Algal Toxins: Challenges in Drinking Water Analysis

Algal Toxins Webinar

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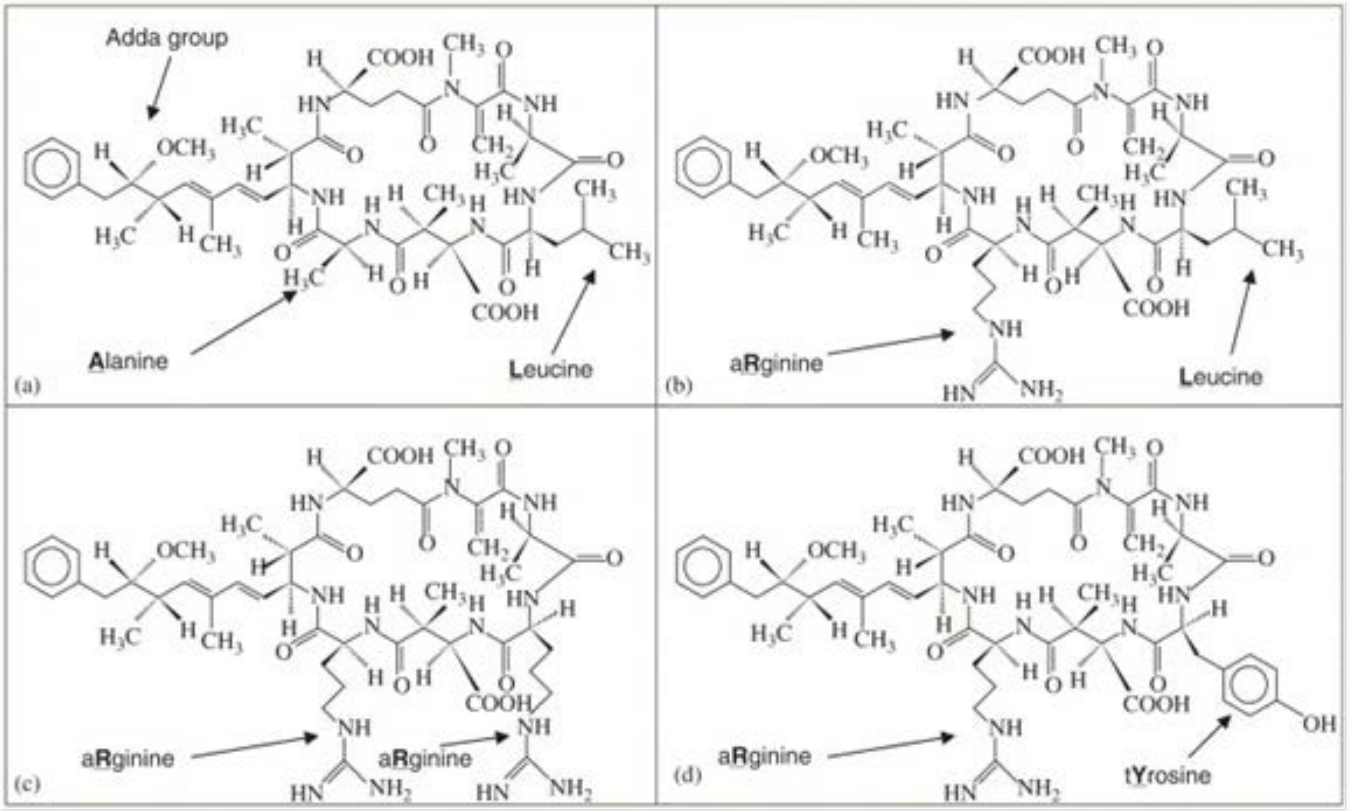
Algal Toxins: Background

- Freshwater cyanobacteria (blue-green algae) produce several toxic compounds
  - Algal toxins have been detected in both source & finished waters
  - While most data relates to cyanobacterial poisoning of animals, there is concern regarding long-term low-level exposure causing chronic health effects in humans
- Due to these health concerns, algal toxins were placed on EPA's Contaminant Candidate List (CCL)
  - Contaminants known, or anticipated, to occur in public water systems
  - Assists prioritization and data collection under UCMR
- WHO guideline drinking water limits: 1 µg/L cylindrospermopsin and microcystins (based on microcystin-LR); 3 µg/L anatoxin-a

