

Foodstuffs — Determination of saxitoxin and dc-saxitoxin in mussels — HPLC method using post column derivatisation

ICS 67.120.30

National foreword

This Draft for Development is the English language version of ENV 14194:2002.

This publication is not to be regarded as a British Standard.

It is being issued in the Draft for Development series of publications and is of a provisional nature because the method has not been validated. It should be applied on this provisional basis, so that information and experience of its practical application may be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the European organization responsible for its conversion into a European Standard. A review of this publication will be initiated 2 years after its publication by the European organization so that a decision can be taken on its status at the end of its three-year life. The commencement of the review period will be notified by an announcement in *Update Standards*.

According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into a European Standard, to extend the life of the prestandard or to withdraw it. Comments should be sent in writing to the Secretary of BSI Technical Committee AW/-/3, Horizontal analysis, at 389 Chiswick High Road, London W4 4AL, giving the document reference and clause number and proposing, where possible, an appropriate revision of the text.

A list of organizations represented on this committee can be obtained on request to its secretary.

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the BSI Standards Catalogue under the section entitled “International Standards Correspondence Index”, or by using the “Find” facility of the BSI Standards Electronic Catalogue.

This Draft for Development, having been prepared under the direction of the Consumer Products and Services Sector Policy and Strategy Committee, was published under the authority of the Standards Policy and Strategy Committee on 16 May 2002

Summary of pages

This document comprises a front cover, an inside front cover, the ENV title page, pages 2 to 11 and a back cover.

The BSI copyright date displayed in this document indicates when the document was last issued.

Amendments issued since publication

Amd. No.	Date	Comments

© BSI 16 May 2002

English version

Foodstuffs - Determination of saxitoxin and dc-saxitoxin in mussels - HPLC method using post column derivatisation

Produits alimentaires - Détermination de la teneur en saxitoxine et en dc-saxitoxine dans les moules - Méthode par chromatographie liquide haute performance après dérivation post-colonne

Lebensmittel - Bestimmung von Saxitoxin und DC-Saxitoxin in Muscheln - HPLC-Verfahren mit Nachsäulenderivatisierung

This European Prestandard (ENV) was approved by CEN on 14 December 2001 as a prospective standard for provisional application.

The period of validity of this ENV is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the ENV can be converted into a European Standard.

CEN members are required to announce the existence of this ENV in the same way as for an EN and to make the ENV available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the ENV) until the final decision about the possible conversion of the ENV into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.



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

Management Centre: rue de Stassart, 36 B-1050 Brussels

Foreword

This document (ENV 14194:2002) has been prepared by Technical Committee CEN /TC 275, "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

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

1 Scope

This European Prestandard specifies a method for the quantitative determination of saxitoxin (STX), dc-saxitoxin (dc-STX) and the qualitative determination of neo-saxitoxin, and the gonyautoxins GTX-2 and GTX-3 in mussels. The method can also be used to identify the N-sulfocarbamoyl toxins C-1, C-2, GTX-5 and GTX-6 after hydrolysis and, if these toxins are present, to exclude false positive results for GTX-2, GTX-3, neo-saxitoxin and saxitoxin. For mussel the limit of quantification is for saxitoxin 0,04 mg/kg mussel meat and for dc-saxitoxin 0,03 mg/kg mussel meat (signal/noise = 10).

The limits of detection for C-1, C-2, GTX-2, GTX-3, GTX-5, GTX-6 and neo-saxitoxin have not been determined.

2 Normative references

This European Prestandard 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 Prestandard 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 3696:1995, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

3 Principle

PSP (Paralytic Shellfish Poisoning) toxins are extracted from mussels homogenate with an acidic aqueous solution. After centrifugation the supernatant is purified by solid phase extraction (SPE) over a C18 clean-up cartridge. Part of the extract is directly injected on the HPLC for toxin determination. After the separation on the analytical column post column derivatisation is used, with oxidation with periodic acid and after acidification the derivatized toxins are detected using fluorimetric detection. HPLC analysis is separated in two parts: a system to separate the saxitoxin group toxins (saxitoxin, dc-saxitoxin and neo-saxitoxin) and a system to separate the GTX group toxins (GTX-2 and GTX-3). Identification after hydrolysis is based on the transformation of the N-sulfo-carbamoyl toxins to their corresponding carbamoyl toxins.

WARNING - PSP toxins are strong neurotoxins. Gloves and safety glasses should be worn at all times, and all standard and sample preparation stages should be carried out in a fume cupboard.

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only water according to grade 1 of EN ISO 3696:1995.

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 Hydrochloric acid, volume concentration $\varphi(\text{HCl})$ 25 %, (acidimetric)

4.4.1 Hydrochloric acid solution, substance concentration $c = 1,0 \text{ mol/l}$

Dilute 130 ml of hydrochloric acid solution (4.4) with 870 ml of water.

4.4.2 Hydrochloric acid solution, $c = 0,1 \text{ mol/l}$

Dilute 100 ml of hydrochloric acid solution (4.4.1) with 900 ml of water.

4.5 Octane sulfonic acid solution, $c = 0,5 \text{ mol/l}$

Dissolve 2,9 g of octane sulfonic acid sodium salt monohydrate in 25,0 ml of water.

4.6 Phosphoric acid, the mass fraction is 85 %

4.6.1 Phosphoric acid solution, $c = 0,5 \text{ mol/l}$

Take 6,7 ml of phosphoric acid (4.6) and dilute to 200,0 ml with water.

4.7 Ammonia solution, $\varphi = 25 \%$ and $\varphi = 1 \%$

For the 1 % solution, dilute 40 ml ammonia solution $\varphi = 25 \%$ with 960 ml of water.

4.8 Periodic acid solution, $c = 0,01 \text{ mol/l}$

Dissolve 1,14 g of periodic acid in water and dilute up to 500 ml with water.

4.9 Acetic acid, 100 %

4.9.1 Acetic acid solution 1, $c = 1 \text{ mol/l}$

Take 57,2 ml of acetic acid solution (4.9) and dilute to 1,0 l with water.

4.9.2 Acetic acid solution 2, $c = 0,2 \text{ mol/l}$

Dilute 200 ml of acetic acid solution 1 (4.9.1) with 800 ml of water.

4.9.3 Acetic acid solution 3, $c = 0,03 \text{ mol/l}$

Dilute 150 ml of acetic acid solution 2 (4.9.2) with 850 ml of water.