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CEE 697z

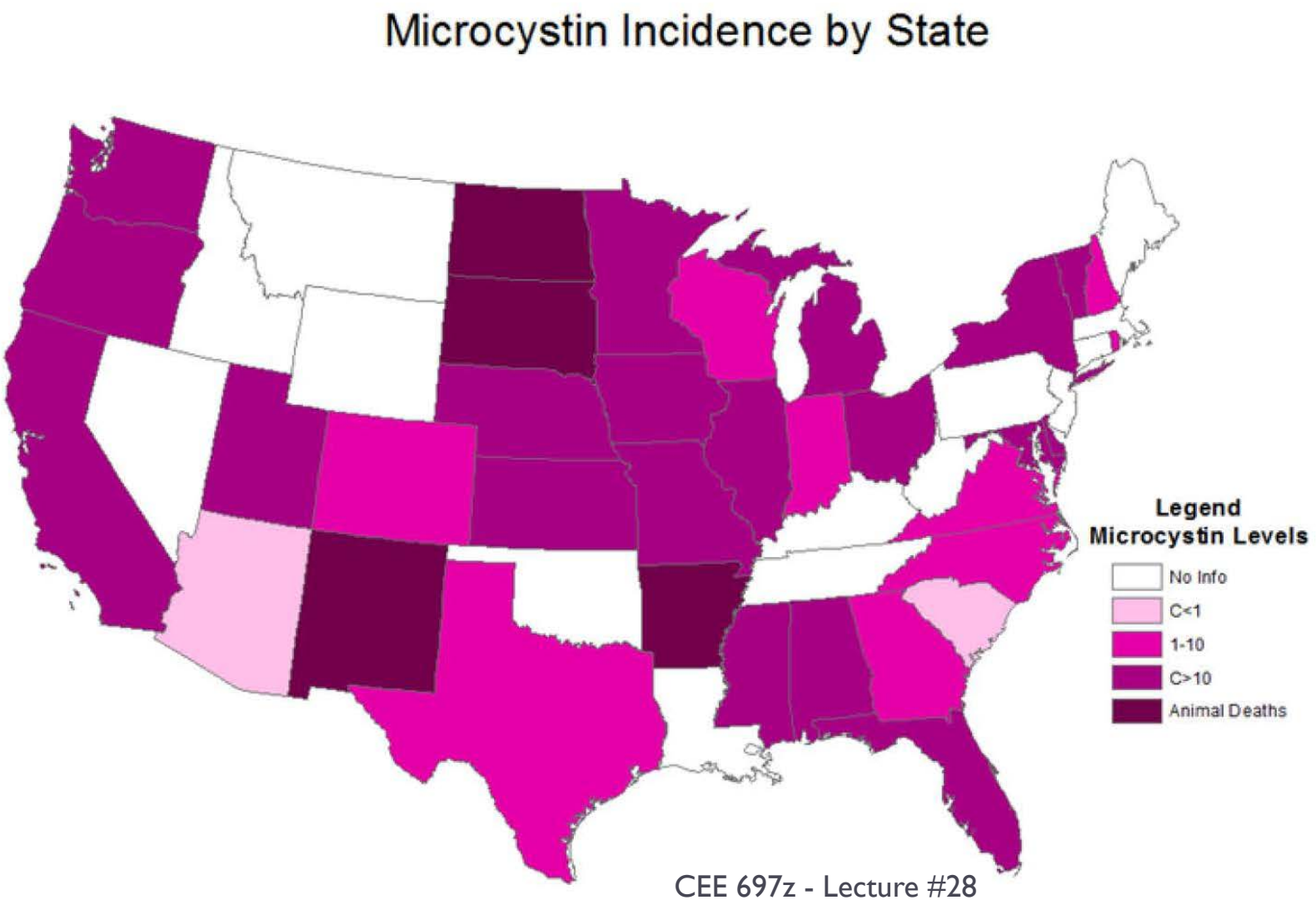
Organic Compounds in Water and Wastewater

