EPA Analytical Methods for Cyanotoxins

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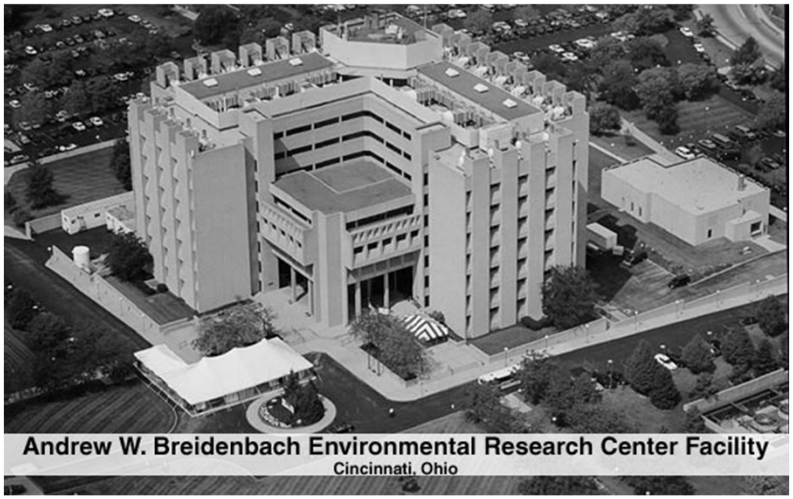
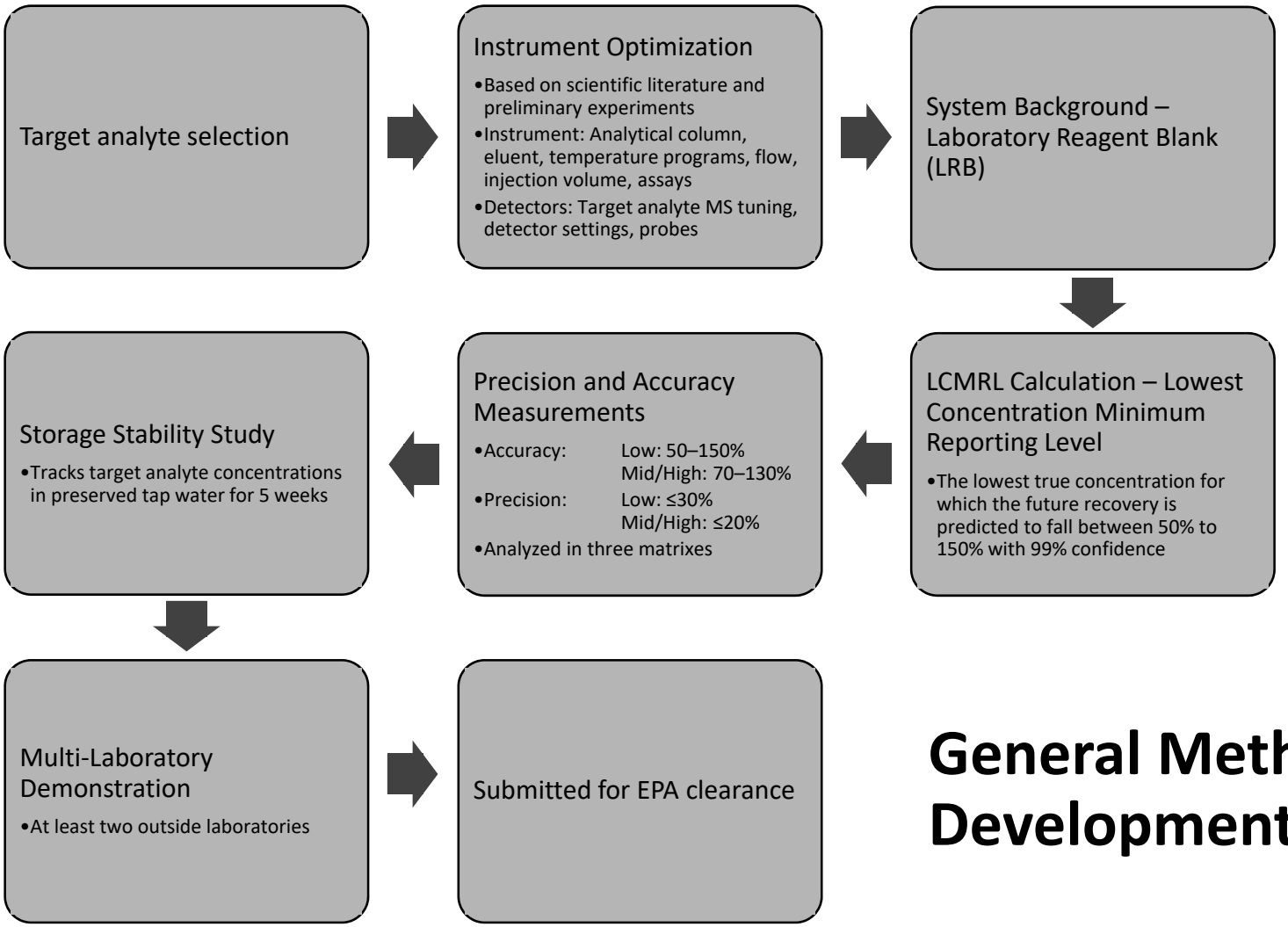
Overview

