SVENSK STANDARD
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Livsmedel – Bestämning av domorinsyra i råa musslor och skaldjur samt kokta musslor med RP-HPLC och användande av UV-detektion

Foodstuffs – Determination of domoic acid in raw shellfish, raw finfish and cooked mussels by RP-HPLC using UV detection

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Europastandarden EN 14176:2017 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN 14176:2017.

Denna standard ersätter SS-EN 14176:2004, utgåva 1.

The European Standard EN 14176:2017 has the status of a Swedish Standard. This document contains the official English version of EN 14176:2017.

This standard supersedes the Swedish Standard SS-EN 14176:2004, edition 1.

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.

Information about the content of the standard is available from the Swedish Standards Institute (SIS), telephone +46 8 555 520 00. Standards may be ordered from SIS Förlag AB, who can also provide general information about Swedish and foreign standards.

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

Har du synpunkter på innehållet i den här standarden, vill du delta i ett kommande revideringsarbete eller vara med och ta fram andra standarder inom området? Gå in på www.sis.se - där hittar du mer information.

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 14176

January 2017

ICS 67.120.30

Supersedes EN 14176:2003

English Version

Foodstuffs - Determination of domoic acid in raw shellfish, raw finfish and cooked mussels by RP-HPLC using UV detection

Produits alimentaires - Dosage de l'acide domoïque dans les coquillages crus, les poissons crus et les moules cuites par CLHP en phase inverse couplée à la détection UV

Lebensmittel - Bestimmung von Domoinsäure in rohen Schalentieren, rohen Fischen und gekochten Miesmuscheln mit RP-HPLC und UV-Detektion

This European Standard was approved by CEN on 7 November 2016.

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, 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.

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

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN 14176:2017 E

Contents

Page

European foreword

This document (EN 14176:2017) 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 July 2017, and conflicting national standards shall be withdrawn at the latest by July 2017.

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 14176:2003.

EN 14176:2017 includes the following significant technical changes with respect to EN 14176:2003:

— the extraction procedure in 6.2 has been revised;

— the chromatographic conditions in 6.3 have been revised;

— the method has been re-validated, and the validation data in Annex A have been revised.

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, 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 the United Kingdom.

Introduction

The amnesic shellfish poisoning (ASP) toxin, domoic acid (DA), belongs to a group of amino acids, called the kainoids, which are classed as neuroexcitants or excitoxins that interfere with the neurotransmission mechanisms in the brain. The toxin can be accumulated in shellfish feeding on a number of toxic *Pseudonitzschia* species. Ingestion of seafood contaminated with DA can lead to an intoxication which symptoms include (among others) abdominal cramps, vomiting, disorientation and memory loss (amnesia) and can become severe in certain cases.

High performance liquid chromatography with ultraviolet detection (HPLC-UV) was the first chemical analytical method for DA and is still the most commonly used for monitoring shellfish. DA detection is possible by its strong absorbance at 242 nm [1].

This European Standard is based on two, comparable procedures. One procedure for the quantitative determination of DA and its isomers e.g. epi-domoic acid (epi-DA) in unsalted raw seafood (Method A) is described in [2]. The other procedure for the quantitative determination of DA and its isomers e.g. epi-DA in cooked mussel (Method B) is described in [3].

Method A uses a single-step extraction with 50 % aqueous methanol and an optional selective clean-up and concentration step with strong anion exchange solid phase extraction (SPE). Taking into account results of the validation procedure, the optional clean-up step of Method A as published under [2] is not described in this standard. Analytes are determined by high performance liquid chromatography (HPLC) under isocratic conditions with ultraviolet absorbance detection.

Method B uses a single-step extraction with 50 % aqueous methanol and an optional heating step which allows a better decanting of the supernatant. However, it has been observed that heating can degrade DA and epi-DA. DA and epi-DA are determined by HPLC with binary gradient and ultraviolet absorbance detection.

Both methods can be applied for the quantitative determination of DA.

WARNING — The use of this standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to take appropriate measures to ensure the safety and health of personnel prior to application of the standard, and fulfil statutory and regulatory requirements for this purpose.

1 Scope

This European Standard specifies methods for the quantitative determination of domoic acid in raw bivalve molluscs and finfish as well as in cooked mussels. The limit of detection is about 10 ng/ml to 80 ng/ml (0,05 mg/kg to 0,4 mg/kg), depending on the UV detector sensitivity. Method A has been validated for the determination of DA in different raw matrices such as mussels, clams, scallops and anchovies, spiked and/or naturally contaminated at levels ranging from 2,7 mg/kg to 85,1 mg/kg. Method B has been validated for the determination of DA at levels ranging from 5 mg/kg to 12,9 mg/kg in cooked blue mussels.

For further information on validation data, see Clause 8 and Annex A.

Laboratory experience has shown that this standard can also be applied to other shellfish species, however, no complete validation study according to ISO 5725 has been carried out so far.

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

DA and epi-DA are extracted from sample tissue with a mixture of methanol and water. The extract is filtered through a membrane filter and measured using HPLC equipment with isocratic (Method A) or gradient (Method B) elution and detection by UV absorption. The amount of DA is calculated by the method of external standard calibration.

WARNING — ASP toxins are neurotoxins which can be taken up by inhalation or orally. Therefore, adequate protection measures are to be applied.

4 Reagents

During the analysis, unless otherwise stated, use only water according to grade 1 of EN ISO 3696.

If not otherwise indicated, all chemicals shall be of pro analysis (p. a.) quality.

