,7	n.a.	5,9	5,3	2,8
Repeatability limit r [$r = 2,8 \times s_r$], µg/kg	4,5	4,2	n.a.	1,5	2,5	3,4
Reproducibility standard deviation s_R, µg/kg	2,4	2,2	n.a.	1,2	2,2	4,5
Reproducibility relative standard deviation, RSD_R, %	12,9	8,2	n.a.	13,0	13,0	10,1
Reproducibility limit R [$R = 2,8 \times s_R$], µg/kg	6,7	6,1	n.a.	3,3	6,2	12,5
Recovery, % [a]	92	91	n.a.	n.a.	n.a.	n.a.
HorRat value, calculated using Predicted Standard Deviation ($PSRD_R$) from Thompson, see [5] and [6]	0,4	0,3	n.a.	0,4	0,4	0,4

[a] Recovery values were derived independently from the analysis of blind duplicate spiked samples. Each laboratory that participated in the collaborative study performed blind duplicate spikes at 20 µg/kg and 30 µg/kg.

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