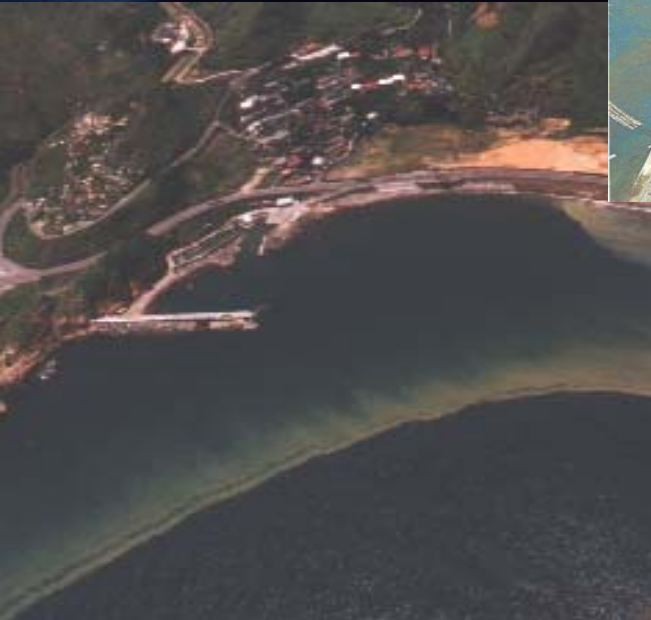
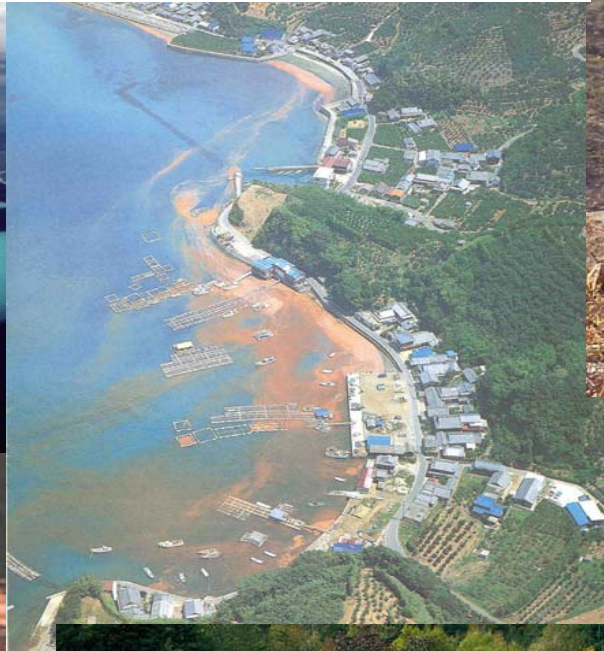


