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Djurfoder – Metoder för provtagning och analys – Bestämning av T-2 och HT-2-toxiner, deoxynivalenol och zearalenon i råvaror och foderblandningar med LC-MS

Animal feeding stuffs: Methods of sampling and analysis – Determination of T-2 and HT-2 toxins, Deoxynivalenol and Zearalenone, in feed materials and compound feed by LC-MS



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EUROPEAN STANDARD

EN 16877

NORME EUROPÉENNE

EUROPÄISCHE NORM

November 2016

ICS 65.120; 71.040.50

English Version

**Animal feeding stuffs: Methods of sampling and analysis -
Determination of T-2 and HT-2 toxins, Deoxynivalenol and
Zearalenone, in feed materials and compound feed by LC-
MS**

Aliments des animaux - Méthodes d'échantillonnage et
d'analyse - Dosage par CL-SM des toxines T-2 et HT-2,
du déoxynivalénol et de la zéaralénone dans les
matières premières pour aliments et les aliments
composés

Futtermittel - Probenahme- und
Untersuchungsverfahren - Bestimmung von T-2- und
HT-2-Toxinen, Deoxynivalenol und Zearalenon in
Einzelfuttermitteln und Mischfuttermitteln mittels LC-
MS

This European Standard was approved by CEN on 26 September 2016.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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SS-EN 16877:2016 (E)

European foreword

This document (EN 16877:2016) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs - Methods of sampling and analysis”, 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of an identical text or by endorsement, at the latest by May 2017, and conflicting national standards shall be withdrawn at the latest by May 2017.

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Introduction

WARNING — The method described in this standard implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this standard to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

SS-EN 16877:2016 (E)**1 Scope**

This method of analysis is applicable to the determination of HT-2 toxin (HT2) in the tested range of 22 µg/kg to 178 µg/kg, T-2 toxin (T2) in the tested range of 7 µg/kg to 50 µg/kg, Deoxynivalenol (DON) in the tested range of 88 µg/kg to 559 µg/kg, and Zearalenone (ZON) in the tested range of 14 µg/kg to 430 µg/kg in cereals and cereal-based compound animal feed. The actual working ranges may extend beyond the tested ranges. It is the responsibility of the laboratory to prove that the limit of quantitation (LOQ) for HT-2 and T-2 toxin is ≤ 10 µg/kg, for DON ≤ 100 µg/kg, and for ZON ≤ 20 µg/kg.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696:1995, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

Finely ground and homogeneous test material is suspended in water. After addition of ethyl acetate the sample is agitated. Then sodium sulphate is added to facilitate phase separation and after a delay the sample is centrifuged to pellet particulate matter at the bottom of the extraction tube. The organic phase is transferred to a clean vial for possible storage. An aliquote of the organic phase is mixed with stable-isotope labelled analogues of the analytes and evaporated to dryness in deactivated glass vials. After reconstitution of the dry extract with organic mobile phase modifier and water, and thorough mixing, the analytes are quantified with a Liquid Chromatography-Mass Spectrometry (LC-MS) system.

4 Reagents

WARNING The method described in this standard implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this standard to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

- 4.1 **Water** (deionized).
- 4.2 **Water** (LC-MS grade, double-distilled or water of grade 1 as defined in EN ISO 3696:1995).
- 4.3 **Methanol** (LC-MS grade).
- 4.4 **Methanol** (p.a.).
- 4.5 **Ethyl acetate** (p.a.).
- 4.6 **Formic acid** (98-100 %, LC-MS grade).
- 4.7 **Acetonitrile** (LC-MS grade).
- 4.8 **Sodium sulfate**, anhydrous, granulated.
- 4.9 **Deoxynivalenol** (DON).
- 4.10 **HT-2 toxin** (HT2).

4.11 T-2 toxin (T2).

4.12 Zearalenone (ZON).

4.13 ¹³C₁₅-Deoxynivalenol (¹³C₁₅-DON).

4.14 ¹³C₂₂-HT-2 toxin (¹³C₂₂-HT2).

4.15 ¹³C₂₄-T-2 toxin (¹³C₂₄-T2).

4.16 ¹³C₁₈-Zearalenone (¹³C₁₈-ZON).

4.17 Multitoxin stock solution:

A mixture containing Deoxynivalenol (4.9), HT-2 toxin (4.10), T-2 toxin (4.11), and Zearalenone (4.12) in neat acetonitrile (4.7) at relevant concentrations.

When preparing this solution the certified purities of the mycotoxin reference materials need to be properly accounted for. In any case the purities shall be $\geq 95\%$.

NOTE 1 3,2 µg/ml DON, 0,5 µg/ml HT-2 toxin, 0,3 µg/ml T-2 toxin, and 0,3 µg/ml ZON in neat acetonitrile have been used during the collaborative study. This solution is stable for three months in the dark at 2–8 °C.

To compare a new stock solution against an old one add 25 µl of each into separate deactivated vials (5.6) and proceed as described in “Test solution” (6.3).

NOTE 2 If 6.4 “Spiking procedure” is executed at least 6 ml of the stock solution are needed.

4.18 Multitoxin working solution:

Dilute Multitoxin stock solution (4.17) with Methanol (4.3) such that the resulting concentration in the working solution is applicable to the calibration range of the different compounds. Only prepare enough volume for one full calibration.

NOTE Adding 188 µl of the Multitoxin stock solution described in 4.17, Note 1 to a 3 ml volumetric flask and making up to the mark with methanol will result in a solution containing 0,2 µg/ml DON, 0,031 µg/ml HT-2 toxin, 0,019 µg/ml T-2 toxin, and 0,019 µg/ml ZON in methanol/acetonitrile (94/6, v/v).

4.19 Multi internal standard (ISTD) stock solution:

A mixture containing ¹³C₁₅-DON (4.13), ¹³C₂₂-HT-2 toxin (4.14), ¹³C₂₄-T-2 toxin (4.15), and ¹³C₁₈-ZON (4.16) in neat acetonitrile (4.7) at the same concentrations as the respective native compounds in the Multitoxin stock solution (4.17).

NOTE This solution is stable for three months in the dark at (2–8) °C.

4.20 Calibration:

To six deactivated glass vials (5.6) add different volumes of the Multitoxin working solution (4.18) such that six equidistant calibration levels across the calibration range result. Proceed as described in 6.3, “Test solution”.

Table 1 below shows example calibration levels using the solution described in the Note to 4.18 above.

Once it has been shown that there is linearity the number of levels may be adjusted to local needs and requirements.