NSAI
Standards

Irish Standard
I.S. EN 14176:2017

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

I.S. EN 14176:2017

Incorporating amendments/corrigenda/National Annexes issued since publication:

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Údarás um Chaighdeáin Náisiúnta na hÉireann

National Foreword

I.S. EN 14176:2017 is the adopted Irish version of the European Document EN 14176:2017, Foodstuffs - Determination of domoic acid in raw shellfish, raw finfish and cooked mussels by RP-HPLC using UV detection

This document does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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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.

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COMITÉ EUROPÉEN DE NORMALISATION
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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)