The Analysis of Algal Toxins Using Various Scan Modes in LC/MS/MS

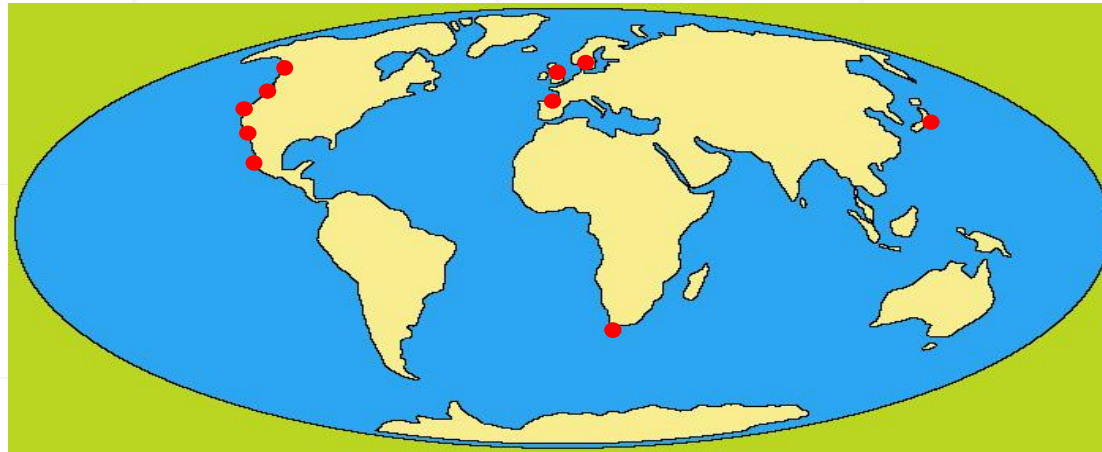


Toxic Algal Blooms

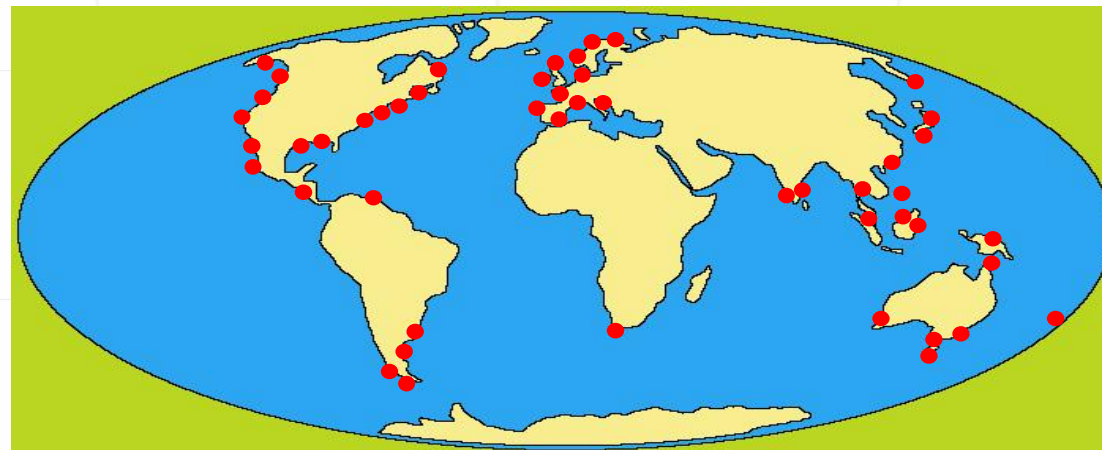


Distribution of Paralytic Shellfish Poisoning events

1970

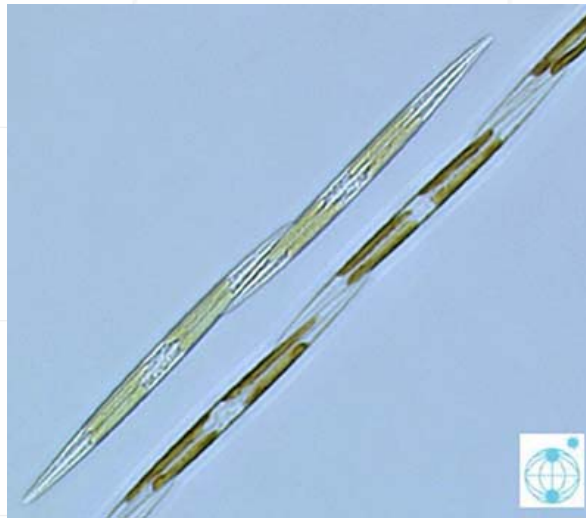


2005



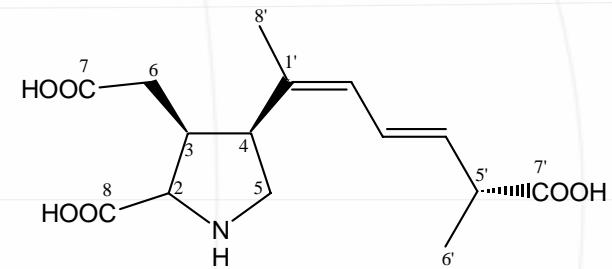
Causative Organisms

Diatoms



Pseudo-Nitzschia pungens

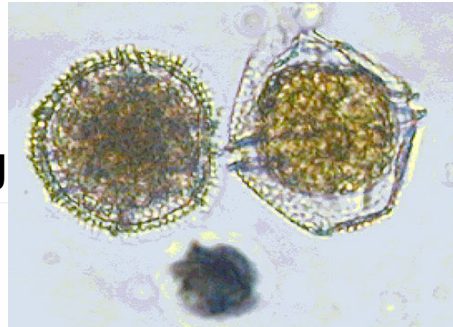
Amnesic Shellfish Poisoning



Domoic acid

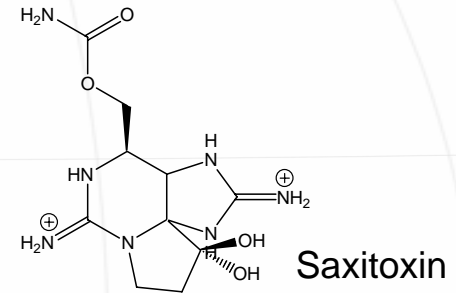
Causative Organisms

Dinoflagellates

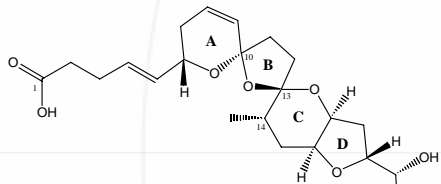


Lingulodinium polyedrum

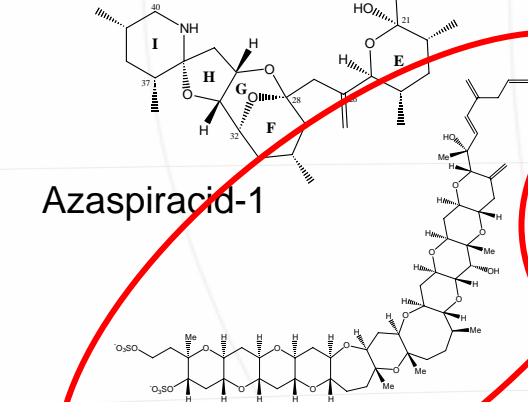
Paralytic Shellfish Poisoning



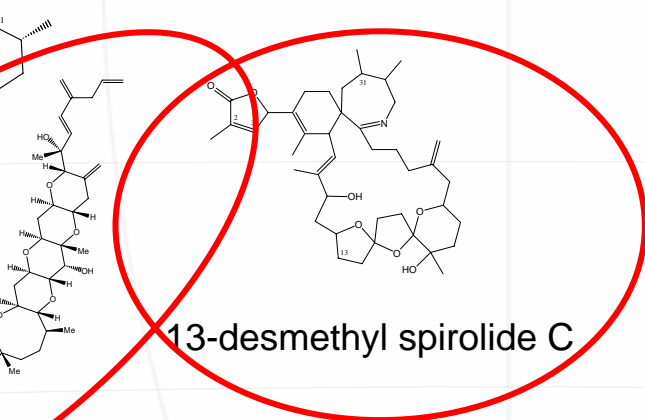
Diarrhetic Shellfish Poisoning



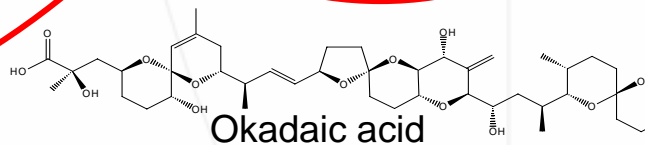
Azaspiracid-1



Yessotoxin

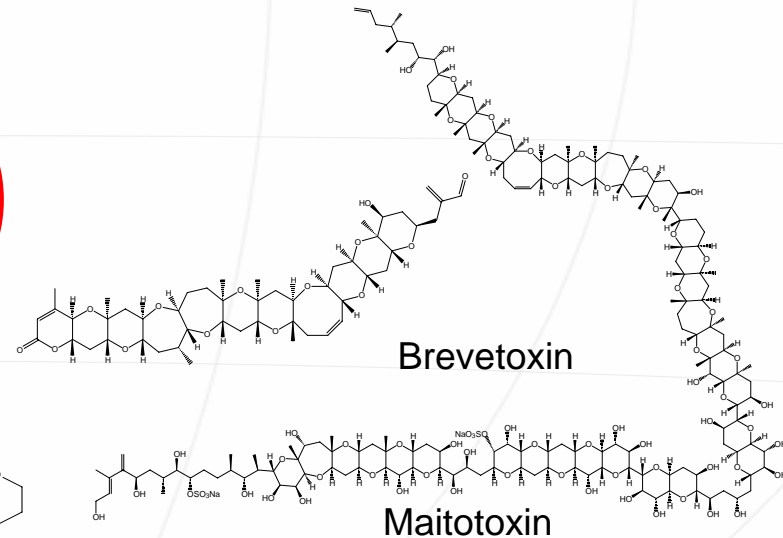


13-desmethyl spirolide C

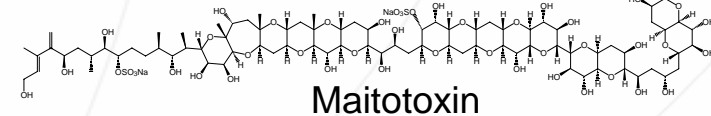


Okadaic acid

Neurotoxic Shellfish Poisoning



Brevetoxin



Maitotoxin

Spirolide determination - MRM

Chromatography

Luna C18 150x3 mm, 3 µm, 100 Å

A: 2mM NH₄HCOO, 50 mM HCOOH

B: 2mM NH₄HCOO, 50 mM HCOOH in 95% ACN

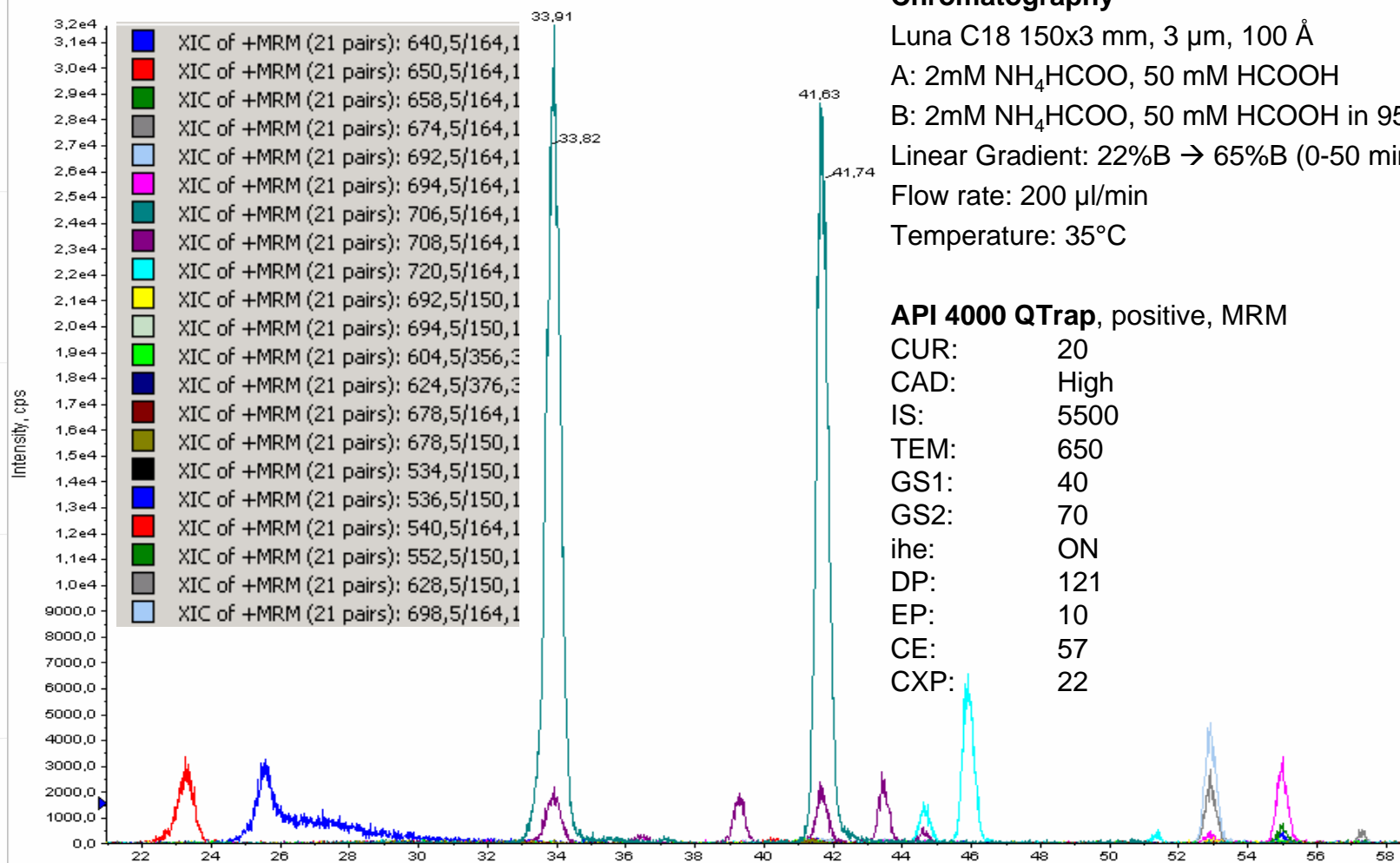
Linear Gradient: 22%B → 65%B (0-50 min)

