Irish Standard
I.S. EN 14526:2017

Foodstuffs - Determination of saxitoxin-group toxins in shellfish - HPLC method using pre-column derivatization with peroxide or periodate oxidation

I.S. EN 14526:2017

Incorporating amendments/corrigenda/National Annexes issued since publication:

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National Foreword

I.S. EN 14526:2017 is the adopted Irish version of the European Document EN 14526:2017, Foodstuffs - Determination of saxitoxin-group toxins in shellfish - HPLC method using pre-column derivatization with peroxide or periodate oxidation

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 14526

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ICS 67.120.30

Supersedes EN 14526:2004

English Version

Foodstuffs - Determination of saxitoxin-group toxins in shellfish - HPLC method using pre-column derivatization with peroxide or periodate oxidation

Produits alimentaires - Détermination de la teneur en toxines du groupe de la saxitoxine dans les coquillages - Méthode par CLHP avec dérivation pré-colonne et par oxydation au peroxyde ou au periodate

Lebensmittel - Bestimmung von Toxinen der Saxitoxingruppe in Schalentieren - HPLC-Verfahren mit Vorsäulenderivatisierung und Peroxid- oder Periodatoxidation

This European Standard was approved by CEN on 7 November 2016.

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COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (EN 14526:2017) 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of an identical text or by endorsement, at the latest by July 2017, and conflicting national standards shall be withdrawn at the latest by July 2017.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 14526:2004.

EN 14526:2017 includes the following significant technical changes with respect to EN 14526:2004:

— the applicability is greater as more samples were tested in interlaboratory studies;

— the extraction procedure in 6.2 has been revised;

— the chromatographic conditions in Clause 7 have been revised;

— guidelines for calculation in presence of several toxins were introduced;

— the method has been additionally validated in several interlaboratory studies, and the precision data in Annex A have been revised.

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

Introduction

Paralytic shellfish poisoning (PSP) toxins are derivatives of saxitoxin. These toxins have been detected in filter-feeding bivalve molluscs in various parts of the world. Paralytic shellfish poisoning is characterized by symptoms varying from slight tingling sensation or numbness around the lips to fatal respiratory paralysis. This document describes an analytical method for the quantification of these PSP toxins by extraction from shellfish tissue followed by several clean-up steps and a separation by high performance liquid chromatography (HPLC) with fluorescence detection (FLD).

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 take appropriate measures to ensure the safety and health of personnel prior to application of the standard, and fulfil statutory and regulatory requirements for this purpose.

1 Scope

This European standard specifies a method [1] for the quantitative determination of saxitoxin (STX), decarbamoyl saxitoxin (dcSTX), neosaxitoxin (NEO), decarbamoyl neosaxitoxin (dcNEO), gonyautoxin 1 and 4 (GTX1,4; sum of isomers), gonyautoxin 2 and 3 (GTX2,3; sum of isomers), gonyautoxin 5 (GTX5 also called B1), gonyautoxin 6 (GTX6 also called B2), decarbamoyl gonyautoxin 2 and 3 (dcGTX2,3; sum of isomers), N-sulfocarbamoyl-gonyautoxin 1 and 2 (C1,2; sum of isomers) and (depending on the availability of certified reference materials (CRMs)) N-sulfocarbamoyl-gonyautoxin 3 and 4 (C3,4; sum of isomers) in (raw) mussels, oysters, scallops and clams. Laboratory experience has shown that it is also be applicable in other shellfish [2], [3] and cooked shellfish products. The method described was validated in an interlaboratory study [4], [5] and was also verified in a EURL-performance test aiming the total toxicity of the samples [6]. Toxins which were not available in the first interlaboratory study [4], [5] as dcGTX2,3 and dcNEO were validated in two additional interlaboratory studies [7], [8]. The lowest validated levels [4], [5], [8], are given in µg toxin (free base)/kg shellfish tissue and also as µmol/kg shellfish tissue and are listed in Table 1.

