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LCMS-8060RX High Performance Liquid Chromatograph Mass Spectrometer

Application News

Extended Evaluation of Method Performance for PFAS & Cyanotoxins Analysis Using a Single LC-MS/MS in Water

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User Benefits

- Using automatic method switching, a single Shimadzu LCMS-8060RX triple quadrupole mass spectrometer can effectively measure both PFAS and cyanotoxins in water following EPA Methods 533, 545, and 544.
- The system sustains high performance over extended operation time and multiple method changes with only a five-minute rinse.
- High performance with lower cost enables labs to respond swiftly to harmful algal blooms without impacting routine workflows.

■ Background

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals widely used in consumer products and industrial applications. Their chemical stability and resistance to degradation have resulted in environmental persistence and accumulation. As a result, regulatory bodies like the US Environmental Protection Agency (EPA) and the European Chemicals Agency (ECHA) are implementing measures to limit their environmental presence [1].

In the U.S., EPA Methods 537.1 and 533 are approved for quantifying specific PFAS in drinking water using solid-phase extraction (SPE) and LC-MS/MS. Method 537.1 targets 18 PFAS compounds [2,3]. Method 533 targets a set of 25 PFAS, including many shorter-chain compounds like ADONA, which are not covered by 537.1. Accounting for the overlap between the two lists, these methods collectively enable the quantification of 30 unique PFAS compounds including GenX and ADONA.

Beyond PFAS, other drinking water contaminants such as cyanotoxins also pose serious health risks. Cyanobacteria (blue-green algae) are photosynthetic organisms found in freshwater and marine environments. Human-driven nutrient enrichment, especially phosphorus and nitrogen, has led to an increase in Harmful Algal Blooms (HABs) globally. These blooms can produce toxins like cylindrospermopsin, anatoxin-a, and microcystins, which impair water quality and may cause effects ranging from skin irritation to severe illness in humans and animals [4].

To address the growing concern over cyanotoxins in drinking water, the US Environmental Protection Agency (EPA) has established analytical protocols—Methods 544 and 545—for their detection in both drinking water and freshwater matrices. EPA Method 544 focuses on the detection of microcystins and nodularin, which are commonly produced by certain species of cyanobacteria [5]. In parallel, EPA Method 545 is designed to target other potent cyanotoxins, specifically cylindrospermopsin and anatoxin-a [6]. Both methods employ liquid chromatography-tandem mass spectrometry (LC-MS/MS) for quantitative analysis.

While dedicated instruments for PFAS and cyanotoxin analysis are ideal to avoid cross-contamination, a single system capable of multi-method testing offers a practical and cost-effective alternative, especially during high-demand or emergency situations. We previously demonstrated accurate and robust analysis of PFAS and cyanotoxins following EPA Methods 537.1, 545, and 544 on the Shimadzu LCMS-8060RX triple quadrupole mass spectrometer with automatic method switching [7]. In this application, we expanded the evaluation to include EPA Methods 533, 545, and 544 on the same platform.

■ Method

Standards preparation: EPA Method 533 was followed to analyze 25 specific PFAS in drinking water, including long-chain and short chain PFAS, all purchased from Wellington Laboratories. Calibration standards (0.5 – 50 µg/L) were prepared using methanol with 20% water as diluent.

EPA Method 545 was followed to analyze cylindrospermopsin and anatoxin-a (Enzo Life Sciences), with uracil-d4 and L-phenylalanine-d5 as internal standards (Toronto Research Chemicals). A series of calibration standards were prepared from 1 µg/mL stock solution using LC-MS grade water containing the sample preservatives (1.0 g/L sodium bisulfate and 0.10 g/L ascorbic acid) as diluent to obtain the final concentrations of 0.02 - 20 µg/L for anatoxin-a and 0.005 - 10 µg/L for cylindrospermopsin.

Figure 1: Shimadzu LCMS-8060RX system

The analytes specified in EPA Method 544, including six microcystins and nodularin, were sourced from Enzo Life Sciences. The internal standard, ethylated D5 microcystin-LR (MC-LR-C2D5), was obtained from Gold Standard Diagnostics. A series of calibration standards were prepared using methanol/water (1:1) as diluent to obtain the final concentrations of 0.5 - 500 µg/L for the various calibration levels.