Flow rate: 200 µl/min

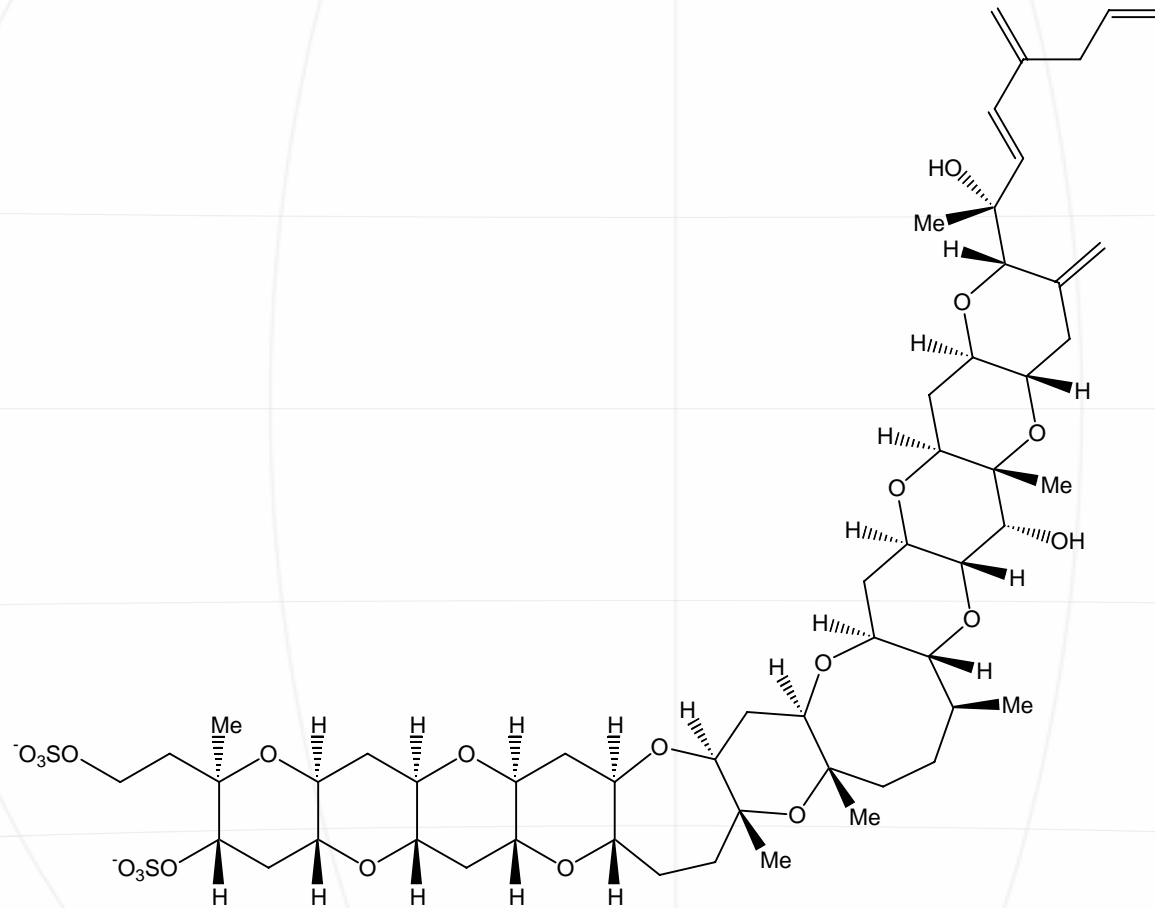
Temperature: 35°C

API 4000 QTrap, positive, MRM

CUR: 20
 CAD: High
 IS: 5500
 TEM: 650
 GS1: 40
 GS2: 70
 ihe: ON
 DP: 121
 EP: 10
 CE: 57
 CXP: 22



Yessotoxin detection – Neutral Loss



yessotoxin

List of Yessotoxin Pseudo-molecular and Fragment Masses (abridgement)

YTX analogs detected by LC-MS³ analysis of a fractionated extract of *Protocecatium reticulatum*

Entry	M_{we}	R_t	$[M - H]^-$	MS^2	MS^3 (see Fig. 5)	$[M - 2H]^{2-}$	Relative intensity ^a	MS^2	MS^3	Structure ¹
1	956 ^c	3.2	955	875	831 , 795	477.3	++	-	-	
2	984	3.3	983	903 , 869, 653, 599	-	-	-	-	-	
3	986	2.2	985	905 , 815 , 771	797 , 772, 645, 627, 583	439.0	+	-	-	
4	992	5.2	991	911	868, 799 , 757, 729, 688, 575	-	-	-	-	17
5	992	6.1	991	911	868 , 657	-	-	-	-	18
6	992	7.1	991	911	827	-	-	-	-	19
7	1008	3.2	1007	927	919, 912, 855 , 759	-	-	-	-	
8	1010	3.3	1009	929 , 922, 850, 799	-	-	-	-	-	
9	1012 ^d	2.7	1011	931	887 , 851, 807 , 696	505.2	+	-	-	
10	1020 ^e	4.4	1019	939 , 799	939	-	-	-	-	
11	1022 ^f	4.1	1021	980, 941 , 925	-	-	-	-	-	
12	1026 ^g	2.8	1025	945 , 875, 847, 786	927 , 758	512.0	+	-	-	
13	1026	3.1	1025	945	927 , 864	-	-	-	-	
14	1038	3.3	1037	957	939 , 877	518.3	++	-	-	
15	1038	4.0	1037	957	929	-	-	-	-	
16	1040	5.4	1039	959 , 929, 847, 598	927	519.5	+	-	-	
17	1042	2.6	1041	961	946, 943 , 917, 915, 881	520.4	+	-	-	
18	1048	5.2	1047	967	924, 907, 895, 855 , 713, 671	-	-	-	-	6
19	1048	5.9	1047	967	924, 895, 855 , 713, 671, 659	-	-	-	-	7
20	1048	6.8	1047	967	883	-	-	-	-	8
21	1062	3.2	1061	981	951	-	-	-	-	
22	1062 ^h	6.3	1061	981 , 924, 855, 713	-	-	-	-	-	
23	1082	3.0	1081	1001	970, 927, 885 , 855, 799, 713	-	-	-	-	
24	1082	3.4	1081	1001	983, 957, 927, 869, 855 , 713	-	-	-	-	
25	1086	8.8	1085	1005 , 868	921, 868 , 851, 822, 799, 773, 657	-	-	-	-	16
26	1090	7.5	1089	1009	981, 967, 925 , 855, 799, 671	-	-	-	-	
27	1118 ⁱ	5.7	1117	1037	924 , 895, 855, 713	-	-	-	-	
28	1120	4.6	1119	1039	959 , 895, 855, 799, 713, 687	559.4	++	-	-	
29	1120 ^j	5.3	1119	1039	1021, 941, 924 , 895, 855, 713	559.4	+	-	-	
30	1134	5.8	1133	1053	967, 925 , 855, 713	-	-	-	-	

The most abundant fragment of all YTXs is the loss of SO₃ from the sulfate groups

