SLOVENSKI STANDARD
SIST EN 14526:2026

01-junij-2026

Nadomešča:
SIST EN 14526:2017

Živila - Določanje toksinov iz skupine saksitoksinov v školjkah - Metoda HPLC z uporabo predkolonske derivatizacije s peroksidno ali perjodatno oksidacijo

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

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

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

Ta slovenski standard je istoveten z: **EN 14526:2026**

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67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.120.30	Ribe in ribji proizvodi	Fish and fishery products

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This European Standard was approved by CEN on 24 November 2025.

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Ref. No. EN 14526:2026 E

Contents

Page

European foreword

This document (EN 14526:2026) 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of an identical text or by endorsement, at the latest by October 2026, and conflicting national standards shall be withdrawn at the latest by October 2026.

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 14526:2017.

EN 14526:2026 includes the following significant technical changes with respect to EN 14526:2017:

— new Figure 2 detailing a schematic overview of procedure added;

— mandatory Clause 3 Terms and definitions added;

— new Clause 12 specifying quality controls added;

— new Clause 13 specifying verification added;

— new Annex B with example chromatograms added;

— new Annex C with alternative calculation methods added and deleted from Clause 11.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye 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).

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1 Scope

This document 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), gonyautoxin 6 (GTX6), decarbamoyl gonyautoxin 2 and 3 (dcGTX2,3; sum of isomers), *N*-sulfocarbamoyl gonyautoxin 2 and 3 (C1,2; sum of isomers) and *N*-sulfocarbamoyl gonyautoxin 1 and 4 (C3,4; sum of isomers) in (raw) mussels, oysters, scallops and clams. Laboratory experience has shown that this document can also be applied to other marine invertebrates [2], [3] and processed products of those species, however, no complete interlaboratory validation study according to ISO 5725-2 [21] has been carried out so far. The method described was validated in an interlaboratory study [4], [5] and was also verified in a European Union Reference Laboratory for Marine Biotoxins (EURLMB)-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 as mass fraction of toxin (free base) in µg/kg shellfish tissue and also as µmol/kg shellfish tissue and are listed in Table 1.

Table 1 — Lowest validated levels

Toxin		mass fraction (free base) µg/kg	molality µmol/kg
saxitoxin (STX) [5]		22[c]	0,07[c]
gonyautoxin 2,3 (GTX2,3) [5]		114[b]	0,29[b]
gonyautoxin 5 (GTX5) [5]		27[c]	0,07[c]
decarbamoyl 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 2,3 (C1,2) [5]		93[c]	0,20[c]
N-sulfocarbamoyl gonyautoxin 1,4 (C3,4) [5]		725[b]	1,48[b]
gonyautoxin 6 (GTX6)	direct [4]	30	0,08
	indirect [9]	834[b]	2,11[b]
decarbamoyl gonyautoxin 2,3 (dcGTX2,3) [8]		271[a]	0,77[a]
decarbamoyl 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 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 fraction (free base) of 30 µg/kg or higher in certain samples. For that reason, the present method is applicable to quantify GTX6 directly, depending on the availability of the standard substance. Whenever GTX6 standard is not commercially available, it is possible to determine GTX6 after hydrolysis of Fraction 2 of the SPE-COOH clean-up, described in 7.4, as NEO. The indirect quantification of GTX6 was validated in two additional interlaboratory studies [7], [8]. A study to compare direct and indirect GTX6 quantification was conducted at the EURLMB [16].

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 (7.4) is applied to Fraction 1 of the SPE-COOH clean-up [10]. A study to compare direct and indirect C3,4 quantification was conducted at the EURLMB [16].

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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)*

EN ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025)*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 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, 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 the 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 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

[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].

toxins NEO, GTX6, GTX1,4 and C3,4 produce three peaks after periodate oxidation but normally the second eluting peak is used for quantification (peroxide oxidation cannot be used for quantification).

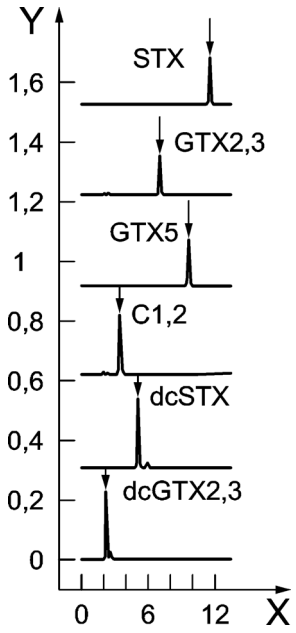
Co-occurrence of different PSP toxins in shellfish can influence the analytical results, because some of the PSP toxins can (partially) lead to the same reaction products (see Table 2, Figure 1 and Annex B). So, the chromatograms shall be carefully interpreted after a SPE C18 clean-up.