System configuration: A Shimadzu LCMS-8060RX triple quadrupole mass spectrometer (**Figure 1**) was utilized for the quantification of PFAS and cyanotoxins in water samples. The system was paired with a Shimadzu LC-40 series front-end liquid chromatography (LC) system, designed for seamless automation, method switching, and high operational efficiency. The configuration included three degassers (one 3-channel and two 5-channel), dual LC-40 solvent delivery pumps with low-pressure gradient (LPGE) modules, an autosampler, a system controller, and a column oven equipped with two switching valves, a 6-port, 2-position valve and a 7-port, 6-position valve.

To address potential PFAS contamination from LC system solvents and consumables, a delay column was positioned after the solvent mixer and before the autosampler to retain background PFAS away from target analytes. As shown in **Figure 2**, the system enables automated switching between EPA Methods 533, 544, and 545. For EPA Method 533 (PFAS analysis), the delay column is included in the flow path to reduce background interference. For cyanotoxin analyses (EPA Methods 544 and 545), the delay column is bypassed to prevent cross-contamination, ensuring accurate and reliable results. This flexible configuration enhances both workflow efficiency and analytical versatility on a single LC-MS platform.

Instrumentation parameters: For this application, we used the EPA 533 method based on the Shimadzu PFAS method package (P/N: 225-45420-91), with minor retention time adjustment based on system volume. Run time was 15 minutes by utilizing the Shim-pack™ GIST C18 column (PN: 227-30008-03). To effectively delay background PFAS contamination, we incorporated the Shim-pack GIST C18 column as a delay column (PN: 227-30015-03).

For EPA Methods 544 and 545, chromatographic separation of analytes and internal standards was achieved within 8 minutes. EPA Method 545 utilized a Shim-pack GIST C18 column (PN: 227-30001-04), while EPA Method 544 employed a Shim-pack Velox™ SP-C18 column (PN: 227-32003-03). Gradient and LC conditions are detailed in **Tables 1 and Table 2.**

To prevent mobile phase contamination when switching between PFAS and cyanotoxin analysis, a simple five-minute rinse (the flow path indicated by the red lines in **Figure 2**) with the appropriate mobile phase has shown to be sufficient. Additional rinsing steps may be included to address matrix-related contamination. This process can be fully automated within the batch sequence, eliminating manual intervention. For example, transitioning from EPA Method 533 to 545 requires only a five-minute flush with the new mobile phase before starting the next analysis.

Table 1. Gradient time program of mobile phases for three EPA methods

Time (min)	%A	%B
EPA Method 533 Mobile phase A: 5 mM ammonium acetate in water Mobile phase B: methanol		
0.00	95	5
1.00	60	40
8.00	5	95
10.00	5	95
10.10	95	5
15.00	95	5
EPA Method 545 Mobile phase A: 0.2% acetic acid in water Mobile phase B: methanol		
0.00	98	2
1.00	80	20
3.50	60	40
3.51	40	60
4.00	40	60
4.01	98	2
8.00	98	2
EPA Method 544 Mobile phase A: 0.2% acetic acid in water Mobile phase B: 0.2% acetic acid in acetonitrile		
0.00	85	15
0.50	85	15
5.00	10	90
6.50	10	90
6.51	85	15
8.00	85	15