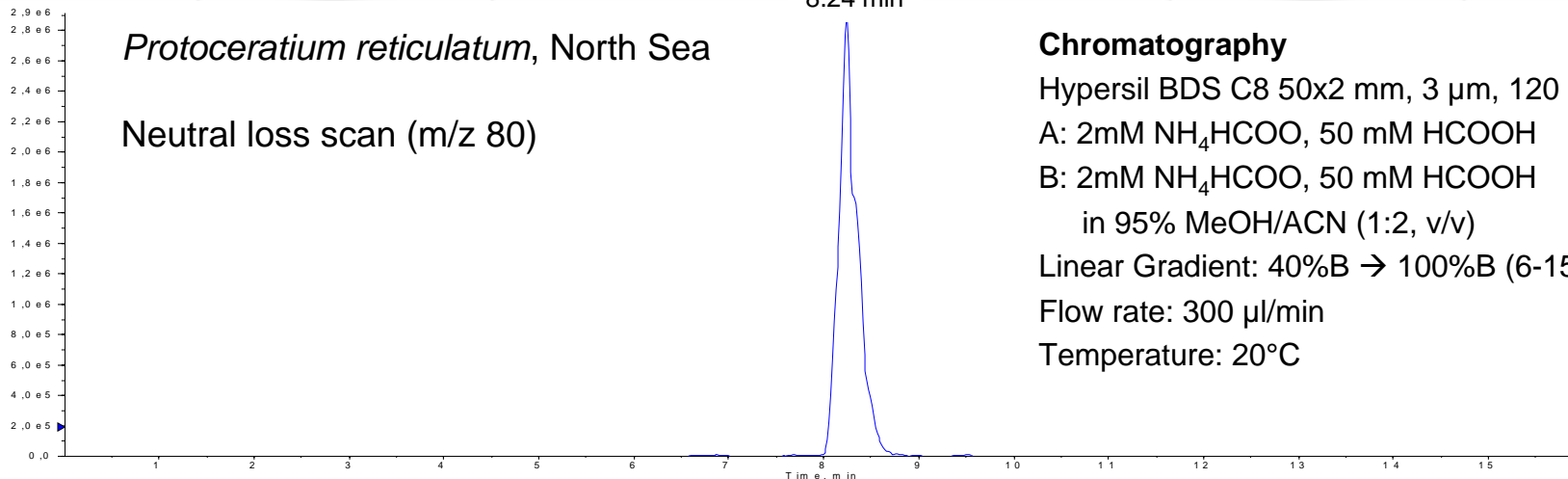
Miles et al. (2005) Harmful Algae 4 : 1075-1091

Yessotoxin detection – Neutral Loss

8.24 min

Protoceratium reticulatum, North Sea

Neutral loss scan (m/z 80)



Chromatography

Hypersil BDS C8 50x2 mm, 3 μm, 120 Å

A: 2mM NH₄HCOO, 50 mM HCOOH

B: 2mM NH₄HCOO, 50 mM HCOOH

in 95% MeOH/ACN (1:2, v/v)

Linear Gradient: 40%B → 100%B (6-15 min)

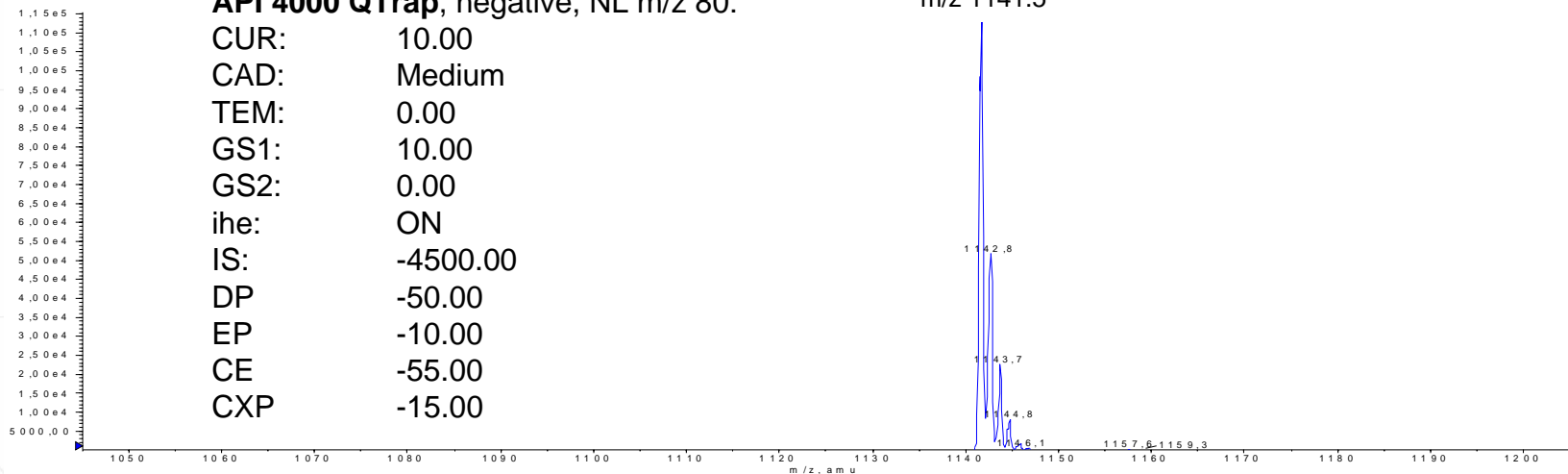
Flow rate: 300 μl/min

Temperature: 20°C

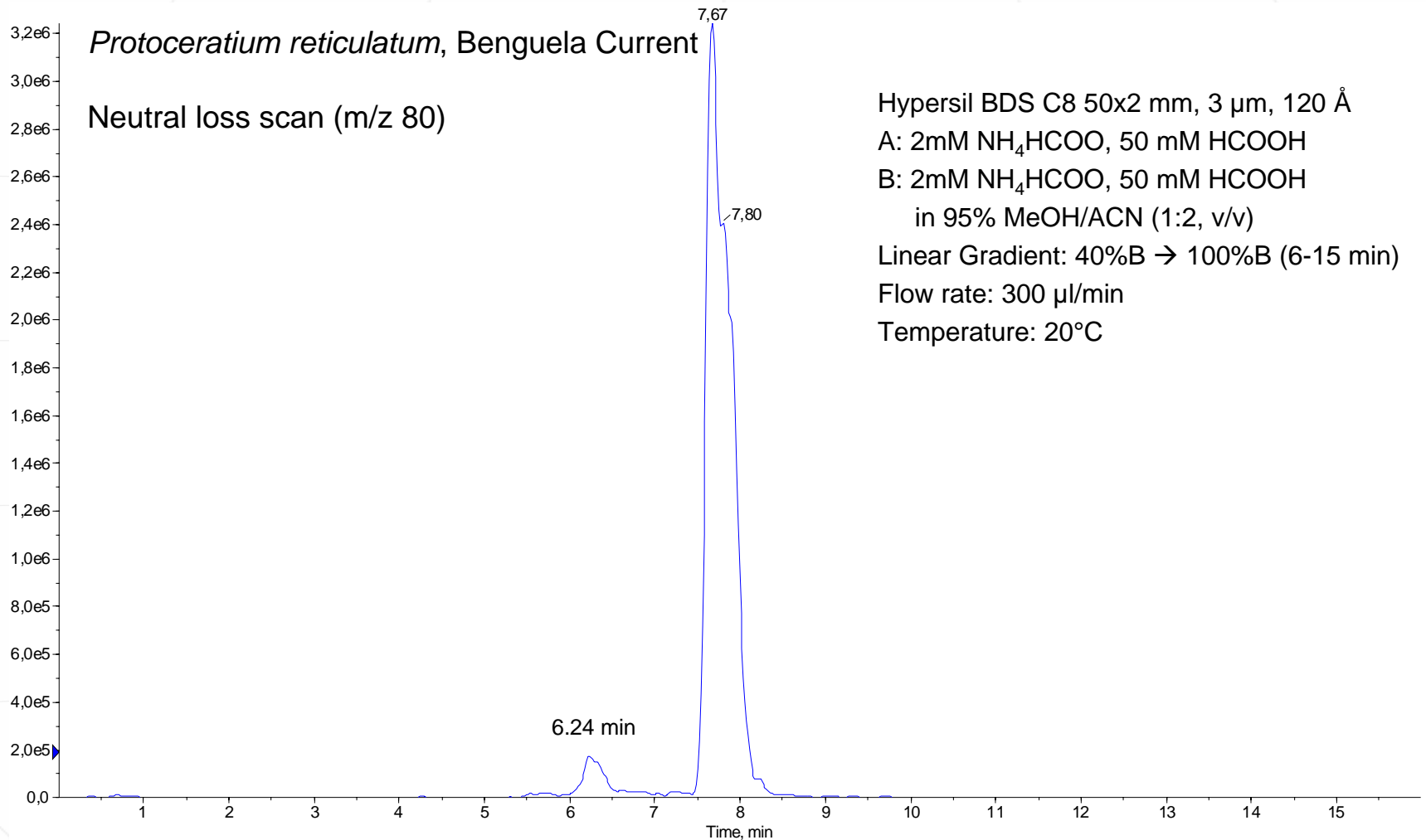
API 4000 QTrap, negative, NL m/z 80:

