Qualitative and Quantitative Analysis of Cyanobacterial Toxins by LC-MS/MS

Outline

1. Relevance of cyanotoxins in food chemistry

2. What are cyanotoxins? - Chemical classes

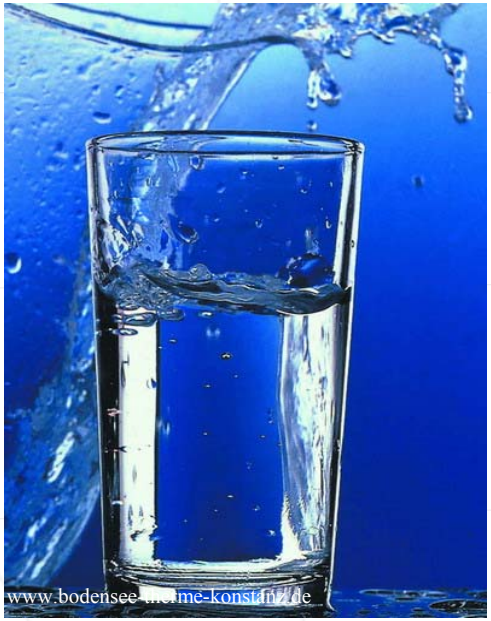
3. Qualitative detection of cyanotoxins by LC-MS/MS

4. Quantitative analysis

5. Summary

Relevance of cyanotoxins in food chemistry

1. potable water

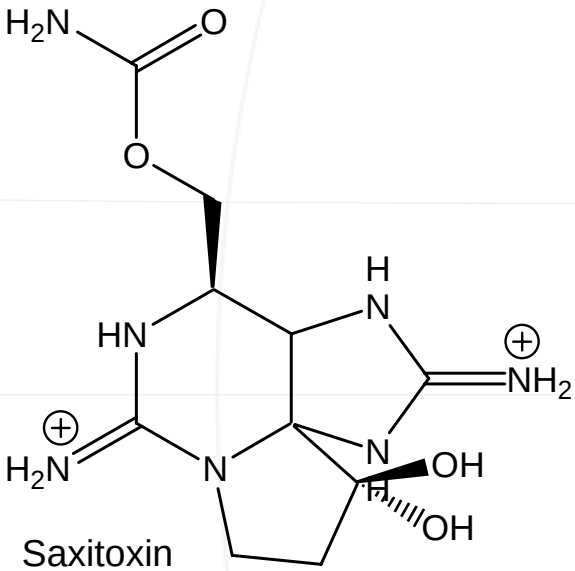


2. dietary supplements - algal preparations

Cyanotoxins – Classes

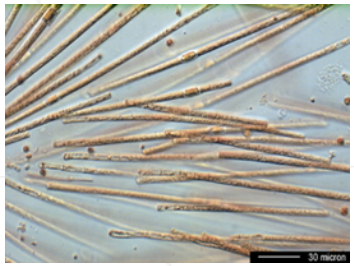
Toxin class	mode of action
PSTs (paralytic shellfish toxins)	sodium channel inhibitors
Anatoxins	nicotinic acetylcholin receptor agonists
Anatoxin-a(s)	acetylcholin esterase inhibitor
Cylindrospermopsins	inhibition of uridine monophosphate synthase complex - hepatotoxins
Microcystins	block of protein serine/threonine phosphatases PP1 and PP2A
Nodularins	block of protein serine/threonine phosphatases PP1 and PP2A

Paralytic Shellfish Toxins (PSTs)

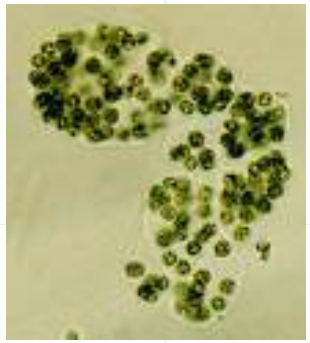


Saxitoxin

Anabaena
circinalis
lemmermannii
Aphanizomenon
flos-aquae
Cylindrospermopsis
raciborskii
Lyngbya
wollei
Microcystis
aeruginosa