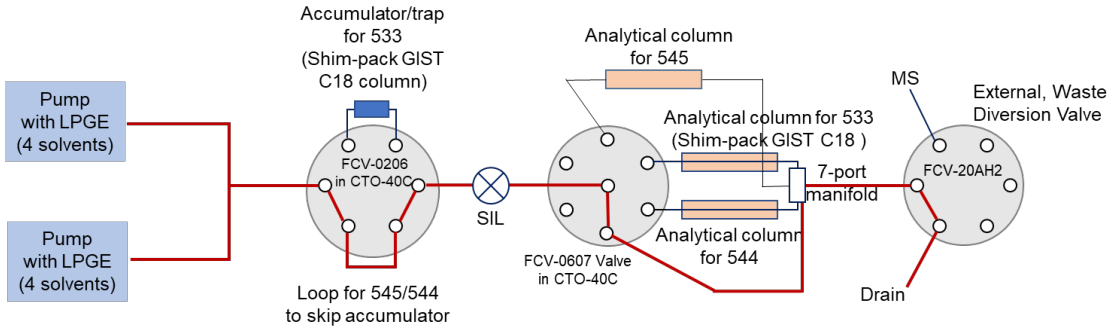

Figure 2: Flow diagram of the integrated LCMS-8060RX for multiple applications (red lines indicate the flow in the rinse step).

Table 2. LC and MS method conditions of the three EPA methods

Parameter	EPA 533	EPA 545	EPA 544
Analytical Column	Shim-pack GIST C18 column, 3 µm, 2.1 x 50mm	Shim-pack GIST C18 column, 2 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 µm, 3.0 x 50mm	Not applicable	Not applicable
Injection Volume	2 µL	20 µL	10 µL
Column Oven Temp.	45 °C	40 °C	40 °C
Flow Rate	0.25 mL/min	0.3 mL/min	0.3 mL/min
Run Time	15 minutes	8 minutes	8 minutes
MS Interface	ESI Negative	ESI Positive	ESI Positive
Nebulizing Gas Flow	3.0 L/min	3.0 L/min	3.0 L/min
Heating Gas Flow	15.0 L/min	10.0 L/min	10.0 L/min
Drying Gas Flow	5.0 L/min	10.0 L/min	10.0 L/min
Interface Temp.	100 ℃	300 ℃	300 ℃
DL Temp.	150 ℃	250 ℃	250 ℃
Heat Block Temp.	250 ℃	400 ℃	400 ℃

Experimental design: Figure 3 presents a comprehensive overview of the batch structure designed to assess system robustness and reliability during method switching. The experiment began with triplicate calibration injections for Method 533 to establish accuracy and reproducibility. This procedure was subsequently repeated for Methods 545 and 544, ensuring that each method was calibrated under consistent conditions.

After completing the calibration for all three methods, continuing calibration checks were conducted for Method 533, followed by the same checks for Methods 545 and 544. This entire sequence was repeated twice to rigorously assess consistency and system stability. The batch comprised a total of 294 injections—including null, solvent blank, rinse, and standard injections—executed over 54 hours of continuous operation. This comprehensive dataset offers a robust evaluation of method-switching performance and overall system reliability.

