

English Version

Animal feeding stuffs - Determination of zearalenone in animal feed - High performance liquid chromatographic method with fluorescence detection and immunoaffinity column clean-up

Aliments des animaux - Dosage de la zéaralénone dans les aliments des animaux - Méthode de chromatographie liquide haute performance avec détection par fluorescence et purification sur colonne d'immuno-affinité

Futtermittel - Bestimmung von Zearalenon in Futtermitteln - Hochleistungsflüssigchromatographisches Verfahren mit Fluoreszenznachweis und Reinigung an einer Immunoaffinitätssäule

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Foreword

This document (EN 15792:2009) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, the secretariat of which is held by NEN.

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of an identical text or by endorsement, at the latest by March 2010, and conflicting national standards shall be withdrawn at the latest by March 2010.

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1 Scope

This Standard is applicable to the determination of zearalenone in animal feed at concentrations from 30 µg/kg to 3 000 µg/kg.

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, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

Zearalenone is extracted from the commodity using organic solvent. The solvent extract is then diluted with phosphate buffered saline to give an aqueous extract which is applied to an immunoaffinity column containing antibodies specific for zearalenone. The analyte is isolated, purified and concentrated on the column and removed from the antibodies with elution solvent. Zearalenone is quantitatively determined by high performance liquid chromatography (HPLC) with fluorescence detection.

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade and only distilled water or water of grade 1 as defined in EN ISO 3696. Solvents shall be of quality for HPLC analysis.

4.1 Acetonitrile

WARNING — Acetonitrile is hazardous and handling shall be carried out inside a fume cupboard. Appropriate safety equipment (lab coat, goggles, gloves) shall be worn.

4.2 Methanol, technical grade

WARNING — Methanol is hazardous and handling shall be carried out inside a fume cupboard. Appropriate safety equipment (lab coat, goggles, gloves) shall be worn. Samples shall be blended using an explosion proof blender.

4.3 Methanol, HPLC grade

WARNING — Methanol is hazardous and handling shall be carried out inside a fume cupboard. Appropriate safety equipment (lab coat, goggles, gloves) shall be worn. Samples shall be blended using an explosion proof blender.

4.4 Sodium chloride

4.5 Disodium hydrogen orthophosphate

4.6 Potassium dihydrogen phosphate

4.7 Potassium chloride

4.8 Hydrochloric acid (32%)

WARNING — Hydrochloric acid is hazardous and handling shall be carried out with the necessary precaution inside a fume cupboard. Appropriate safety equipment (lab coat, goggles, gloves) shall be worn.

4.9 Phosphate buffered saline (PBS)

Dissolve 8 g sodium chloride (4.4), 1,2 g disodium hydrogen orthophosphate (4.5), 0,2 g potassium dihydrogen phosphate (4.6) and 0,2 g potassium chloride (4.7) in 1 l of distilled water. Adjust the pH to 7,4 with hydrochloric acid (4.8).

NOTE Commercially available phosphate buffered saline tablets with equivalent properties may be used.

4.10 Extraction solvent, methanol/water = 75+25 parts by volume

Mix 75 parts per volume methanol (4.2) with 25 parts per volume of water

4.11 Washing solvent, methanol/PBS = 15 + 85 parts by volume

Mix 15 parts per volume methanol (4.3) with 85 parts per volume PBS (4.9).

4.12 Injection solvent for HPLC analysis, methanol/water = 50+50 parts by volume

Mix 50 parts per volume methanol (4.3) with 50 parts per volume water.

4.13 HPLC mobile phase, methanol/water = 75+25 parts by volume

Mix 75 parts per volume methanol (4.3) and 25 parts per volume water. Mix well and degas.

4.14 Zearalenone, minimum purity of 98 %

WARNING — Zearalenone is an oestrogenic compound and shall 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.

4.15 Zearalenone (ZON) stock solution

10 µg Zearalenone per millilitre of Acetonitrile.

May be prepared by the following: Add 4,0 ml of acetonitrile (4.1) to 5 mg of zearalenone (4.14) for a standard solution of 1,25 mg/ml. Dilute 800 µl of the 1,25 mg/ml standard solution to 5,0 ml with acetonitrile (4.1) for a standard solution of 200 µg/ml. Dilute 250 µl of the 200 µg/ml standard solution to 5,0 ml of acetonitrile (4.1) to create the stock solution of 10 µg/ml.

