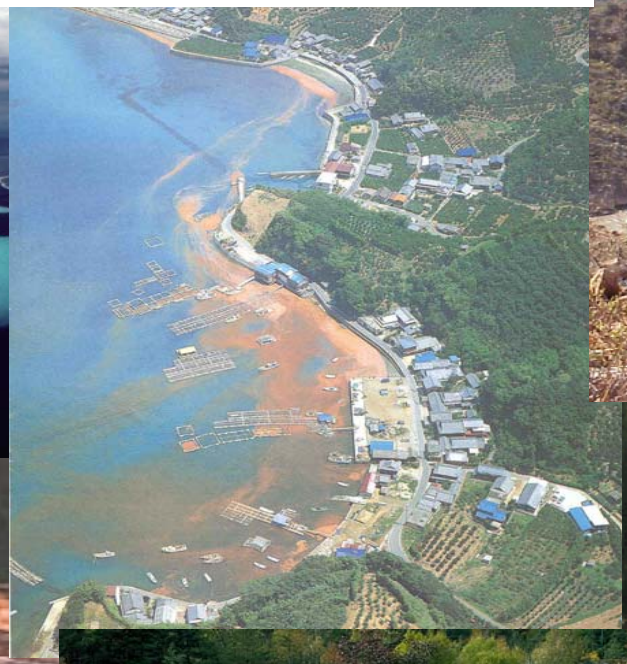
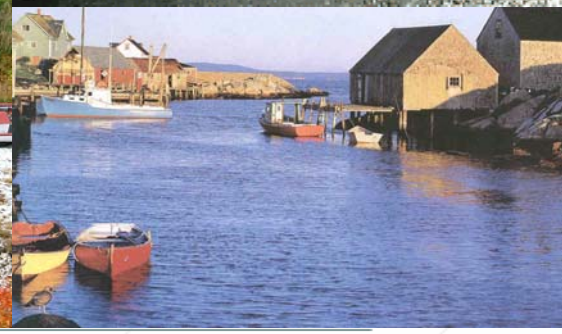
The Analysis of Algal Toxins Using Various Scan Modes in LC/MS/MS

