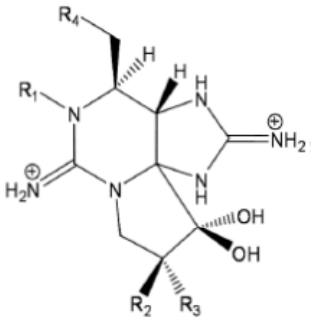
Toxins

Definition

1. A toxin is a poisonous substance produced within living cells or organisms; man-made substances created by artificial processes are thus excluded.

2. Toxins usually consist of an amino acid chain which can vary in molecular weight between a couple of hundred (peptides) and one hundred thousand (proteins). They may also be low-molecular organic compounds. Toxins are produced by numerous organisms, e.g., bacteria, fungi, algae and plants. Toxins vary greatly in their severity, ranging from usually minor and acute to almost immediately deadly. Many of them are extremely poisonous, with a toxicity that is several orders of magnitude greater than chemical warfare nerve agents.

Shellfish toxin analysis



With increasing sea temperatures the number of alagal blooms are increasing especially in over fertilized areas like the Baltic sea. These algae are consumed by shellfish filter feeders who, therefore, accumulate toxins produced by dinoflagellates , diatoms and cyanobacteria. Paralytic shellfish poisoning (PSP) toxins often referred to as Saxitoxins, can reach lethal levels in shellfish, and detection of saxitoxin in mussels, clams and scallops frequently leads to closures of commercial and recreational shellfish harvesting, especially in California, Oregon, Washington, and New England.

PSP toxins are powerful sodium channel blockers that can cause respiratory insufficiency and death. An AOAC mouse bioassay used to determine PSP's only determines the total toxicity of the sample. Chromatographic separation is used to identify individual toxins. Here an analytical method for the determination of paralytic shellfish toxins using hydrophilic interaction liquid chromatography is described. This method provides a sensitive and selective tool which can be employed with either a fluorescence detector or a mass spectrometric detector. The method can be used for various phytoplankton and the routine analysis of seafood.

There are a number of other toxins assosiated with algae and cyanobacteria like anatoxins and β-N-methylamino-L-alanine (BMAA) where methods using the ZIC®-HILIC column are available in the scientfic literature.

Determination of Paralytic Shellfish Toxins

SeQuant® ZIC®-HILIC

Column:	SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 250×4.6 mm	(1.50458.0001)

Recommended solvents and reagents

Acetonitrile	Hypergrade for LC-MS LiChrosolv®	(1.00029)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q® water purification system	(1.15333)
Formic Acid	98-100% for analysis EMSURE® ACS,Reag. Ph Eur	(1.00264)
Ammonia	25% solution for analysis EMSURE®	(1.05432)
Periodic Acid	for analysis EMSURE®	(1.00524)
Nitric Acid	69% for analysis EMSURE® ACS,Reag. Ph Eur	(1.01799)
Hydrochloric acid:	32% for analysis EMSURE®	(1.00319)
Ammonium Formate	Use ACS grade or HPLC grade.	

Recommended filtration tools

Mobile phase filtration:

PTFE coated with funnel, base, stopper clamp	(XX1004720)
Omnipore PTFE membrane filter 0.45µm	(JHWP04700)

Determination of Paralytic Shellfish Toxins using Fluorescence Detection

SeQuant® ZIC®-HILIC

Column: SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 250x4.6 mm (1.50458.0001)

Mobile phase: A: 10 mM ammonium formate and 10 mM formic acid in Milli-Q® water (100%)
B: Acetonitrile and Milli-Q® water with I_{tot} 8mM with ammonium formate (80:20 v/v)

Gradient:

Time (min)	Solution A (%)	Solution B (%)	Elution
0-24.0	18	82	Isocratic
24.1-35.0	30	70	Isocratic
35.1-50.0	35	65	Isocratic
50.1-70.0	18	82	equilibration

Detection: Fluorescence detection (Ex=350nm, Em=395mn).
Post-column oxidation with 10 mM periodic acid and 550 mM ammonia in water (flow rate 0.3 mL/min).
Nitric acid (0.75 M; flow rate 0.3 mL/min) was used to lower the pH value to 2–3.

