SLOVENSKI STANDARD
SIST EN 14176:2004

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Živila - Določevanje domojske kisline v školjkah s HPLC

Foodstuffs - Determination of domoic acid in mussels by HPLC

Lebensmittel - Bestimmung von Domoinsäure in Muscheln mit HPLC

Produits alimentaires - Dosage de l'acide domoique dans les moules par CLHP

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Foodstuffs - Determination of domoic acid in mussels by HPLC

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Lebensmittel - Bestimmung von Domoinsäure in Muscheln mit HPLC

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

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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 Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Management Centre: rue de Stassart, 36 B-1050 Brussels

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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of an identical text or by endorsement, at the latest by June 2004, and conflicting national standards shall be withdrawn at the latest by June 2004.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.

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 $c(HCl) = 0,1$ mol/l

4.3 Phosphoric acid solution, mass fraction $w(H_3PO_4) = 8,5$ %

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 pH ≈ 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.

5 Apparatus

5.1 General

Usual laboratory equipment and, in particular, the following:

5.2 Blender or household-type grinder

5.3 Membrane filter, of pore size of approximately 0,45 µm, attached to a 5 ml glass or disposable plastic syringe.

5.4 Centrifuge, high speed, with timer, capable of 700 *g* using 50 ml to 100 ml centrifuge tubes.

5.5 Centrifuge tubes, of a capacity from 50 ml to 100 ml.

5.6 HPLC apparatus, comprising the following:

5.6.1 High performance liquid chromatograph, pump able to deliver up to 1,5 ml/min constant flow rate, equipped with an injection system with a volume of e.g. 20 µl.

5.6.2 Analytical reverse-phase separating column, for example octyldecylsilane, which ensures a baseline resolved resolution of the domoic acid peak from all other peaks.

A column with the following characteristics shows satisfying results:

— stainless steel (or other suitable material);

— a length of 150 mm;

— an inner diameter of 4,6 mm;

— a stationary phase with particle size of 5 µm.

NOTE The type of reverse-phase column used does not significantly affect the results if the acetonitrile concentration in the mobile phase is adjusted so that domoic acid elutes in a reasonable time (approximately 8 min) and away from interfering materials in the early part of the chromatogram.

5.6.3 Ultra violet detector, set at 242 nm and fitted with a flow cell.

5.6.4 Data system

6 Sampling

It is important that the laboratory receives a sample which is truly representative and has not been damaged or changed during transport or storage.

7 Procedure

7.1 General

The whole analytical procedure shall be performed in one working day. Refrigerate the samples and extracts at 4 °C to 7 °C when they are not being handled.

IMPORTANT — Domoic acid in the sample extract is instable. Care shall be taken that the requirements of this standard concerning temperature, acidity and time dependent actions are strictly followed.

NOTE Domoic acid in acidic extracts slowly decomposes if left at room temperature (30 % to 50 % decrease in domoic acid content when extracts are kept at room temperature for four to five days). When extracts have been refrigerated at 4 °C, there is no significant change after three weeks [2].

7.2 Preparation of the test sample

Thoroughly clean the outside of the mussels with fresh water. Slightly move both shells across each other using the fingers of one hand so that a narrow opening occurs between both shells. Enter this narrow opening with a small sharp knife until the point of the knife reaches the inside wall of one shell. Cut the adductor muscle by moving the knife through the mussel while the point of the knife always keeps into contact with the inside wall of one shell. Lift one shell. Rinse the inside with fresh water to remove sand or other foreign material. Cut the mussel out of the other shell using the knife.

Do not use heat or anaesthetics before opening the shell, and do not cut or damage the body of the mussel at this stage. Deposit 100 g to 150 g mussel tissue over a sieve for 5 min. Pick out the pieces of the shell and discard the drainings. Grind the mussel tissue in a blender or grinder (5.2) until it is homogeneous.

7.3 Extraction and clean up

Weigh approximately 50 g homogenate to the nearest 0,1 g in a clean 250 ml beaker. Add 50 ml of hydrochloric acid (4.2) and stir thoroughly. Quickly heat the mixture to boiling (within 10 min) and continue gentle boiling, with stirring for exactly 5 min. Immediately transfer the hot beaker and the contents on an ice bath and let it cool to room temperature in approximately 10 min. Transfer the cooled mixture to a 100 ml graduated cylinder with stopper or a volumetric flask and dilute to 100 ml with hydrochloric acid (4.2). Place the glass stopper and shake to homogeneity. Transfer a portion of at least 50 ml homogeneous mixture to the centrifuge tube (5.5). Centrifuge 5 min ± 5 s at 700 g. Use a volumetric pipette to transfer 5,0 ml clear solution to a 50 ml volumetric flask. Dilute to the mark with water and mix well. Filter 1 ml to 2 ml of diluted solution through the filter (5.3) and collect the filtrate in a suitable vial for HPLC determination. When the domoic acid content is too high for quantification dilute the final solution with water until it is possible to quantify the domoic acid concentration.

NOTE 1 Increased boiling time leads to decreased recovery (by 7 % after 10 min heating relative to 5 min heating, see [2]).

NOTE 2 Room temperature cooling and storage leads to decreased recovery. Recoveries are significantly improved if the hot acid extracts are cooled quickly in a refrigerator, freezer, or ice bath, and if sample solutions are always refrigerated when not being analyzed [2].

7.4 Determination

Inject replicate portions of domoic acid standard solution (4.6), e.g. 20 µl onto the HPLC column until the peaks, measured as height or area, for three consecutive injections do not vary by more than 3 %. Make alternate, duplicate injections of test samples and standards.

7.5 Identification

The domoic acid peak can be identified by injecting a test sample solution spiked with a standard solution.

8 Calculation

Calculate the mass fraction of domoic acid, w, in micrograms per gram, present in the sample using equation (1):

$$w = \frac{P_t \times m_{st}}{P_s \times m_s} \quad (1)$$

where:

P_t is the domoic acid peak of the test sample, in units of height or area;

P_s is the domoic acid peak of the standard solution, in units of height or area;

m_{st} is the mass of domoic acid standard in nanograms, injected onto the HPLC column;

m_s is the mass of the test sample in milligrams, injected onto the HPLC column.

9 Precision

9.1 General

Details of the interlaboratory test on the precision of the method are summarised in annex A . The values derived from the interlaboratory tests may not be applicable to analyte concentration ranges and matrices other than given in annex A.

9.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible interval will exceed the repeatability limit r in not more than 5 % of the cases.

The values are:

$\bar{x}$ = 10,1 µg/g	r = - µg/g
$\bar{x}$ = 13,3 µg/g	r = - µg/g
$\bar{x}$ = 75,3 µg/g	r = 10 µg/g
$\bar{x}$ = 186 µg/g	r = 9,8 µg/g

9.3 Reproducibility

The absolute differences between two single test results found on identical test material reported by two laboratories will exceed the reproducibility limit R in not more than 5 % of the cases.

The values are:

$\bar{x}$ = 10,1 µg/g	R = 5,5 µg/g
$\bar{x}$ = 13,3 µg/g	R = 5,1 µg/g
$\bar{x}$ = 75,3 µg/g	R = 16 µg/g
$\bar{x}$ = 186 µg/g	R = 67 µg/g

10 Test report

The test report shall contain the following data:

— all information necessary for the identification of the sample (kind of sample, origin of sample, designation);