SLOVENSKI STANDARD
oSIST prEN 16923:2015

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Živila - Določevanje toksinov T-2 in HT-2 v žitu in žitnih proizvodih za dojenčke in majhne otroke z LC-MS/MS po čiščenju s SPE

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

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

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

Ta slovenski standard je istoveten z: **prEN 16923**

ICS:

67.060	Žita, stročnice in proizvodi iz njih	Cereals, pulses and derived products
67.230	Predpakirana in pripravljena hrana	Prepackaged and prepared foods

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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

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

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

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European foreword

This document (prEN 16923:2015) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

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 — Suitable precaution and protection measures need to be taken when carrying out working steps with harmful chemicals. The hazardous substances ordinance (EU) 1907/2006 [3] should be taken into account as well as appropriate National statements e.g. such as in Bibliographical Reference [4].

WARNING — 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 — 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 the content 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].

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 [6]. The content is 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 Stock solution of T-2 toxin, mass concentration ρ = 100 µg/ml, in acetonitrile.

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

4.3 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.

4.4 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.

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

Pipette 25 µl of each T-2 toxin and HT-2 toxin stock solution (4.1 and 4.2), respectively, into a 5 ml volumetric flask, and dilute up to the mark with injection solution (4.10).

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

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

Dilute 200 µl of the internal standard solutions (4.3 and 4.4) with injection solution (4.10) in a 5 ml volumetric flask.

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

4.7 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 (4.5) µl	Mixed internal standard solution (4.6) µl	Injection solution (4.10) 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

4.8 Acetonitrile, HPLC quality.

4.9 Methanol, HPLC quality.

4.10 Injection mixture.

Mix 20 parts of acetonitrile (4.8) and 80 parts of water (20+80, v+v).

4.11 Extraction mixture.

Mix 84 parts of acetonitrile (4.8) and 16 parts of water (84+16, v+v).

4.12 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 (5.16).

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

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

4.15 Aluminium oxide (neutral, for liquid chromatography).

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

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

4.18 Cyclohexane, analytical quality, (optional).

4.19 Preparation of the siliconization reagent, (optional).

Add e.g. 50 ml Surfasil™ (4.17) to 950 ml cyclohexane (4.18).

4.20 Formic acid, HPLC quality.

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

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

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

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.**

5.8 Syringe filters (0,45 µm), or centrifugal filters, (e.g. Durapore® PVDF (0,45 µm), or Millipore Ultrafree-MC® [2] 0,5 ml), fitting with centrifuge for Eppendorf vessels.

5.9 Folded filter, 595 filters.

5.10 Laboratory centrifuge.

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.

2) Ultra Turrax® and Surfasil ™ are trade names of products 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.

5.11 Cartridges (6 ml), made from polypropylene (PP) and corresponding frits from polyethylene (PE).

5.12 SPE vacuum/elution station.

5.13 Laboratory shaker, e.g. overhead shaker.

5.14 Laboratory blender, e.g. Ultra Turrax®[2)].

5.15 Test tubes, suitable for a volume up to 10,0 ml.

5.16 Siliconized test tubes (optional).

After thorough cleaning of the test tubes (5.15), fill up to the top with the diluted siliconization reagent (4.19) and allow them to stand for 1 min. Then, pouring out the reagent solution, make sure to collect it for repeated usage. Afterwards rinse the tubes with cyclohexane (4.18) and acetonitrile (4.8) or methanol (4.9) successively in this order. The rinsing solutions may be used again. Finally rinse the tubes twice with double-distilled water and allow them to dry.

WARNING — Surfasil™[2)] being a chloride silane solvent, readily reacts with water by forming hydrochloric acid vapour. Therefore never rinse tubes with water directly after derivatization.

Tubes that are not siliconized, such as made from polypropylene, may be used, if formally proved suitable.