Cyanotoxins

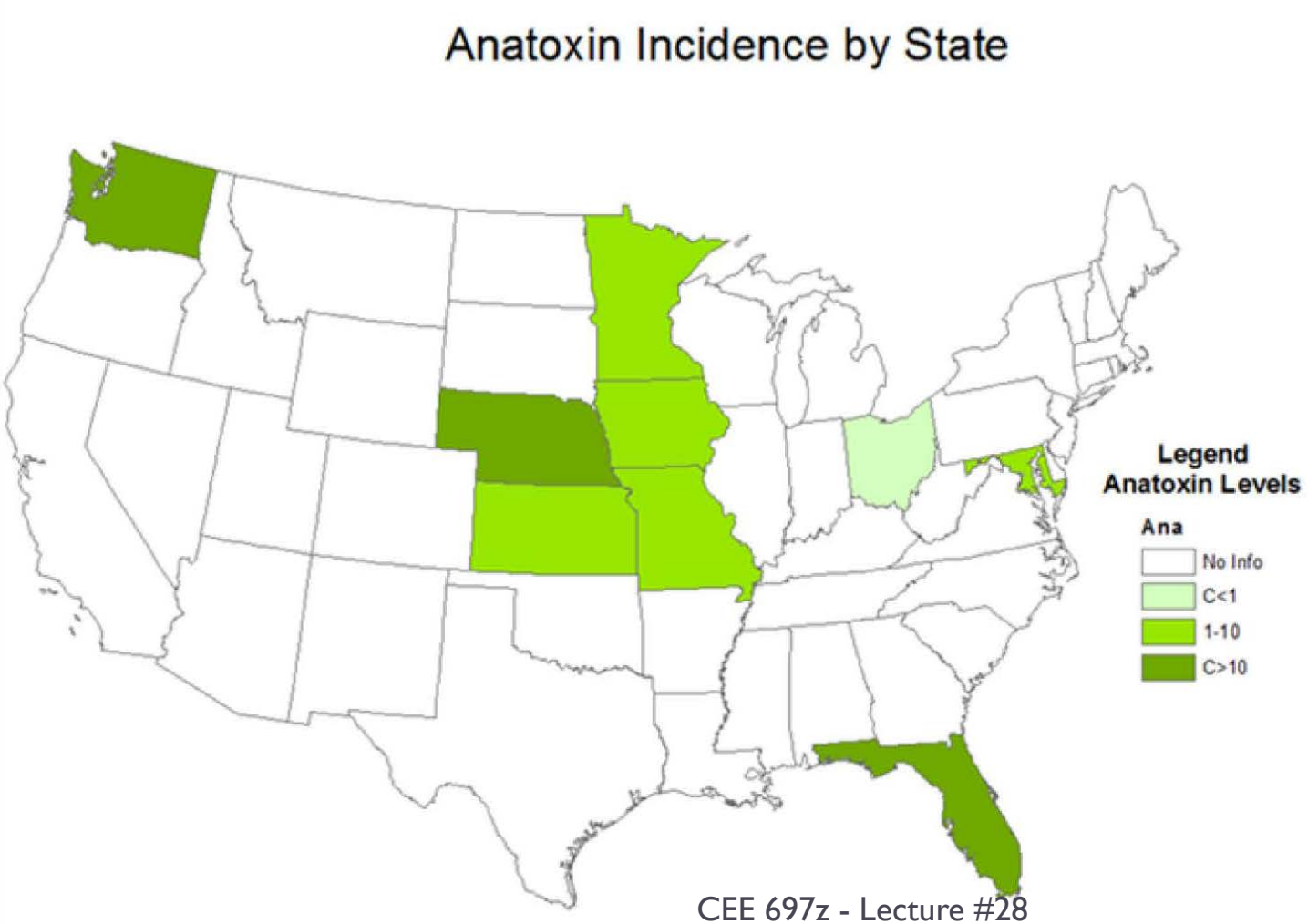
Occurrence & Chemical Analysis

Lecture #28

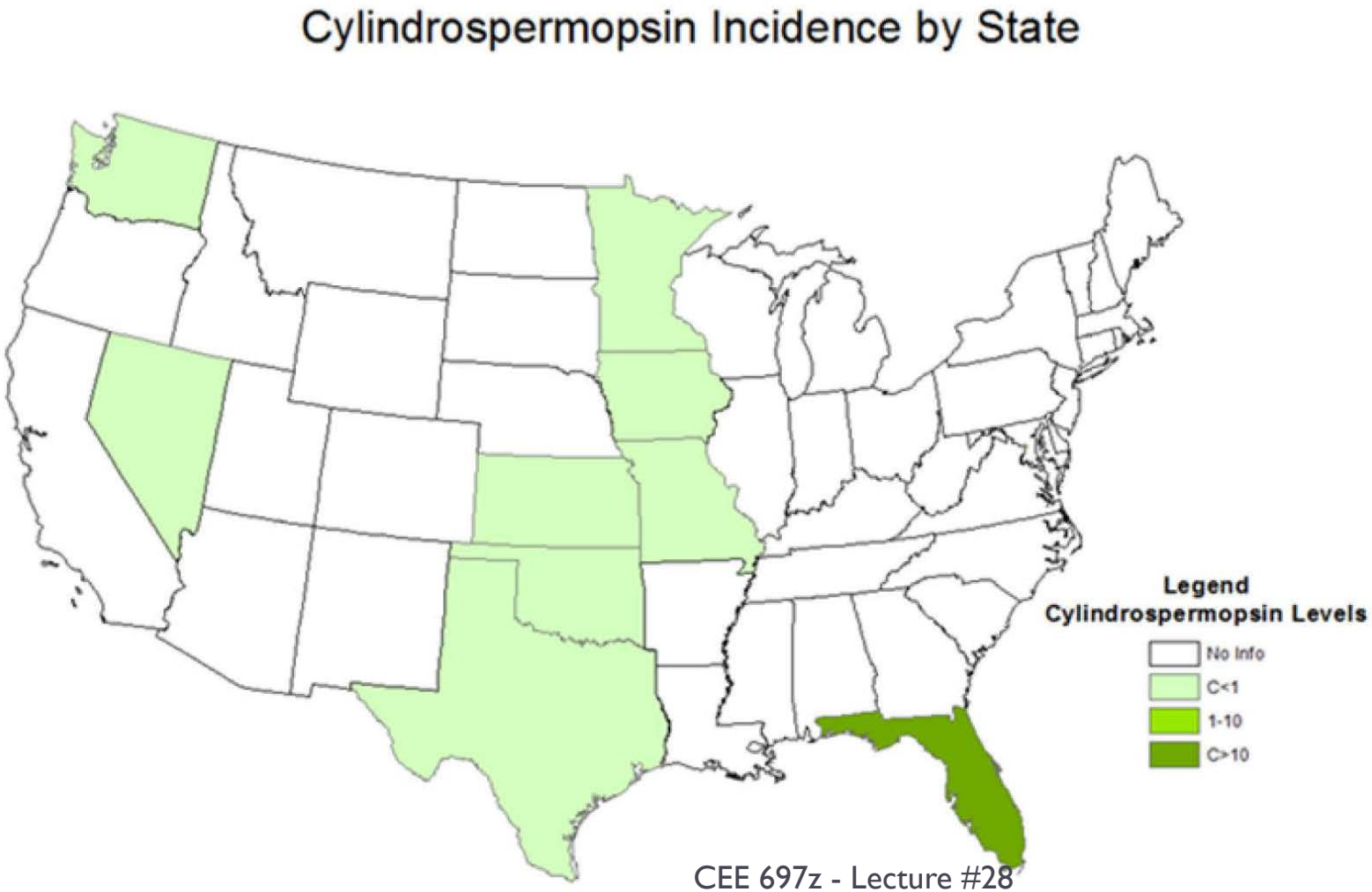
Microcystin Occurrence in US



Anatoxin


Anatoxin Incidence by State
Legend
Anatoxin Levels
Ana
No Info
C<1
1-10
C>10

Cylindrospermopsin



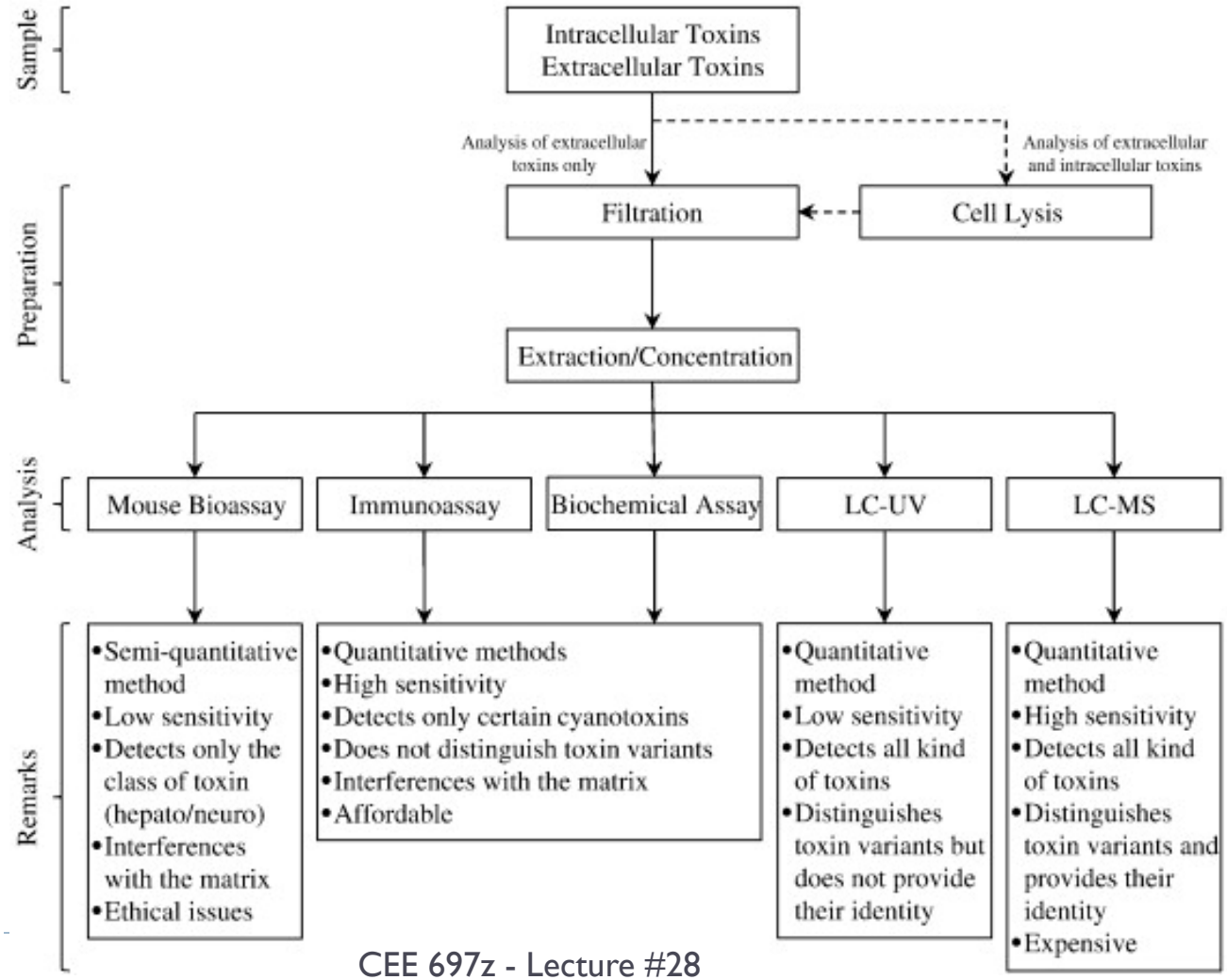
Analysis

From: "*Analytical Methods for Cyanotoxin Detection and Impacts on Data Interpretation*", Loftin et al., 2010, USG

	Freshwater Cyanotoxins				
	Anatoxins	**Cylindrospermopsins**	**Microcystins**	**Nodularins**	**Saxitoxins**
Bioloigcal Assays (Class Specific Methods at Best):					
Mouse	Y	Y	Y	Y	Y
PPIA	N	N	Y	N	N
Neurochemical	Y	N	N	N	Y
ELISA	?	Y	Y	Y	Y
Chromatographic Methods (Compound Specific Methods):					
Gas Chromatography:					
GC/FID	Y	N	N	N	N
GC/MS	Y	N	N	N	N
Liquid Chromatography:					
LC/UV (or HPLC)	Y	Y	Y	Y	Y
LC/FL	Y	N	N	N	Y
Liquid chromatography combined with mass spectrometry can analyze cyanotoxins very specifically.					
LC/IT MS	Y	Y	Y	Y	Y
LC/TOF MS	Y	Y	Y	Y	Y
LC/MS	Y	Y	Y	Y	Y
LC/MS/MS	Y	Y	Y	Y	Y

Analysis

Fig. 5 Overview of sample preparation and analytical methods for the detection of cyanotoxins.