CUR:	10.00
CAD:	Medium
TEM:	0.00
GS1:	10.00
GS2:	0.00
ihe:	ON
IS:	-4500.00
DP:	-50.00
EP:	-10.00
CE:	-55.00
CXP:	-15.00

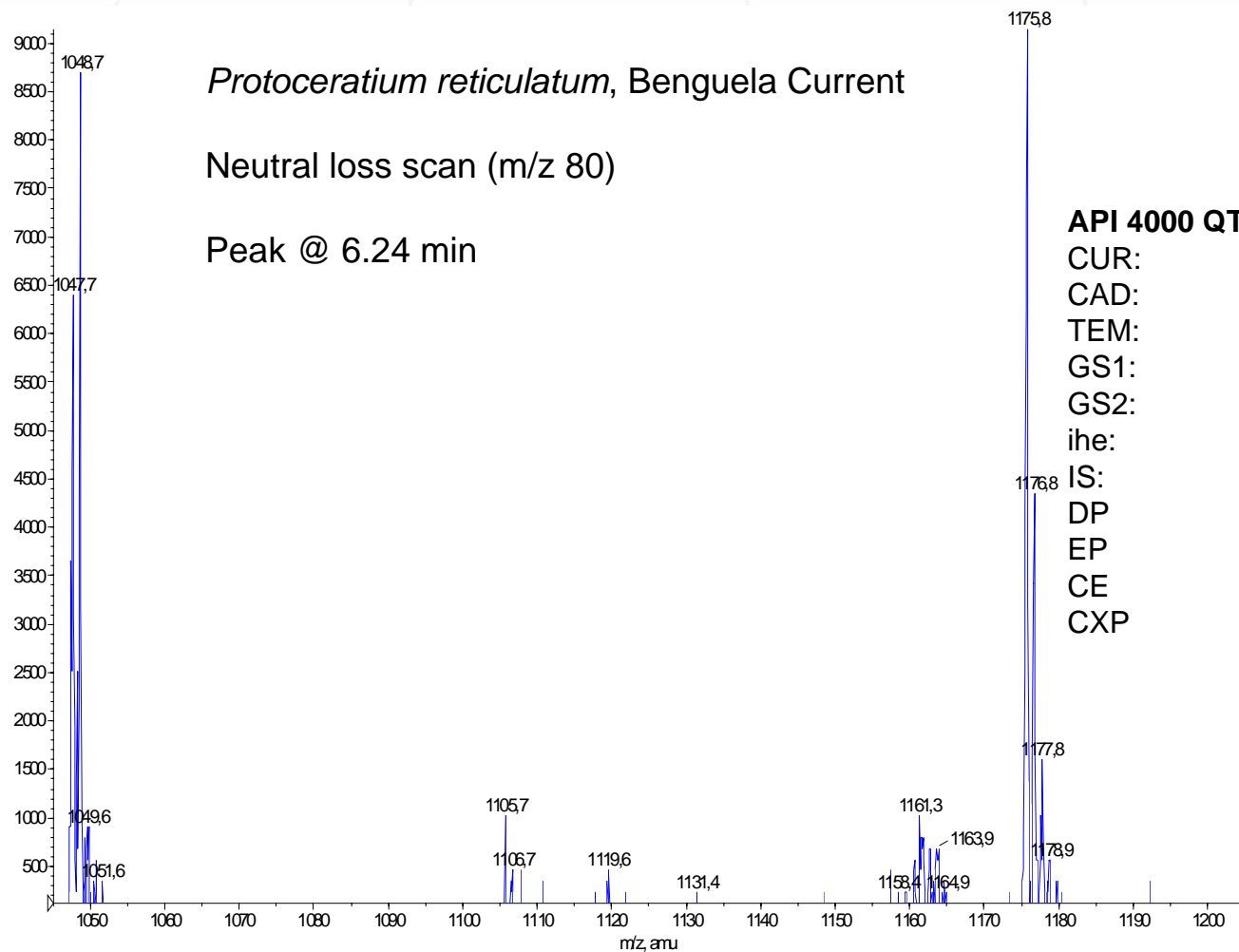
m/z 1141.5



Yessotoxin detection – Neutral Loss



Yessotoxin detection – Neutral Loss



Protoceratium reticulatum, Benguela Current

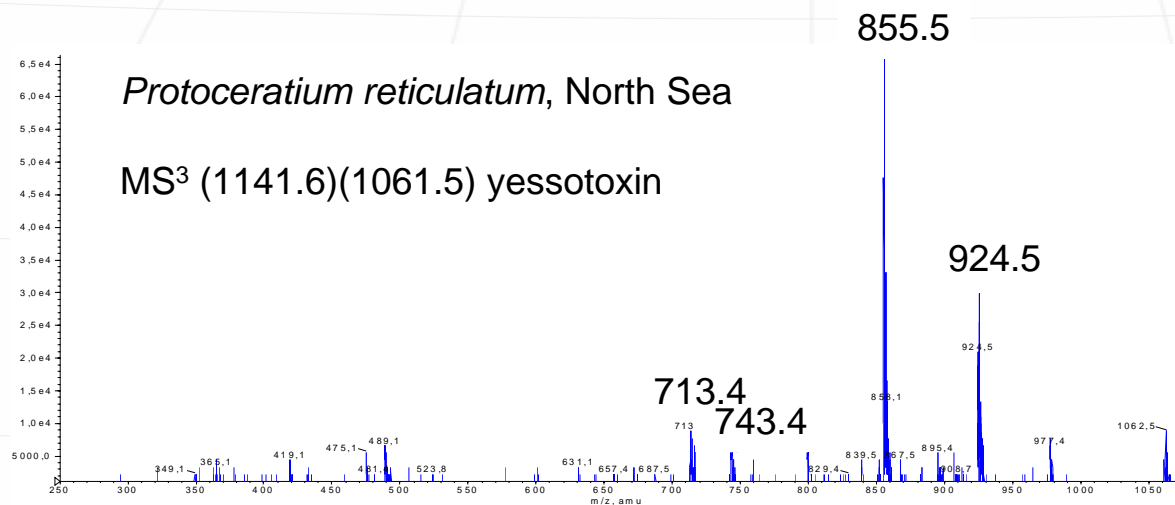
Neutral loss scan (m/z 80)

Peak @ 6.24 min

API 4000 QTrap, negative, NL m/z 80:

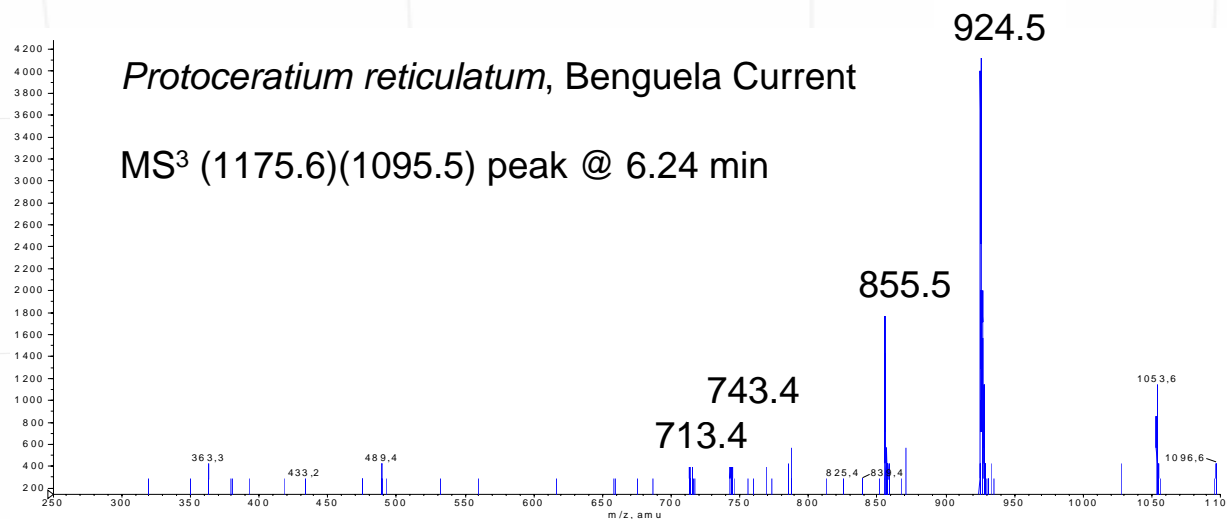
CUR:	10.00
CAD:	Medium
TEM:	0.00
GS1:	10.00
GS2:	0.00
ihe:	ON
IS:	-4500.00
DP:	-50.00
EP:	-10.00
CE:	-55.00
CXP:	-15.00

Yessotoxin confirmation – MS³



API 4000 QTrap, negative, MS3:

CUR:	10
CAD:	Medium
TEM:	0
GS1:	10
GS2:	0
ihe:	ON
IS:	-4500
DP	-50
AF2	150
CES	0
CE	-55



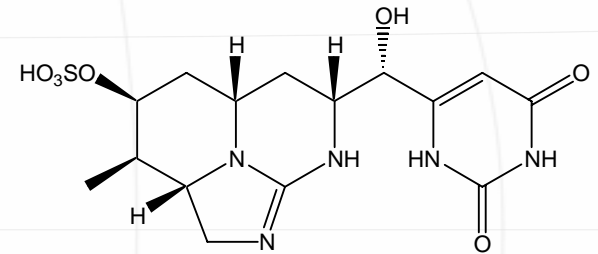
Causative Organisms

Cyanobacteria
(Blue-green algae)



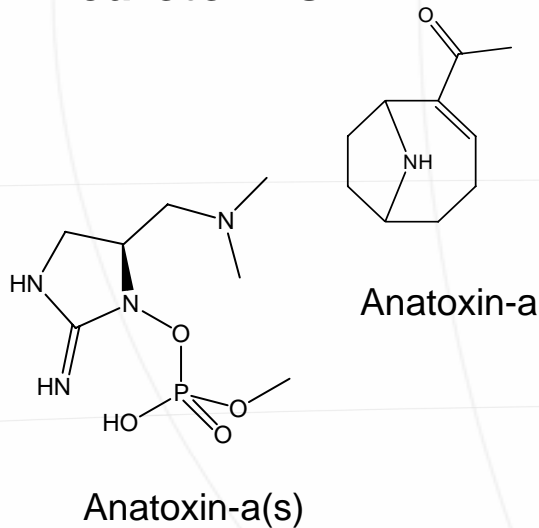
Anabaena flos-aquae

Hepatotoxins



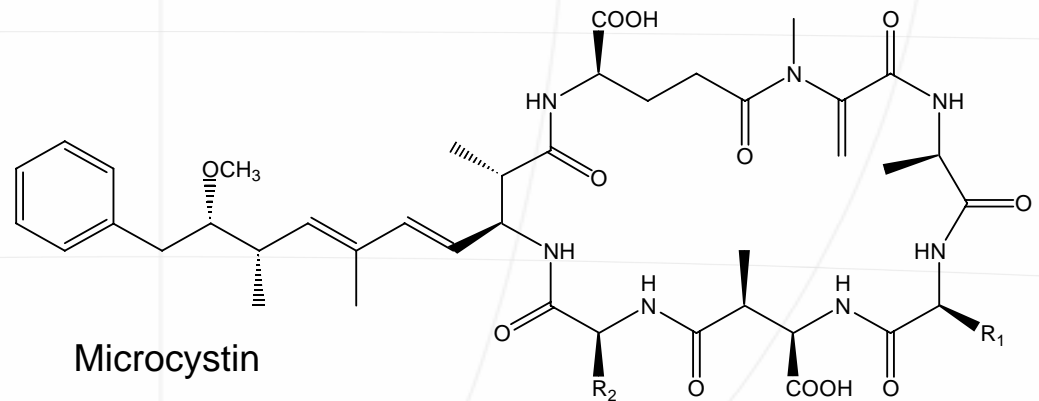
Cylindrospermopsin

Neurotoxins



Anatoxin-a

Anatoxin-a(s)



Microcystin

Cyanotoxin detection – Precursor Scan

Aim:

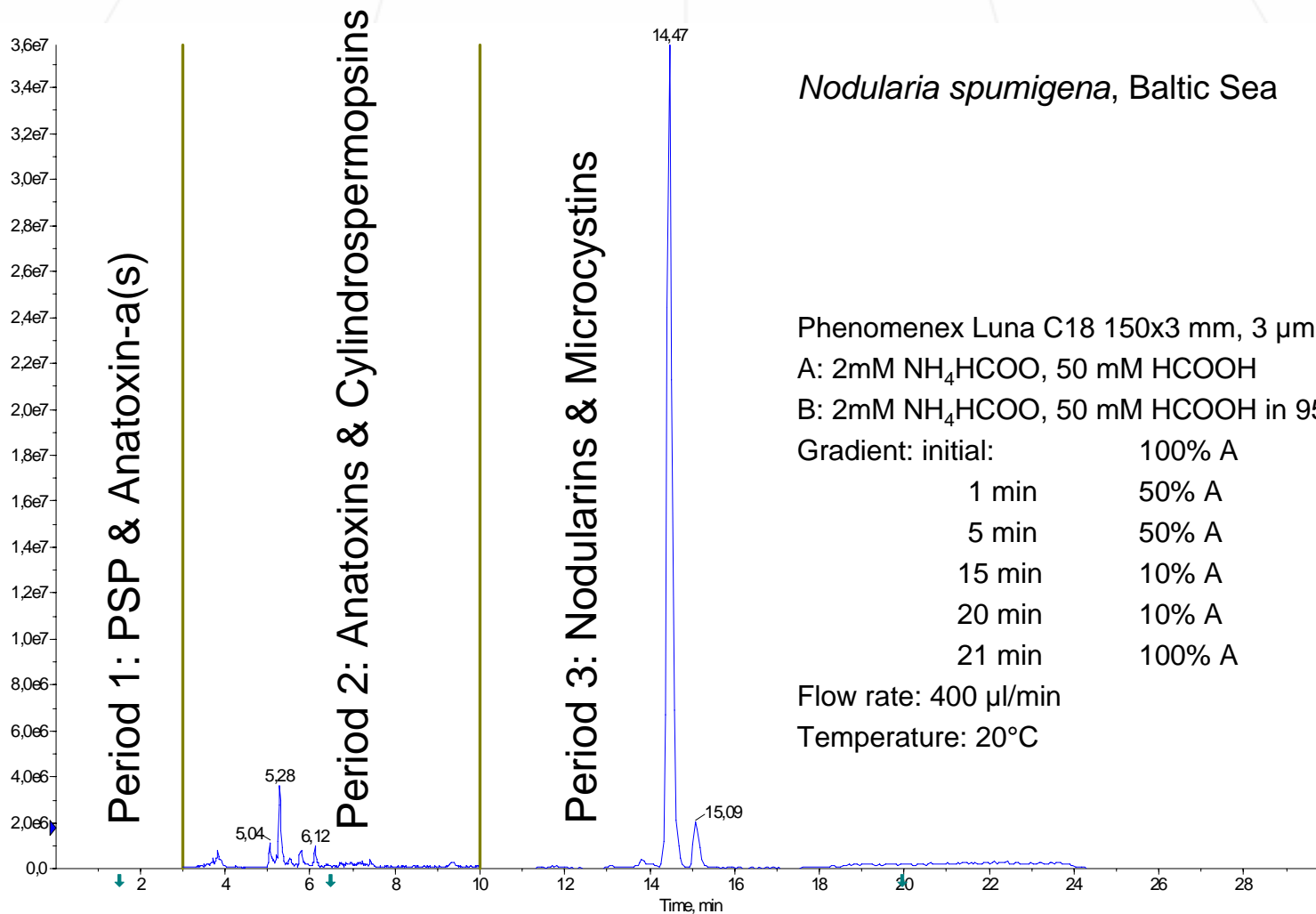
Survey method for the qualitative detection of cyanobacterial freshwater toxins