- Method development
- EPA methods used for cyanotoxin analysis
- Comparing techniques





General Method Development

Microcystins DW Methods Overview

Summary Options	ELISA-Field (Tube/Strips)	ELISA-Lab	LC-MS/MS
Scope	"Total Microcystins and Nodularins"	"Total Microcystins and Nodularins" (EPA Method 546)	6 Specific Microcystin Congeners and Nodularin-R (EPA Method 544)
Approx. Limit of Quantification (LOQ)	~0.5 – 1 ug/L	~ 0.3 µg/L	~ 0.02 µg/L
Time to Result	10 – 60 minutes	1 – 4 hours	< one day

Cylindrospermopsin and Anatoxin-a DW Methods Overview

Summary Options	ELISA-Lab	LC-MS/MS
Scope	Cylindrospermopsin and Anatoxin-a	Cylindrospermopsin and Anatoxin-a
Approx. Limit of Quantification (LOQ)	~ 0.3 and 1.0 µg/L	~ 0.06 and 0.02 µg/L
Time to Result	1 – 4 hours	< one day

LC-MS/MS

- EPA finished water methods
  - EPA Method 544 – six selected microcystins and nodularin-R
  - EPA Method 545 – cylindrospermopsin and anatoxin-a

LC-MS/MS

- EPA ambient water methods
  - Single Laboratory Validated Method for Determination of Cylindrospermopsin and Anatoxin-a in Ambient Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) (Nov 2017, EPA 600-R-17-130)
  - Single Laboratory Validated Method Determination of Microcystins and Nodularin in Ambient Freshwaters by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) (Nov 2017, EPA 600-R-17-344)
    - thirteen selected microcystins and nodularin-R

LC-MS/MS EPA Method 544 (Selected Microcystins and Nodularin-R)[1]

Parameter	Method Description	Parameter	Method Description
Reporting Limit	0.0029–0.022 µg/L (LCMRL)	Sample Preparation	Cell lysing, SPE, concentration
Sample Collection	500 mL in glass	Quality Control	LRB, precision and accuracy demonstrations, MRL confirmation, QCS, calibration checks, surrogate standard, laboratory fortified blank, laboratory fortified sample matrix and duplicate, field duplicate
Preservation	Refrigerated samples, frozen extracts, Trizma buffer, ascorbic acid dechlorination, 2-chloroacetamide microbial inhibition, EDTA, 28-day extract and sample hold time		

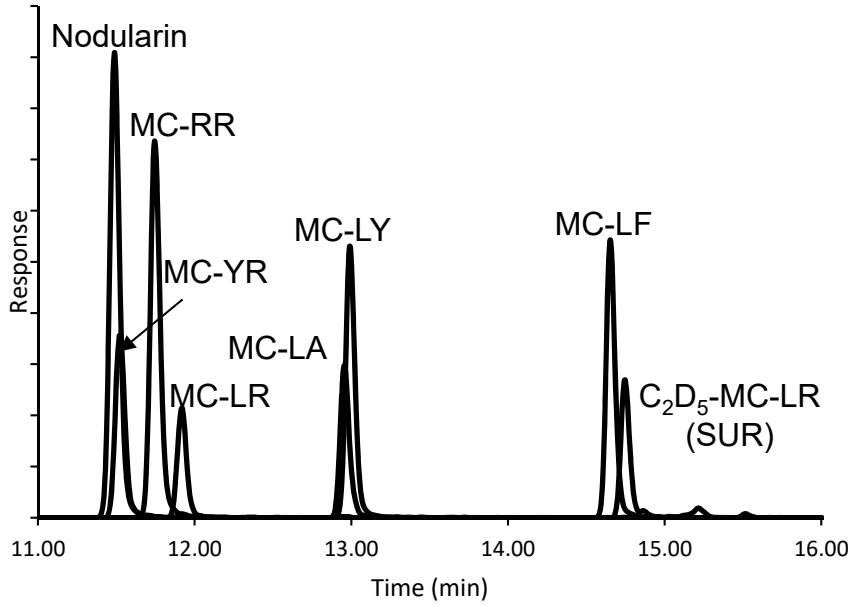
[1]*EPA Method 544: Determination of microcystins and nodularin in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS)*; EPA Document No. 600-R-14-474; U.S. Environmental Protection Agency, ORD/NERL: Cincinnati, OH, 2015.