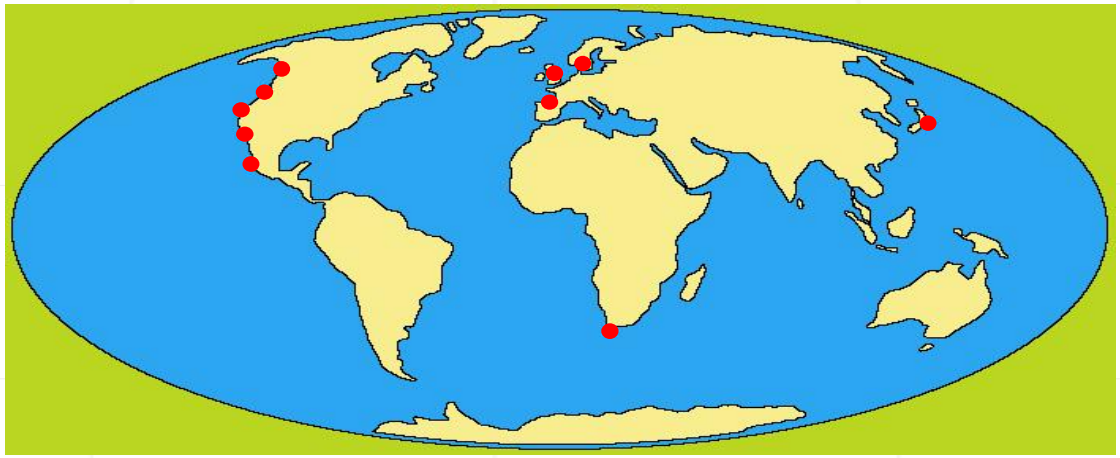
Toxic Algal Blooms



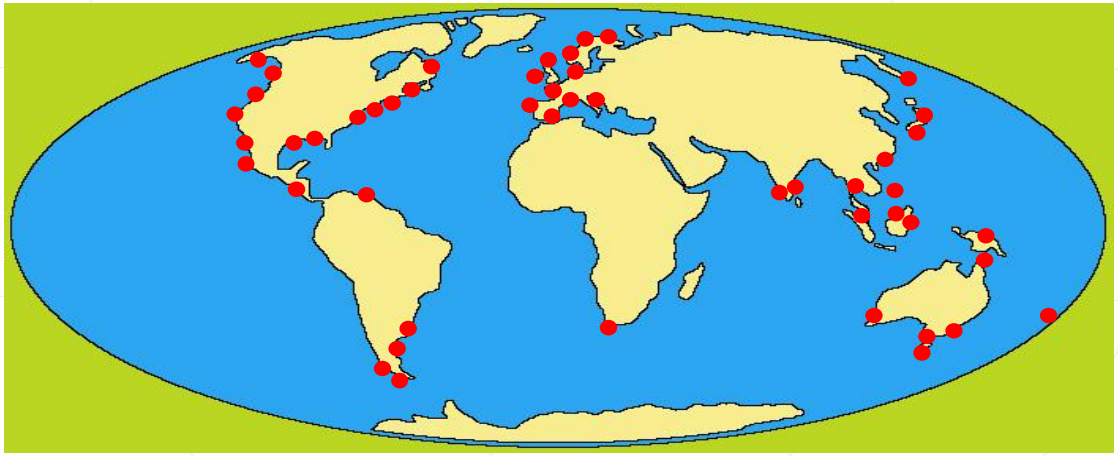


Distribution of Paralytic Shellfish Poisoning events

1970

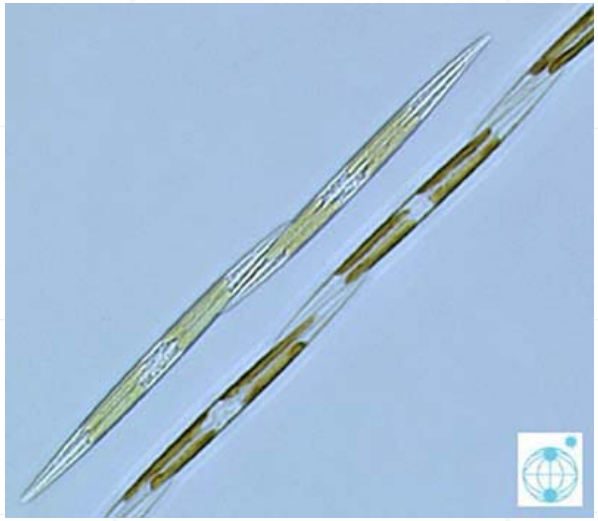


2005



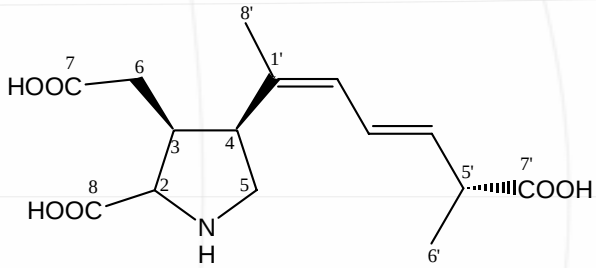
Causative Organisms

Diatoms



Pseudo-Nitzschia pungens

Amnesic Shellfish Poisoning

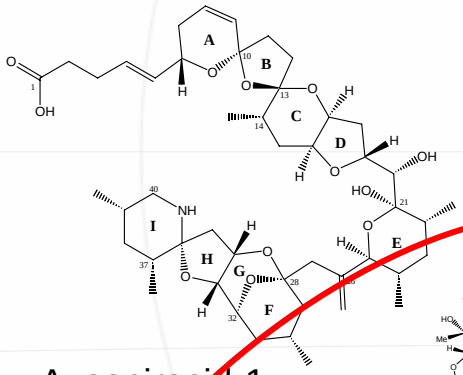


Domoic acid

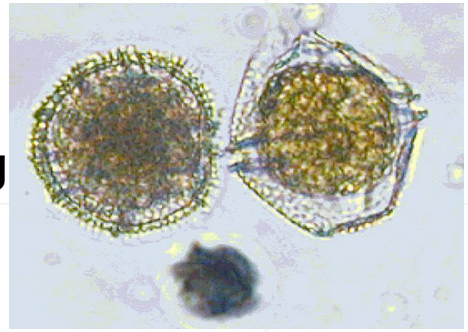
Causative Organisms

Dinoflagellates

Diarrhetic Shellfish Poisoning

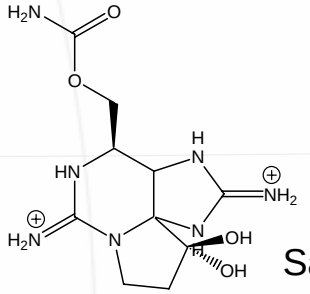


Azaspiracid-1



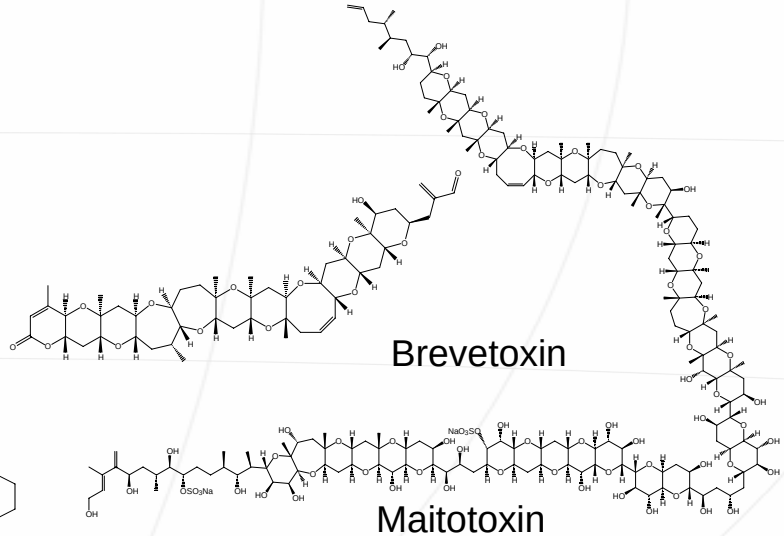
Lingulodinium polyedrum

Paralytic Shellfish Poisoning



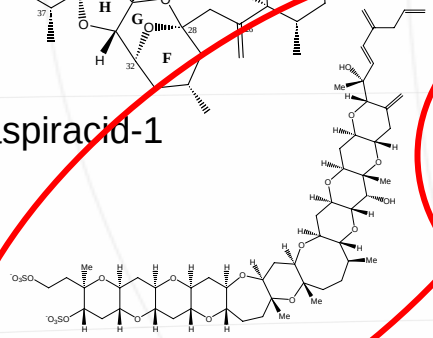