Prerequisites:

All toxins soluble in the same extraction solvent

Characteristic fragment for each toxin group

Toxin group (not single compound!) separation



Nodularia spumigena, Baltic Sea

Phenomenex Luna C18 150x3 mm, 3 μ m, 100 Å

A: 2mM NH₄HCOO, 50 mM HCOOH

B: 2mM NH₄HCOO, 50 mM HCOOH in 95% MeOH

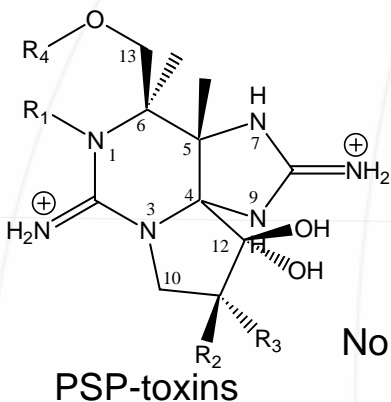
Gradient: initial:	100% A
1 min	50% A
5 min	50% A
15 min	10% A
20 min	10% A
21 min	100% A

Flow rate: 400 μ l/min

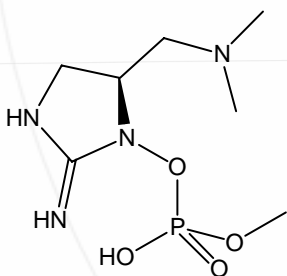
Temperature: 20°C

Hiller et al. J. Mass Spectrom. submitted

Period 1: PSP & Anatoxin-a(s)



No characteristic fragment



Only one toxin known

Mass transtions:

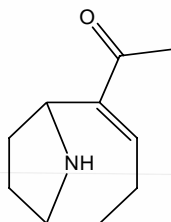
412 → 332 / 412 → 314 (GTX1, GTX4, C3, C4)
 396 → 316 / 396 → 298 (GTX2/3, B2, C1, C2)
 380 → 300 / 380 → 282 (B1)
 369 → 289 (dcGTX1/4)
 353 → 273 (dcGTX2/3)
 316 → 298 (NEO, GTX2/3, B2, C1, C2)
 316 → 220 (NEO)
 300 → 282 / 300 → 204 (STX, B1)
 273 → 255 (dcNEO, dcGTX2, dcGTX3)
 257 → 239 (dcSTX)
 253 → 235 / 253 → 159 (ANAS)

API 4000 QTrap, positive, MRM:

IS: 5000 V
 CAD: high level
 TEM.: 550 °C
 GS 1: 50 L h-1
 GS 2: 70 L h-1
 CUR: 25 L h-1
 CE: 30 eV
 DP: 40 eV

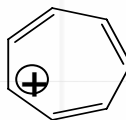
Period 2: Anatoxins & Cylindrospermopsins

Experiment 1



Anatoxin-a

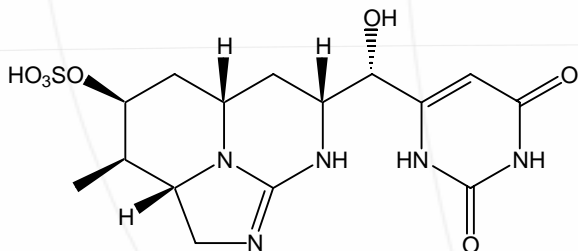
characteristic fragment: m/z 91



API 4000 QTrap, positive precursor ion (m/z): 91.0 scan range (m/z): 100-300 amu

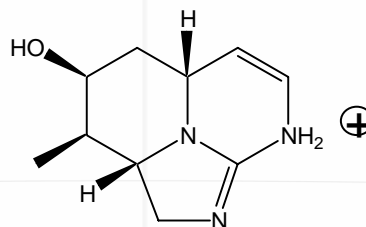
CUR:	25
CAD:	High
IS:	5200
TEM:	550
GS1:	50
GS2:	70
ihe:	OFF
DP:	80
EP:	10
CE:	30
CXP:	12

Experiment 2



Cylindrospermopsin

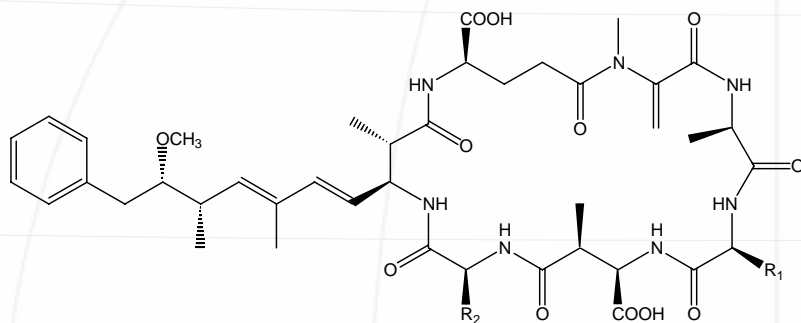
characteristic fragment: m/z 194



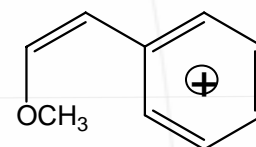
API 4000 QTrap, positive precursor ion (m/z): 194.0 scan range (m/z): 350-450 amu