To determine the exact concentration record the absorption curve of this 10 µg/ml stock solution with the spectrophotometer (5.26) in the range of 200 nm to 300 nm in a 1 cm quartz cell with acetonitrile (4.1) as reference. Determine the absorption of the second maximum at $\lambda = 274$ nm. Calculate the mass concentration of zearalenone, ρ_{zon} , in micrograms per millilitre using equation 1:

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

where:

- A_{max}

is the absorption determined at the second maximum of the absorption curve (here: at 274 nm)
- M

is the molar mass of zearalenone ($M = 318,4$ g/mol);
- κ

is the molar absorption coefficient of zearalenone in acetonitrile (4.1) (here: $1\,262\text{ m}^2/\text{mol} \pm 1\text{ m}^2/\text{mol}$ [1]);
- d

is the optical path length of the quartz cell in centimetres (here: 1 cm).

Store standard solutions at below $-18\text{ }^{\circ}\text{C}$.

4.16 ZON spiking solution

The calibrated stock solution, see (4.15). This solution is stable for 2 months if stored at below $-18\text{ }^{\circ}\text{C}$.

4.17 ZON working solution

Transfer an aliquot of the calibrated stock solution (4.15), equivalent to $10\text{ }\mu\text{g}$ of ZON, into a volumetric flask (5.11). Add acetonitrile (4.1) to make the total volume up to 5 ml. This is a $2\text{ }\mu\text{g/ml}$ working solution. This solution is stable for 2 months if stored at below $-18\text{ }^{\circ}\text{C}$.

4.18 ZON Calibration solutions for HPLC

Prepare 5 HPLC calibration solutions in separate 10 ml volumetric flasks (5.11) by pipetting the volumes shown in Table 1 below. Make up each standard to volume (10 ml) with injection solvent for HPLC (4.12).

Table 1 — Preparation of calibration solutions

Calibration solution	Volume of ZON working solution (4.17) μl	Mass concentration of calibration solution ng/ml
1	50	10
2	250	50
3	450	90
4	650	130
5	850	170

The procedures above for standard preparation can be performed either by the use of pipettes or calibrated glassware as available.

4.19 Immunoaffinity column

The immunoaffinity (IA) column contains antibodies raised against zearalenone. The column shall have a capacity of not less than $1\,500\text{ ng}$ of zearalenone and a recovery of not less than 70% when 75 ng of zearalenone are applied in 10 ml of washing solvent (4.11).

5 Apparatus

Usual laboratory equipment and in particular the following:

- 5.1 **Analytical balance**, with $d=0,001$ g or better
- 5.2 **Horizontal or vertical shaker**
- 5.3 **Homogeniser/ High Speed Blender**
- 5.4 **Vortex Mixer**, or equivalent
- 5.5 **pH meter**
- 5.6 **Mill (various screens)**
- 5.7 **Tumble mixer**
- 5.8 **Glass vials**, various sizes
- 5.9 **Graduated pipettes**, with volumes of 5 ml and 50 ml
- 5.10 **Graduated cylinders with and without stoppers**, with volumes of 5 ml and 250 ml
- 5.11 **Volumetric flasks**, with volumes of 3 ml, 5 ml and 10 ml
- 5.12 **Beaker**, 250 ml
- 5.13 **Conical or screw cap flasks**, with volumes of 100 ml and 250 ml to 500 ml
- 5.14 **Glass funnels**, of appropriate size
- 5.15 **Folded filters**, cellulose (ca. 30 μm pore size) for the glass funnels (5.14)
- 5.16 **Filter disks**, binder-free glass microfibre ($< 2 \mu\text{m}$ pore size) of appropriate size for the solvent vacuum filtration system (5.22)
- 5.17 **Pipettors or gas-tight glass syringes**, with a volume of 100 μl , 500 μl and 1 000 μl
- 5.18 **Vacuum manifold or Automated SPE Vacuum System**, capable of accommodating the immunoaffinity columns
- 5.19 **Reservoirs**, of appropriate volume with attachments to fit the immunoaffinity columns
- 5.20 **Plastic syringes**, 5 ml
- 5.21 **Vacuum pump**, capable of generating sufficient vacuum for the solvent vacuum filtration system (5.22)
- 5.22 **Solvent vacuum filtration system**, fitted with glass microfibre filter (5.16)

5.23 HPLC syringe filter unit, polyamide (nylon) with 0,45 µm pore size

5.24 Ultrasonic bath

5.25 HPLC apparatus, comprising the following:

5.25.1 Injection system, manual or autosampler, with loop suitable for 100 µl to 300 µl injections

5.25.2 Pump, isocratic, pulsation-free, capable of maintaining a volume flow rate of 0,5 ml/min to 1,5 ml/min

5.25.3 Analytical reversed phase HPLC column,

Generally every RP-column is suitable that allows a sufficient separation of zearalenone from other interfering components. For example Phenomenex ODS3-Prodigy (150 mm x 4,6 mm i.d.), 5 µm particle size, 250 Å pore size, or Spherisorb ODS2-Excel (250 mm x 4,6 mm i.d.), 5 µm particle size, 250 Å pore size have been found to be suitable.