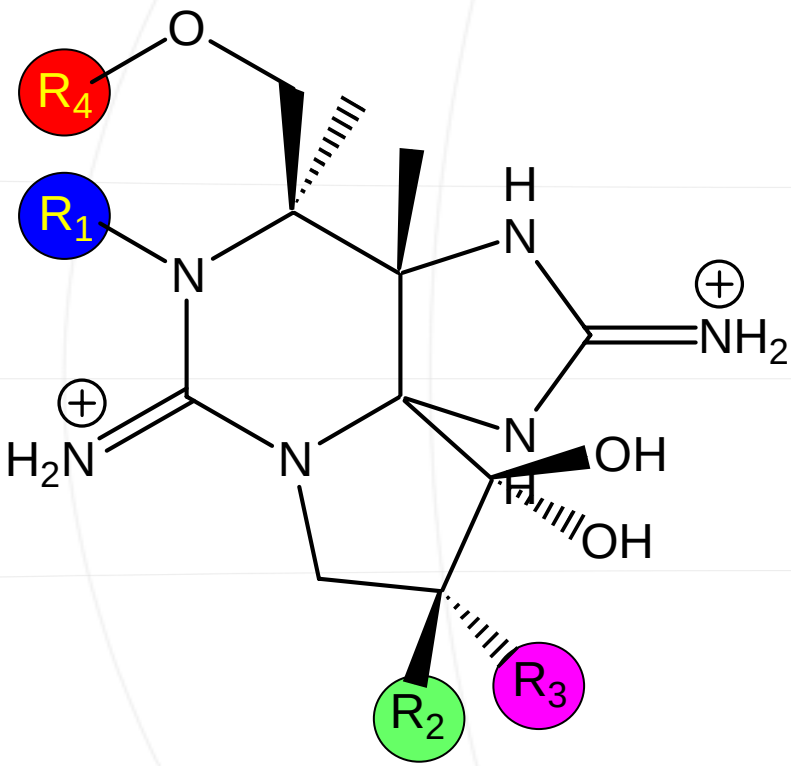
Aphanizomenon flos-aquae



Microcystis aeruginosa

Anabaena circinalis

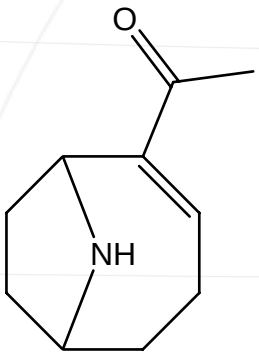
Paralytic Shellfish Toxins (PSTs)



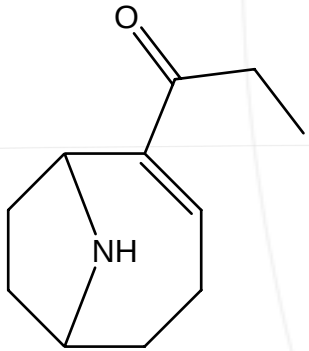
Toxin	R1	R2	R3	R4
STX	H	H	H	$CO-NH_2$ (Carbamoyl-)
NEO	OH	H	H	
GTX1	OH	H	OSO_3^-	
GTX2	H	H	OSO_3^-	
GTX3	H	OSO_3^-	H	
GTX4	OH	OSO_3^-	H	
B1= GTX5	H	H	H	$CO-NH-SO_3^-$ (N-Sulfocarbamoyl-)
B2= GTX6	OH	H	H	
C3	OH	H	OSO_3^-	
C1	H	H	OSO_3^-	
C2	H	OSO_3^-	H	
C4	OH	OSO_3^-	H	
dc-STX	H	H	H	H (Decarbamoyl-)
dc-NEO	OH	H	H	
dc-GTX1	OH	H	OSO_3^-	
dc-GTX2	H	H	OSO_3^-	
dc-GTX3	H	OSO_3^-	H	
dc-GTX4	OH	OSO_3^-	H	

STX = Saxitoxin
NEO = Neosaxitoxin
GTX = Gonyautoxin

Anatoxin-a



Anatoxin-a

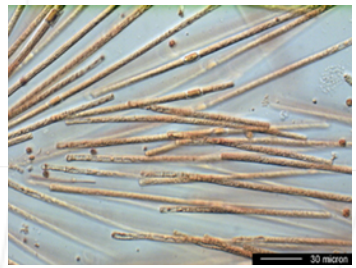


Homoanatoxin-a

Anabaena circinalis

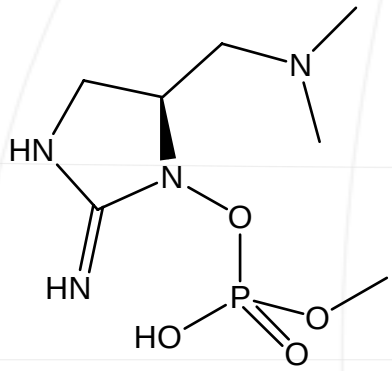
Anabaena flos-aquae

Anabaena
circinalis
flos-aquae
Aphanizomenon
flos-aquae
Planktothrix
formosa



Aphanizomenon flos-aquae

Anatoxin-a(s)



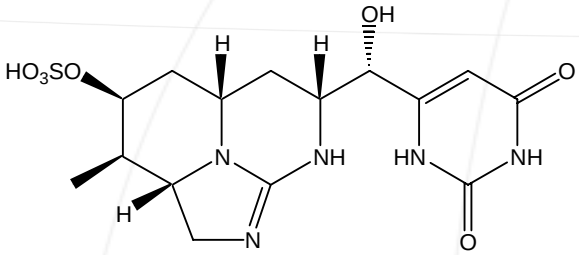
Anatoxin-a(s)

