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**Foodstuffs – Determination of domoic acid in  
mussels by HPLC**

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EUROPEAN STANDARD  
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EUROPÄISCHE NORM

**EN 14176**

December 2003

ICS 67.120.30

English version

## Foodstuffs - Determination of domoic acid in mussels by HPLC

Produits alimentaires - Dosage de l'acide domoïque dans  
les moules par CLPH

Lebensmittel - Bestimmung von Domoinsäure in Muscheln  
mit HPLC

This European Standard was approved by CEN on 1 October 2003.

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## EN 14176:2003 (E)

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## Foreword

This document (EN 14176:2003) 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of an identical text or by endorsement, at the latest by June 2004, and conflicting national standards shall be withdrawn at the latest by June 2004.

Annex A in this document is informative.

**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 establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.**

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

## EN 14176:2003 (E)

### 1 Scope

This European Standard specifies a method for the determination of domoic acid in mussels using high performance liquid chromatography (HPLC).

The method has been successfully validated in an interlaboratory study according to AOAC guidelines on mussels containing 14,1 µg/g (spiked sample) to 186 µg/g (naturally contaminated sample) domoic acid.

Laboratory experiences show that the method is also applicable on the common cockle (*Cerastoderma edule*), the peppery furrow shell (*Scrobicularia plana*), clams (*Venerupis pullastra*, *Ruditapes decussata*), oyster (*Crassostrea japonica*) and razor clams (*Ensis spp.*, *Solen spp.*) [1].

### 2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*.

### 3 Principle

Domoic acid is extracted by boiling homogenized mussel tissue with a solution of hydrochloric acid. The mixture is cooled, diluted to a known volume and then centrifuged. The supernate is diluted, filtered, and analysed by reverse-phase HPLC with ultraviolet detection.

### 4 Reagents

#### 4.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 as defined in EN ISO 3696. Solvent shall be of quality for HPLC analysis.

**4.2 Hydrochloric acid solution**, substance concentration  $c(\text{HCl}) = 0,1 \text{ mol/l}$

**4.3 Phosphoric acid solution**, mass fraction  $w(\text{H}_3\text{PO}_4) = 8,5 \%$

#### 4.4 Acetonitrile

#### 4.5 Mobile phase

Mix 2 volume parts of phosphoric acid (4.3) with 873 volume parts of water. Check to ensure that  $\text{pH} \approx 2,5$ . Add 125 ml acetonitrile (4.4), mix and degas this solution before use. Perform a preliminary analysis of the domoic acid standard solution (4.6) and adjust the acetonitrile concentration as necessary to give a domoic acid retention time of approximately 8 min under method conditions.

#### 4.6 Domoic acid standard solution

Prepare a standard solution at a precisely known concentration in the region of 1 µg/ml. If necessary dilute with water. Refrigerate the solution when not in use. Warm the solution to room temperature before use.