LC-MS/MS EPA Method 545 (Cylindrospermopsin and Anatoxin-a)[1]

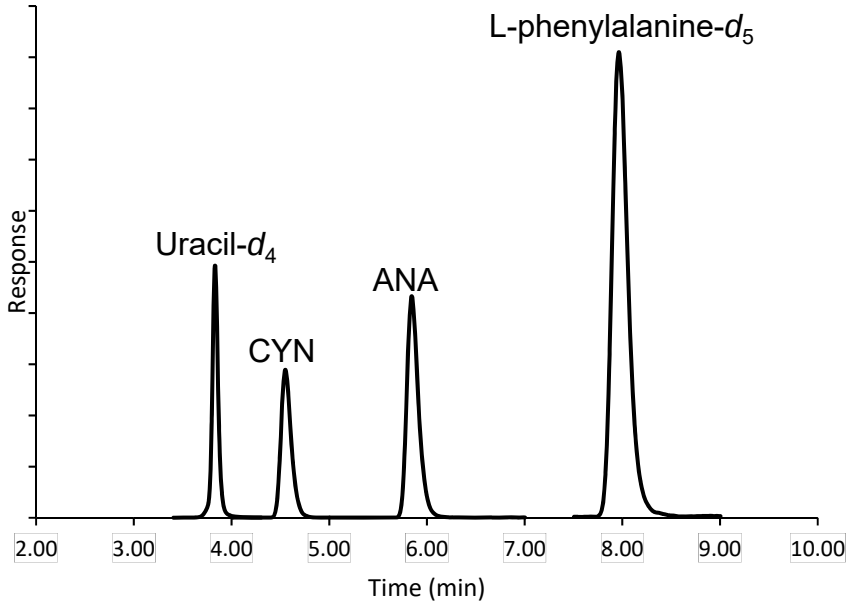
Parameter	Method Description	Parameter	Method Description
Reporting Limit	0.063 and 0.018 µg/L (LCMRL)	Sample Preparation	Cell lysing, filtration
Sample Collection	At least 10 mL in glass	Quality Control	LRB, precision and accuracy demonstrations, MRL confirmation, QCS, calibration checks, internal standards, laboratory fortified sample matrix and duplicate, field duplicate
Preservation	Refrigerated, ascorbic acid dechlorination, sodium bisulfate microbial inhibition, 28-day hold time		

[1]*EPA Method 545: Determination of cylindrospermopsin and anatoxin-a in drinking water by liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS)*; EPA Document No. 815-R-15-009; U.S. Environmental Protection Agency, OW/OGWDW/SRMD/TSC: Cincinnati, OH, 2015.

LC-MS/MS Chromatograms



EPA Method 544



EPA Method 545

Enzyme-Linked Immunosorbent Assay (ELISA)