Saxitoxin

Neurotoxic Shellfish Poisoning

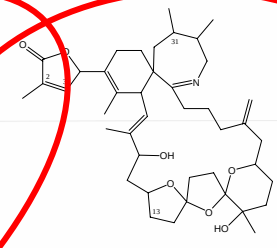


Brevetoxin

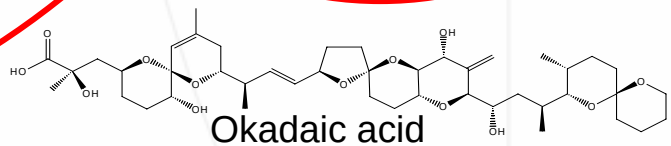
Maitotoxin



Yessotoxin

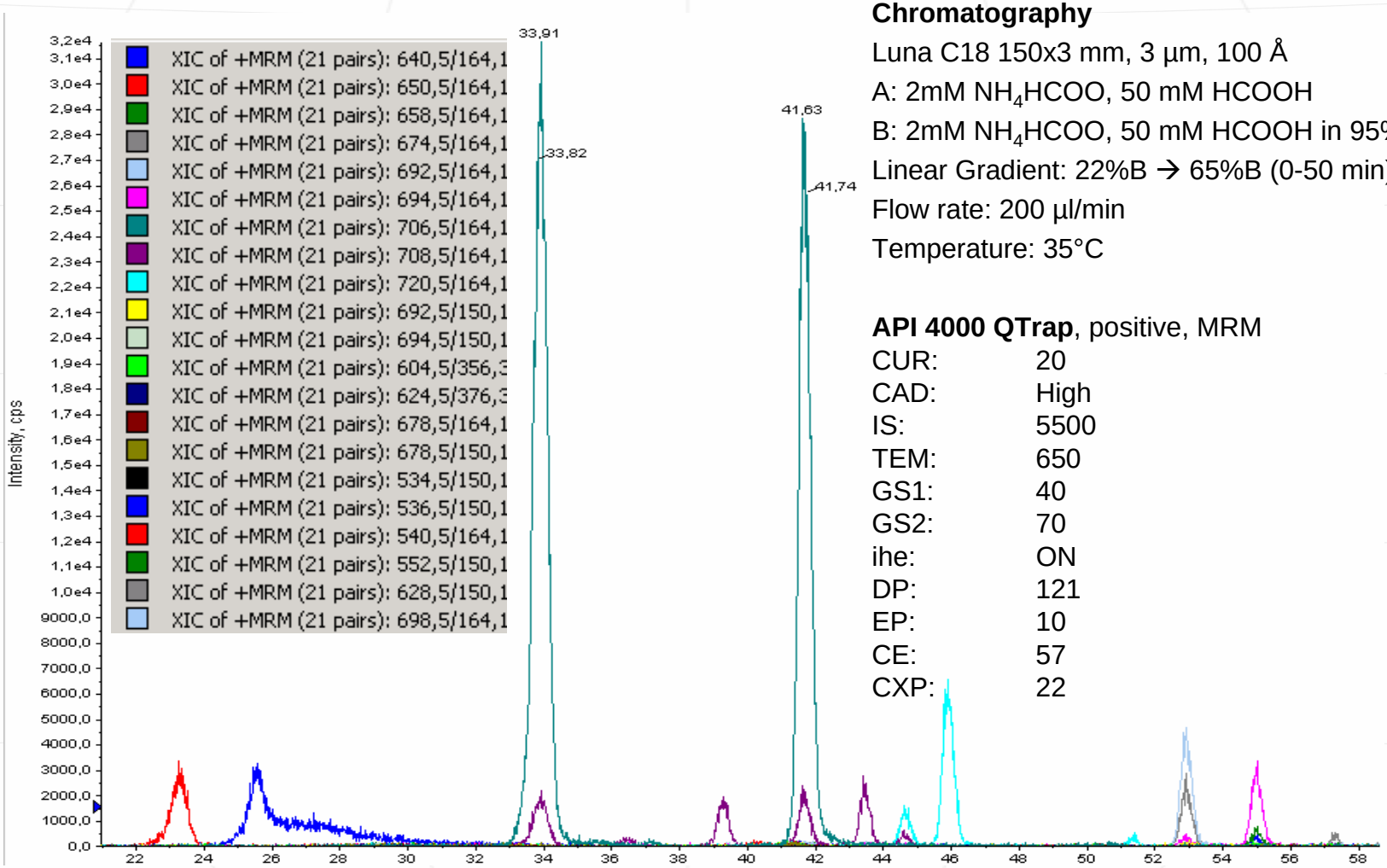


13-desmethyl spirolide C



Okadaic acid

Spirolide determination - MRM



Chromatography

Luna C18 150x3 mm, 3 µm, 100 Å

A: 2mM NH_4HCOO, 50 mM HCOOH

B: 2mM NH_4HCOO, 50 mM HCOOH in 95% ACN

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

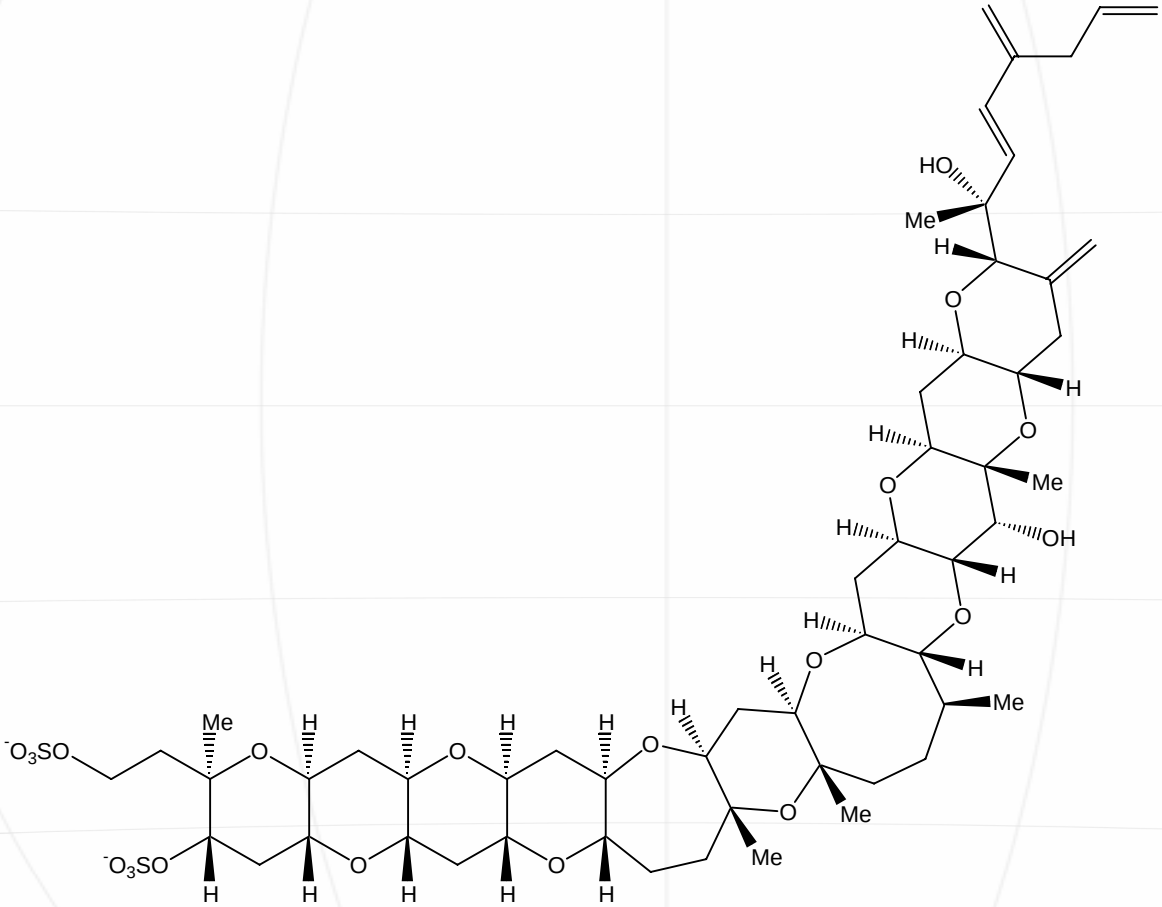
Flow rate: 200 µl/min

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)

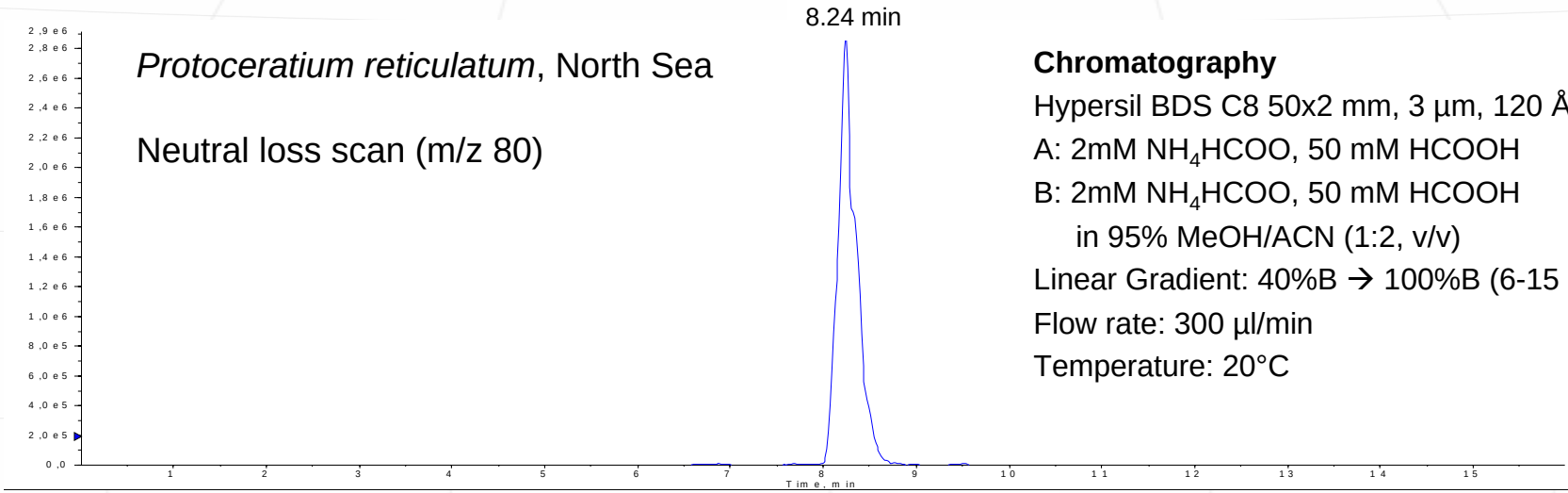
YTX analogs detected by LC–MS^3 analysis of a fractionated extract of *Protoceratium reticulatum*