Anabaena
flos-aquae
lemmermannii

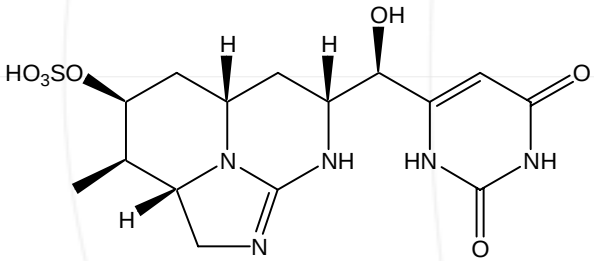
Anabaena flos-aquae

Anabaena lemmermannii

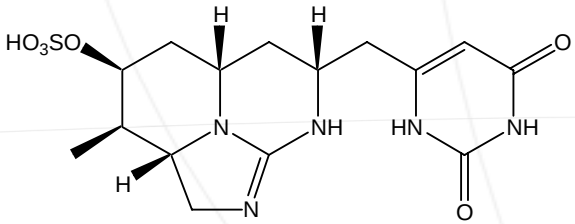
Cylindrospermopsins



Cylindrospermopsin



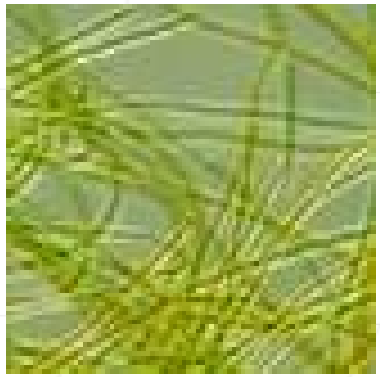
7-epi-Cylindrospermopsin



deoxy-Cylindrospermopsin

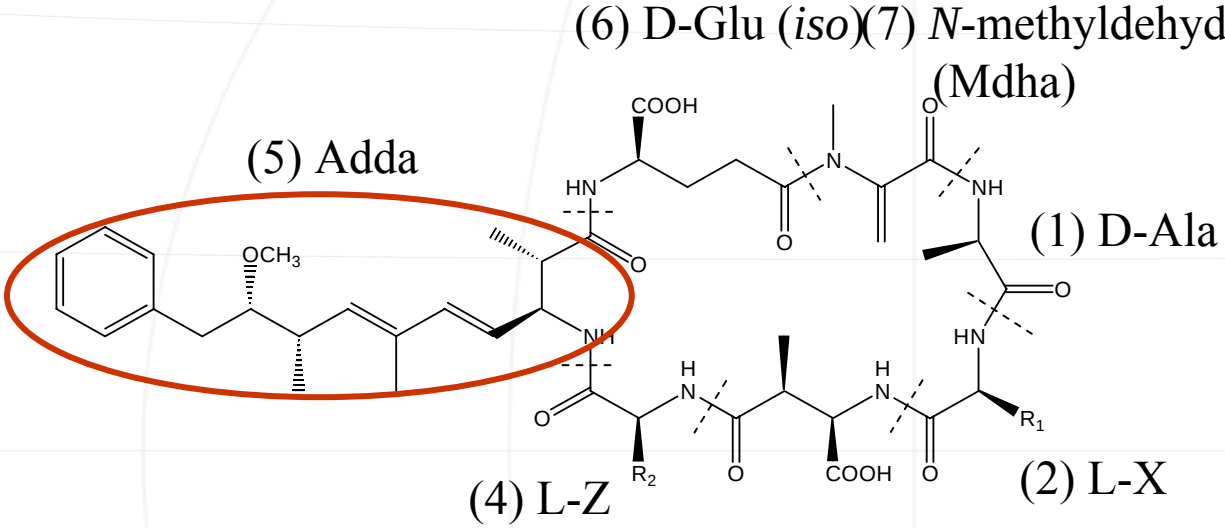
Aphanizomenon
flos-aquae
ovalisporum
Cylindrospermopsis
raciborskii
Umezakia
natans

Umezakia natans



Cylindrospermopis raciborskii

Microcystins



Anabaena
- *circinalis*
- *flos-aquae*

Anabaenopsis
- *milleri*

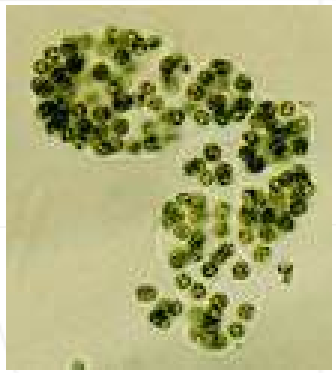
Aphanizomenon
- *ovalisporum*

Microcystis
- *aeruginosa*
- *botrys*
- *viridis*

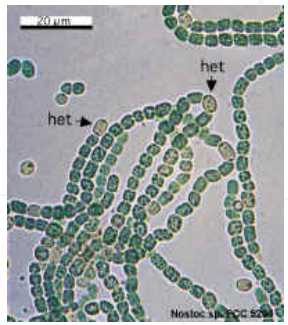
Planktothrix
- *agardhii*
- *mugeotii*
- *rubescens*

Nostoc spp.