5.25.4 Pre-column (optional), appropriate for the analytical column used

5.25.5 Fluorescence detector, fitted with a flow cell and suitable for measurements with excitation wavelength of 274 nm, and emission of 446 nm

5.25.6 Data system, integrator or PC workstation

5.26 UV spectrophotometer, for checking the concentration of the stock solution (4.15)

6 Procedures

6.1 Sample preparation

It is important that the laboratory receives a sample which is truly representative and has not been damaged or changed during transport or storage. Samples should be taken and prepared in accordance with European legislation where applicable [2]. Samples should be finely ground and thoroughly mixed using a mill (5.6) and a tumble mixer (5.7) or another process that has been demonstrated to give complete homogenisation before a test portion is removed for analysis.

In all instances if the sample has been frozen allow it to thaw completely before sampling. Stir the sample thoroughly before removing an analytical test portion.

6.2 Extraction

Weigh 20,00 g (recorded to 2 decimal places) test portion into a screw cap flask of 250 ml to 500 ml (5.13). Add 150 ml extraction solvent (4.10). Mix briefly by hand to obtain a homogeneous suspension then either shake for 1 h on a shaker (5.2), or sonicate for 15 min in an ultrasonic bath (5.24) and shake on a shaker (5.2) for another 15 min.

Filtrate extract through folded filter paper (5.15) and collect the extract in a screw cap flask of 100 ml (5.13). Transfer exactly 30 ml (or 3 ml in case of results above 500 µg/kg, see section 8) of the filtered extract into a 250 ml graduated cylinder with stopper (5.10). Dilute the extract in the cylinder with PBS (4.9) to the 150 ml mark. Mix and filtrate approximately 20 ml of this diluted extract through a glass microfiber filter (5.16) into a glass beaker by applying a slight vacuum (5.22). Do not apply too strong a vacuum in the beginning of the filtration process, as this can lead to turbid filtered extracts after filtration. Discard these 20 ml and filtrate another approximately 70 ml for analysis.

Proceed immediately with the immunoaffinity column cleanup procedure (6.3).

6.3 Immunoaffinity Clean-up

Connect the immunoaffinity column (4.19) to the vacuum manifold (5.18) and attach a reservoir (5.19) to the top of the immunoaffinity column. Precondition the immunoaffinity column (4.19) with 20 ml of PBS (4.9) using a flow rate of 3 ml/min to 5 ml/min. Pipette 50 ml of the filtered and diluted sample extract (6.2) into the reservoir. Let the extract pass through the column by gravity at a steady flow rate until all the extract has passed and the last solvent portion reaches the frit of the column. The flow rate should be 1 drop/s to 2 drops/s.

After the extract has passed through the column wash the column with 5 ml of washing solvent (4.11) followed by 15 ml of water at a rate of 1 drop/s to 2 drops/s.

Remove residual water from the column (4.19) by passing 3 ml of air or nitrogen through the column (1 s to 2 s). Discard all the eluates from this stage of the clean-up procedure.

6.4 Preparation of the test solution

Place a 5 ml graduated cylinder (5.10) or a 3 ml volumetric flask (5.11) under the column and pass 0,75 ml of methanol (4.3) 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 another 0,75 ml of methanol (4.3) and continue to collect the eluate. Carefully pass air through the column in order to collect any residual methanol.

Fill the graduated cylinder/volumetric flask to the 3 ml mark with water and mix. After mixing check volume again and adjust if necessary. In case of turbid samples filtrate the test solution through a HPLC syringe filter unit (5.23) with a plastic syringe (5.20) before injection.

NOTE Alternatively to the manual procedure (6.4) the immunoaffinity clean-up and elution may be performed with an automatic sample preparation unit, provided that volumes and flow rates remain unchanged.