Table 1 — Lowest validated levels

Toxin		**µg/kg**	**µmol/kg**
saxitoxin (STX) [5]		22[c]	0,07[c]
gonyautoxin 2,3 (GTX2,3) [5]		114[b]	0,29[b]
gonyautoxin 5 (GTX5, B1) [5]		27[c]	0,07[c]
dc-saxitoxin (dcSTX) [5]		8[c]	0,03[c]
neosaxitoxin (NEO) [5]		33[c]	0,10[c]
gonyautoxin 1,4 (GTX1,4) [5]		61,4[c]	0,15[c]
N-sulfocarbamoyl-gonyautoxin 1,2 (C1,2) [5]		93[c]	0,20[c]
N-sulfocarbamoyl-gonyautoxin 3,4 (C3,4) [5]		725[b]	1,48[b]
gonyautoxin 6 (GTX6, B2)	Direct [4] Indirect [9]	30 834[b]	0,08 2,11[b]
dc-gonyautoxin 2,3 (dcGTX2,3) [8]		271[a]	0,77[a]
dc-neosaxitoxin (dcNEO) [8]		594[b]	2,18[b]

[a] lowest spiked level; mean recovery: 58 % [8]

[b] lowest concentration tested

[c] lowest concentration tested with a HorRat < 2 [4], [5]

A quantitative determination of GTX6 (B2) was not included in the first interlaboratory study but several laboratories detected this toxin directly after solid phase extraction with ion-exchange (SPE-COOH) clean-up and reported a mass concentration of 30 µg/kg or higher in certain samples. For that reason, the present method is applicable to quantify GTX6 (B2) directly, depending on the availability of the standard substance. Currently it is possible to determine GTX6 after a hydrolysis of Fraction 2 of the SPE-COOH clean-up, described in 6.4 as NEO. The indirect quantification of GTX6 was validated in two additional interlaboratory studies [7], [8].

A quantitative determination of C3,4 was included in the first interlaboratory study. The present method is applicable to quantify C3,4 directly, depending on the availability of the standard substance.

If no standard substances are available, C3,4 can only be quantified as GTX1,4 if the same hydrolysis protocol used for GTX6 (6.4) is applied to Fraction 1 of the SPE-COOH clean-up, see [10].

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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)*

3 Principle

WARNING — PSP toxins are neurotoxins which can be taken up by inhalation or orally. Therefore, adequate protection measures are to be applied.

Paralytic Shellfish Poisoning (PSP) toxins are extracted from shellfish tissue homogenate by heating with acetic acid. After centrifugation the supernatant is purified by solid phase extraction (SPE) using a C18 clean-up cartridge. It is analysed by HPLC after oxidation with periodate or peroxide with fluorescence detection. Most toxins (STX, C1,2, GTX5 (B1), dcSTX, GTX2,3 and dcGTX2,3) can be quantified after SPE-C18 clean-up[1)].

Oxidation of PSP toxins leads to several reaction products that are separated by reversed phase HPLC and detected by fluorescence detection. The obtained reaction products for PSP toxins after oxidation with peroxide and periodate are listed in Table 2. Additionally, the corresponding chromatograms are shown in Figure 1.

The gonyautoxins GTX2 and GTX3 as well as GTX1 and GTX4 and decarbamoyl gonyautoxins dcGTX2 and dcGTX3 and the N-sulfocarbamoyl-gonyautoxins C1 and C2 as well as C3 and C4 are structural isomers and lead with both oxidation modes to the same reaction products. The amount of structural isomers is determined as sum of both toxins.

STX reacts to a single specific oxidation product regardless of the kind of oxidation reaction (whether peroxide or periodate). The same is valid for GTX2,3 as well as GTX5 (B1) and C1,2. In contrast, dcSTX and dcGTX2,3 produce each two different oxidation products in both oxidation reactions, see also Table 2. The toxin dcNEO is oxidized into two oxidation products only with the periodate oxidation. Each of the toxins NEO, GTX6 (B2), GTX1,4 and C3,4 produce three peaks after periodate oxidation but only the second eluting peak is used for quantification (peroxide oxidation cannot be used for quantification).

Co-occurrence of different PSP toxins in shellfish can influence the analytical results, because some of the PSP toxins can (partially) lead to the same reaction products (see Table 2). So the chromatograms shall be carefully interpreted after a SPE C18 clean-up.

1) This document is based on a procedure described by Lawrence et al. [4] and was also published as AOAC Official Method 2005.06 [1].