Planktothrix agardhii

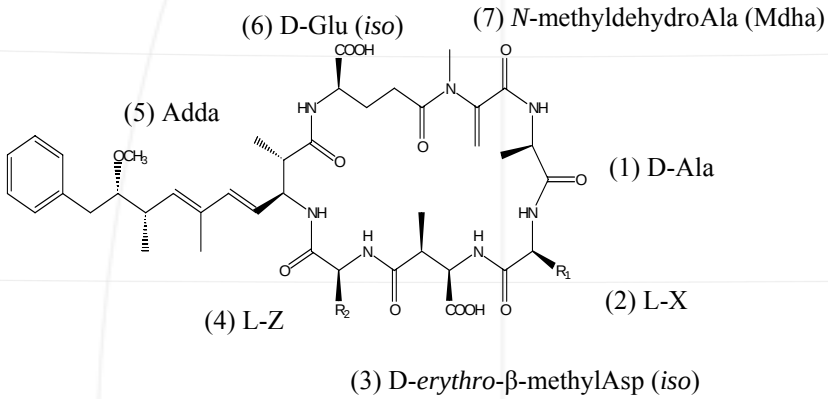


Microcystis aeruginosa



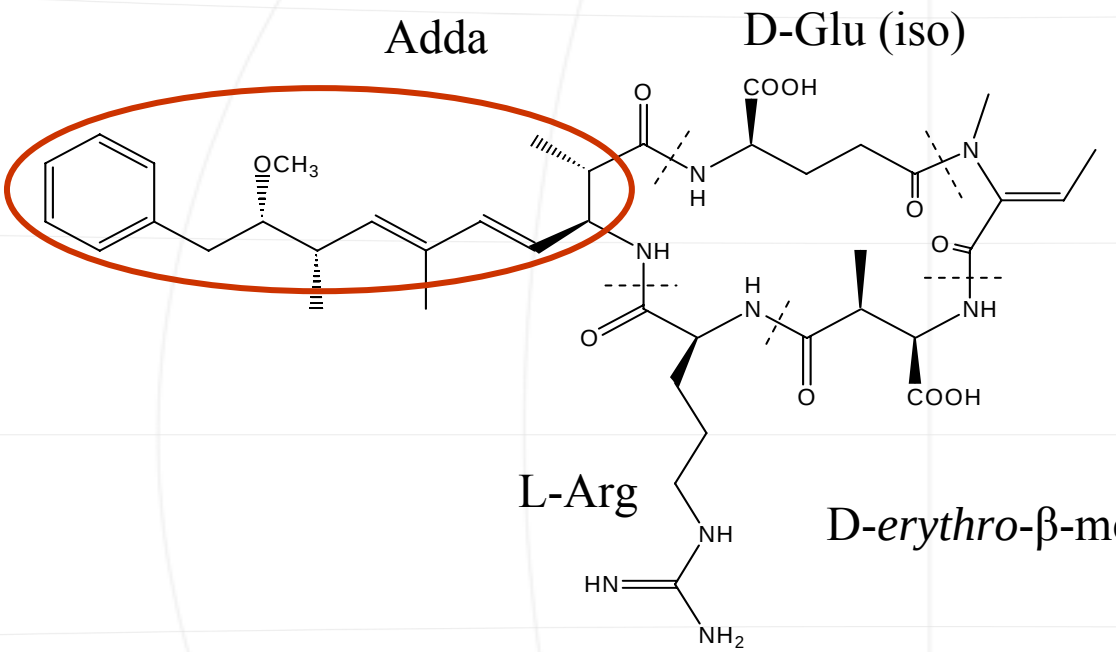
Nostoc

Microcystins

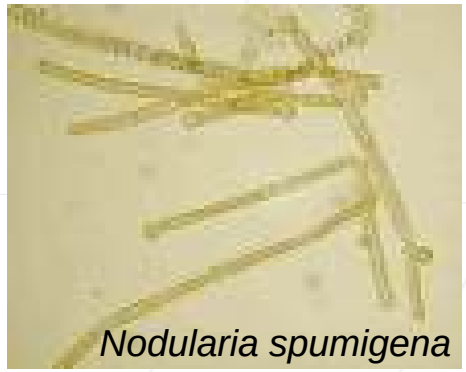


AA 1	AA 2	AA 3	AA 4	AA 5	AA 6	AA 7
D-Ala	**L-Leu**	**D-MeAsp**	**L-Arg**	**Adda**	**D-Glu**	**Mdha**
D-Ser	L-Ala	D-Asp	L-Aib	ADMAdda	D-MeGlu	Dha
	L-Glu		L-Ala	DMAdda	$OC_2H_3(CH_3)$OH-Glu	Dhb
	L-GluMe		L-Glu	(6Z)Adda		L-Ala
	L-Har		L-GluMe			L-MeSer
	L-Hil		L-Har			L-Ser
	L-Hph		L-Hph			Mdhb
	L-Hty		L-Hty			MeLan
	L-Met		L-Leu			
	L-Met(O)		L-Met			
	L-Phe		L-Met(O)			
	L-ThTyr		L-Phe			
	L-Trp		L-Trp			
	L-Tyr		L-Tyr			
			L-Val			