Reference materials (certified, if available) and standard substances originating from other sources as indicated may also be used if well-characterized and with a well-defined mass concentration.

If not already specified, stability of solutions should be determined by the laboratory.

4.1 **Methanol**, HPLC quality

4.2 **Acetonitrile**, HPLC quality

4.3 **Extraction solvent,** methanol/water 50:50 v/v

4.4 **Acetonitrile/water**, 10:90 v/v (Method A)

4.5 **Trifluoroacetic acid (TFA),** spectrophotometric grade ≥ 99 % (Method A)

4.6 **Formic acid,** mass concentration ≥ 98 % (Method B)

4.7 Eluents

4.7.1 Eluent 1 (isocratic conditions)

Aqueous 10 % v/v acetonitrile (4.4) with 0,1 % v/v TFA (4.5). For single pump systems, mix 100 ml acetonitrile with approximately 400 ml water, add 1,0 ml TFA, and dilute to 1 l with water.

4.7.2 Eluent 2 (gradient conditions)

Mix 100 ml acetonitrile (4.2) with 900 ml water and adjust pH to 2,5 using formic acid (4.6).

4.7.3 Eluent 3 (gradient conditions)

Mix 300 ml acetonitrile (4.2) with 700 ml water and adjust pH to 2,5 using formic acid (4.6).

4.8 Standard substances

4.8.1 Domoic acid as certified calibration solution[1)]

Sealed ampoules should be stored in the dark in a refrigerator (at approximately +4 °C). Do not freeze the solution. Prior to opening, each ampoule should be at room temperature. Once the ampoule has been opened, accurate aliquots should be removed using calibrated volumetric equipment and transferred to other amber glass vial for dilution and/or analysis as soon as possible. Closed vials should be stored in the dark in a refrigerator (at approximately +4 °C) for no more than 3 months.

NOTE Epi-DA is contained as a minor component in the certified calibration solution from the Institute for Marine Biosciences, National Research Council of Canada, Halifax, Nova Scotia-Canada[1)]

4.8.2 **Domoic acid, as crystalline powder,** purity of > 95 %

4.9 Standard solutions

4.9.1 General

Either use commercially available certified calibration solutions (4.8.1) or prepare calibration solutions by dissolving crystalline DA powder (4.8.2) and subsequently dilute. Both procedures have been proven to lead to successful validation data.

4.9.2 Stock solution

Weigh (5.1) DA crystalline powder (4.8.2) into a volumetric flask and dissolve in methanol to a final concentration of 500 µg/ml. Closed vials should be stored in the dark in a refrigerator (at approximately +4 °C).

4.9.3 Standard solution

Dilute the stock solution (4.9.2) with methanol to a final concentration of 50 µg/ml. Check the mass concentration of this solution by comparing with certified calibration solutions (4.8.1).

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1) Information on suitable calibration solutions are e. g. available on http://aesan.msssi.gob.es/en/CRLMB/web/home.shtml and http://aesan.msssi.gob.es/en/CRLMB/web/estandares_materiales_referencia/materiales_referencia.shtml. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of the products referred to in the websites or available from Canada. Equivalent products may be used if they can be shown to lead to the same results.

4.9.4 Calibration solutions

Prepare calibration solutions of appropriate mass concentrations of DA and epi-DA, either by diluting the standard solution (4.9.3) or by diluting the certified calibration solution (4.8.1).

For the validation of Method A with isocratic elution, calibration solutions were prepared in the range of 0,2 µg/ml to 25 µg/ml by diluting the certified calibration solution (4.8.1) with acetonitrile:water, 10:90 v/v (4.4).

For the validation of Method B with gradient elution, calibration solutions were prepared in the range of 0,5 µg/ml to 10 µg/ml by diluting the standard solution (4.9.3) with a methanol/water solution (4.3).

Keep solutions in the dark and refrigerated (at approximately + 4 °C) when not in use. Do not store them for more than 3 months. Do not freeze the solutions. Warm up solutions to room temperature before use.

4.10 Reference material[2)]

Mussel tissue reference material should be stored according to the manufacturers specifications. Each bottle should be allowed to warm to room temperature prior to opening and the contents thoroughly mixed by vortexing for a minimum of 2 min. When a bottle is opened the entire contents should be used immediately. The reference material can be used to test the accuracy of an existing analytical procedure. Extraction of the reference material should be performed according to the procedure described in 6.2.1 or 6.2.2.

5 Apparatus

Use usual laboratory apparatus and, in particular, the following:

5.1 Analytical balance, capable of weighing to the nearest 0,1 mg

5.2 Balance, capable of weighing to the nearest 0,01 g

5.3 Homogenizer (e.g. grinding or blending machine)

5.4 Mechanical mixer, high speed at 8 000 min^{-1} to 45 000 min^{-1} (e.g. Ultra turrax)

5.5 Centrifuge, capable of effectively separating the liquid and solid phase, e.g. 3 000 g [3)] (refrigerated at + 4 °C, if possible)

5.6 Centrifuge tubes, nominal volume 30 ml to 50 ml, with screw caps

5.7 Membrane filter, methanol compatible with a pore size of 0,2 µm or 0,45 µm, e.g. surfactant-free cellulose acetate with glass fibre pre-filter

5.8 Adjustable automatic pipettes, covering the range from 20 µl to 1000 µl

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2) Suitable reference material is e. g. available from the Institute for Marine Biosciences, National Research Council of Canada, Halifax, Nova Scotia-Canada. Epi-DA is contained as a minor component in this certified reference material. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

3) $g = 9{,}81\ m \cdot s^{-2}$.