SVENSK STANDARD
SS-EN 16923:2017

Fastställd/Approved: 2017-06-09
Publicerad/Published: 2017-06-20
Utgåva/Edition: 1
Språk/Language: engelska/English
ICS: 67.060; 67.230

SWEDISH STANDARDS INSTITUTE

Livsmedelsanalyser – Bestämning av T-2-toxin och HT-2-toxin i spannmål och spannmålsbaserade livsmedel avsedda för spädbarn och småbarn med LC-MS/MS efter SPE-upprening

Foodstuffs – Determination of T-2 toxin and HT-2 toxin in cereals and cereal products for infants and young children by LC-MS/MS after SPE cleanup

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The European Standard EN 16923:2017 has the status of a Swedish Standard. This document contains the official version of EN 16923:2017.

Upplysningar om sakinnehållet i standarden lämnas av SIS, Swedish Standards Institute, telefon 08-555 520 00. Standarder kan beställas hos SIS Förlag AB som även lämnar allmänna upplysningar om svensk och utländsk standard.

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Denna standard är framtagen av kommittén för Livsmedel och foder, SIS/TK 435.

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 16923

May 2017

ICS 67.060; 67.230

English Version

Foodstuffs - Determination of T-2 toxin and HT-2 toxin in cereals and cereal products for infants and young children by LC-MS/MS after SPE cleanup

Produits alimentaires - Dosage des toxines T-2 et HT-2 dans les céréales et les produits céréaliers pour nourrissons et enfants en bas âge par CL-SM/SM après purification par SPE

Lebensmittel - Bestimmung von T-2-Toxin und HT-2-Toxin in Getreide und Säuglings- und Kleinkindernahrung auf Getreidebasis mit LC-MS/MS nach SPE-Reinigung

This European Standard was approved by CEN on 27 February 2017.

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

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European foreword

This document (EN 16923:2017) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by November 2017, and conflicting national standards shall be withdrawn at the latest by November 2017.

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Introduction

The mycotoxin T-2 toxin and its metabolite HT-2 toxin belong to the group of trichothecenes which are produced by various Fusarium species. Cereals like maize, wheat, barley, oats, and rye are most likely to be affected.

WARNING 1 — Suitable precaution and protection measures need to be taken when carrying out working steps with harmful chemicals. The latest version of the hazardous substances ordinance, Regulation (EC) No 1907/2006 [3], should be taken into account as well as appropriate National statements e.g. such as in [4].

WARNING 2 — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

WARNING 3 — T-2 toxin and its metabolite HT-2 toxin are known to have carcinogenic effects.

1 Scope

This European Standard describes a method for the determination of T-2 toxin and HT-2 toxin in cereals and cereal based products e.g. oats, intended for nutrition of infants and young children by high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS) after cleanup by solid phase extraction (SPE) [5].

The method has been validated for HT-2 toxin in oat flour at levels of 9,3 µg/kg and 28,1 µg/kg, oat flakes at levels of 16,5 µg/kg and 21,4 µg/kg, and breakfast cereals (containing oat flakes) at a level of 8,1 µg/kg and for T-2 toxin in oat flour at levels of 4,4 µg/kg and 8,3 µg/kg, oat flakes at levels of 4,9 µg/kg and 6,6 µg/kg and breakfast cereals (containing oat flakes) at a level of 3,5 µg/kg.

Laboratory experiences [6] have shown that the method is also applicable to highly swelling materials (dry cereal based porridges and modified starches), but these were not examined in the method validation study. Details are outlined in 6.3.

The method can also be applied to oat-by-products at higher levels of T-2- and HT-2 toxin. In this case, the dilution steps need to be considered [6].

The method can also be applied to cereals and cereal products for infants and young children based on e.g. wheat, barley, and rice. In this case, the method needs to be in-house-validated for each material. At the time of the interlaboratory study, planned range was 10 µg/kg to 100 µg/kg, and it is known from the pre-study that the method works well in the whole range, although final validation was only done in the range from 3,5 µg/kg to 28,1 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, *Water for analytical laboratory use - Specification and test methods (ISO 3696)*

3 Principle

T-2 toxin and HT-2 toxin are extracted with acetonitrile-water mixture and by shaking manually or with a laboratory blender. A solid phase extraction column or a pass through column is used to clean up and concentrate the filtered and diluted extract, see also [7]. The toxins are determined by HPLC coupled with tandem mass spectrometry.

