SLOVENSKI STANDARD
SIST EN 15791:2009

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Krma - Določevanje deoksinivalenola v krmi - Metoda HPLC z imunoafinitetnim kolonskim čiščenjem

Foodstuffs - Determination of Deoxynivalenol in animal feed - HPLC method with immunoaffinity column clean-up

Futtermittel - Bestimmung von Deoxynivalenol in Futtermitteln - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule

Produits alimentaires - Dosage du désoxynivalénol dans les aliments pour animaux - Méthode de chromatographie liquide haute performance avec détection UV et purification sur colonne d'immuno-affinité

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English Version

Foodstuffs - Determination of Deoxynivalenol in animal feed - HPLC method with immunoaffinity column clean-up

Produits alimentaires - Dosage du désoxynivalénol dans les aliments pour animaux - Méthode de chromatographie liquide haute performance avec détection UV et purification sur colonne d'immuno-affinité

Futtermittel - Bestimmung von Deoxynivalenol in Futtermitteln - HPLC-Verfahren mit Reinigung an einer Immunoaffinitätssäule

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Foreword

This document (EN 15791:2009) 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of an identical text or by endorsement, at the latest by March 2010, and conflicting national standards shall be withdrawn at the latest by March 2010.

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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.

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1 Scope

This Standard is applicable to the determination of deoxynivalenol (DON) in animal compound feed at concentrations of 150 µg/kg up to at least 4 000 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

Deoxynivalenol (DON) is extracted from the commodity using water. The aqueous extract is then cleaned up with an immunoaffinity column to remove impurities from the sample. Subsequently DON is quantitatively determined by HPLC with UV detection.

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade and only double-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 **Deoxynivalenol (DON),** with a minimum purity of 97 %

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.

4.3 Methanol

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

4.4 Glacial acetic acid

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

4.5 Mobile Phase

Mix 15 parts per volume of methanol (4.3) with 84,9 parts per volume of water and 0,1 parts of glacial acetic acid (4.4). The exact amount of methanol used and whether acetic acid will have to be used depends on the HPLC column chosen for analysis and must be adjusted if necessary. Degas this solution before use.

4.6 Wash Solvent

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

4.7 DON stock solution

250 µg Deoxynivalenol per ml of Acetonitrile.

May be prepared by the following: Add 4,0 ml of acetonitrile (4.1) to 5 mg of DON (4.2) for a solution of 1,25 mg/ml. Dilute 1 000 µl of the 1,25 mg/ml solution to 5,0 ml with acetonitrile for the stock solution of 250 µg/ml. Dilute 200 µl of the 250 µg/ml stock solution in a 2,0 ml volumetric flask (5.11) with acetonitrile to create a diluted stock solution of 25 µg/ml.

To determine the exact concentration record the absorption curve of this 25 µg/ml diluted stock solution with the spectrophotometer (5.15) in the range of 200 nm to 270 nm in a 1 cm quartz cell with acetonitrile (4.1) as reference. Determine the absorption at 220 nm. Calculate the mass concentration of deoxynivalenol, ρ_{Don}, in micrograms per millilitre using equation 1:

$$\rho_{DON}(\sim 25\mu g/ml) = \frac{A_{\max} \times M \times 100}{\kappa \times d} \quad (1)$$

where:

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

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

κ is the molar absorption coefficient of deoxynivalenol in acetonitrile (4.1), (here: 681 m^2/mol ± 12,6 m^2/mol [1]);

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

Calculate the exact concentration of the 250 µg/ml stock solution by the following equation:

$$\rho_{DON}(\sim 250\mu g/ml) = \rho_{DON}(\sim 25\mu g/ml) \times 10 \quad (2)$$

Stock solution may be stored in the dark for up to 3 months at 4°C to 8°C or at least 6 months at below -18 ºC.

NOTE Stock solution preparation can be carried out gravimetrically by accurately weighing the DON standard material and the solvent used to dissolve it.