Entry	M_{wt}	R_t	$[M-H]^-$	MS^2	MS^3 (see Fig. 5)	$[M-2H]^{2-}$	Relative intensity[a]	MS^2	MS^3	Structure[b]
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 SO_3 from the sulfate groups

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

Yessotoxin detection – Neutral Loss



Protoceratium reticulatum, North Sea

Neutral loss scan (m/z 80)

Chromatography

Hypersil BDS C8 50x2 mm, 3 µm, 120 Å

A: 2mM NH_4HCOO, 50 mM HCOOH

B: 2mM NH_4HCOO, 50 mM HCOOH
in 95% MeOH/ACN (1:2, v/v)

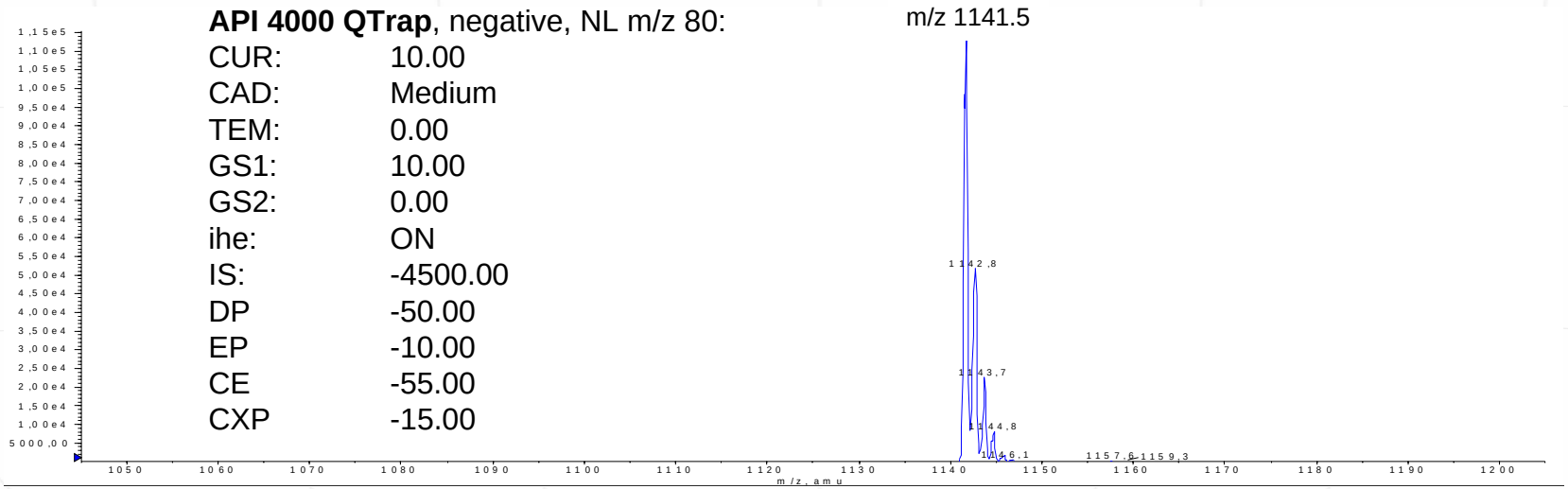
Linear Gradient: 40%B → 100%B (6-15 min)