6.5 Spiking procedure

To determine the recovery spike a zearalenone-free representative material with the ZON spiking solution (4.16). 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 determination

7.1 Calibration graph

Prepare a calibration graph at the beginning of every day of analyses using the ZON calibration solutions (4.18). Establish the calibration curve prior to the analyses of test samples by plotting the concentration of ZON [ng/ml] (x-axis) against the peak signal as area or height (y-axis), determine slope and possible intercept with linear regression, and check for linearity using appropriate diagnostics.

7.2 Determination of zearalenone in test solutions

Inject aliquots of the test solutions (6.4) into the chromatograph using the same conditions used for the preparation of the calibration graph.

7.3 Peak identification

Identify the zearalenone peak in the test solution by comparing the retention time with that of the nearest HPLC calibration solution (4.18) injected during the HPLC analyses batch. The concentration of zearalenone in the test solution must fall within the calibration range. If the zearalenone level in the

solution exceeds the concentration of the highest calibration solution the test solution shall be diluted with HPLC diluent to bring it within calibration range and be reanalysed. The dilution factor must be incorporated into all subsequent calculations.

7.4 HPLC operating conditions

When the column specified in 5.25.3 and the mobile phase specified in 4.13 were used, the following settings were found to be appropriate.

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

8 Calculations

Determine from the calibration graph the mass concentration in ng/ml of the zearalenone in the aliquot of test solution injected onto the HPLC column.

Calculate the mass fraction of zearalenone, w_{ZON} , in nanograms per gram or micrograms per kilogram to one decimal place using the equation below:

$$w_{ZON} = c_{ZON} \times \frac{V_5}{V_4} \times \frac{V_3}{V_2} \times \frac{V_1}{m_s} \tag{2}$$

where

- c_{ZON} is the mass concentration of Zearalenone as determined from calibration (7.1);
- V_5 is the volume of the test solution (3,0 ml; 6.4);
- V_4 is the volume of the aliquot of diluted filtered extract applied to the immunoaffinity column (50 ml; 6.3);
- V_3 is the total volume of the diluted filtered extract (150 ml; 6.2);
- V_2 is the volume of the aliquot of extract used for dilution (30 ml or 3 ml; 6.2);
- V_1 is the total volume of the extraction solvent (150 ml; 6.2);
- m_s is the mass of the extracted sample ($m_s = 20$ g);

The above equation can be simplified if the described masses and volumes have been used:

$$w_{ZON} = c_{ZON} \times 2.25 \text{ (30 ml of extract were diluted)} \tag{3}$$

Should the above calculation render a value above 500 then a new dilution of 3 ml of the sample extract should be prepared (see section 6.2). The simplified equation is then as follows:

$$w_{ZON} = c_{ZON} \times 22.5 \text{ (3 ml of extract were diluted)} \tag{4}$$

9 Precision

9.1 Interlaboratory study

Details of an interlaboratory study on the precision of the method are given in [3]. The values derived from this interlaboratory study may not be applicable to concentration ranges and/or matrices other than those shown.

9.2 Repeatability

The absolute difference between two single test results found on identical test materials 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.

The values are:

$$\bar{x} = 31,5 \text{ } \mu\text{g/kg} \quad r = 5,0 \text{ } \mu\text{g/kg} \text{ (naturally contaminated)}$$

$$\bar{x} = 69,7 \text{ } \mu\text{g/kg} \quad r = 13,8 \text{ } \mu\text{g/kg} \text{ (naturally contaminated)}$$

$$\bar{x} = 307 \text{ } \mu\text{g/kg} \quad r = 81,7 \text{ } \mu\text{g/kg} \text{ (naturally contaminated)}$$

$$\bar{x} = 87,4 \text{ } \mu\text{g/kg} \quad r = 19,1 \text{ } \mu\text{g/kg} \text{ (fortified)}$$

$$\bar{x} = 126 \text{ } \mu\text{g/kg} \quad r = 29,8 \text{ } \mu\text{g/kg} \text{ (fortified)}$$

9.3 Reproducibility

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

The values are:

$$\bar{x} = 31,5 \text{ } \mu\text{g/kg} \quad R = 15,2 \text{ } \mu\text{g/kg} \text{ (naturally contaminated)}$$

$$\bar{x} = 69,7 \text{ } \mu\text{g/kg} \quad R = 31,8 \text{ } \mu\text{g/kg} \text{ (naturally contaminated)}$$

$$\bar{x} = 307 \text{ } \mu\text{g/kg} \quad R = 133 \text{ } \mu\text{g/kg} \text{ (naturally contaminated)}$$

$$\bar{x} = 87,4 \text{ } \mu\text{g/kg} \quad R = 52,5 \text{ } \mu\text{g/kg} \text{ (fortified)}$$

$$\bar{x} = 126 \text{ } \mu\text{g/kg} \quad R = 62,6 \text{ } \mu\text{g/kg} \text{ (fortified)}$$

10 Test report

The test report shall contain the following data:

- a) 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) whether the repeatability has been verified;
- h) particular points observed in the course of the test;
- i) operations not specified in the method or regarded as optional, which might have effected the results.