Merel, S., Walker, D., Chicana, R., Snyder, S., Baures, E. and Thomas, O. (2013) State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environment International 59, 303-327.

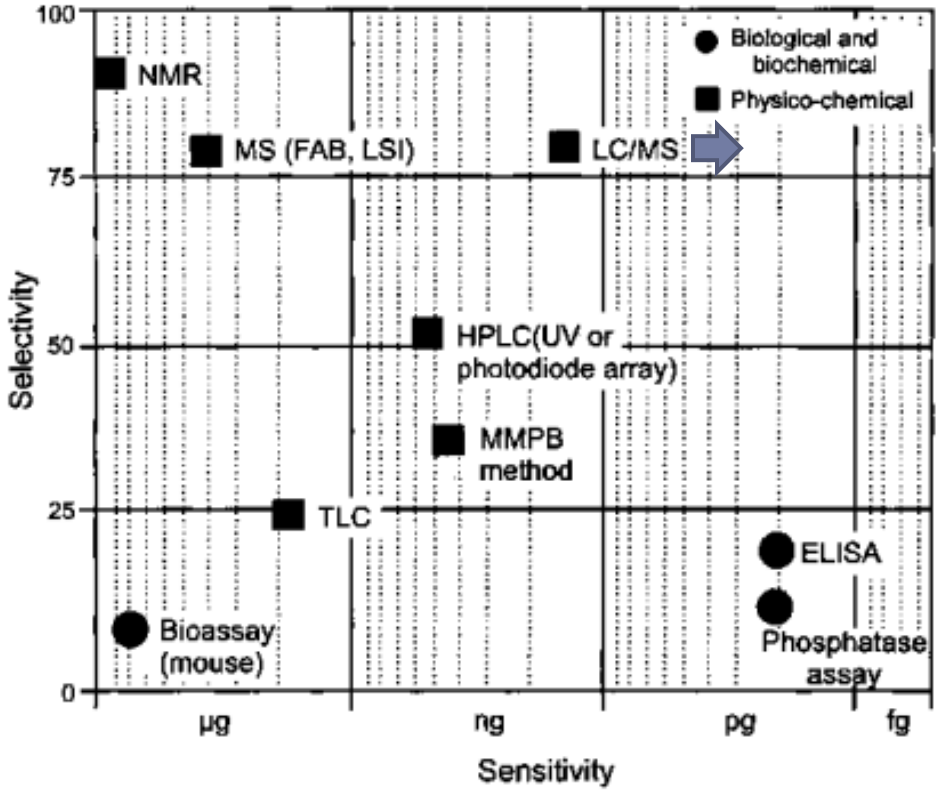
Analytical Specificity

From: "*Analytical Methods for Cyanotoxin Detection and Impacts on Data Interpretation*", Loftin et al., 2010, USGS

	Specificity
Biological Assays (Class Specific Methods at Best):	
Mouse	Non-specific, test must be rapid therefore endpoint usually death.
PPIA	Of the freshwater cyantoxins, only microcystins are known to inhibit protein phosphatase.
Neurochemical	Of the freshwater cyanotoxins, only anatoxins and saxitoxins are known to inhibit neurochemical processes.
ELISA	Compound and toxin class specificity dependent on antibody or mix of antibodies used.
Chromatographic Methods (Compound Specific Methods):	
Gas Chromatography:	
GC/FID	Only the anatoxins have been routinely measured. Derivitization is typically required.
GC/MS	Only the anatoxins have been routinely measured. Derivitization is typically required.
Liquid Chromatography:	
LC/UV (or HPLC)	Variable. Subject to interference with co-eluting matrix.
LC/FL	Variable. Subject to interference with co-eluting matrix.
Liquid chromatography combined with mass spectrometry can analyze cyanotoxins very specifically.	
LC/IT MS	Second in compound specificity only to LC/TOF MS.
LC/TOF MS	Accurate mass capability makes this technique the most specific.
LC/MS	Weaker cousin of LC/MS/MS. Fourth most specific.
LC/MS/MS	Third most specific technique routinely employed

Selectivity & Sensitivity

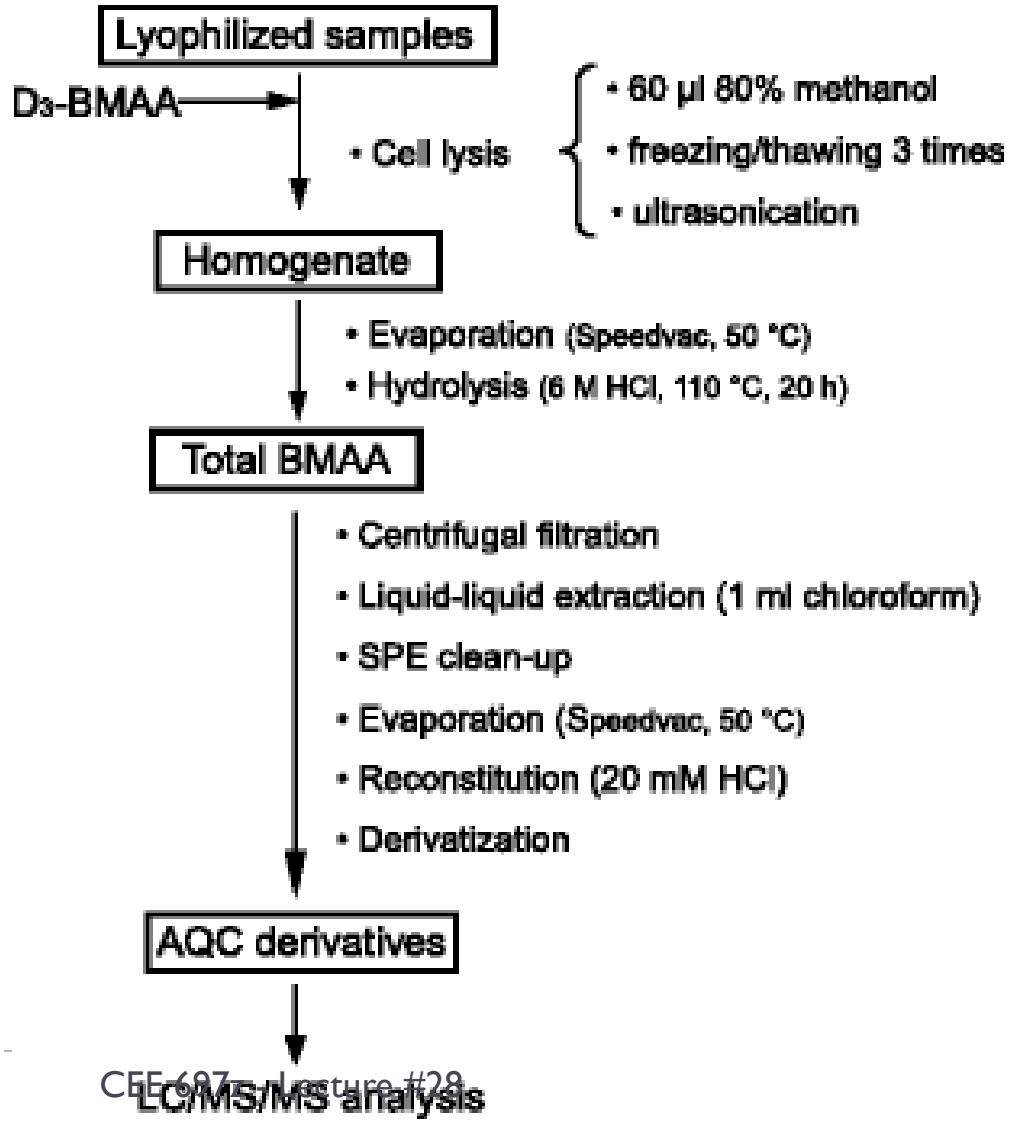
Figure 13.1 Relationship between sensitivity and selectivity of analytical methods for microcystins (see text for explanation of methods)



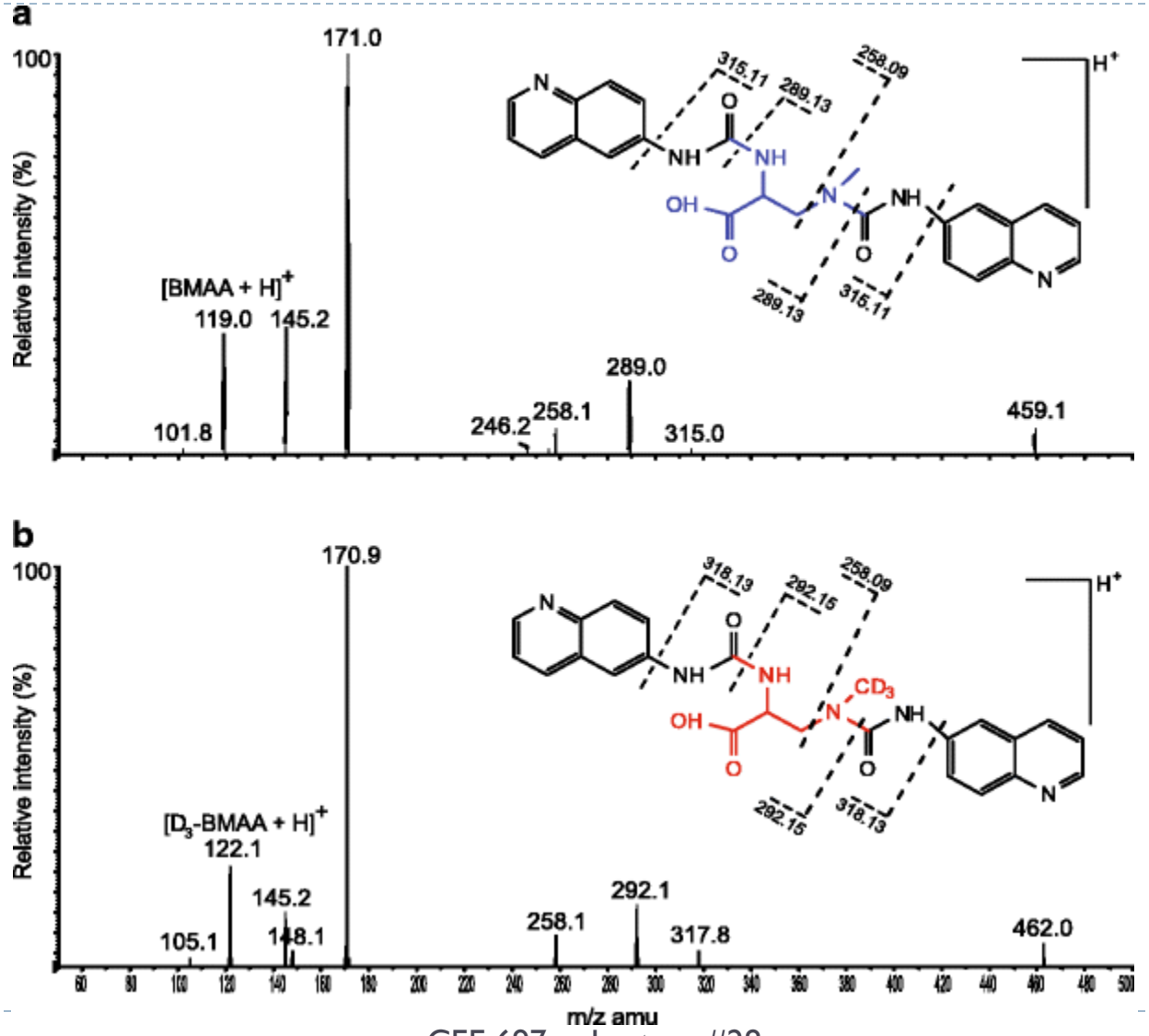
Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management, Edited by Ingrid Chorus and Jamie Bartram , 1999 WHO

Example: Analysis of BMAA I

Sample preparation scheme for determining total BMAA in cyanobacteria (D_3-BMAA internal standard was added only to samples for quantification)



Analysis of BMAA II



ESI-MS/MS product ion spectra of (**a**) BMAA and (**b**) D_3-BMAA

WRF Study on Methods

- 2007, "Determination and Significance of Emerging Algal Toxins
  - Nicholson et al.
- Analytes
  - Anatoxin-a
  - Saxitoxin (STX) & derivatives (PSTs)
  - Cylindrospermopsin (CYN)

Preferred Method

- LC/MS/MS selected
- HILIC for highly polar PSTs
  - Hydrophilic interaction liquid chromatography
- Prior SPE
  - procedure

Both C_{18} and Carbograph SPE cartridges were conditioned by passing 5 mL of methanol followed by 5 mL of MilliQ water through the cartridges. Samples (20 mL) were loaded onto the C_{18} SPE cartridge and passed through at a flow rate of 1 mL/min. The cartridge was eluted with 10 mL of 5% acetic acid in methanol at a flow rate of 1 mL/min. The pH was adjusted to 6.0 with a solution of NaOH (1.0 M); this solution was applied to the Carbograph column and allowed to drain at 1 mL/min, followed by washing with 5 mL of MilliQ water. The final elution was done with two 5 mL volumes of 5% formic acid in methanol at a flow rate of 1 mL/min. The combined eluates were evaporated to dryness under N_2 and redissolved in 200 µL of MilliQ water for HILIC/MS/MS analysis.

Selection of SPE phase

Table 6.3
Preliminary evaluation of different SPE cartridges for concentrating mixtures of toxins

Chromatographic property	Solid phase extractor				
	C_2	C_8	C_{18}	Carbographs	HLB Oasis
CYN retention capacity	low	low	very low	high	low*
Anatoxin-a retention capacity	high	high	high	high	low*
STX retention capacity	high	high	high	high	low
CYN elution capacity	very low	very low	very low	high	high
Anatoxin-a elution capacity	very high	very low	very low	high	high
STX elution capacity	high	very low	very low	very high	very low
Overall performance	moderate	low	low	good	low

Note. Very low < 30%, low < 50%, high > 70% and very high >85%
* we have re-evaluated the CYN and anatoxin-a solid phase concentration using HLB Oasis SPE Cartridges of high capacity (500 mg) and found that it is suitable to concentrate CYN and anatoxin-a but not suitable for STX

Carbograph is non-porous graphitized carbon black. Its surface contains oxygen complexes forming positively charged chemical heterogeneities on surface offering rapid both primary reversed-phase and secondary anion exchange interaction

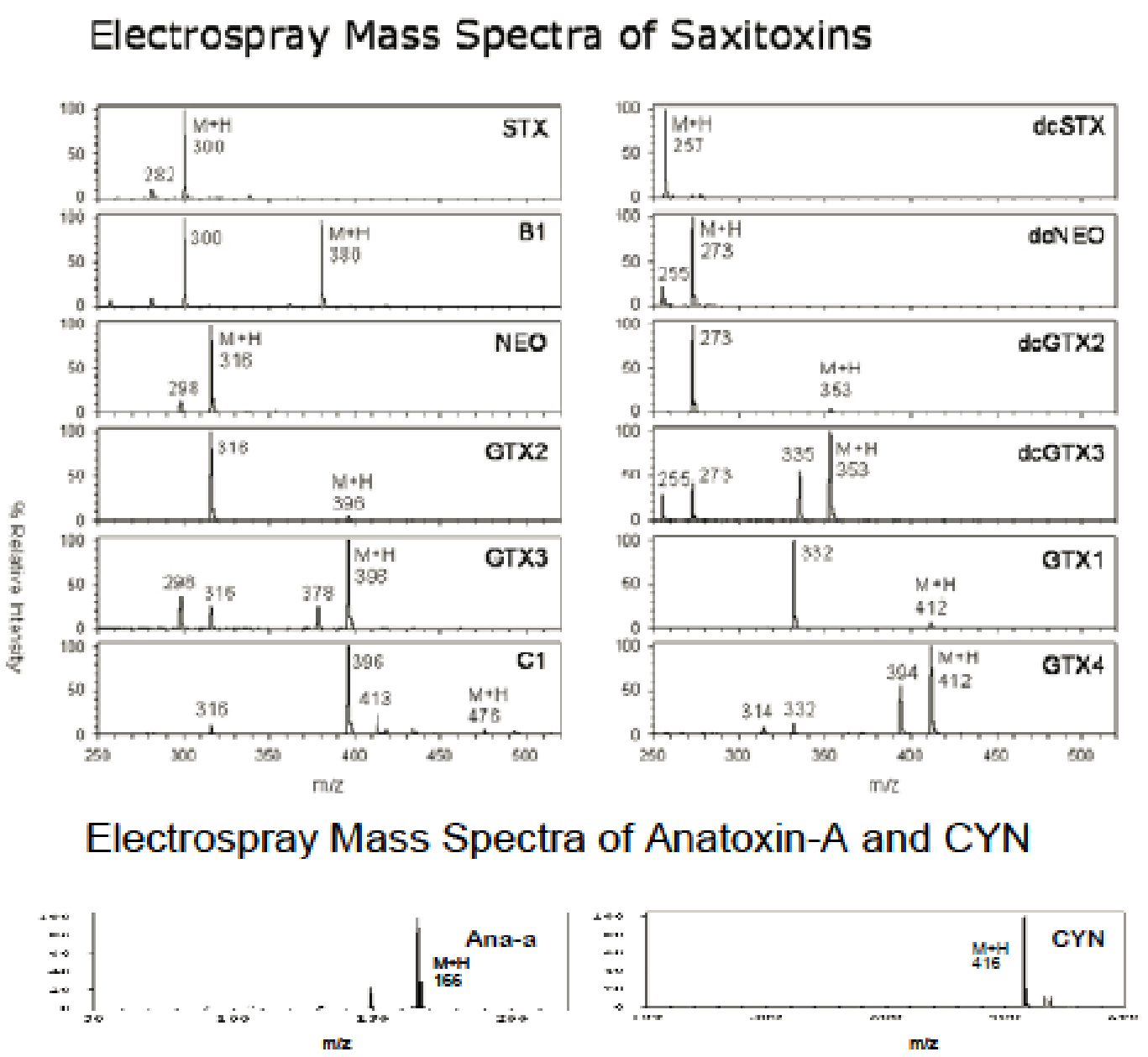


Figure 6.2 Mass spectra of toxins examined in this project

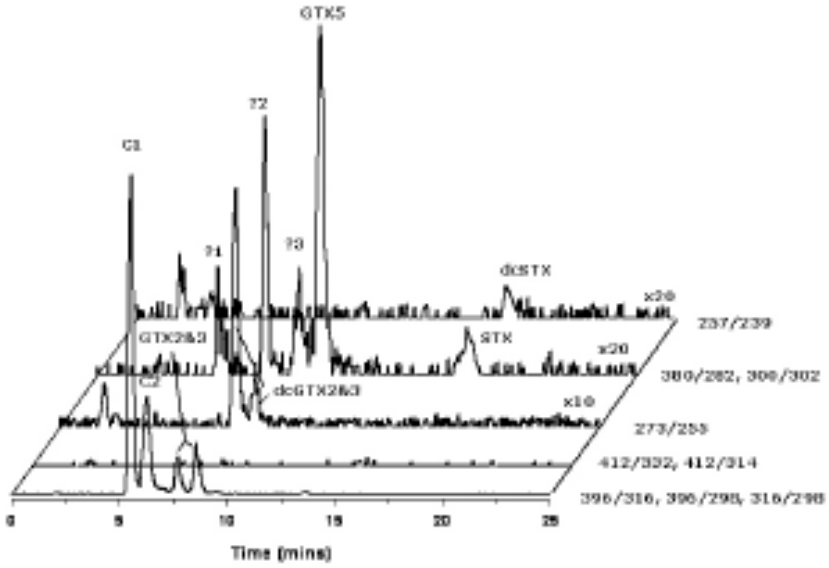


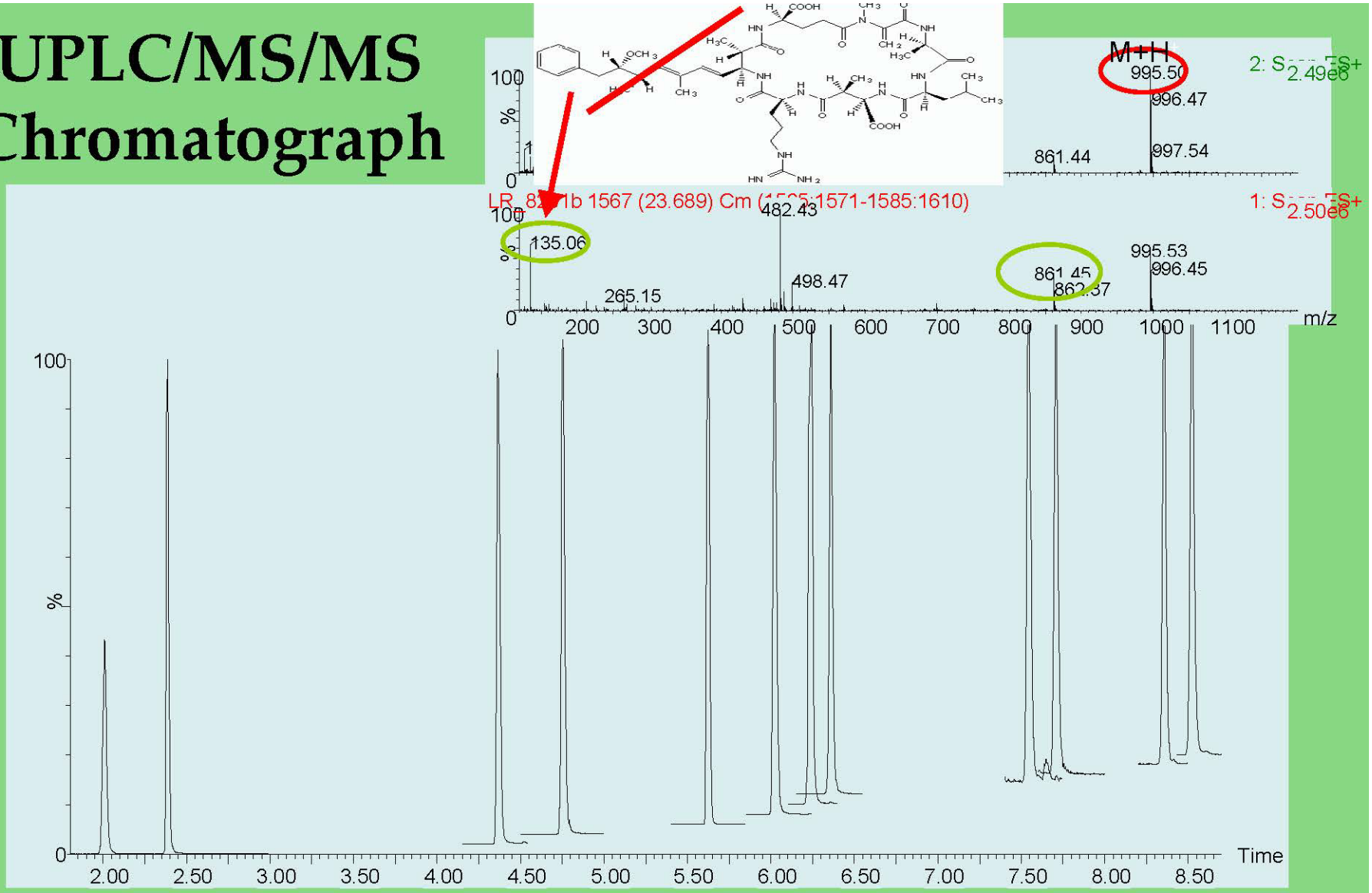
Figure 6.3 HILIC-Mass spectra of saxitoxins in water from Burrinjuck Dam

Table 6.6
Ions monitored for saxitoxins, cylindrospermopsin and anatoxin-a using HILIC/MS/MS methodology

Toxin	SIM Ions *m/z* (%RI)		SRM Ions *m/z*→*m/z* (%RI)	
STX	300 (100)		300/282 (100),	300/204 (70)
neoSTX	316 (100)		316/298 (100)	
GTX2	316 (100),	396 (25)	396/316 (100),	316/298 (30),
GTX3	396 (100),	316 (30)	396/298 (100),	396/316 (20),
GTX1	332 (100),	412 (30)	412/332 (100),	412/314 (1)
GTX4	412 (100),	332 (30)	412/314 (100),	412/332 (40)
GTX5 (B1)	380 (100),	300 (40)	380/300 (100),	380/282 (15),
GTX6 (B2)	396 (100),	316 (30)	396/316 (100),	396/298 (40),
C1	396 (100),	476 (20)	396/316 (100),	396/298 (40),
C2	396 (100),	476 (20)	396/298 (100),	396/316 (20),