Flow rate: 300 µl/min

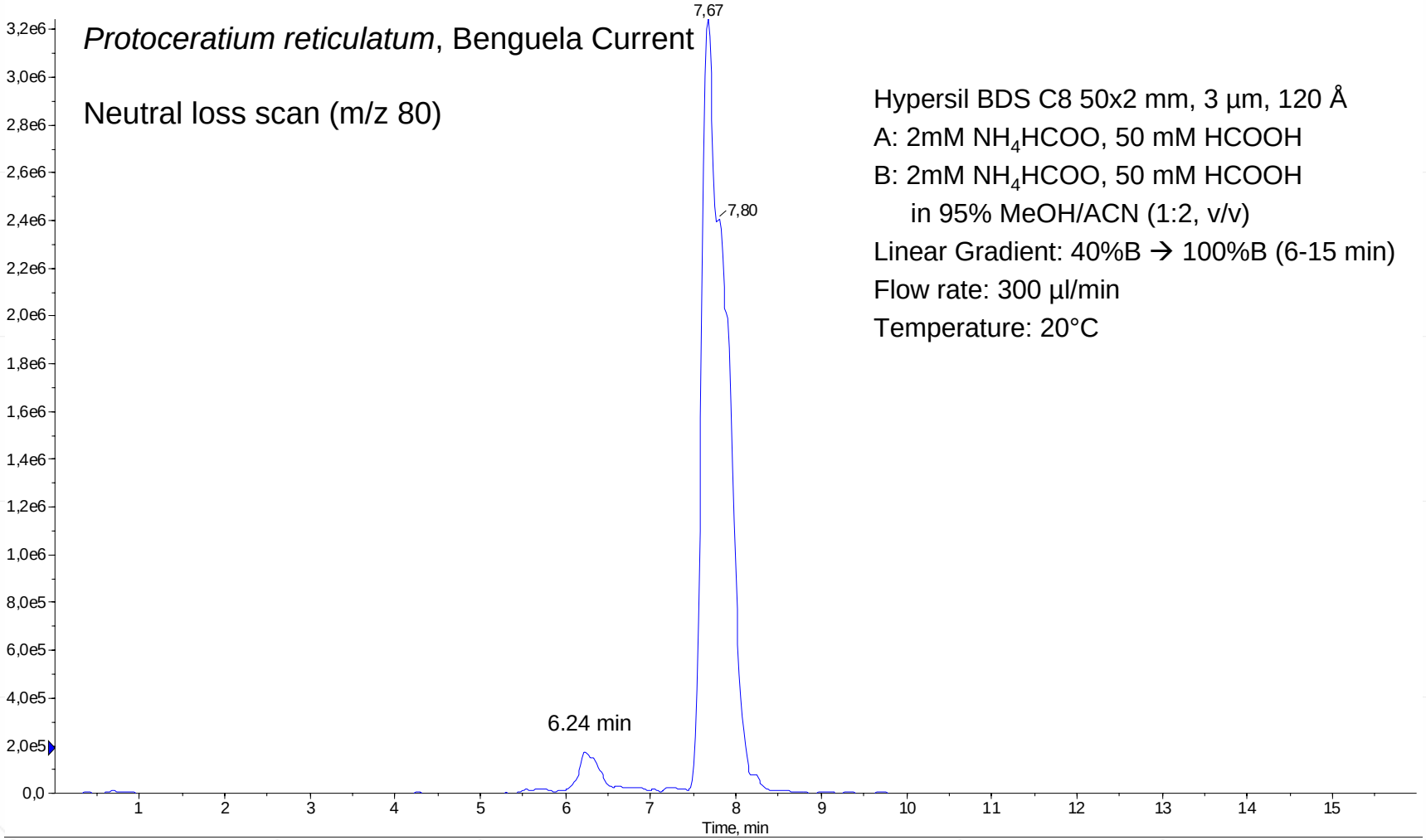
Temperature: 20°C

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

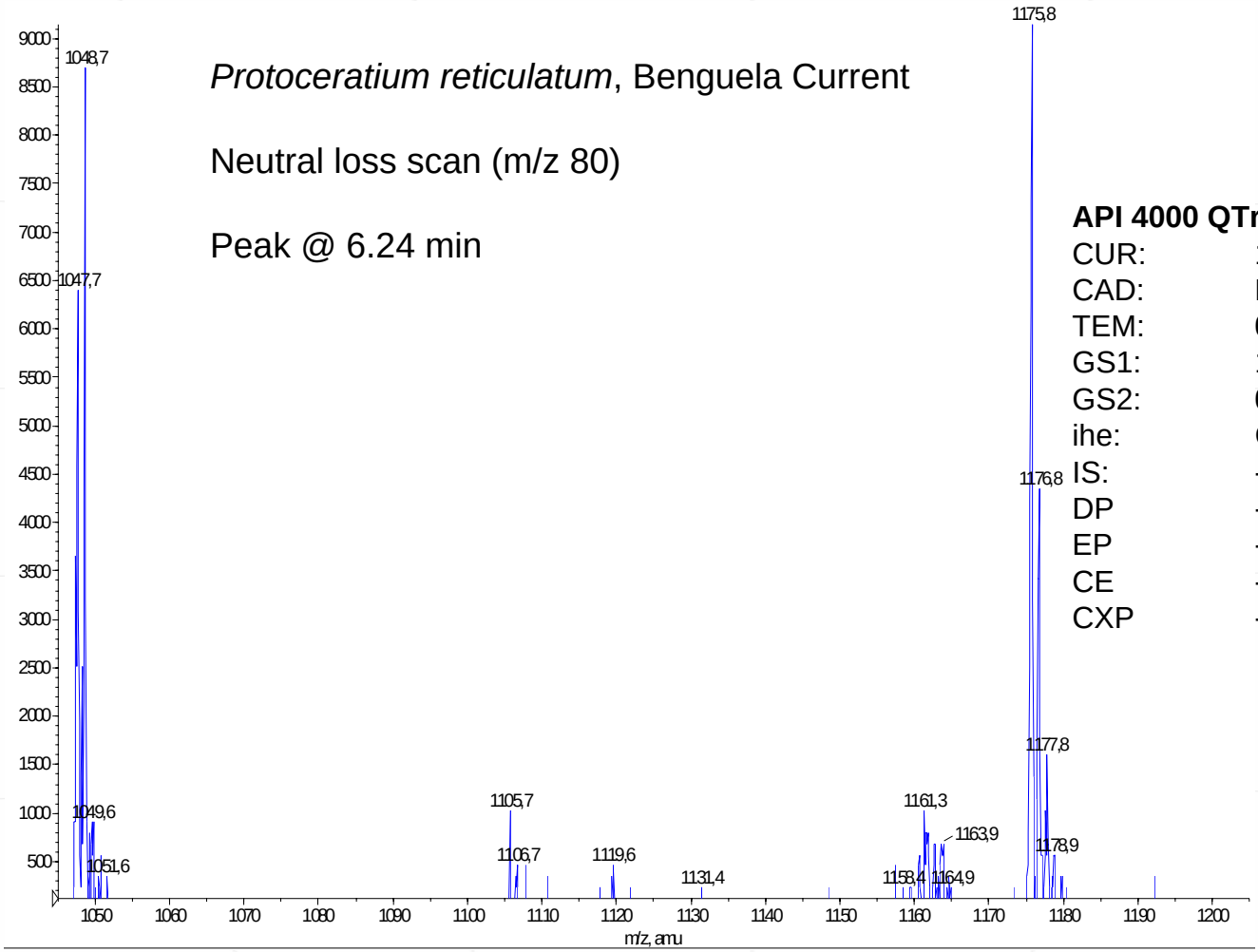


Yessotoxin detection – Neutral Loss



Hypersil BDS C8 50x2 mm, 3 µm, 120 Å
A: 2mM NH_4HCOO, 50 mM HCOOH
B: 2mM NH_4HCOO, 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

Yessotoxin detection – Neutral Loss



Protoceratium reticulatum, Benguela Current

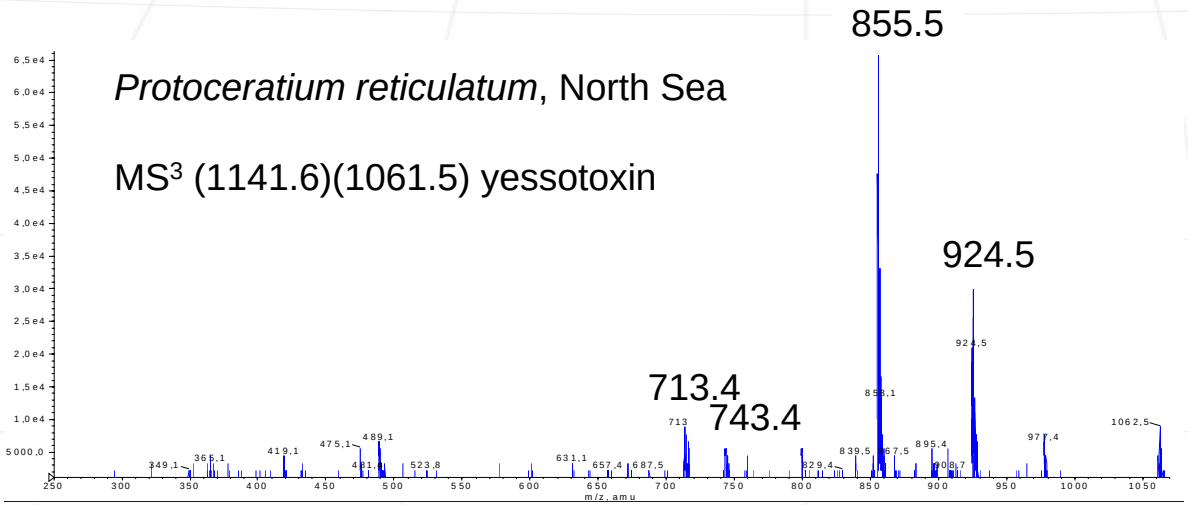
Neutral loss scan (m/z 80)

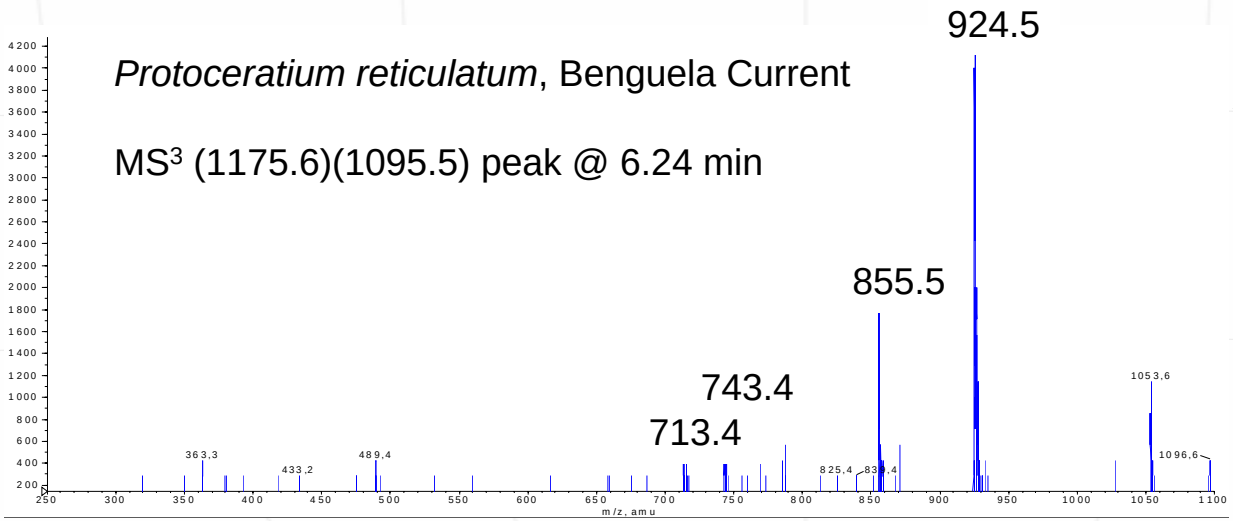
Peak @ 6.24 min

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 confirmationtion – MS^3





API 4000 QTrap, negative, MS3:

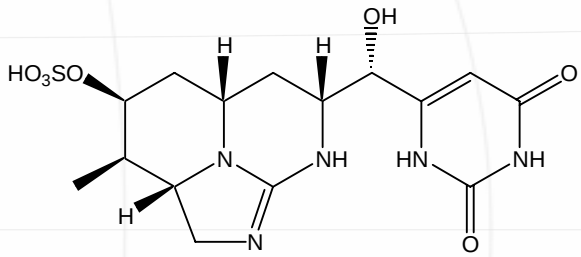
CUR:	10
CAD:	Medium
TEM:	0
GS1:	10
GS2:	0
ihe:	ON
IS:	-4500
DP	-50
AF2	150
CES	0
CE	-55

Causative Organisms

Cyanobacteria
(Blue-green algae)

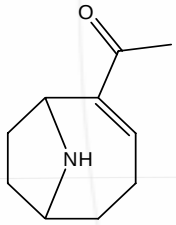
Anabaena flos-aquae

Hepatotoxins

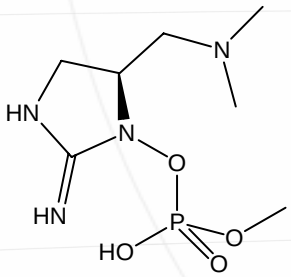


Cylindrospermopsin

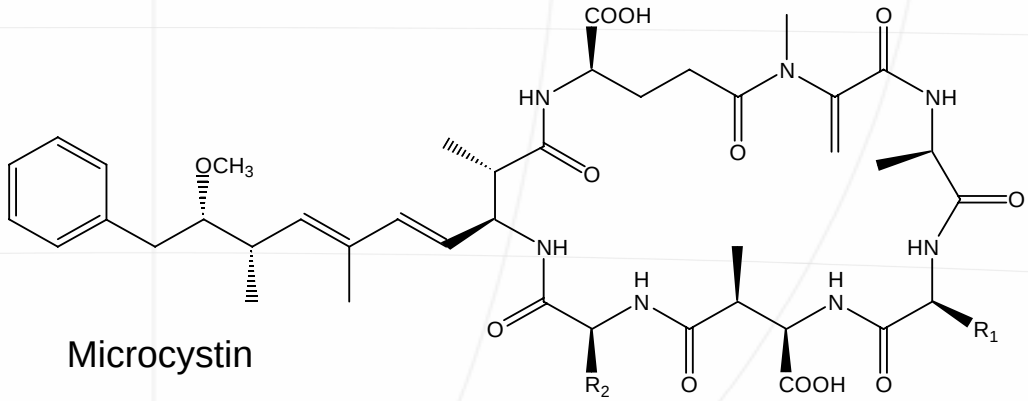
Neurotoxins



Anatoxin-a



Anatoxin-a(s)



Microcystin

Cyanotoxin detection – Precursor Scan

Aim:

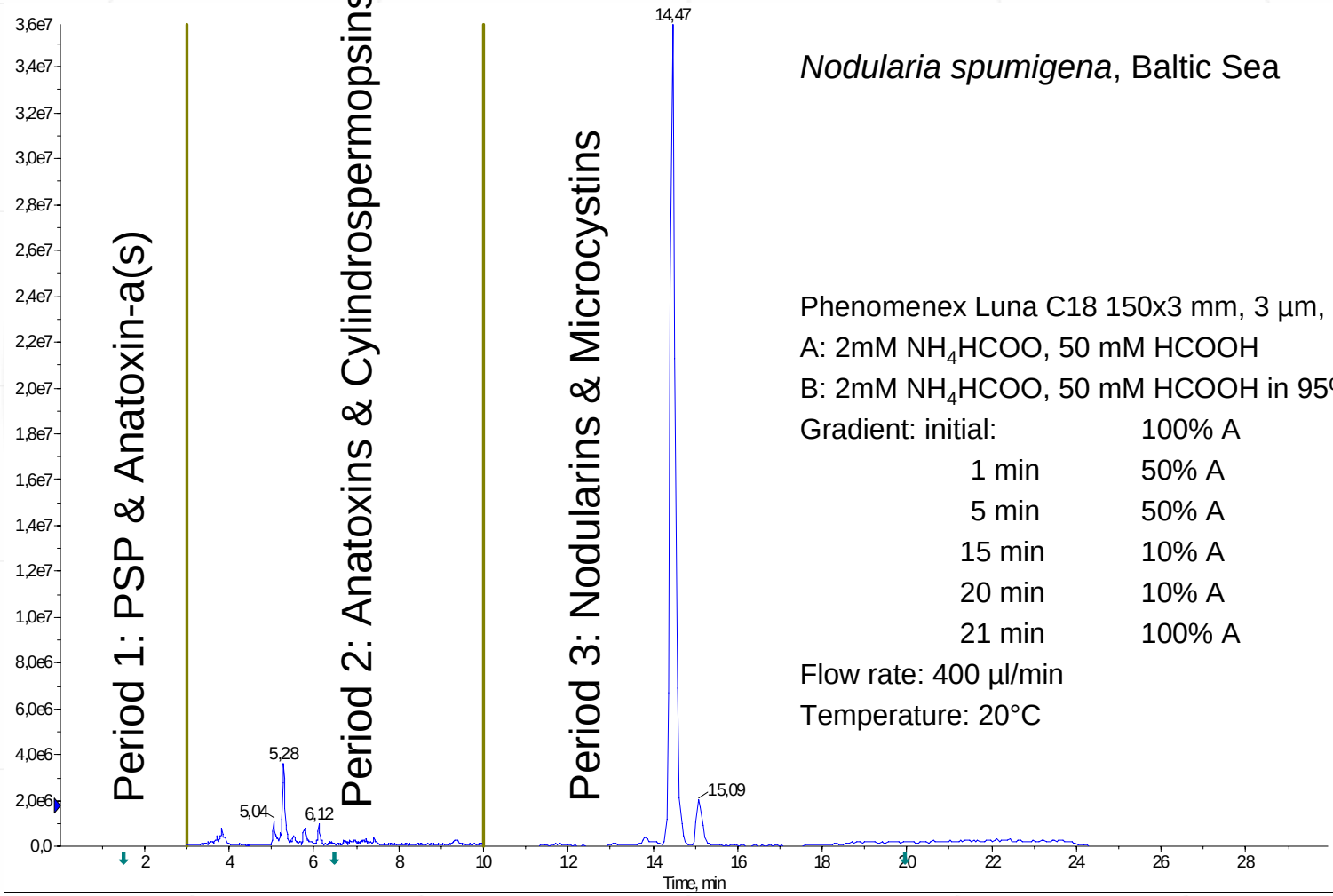
Survey method for the qualitative detection of cyanobacterial freahwater toxins

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 µm, 100 Å

A: 2mM NH_4HCOO, 50 mM HCOOH

B: 2mM NH_4HCOO, 50 mM HCOOH in 95% MeOH

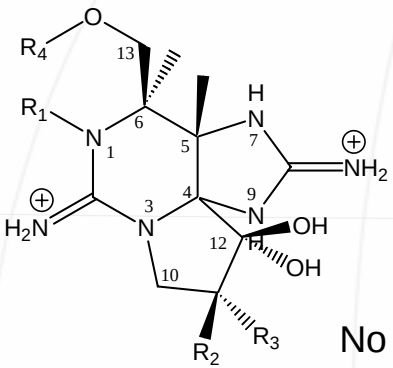
Gradient: initial:	100% A
1 min	50% A
5 min	50% A
15 min	10% A
20 min	10% A
21 min	100% A

Flow rate: 400 µl/min

Temperature: 20°C

Hiller et al. J. Mass Spectrom. submitted

Period 1: PSP & Anatoxin-a(s)

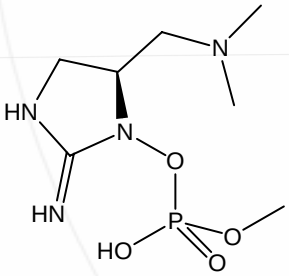


PSP-toxins

No characteristic fragment

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)



Anatoxin-a(s)

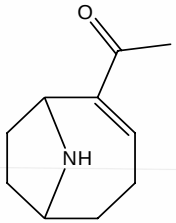
Only one toxin known

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

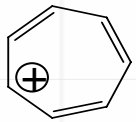
Period 2: Anatoxins & Cylindrospermopsins

Experiment 1



Anatoxin-a

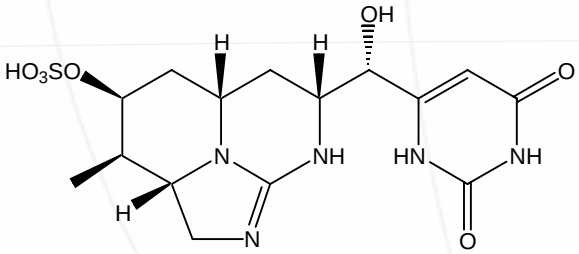
characteristic fragment: m/z 91



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

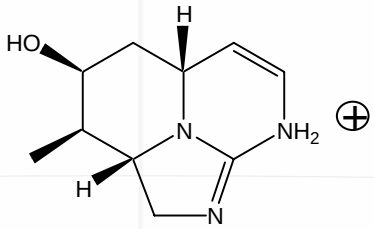
CUR:	25
CAD:	High
IS:	5200
TEM:	550
GS1:	50
GS2:	70
ihe:	OFF
DP:	80
EP:	10
CE:	30
CXP:	12

Experiment 2



Cylindrospermopsin

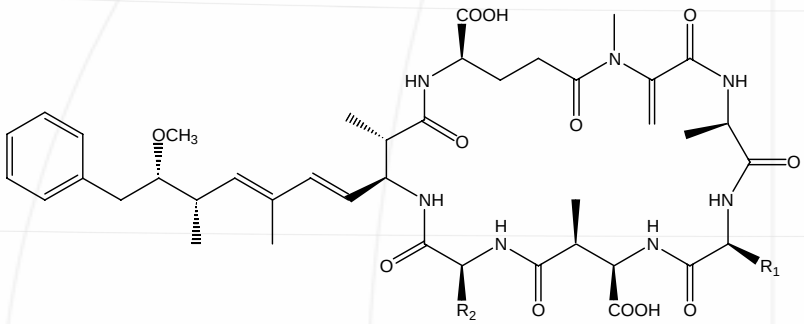
characteristic fragment: m/z 194



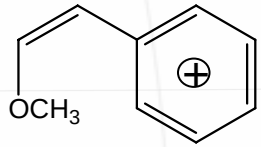
API 4000 QTrap, positive
precursor ion (*m/z*): 194.0
scan range (*m/z*): 350-450 amu