Annex A
(informative)

Precision data

The following data were obtained in interlaboratory studies [3] according to AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis [4].

Table A.1 — Precision data

Sample	Animal feed					
Year of inter-laboratory study	2005	2005	2005	2005	2005	2005
Number of laboratories	14	15	15	15	15	15
Number of laboratories retained after eliminating outliers	14	15	15	12	14	15
Number of outliers (laboratories)	0	0	0	3	1	0
Number of accepted results	14	15	15	12	14	15
Mean value \bar{x} , µg/kg	87,4	126,0	<20	31,5	69,7	307
Repeatability standard deviation s_r , µg/kg	6,8	10,7	n.a. ^a	1,8	5,0	29,2
Coefficient of variation of repeatability $CV(r)$, %	7,8	8,5	n.a. ^a	5,7	7,1	9,5
Repeatability limit r , µg/kg ^b	19,1	29,8	n.a. ^a	5,0	13,8	81,7
Reproducibility standard deviation s_R , µg/kg	18,7	22,4	n.a. ^a	5,5	11,4	47,5
Coefficient of variation of reproducibility $CV(R)$, %	21,4	17,7	n.a. ^a	17,3	16,3	15,5
HorRat value	0.9	0.8	n.a. ^a	0.6	0.7	0.8
Reproducibility limit R , µg/kg ^c	52,5	62,6	n.a. ^a	15,2	31,8	133
Recovery, %	75	72	n.a. ^a	n.a.	n.a.	n.a.
a - n.a.: not applicable b - $r = 2,8 \times s_r$ c - $R = 2,8 \times s_R$						

Table A.2 – Material composition

Test Material	Ingredient	Amount (kg)	Composition (in increasing amounts)
Blank	Compound pig feed	4,4	peas, soy, wheat, barley, tapioca, cabbage seeds , animal fat, corn, calcium carbonate
	Compound rabbit feed	2,2	wheat, alfalfa, sunflower seeds, cabbage seeds, straw molasses, barley, roasted soy
	Compound horse feed	2,2	Barley flakes, oat, cornflakes, oil, alfafla, pellets of grass fibres
	Compound chicken feed	2,2	barley, wheat, oat, several cereals, oil corn
	Alfalfa	1,0	alfalfa
Level 1	Compound pig feed	3,0	peas, soy, wheat, barley, tapioca, cabbage seeds , animal fat, corn, calcium carbonate
	Corn	2,6	corn, blank
	Compound rabbit feed	1,5	wheat, alfalfa, sunflower seeds, cabbage seeds, straw molasses, barley, roasted soy
	Corn	0,6	corn, contaminated
	Wheat	0,2	wheat, contaminated
Level 2	Corn	2,5	corn, blank
	Compound pig feed	1,5	peas, soy, wheat, barley, tapioca, cabbage seeds , animal fat, corn, calcium carbonate
	Compound rabbit feed	1,5	wheat, alfalfa, sunflower seeds, cabbage seeds, straw molasses, barley, roasted soy
	Compound horse muesli	0,5	oat, barley flakes, cornflakes, peas, molasses, plant oil
	Compound dog feed	0,5	meat 14%, cereals14%, sugar, oil, minerals, carrots 4%
	Alfalfa	0,5	Alfalfa (luzerne)
	Corn	0,5	corn, contaminated
Level 3	Level 2	2	(see “Level 2”)
	Compound pig feed	2	peas, soy, wheat, barley, tapioca, cabbage seeds , animal fat, corn, calcium carbonate

Bibliography

- [1] Krska R., Welzig E., Josephs R. D. et al., 2003. Purity Assessment of Crystalline Zearalenone. J. AOAC Int, 2003. 86(4), 722-728
- [2] Commission Regulation EC (No) 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs
- [3] Arranz I., Mischke C., Stroka J., et al., Liquid Chromatographic Method for the Quantification of Zearalenone in Baby Food and Animal Feed: Interlaboratory Study. J AOAC Int, 2007. 90(6): 1598-1609
- [4] AOAC International 1995, AOAC Official Methods Program, p. 23-51