Nodularins



Nodularia spumigena



Qualitative detection of cyanotoxins

Aim:

Survey method for the *qualitative* detection of cyanobacterial freshwater toxins

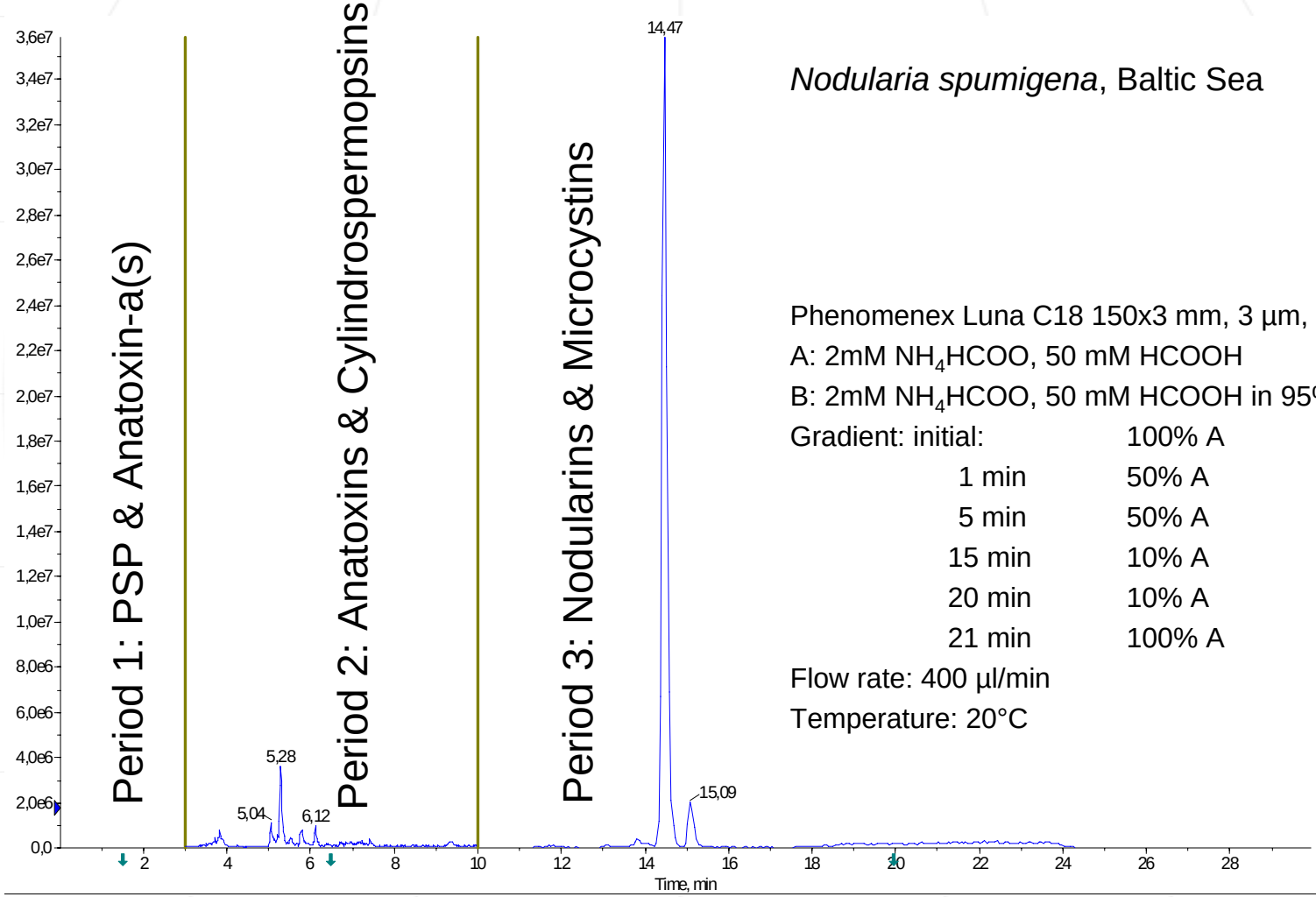
Prerequisites:

All toxins soluble in the same extraction solvent

Characteristic fragment for each toxin group

Toxin group (not single compound!) separation

Qualitative detection of cyanotoxins



Nodularia spumigena, Baltic Sea

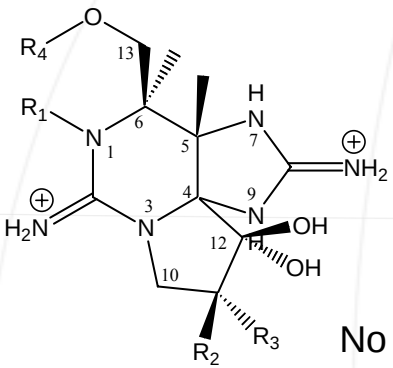
Phenomenex Luna C18 150x3 mm, 3 µm, 100 Å
A: 2mM NH_4HCOO, 50 mM HCOOH
B: 2mM NH_4HCOO, 50 mM HCOOH in 95% MeOH

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. (2007) J. Mass Spectrom. 42(9), 1238-1250

Period 1: PSP & Anatoxin-a(s)

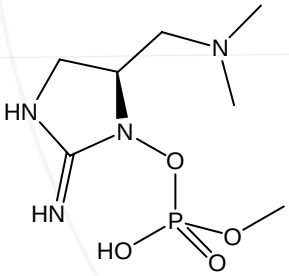


PSP-toxins

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)

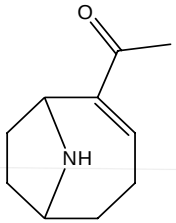
Only one toxin known

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

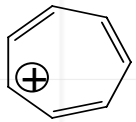
Period 2: Anatoxins & Cylindrospermopsins

Experiment 1



Anatoxin-a

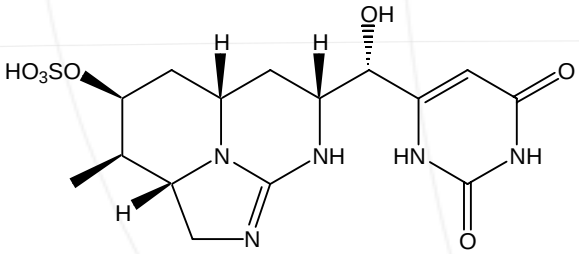
characteristic fragment: m/z 91



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

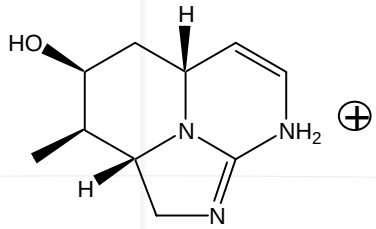
CUR:	25
CAD:	High
IS:	5200
TEM:	550
GS1:	50
GS2:	70
ihe:	OFF
DP:	80
EP:	10
CE:	30
CXP:	12

Experiment 2



Cylindrospermopsin

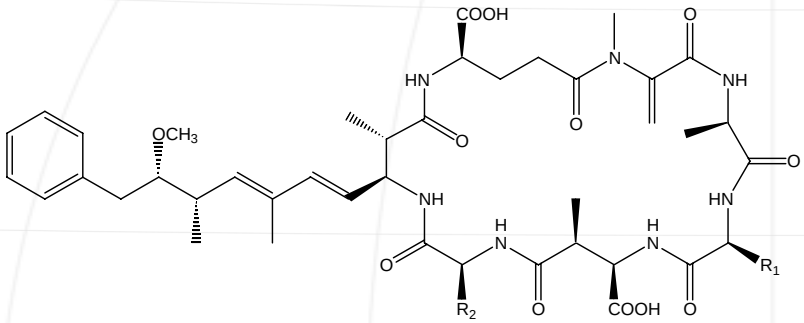
characteristic fragment: m/z 194



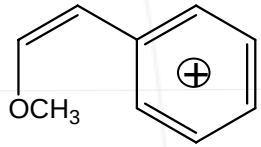
API 4000 QTrap, positive
precursor ion (*m/z*): 194.0
scan range (*m/z*): 350-450 amu

