State-of-the-Art Liquid Chromatography-Tandem Mass Spectrometry Detection Methods for Shellfish Toxins (ASP, DSP, PSP)

Outline

1. Introduction

2. Toxins

3. Mass spectrometry
   - functional principle
   - ionization
   - mass analyzers
   - quadrupoles
   - triple quad scan modes

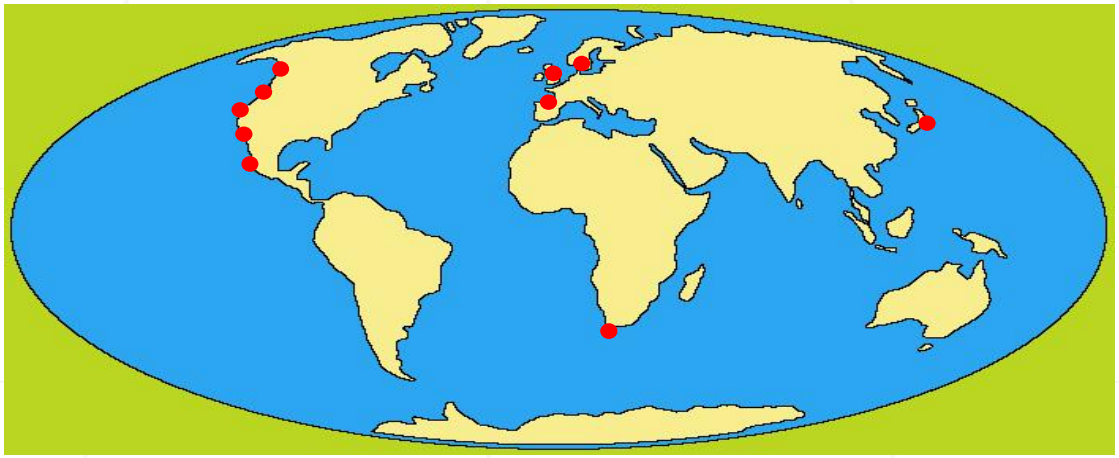
4. Algal toxin MS/MS methods
   - spirolides
   - yessotoxins
   - PSTs
   - multi toxin method

5. Conclusions

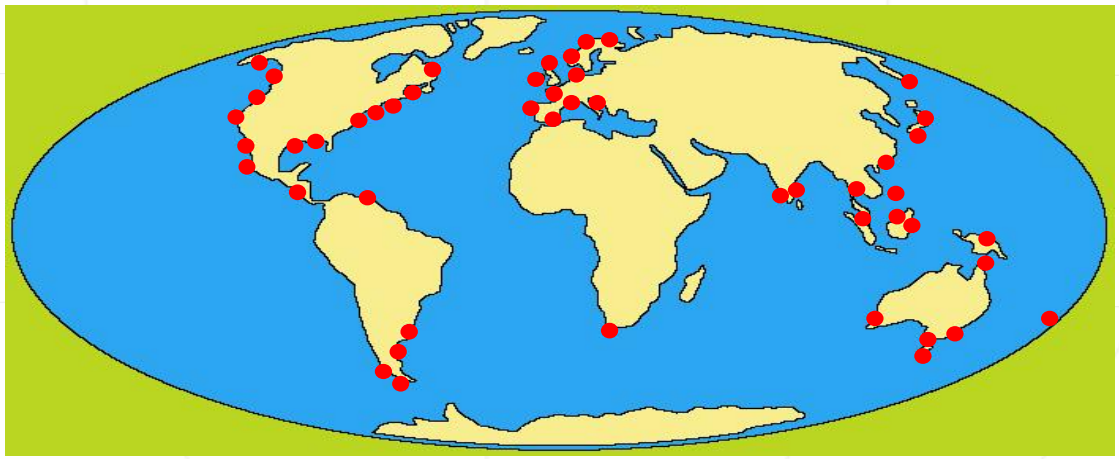
Bernd Krock 2007

Global Distribution of Paralytic Shellfish Poisoning Events

1970

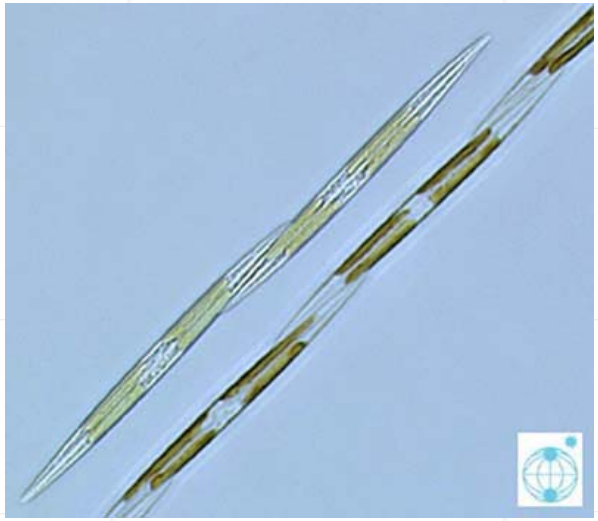


2005



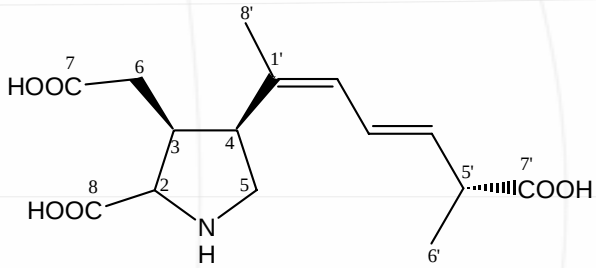
Toxins

Diatoms



Pseudo-Nitzschia pungens

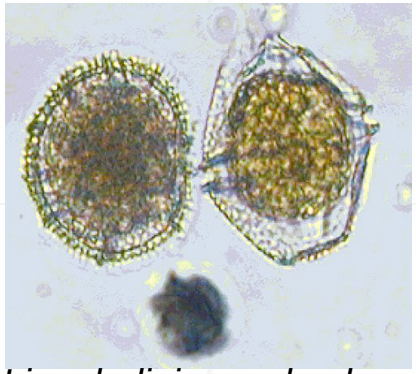
Amnesic Shellfish Poisoning



Domoic acid

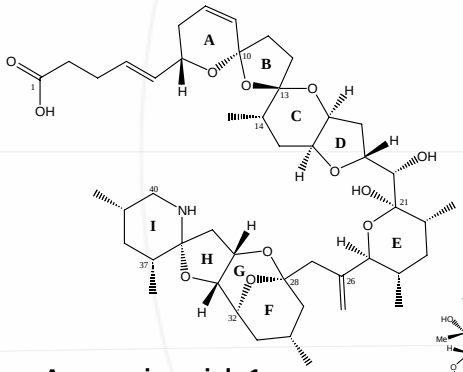
Toxins

Dinoflagellates

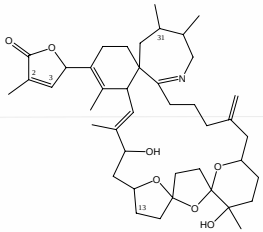


Lingulodinium polyedrum

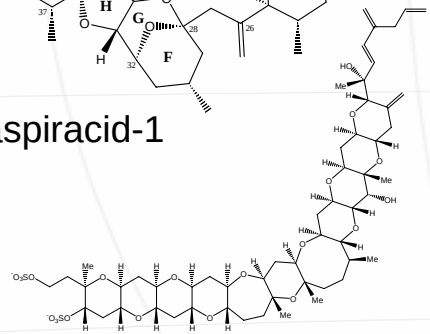
Diarrhetic Shellfish Poisoning



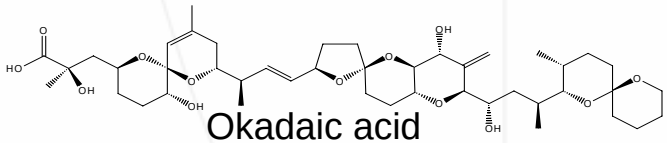
Azaspiracid-1



13-desmethyl spirolide C

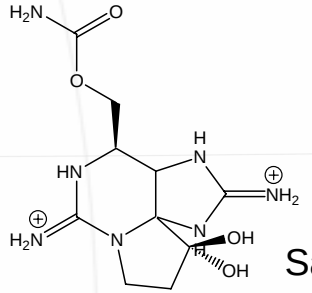


Yessotoxin



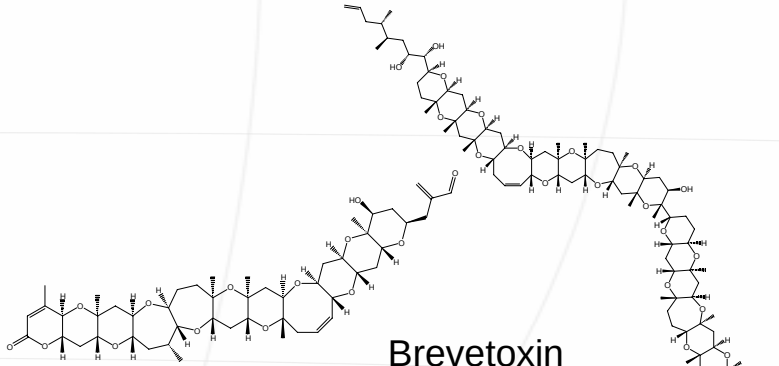
Okadaic acid

Paralytic Shellfish Poisoning

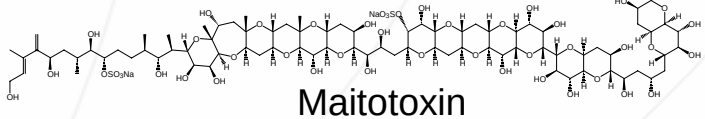


Saxitoxin

Neurotoxic Shellfish Poisoning



Brevetoxin



Maitotoxin

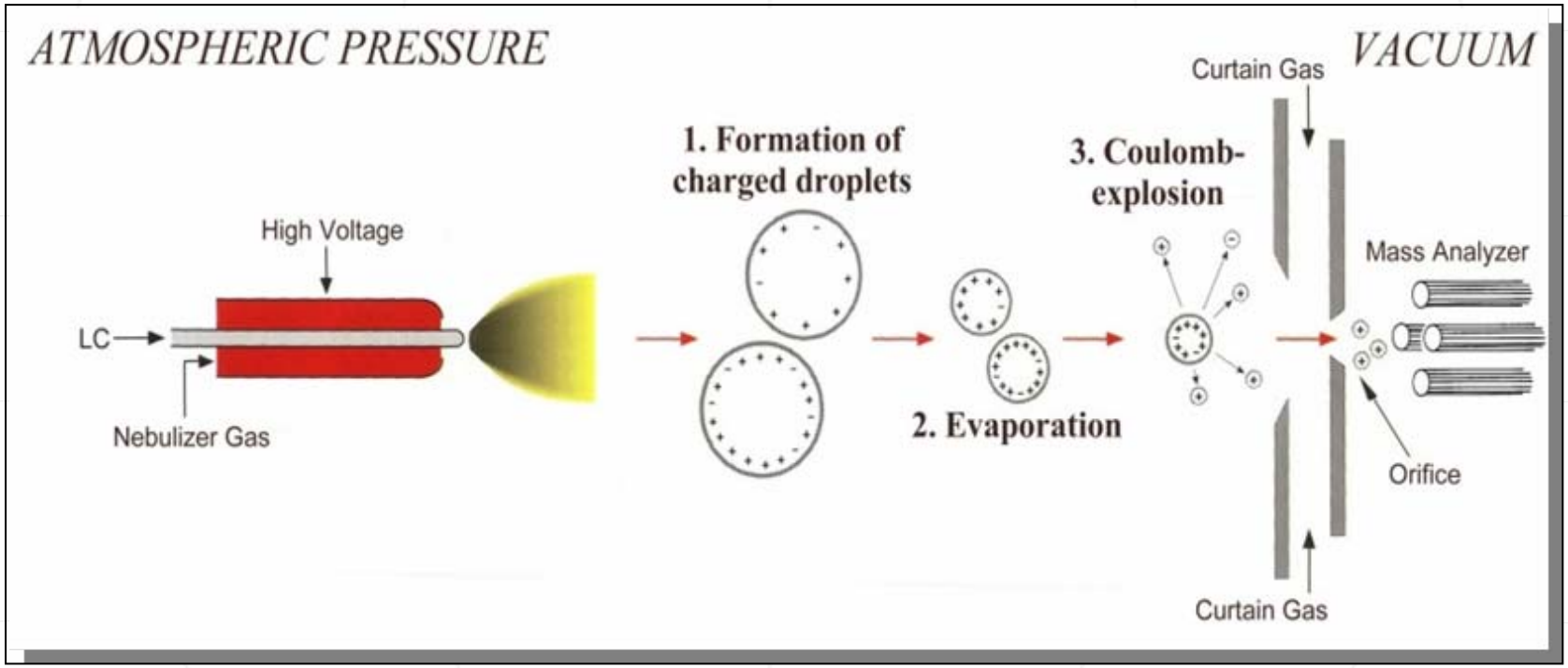
Functional Principle

Mass spectrometry is the determination of mass to charge (m/z) ratio of any compound

Process	Instrumentation
1. Ionization	Ion source
2. Acceleration	Interface
3. Mass separation	Mass analyzer
4. Ion detection	Electron multiplier

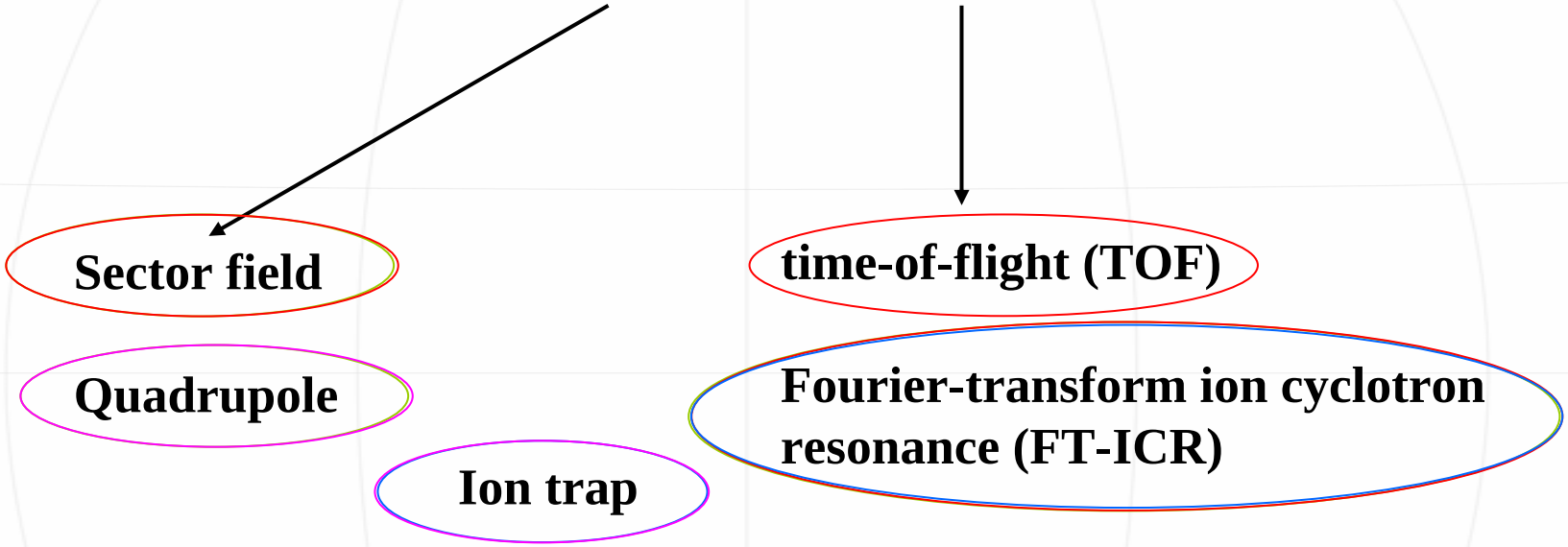
Ionization

Electrospray ion source



Mass Analyzers

2 categories: **scanning and non-scanning analyzers**



Acurate quantitation

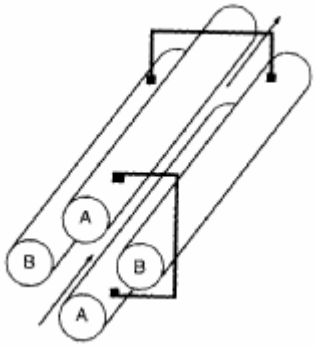
Exact mass measurement

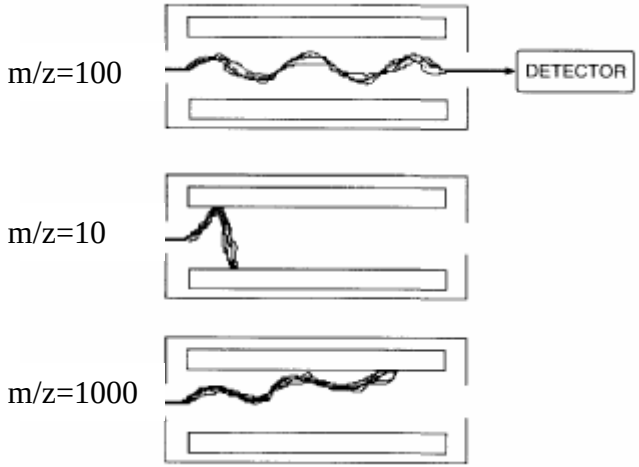
Structural information

cost

Quadrupoles

Quadrupole

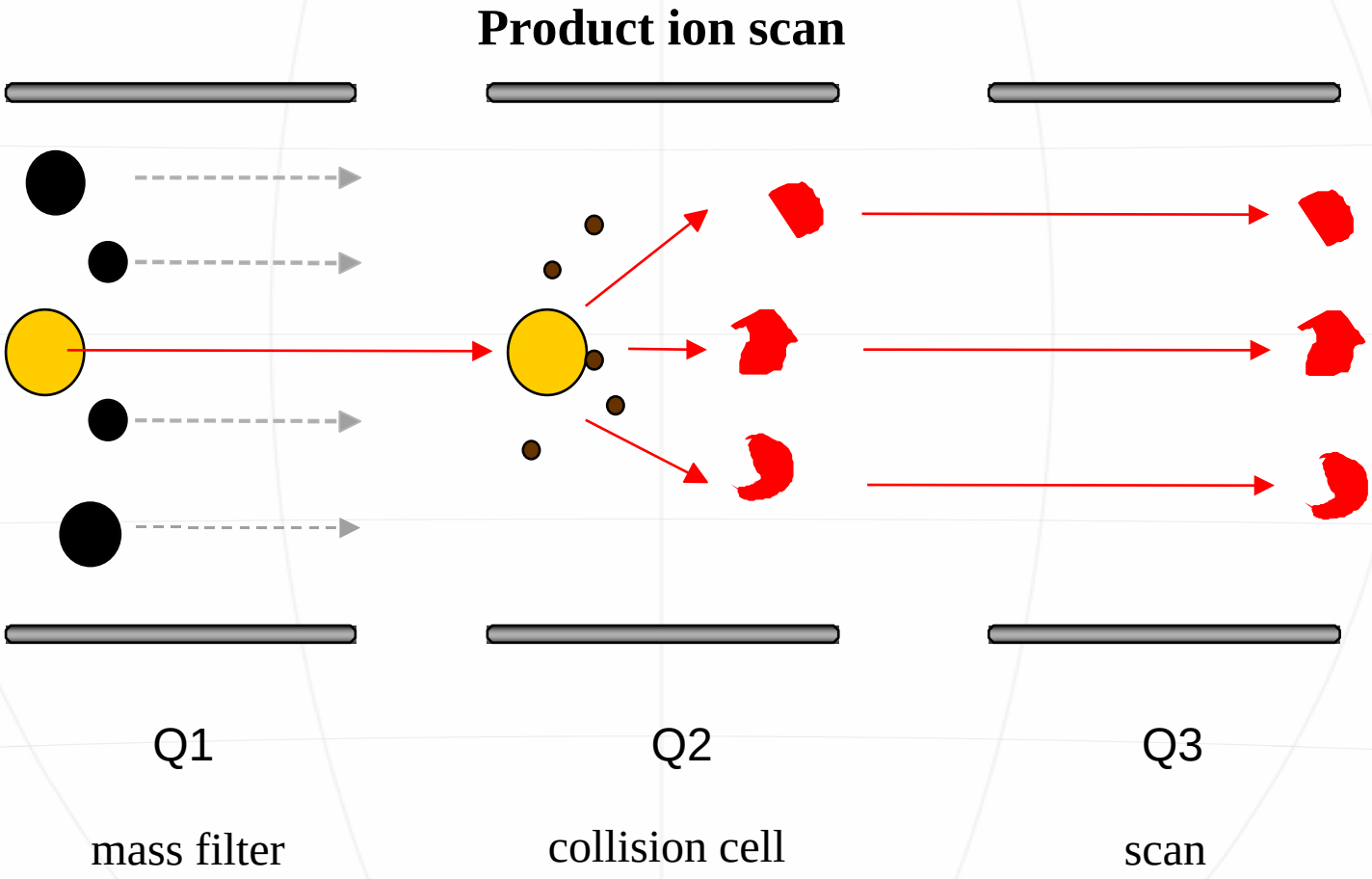




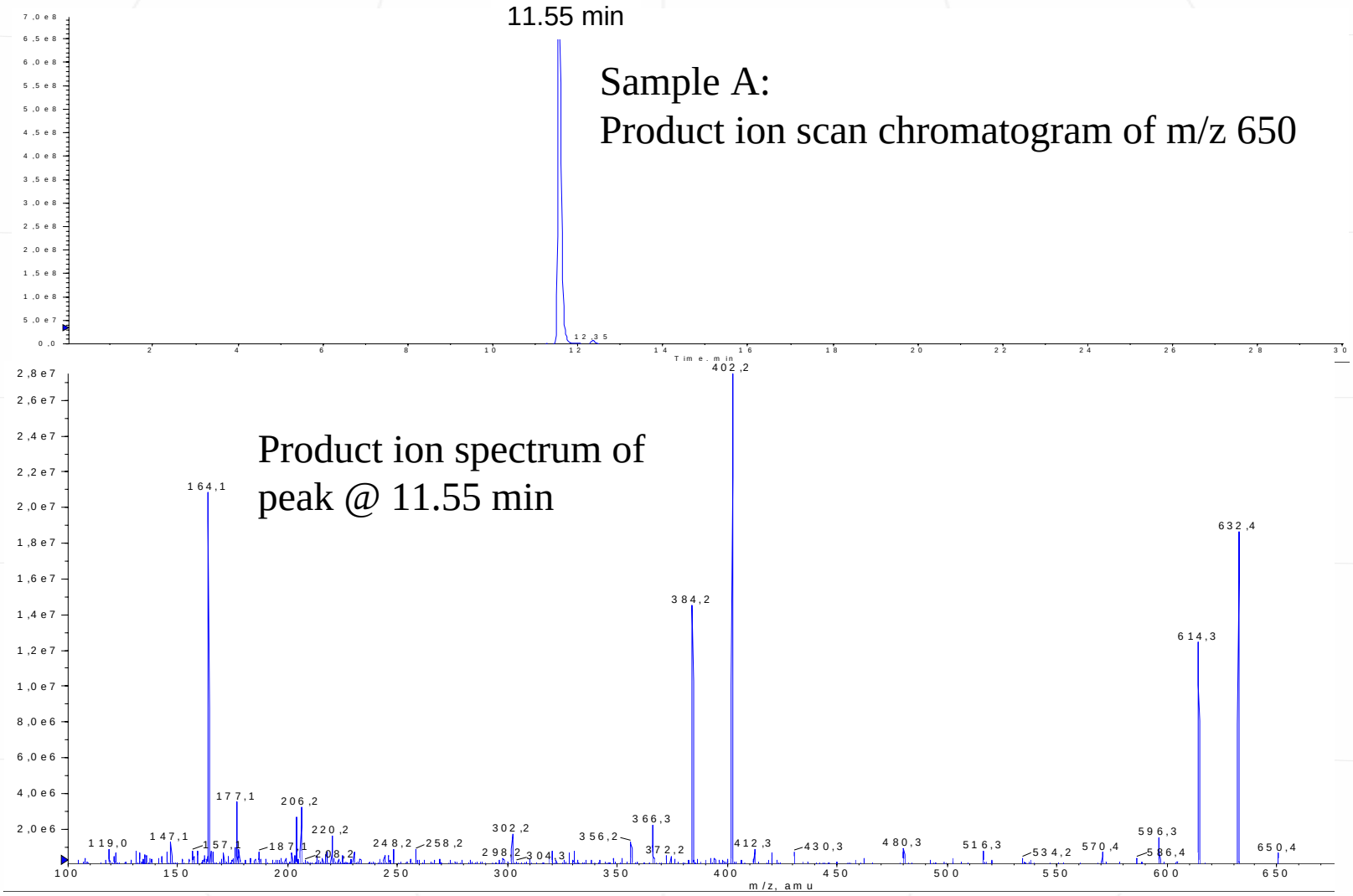
The magnetic frequencies of the quadrupole rods are modulated that way, that only a certain m/z value hits the detector at a time

Advantage: robustness
reproducible mass spectra
linear response over several orders of magnitude

Triple Quad Scan Modes

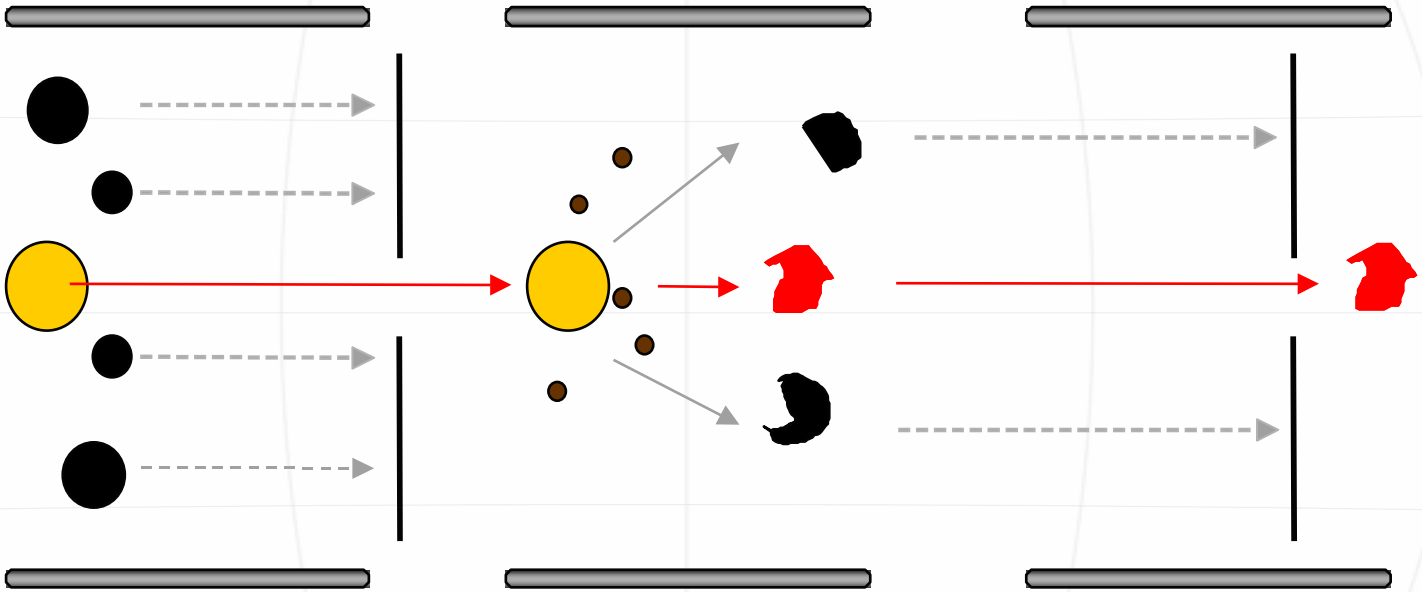


Triple Quad Scan Modes



Triple Quad Scan Modes

Multiple Reaction Monitoring (MRM)

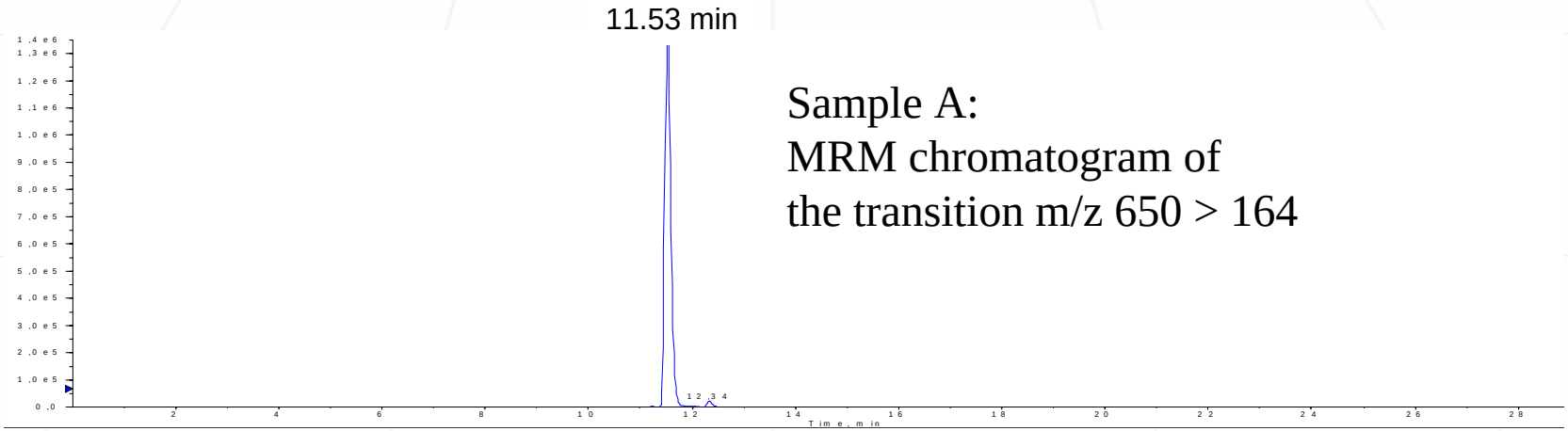


Q1
mass filter

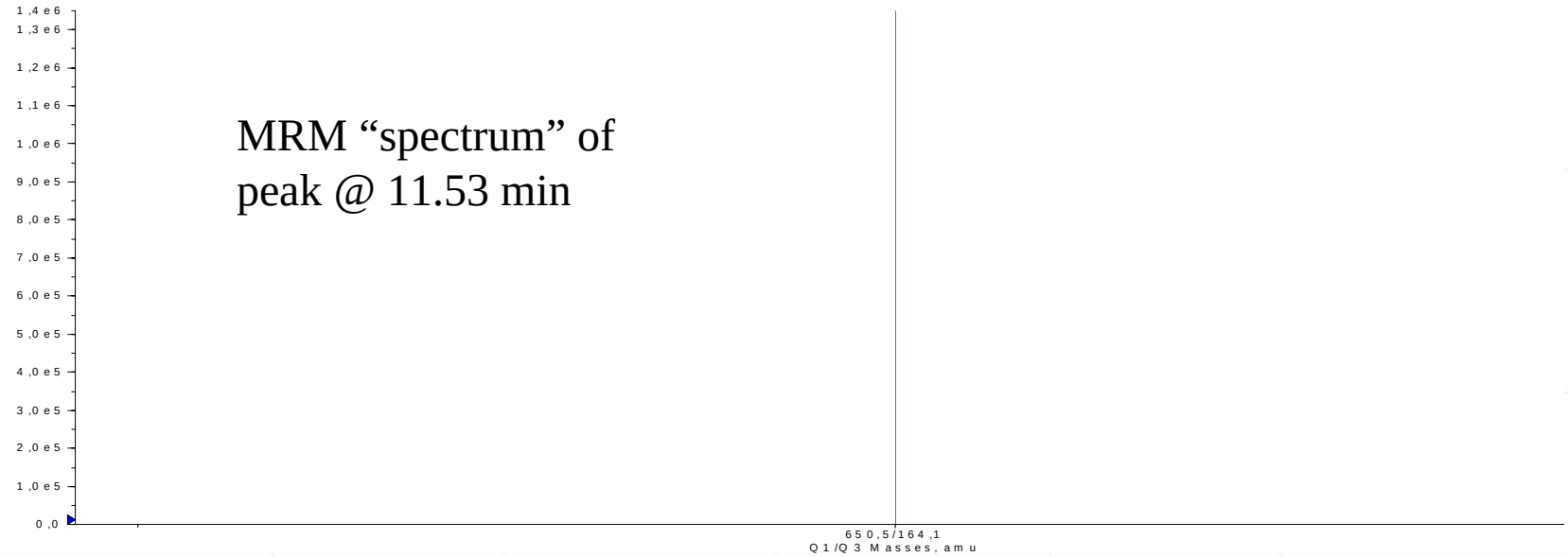
Q2
collision cell

Q3
mass filter

Triple Quad Scan Modes

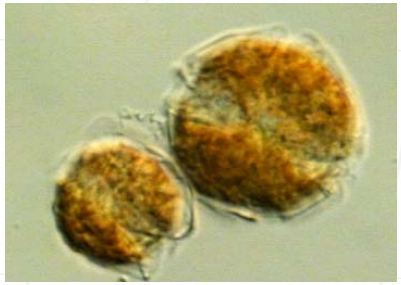


Sample A:
MRM chromatogram of
the transition m/z 650 > 164



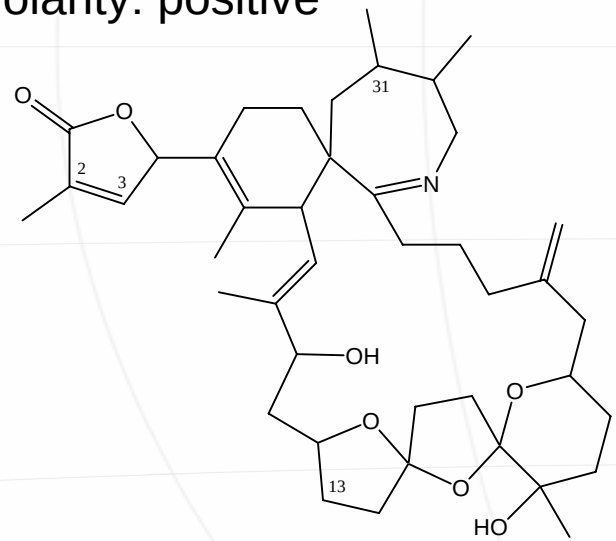
MRM “spectrum” of
peak @ 11.53 min

Example: Spirolides

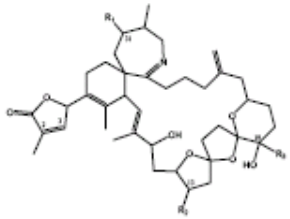
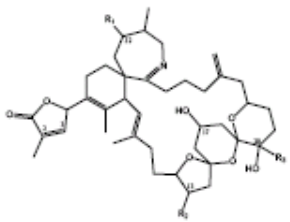


Alexandrium ostenfeldii

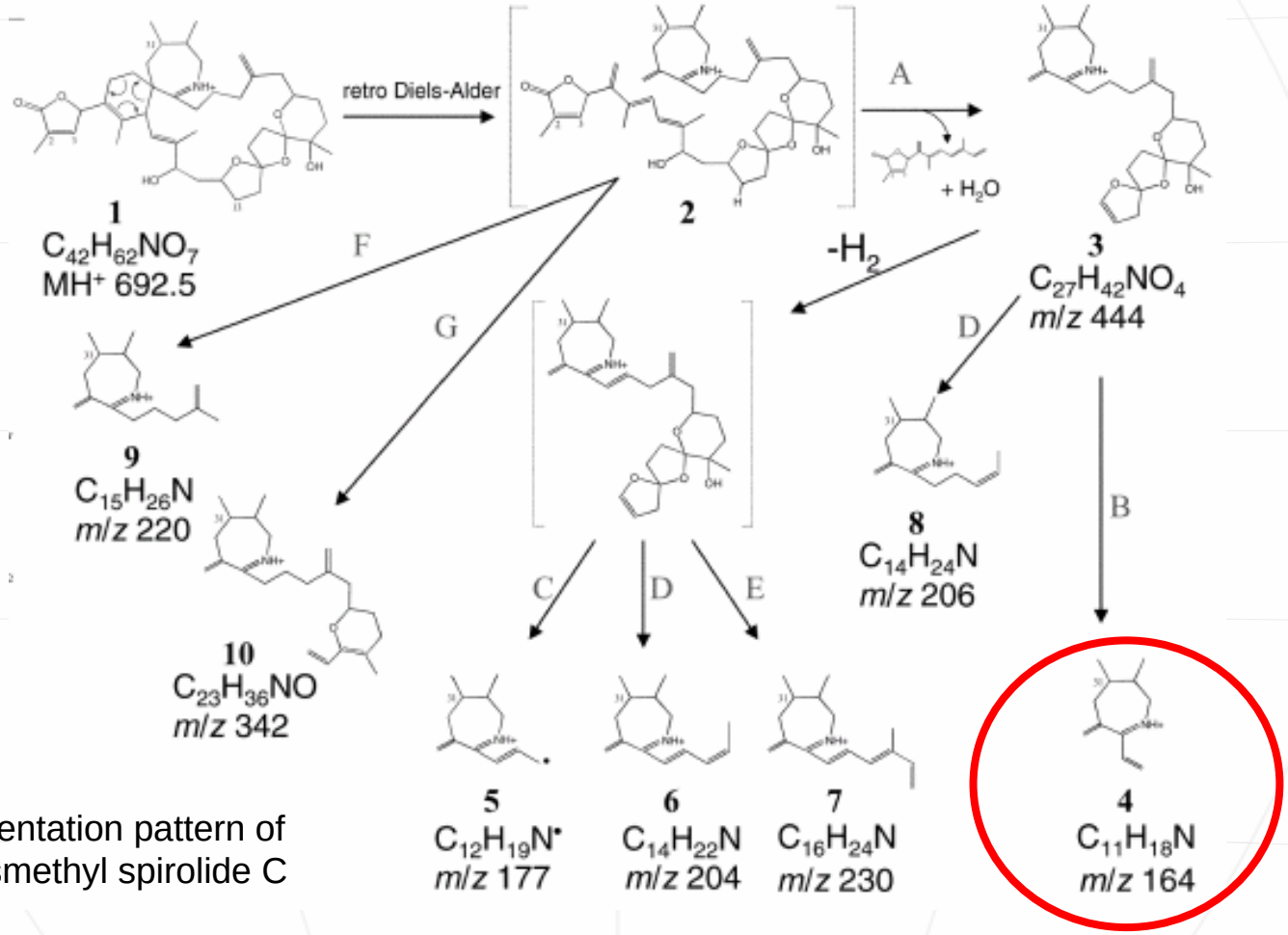
Polarity: positive



13-desmethyl spirolide C

	Spirolide	R1	R2	R3	$\Delta^{2,3}$	MW
	A	H	CH_3	CH_3	√	691,5
	B	H	CH_3	CH_3	-	693,5
	C	CH_3	CH_3	CH_3	√	705,5
	13-desMe-C	CH_3	H	CH_3	√	691,5
	13,19-didesMe-C	CH_3	H	H	√	677,5
	D	CH_3	CH_3	CH_3	-	707,5
	13-desMe-D	CH_3	H	CH_3	-	693,5
	G	CH_3	H	H	√	691,5
	20-Me-G	CH_3	H	CH_3	√	705,5

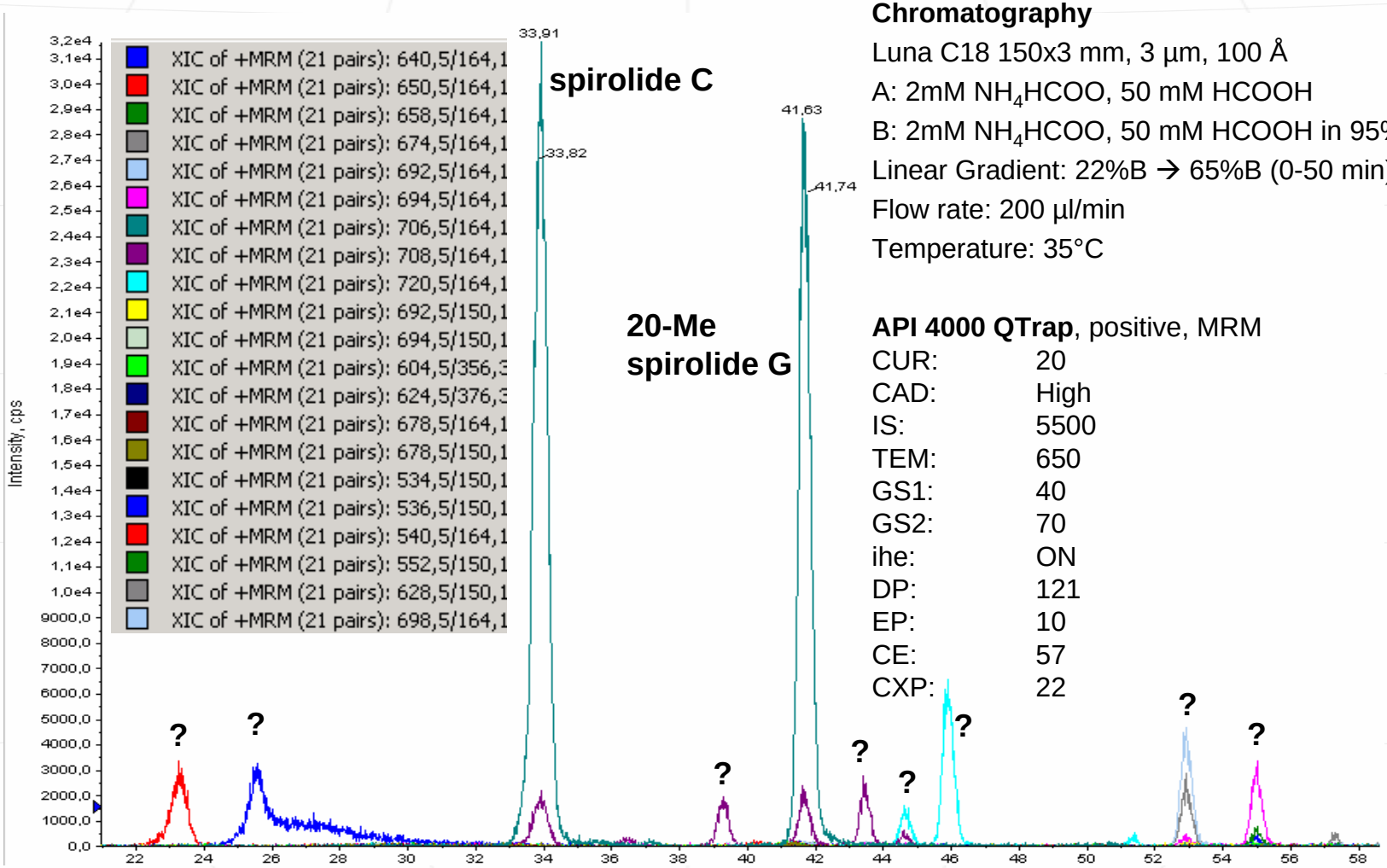
Example: Spirolides



Fragmentation pattern of
13-desmethyl spirolide C

Sleno et al. (2004) Anal Bioanal Chem 378 : 969-976

Example: Spirolides



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

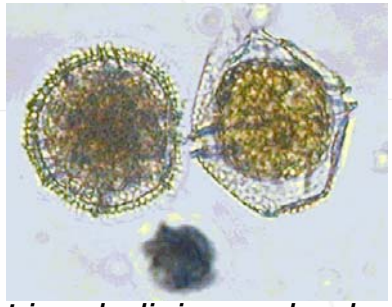
Temperature: 35°C

API 4000 QTrap, positive, MRM

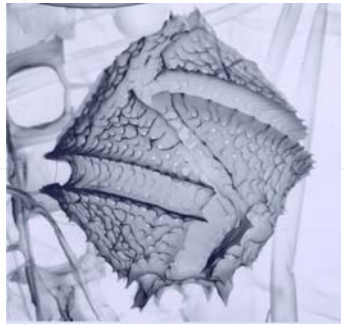
CUR:	20
CAD:	High
IS:	5500
TEM:	650
GS1:	40
GS2:	70
ihe:	ON
DP:	121
EP:	10
CE:	57
CXP:	22

Example: Yessotoxins

Protoceratium reticulatum



Lingulodinium polyedrum



Gonyaulax spinifera

Polarity: negative

yessotoxin

Gonyaulax spinifera
Lingulodinium polyedrum
Protoceratium reticulatum

Example: Yessotoxins

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

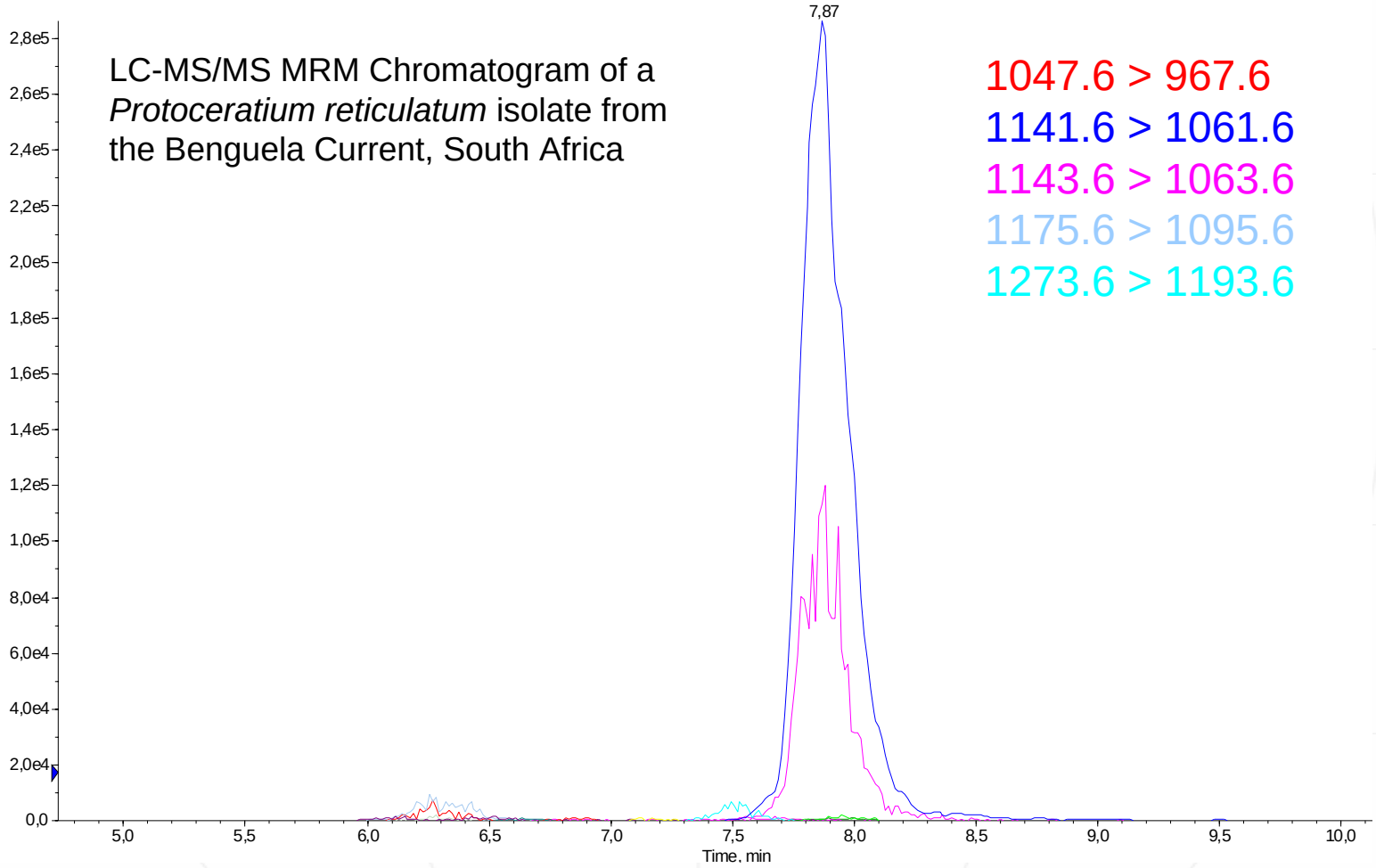
Entry	M_{wt}	R_t	$[M-H]^-$	MS^2	MS^3 (see Fig. 5)	$[M-2H]^{2-}$	Relative intensity[a]	MS^2	MS^3	Structure[b]
1	956[c]	3.2	955	**875**	**831**, 795	477.3	++	–	–	
2	984	3.3	983	**903**, 869, 653, 599	–	–	–	–	–	
3	986	2.2	985	905, **815**, 771	**797**, 772, 645, 627, 583	439.0	+	–	–	
4	992	5.2	991	**911**	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

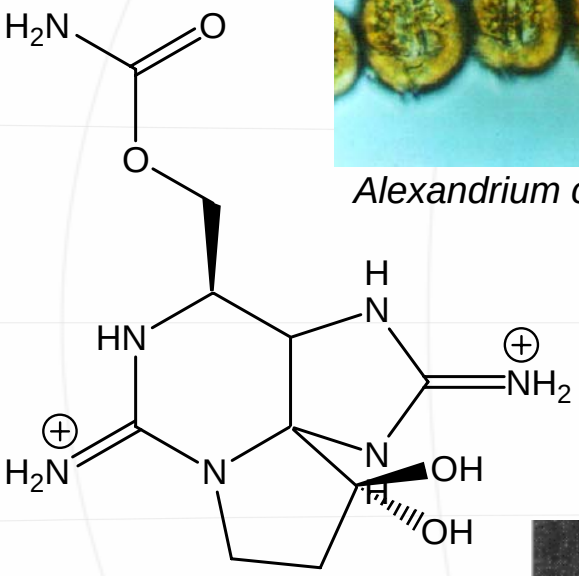
Example: Yessotoxins

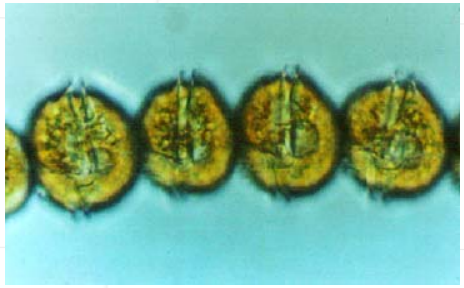
LC-MS/MS MRM Chromatogram of a *Protoceratium reticulatum* isolate from the Benguela Current, South Africa



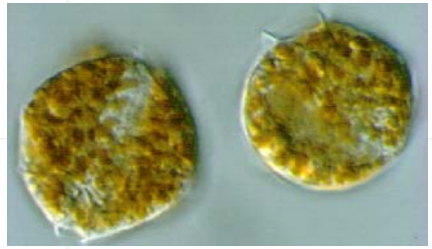
1047.6 > 967.6
1141.6 > 1061.6
1143.6 > 1063.6
1175.6 > 1095.6
1273.6 > 1193.6

Example: PSP

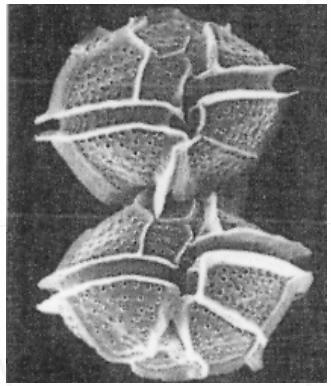




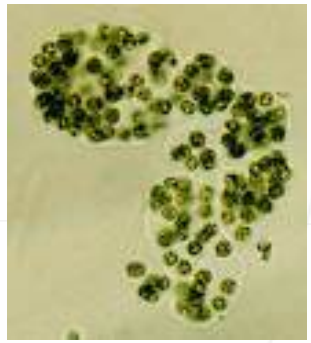
Alexandrium catenella



Alexandrium tamarense



Pyrodinium bahamense



Microcystis aeruginosa

Alexandrium
- *andersonii*
- *catenella*
- *fundyense*
- *minutum*
- *peruvianum*
- *tamarense*
- *tamiyavanichii*

Pyrodinium
- *bahamense*

Gymnodinium
- *catenatum*

Anabaena
- *circinalis*
- *lemmermannii*

Aphanizomenon
- *flos-aquae*

Cylindrospermopsis
- *raciborskii*

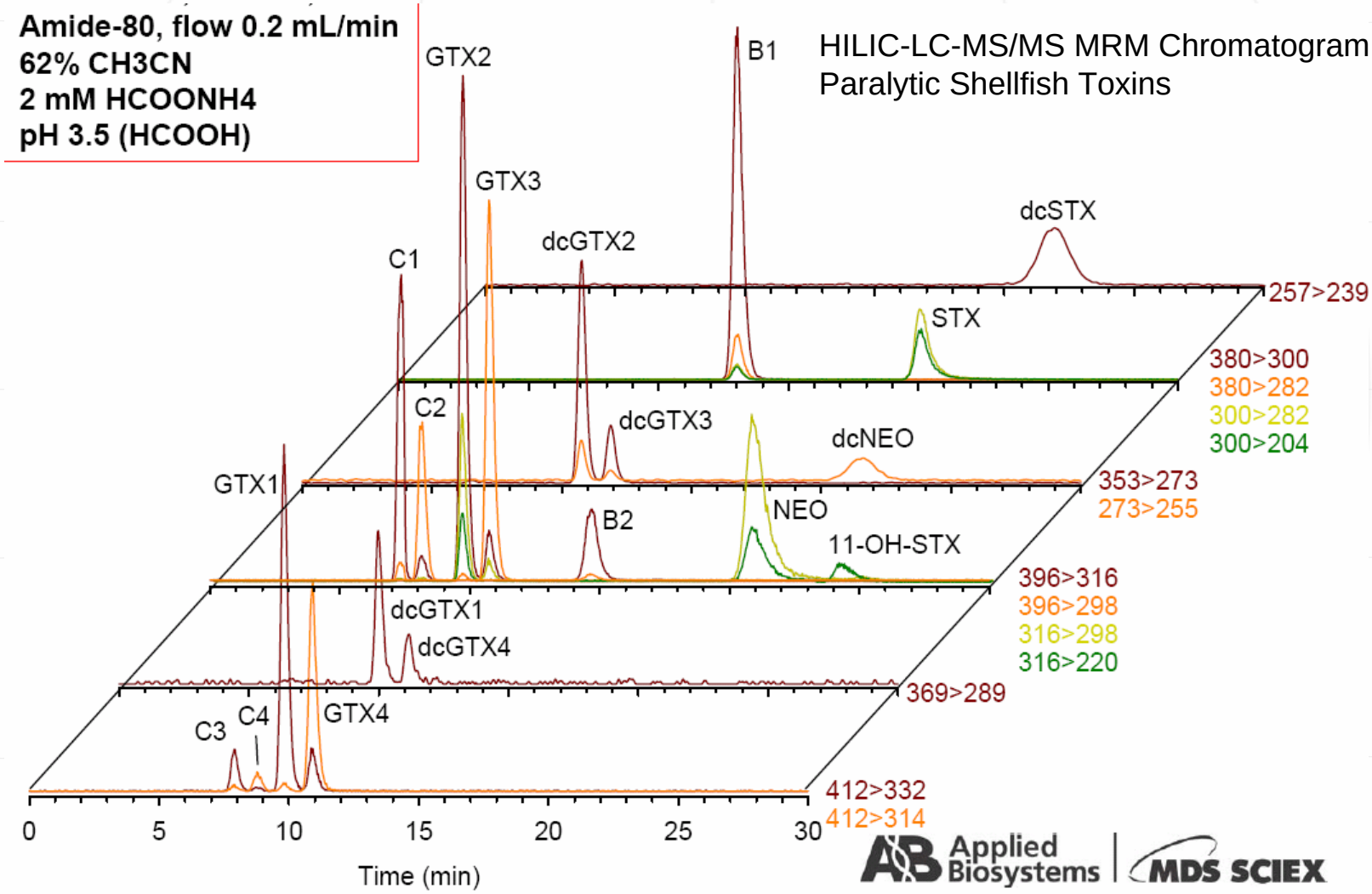
Lyngbya
- *wollei*

Microcystis
- *aeruginosa*

Example: PSP

Toxin	R1	R2	R3	R4	MW	Ions formed
STX	H	H	H	$CO\text{-}NH_2$ (Carbamoyl-)	301	$[M\text{-}H^+]^+ = 300$
NEO	OH	H	H		317	$[M\text{-}H^+]^+ = 316$
GTX1	OH	H	OSO_3^-		412	$M^+ = 412$
GTX2	H	H	OSO_3^-		396	$M^+ = 396$
GTX3	H	OSO_3^-	H		396	$M^+ = 396$
GTX4	OH	OSO_3^-	H		412	$M^+ = 412$
B1= GTX5	H	H	H	$CO\text{-}NH\text{-}SO_3^-$ (N-Sulfocarbamoyl-)	380	$M^+ = 380$
B2= GTX6	OH	H	H		396	$M^+ = 396$
C3	OH	H	OSO_3^-		492	$[M\text{-}SO_3^-]^+ = 412$
C1	H	H	OSO_3^-		476	$[M\text{-}SO_3^-]^+ = 396$
C2	H	OSO_3^-	H		476	$[M\text{-}SO_3^-]^+ = 396$
C4	OH	OSO_3^-	H		492	$[M\text{-}SO_3^-]^+ = 412$
dc-STX	H	H	H	H (Decarbamoyl-)	258	$[M\text{-}H^+]^+ = 257$
dc-NEO	OH	H	H		274	$[M\text{-}H^+]^+ = 273$
dc-GTX1	OH	H	OSO_3^-		369	$M^+ = 369$
dc-GTX2	H	H	OSO_3^-		353	$M^+ = 353$
dc-GTX3	H	OSO_3^-	H		353	$M^+ = 353$
dc-GTX4	OH	OSO_3^-	H		369	$M^+ = 369$

Example: PSP

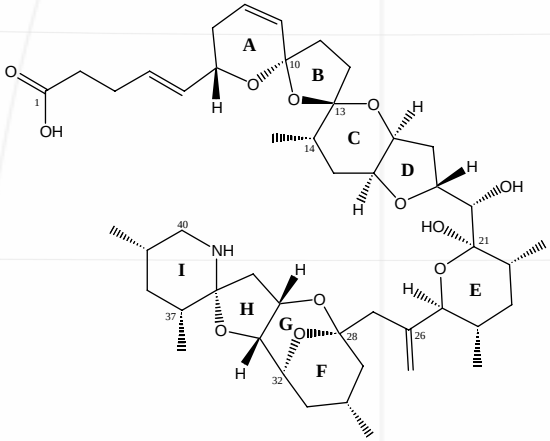

Amide-80, flow 0.2 mL/min
62% CH3CN
2 mM HCOONH4
pH 3.5 (HCOOH)
HILIC-LC-MS/MS MRM Chromatogram of Paralytic Shellfish Toxins
GTX2
B1
GTX3
dcSTX
C1
dcGTX2
257>239
STX
380>300
380>282
300>282
300>204
C2
dcGTX3
dcNEO
GTX1
353>273
273>255
B2
NEO
11-OH-STX
396>316
396>298
316>298
316>220
dcGTX1
dcGTX4
369>289
C3
C4
GTX4
412>332
412>314
0
5
10
15
20
25
30
Time (min)
Applied Biosystems
MDS SCIEX

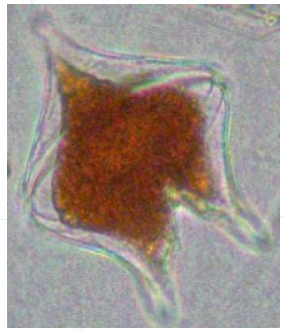
Multi Method

Definition:	Multi methods aim to analyze toxins of as many as possible different classes
Prerequisites:	extractability under same conditions elution with same solvent system
Problems:	each compound class requires individual MS parameters: (curtain gas, ion source temperature, ionization voltage, auxiliary gas flows, ion polarity, quadrupol voltages, fragmentation energy)
Limitations:	limited amount single compounds group elution of every toxin class required
Summary:	Multimethods are a compromise between a number of toxins to be analyzed and sensivity

Multi Method

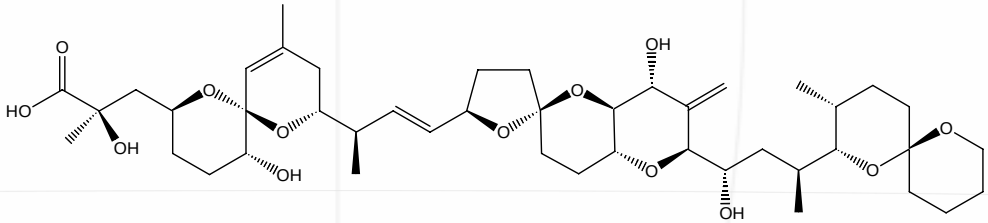
1. ASP
2. Gymnodimine
3. Spirolides
4. Azaspiracids
5. Dinophysistoxins
6. Pectenotoxins
7. Yessotoxins



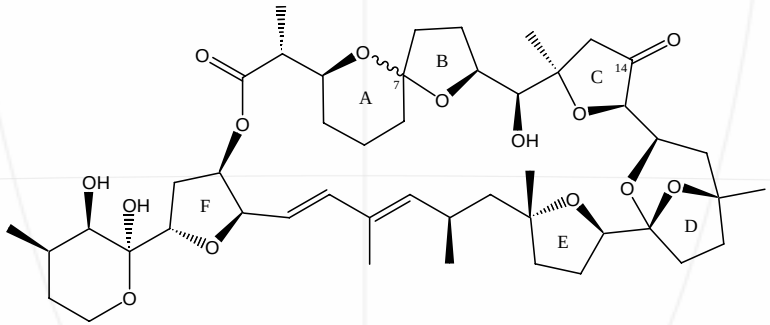


?

Protoperidinium crassipes

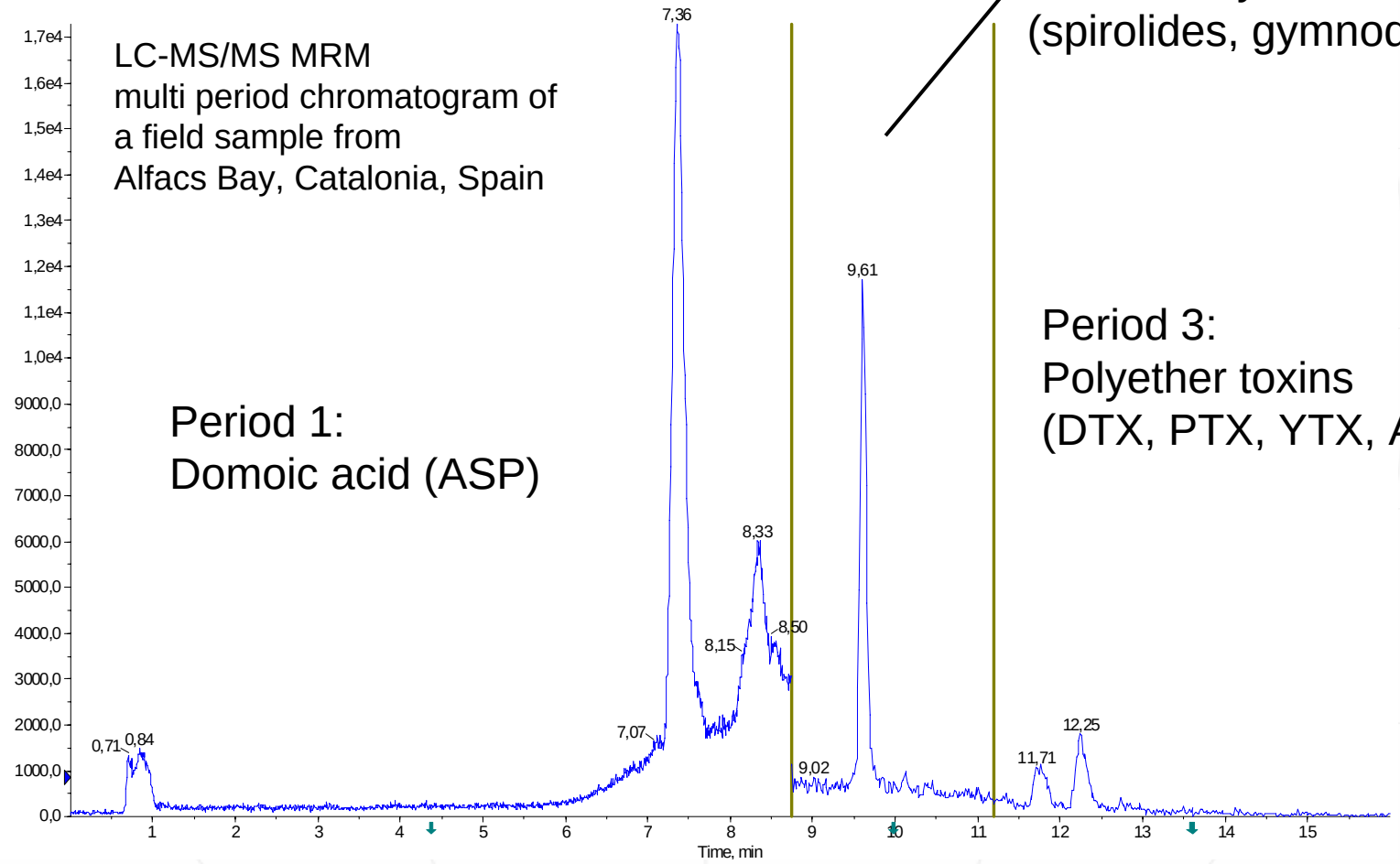


Prorocentrum lima



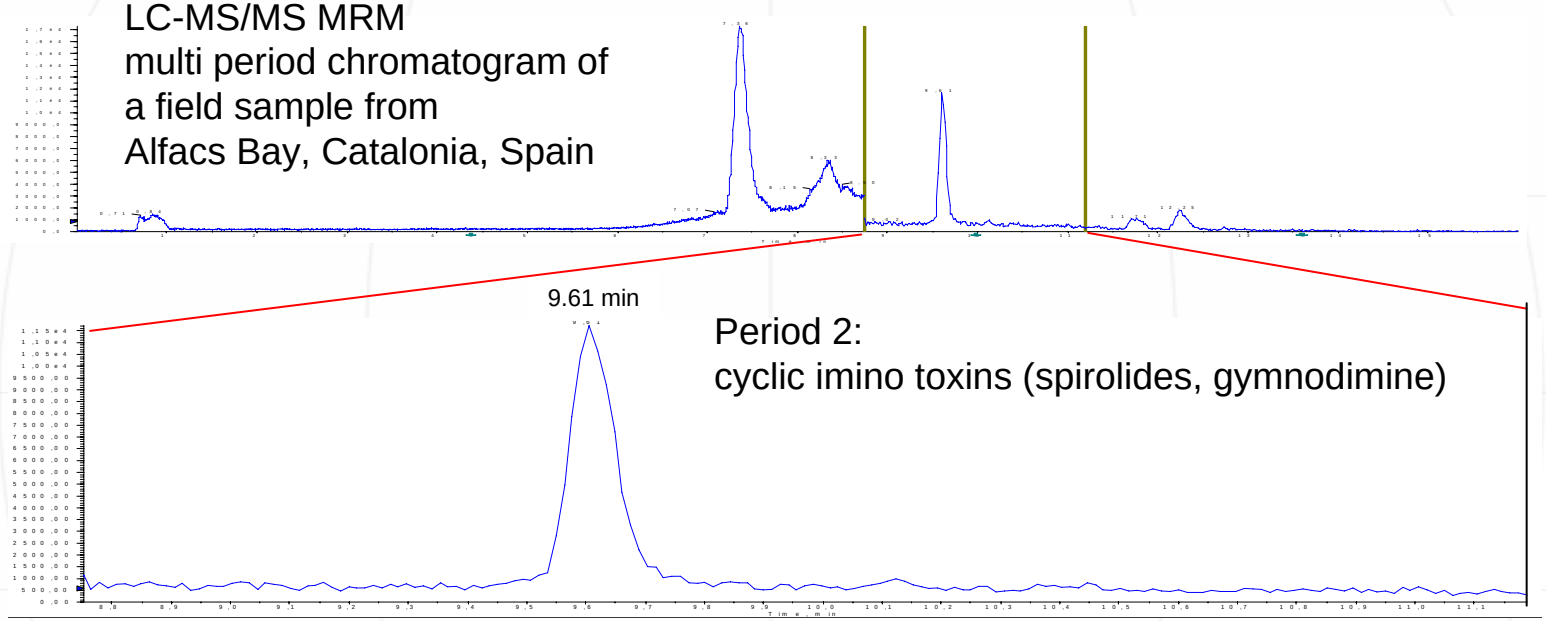
Dinophysis acuminata

Multi Method



Multi Method

LC-MS/MS MRM
multi period chromatogram of
a field sample from
Alfacs Bay, Catalonia, Spain

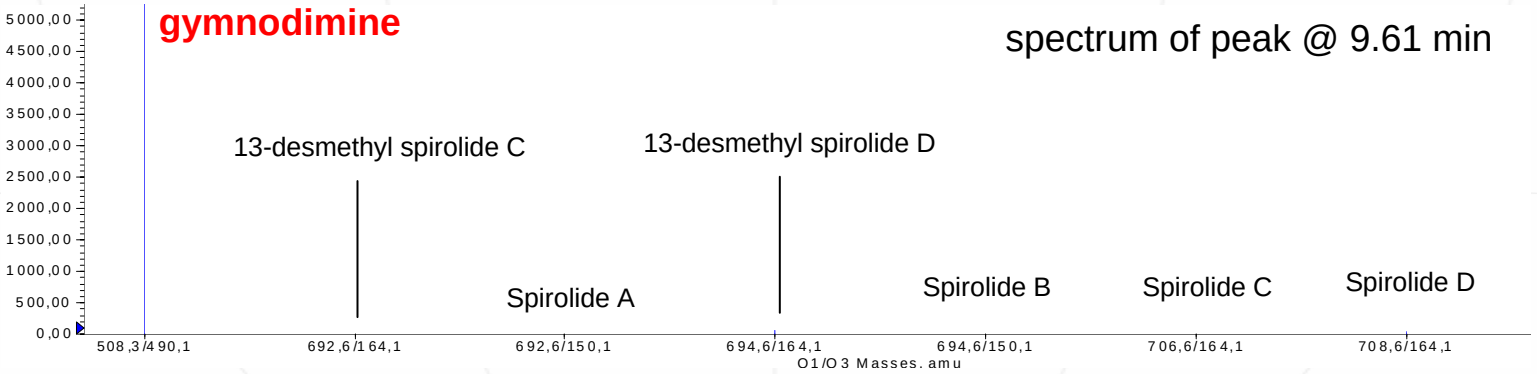


9.61 min

Period 2:
cyclic imino toxins (spirolides, gymnodimine)

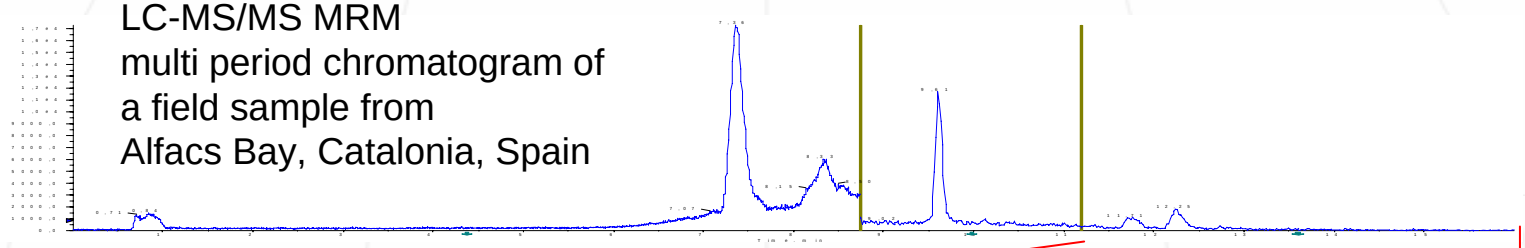
gymnodimine

spectrum of peak @ 9.61 min

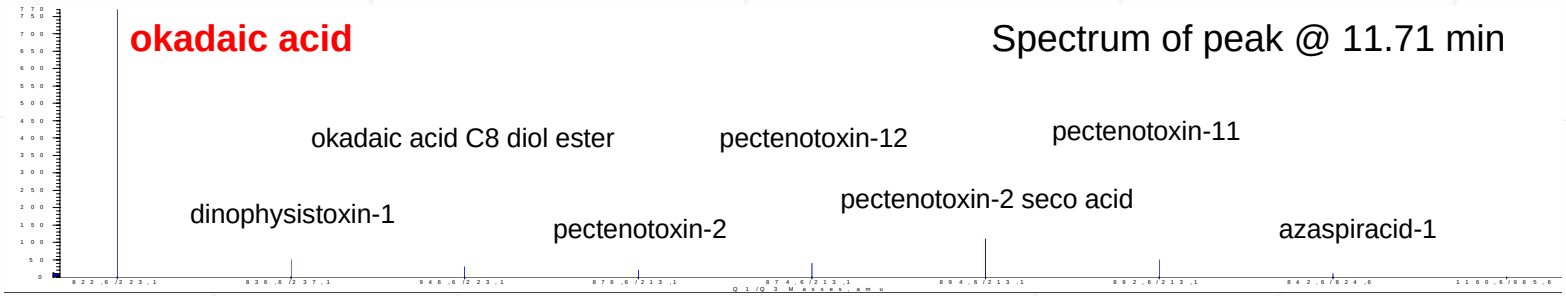


Multi Method

LC-MS/MS MRM
multi period chromatogram of
a field sample from
Alfacs Bay, Catalonia, Spain



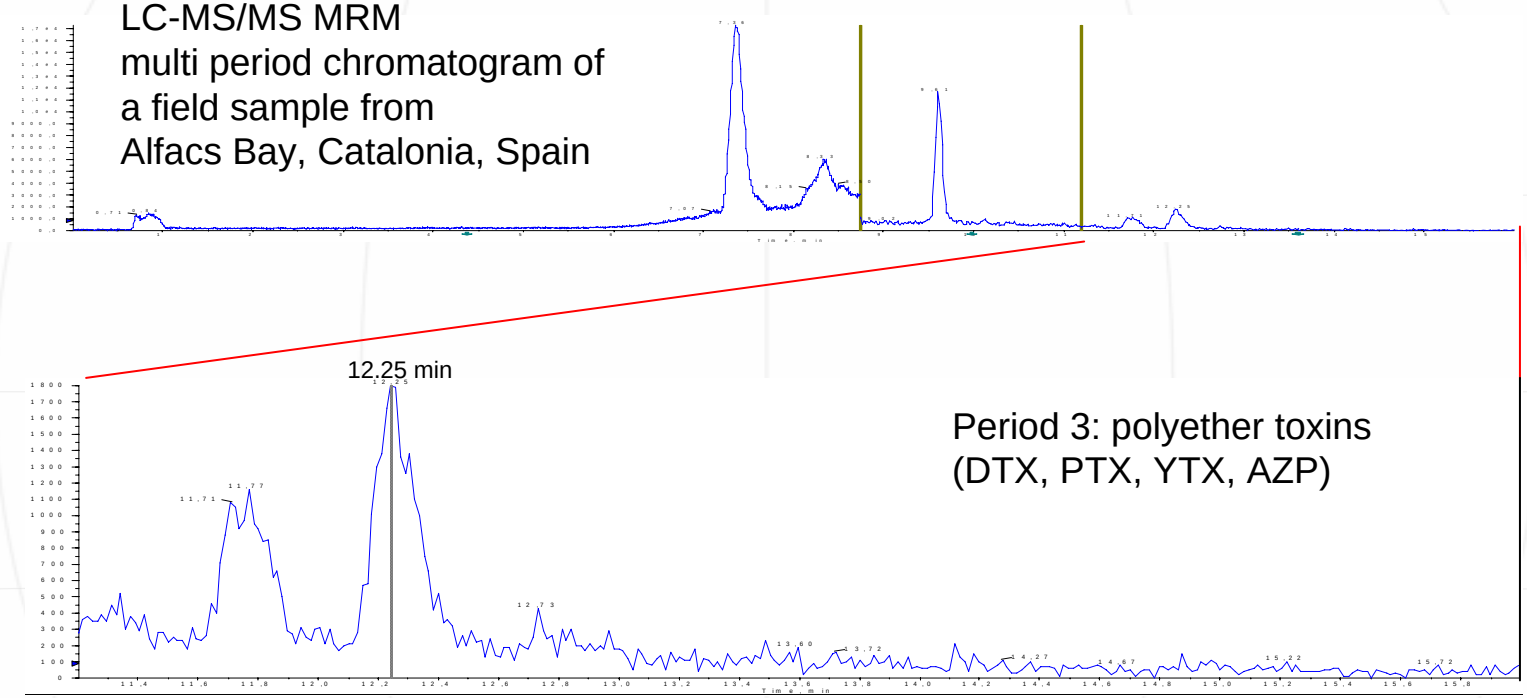
Period 3: polyether toxins
(DTX, PTX, YTX, AZP)

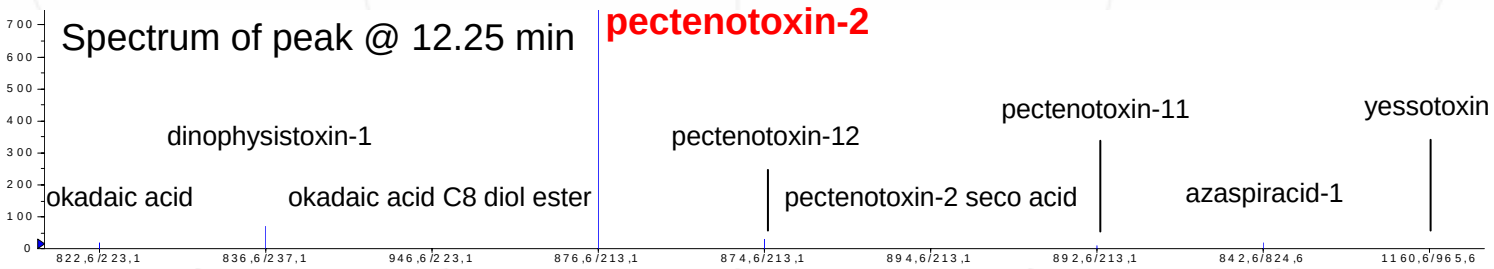


Spectrum of peak @ 11.71 min

Multi Method

LC-MS/MS MRM
multi period chromatogram of
a field sample from
Alfacs Bay, Catalonia, Spain





1. The mass analyzer of choice for quantitation of algal toxins by LC-MS is the quadrupole (or better: triple quad)

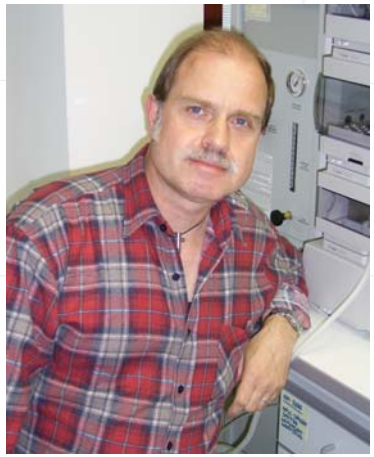
2. Ionization of algal toxins is best achieved by electrospray ionization (ESI) with acidic and basic modifiers (formic acid and ammonium formate, respectively) in the mobile phase

3. Most toxin classes can be identified by characteristic group fragments of the individual components

4. Hydrophilic Interaction (HILIC) stationary phases for the first time allow to analyze very polar PSTs by LC-MS

5. Multi methods are a good tool for screening a defined amount of different toxins, but do not give an overall toxin response

Thanks to…



Wolfgang Drebing, AWI

…and for your attention!