SLOVENSKI STANDARD
oSIST prEN 17252:2018

01-julij-2018

Živila - Določevanje fomopsina A v semenih volčjega boba in predelanih proizvodih z LC-MS/MS

Foodstuffs - Determination of phomopsin A in lupin seeds and lupin derived products by LC-MS/MS

Lebensmittel - Bestimmung von Phomopsin A in Lupinensamen und Lupinenerzeugnissen mit LC-MS/MS

Produits alimentaires - Détermination de la teneur en phomopsine A dans les graines de lupin et les produits dérivés du lupin par CL-SM/SM

Ta slovenski standard je istoveten z: **prEN 17252**

ICS:

67.060	Žita, stročnice in proizvodi iz njih	Cereals, pulses and derived products

oSIST prEN 17252:2018 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 17252

May 2018

ICS 67.060

English Version

Foodstuffs - Determination of phomopsin A in lupin seeds and lupin derived products by LC-MS/MS

Produits alimentaires - Détermination de la teneur en phomopsine A dans les graines de lupin et les produits dérivés du lupin par CL-SM/SM

Lebensmittel - Bestimmung von Phomopsin A in Lupinensamen und Lupinenerzeugnissen mit LC-MS/MS

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 275.

If this draft becomes a European Standard, 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.

This draft European Standard was established by CEN 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, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Warning : This document is not a European Standard. It is distributed for review and comments. It is subject to change without notice and shall not be referred to as a European Standard.

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

European foreword

This document (prEN 17252:2018) 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

Phomopsins are mycotoxins produced by the fungus *Diaporthe toxica*. There are several phomopsins of which phomopsin A is the major toxic congener. The main host of the fungus are lupins (*Lupinus L.*). Lupin seeds are being used as food ingredient and therefore phomopsin A might occur in food ingredients and food products containing lupin seeds or lupin flour.

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 (EU) 1907/2006, [3] should be taken into account as well as appropriate National statements.

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.

1 Scope

This document describes a procedure for the determination of phomopsins in lupin seeds and lupin-derived products based on liquid chromatography with tandem mass spectrometry (LC-MS/MS). Several phomopsins exist, i.e. phomopsin A, B, C and D, but the method only deals with the quantitative measurement of phomopsin A due to lack of commercially available analytical reference standards for the other phomopsins.

The method has been validated for phomopsin A in naturally contaminated lupin seeds, lupin flour and crisp bread at levels ranging from approximately 5 µg/kg to 60 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:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

4 Principle

The phomopsins are extracted from the homogenized sample material by shaking with a mixture of acetonitrile/water/acetic acid (80+19+1, v+v+v). After centrifugation, an aliquot of the extract is diluted with water, optionally filtered, and analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Phomopsins are quantified by multi-level matrix-matched calibration.

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 LC analysis, unless otherwise specified.

5.1 Water, deionised.

5.2 Water, LC-MS grade.

5.3 Acetonitrile, p.a.

5.4 Methanol, LC-MS grade.

5.5 Acetic acid, purity greater than $w \geq 98$ %.

5.6 Ammonium formate, p.a.

5.7 Extraction solution acetonitrile/water/acetic acid, (80+19+1, v+v+v).

Mix 800 ml of acetonitrile (5.3), 190 ml of water (5.1 or 5.2) and 10 ml of acetic acid (5.4) in a bottle of 1000 ml. This solution is stable for 3 months if stored at room temperature.

5.8 Phomopsin A, isolated from *Phomopsis leptostromiformis.*

5.9 Phomopsin A stock solution (STD 1), mass concentration ρ = 500 mg/l.

Accurately weigh between 5 mg and 6 mg of the phomopsin A standard (5.8) into an amber-coloured glass bottle of 30 ml. Add a volume of methanol (5.4) to produce a solution with a concentration of 500 mg/l. Take into account the weight and the purity of the standard. The solution is stable for 3 months if stored in the refrigerator at 4 °C.

5.10 Standard solution of phomopsin A (STD 2), ρ = 10 mg/l.

Pipette 100 µl of the standard solution (STD 1) (5.9) into a calibrated volumetric flask of 5 ml and make up the volume with methanol (5.4). The solution is stable for 3 months if stored in the refrigerator at 4 °C.

5.11 Standard solution of phomopsin A (STD 3), ρ = 250 µg/l.

Pipette 250 µl of the standard solution (STD 2) (5.10) into a calibrated volumetric flask of 10 ml and make up to the volume with methanol (5.4).

5.12 Intermediate solutions for preparation of the matrix-matched standards.

To seven glass vials (6.9) add different volumes of the standard solution of phomopsin A (5.11) and methanol (5.4) according to Table 1. Close with screw cap and mix. Prepare these solutions freshly for each batch of analysis.

Table 1 — Intermediate standard solutions of phomopsin A in methanol

Intermediate solution	Standard solution STD 3 (5.11)	Methanol	Mass concentration
no	µl	µl	µg/l
1	25	975	6,25
2	50	950	12,5
3	100	900	25
4	200	800	50
5	350	650	87,5
6	500	500	125
7	650	350	162,5

5.13 Matrix matched calibration solutions

Prepare matrix-matched calibration solutions in vials (6.9) according to Table 2.

The matrix matched calibration solutions may also be prepared directly in auto sampler vials with insert or filter vials. In that case, proportionally reduce the volumes indicated in Table 2.

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