

**Loomasööt. Ohratoksiin A määramine loomasöödas koos immunoafiinsuskolonnis puhastamisega ja kõrgefektiivse vedelikkromatograafiga koos fluoresentsenti määramisega**

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

## EESTI STANDARDI EESSÕNA

## NATIONAL FOREWORD

See Eesti standard EVS-EN 16007:2011 sisaldab Euroopa standardi EN 16007:2011 ingliskeelset teksti.	This Estonian standard EVS-EN 16007:2011 consists of the English text of the European standard EN 16007:2011.
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EUROPEAN STANDARD

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NORME EUROPÉENNE

EUROPÄISCHE NORM

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English Version

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

This European Standard was approved by CEN on 25 June 2011.

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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of an identical text or by endorsement, at the latest by February 2012, and conflicting national standards shall be withdrawn at the latest by February 2012.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**, CH<sub>3</sub>OH, technical grade.

**4.2 Methanol**, CH<sub>3</sub>OH, 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**, Na<sub>2</sub>HPO<sub>4</sub>\*12 H<sub>2</sub>O .

**4.7 Acetonitrile**, CH<sub>3</sub>CN, HPLC grade.

**4.8 Glacial acetic acid**, CH<sub>3</sub>COOH, 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**, C<sub>6</sub>H<sub>5</sub>CH<sub>3</sub>, analytical grade.