SLOVENSKI STANDARD

SIST EN 15792:2009

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Krma - Doloļ evanje zearalenona v krmi - Metoda HPLC s fluorescenļ no detekcijo in imunoafinitetnim kolonskim ļ iģļ enjem

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

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

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

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

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Management Centre: Avenue Marnix 17, B-1000 Brussels

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of an identical text or by endorsement, at the latest by March 2010, and conflicting national standards shall be withdrawn at the latest by March 2010.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

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

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 m^2/mol ± 1 m^2/mol [1]);

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

Store standard solutions at below –18 °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 °C.

4.17 ZON working solution

Transfer an aliquot of the calibrated stock solution (4.15), equivalent to 10 µg 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 µg/ml working solution. This solution is stable for 2 months if stored at below –18 °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 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 glas microfibre (< 2 µm pore size) of appropriate size for the solvent vacuum filtration system (5.22)

5.17 Pipettors or gas-tight glas 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)