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

Period 3: Microcystins & Nodularins

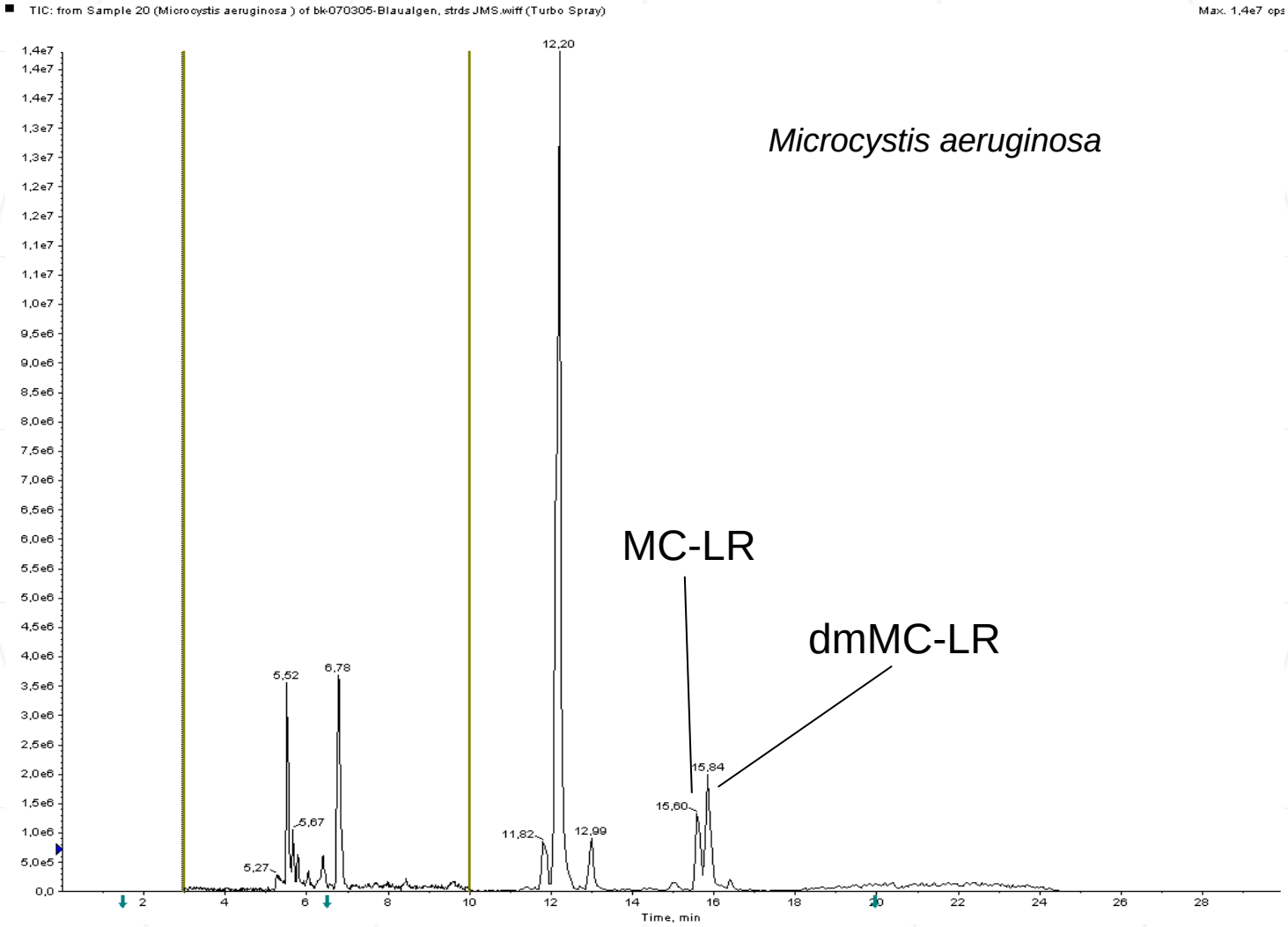


characteristic fragment: m/z 135

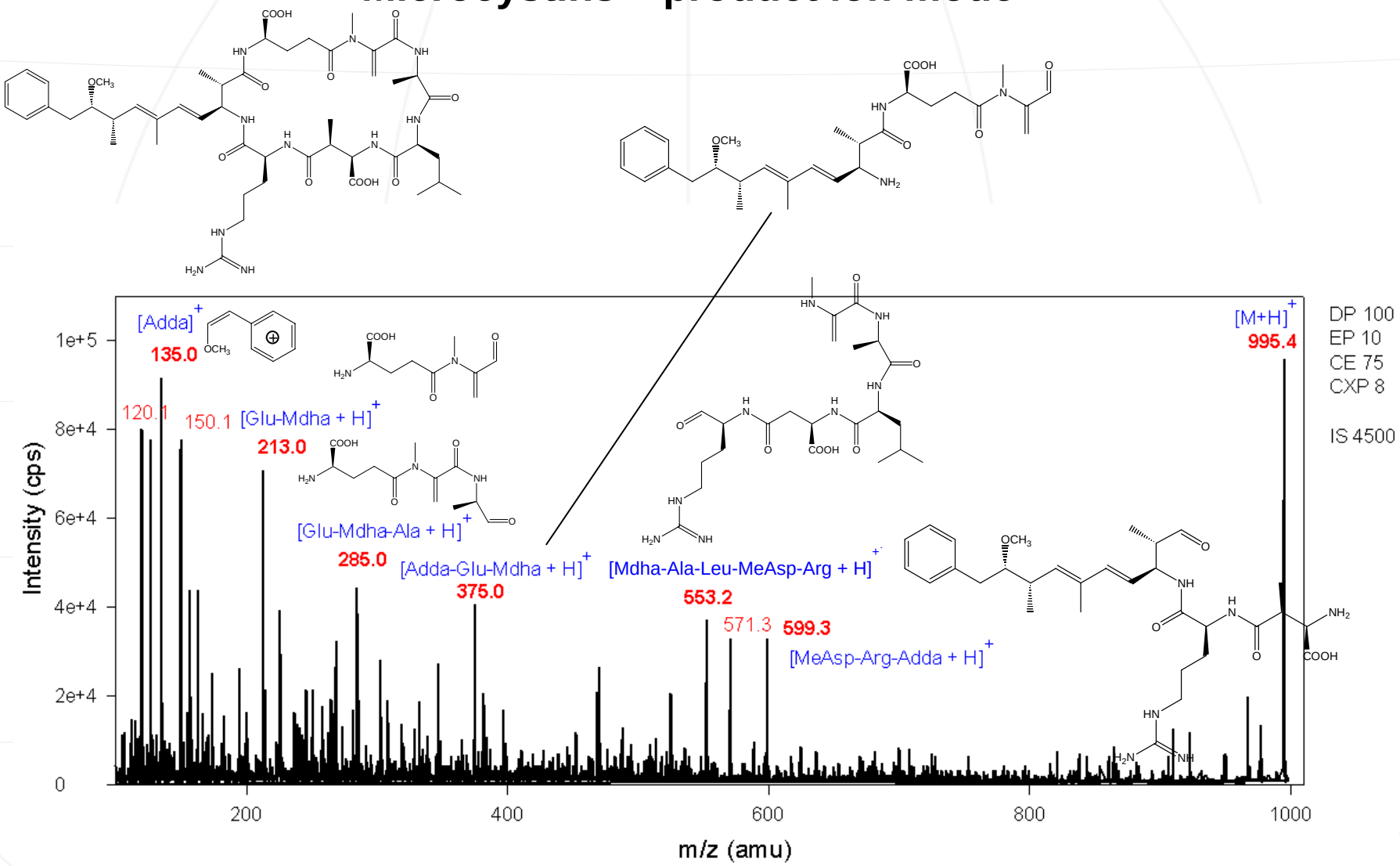


	experiment 1	experiment 2	experiment 3	experiment 4
scan range (*m/z*)	400 - 575	400 - 575	900 - 1150	800 - 850
protonated fragment ions $[M+H]^+$ / $[M+2H]^{2+}$	$[M+2H]^{2+}$	$[M+2H]^{2+}$	$[M+H]^+$	$[M+H]^+$
collision energy (eV)	17	35	60	90
declustering potential (V)	46	40	60	175
cyanobacterial toxins: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

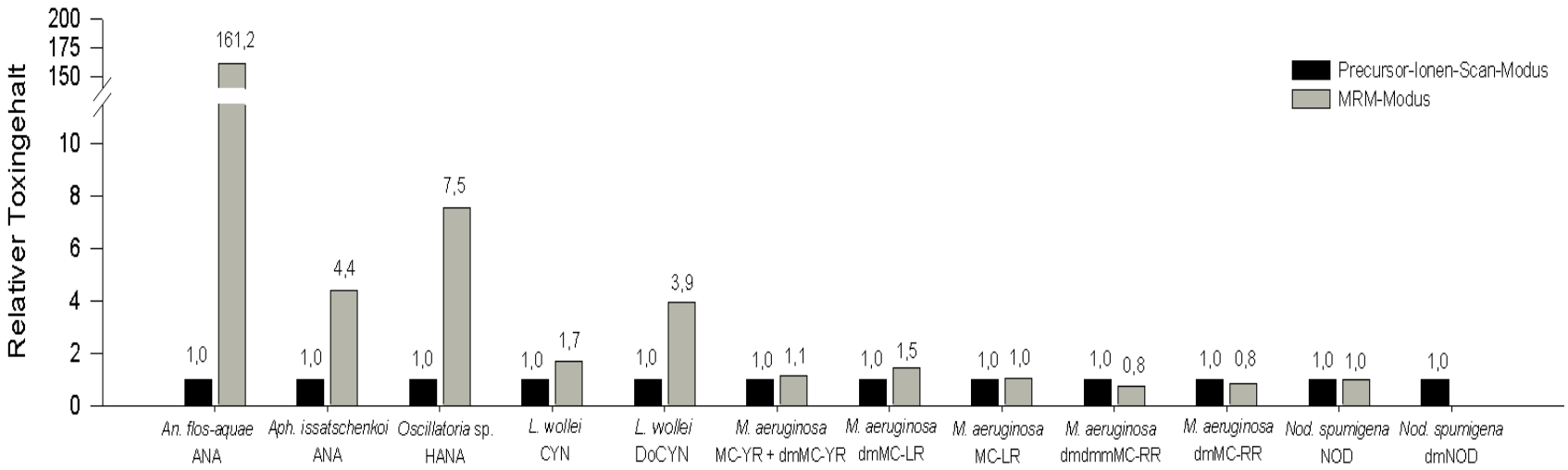
Microcystins – precursor mode



Microcystins – product ion mode



Quantitation – product ion mode



Take home messages

1. precursor ion mode is a powerful tool to detect known and unknown structural variants of cyanotoxins

2. the presented method covers all to date known limnic cyanotoxin classes

3. putative cyanotoxins have to be confirmed by independent methods (product ion spectra, immuno assays, etc.)

4. Quantitative analysis in the precursor ion mode is in good agreement with MRM for microcystins and nodularins, but understimates values for anatoxins and cylindrospermopsins

Thanks to…

Susann Hiller, Friedrich-Schiller-Universität Jena

…and for your attention!