Irish Standard
I.S. EN 16204:2012

Foodstuffs - Determination of lipophilic algal toxins (okadaic acid group toxins, yessotoxins, azaspiracids, pectenotoxins) in shellfish and shellfish products by LC-MS/MS

I.S. EN 16204:2012

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English Version

Foodstuffs - Determination of lipophilic algal toxins (okadaic acid group toxins, yessotoxins, azaspiracids, pectenotoxins) in shellfish and shellfish products by LC-MS/MS

Produits alimentaires - Dosage des toxines algales lipophiles (toxines du groupe acide okadaïque, yessotoxines, azaspiracides, pecténotoxines) dans les coquillages et les produits à base de coquillages par CL-SM/SM

Lebensmittel - Bestimmung der lipophilen Algentoxine (Okadasäuregruppen-Toxine, Yessotoxine, Azaspirosäuren, Pectenotoxine) in Schalentieren und Schalentiererzeugnissen mit LC-MS/MS

This European Standard was approved by CEN on 20 April 2012.

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

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Foreword

This document (EN 16204:2012) 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of an identical text or by endorsement, at the latest by November 2012, and conflicting national standards shall be withdrawn at the latest by November 2012.

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

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Introduction

Lipophilic marine biotoxins are the most frequently occurring algal toxins in Europe and are produced by certain marine dinoflagellates. They can accumulate in filter-feeding bivalves reaching highly toxic levels and, after consumption, may cause harm in humans such as nausea, vomiting and diarrhoea.

Commission Regulation 15/2011 stipulates LC-MS/MS as the reference methodology and refers to a method validated by the EU-RL Network. The method presented in EN 16204 is proposed as an alternative method to the one validated by EU-RL.

1 Scope

This European Standard specifies a multi-reference method for the determination of lipophilic algal toxins (fat-soluble algal toxins produced by some dinoflagellates) in raw shellfish and shellfish products including cooked shellfish, by liquid chromatography coupled to tandem mass spectrometry LC-MS/MS [1], [2], [3]. This method has been validated in an inter-laboratory study consisting of three parts via the analysis of both naturally contaminated homogenates of blue mussel and spiked extracts of blue mussel, oyster and clam. For further information on the validation, see Annex A. Additional studies have investigated further matrices (see [4], [5]).

The detection limit for toxins of the okadaic acid group, azaspiracids and pectenotoxins was determined to be 6 µg/kg shellfish meat and for yessotoxins 10 µg/kg shellfish meat.

Quantitative determination of okadaic acid (OA), pectenotoxin-2 (PTX-2), azaspiracid-1 (AZA-1) and yessotoxin (YTX) can be carried out directly by means of standard substances available commercially. Assuming an equal response factor, okadaic acid is used for the indirect quantitative determination of the two dinophysistoxins dinophysistoxin-1 (DTX-1) and dinophysistoxin-2 (DTX-2); likewise azaspiracid-1 (AZA-1) is used for the indirect quantitative determination of azaspiracid-2 (AZA-2) and azaspiracid-3 (AZA-3), while YTX is used for homo-yessotoxin, 45-OH-yessotoxin and 45-OH-homo-yessotoxin, and PTX-2 for pectenotoxin-1 (PTX-1).

The limit of quantification (LOQ) for toxins of the okadaic acid group, azaspiracids and pectenotoxins was determined to be 20 µg/kg shellfish meat and for yessotoxins 35 µg/kg shellfish meat.

By means of hydrolysis [6], the esters of okadaic acid, DTX-1 and DTX-2 can also be determined quantitatively as the corresponding free acids.

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 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

3 Principle

Remove the shellfish meat from the shell and homogenize the total shellfish meat. Extraction is carried out with aqueous methanol (φ = 80 %). Separation is performed on a HPLC reverse-phase column provided with a binary gradient and detection is carried out by means of tandem mass spectrometry using triple quadrupole technology. The concentration of lipophilic toxins is determined by means of external calibration.

4 Reagents

If not otherwise specified, reagents of analytical grade and solvents suitable for LC-MS/MS shall be used. Water shall be distilled in glass vessels or demineralised before use, or shall be of equivalent purity according to EN ISO 3696:1995. Since the use of this method involves reagents harmful to health, appropriate precautionary and protective measures such as avoiding skin contact and using an extractor hood shall be taken.

4.1 Aqueous methanol (φ= 80 %).

NOTE The validation data of this method have been elaborated with 80 % aqueous methanol. However, it has been shown (see [4], [5]) that equivalent results can be obtained when using 100 % methanol.

4.2 Acetonitrile