C3	492 (100),	412 (30)	412/332 (100),	412/314 (20)
C4	492 (100),	412 (30)	412/314 (100),	412/332 (10)
dcSTX	257 (100)		257/239 (100)	
dcneoSTX	273 (100)		273/255 (100)	
dcGTX2	273 (100),	353 (70)	353/273 (100),	273/255 (30)
dcGTX3	353 (100),	273 (20)	353/273 (100),	273/255 (30)
dcGTX1	289 (100),	369 (50)	369/289 (100)	
dcGTX4	369 (100),	289 (1)	369/289 (100)	
ANTX-a	166 (100),	149 (30)	166/91 (100),	166/131 (30),
CYN	416 (100),	433 (30),	416/194 (100),	416/176 (50),

SIM: Selected ion monitoring; SRM: Selected reactant monitoring; %RI: Percentage relative intensity of ions

UPLC/MS/MS Chromatograph



1=Cylindrospermopsin, **2**=Anatoxin-a, **3**=Cyclo (Arg-Ala-Asp-D-Phe-Val) (IStd), **4**=[Leu^5]-Enkephalin (IStd), **5**= Microcystin RR, **6**=Nodularin, **7**=Microcystin YR, **8**=Microcystin LR, **9**=Microcystin LA, **10**=Microcystin LY, **11**=Microcystin LW, and **12**=Microcystin LF

- To next lecture