5.17 Concentration evaporator workstation, e.g. TurboVap®LV[3)] Zymark, or similar.

5.18 Membrane filters for aqueous solutions (pore size 0,45 µm).

5.19 LC-MS/MS system with the following components:

5.19.1 HPLC pump.

5.19.2 Injection system.

5.19.3 HPLC column, e.g. octadecylsilane (ODS), that ensures base line separation to distinguish peaks of the T-2 toxin and HT-2 toxin from all other signals, 150 mm length, 2,00 mm inner diameter, particle size 5 µm, suitable reversed-phase pre-column.

Columns of different dimensions may also be used.

5.19.4 Column thermostat.

5.19.5 Tandem mass spectrometer (MS/MS).

5.19.6 Data evaluation system.

6 Procedure

6.1 Preparation of the test sample

Use a sample that is ground and homogenized to carry out analysis.

3) TurboVap®LV[3)] Zymark 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.

6.2 Preparation of the solid phase column

Mix 42 g of activated charcoal (4.14) with 30 g of neutral Al_2O_3 (4.15) and 18 g of *Celite 545* (4.16) in a glass vessel (500 ml) and homogenize with a shaker (5.13) for 1 h (ratio 7:5:3 activated charcoal/neutral Al_2O_3/*Celite 545*; m/m/m). Place the homogenized mixture, 0,5 g respectively, in empty 6 ml cartridges provided with three PE frits (2 frits below, and one on top for covering).

Alternatively, commercially available SPE-columns may be used. For this reason, clean up procedure shall be checked for recovery and shall be optimized if necessary.

6.3 Extraction of T-2 toxin and HT-2 toxin

Weigh 25,0 g of the homogenized and finely ground sample (6.1) with an accuracy of 0,1 g into a 250 ml beaker/Erlenmeyer flask, or into a 250 ml centrifuge tube (5.7), add 100 ml of the extraction mixture (4.11) and close the vessel. Shake the mixture with a shaker (5.13) for 1 h at room temperature.

Alternatively, use a laboratory blender (5.14) for extraction. In this case, homogenize the mixture for 3 min at a great speed first.

After extraction, pass sufficiently more than 10 ml extract through a folded filter (5.9) into a glass vessel. Centrifuge this portion at 2500 × *g* and room temperature for 10 min, Remove 10 ml of the upper solution of the centrifugate.

If highly swelling food matrices are analysed, increase the water content in the extraction medium up to 200 % or alternatively reduce the weight of the sample amount down to 50 % of the described amount. To prevent clogging of the swelling material, add the same amount of sea sand (4.23) than the sample weight.

Take volume and/or weight adjustments into account in the final calculation.

6.4 Clean-up by solid phase filtration

Plug the prepared column containing 0,5 g of activated charcoal/*Celite* (6.2) on the SPE station (5.12), and place a test tube (5.15) beneath to collect the eluate. Elute 5,0 ml of the extract (6.3) through the SPE-column and collect the eluate. Apply a low vacuum in order to obtain an elution speed of 1 drop to 2 drops per s. Rinse the cartridge again with 5 ml of extraction mixture (4.11), and collect that eluate also.

Add 25 µl of internal mixed standard solution (4.6) to the combined eluates, and evaporate to dryness with nitrogen (4.13) using a concentration evaporator workstation (at 45 °C for 30 min, and 10 psi gas pressure).

Re-dissolve the residue in 500 µl of injection mixture (4.10) by shaking turbulently (5.4) for 60 s, and, if necessary, apply an ultrasonic bath (5.3) for 5 min at room temperature. The solution should be filtered through syringe filters (5.8) or centrifugal filters (0,45 µm, 0,5 ml) at minimum 10000 *g* in order to discard any micro-particles to give the injection solution.

If necessary, the process of sample purification may be interrupted without analyte loss by storing the sample extracts (evaporated to dryness) at 4 °C for several days, or at −18 °C for two weeks.

6.5 Determination by LC-MS/MS

Depending on the LC-MS/MS system, inject e.g. 10 µl to 25 µl of the injection solution derived from 6.4.

Set up a suitable measuring system by weighing in analytes and optimizing the separation and detection parameters. Annex A lists some example chromatograms, and Annex B lists some suitable parameters.

The adopted measuring systems shall meet with the following requirements as in Table 2: