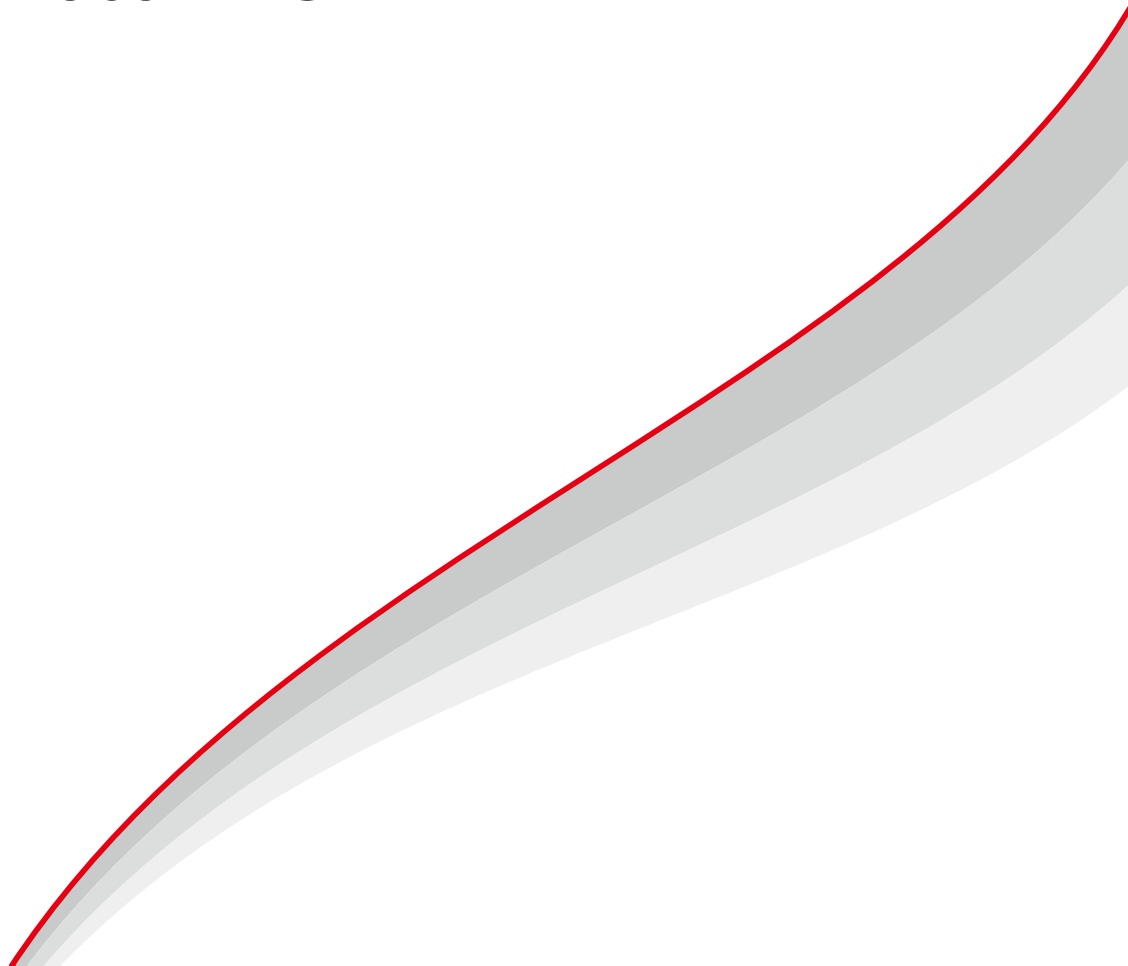


Extended Performance Evaluation: Single LC-MS/MS for PFAS & Cyanotoxins in Drinking Water

Xiaomeng (Kate) Xia, Ruth Marfil-Vega,

Evelyn Wang, Toshiya Matsubara



What keeps you up at night?



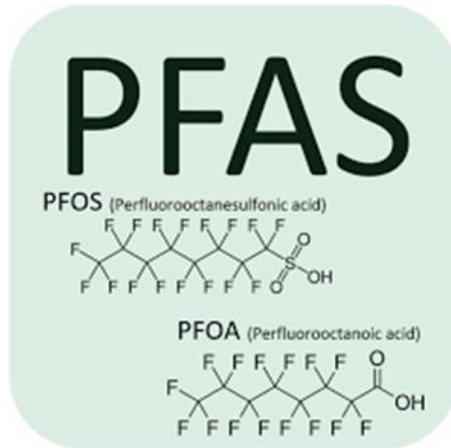
What keeps you up at night?



Not being able to provide safe water

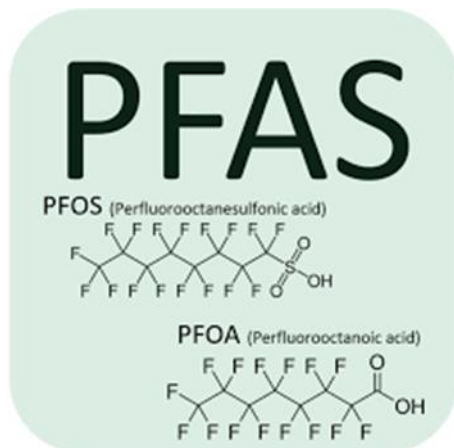
What keeps you up at night?

Compliance testing



What keeps you up at night?

Compliance testing



Cyanotoxins



Emergency Response

Multi Analysis Solution for Drinking Water



Shimadzu LCMS-8060 RX system

ESI (-)

PFAS

PFOS (Perfluorooctanesulfonic acid)



PFOA (Perfluorooctanoic acid)



❑ **Method 537.1**

18 PFAS

❑ **Method 533**

29 PFAS

Cyanotoxins

❑ **Method 544**

Microcystins and Nodularin

❑ **Method 545**

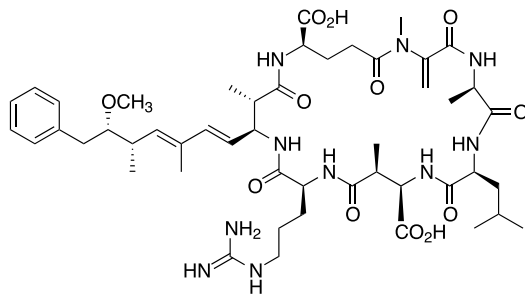
Cylindrospermopsin and Anatoxin-a

EPA Method 537.1 and 533 for PFAS

- EPA Method 537.1 measures 18 PFAS compounds in drinking water.
- EPA Method 533 covers 25 PFAS compounds in drinking water.
- Utilizes solid-phase extraction (SPE) for sample preparation and LC-MS/MS for detection.
- Capable of detecting PFAS at trace levels (ng/L or ppt range).
- Supports compliance with environmental and public health guidelines.

Targets	537.1	533
PFBS	x	x
PFHxA	x	x
HFPO-DA	x	x
PFHpA	x	x
PFHxS	x	x
ADONA	x	x
PFOA	x	x
PFOS	x	x
PFNA	x	x
9Cl-PF3ONS	x	x
PFDA	x	x
PFUNA	x	x
11Cl-PF3OUdS	x	x
PFDoA	x	x
NMeFOSAA	x	
NEtFOSAA	x	
PFTTrDA	x	
PFTA	x	
4:2FTS		x
6:2FTS		x
8:2FTS		x
NFDHA		x
PFBA		x
PFEESA		x
PFHpS		x
PFMBA		x
PFMPA		x
PFPeA		x
PFPeS		x

EPA Method 544 and EPA Method 545



Microcystin-LR

Targets (EPA 544)

MC-LR

MC-RR

MC-YR

Nodularin

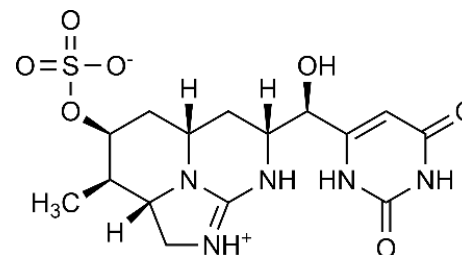
MC-LF

MC-LA

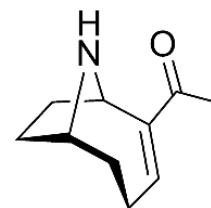
MC-LY

- EPA Method 544 targets **six microcystins and nodularin** in drinking water.
- Microcystins and nodularin are highly potent **hepatotoxins** produced by different species of cyanobacteria.

