



BSI Standards Publication

Animal feeding stuffs — Determination of Ochratoxin A in animal feed by immunoaffinity column clean- up and High Performance Liquid Chromatography with fluorescence detection

National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/10, Animal feeding stuffs.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Animal feeding stuffs - Determination of Ochratoxin A in animal feed by immunoaffinity column clean-up and High Performance Liquid Chromatography with fluorescence detection

Aliments pour animaux - Dosage de l'ochratoxine A dans les aliments pour animaux par purification sur colonne d'immuno-affinité et chromatographie liquide à haute performance avec détection par fluorescence

Futtermittel - Bestimmung von Ochratoxin A in Tierfutter durch Reinigung an einer Immunoaffinitätssäule und Hochleistungs-Flüssig-Chromatographie mit Fluoreszenzdetektion

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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 16007:2011) 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of an identical text or by endorsement, at the latest by February 2012, and conflicting national standards shall be withdrawn at the latest by February 2012.

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1 Scope

This European Standard specifies a method for the determination of Ochratoxin A (OTA) in cereal based animal feed using immunoaffinity for clean-up followed by liquid-chromatography with fluorescence detection.

NOTE The validated mass fraction range was 39 µg/kg to 338 µg/kg OTA.

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 1042, *Laboratory glassware — One-mark volumetric flasks (ISO 1042:1998)*

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

3 Principle

OTA is extracted from the test material with a mixture of methanol – 3% aqueous sodium bicarbonate solution. The extract is filtered, diluted with PBS and purified using immunoaffinity columns (IAC). The purified OTA is eluted from the IAC using first methanol and then water, brought to a defined volume with water and quantified by HPLC with fluorescence detection.

4 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade. Solvents shall be of HPLC or better quality.

4.1 Methanol, CH₃OH, technical grade.

4.2 Methanol, CH₃OH, HPLC grade.

4.3 Water, water (EN ISO 3696 grade 1 (HPLC grade)) and water (EN ISO 3696 grade 3), or equivalent.

4.4 Potassium chloride, KCl.

4.5 Sodium chloride, NaCl.

4.6 Disodium hydrogenphosphate dodecahydrate, Na₂HPO₄*12 H₂O .

4.7 Acetonitrile, CH₃CN, HPLC grade.

4.8 Glacial acetic acid, CH₃COOH, 96% minimum.

4.9 Solution of acetonitrile/ glacial acetic acid, acetonitrile (4.7) and glacial acetic acid (4.8) in proportion of 99/1 (v/v).

4.10 Toluene, C₆H₅CH₃, analytical grade.

4.11 Toluene/Acetic acid solution, Toluene (4.10) / Glacial acetic acid (4.8) in proportion of 99/1 (v/v).

4.12 Sodium hydrogen carbonate, NaHCO_3 , minimum 99% purity.

4.13 PBS concentrate, Phosphate buffered saline concentrate.

Dissolve the following in 1 800 ml of water (EN ISO 3696 grade 1):

- 4 g KCl (4.4);
- 160 g NaCl (4.5);
- 72 g $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ (4.6).

4.14 PBS Ready to use.

Dilute 100 ml of PBS concentrate (4.13) to 1 000 ml with water (EN ISO 3696 grade 1). Adjust to pH 7,4 with 1 mol/l HCl and make up to 2 000 ml with water (EN ISO 3696 grade 1),

or

PBS tablets, Phosphate buffered saline tablets,

One tablet dissolved in 200 ml of water (EN ISO 3696 grade 1) yields 0,01 mol/l phosphate buffer, 0,002 7 mol/l potassium chloride and 0,137 mol/l sodium chloride, pH 7,4, at 25°C (e.g. Sigma P4417).

4.15 3% aqueous sodium hydrogen carbonate solution.

Add 30 g of sodium hydrogen carbonate (4.12) to 1 000 ml of water (EN ISO 3696 grade 3).

4.16 Extraction solvent, methanol / 3% aqueous sodium hydrogen carbonate solution in proportion of 50/50 (v/v).

Add 500 ml of methanol (4.1) to 500 ml of 3% aqueous sodium hydrogen carbonate solution (4.15). Mix well.

4.17 Aqueous solution of glacial acetic acid.

Add 30 ml of glacial acetic acid (4.8) to 870 ml of water (EN ISO 3696 grade 1) and filter.

4.18 HPLC mobile phase, acetonitrile / methanol / aqueous solution of glacial acetic acid in proportion of 35/35/30 (v/v/v).

Mix 1 050 ml of methanol (4.2) with 1 050 ml of acetonitrile (4.77) and with 900 ml of aqueous solution of glacial acetic acid (4.1717). Mix well and degas.

4.19 OTA, ochratoxin A.

OTA in pure form as crystals, dried film, powder or in solution.

4.20 OTA Stock Solution.

Prepare from the OTA (4.19) a solution of 20 µg/ml in toluene/acetic acid solution (4.11).

To determine the exact concentration, record the absorption curve of this solution between a wavelength of 300 nm and 370 nm in a 1 cm quartz cell with toluene/acetic acid solution (4.11) as reference using the UV-