



SLOVENSKI STANDARD

SIST EN 16923:2023

01-februar-2023

Nadomešča:
SIST EN 16923:2017

Živila - Določevanje toksinov T-2 in HT-2 v žitu in žitnih proizvodih za dojenčke in majhne otroke s HPLC-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 SPE clean up and HPLC-MS/MS

Lebensmittel - Bestimmung von T-2-Toxin und HT-2-Toxin in Getreide und Säuglings- und Kleinkindernahrung auf Getreidebasis mit HPLC-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 SPE et CLHP-SM/SM

Ta slovenski standard je istoveten z: EN 16923:2022

ICS:

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

SIST EN 16923:2023 en,fr,de

EUROPEAN STANDARD

EN 16923

NORME EUROPÉENNE

EUROPÄISCHE NORM

November 2022

ICS 67.060; 67.230

Supersedes EN 16923:2017

English Version

Foodstuffs - Determination of T-2 toxin and HT-2 toxin in cereals and cereal products for infants and young children by SPE clean up and HPLC-MS/MS

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 purification par SPE et CLHP-SM/SM

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

This European Standard was approved by CEN on 9 October 2022.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and United Kingdom.



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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Contents	Page
European foreword.....	3
Introduction	4
1 Scope	5
2 Normative references.....	5
3 Terms and definitions	5
4 Principle	5
5 Reagents	5
6 Apparatus and equipment.....	8
7 Procedure.....	9
7.1 Preparation of the test sample.....	9
7.2 Preparation of the solid phase column	9
7.3 Extraction of T-2 toxin and HT-2 toxin.....	9
7.4 Clean-up by solid phase filtration	10
7.5 LC-MS/MS-analysis	10
7.6 Identification	10
8 Calculation.....	11
9 Precision.....	12
9.1 General.....	12
9.2 Repeatability.....	12
9.3 Reproducibility.....	12
10 Test report.....	13
Annex A (informative) Example chromatograms (API 4000™)	14
Annex B (informative) Example conditions for suitable LC-MS/MS systems	18
B.1 System settings for SCIEX API 4000™ and SCIEX API 4000™ QTrap.....	18
B.1.1 Settings for chromatography	18
B.1.2 Detector parameters.....	18
B.2 System settings for SCIEX API 2000.....	20
B.2.1 Settings for chromatography	20
B.2.2 Detector parameters.....	21
B.3 System settings for SCIEX API 3000™	22
B.3.1 Settings for chromatography	22
B.3.2 Detector parameters.....	22
B.4 System settings for Micromass Quattro LC	23
B.4.1 Settings for chromatography	23
B.4.2 Detector parameters.....	24
Annex C (informative) Precision data.....	25
Bibliography.....	27

European foreword

This document (EN 16923:2022) 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 May 2023, and conflicting national standards shall be withdrawn at the latest by May 2023.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 16923:2017.

In comparison with the previous edition, the following technical modifications have been made:

— the second elution step in the solid phase extraction in 7.4 is more clearly described.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

[SIST EN 16923:2023](https://standards.iteh.ai/catalog/standards/sist/b081bc41-c0dc-41fa-ad29-da004abc04a0/sist-en-16923-2023)

<https://standards.iteh.ai/catalog/standards/sist/b081bc41-c0dc-41fa-ad29-da004abc04a0/sist-en-16923-2023>

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.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

SIST EN 16923:2023

<https://standards.iteh.ai/catalog/standards/sist/b081bc41-c0dc-41fa-ad29-da004abc04a0/sist-en-16923-2023>

1 Scope

This document 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 7.3.

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 are referred to in the text in such a way that some or all of their content constitutes requirements 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 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696)*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp/ui>
- IEC Electropedia: available at <https://www.electropedia.org/>

4 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.

5 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.

EN 16923:2022 (E)

5.1 Acetonitrile, HPLC grade.

5.2 Methanol, HPLC grade.

5.3 Solvent mixture.

Mix 20 parts of acetonitrile (5.1) and 80 parts of water (20+80, v+v).

5.4 Extraction mixture.

Mix 84 parts of acetonitrile (5.1) and 16 parts of water (84+16, v+v).

5.5 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 (6.18).

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

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

5.8 Aluminium oxide (neutral, for liquid chromatography).

5.9 Finely ground/pulverized diatomaceous earth (diatomite, kieselgur), e.g. Celite® 545¹.

5.10 Siliconization reagent, e.g. Surfasil™² (optional).

5.11 Cyclohexane, analytical quality, (optional).

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

Add e.g. 50 ml of a siliconization reagent (5.10) to 950 ml cyclohexane (5.11).

5.13 Formic acid, HPLC quality.

5.14 Ammonia solution, substance concentration $c(\text{NH}_3) = 13,4 \text{ mol/l}$ or mass concentration $\rho(\text{NH}_3) = 250 \text{ g/l}$.

5.15 Ammonium acetate ($\text{CH}_3\text{CO}_2\text{NH}_4$), LC-MS/MS quality.

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

5.17 Stock solution of T-2 toxin, mass concentration $\rho = 100 \text{ µg/ml}$, in acetonitrile.

T-2-toxin, e.g. crystalline or as a film, purity greater than 98 % mass fraction, or as certified standard solution.

5.18 Stock solution of HT-2 toxin, $\rho = 100 \text{ µg/ml}$, in acetonitrile.

¹ Celite® 545 is a trade name of a product commercially available from various suppliers. This information is given for the convenience of users of this document 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 Surfasil™ is a trade name of a product commercially available from various suppliers. This information is given for the convenience of users of this document 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.