https://www.shimadzu.com/an/sites/shimadzu.com/an/files/pim/pim_document_file/applications/application_note/24186/an_02-ssi-lcms-155-en.pdf



Cylindrospermopsin



Anatoxin-a

Targets (EPA 545)

Cylindrospermopsin

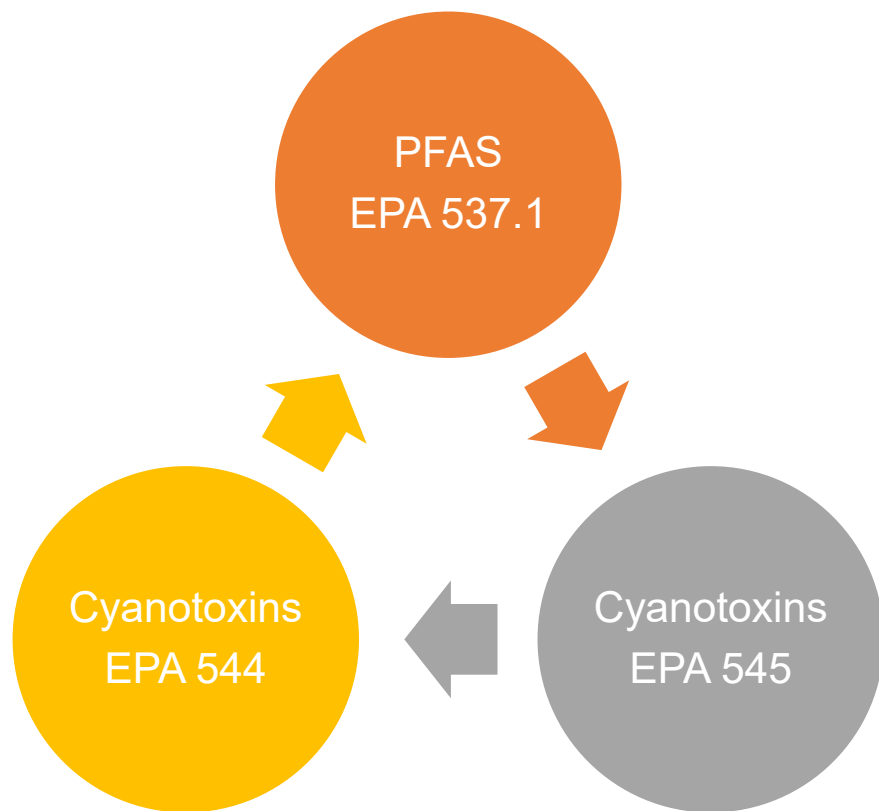
Anatoxin-a

- EPA Method 545 targets **cylindrospermopsin and anatoxin-a** in drinking water.
- Cylindrospermopsin can harm the liver, kidneys, and other organs.
- Anatoxin-a may cause muscle twitching, loss of coordination, and rapid death.

https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/pim/pim_document_file/ssi/applications/application_note/22645/SSI-LCMS153_Quantitation_Cylindrospermopsin_Anatoxin-a_Water_LCMS-8060NX_EPA-Method-545.pdf

Multi Analysis Solution for Drinking Water

Goal: Automatic Method Switching without Human Intervention

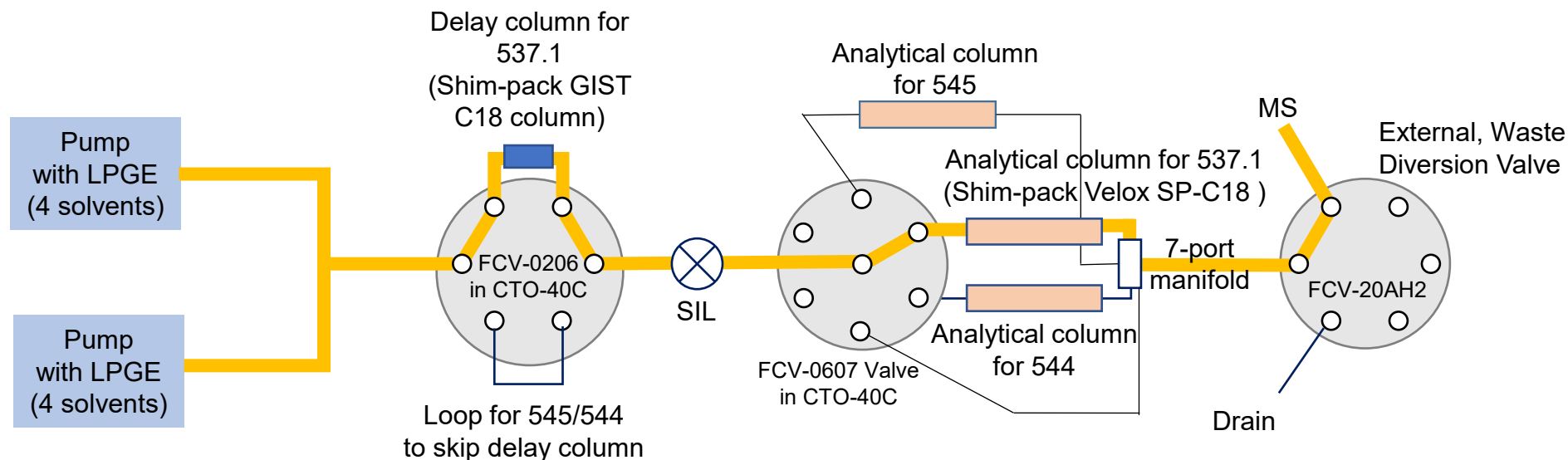


Our Approach:

1. **LPGE Mobile Phase Selection**
2. **Column Switching**
3. **Method Package**

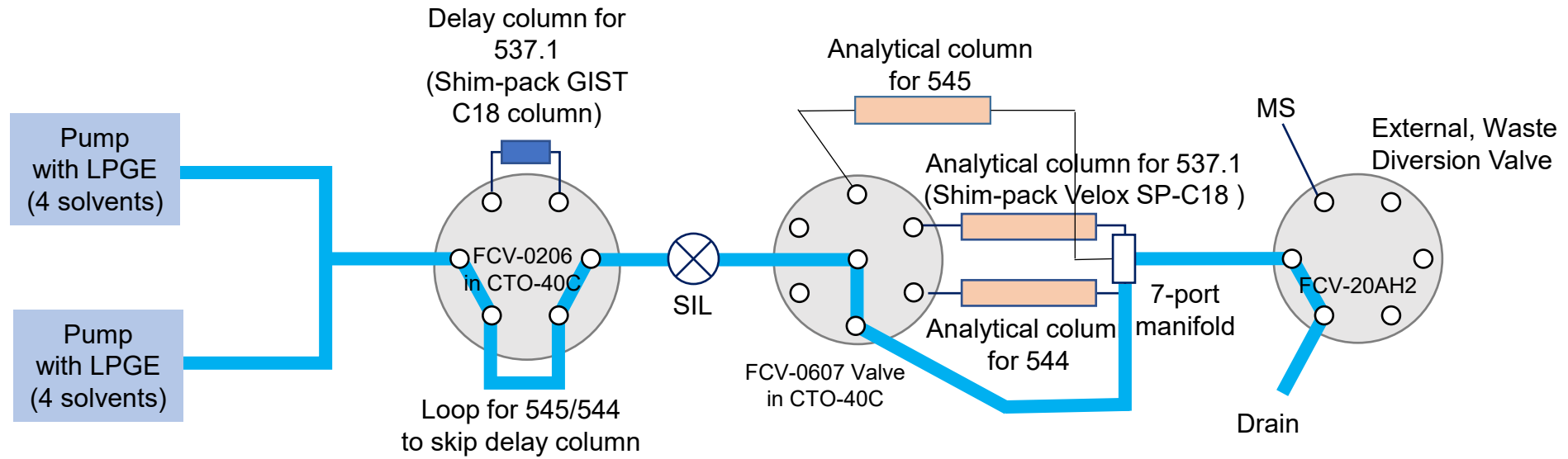
Easy Switch Between Methods

Customized Flow Diagram – EPA 537.1



- Shorten Analysis Time for EPA 537.1 – 30 minutes down to 18 minutes
- Solvent and column switching enables automatic method switching

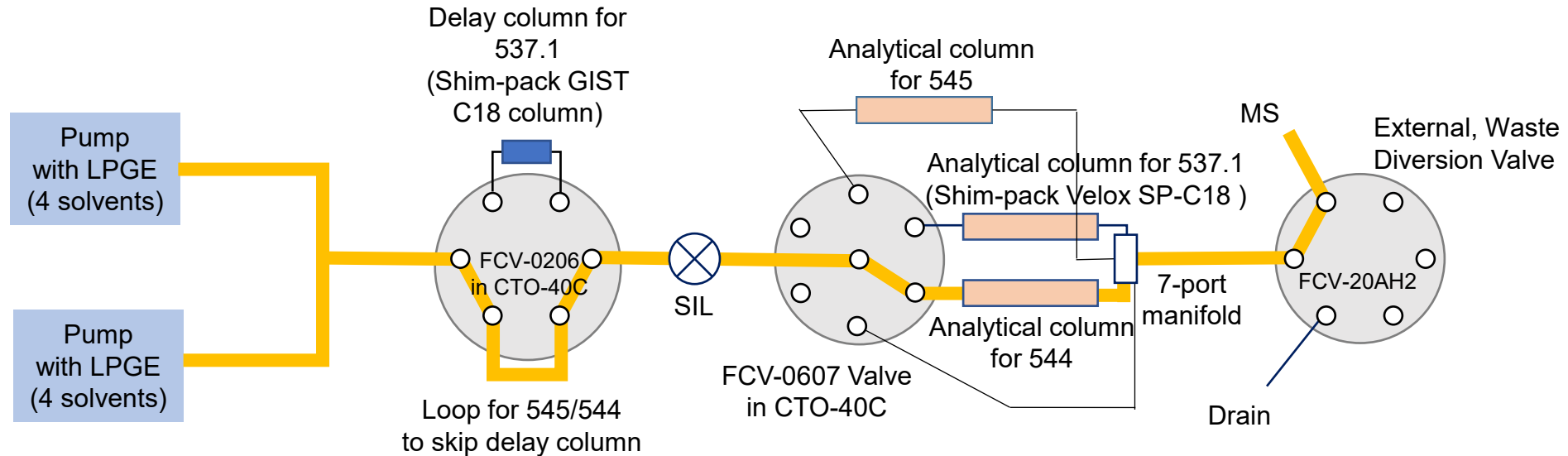
Rinse Method



- Between method transitions, the system was rinsed with the corresponding mobile phases for five minutes
- 50% mobile phase A + 50% mobile phase B, flow rate 1.0 mL/min

Easy Switch Between Methods

Customized Flow Diagram – EPA 544



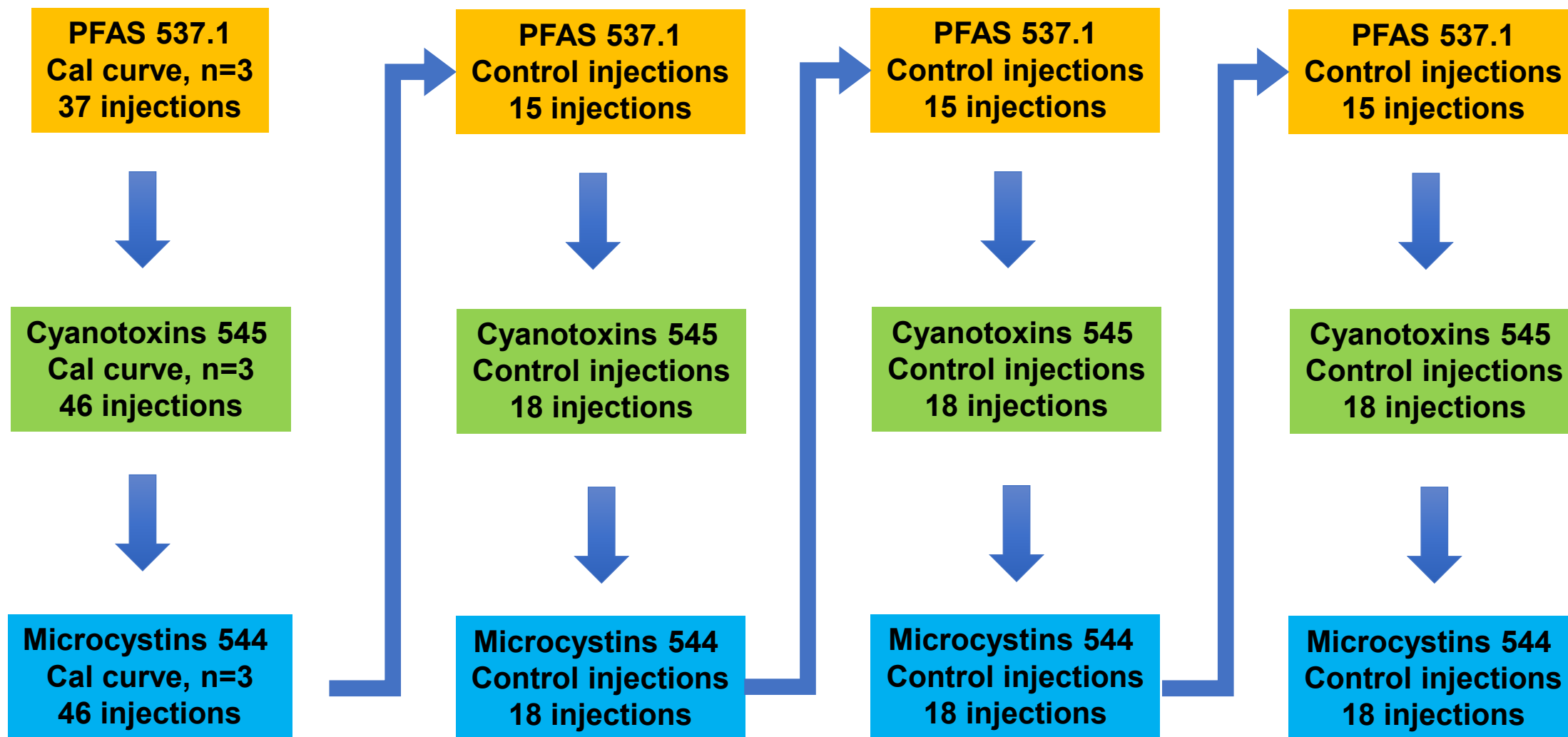
- Shorten Analysis Time for EPA 544 – 26 minutes down to 8 minutes
- Solvent and column switching enables automatic method switching

Method Conditions Comparison

Parameter	EPA 537.1	EPA 533	EPA 545	EPA 544
LCMS	Shimadzu LCMS-8060RX	Shimadzu LCMS-8060RX	Shimadzu LCMS-8060RX	Shimadzu LCMS-8060RX
Analytical Column	Shim-pack Velox SP-C18 column, 2.7 µm, 2.1 x 50mm	Shim-pack GIST C18 column, 3.0 µm, 2.1 x 50mm	Shim-pack GIST C18 column, 2.0 µm, 2.1 x 100mm	Shim-pack Velox SP-C18 column, 2.7 µm, 2.1 x 100mm
Delay Column	Shim-pack GIST C18 column, 5.0 µm, 3.0 x 50mm	Shim-pack GIST C18 column, 5.0 µm, 3.0 x 50mm	Not applicable	Not applicable
Injection Volume	2 µL	2 µL	20 µL	10 µL
Column Oven Temp.	45 °C	45 °C	40 °C	40 °C
Mobile Phase	A: 5 mM Ammonium Acetate in LCMS Grade Water	A: 5 mM Ammonium Acetate in LCMS Grade Water	A: 0.2% Acetic Acid in LCMS Grade Water	A: 0.2% Acetic Acid in LCMS Grade Water
	B: Methanol	B: Methanol	B: Methanol	B: 0.2% Acetic Acid in Acetonitrile
Flow Rate	0.25 mL/min	0.25 mL/min	0.3 mL/min	0.3 mL/min
Run Time	18 minutes	15 minutes	8 minutes	8 minutes
MS Interface	ESI Negative	ESI Negative	ESI Positive	ESI Positive

Experimental Approach

294 total injections = 54 hours of non-stop operation!!