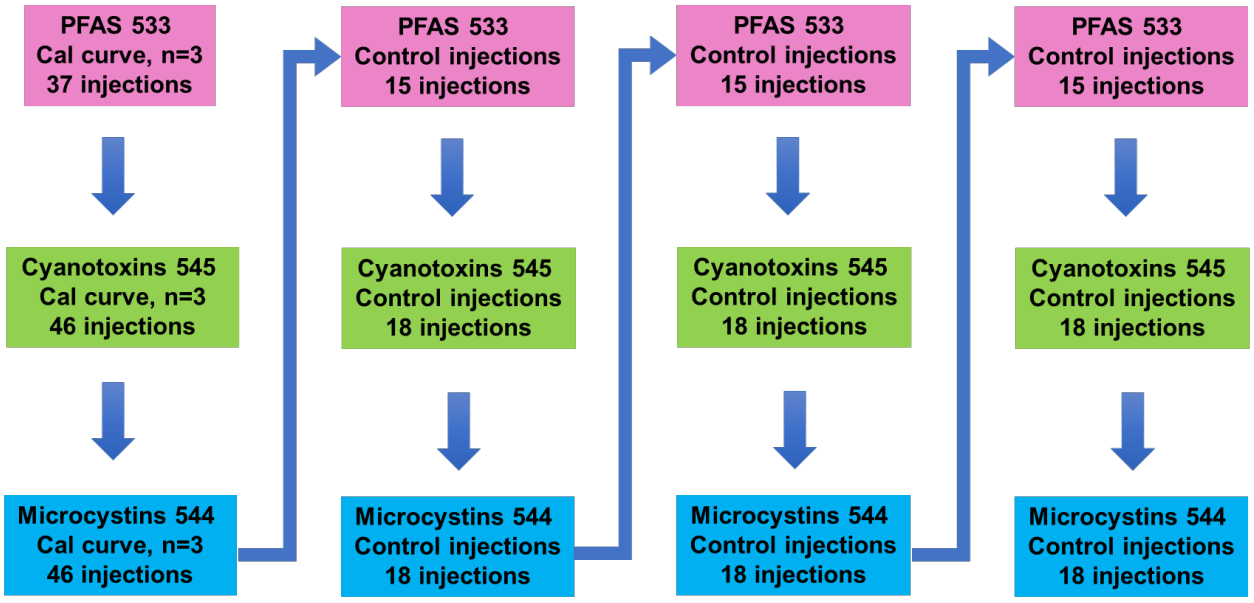

Figure 3: Batch structure designed to assess the performance and reliability of running three analytical methods on a single system.

■ Results and Discussion

Chromatographic separation: The combination of the Shim-pack GIST C18 column and optimized gradient conditions enabled effective retention and baseline separation of most of the 25 PFAS compounds and the internal standards within a 15-minute run, as shown in **Figure 4A**. Although some analytes—such as NFDHA and 4:2 FTS, and PFHxS and PFHpA—exhibited overlapping chromatographic peaks, their distinct MRM transitions ensured accurate identification and quantification by mass spectrometry.

Figures 4B and 4C display chromatograms for EPA Methods 545 and 544, respectively. For Method 545, complete separation of cylindrospermopsin, anatoxin-a, and both internal standards was achieved using an 8-minute gradient with the Shimadzu GIST C18 column. In Method 544, the analytes were effectively retained, enabling baseline separation of most of the seven cyanotoxins and the internal standard within 8 minutes. While MC-LA and MC-LY exhibited overlapping signals, their distinct MRM transitions enabled accurate identification and quantification via mass spectrometry.

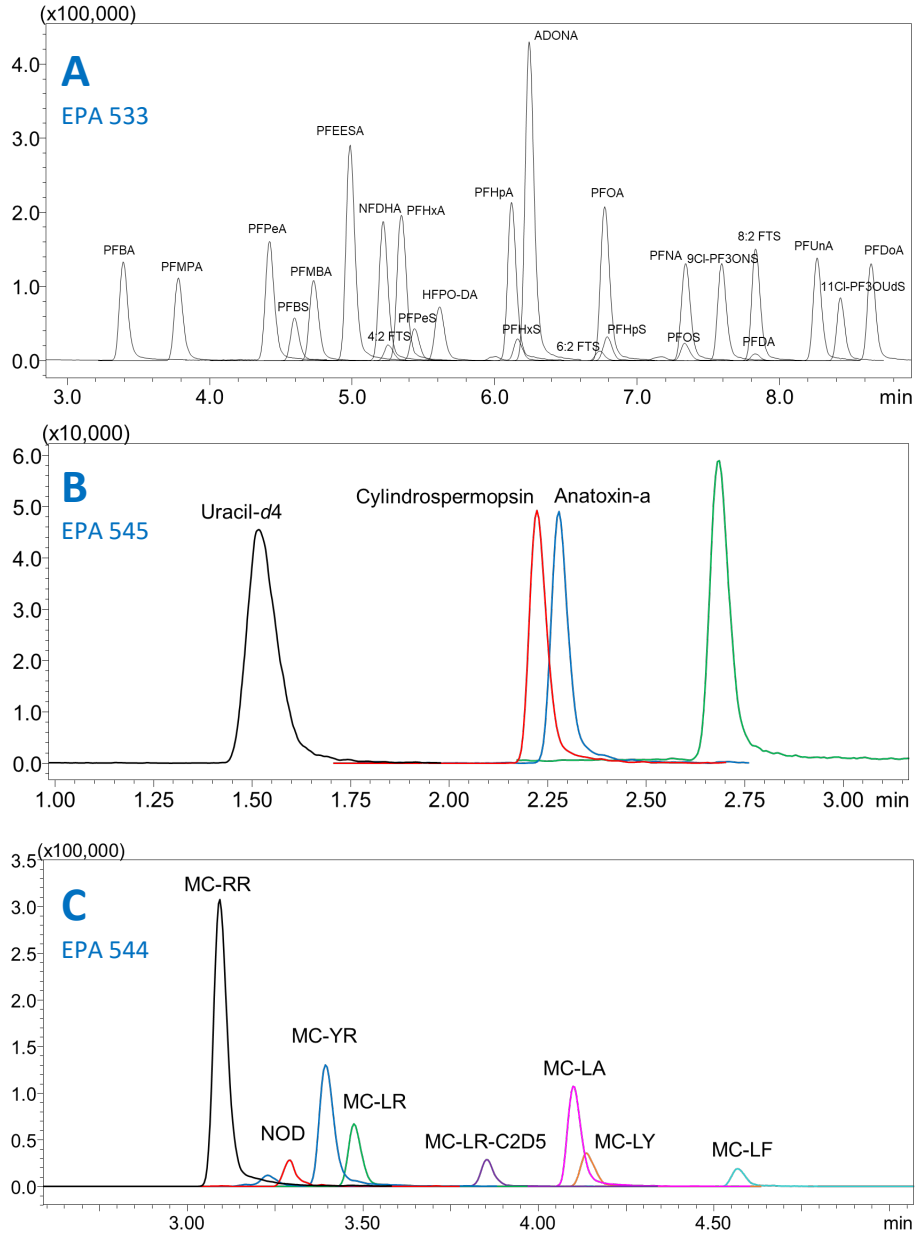

Figure 4: MRM quantifier ion mass chromatograms of the three EPA methods. **A**: EPA Method 533. **B**: EPA Method 545. **C**: EPA Method 544.

Calibrations: Linear calibration curves were successfully established for 25 PFAS compounds across the 0.5 – 50 µg/L range, following EPA Method 533. **Table 3** summarizes the linearity and accuracy based on triplicate calibration injections. All analytes demonstrated excellent linearity, with R^2 values exceeding 0.99 across the full range. This strong correlation confirms the method's reliability for precise PFAS quantification. Injection accuracy ranged from 80% to 120%, consistently falling within acceptable limits. Additionally, the relative standard deviation (%RSD) at each calibration level was below 10%, underscoring the robustness and reproducibility of the analytical system.

A linear calibration curve for anatoxin-a was established over the range of 0.02 – 20 µg/L, while cylindrospermopsin showed linearity across 0.005 – 10 µg/L. Both analytes demonstrated excellent linearity, with R^2 values exceeding 0.998. Injection accuracy ranged from 80% to 120%, and the %RSD for all calibration replicates remained below 15%, confirming the precision and reproducibility of the method.

For microcystins and nodularin, either linear or quadratic calibration curves were established, covering a broad concentration range of 0.5 – 500 µg/L. All seven analytes exhibited R^2 values greater than 0.99, confirming excellent linearity throughout the calibration range. Injection accuracy remained within 80% – 130%, and %RSD for all calibration levels were below 15%.

These results confirm the method's reliability for accurate quantification of PFAS and cyanotoxins across a wide range of concentrations. Representative calibration curves for key analytes from each method are illustrated in **Figure 5**.

Table 3: Summary of results from the triplicate calibration injections for the three EPA methods

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	9Cl-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	11Cl-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				