Table 2 — Reaction products after oxidation with periodate and peroxide

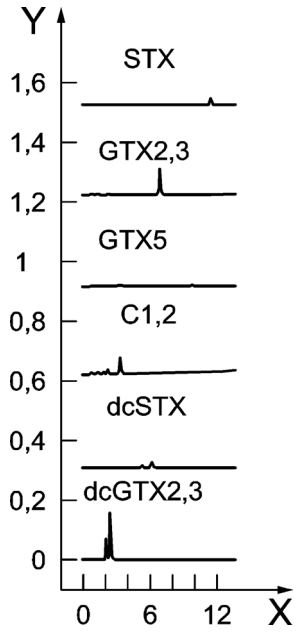
Toxin	Oxidation products and HPLC-eluting order		Intensity		Oxidation product at the same retention time as	
	peroxide[b]	periodate[b]	peroxide	periodate	peroxide[b]	periodate[b]
STX	one	one	++	+	NEO[a] (3)	NEO (3); GTX6 (3)
dcSTX	first (1)	first (1)	++	-		dcNEO (1)
	second (2)	second (2)	+	+	NEO[a] (2)	NEO (2); GTX6 (2); dcNEO (2)
NEO	no	first (1)	—	+		GTX6 (1)
	second (2)	second (2)	-	++	dcSTX (2)	GTX6 (2); dcSTX (2); dcNEO (2)
	third (3)	third (3)	-	+	STX	STX; GTX6 (3)
C1,2	one	one	++	+		
C3,4	no	first (1)	—	+		GTX1,4 (1)
	no	second (2)	—	++		GTX1,4 (2); dcGTX2,3 (2)
	no	third (3)	—	+		GTX1,4 (3); GTX2,3
GTX1,4	no	first (1)	—	+		C3,4 (1)
	no	second (2)	—	++		C3,4 (2); dcGTX2,3 (2)
	third (3)	third (3)	-	++	GTX2,3	C3,4 (3); GTX2,3
GTX2,3	one	one	++	++	GTX1,4[a] (3)	C3,4 (3); GTX1,4 (3)
GTX5	one	one	++	-		
GTX6	no	first (1)	—	+		NEO (1)
	no	second (2)	—	++		NEO (2); dcSTX (2); dcNEO (2)
	no	third (3)	—	-		NEO (3); STX
dcGTX2,3	first (1)	first (1)	++	+		
	second (2)	second (2)	+	++		C3,4 (2); GTX1,4 (2)
dcNEO	first (1)	first (1)	-	++		dcSTX (1)
	second (2)	second (2)	-	+	dcSTX (2)	dcSTX (2); NEO (2); GTX6 (2)

Intensity: — not visible
- very low
+ low
++ high

[a] High concentration of the indicated toxin can influence the quantification by simulating an increased content.
[b] Numbers in brackets are the elution order.

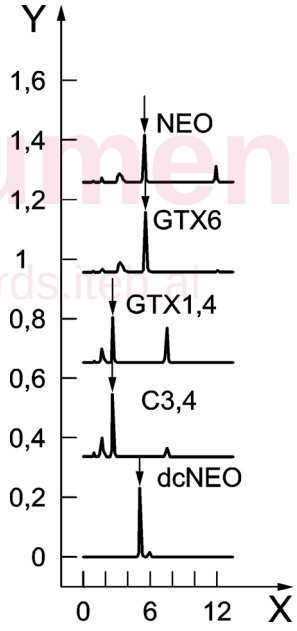


a) non-N1-hydroxylated toxins: peroxide



b) non-N1-hydroxylated toxins: periodate

c) N1-hydroxylated toxins: peroxide



d) N1-hydroxylated toxins: periodate

Key

Y detection response (V)

X time (min)

Figure 1 — Reaction products after derivatization with peroxide and periodate (peaks for quantification are marked with arrows)

For the quantitative determination of most N1-hydroxylated toxins, a fractionation by SPE-COOH clean-up is necessary (shown in Table 4) because the oxidation products of some PSP toxins (NEO and GTX6, GTX1,4 and C3,4) are identical. This step separates the PSP toxins into three distinct groups, namely the C toxins, the GTX toxins and the saxitoxin group by elution with mobile phases of different ionic strength. For example, with Bakerbond[2] Carboxylic Acidsilane (6.11), the C toxins (C1,2 and C3,4) elute unretained with water in Fraction 1, the GTX toxins (GTX2,3, GTX1,4, GTX5, GTX6 and dcGTX2,3) elute with 0,05 mol/l NaCl in Fraction 2 while the saxitoxin group (STX, NEO, dcNEO and dcSTX) requires 0,3 mol/l NaCl for elution in Fraction 3. These fractions can be analysed by HPLC-FLD after oxidation with periodate or peroxide.