HT-2 toxin, e.g. crystalline or as a film, purity greater than 98 % mass fraction, or as certified standard solution.

5.19 Internal standard solution of [$^{13}\text{C}_{24}$]-T-2 toxin, $\rho = 25 \mu\text{g/ml}$, in acetonitrile.

Stock solution of T-2 toxin, mass concentration $\rho = 25 \mu\text{g/ml}$, in acetonitrile, taking into account the certified purity and/or concentration.

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

5.20 Internal standard solution of [$^{13}\text{C}_{22}$]-HT-2 toxin, $\rho = 25 \mu\text{g/ml}$, in acetonitrile.

Stock solution of HT-2 toxin, $\rho = 25 \mu\text{g/ml}$, in acetonitrile, taking into account the certified purity and/or concentration.

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

5.21 Mixed standard solution, $\rho = 500 \text{ ng/ml}$.

Pipette $25 \mu\text{l}$ of each T-2 toxin and HT-2 toxin stock solution (5.17 and 5.18), respectively, into a 5 ml volumetric flask, and dilute up to the mark with solvent mixture (5.3).

This solution can be stored at $-18 \text{ }^\circ\text{C}$ for 12 months.

5.22 Mixed internal standard solution, $\rho = 1000 \text{ ng/ml}$.

Dilute $200 \mu\text{l}$ of internal standard solution of [$^{13}\text{C}_{24}$]-T-2 toxin (5.19) and $200 \mu\text{l}$ of internal standard solution of [$^{13}\text{C}_{22}$]-HT-2 toxin (5.20) with solvent mixture (5.3) in a 5 ml volumetric flask.

This solution can be stored at $-18 \text{ }^\circ\text{C}$ for 6 months.

5.23 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 (5.21) μl	Mixed internal standard solution (5.22) μl	Solvent mixture (5.3) 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

6 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following.

- 6.1 **Laboratory balance**, accuracy of 0,01 g.
- 6.2 **Analytical balance**, accuracy of 0,1 mg.
- 6.3 **Ultrasonic bath**.
- 6.4 **Laboratory shaker for test tubes**.
- 6.5 **Manual dispensers, microlitre syringes or microlitre pipettes** for 10 µl to 5 ml.
- 6.6 **Dispenser**, suitable for 20 ml.
- 6.7 **250 ml-Erlenmeyer flasks with stoppers, or 250 ml-centrifuge tubes**.
- 6.8 **Syringe filters (0,45 µm)**, or centrifugal filters (e.g. Durapore® PVDF (0,45 µm), or Millipore Ultrafree-MC® 0,5 ml), fitting with centrifuge for reaction vessels, e.g. Eppendorf®³ vessels.
- 6.9 **Folded filter**, pore size 4 µm to 7 µm, diameter 100 mm.
- 6.10 **Laboratory centrifuge**.
- 6.11 **Cartridges** (6 ml), made from polypropylene (PP) and corresponding frits from polyethylene (PE), or commercially available SPE columns, e.g. CHROMABOND® Carbon/Alox/Celite®¹, 6 ml, 500 mg.
- 6.12 **SPE vacuum/elution station**.
- 6.13 **Laboratory shaker**, e.g. overhead shaker.
- 6.14 **Laboratory blender**, e.g. Ultra Turrax®³.
- 6.15 **Test tubes**, suitable for a volume up to 10,0 ml.
- 6.16 **Siliconized test tubes** (optional).

After thorough cleaning of the test tubes (6.15), fill up to the top with the diluted siliconization reagent (5.12) 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 (5.11) and acetonitrile (5.1) or methanol (5.2) 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™, 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 those made from polypropylene, may be used, if formally proved suitable.

³ Durapore® PVDF, Millipore Ultrafree-MC®, Ultra Turrax®, TurboVap®LV Zymark and Surfasil™ are trade names of products commercially available from various suppliers. Eppendorf® vessel is an example of a product commercially available from Eppendorf, Chromabond® is the trade name of a product, commercially available from by Macherey-Nagel. This information is given for the convenience of users of this document 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.

6.17 Concentration evaporator workstation, e.g. TurboVap® Zymark³, or similar.

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

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

6.19.1 HPLC pump.

6.19.2 Injection system.

6.19.3 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.

6.19.4 Column thermostat.

6.19.5 Tandem mass spectrometer (MS/MS).

6.19.6 Data evaluation system.

7 Procedure

7.1 Preparation of the test sample

Grind and homogenize the sample to particle sizes less than 1 mm before analysis.

7.2 Preparation of the solid phase column

Mix 42 g of activated charcoal (5.7) with 30 g of neutral Al₂O₃ (5.8) and 18 g of *Celite 545* (5.9) in a glass vessel (500 ml) and homogenize with a shaker (6.13) for 1 h (ratio 7:5:3 activated charcoal/neutral Al₂O₃/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 [7].

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

Weigh 25,0 g of the homogenized and finely ground sample (7.1) with an accuracy of 0,1 g into a 250 ml beaker/Erlenmeyer flask, or into a 250 ml centrifuge tube (6.7), add 100 ml of the extraction mixture (5.4) and close the vessel. Shake the mixture with a shaker (6.13) for approximately 1 h at room temperature.

Alternatively, use a laboratory blender (6.14) for extraction. In this case, homogenize the mixture for 3 min at a great speed.

After extraction, pass slightly more than 10 ml extract through a folded filter (6.9) into a glass vessel. Centrifuge this portion at 2 500 × *g* at 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 e.g. sea sand (5.16) as the sample weight.

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