Sample preparation:

Extraction of PSP toxins from shellfish material:
The sample material (2 g) was homogenized with 3 mL of hydrochloric acid (0.2 M) using an ultra sonic probe. The extracts were heated for 15 min at 90 degrees Celsius and the suspension was centrifuged for 10 min (14000 rpm), afterwards the supernatant was passed through a 0.45 µm nylon filter.
The treatment with HCl induced a conversion of N-sulfocarbamoyl toxins to the corresponding carbamoyl toxins.

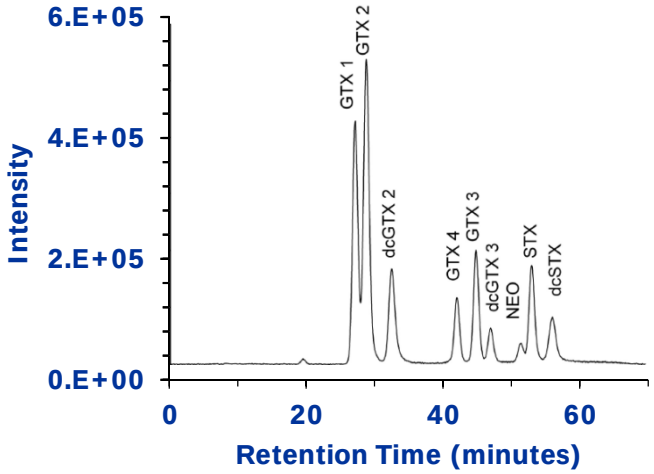
Extraction of the PSP toxins from Alexandrium catenella and Gymnodinium catenatum:
Aqueous acetic acid (2 mL, 0.03 M) was added to a suspension of algae cells (1 mL).
The suspension was then homogenized with an ultra sonic probe and centrifuged for 10 min (14000 rpm).
The supernatant was passed through a 0.45 µm nylon filter and the extracts obtained were used as a qualitative PSP standard solution during the development and optimization of the HILIC method.

Determination of Paralytic Shellfish Toxins

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 250x4.6 mm	(1.50458.0001)
Detection:	Fluorescence detection (Excitation=350nm, Emission=395nm)	
Mobile Phase (v/v):	A: 10 mM Ammonium formate and 10 mM formic acid in Milli-Q® water (100%) B: acetonitrile and Milli-Q® water with I_{tot} 8 mM Ammonium formate (80:20)	
Gradient:	See gradient table on page 47	
Flow Rate:	0.7 mL/min	
Sample:	GTX 1 (10.20 ng), GTX 2 (0.58 ng), dcGTX 2, (0.50 ng), GTX 4 (3.60 ng), GTX 3 (0.20 ng), dcGTX 3 (0.14 ng), NEO (2.05 ng), STX (0.72 ng), and dcSTX (0.79 ng).	
Temperature:	Ambient	



Chromatogram reprinted from J. Sep Sci. 2007, 30, 1821-1826, with permission from Wiley-VCH Verlag GmbH&Co., KGaA, Weinheim, Germany).

Chromatographic Data

No.	Compound	Time (min)
	Void volume (t0)	3.5
1	Gonyautoxin 1 (GTX 1)	27.1
2	Gonyautoxin 2 (GTX 2)	28.6
3	Decarbamoyl-gonyautoxin 2 (dcGTX 2)	32.3
4	Gonyautoxin 4 (GTX 4)	41.8
5	Gonyautoxin 3 (GTX 3)	44.7
6	Decarbamoyl-gonyautoxin 3 (dcGTX 3)	46.6
7	Neosaxitoxin (NEO)	51.4
8	Saxitoxin (STX)	53.0
9	Decarbamoyl-saxitoxin (dcSTX)	55.9

HILIC-MS/MS - Paralytic Shellfish Toxins

SeQuant® ZIC®-HILIC

Column: SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 250x4.6 mm (1.50458.0001)