5 Reagents

If not otherwise specified, reagents of pro analysis and solvents suitable for HPLC-FLD shall be used.

Water shall be distilled in glass vessels or demineralized before use or shall be of equivalent purity according to EN ISO 3696.

If not already specified, stability of solutions should be determined by the laboratory.

5.1 Methanol, HPLC quality.

5.2 Acetonitrile, HPLC quality.

5.3 Ammonium formate:

5.3.1 Ammonium formate solution, substance concentration[3] c = 0,3 mol/l.

Dissolve 1,892 g of ammonium formate (crystalline powder) (5.3) in 100 ml of water.

5.4 Glacial acetic acid:

5.4.1 Acetic acid solution 1, mass fraction $w \approx 1$ %.

Dilute 1 ml of glacial acetic acid (5.4) to 100 ml with water.

5.4.2 Acetic acid solution 2, $c \approx 0{,}1$ mol/l.

Dilute 572 µl of glacial acetic acid (5.4) to 100 ml with water.

5.4.3 Acetic acid solution 3, $c \approx 0{,}1$ mmol/l.

Dilute 100 µl of acetic acid solution 2 (5.4.2) to 100 ml with water.

5.4.4 Acetic acid solution 4, mass fraction $w \approx 0{,}6$ %.

Dilute 0,6 ml of glacial acetic acid (5.4) to 100 ml with water.

[2] Bakerbond Carboxylic Acidsilane is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.
[3] Throughout the document, the term “substance concentration” commonly used in the laboratory is used for the symbol c instead of the normative term “amount-of-substance concentration” according to EN ISO 80000-9:2019, 9-12.1.

5.5 Ammonium acetate:

5.5.1 Ammonium acetate solution 1, c = 0,1 mol/l.

Dissolve 0,77 g of ammonium acetate (5.5) to 100 ml with water.

5.5.2 Ammonium acetate solution 2, c = 0,01 mol/l.

Dilute 10 ml of ammonium acetate solution 1 (5.5.1) to 100 ml with water.

5.6 Sodium chloride:

5.6.1 Sodium chloride solution 1, c = 0,05 mol/l.

Dissolve 0,29 g of sodium chloride (5.6) to 100 ml with water.

5.6.2 Sodium chloride solution 2, c = 0,3 mol/l.

Dissolve 1,75 g of sodium chloride (5.6) to 100 ml with water.

5.7 Hydrochloric acid, c = 1 mol/l.

5.8 Disodium hydrogenphosphate or disodium hydrogenphosphate 7-hydrate:

5.8.1 Disodium hydrogenphosphate solution, c = 0,3 mol/l.

Dissolve 4,26 g of disodium hydrogenphosphate (5.8) to 100 ml in water or dissolve 8,04 g of disodium hydrogenphosphate 7-hydrate (5.8) to 100 ml in water.

5.9 Sodium hydroxide:

5.9.1 Sodium hydroxide solution 1, c = 1 mol/l.

Dissolve 4 g of sodium hydroxide (5.9) to 100 ml with water.

5.9.2 Sodium hydroxide solution 2, c = 0,2 mol/l.

Dilute 10 ml of sodium hydroxide solution 1 (5.9.1) to 50 ml with water.

5.10 Hydrogen peroxide, w = 30 %:

5.10.1 Hydrogen peroxide solution, $w \approx 10$ %.

Dilute 3 ml of hydrogen peroxide solution (5.10), of mass fraction w = 30 % with 6 ml of water. Prepare fresh every day. Keep both solutions in the dark at approximately +4 °C.

5.11 Periodic acid:

5.11.1 Periodic acid solution 1, c = 0,1 mol/l.

Dissolve 0,2279 g of periodic acid (5.11) in 10 ml of water.

5.11.2 Periodic acid solution 2, c = 0,034 mol/l.

Dilute 3,4 ml of periodic acid solution 1 (5.11.1) with 6,6 ml of water. Keep in a refrigerator in the dark at approximately +4 °C. Prepare fresh every day.

5.12 Periodate oxidation reagent

Mix one volume part of periodic acid solution 2 (5.11.2) with one volume part of disodium hydrogenphosphate solution (5.8.1) and one volume part of ammonium formate solution (5.3.1). Bring the mixture to pH 8,2 ± 0,3 by drop wise adding sodium hydroxide solution 2 (5.9.2) and check the pH by using a pH meter (6.16) or pH indicator paper (6.15). Prepare fresh every day of analysis.

5.13 PSP toxin standard substances:

5.13.1 PSP toxin stock solutions

For convenience, standard substances can be combined into four or more mixtures by appropriate dilution of standard solutions in water. Table 3 shows suitable concentration for each PSP toxin in four stock solution mixtures. Keep the solutions in the dark at approximately +4 °C and check the mass concentrations regularly after 2 weeks or keep in the dark at approximately –18 °C or below and check the mass concentrations regularly after 6 months.