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

Period 3: Microcystins & Nodularins

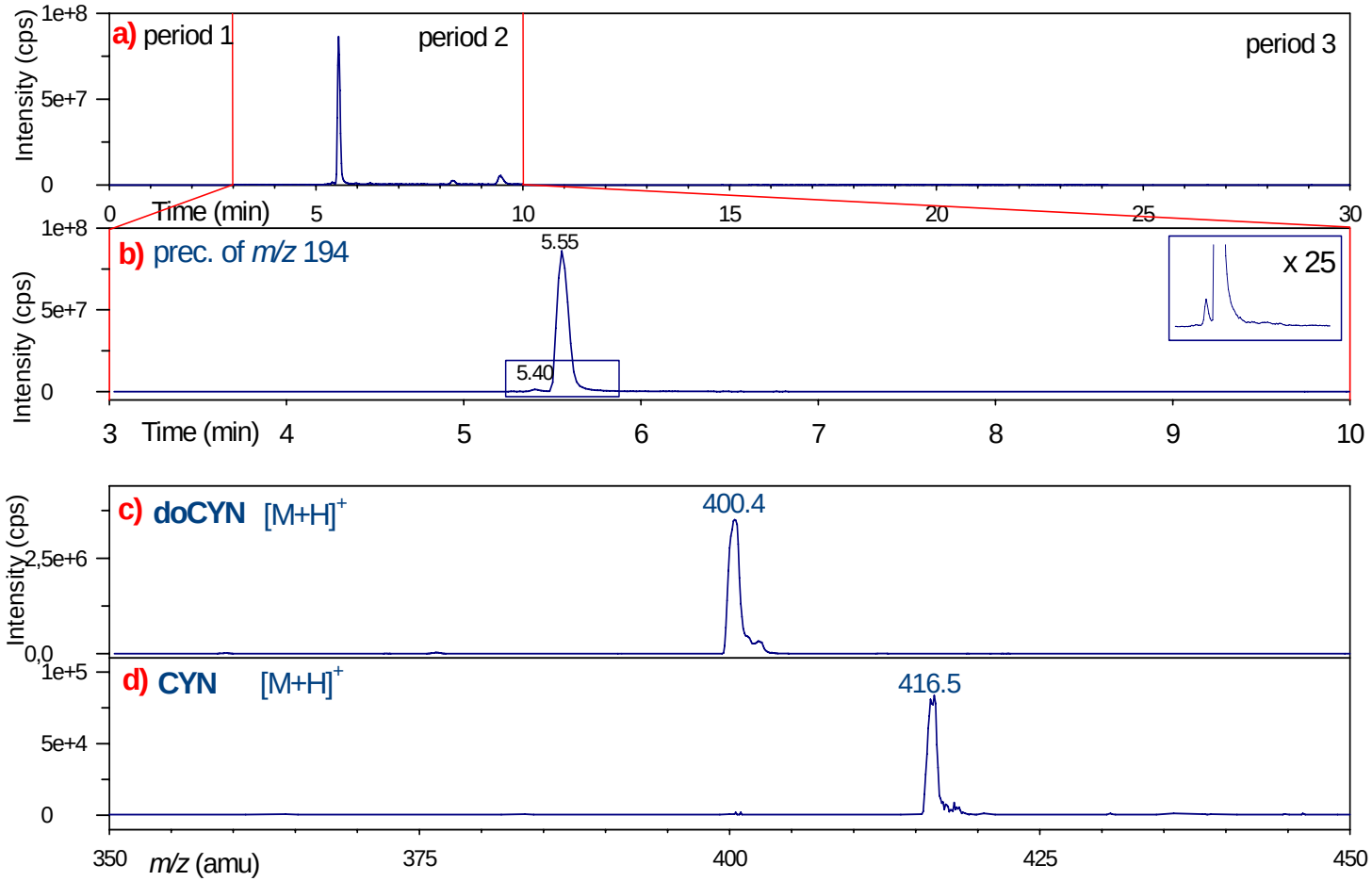


characteristic fragment: m/z 135



	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

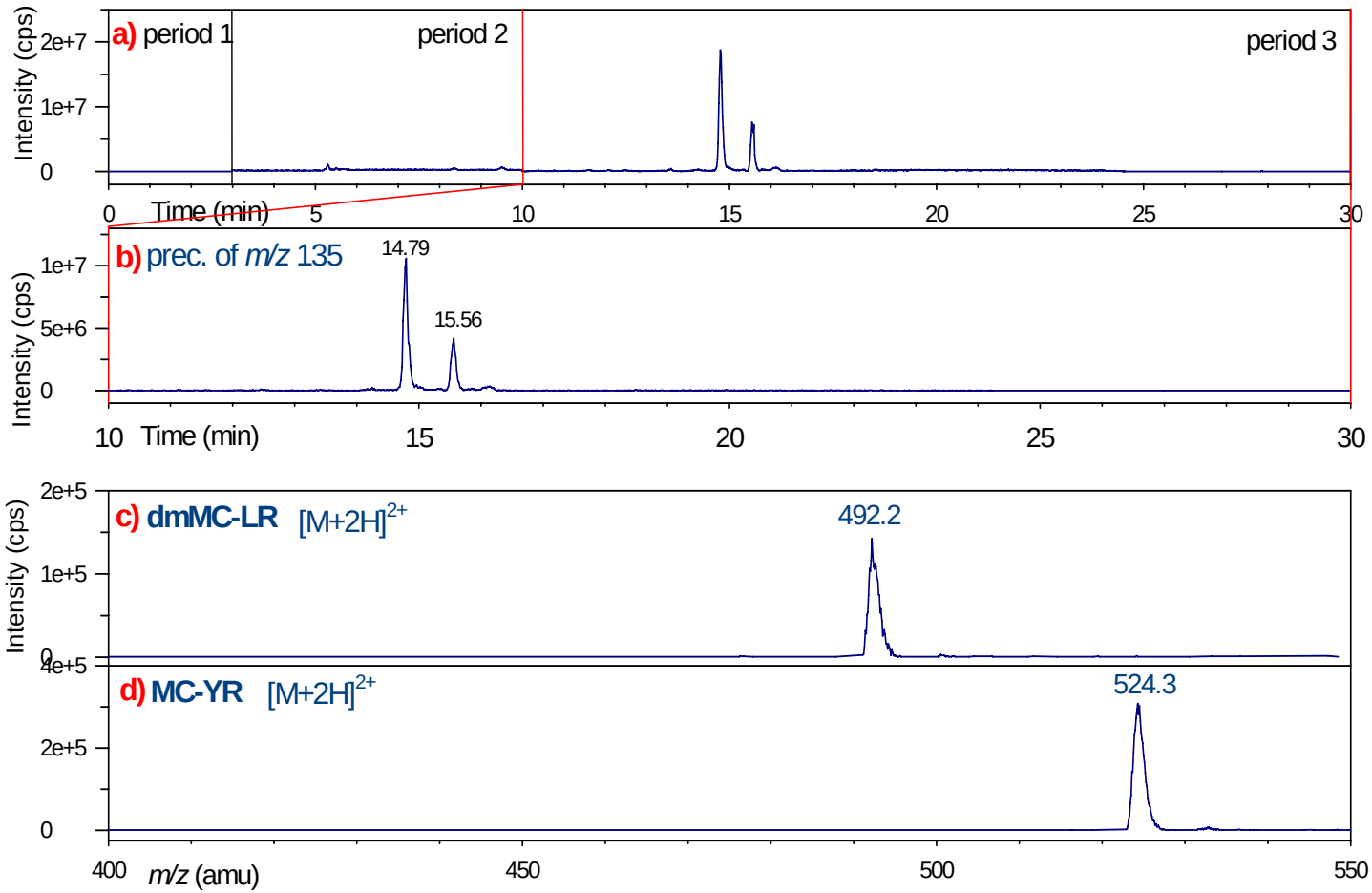


MS parameters for detection of CYNs

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!