Mobile phase: A: 10 mM ammonium formate and 10 mM formic acid in Milli-Q® water (100%)
B: Acetonitrile and Milli-Q® water with I_{tot} 8mM with ammonium formate (80:20 v/v)

Gradient:

Time (min)	Solution A (%)	Solution B (%)	Elution
0.0	20	80	Initial
5.0	35	65	Gradient
10.0	40	60	Gradient final
20.0	40	60	Isocratic
25.1	20	80	Equilibration
40.0	20	80	Equilibration

Sample preparation:

Extraction of PSP toxins from shellfish material:
The sample material (2 g) was homogenized with 3 mL of hydrochloric acid (0.2 M) using an ultra sonic probe. The extracts were heated for 15 min at 90 degrees Celsius and the suspension was centrifuged for 10 min (14000 rpm), afterwards the supernatant was passed through a 0.45 µm nylon filter.
The treatment with HCl induced a conversion of N-sulfocarbamoyl toxins to the corresponding carbamoyl toxins.

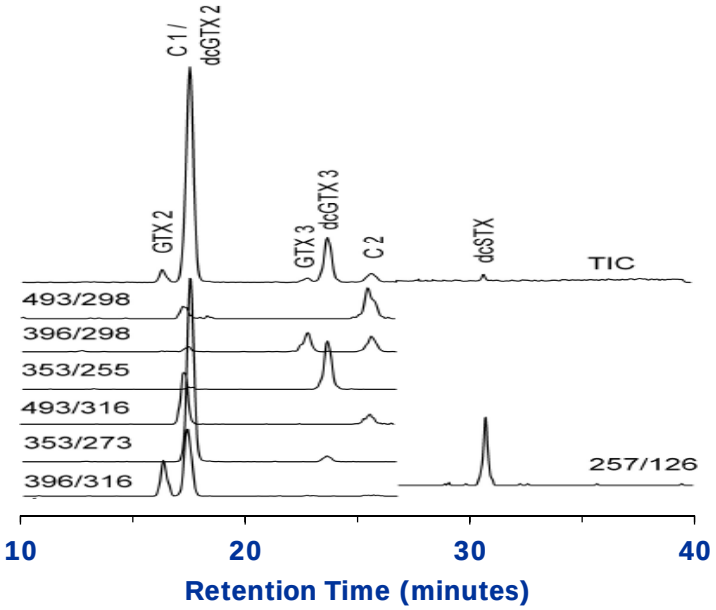
Extraction of the PSP toxins from Alexandrium catenella and Gymnodinium catenatum:
Aqueous acetic acid (2 mL, 0.03 M) was added to a suspension of algae cells (1 mL).
The suspension was then homogenized with an ultra sonic probe and centrifuged for 10 min (14000 rpm).
The supernatant was passed through a 0.45 µm nylon filter and the extracts obtained were used as a qualitative PSP standard solution during the development and optimization of the HILIC method.

HILIC-MS/MS - Paralytic Shellfish Toxins

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 250x4.6 mm	(1.50458.0001)
Detection:	ESI-MS/MS, details given in table below	
Flow Rate:	0.7 mL/min.	
Mobile Phase (v/v):	A: 10 mM Ammonium formate and 10 mM formic acid in water (100 %) B: 80% ACN and 20% water v/v I_{tot} 8 mM Ammonium formate.	
Gradient:	See gradient table on page 49	
Temperature:	Ambient	
Sample:	Gymnodinium catenatum extract, Baja California, Mexico	



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Chromatographic Data

No.	Compound	Time (min)	Transition (m/z)
1	Void volume (t0)	3.5	
2	Gonyautoxin 2 (GTX 2)	16.7	396→316
3	Saxitoxin C1 (C1)	17.7	493→316
4	Decarbamoyl-gonyautoxin 2 (dcGTX 2)	17.9	353→373
5	Gonyautoxin 3 (GTX 3)	23.0	396→298
6	Decarbamoyl-gonyautoxin 3 (dcGTX 3)	24.0	353→255
7	Saxitoxin C2 (C2)	25.7	493→298
8	Decarbamoyl-saxitoxin (dcSTX)	31.3	257→126