Table 3 — Example of suitable compositions and concentrations for each PSP toxin in four stock solution mixtures

Stock solution mixtures		Toxin concentrations	
		(free base) µg/ml	nmol/ml
Mix 1	GTX1,4	0,41	1,0
	NEO	0,32	1,0
Mix 2	GTX2,3	0,40	1,0
	GTX5	0,38	1,0
	STX	0,30	1,0
	dcSTX	0,26	1,0
	dcGTX2,3	0,35	1,0
	C1,2	0,48	1,0
Mix 3	dcNEO	0,27	1,0
Mix 4	GTX6	0,40	1,0
	C3,4	0,49	1,0

NOTE 1 Ampoules containing separately GTX1,4, NEO, GTX2,3, GTX5, STX, dcSTX, dcGTX2,3, C1,2, dcNEO, GTX6, C3,4 standard substances in aqueous hydrochloric acid or aqueous acetic acid with concentrations ranging from 10 µmol/l to 200 µmol/l are currently commercially available[4].

[4] Suitable calibration solutions can be obtained from the National Research Council Canada, Halifax, Canada. Further information on suitable calibration solutions is e. g. available on the homepage of the European Reference Laboratory on Marine Biotoxins https://www.aesan.gob.es/en/CRLMB/. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of this source of supply. Equivalent products may be used if they can be shown to lead to the same results.

NOTE 2 For chromatograms for the mixtures in Table 3, see Annex B.

Some of the standard substances can be contaminated with other PSP toxins; therefore, the impurities shall be taken into account for calibration purposes (by quantifying impurities, running different calibration curves or including it in uncertainty measurements).

5.13.2 PSP toxin calibration solutions

Prepare a calibration with at least five points for the determination of PSP toxins for example evenly distributed points in the range of (20 – 400) pmol/ml diluted from the PSP stock solution (5.13.1) with 0,1 mmol/l of acetic acid solution 3 (5.4.3). This range corresponds to (60 – 1191) µg STX 2HCl eq/kg in a sample in the C18 fraction for PSP toxins with a TEF of 1. PSP toxin calibration solutions may be also prepared by diluting stock solution mixtures with water. Keep in the dark at –18 °C and check the mass concentration regularly after 6 months.

NOTICE —It is important to keep diluted standard solutions in plastic vials or in deactivated glass containers which can e.g. be achieved by soaking the vials overnight in sodium hydroxide, rinsed with water followed by methanol (5.1), and dried.

For the interlaboratory study described in Clause A.1 [4], [5], three calibration points were used. However, in order to increase the robustness of the method, it is advised to use at least five calibration points.

NOTE Another method to prepare the calibration solution is to implement this in the oxidation step (7.5.2 and 7.5.3). Different aliquots from the PSP toxin stock solution are used and made up to 100 µl final volume with 0,1 mmol/l acetic acid solution 3 (5.4.3).

5.13.3 PSP-solution for checking the efficiency of the cartridges

Prepare solutions of toxins of the appropriate mass concentration for checking the recovery of the toxins on the SPE-cartridges.

One option is to prepare each standard mix in 0,6 % acetic acid solution (5.4.4) to check recovery for SPE-C18 and prepare each standard mixing water adjusted to pH 6,5 ± 0,3 to check SPE-COOH.

A second option is to prepare the mix(es) which contain non-N1-hydroxylated toxins (mixture 2 according to Table 3) in 0,6 % acetic acid solution 4 (5.4.4); Toxins quantified after peroxide oxidation: dcGTX2,3; C1,2; dcSTX; GTX2,3; GTX5 and STX will be only checked after SPE-C18.

For all other toxins checks will be done after both SPE-C18 and SPE-COOH clean-ups. For this, standard mix(es) which contain N1-hydroxylated toxins (mixtures 1, 3 and 4 according to Table 3) will be passed through both clean-ups sequentially after the SPE-C18 the pH is adjusted to 6,5 ± 0,3 and recovery will be evaluated after each clean-up. For example, the standards used for these checks can have a 0,8 µM concentration.

5.14 Matrix modifier (MM) for periodate oxidation

Use a blank extract (PSP free) from oysters as described in 7.1 and 7.2. If kept frozen at –20 °C, this initial PSP-free crude oyster extract is stable and can be used within at least two months. For use as matrix modifier, clean-up according to 7.3.1 and adjust the extract to pH 6,5 ± 0,3 with sodium hydroxide solution 1 (5.9.1). Filter the supernatant using a 0,45 µm filter (6.18) and keep the obtained matrix modifier in a refrigerator. Analyse the matrix modifier for PSP toxins by periodate and peroxide oxidation to ensure absence of toxins before use. It should be prepared every two weeks (i.e. again cleaned up from the crude extract).