CUR:	25
CAD:	High
IS:	5200
TEM:	550
GS 1:	50
GS 2:	70
CE:	50
DP:	80

Period 3: Microcystins & Nodularins

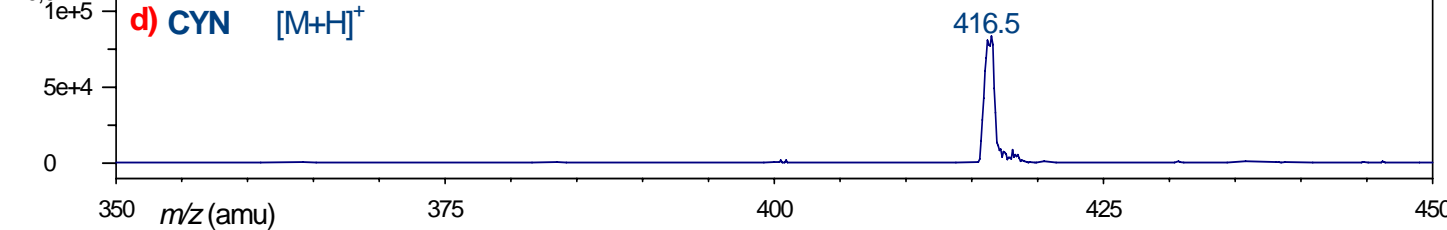
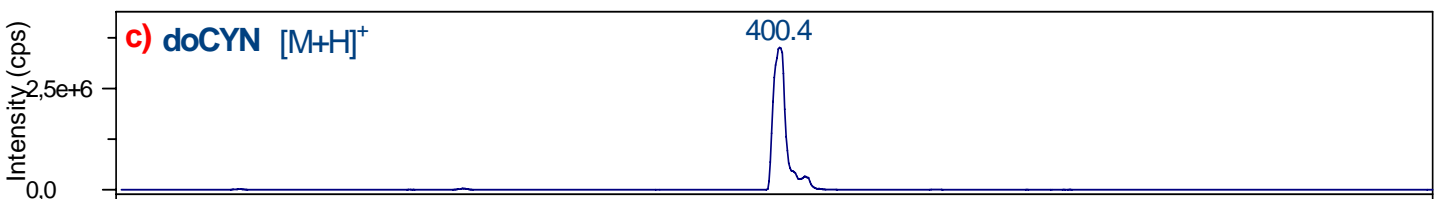
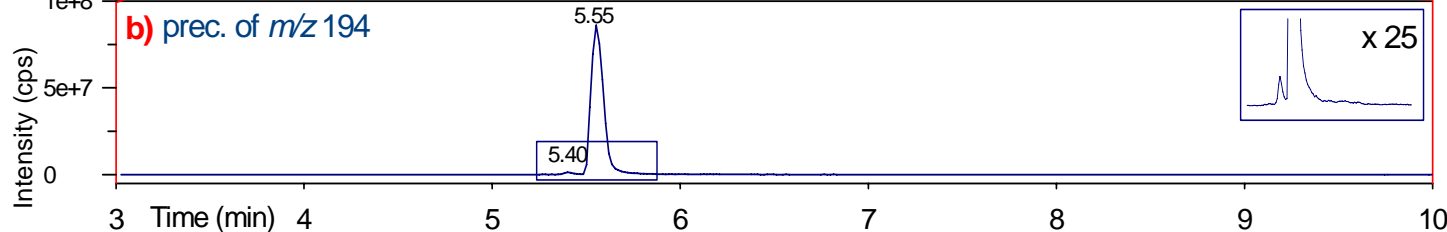
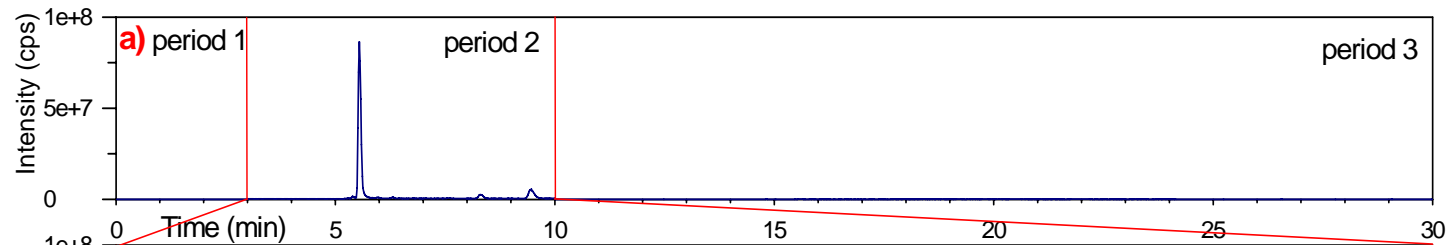


characteristic fragment: m/z 135



	experiment 1	experiment 2	experiment 3	experiment 4
scan range (m/z)	400 - 575	400 - 575	900 - 1150	800 - 850
protonated fragment ions [M+H] ⁺ / [M+2H] ²⁺	[M+2H] ²⁺	[M+2H] ²⁺	[M+H] ⁺	[M+H] ⁺
collision energy (eV)	17	35	60	90
declustering potential (V)	46	40	60	175
cyanobacterial toxins: microcystins / nodularins	microcystins	microcystins	microcystins	nodularins
number of Arg residues within the microcystin peptide	1, exceptional 0	2	0	

Lyngbya wollei, Australia

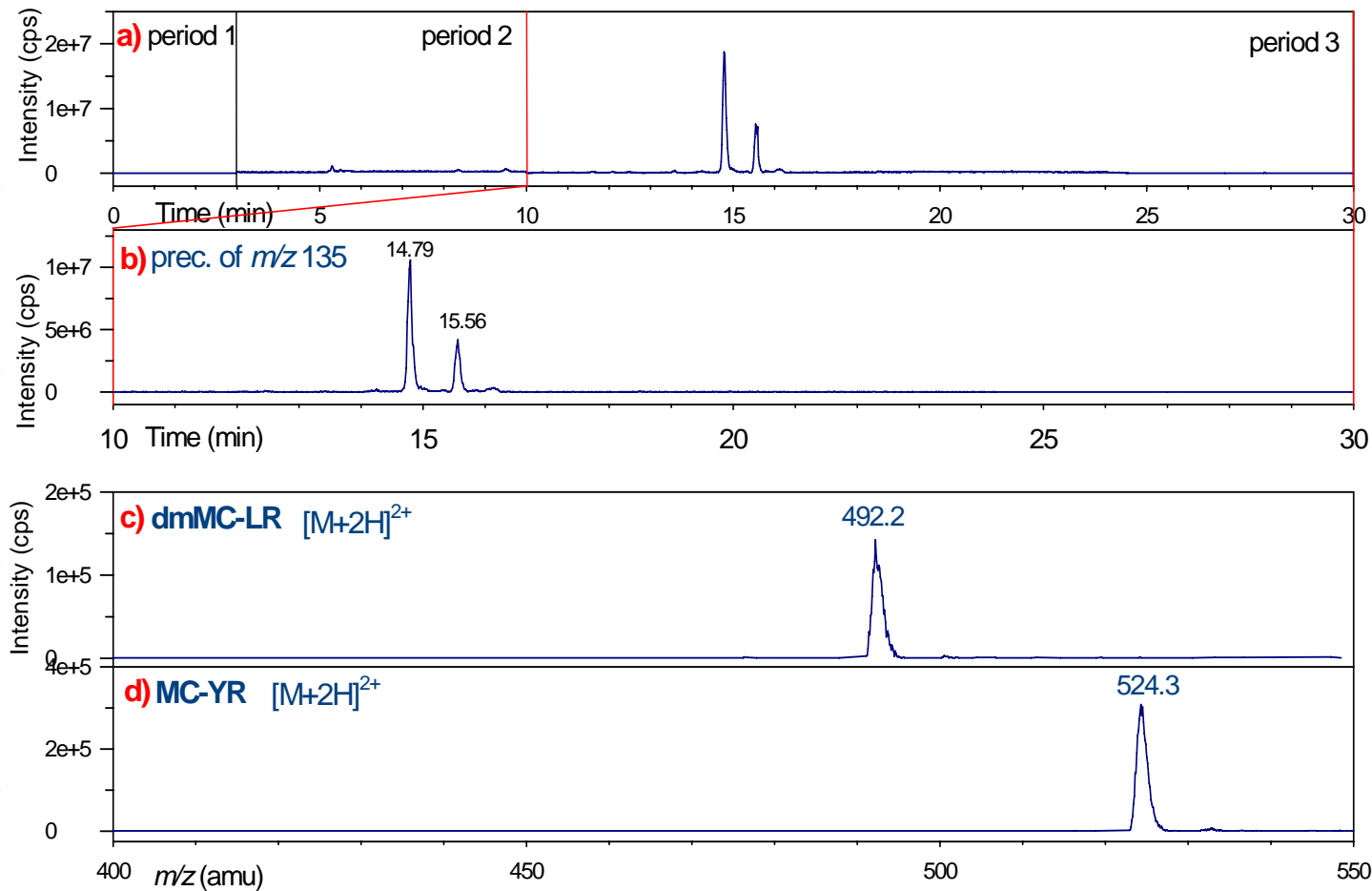


MS parameters
for detection of
CYNs

Precursor Ion mode
protonated ion:
 $[M+H]^+$
precursor ion (m/z):
194.0
scan range (m/z):
350-450 amu

IS: 5200 V
CAD: high level
Temp.: 550 °C
Gas 1: 50 L h⁻¹
Gas 2: 70 L h⁻¹
CUR: 25 L h⁻¹
CE: 50 eV
DP: 80 eV

Unknown cyanobacterial sample



MS parameters
for detection of
MCs

Precursor Ion mode
protonated ion:
 $[M+2H]^{2+}$
precursor ion (m/z):
135.0
scan range (m/z):
400-550 amu

IS: 5500 V
CAD: high level
Temp.: 550 °C
Gas 1: 50 L h⁻¹
Gas 2: 70 L h⁻¹
CUR: 15 L h⁻¹
CE: 35 eV
DP: 40 eV

Thanks to...



Susann Hiller, Friedrich-Schiller-Universität Jena



Wolfgang Drebing, AWI

...and for your attention!