Nederlandse

Ontwerp

norm

NEN-EN 17252

Voedingsmiddelen - Bepaling van phomopsine A in lupinezaden en van lupine afgeleide producten met LC-MS/MS

Publicatie uitsluitend voor commentaar

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

mei 2018
ICS 67.060

Commentaar vóór 2018-06-26

Als Europees normontwerp is gepubliceerd: prEN 17252:2018,IDT

Definitief vastgestelde normen zullen als Nederlandse norm gelden. Daarom wordt dit normontwerp in Nederland voor commentaar gepubliceerd. Op het ontwerp ingebracht commentaar zal aan de bevoegde normcommissie worden voorgelegd die hiermee rekening zal houden bij de bepaling van de Nederlandse stem. Indien er geen bezwaar bij NEN wordt gebracht, kan dat leiden tot ongewijzigde definitieve vaststelling van het ontwerp als norm.

Van Europese normen bestaan drie officiële versies: Engels, Frans en Duits. Voor Nederland zal de Engelse versie gelden. Daarnaast kan er gekozen worden voor een andere geautoriseerde versie in het Nederlands.

Normcommissie 370275 'Voedingsmiddelenanalyse - Horizontale methoden'

©2018 Koninklijk Nederlands Normalisatie-instituut
Postbus 5059, 2600 GB Delft
Telefoon (015) 2 690 390, Fax (015) 2 690 190

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

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

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

Ref. No. prEN 17252:2018 E

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.

Table 2 — Matrix matched calibration solutions of phomopsin A in blank matrix extract

Calibration solution	Mass concentration	Blank extract	Phomopsin A intermediate solutions (5.12) see Table 1	Water (5.2)	Equivalent to mass fraction in sample
no	µg/l	µl	µl	µl	µg/kg
0	0	500	0	500	0
1	0,3125	450	50 µl no 1	500	2,5
2	0,625	450	50 µl no 2	500	5,0
3	1,25	450	50 µl no 3	500	10,0
4	2,5	450	50 µl no 4	500	20,0
5	4,375	450	50 µl no 5	500	35,0
6	6,25	450	50 µl no 6	500	50,0
7	8,125	450	50 µl no 7	500	65,0

6 Apparatus and equipment

Usual laboratory glassware and equipment, in particular, the following:

6.1 Conical polypropylene screw cap centrifuge tubes, 50 ml with caps.

6.2 Volumetric flasks, 5 ml and 10 ml.

6.3 Analytical balance, accuracy 0,1 mg.

6.4 Laboratory balance, accuracy 0,01 g.

6.5 Pipettes, e.g. 10 µl to 1000 µl, for organic solvents.

6.6 Adjustable mechanical vertical or horizontal shaker or rotary tumbling machine.

6.7 Laboratory shaker.

6.8 Centrifuge, capable of generating a relative centrifugal force of 3 500 *g*.

6.9 Vials, 1,5 ml to 2 ml, used for intermediate solutions (5.12), made of glass or polypropylene, with screw cap.

6.10 Syringe filter, 0,20 µm to 0,45 µm, nylon or PTFE (for optional filtration of final extracts).

6.11 Auto sampler vials, of appropriate size for the auto sampler in use, e.g. glass with insert vials, or filter vials (polytetrafluoroethylene (PTFE) 0,45 µm), with crimp cap or equivalent.

6.12 LC-MS/MS system, with the following components:

6.12.1 LC pump, capable of delivering a binary gradient at flow rates appropriate for the analytical column in use with sufficient accuracy.

NEN
CONNECT

ALTIJD DE ACTUELE NORM IN UW BEZIT HEBBEN?

Nooit meer zoeken in de systemen en uzelf de vraag stellen: ‘Is NEN-EN 17252:2018 Ontw. en de laatste versie?’

Via het digitale platform NEN Connect heeft u altijd toegang tot de meest actuele versie van deze norm. Vervallen versies blijven ook beschikbaar. **U en uw collega’s** kunnen de norm via NEN Connect makkelijk raadplagen, online en offline.

Kies voor slimmer werken en bekijk onze mogelijkheden op **www.nenconnect.nl**.

Heeft u vragen?

Onze Klantenservice is bereikbaar maandag tot en met vrijdag, van 8.30 tot 17.00 uur.

Telefoon: 015 2 690 391
E-mail: klantenservice@nen.nl