SLOVENSKI STANDARD
SIST EN 14524:2005

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Foodstuffs - Determination of okadaic acid in mussels - HPLC method with solid phase extraction clean-up, derivatization and fluorimetric detection

Lebensmittel - Bestimmung von Okadasäure in Muscheln - HPLC-Verfahren mit Reinigung durch Festphasenextraktion, Derivatisierung und fluorimetrischer Bestimmung

Produits alimentaires - Dosage de l'acide okadaique dans les moules - Méthode par CLHP avec purification par extraction sur phase solide, dérivation et détection fluorimétrique

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ICS:

67.120.30	Ribe in ribji proizvodi	Fish and fishery products

SIST EN 14524:2005 **en**

2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

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EUROPEAN STANDARD

NORME EUROPÉENNE

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Foodstuffs - Determination of okadaic acid in mussels - HPLC method with solid phase extraction clean-up, derivatization and fluorimetric detection

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This European Standard was approved by CEN on 21 May 2004.

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Foreword

This document (EN 14524:2004) 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of an identical text or by endorsement, at the latest by February 2005, and conflicting national standards shall be withdrawn at the latest by February 2005.

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

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.

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1 Scope

This document specifies a method for the quantitative determination of the content of okadaic acid in mussels and mussel products. The content of okadaic acid is determined as free extractable acid of mussel hepatopancreas. Okadaic acid, a fat-soluble toxin from dinophysis algae, is a main component of dinophysis toxins.

The method has been validated in an interlaboratory study according to ISO general principles on assessing accuracy of measurement methods and results. The limit of determination of this method (signal/noise = 10) is 100 µg/kg for okadaic acid in mussel hepatopancreas. The method has been validated for okadaic acid in cooked mussels at levels of 441 µg/kg to 1 467 µg/kg.

Laboratory experiences have shown that this method can also be used to determine other dinophysis toxins, e.g. dinophysis toxins 1, 2 and 3 (DTX-1, DTX-2 and DTX-3), see [1], [2], [3], [4], [5] and [6]

2 Normative references

The following referenced documents are indispensable for the application of this document. 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 3696:1987).*

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3 Principle

Mussel hepatopancreas is separated from cooked mussels and homogenized. The toxins are extracted using methanol, derivatized with 9-anthryldiazomethane and the extract is cleaned up using a solid phase extraction (SPE) cartridge with silica gel. Chromatographic separation is performed on a HPLC-gradient system, followed by fluorescence measurement of the 9-anthryldiazomethyl ester of the toxin at 412 nm with excitation at 365 nm. Determination of okadaic acid is performed using external standards.

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4 Reagents and materials

4.1 General

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

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4.2 Sodium sulfate, anhydrous

4.3 Nitrogen gas, volume fraction $\varphi \geq 99{,}999$ %

4.4 Acetone

4.5 Acetonitrile

4.6 Chloroform, stabilised with 2-methyl-2-butene

4.7 Ethyl acetate

4.8 Dichloromethane, stabilised with 2-methyl-2-butene

4.9 Methanol

4.10 Methanol solution, $\varphi = 80$ %

4.11 n-hexane

4.12 9-anthryldiazomethane (ADAM)

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Weigh solid ADAM in discrete portions (e.g. 1 mg), store at ≤ -18 °C and dissolve in ethyl acetate just before use.

4.13 Derivatization solution, mass concentration $\rho = 1{,}5$ g/l in ethyl acetate (4.7)

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4.14 Solvent mixture of n-hexane and dichloromethane

Mix 1 part per volume of n-hexane (4.11) with 1 part per volume of dichloromethane (4.8).

4.15 Solvent mixture of dichloromethane and acetone

Mix 9 parts per volume of dichloromethane (4.8) with 1 part per volume of acetone (4.4).

4.16 Solvent mixture of acetonitrile and dichloromethane

Mix 5 parts per volume of acetonitrile (4.5) with 1 part per volume of dichloromethane (4.8).

