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English version

Foodstuffs - Determination of saxitoxin and dc-saxitoxin in mussels - HPLC method using pre-column derivatization with peroxide or periodate oxidation

Produits alimentaires - Détermination de la teneur en saxitoxine et en dc-saxitoxine dans les moules - Méthode par CLHP avec dérivation pré-colonne et par oxydation au peroxide ou au periodate

Lebensmittel - Bestimmung von Saxitoxin und DC-Saxitoxin in Muscheln - HPLC-Verfahren mit Vorsäulenderivatisierung mit Peroxid- oder Periodatoxdation

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Foreword

This document (EN 14526:2004) 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of an identical text or by endorsement, at the latest by February 2005, and conflicting national standards shall be withdrawn at the latest by February 2005.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.

Introduction

This document is based on a procedure described by Lawrence and Ménard [1]. In this original procedure, the C_{18} solid phase extraction is followed by a solid phase extraction with a weak cation exchanger. In the latter step, three different groups of shellfish toxins are being separated by elution with mobile phases of different ionic strength and polarity. The different groups of toxins are then derivatized with periodate or peroxide depending on the peak responses of the different toxins to the respective derivatization reactions. The method described in this document is specifically for saxitoxin and decarbamoyl (dc)-saxitoxin, so only the C_{18} clean up is applied.

The method described in this document offers the option to choose between an extraction with acetic acid (HAc) or an extraction with hydrochloric acid (HCl). The analyst's choice depends on his desires and final needs.

HCl extraction with boiling leads to partial hydrolysis of the PSP toxins, leading to conversion of some of the PSP toxins (C-1, C-2, GTX-5) into more toxic analogues (GTX-2, GTX-3, saxitoxin). This process also occurs more or less in the human stomach. This extraction can therefore be considered a reasonable approach to estimate potential toxicity.

HAc extraction without boiling is a milder extraction procedure, which leaves the toxin profile of the sample practically intact. This extraction procedure can be applied if it is considered more important to estimate the actual toxin profile. The HAc extraction has been applied in the development of (certified) reference material for PSP toxins [2] and [3].

1 Scope

This document specifies a method for the quantitative determination of saxitoxin (STX) and decarbamoyl saxitoxin (dc-STX) in mussels. It may also be applicable in other shellfish, for example scallops. The limit of determination of this method (signal/noise = 10) is 0,006 mg/kg for saxitoxin and 0,02 mg/kg for dc-saxitoxin in mussel meat. The method has been tested for saxitoxin at levels at 0,4 mg/kg and 0,5 mg/kg and for dc-saxitoxin at levels at 0,4 mg/kg and 1,6 mg/kg.

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)*

3 Principle

Paralytic Shellfish Poisoning (PSP) toxins are extracted from mussel meat homogenate with acetic acid (HAc) or with hydrochloric acid (HCl). After centrifugation the supernatant is purified by solid phase extraction (SPE) over a C_{18} clean-up cartridge. Part of the extract is derivatized with hydrogen peroxide.

WARNING — PSP toxins are strong neurotoxins. 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.

Derivatization of STX and dc-STX leads to several oxidation products that are separated by reverse phase HPLC with fluorescence detection. Table 1 gives the reaction products for STX and dc-STX after derivatization with hydrogen peroxide.

Table 1 — Reaction products after derivatization with hydrogen perioxide

Toxin	Eluting order	Intensity	Peak name of reaction product
STX	first	+	dc-STX oxid. (1)
	second	+	dc-STX oxid. (2)
	third	++	STX oxid.
dc-STX	first	++	dc-STX oxid. (1)
	second	+	dc-STX oxid. (2)

The method described in this document is suitable for the quantitative determination of the PSP toxins STX and dc-STX. The following should however be considered.

Co-occurrence of different PSP toxins in mussels could influence the analytical results, because some of the PSP toxins could (partially) lead to the same reaction products. So the chromatograms shall be carefully interpreted.

The analytical result obtained for STX with hydrogen peroxide oxidation is not influenced by the presence of other toxins.

The analytical result obtained for dc-STX with hydrogen peroxide oxidation is slightly influenced by the presence of STX, because the "dc-STX oxid. (1)" reaction product of STX is the same as the main reaction product of dc-STX (see Table 1). The contribution of STX to the dc-STX peak is approximately 4 % of the peak area of the STX peak [4] and [5].

If oxidised with peroxide, the gonyautoxins GTX-2 and GTX-3 lead to the same reaction product, whereas GTX-1 and GTX-4 can not be detected. If oxidised with periodate, GTX-1, GTX-2, GTX-3 and GTX-4 lead to the same reaction products. GTX-5 can be detected with peroxide oxidation, neo-STX only with periodate oxidation.

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only water according to grade 1 of EN ISO 3696. All chemicals shall be of pro analysis (p.a.) quality, unless otherwise indicated. Reference materials (calibrants of the toxins) originating from other sources than indicated may also be used if well-characterised and with a well-defined mass concentration.

4.2 Methanol

4.3 Acetonitrile, HPLC quality

4.4 Ammonium formate

4.4.1 Ammonium formate solution 1, substance concentration c = 0,3 mol/l

Dissolve 18,9 g ammonium formate (4.4) in water and make up to 1 l with water.

4.4.2 Ammonium formate solution 2, c = 0,1 mol/l

Dilute 350 ml of ammonium formate solution 1 (4.4.1) with 700 ml of water. Adjust to pH 6 with acetic acid (pH paper) and filter over a membrane filter (5.15). Prepare fresh every 2 days of analysis.

4.5 Hydrochloric acid solution

4.5.1 General

volume fraction $\varphi \approx 25$ % (acidimetric)

4.5.2 Hydrochloric acid solution 1, c = 5 mol/l

Dilute 100 ml of hydrochloric acid solution (4.5) with 54 ml of water.

4.5.3 Hydrochloric acid solution 2, c = 0,1 mol/l

Dilute 5 ml of hydrochloric acid solution 1 (4.5.2) with 245 ml of water.

4.6 Sodium hydroxide

4.6.1 Sodium hydroxide solution 1, c = 5 mol/l

Dissolve 20 g of sodium hydroxide (4.6) in 100 ml of water.