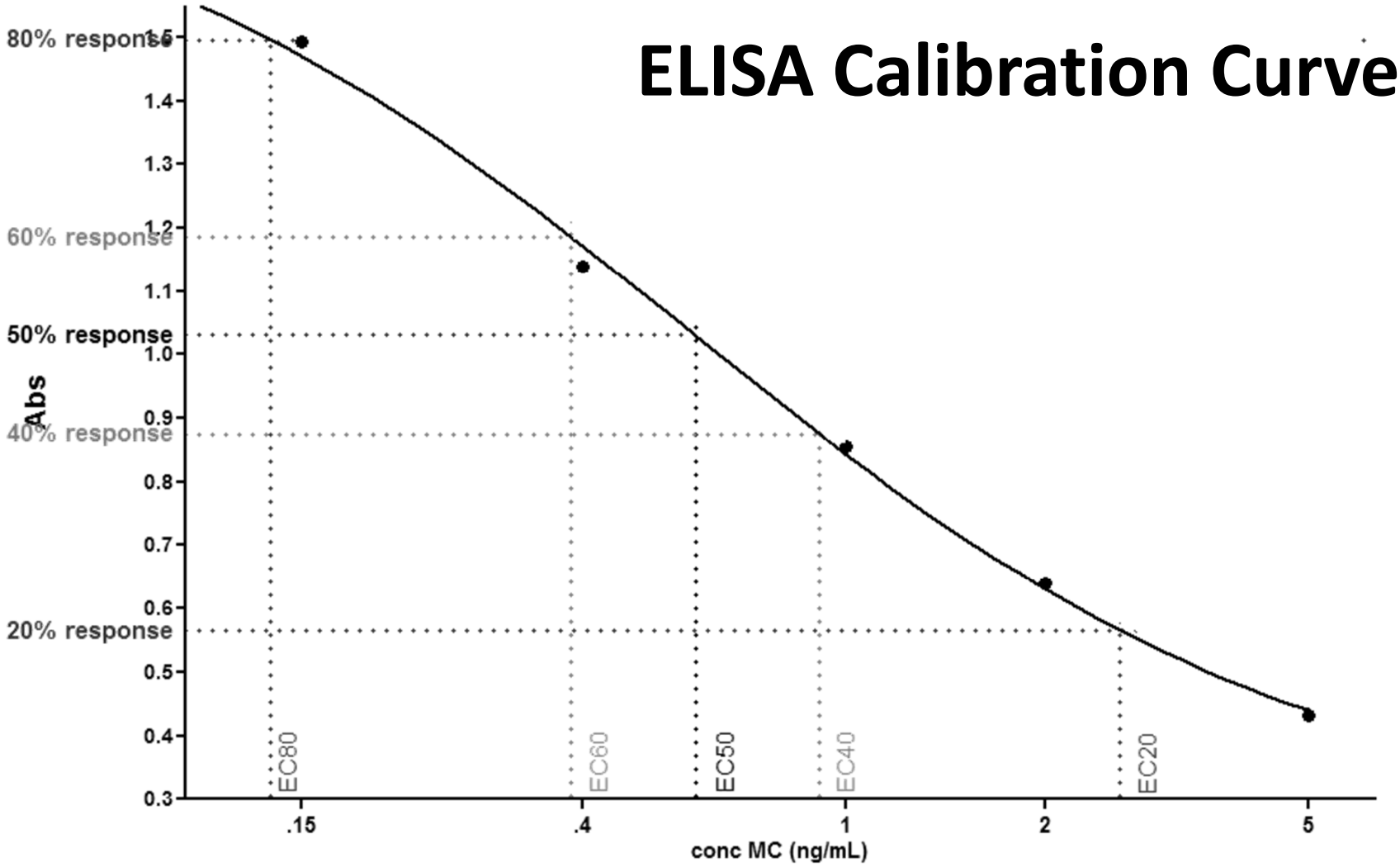
- ELISA is commonly used to detect cyanotoxins
  - Separate assays are used to detect individual or groups of cyanotoxins
- Adda-ELISA results quantify “total microcystins and nodularins”
  - Based on the Adda portion of the molecules
- Calibration curve based on four-parameter logistic function (sigmoidal curve)

Adda-ELISA EPA Method 546 (Total Microcystins and Nodularins)[1]

Parameter	Method Description	Parameter	Method Description
Reporting Limit	0.26 µg/L (MC-LR, LCMRL)	Sample Preparation	Cell lysing, filtration
Sample Collection	<100 mL in glass or PTEG	Quality Control	LRB, precision and accuracy demonstrations, MRL confirmation, QCS, calibration verification, laboratory fortified sample matrix and duplicate
Preservation	Refrigerated then frozen, sodium thiosulfate dechlorination, 14-day hold time		

[1]*EPA Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Source Water by Adda Enzyme-Linked Immunosorbent Assay*; EPA Document No. 815-B-16-011; U.S. Environmental Protection Agency, OW/OGWDW/SRMD/TSC: Cincinnati, OH, 2016.

ELISA Calibration Curve



Microcystin Analytical Comparisons

Analysis	Advantages	Limitations
EPA Method 544 -or- Other Microcystin LC-MS/MS Analyses	• Sensitive • Speciates microcystins	• Standards not available for all microcystin congeners (limited target analyte list) • Instrument limitations considering number of congeners
EPA Method 546 ADDA-ELISA	• Cost effective • Provides "total" concentration (single number) • Faster turnaround for results	• Does not speciate microcystins • Non-typical calibration • Technique is important

Method 544 and Method 546 Results

- Method results may differ from each other
  - M544 was developed and validated to detect only six microcystin congeners and nodularin-R
  - M546 was developed and validated to detect the Adda portion of microcystins and nodularins with varying degrees of assay recognition (cross-reactivities) using MC-LR as the calibration standard
- It is important to understand what is being measured by each technique for proper application

Conclusions

- EPA cyanotoxin methods underwent rigorous method development processes
- Several methods are available for the analysis of various cyanotoxins
- EPA Methods meet typical DW method validation and performance acceptance criteria
- Results are dependent on the analysis being used

Questions?

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