4.17 Mixture of methanol and ethyl acetate

Mix 1 part per volume of methanol (4.9) with 1 part per volume of ethyl acetate (4.7).

4.18 HPLC mobile phase solvent A

Mix 800 ml of methanol (4.9) with 200 ml of ethyl acetate (4.7).

4.19 HPLC mobile phase solvent B

Mix 700 ml of acetonitrile (4.5) with 300 ml of water.

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4.20 Okadaic acid

WARNING — Okadaic acid is toxic. Gloves and safety glasses shall be worn at all times, and all standard and sample preparation stages shall be carried out in a fume cupboard.

4.21 Okadaic acid stock solution in methanol

Dilute okadaic acid with methanol (4.9) to a concentration of 1,0 µg/ml. This solution can be stored at -18 °C for at least 6 months.

4.22 Okadaic acid methylanthrylester

NOTE For the availability of this substance, contact your National Standardization Institute. It can for example be obtained from SIGMA-ALDRICH [1)].

4.23 Solution of okadaic acid methylanthrylester in methanol

Dilute okadaic acid methylanthrylester with methanol (4.9) to a concentration of 0,1 µg/ml. This corresponds to 1 600 µg/kg mussel hepatopancreas (in samples). Store the solution in a brown bottle. At ≤ -18 °C the solution is stable for at least 6 months.

4.24 Mussels free of okadaic acid and related compounds

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5 Apparatus

5.1 General

Usual laboratory apparatus, and in particular:

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5.2 Instrument for separation of hepatopancreas, e.g. scalpel

5.3 Blender or homogenizer

5.4 Centrifuge, capable of a centrifugal force up to 3 000 *g,* with suitable tubes, e.g. 20 ml

5.5 Pear shaped flask, e.g. 10 ml and 25 ml capacity

5.6 Glass sample tubes with stoppers, e.g. 10 ml capacity

5.7 One-mark volumetric flask, 20 ml capacity

5.8 Analytical balance, accurate to the nearest 0,1 mg

5.9 Solid phase extraction (SPE) cartridges, (3 ml) filled with 650 mg silica gel with solvent reservoir (approx. 10 ml), commercially available

5.10 Suitable pipettes of various volumina

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1) This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN. Equivalent products may be used if they can be shown to lead to the same results.

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5.11 Apparatus for solvent removal using nitrogen gas

5.12 HPLC apparatus, comprising the following

5.12.1 HPLC pump, for gradient elution at e.g. 0,8 ml/min constant flow rate

5.12.2 Injection system, capable to deliver an injection volume of e.g. 20 µl

5.12.3 Analytical reverse-phase separating column which ensures a baseline resolution of the peaks of the derivatives from all other peaks, for example:

— RP-C_{18}

— a length of 250 mm;

— an inner diameter of 4 mm;

— a stationary phase with particle size of 5 µm.

Columns of other dimensions may also be used.

5.12.4 Fluorescence detector, fitted with a flow cell and set at 365 nm (excitation) and 412 nm (emission).

Specific properties of the detector shall be regarded.

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5.12.5 Data system

5.13 Rotary evaporator, with evaporation flask and water bath

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5.14 Laboratory shaker for tubes

5.15 Ultrasonic bath

5.16 Glass funnel, with paper filter, 90 mm diameter

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. Samples shall be deep frozen until use or shall be extracted immediately.

NOTE Storage of mussel samples in frozen state at –18 °C has no negative influence on the result of this method.

7 Procedure

7.1 Preparation of test sample

Transfer fresh mussels into boiling water and cook for 10 min. Separate 20 g to 30 g of hepatopancreas material from approximately 1 kg of mussels (including the shells). The hepatopancreas is the green or brown part of the mussel. If only smaller sample amounts of hepatopancreas material are available, the whole material has to be prepared. Homogenize the hepatopancreas material with the homogenizer or blender (5.3) and start immediately with the preparation or otherwise store the homogenate at –18 °C.