4 Reagents

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696, unless otherwise specified. Solvents shall be of quality for HPLC analysis, unless otherwise specified.

4.1 Acetonitrile, HPLC grade.

4.2 Methanol, HPLC grade.

4.3 Solvent mixture.

Mix 20 parts of acetonitrile (4.1) and 80 parts of water (20+80, v+v).

4.4 Extraction mixture.

Mix 84 parts of acetonitrile (4.1) and 16 parts of water (84+16, v+v).

4.5 Eluent for LC-MS/MS.

Examples of eluents suitable for LC-MS/MS systems are given in Annex B. Filter the solution through a membrane filter (5.18).

4.6 Nitrogen, purity of at least 99,9 %.

4.7 Activated charcoal for column chromatography (particle size: 63 µm to 200 µm).

4.8 Aluminium oxide (neutral, for liquid chromatography).

4.9 Finely ground/pulverized diatomaceous earth (diatomite, kieselgur), e.g. Celite® 545.

4.10 Siliconization reagent, e.g. SurfaSil™[1)] (optional).

4.11 Cyclohexane, analytical quality, (optional).

4.12 Preparation of the diluted siliconization reagent, (optional).

Add e.g. 50 ml of a siliconization reagent (4.10) to 950 ml cyclohexane (4.11).

4.13 Formic acid, HPLC quality.

4.14 Ammonia solution, substance concentration $c(NH_3)$ = 13,4 mol/l or mass concentration $\rho(NH_3)$ = 250 g/l.

4.15 Ammonium acetate ($CH_3CO_2NH_4$), LC-MS/MS quality.

4.16 Anti-clogging material, such as washed sea sand, glass beads, or polyethylene beads, (optional).

4.17 Stock solution of T-2 toxin, mass concentration ρ = 100 µg/ml, in acetonitrile.

4.18 Stock solution of HT-2 toxin, ρ = 100 µg/ml, in acetonitrile.

4.19 Internal standard solution of [$^{13}C_{24}$]-T-2 toxin, ρ = 25 µg/ml, in acetonitrile.

Other suitable isotopic labelled standards of T-2 toxin than the [$^{13}C_{24}$]-T-2 toxin may be used.

4.20 Internal standard solution of [$^{13}C_{22}$]-HT-2 toxin, ρ = 25 µg/ml, in acetonitrile.

Other suitable isotopic labelled standards of HT-2 toxin than the [$^{13}C_{22}$]-HT-2 toxin may be used.

4.21 Mixed standard solution, ρ = 500 ng/ml.

Pipette 25 µl of each T-2 toxin and HT-2 toxin stock solution (4.17 and 4.18), respectively, into a 5 ml volumetric flask, and dilute up to the mark with solvent mixture (4.3).

This solution can be stored at −18 °C for 12 months.

1) Surfasil ™ is a trade name of a product commercially available from various suppliers. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the products named. Equivalent products may be used if they can be shown to lead to the same results.

4.22 Mixed internal standard solution, ρ = 1000 ng/ml

Dilute 200 µl of the internal standard solutions (4.19 and 4.20) with solvent mixture (4.3) in a 5 ml volumetric flask.

This solution can be stored at – 18 °C for 6 months.

4.23 Calibration solutions.

For the calibration of the measuring system, prepare calibration solutions within a range from 5 ng/ml to 100 ng/ml.

Prepare e.g. the following calibration solutions as outlined in Table 1:

Table 1 — Examples of suitable calibration solutions

Calibration solution	Mass concentration per analyte ng/ml	Mass concentration per isotope labelled analyte ng/ml	Mixed standard solution (4.21) µl	Mixed internal standard solution (4.22) µl	Solvent mixture (4.3) µl
IS-Blank	0	50	–	50	950
1	5	50	10	50	940
2	10	50	20	50	930
3	20	50	40	50	910
4	40	50	80	50	870
5	60	50	120	50	830
6	80	50	160	50	790
7	100	50	200	50	750

5 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following.

5.1 Laboratory balance, accuracy of 0,01 g.

5.2 Analytical balance, accuracy of 0,1 mg.

5.3 Ultrasonic bath.

5.4 Laboratory shaker for test tubes.

5.5 Manual dispensers, microlitre syringes or microlitre pipettes for 10 µl to 5 ml.

5.6 Dispenser, suitable for 20 ml.

5.7 250 ml-Erlenmeyer flasks with stoppers, or **250 ml-centrifuge tubes.**