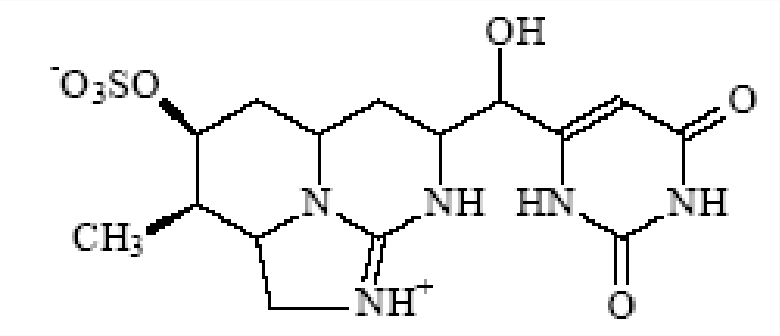
Algal Toxins: Background

- EPA convened a meeting in 2001 to discuss algal toxins as emerging contaminants
  - Developed a prospective list of contaminants for analytical method development
    - Four microcystins: MC-RR, MC-YR, MC-LR, MC-LA
    - Cylindrospermopsin
    - Anatoxin-a

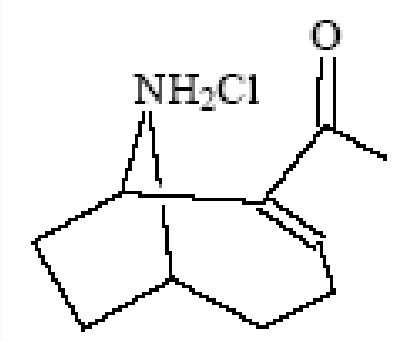
Algal Toxins: Structures
Microcystins



Algal Toxins: Structures
Cylindrospermopsin and Anatoxin-a



Cylindrospermopsin



Anatoxin-a

EPA Drinking Water Methods

- All drinking waters are not the same!
  - Variable geographic source waters
- Selective, sensitive, and robust
  - Selective detection for unambiguous identification
  - Enzyme-linked immunoassays: sensitive but not selective
  - UV: universal detection, not selective
  - MS: selective detection

EPA Drinking Water Methods

- Preservation of samples
  - Sequester oxidants (e.g., free chlorine)
  - Prevent microbial degradation
- Quantitative analysis/QC
  - Internal standardization
  - Extraction surrogate

One Method or Two?

- A single method is desirable, but may not be practical
- Structural dissimilarities and physical properties indicate development of separate methods:
  - Microcystins
    - Cyclic structure is stable
    - LC-MS/MS analysis. Ionization results in a cascade of fragments – relatively weak signal
    - Requires pre-concentration using solid phase extraction (SPE) to increase sensitivity
    - Co-extracted organic matter causes serious matrix effects; impacts recoveries. Clean-up to remove organic matter is essential!

One Method or Two?

- Cylindrospermopsin and Anatoxin-a
  - Too polar to extract/concentrate using SPE
  - Direct injection LC-MS/MS. Both yield excellent product ions; good sensitivity
  - Matrix effects can still be a potential issue

Additional Analytical Challenges

- Algal toxins are derived from natural products
  - Lack of certified standards
  - Lot-to-lot variability (purity and mass supplied - µg quantities)
  - Lack of isotopically-labeled analogs to serve as internal standards/surrogates

Microcystins: LC-MS/MS Method Development

HPLC: Methanol/Water mobile phase acidified with 10mM formic acid (Microcystin pKa's are ~3.5; acidic mobile phase facilitates ionization)

MS: Triple quadrupole MS; ESI+ ionization mode

Capillary Voltage	1.5 kV
Source Temperature	115 ^{o}C
Desolvation Temperature	350 ^{o}C
Desolvation Gas Flow	700 L/hr
Cone Gas Flow	50 L/hr
Collision Cell Pressure	2 e-3 mbar

Microcystins: LC-MS/MS Method Development

Maximal sensitivity favors the $[M+2H^+]^{2+}$ precursor for Microcystin-RR and the $[M+H^+]^+$ precursor for Microcystins-YR and –LR. Product ion at m/z 135 represents the unique Adda fragment. Microcystin-LA yielded weak fragment at m/z 135.

Analyte	Precursor m/z	Cone Voltage, V	Product Ion m/z	Collision Energy, eV
Microcystin-RR	520	35	135	30
Nodularin	825	60	135	55
Microcystin-YR	1045	65	135	60
Microcystin-LR	995	70	135	70
Microcystin-LA	910	35	776	20

Microcystins: LC-MS/MS Method Development

- Calibration
  - 50-fold range (0.01 to 0.5 µg/mL)
  - Solid phase extraction: One-liter sample concentrated to 1-mL final volume, resulting in 1000-fold concentration factor to the µg/L level.

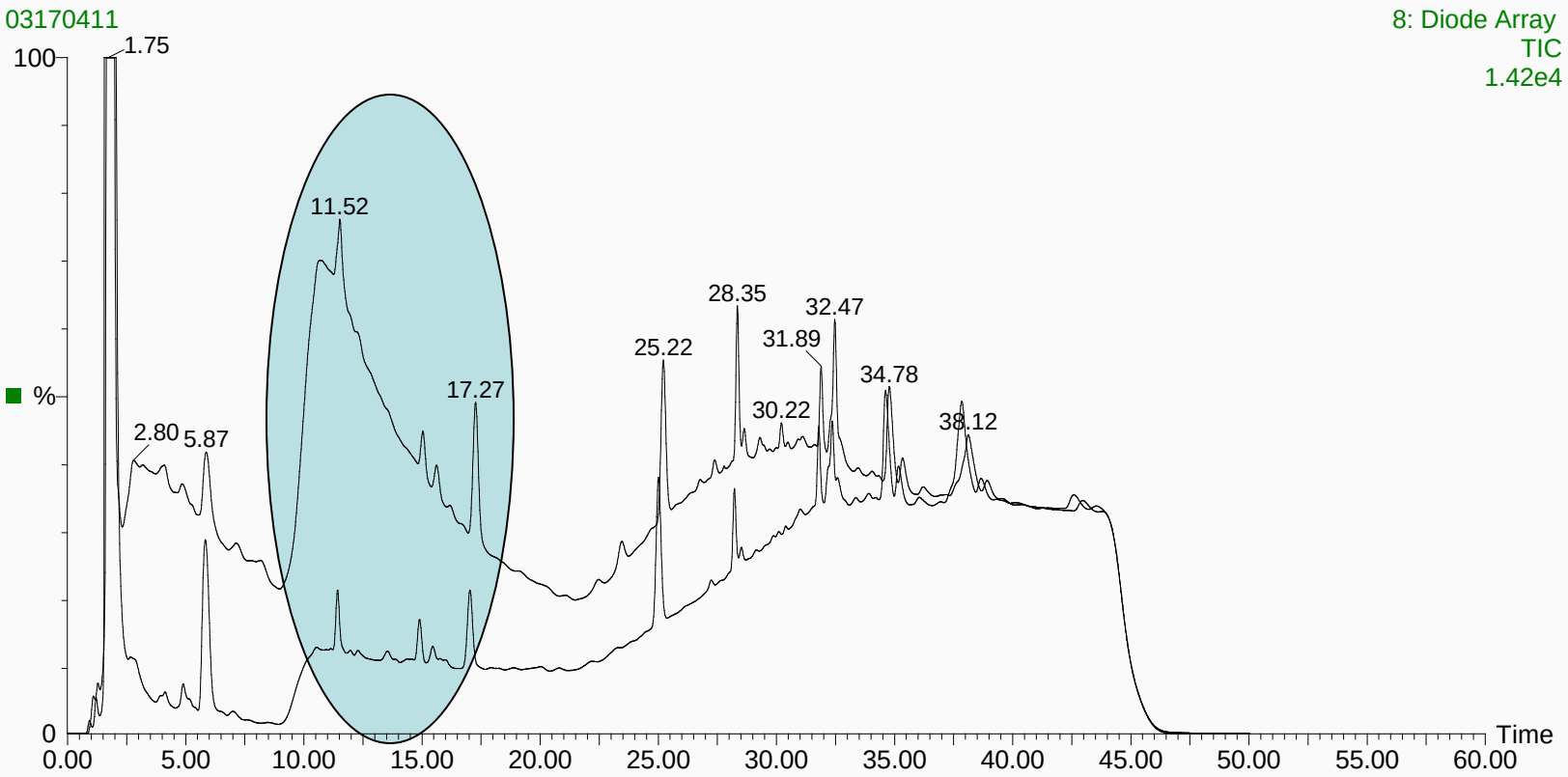
Microcystins: LC-MS/MS Method Development

Solid Phase Extraction

Analyte	Oasis HLB cartridges (n = 5) (5/4/06)	3M Empore Styrene-DVB disks (n = 5) (5/2/06)
Microcystin-RR	101 ± 4.8%	96.8 ± 3.6%
Microcystin-YR	97.2 ± 3.5%	87.7 ± 4.2%
Microcystin-LR	96.1 ± 2.6%	88.6 ± 3.3%
Microcystin-LA	**47.1 ± 33%**	**73.9 ± 9.0%**

Note: C18 SPE media yields low recoveries for MC-RR

Microcystins: LC-MS/MS Method Development



Summary

- Development of a robust, sensitive, and selective drinking water method for the analysis of algal toxins entails numerous challenges:
  - Must be applicable to finished drinking waters throughout the nation
  - Need certified standards; confidence in lot-to-lot consistency
  - Need isotopically-labeled, or other appropriate, internal standards and extraction surrogates
  - Solid phase extraction: Need concentration of the contaminants of interest with minimal co-extraction of unwanted interferents