4.8 DON spiking solution

Pipette an aliquot of the calibrated DON stock solution (4.7), equivalent to 500 µg DON, into a 5 ml volumetric flask (5.11). Make up to the mark with acetonitrile (4.1). This will result in the spiking solution of 100 µg/ml.

4.9 DON working solution

Pipette an aliquot of the calibrated diluted DON stock solution (4.7), equivalent to 50 µg DON, into a 5 ml volumetric flask (5.11). Make up to the mark with acetonitrile (4.1). This will result in the DON working solution of 10 µg/ml.

4.10 DON Calibration solutions

Calibration solutions are prepared from the 10 µg/ml DON working solution (4.9). For example, add the volumes of 10 µg/ml DON working solution (4.9) shown in the table below into 10 ml volumetric flasks (5.11). Fill the flasks up to the mark with mobile phase (4.5). Deviations are permissible as long as the lowest level is above the limit of detection, the highest level does not lead to saturation of the detector signal, and there are at least two more levels equidistantly in between.

Table 1 — Preparation of standard solutions

Calibration solution	DON Working solution (4.9) (µl)	DON concentration ng/ml
1	450	450
2	375	375
3	300	300
4	225	225
5	150	150
6	75	75

4.11 DON immunoaffinity clean-up columns

The immunoaffinity (IA) column contains antibodies raised against deoxynivalenol. The column shall have a capacity of not less than 2 500 ng of DON and shall give a recovery of not less than 70% when 25 ng of DON are applied in 1 ml to-2 ml of water (depending on manufacturer's instructions).

5 Apparatus

Usual laboratory equipment and in particular the following:

5.1 Analytical balance, with d=0,001g for sample weighing, with d=0,01mg for gravimetrical preparation of the DON stock solution (4.7)

5.2 Homogeniser/ High Speed Blender

5.3 Laboratory shaker

5.4 Vortex Mixer, or equivalent

5.5 Mill (various screens)

5.6 Tumble mixer

5.7 Screw cap flasks, with volumes of 250 ml and 500 ml

5.8 Funnels, of appropriate size

5.9 Filter, cellulose with ca. 30 µm pore size

5.10 Filter, binder-free glass microfiber with ca. 2 µm pore size

5.11 Volumetric flasks, with volumes of 2 ml, 5 ml, and 10 ml

5.12 Graduated pipettes, with volumes of 1 ml and 5 ml

5.13 Adjustable Pipettors or gas-tight glass syringes, with volumes of 100 µl and 1 000 µl

5.14 HPLC system consisting of:

5.14.1 Pump, capable at least of generating binary gradients, pulsation-free, at flows appropriate for the analytical column

5.14.2 Analytical column

Any column which allows for sufficient separation of deoxynivalenol from other interfering components is suitable. Examples are: Phenomenex ODS3-Prodigy (15 cm x 4,6 mm i.d.), 5 µm particle size, 100 Å pore size, Octadecylsilane (ODS) 250 mm x 4,6 mm I.D., 3 µm particle size, 80 Å pore size, Octadecyl (C18) 250 mm x 4,6 mm I.D., 5 µm particle size, 180 Å pore size

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

5.14.4 Autosampler, capable of injecting appropriate volumes with sufficient repeatability

5.14.5 UV detector, capable of measuring at 220 nm

5.14.6 Data collection system

5.15 UV spectrophotometer, for checking the concentration of the DON stock solution (4.7)

5.16 Reservoirs, of appropriate size with adaptors to fit the immunoaffinity columns

5.17 Glass vials, of appropriate size for autosampler (5.14.4) but with minimum volume of 2,0 ml

5.18 Syringe filter unit, polyamide (nylon) with 0,45 µm pore size

5.19 Evaporator, capable of maintaining 50ºC with a steady stream of air or nitrogen

6 Procedure

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 (5.5) and a tumble mixer (5.6) 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. Mix the sample thoroughly before removing an analytical test portion.