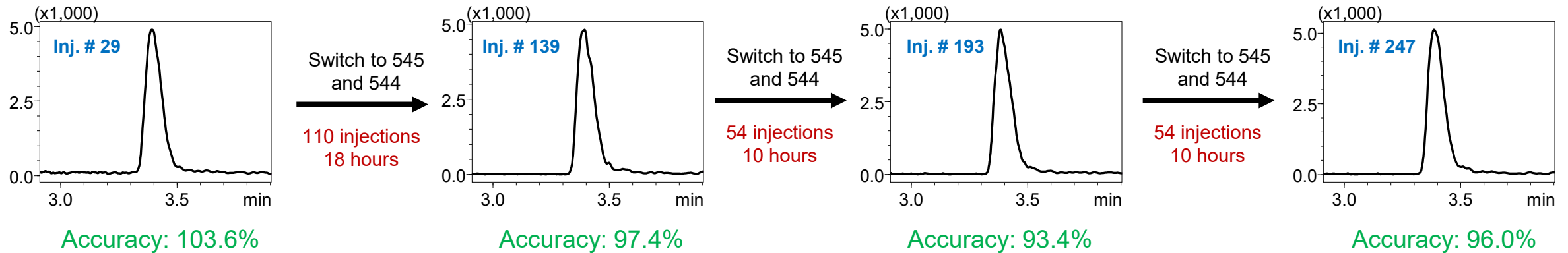


Calibration Information for EPA 537.1, 545, and 544 n=3

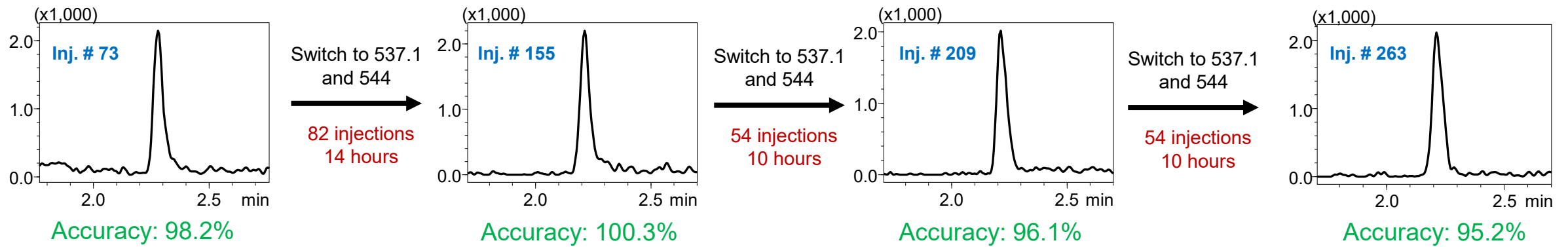
Name	Cal range (µg/L)	R ²	Calibrator Accuracies (%)	Name	Cal range (µg/L)	R ²	Calibrator Accuracies (%)
EPA Method 537.1							
PFBS	0.5 – 50	0.9999	92.6 – 101.8	9CI-PF3ONS	0.5 – 50	0.9995	93.6 – 106.2
PFHxA	0.5 – 50	0.9995	96.7 – 103.9	PFDA	0.5 – 50	0.9995	94.9 – 104.4
HFPO-DA	0.5 – 50	0.9995	94.4 – 104.1	NMeFOSAA	0.5 – 50	0.9987	87.5 – 107.1
PFHpA	0.5 – 50	0.9998	95.8 – 103.7	PFUnA	0.5 – 50	0.9997	94.8 – 104.0
PFHxS	0.5 – 50	0.9996	94.9 – 108.6	NEtFOSAA	0.5 – 50	0.9994	91.8 – 108.9
ADONA	0.5 – 50	0.9995	96.7 – 104.5	11CI-PF3OUdS	0.5 – 50	0.9998	88.5 – 107.8
PFOA	0.5 – 50	0.9995	96.8 – 103.0	PFDaA	0.5 – 50	0.9998	96.7 – 103.6
PFOS	0.5 – 50	0.9996	92.0 – 102.8	PFTTrDA	0.5 – 50	0.9994	94.6 – 106.9
PFNA	0.5 – 50	0.9997	96.7 – 102.3	PFTA	0.5 – 50	0.9996	94.0 – 105.3
EPA Method 545							
Anatoxin-a	0.02 – 20	0.9974	85.1 – 117.4	Cylindrospermopsin	0.005 – 10	0.9996	94.2 – 109.6
EPA Method 544							
MC-LR	5.0 – 500	0.9987	82.6 – 111.2	MC-LF	5.0 – 500	0.9997	84.1 – 127.7
MC-RR	0.5 – 500	0.9976	92.9 – 114.5	MC-LA	1.0 – 100	0.9987	83.5 – 117.7
MC-YR	0.5 – 100	0.9940	85.9 – 111.3	MC-LY	2.0 – 200	0.9964	85.5 – 114.9
Nodularin	5.0 – 500	0.9986	82.8 – 113.6				

Chromatogram of Representative Compounds Before and After Other Methods

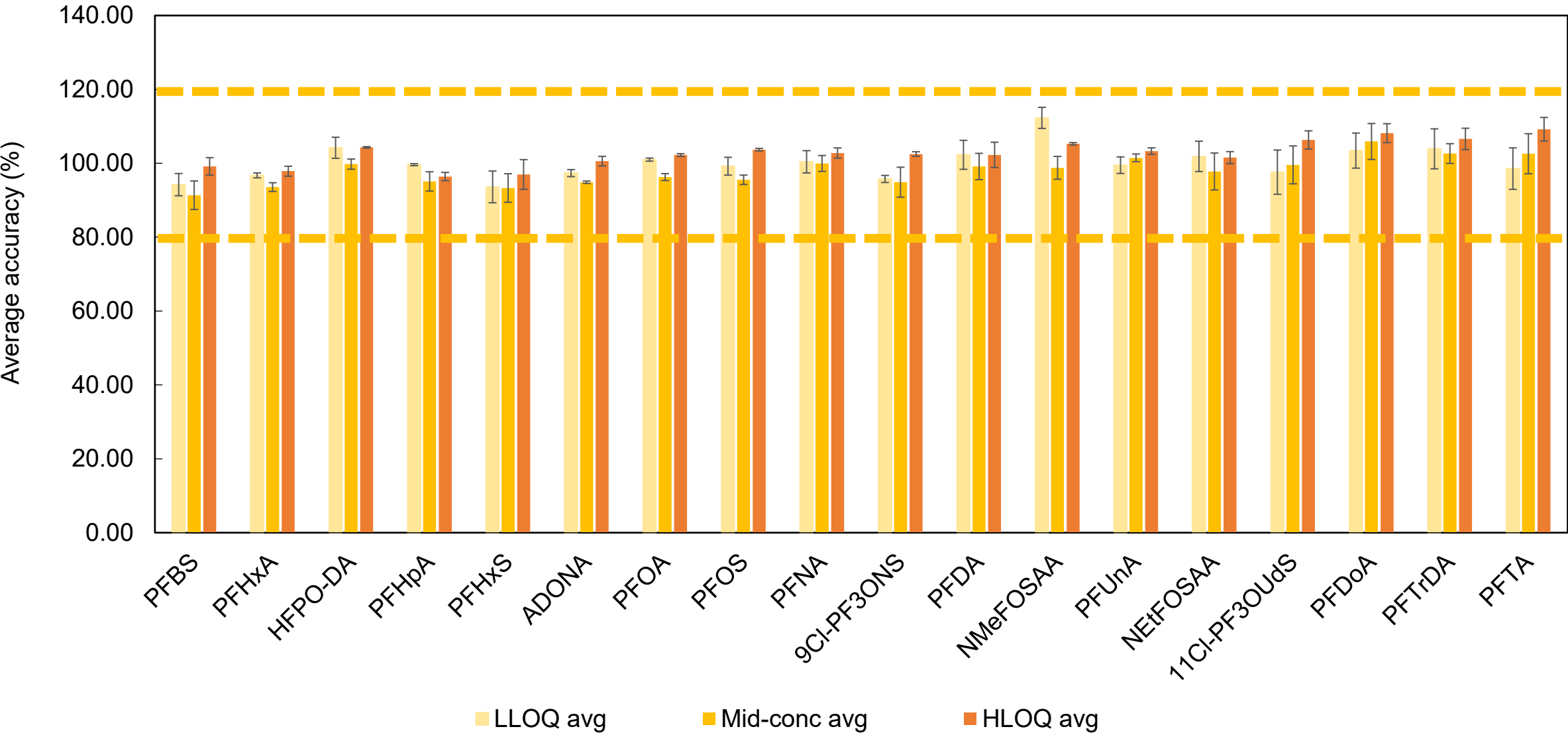
PFBS (-) @ LOQ in EPA Method 537.1



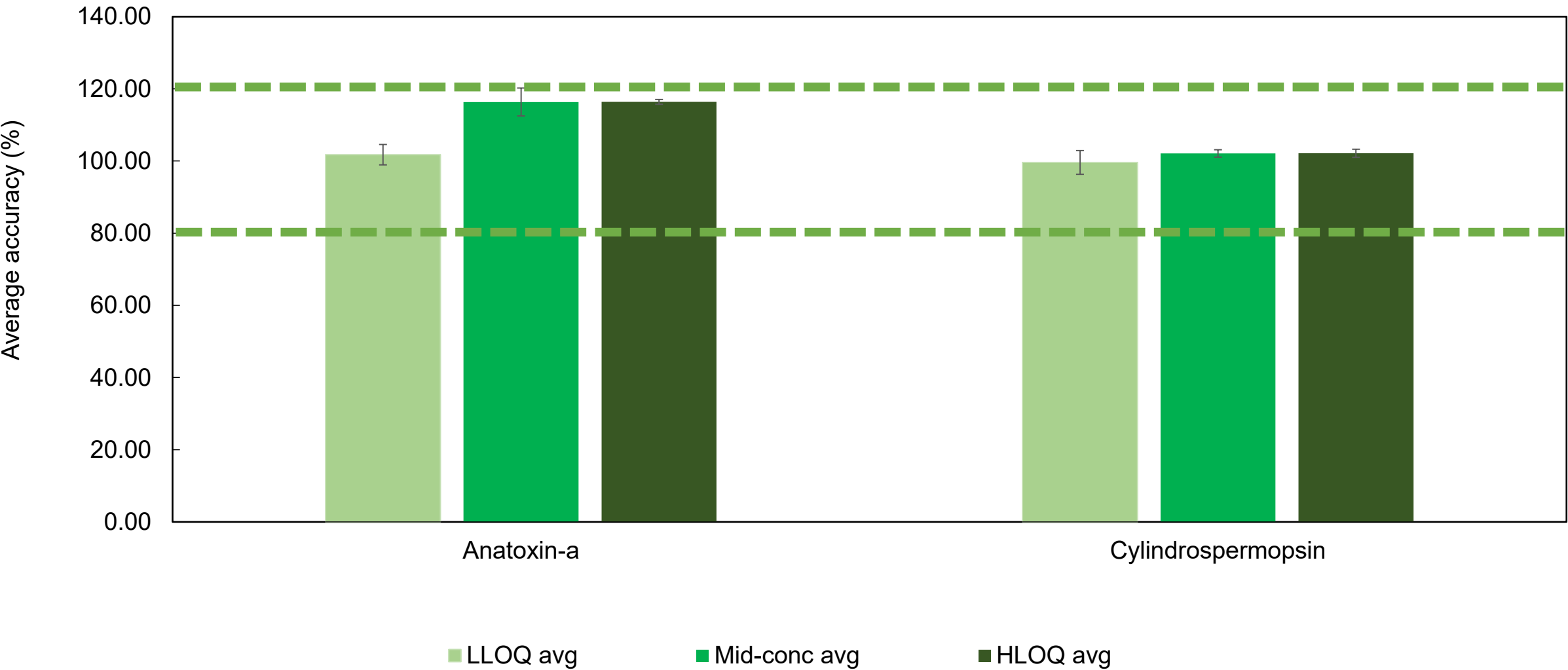
Cylindrospermopsin (+) @ LOQ in EPA Method 545



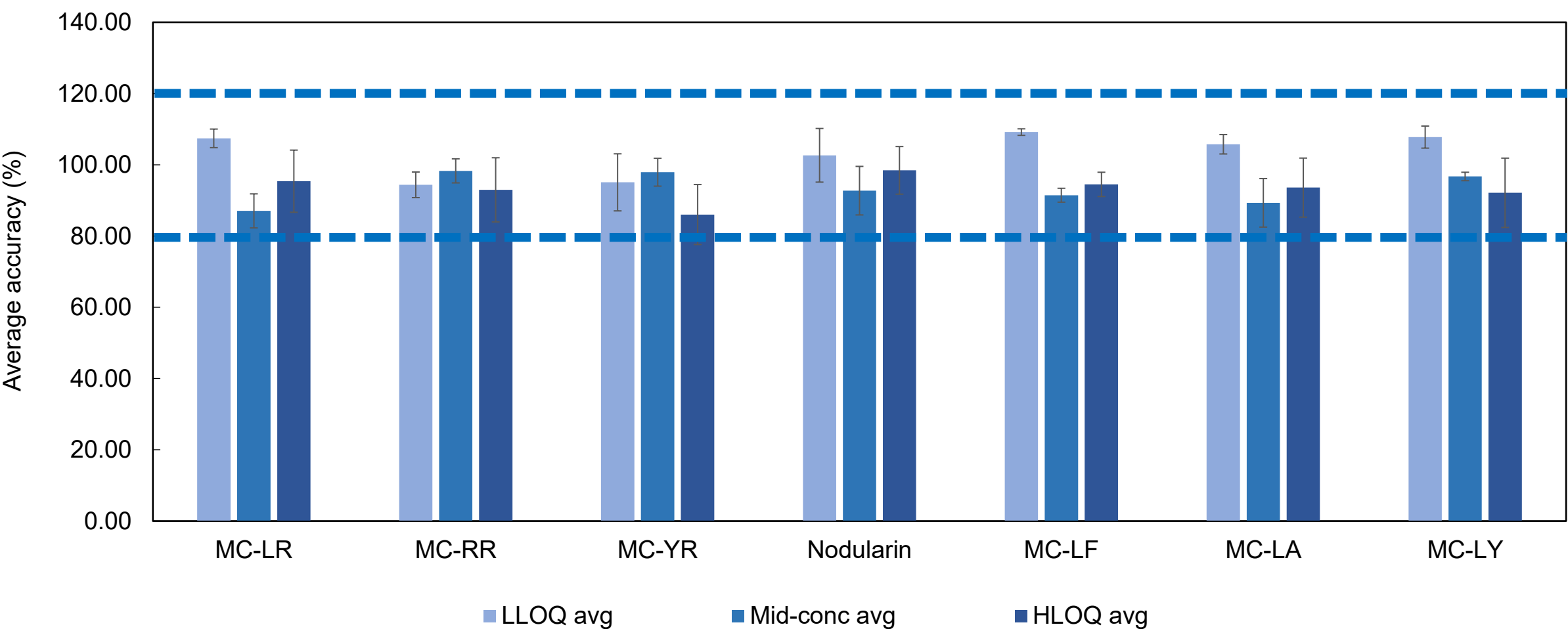
Continuing Calibration Check for PFAS (EPA 537.1)



Continuing Calibration Check for Anatoxin-a & Cylindrospermopsin (EPA 545)

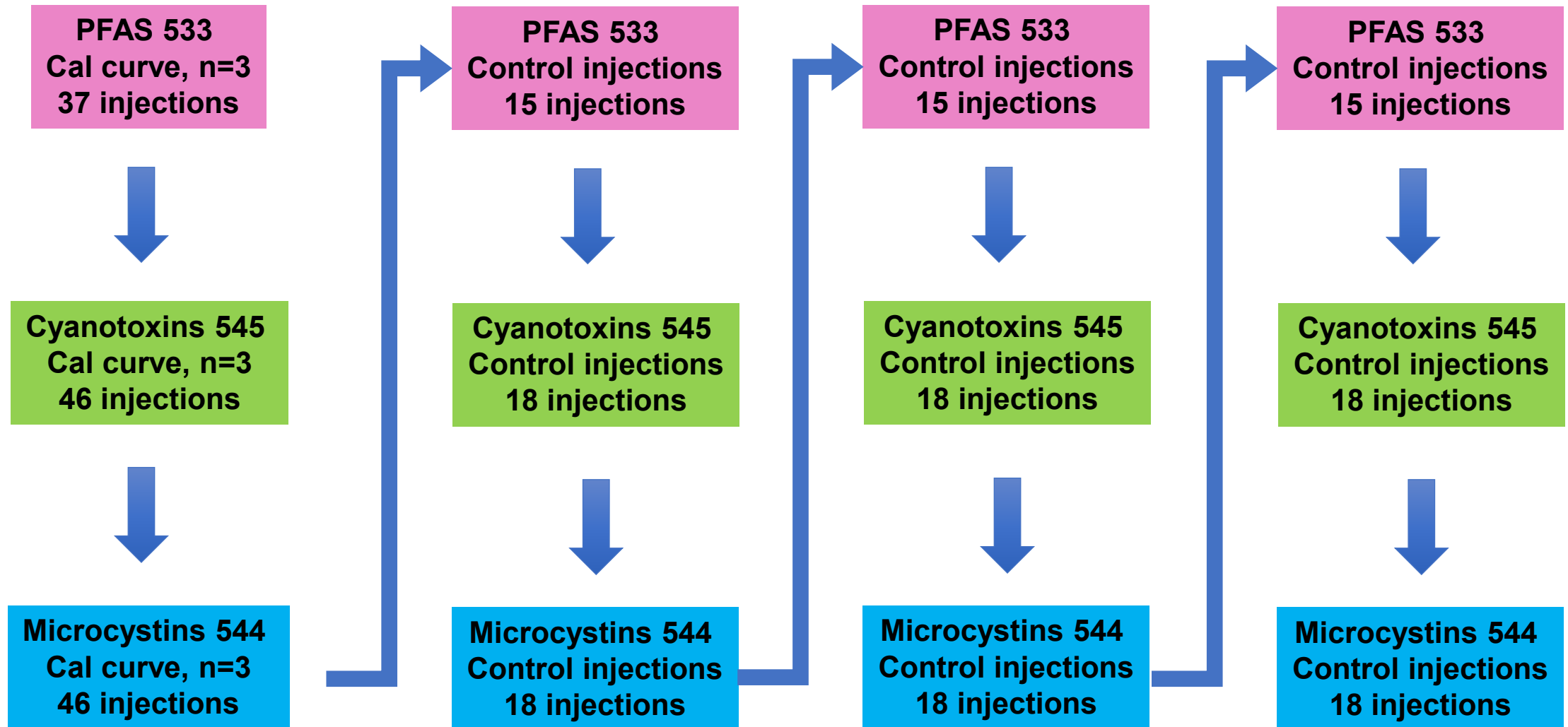


Continuing Calibration Check for Microcystins and Nodularin (EPA 544)



Experimental Approach

294 total injections = 54 hours of non-stop operation!!

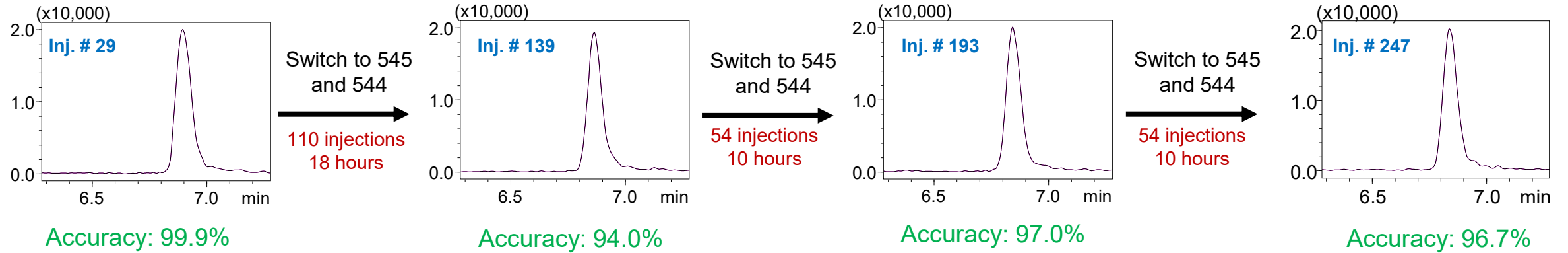


Calibration Information for EPA 533, 545, and 544 n=3

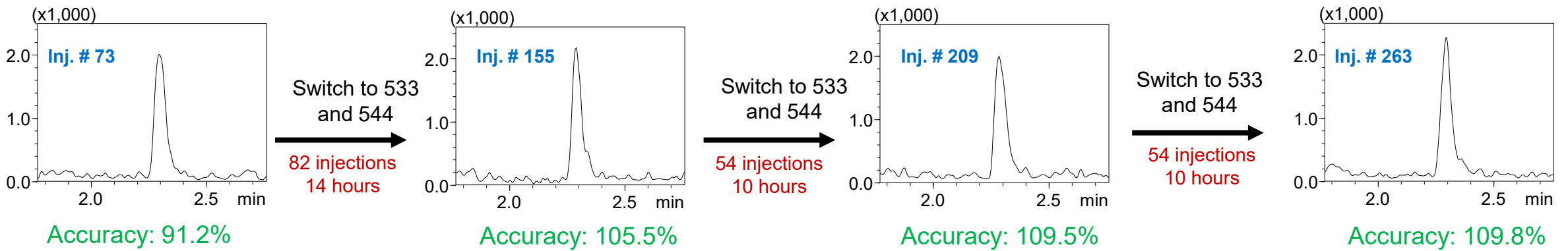
Name	Cal range (µg/L)	R ²	Calibrator Accuracies (%)	Name	Cal range (µg/L)	R ²	Calibrator Accuracies (%)
EPA Method 533							
PFBA	0.5 – 50	0.9986	95.2 – 106.5	ADONA	0.5 – 50	0.9956	91.0 – 114.6
PFMPA	0.5 – 50	0.9983	93.3 – 109.7	6:2 FTS	0.5 – 50	0.9987	87.9 – 110.7
PFPeA	0.5 – 50	0.9984	93.6 – 108.8	PFOA	0.5 – 50	0.9979	92.8 – 107.9
PFBS	0.5 – 50	0.9977	92.8 – 111.1	PFHpS	0.5 – 50	0.9976	86.5 – 111.7
PFMBA	0.5 – 50	0.9980	94.4 – 110.2	PFNA	0.5 – 50	0.9989	93.1 – 109.5
PFEESA	0.5 – 50	0.9964	92.8 – 112.3	PFOS	0.5 – 50	0.9950	86.2 – 115.9
NFDHA	0.5 – 50	0.9977	92.9 – 110.7	9CI-PF3ONS	0.5 – 50	0.9978	89.8 – 111.9
4:2 FTS	0.5 – 50	0.9993	88.7 – 107.2	8:2 FTS	0.5 – 50	0.9987	87.0 – 111.5
PFHxA	0.5 – 50	0.9985	94.2 – 109.5	PFDA	0.5 – 50	0.9964	88.5 – 118.9
PFPeS	0.5 – 50	0.9983	91.1 – 112.9	PFUnA	0.5 – 50	0.9976	88.1 – 118.5
HFPO-DA	0.5 – 50	0.9979	93.2 – 110.2	11CI-PF3OUdS	0.5 – 50	0.9966	92.2 – 117.9
PFHpA	0.5 – 50	0.9976	95.0 – 110.3	PFDoA	0.5 – 50	0.9981	94.7 – 110.4
PFHxS	0.5 – 50	0.9985	87.1 – 109.1				
EPA Method 545							
Anatoxin-a	0.02 – 20	0.9985	91.8 – 109.7	Cylindrospermopsin	0.005 – 10	0.9997	90.8 – 112.6
EPA Method 544							
MC-LR	5.0 – 500	0.9990	80.2 – 123.9	MC-LF	2.0 – 500	0.9925	83.6 – 124.6
MC-RR	0.5 – 500	0.9960	86.5 – 111.4	MC-LA	0.5 – 100	0.9963	86.2 – 111.0
MC-YR	0.5 – 100	0.9940	84.3 – 119.4	MC-LY	2.0 – 500	0.9988	80.4 – 123.2
Nodularin	5.0 – 500	0.9993	81.5 – 116.1				

Chromatogram of Representative Compounds Before And After Other Methods

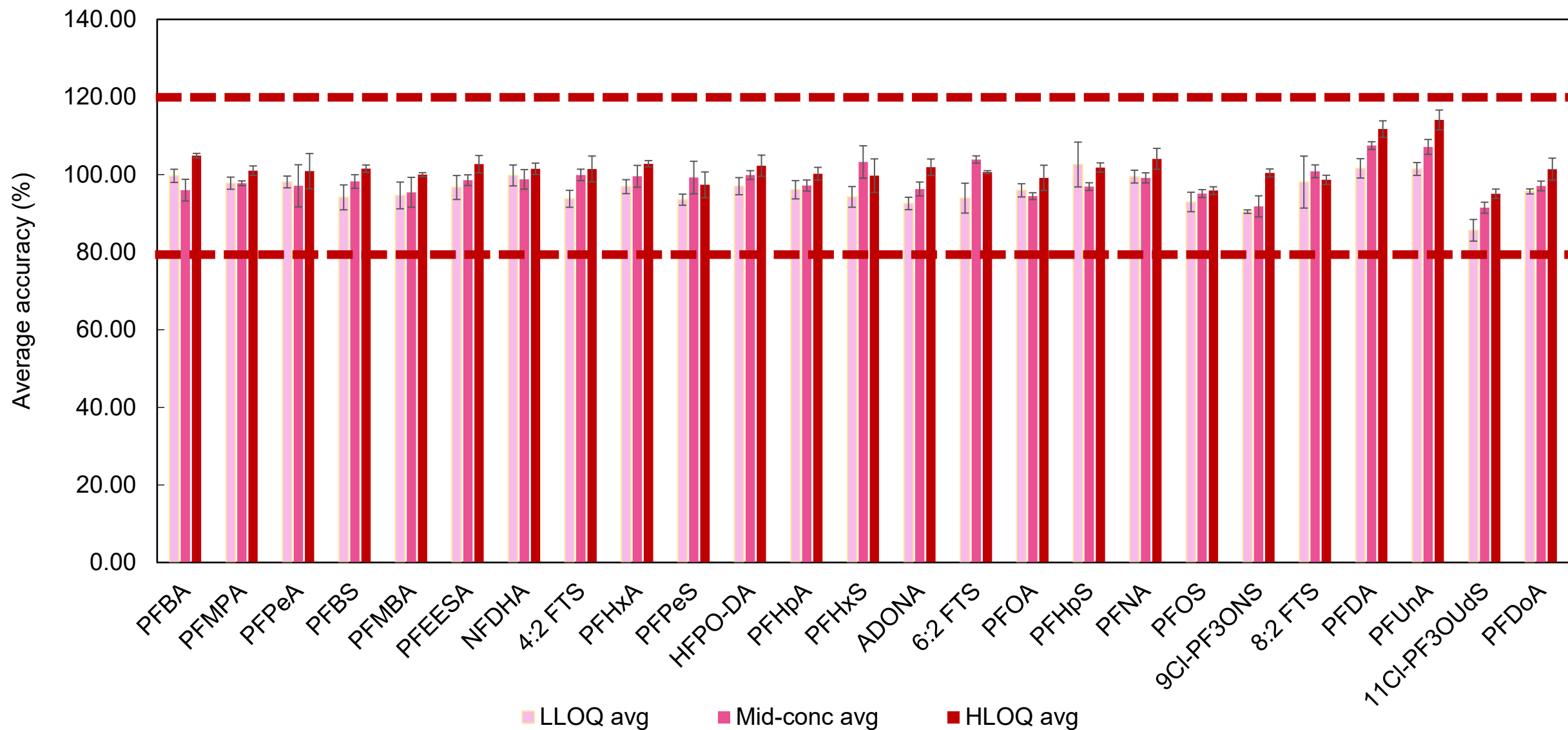
PFOA @ LOQ in EPA Method 533



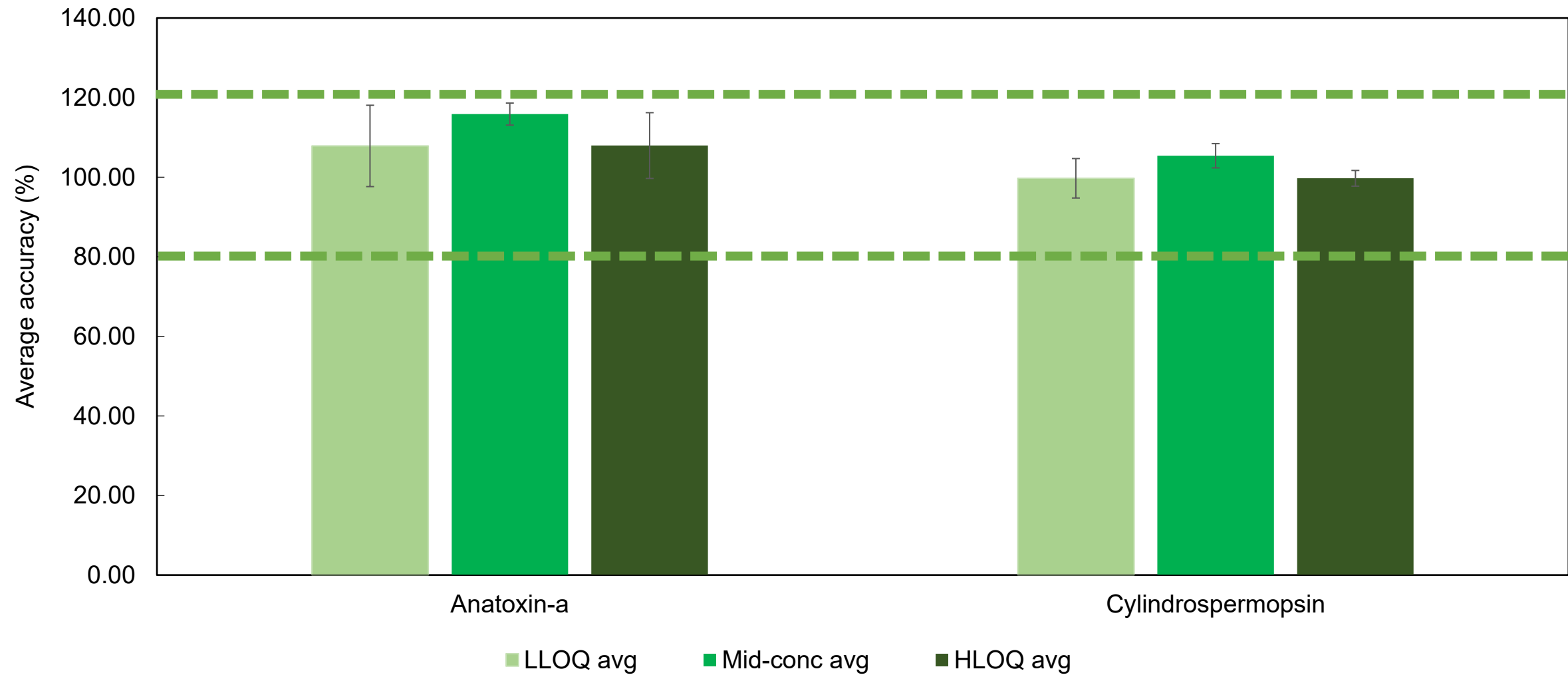
Anatoxin-a @ LOQ in EPA Method 545



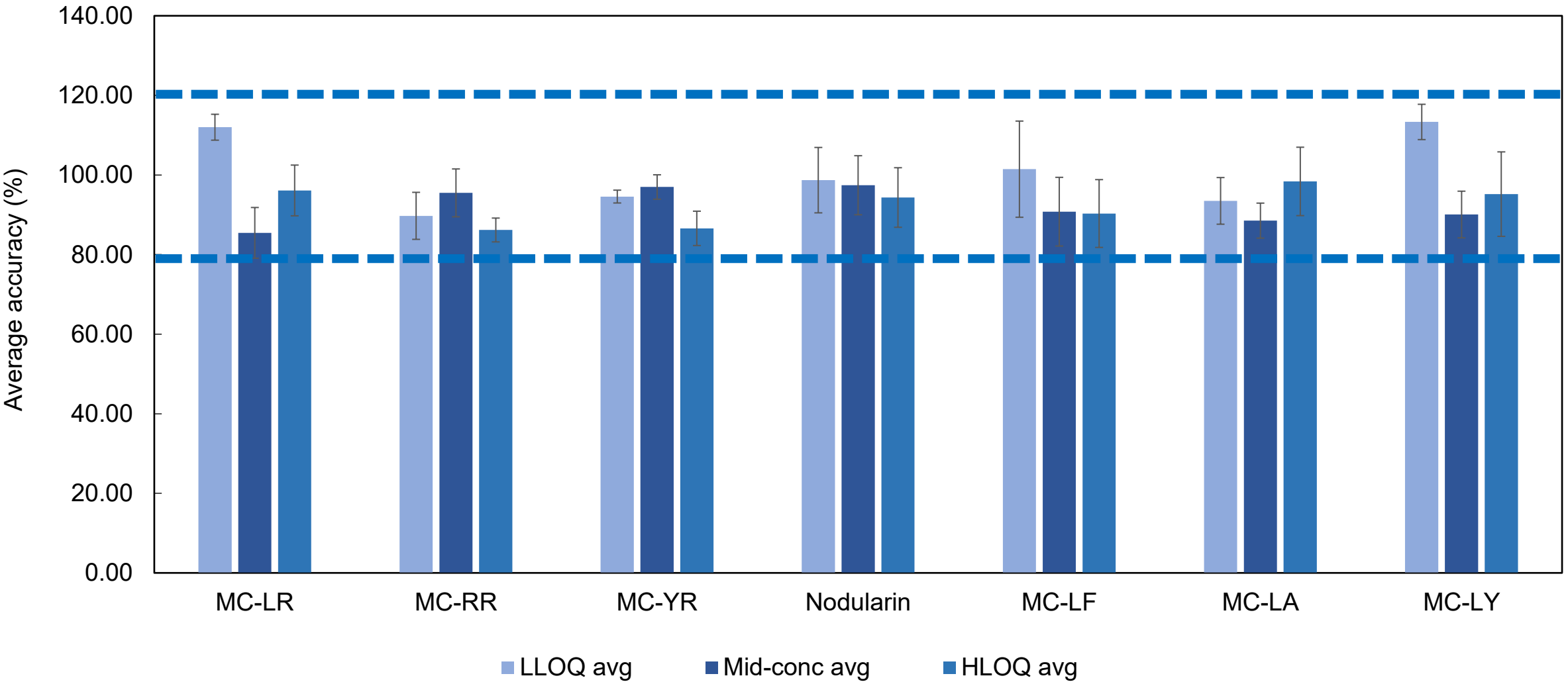
Continuing Calibration Check for PFAS (EPA 533)



Continuing Calibration Check for Anatoxin-a & Cylindrospermopsin (EPA 545)

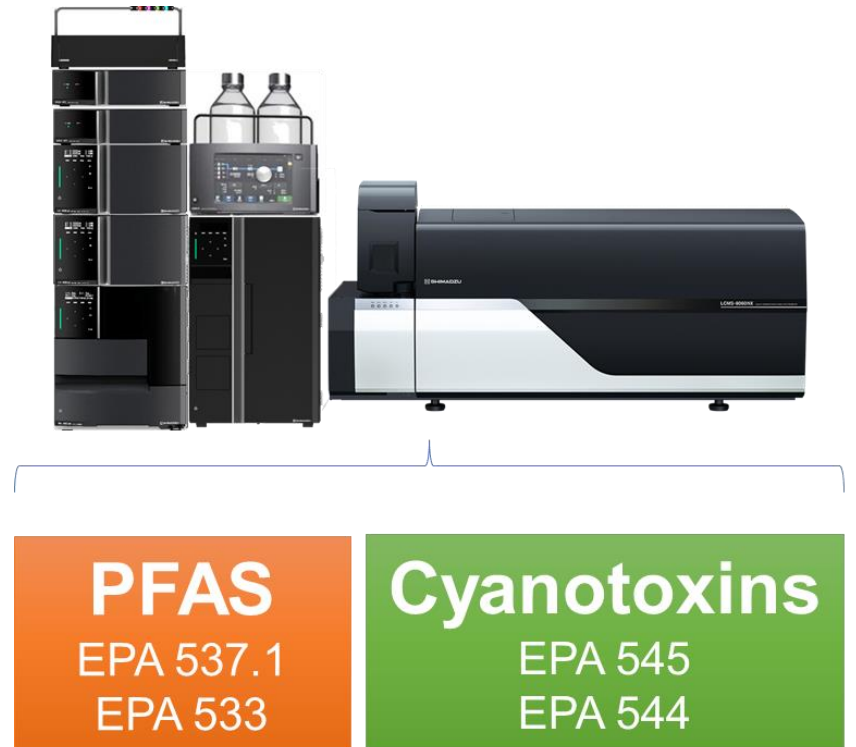


Continuing Calibration Check for Microcystins and Nodularin (EPA 544)

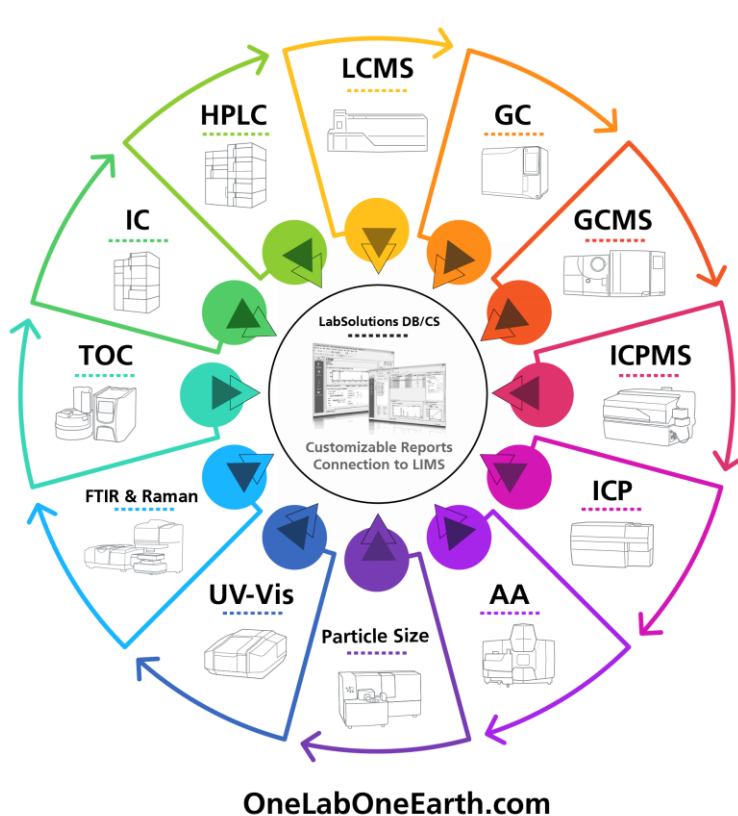


Conclusions

- Enabled quantification of PFAS (EPA 537.1, 533), microcystins/nodularin (EPA 544), and cylindrospermopsin/anatoxin-a (EPA 545) on a single LC-MS/MS system.
- Achieved high sensitivity, accuracy, and reproducibility across extended runs.
- Employed a 5-minute automated rinse to prevent carryover and mobile phase cross-contamination.
- Supported rapid response to HABs without interrupting PFAS workflows.
- Improved throughput and method robustness via automation.



HPLC	Carbamate Diquat Glyphosate
IC	Anions Chromium VI Ammonia
TOC	Nitrogen Organic Carbon Phosphorous
FTIR & Raman	Microplastics
UV-Vis	Chlorine UV254 Others
Particle Size	Solids



LCMS	Cyanotoxins PFAS Pesticides Emerging Contaminants Unknowns (QTOF)
GC	HAAs Herbicides Pesticides
GCMS	Phenols Other Organics PCBs
GCMS	1,4-dioxane Dioxins & Furans Nitrosamines Other Organics Microplastics
ICPMS	Semivolatiles Taste & Odor Volatiles THMs
ICPMS	Metals
ICP	Metals
AA	Metals

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