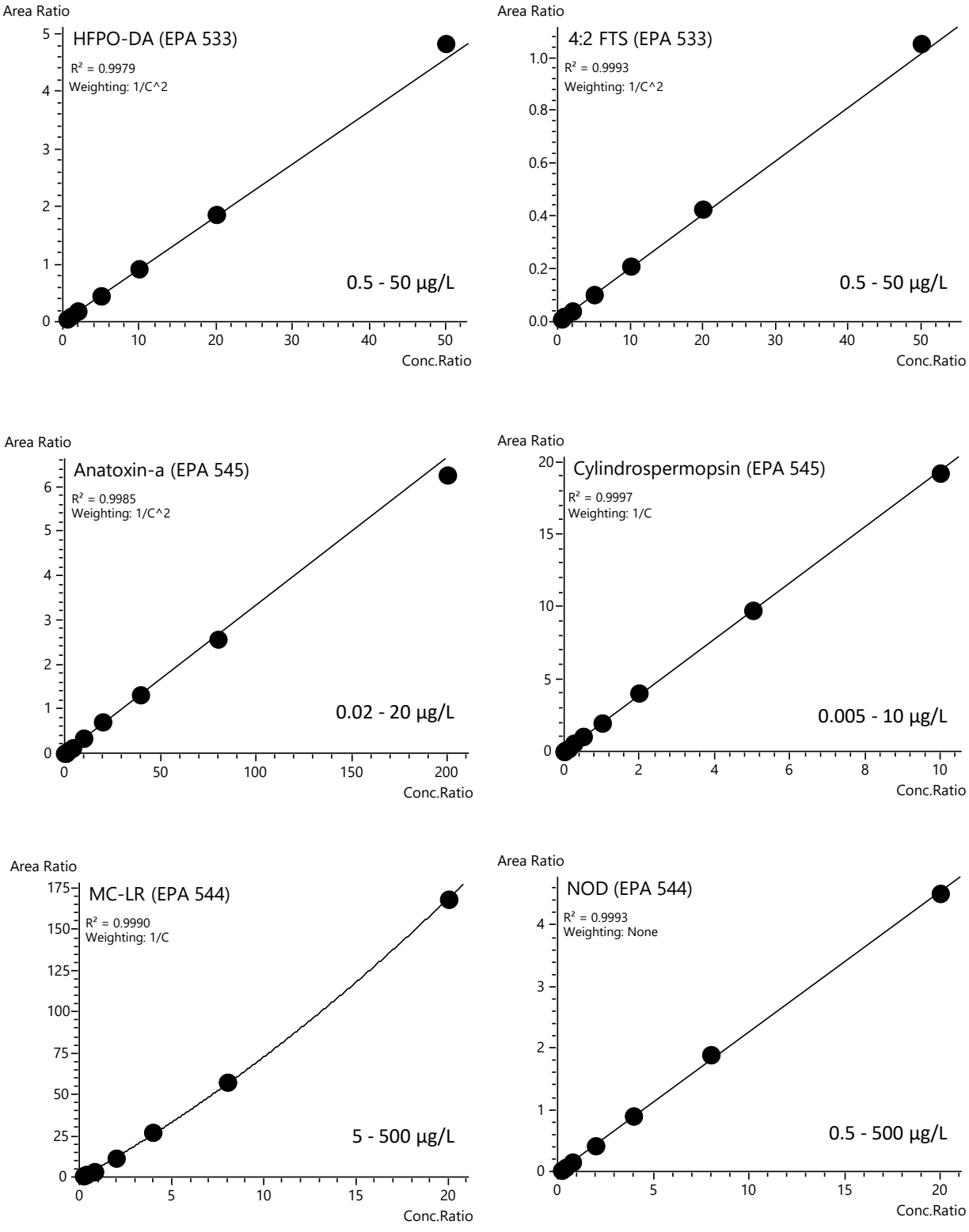

Figure 5: Calibration curves for representative compounds in the three EPA methods.

Continuing calibration check: Continuing calibration checks were performed after triplicate calibration injections for each method, starting with Method 533, followed by Methods 545 and 544. This sequence was repeated three times to assess rinse effectiveness and system consistency.

Figure 6 presents the results, showing accuracy across the lower limit of quantitation (LLOQ), mid-concentration, and higher limit of quantitation (HLOQ). The y-axis represents average accuracy, with error bars showing %RSD values. All analytes across the three methods maintained accuracies between 80 - 120%, with %RSD below 15%, confirming system reliability and stability. These findings highlight the effectiveness of the rinsing procedure in preventing carryover and maintaining system performance across different analytical methods.

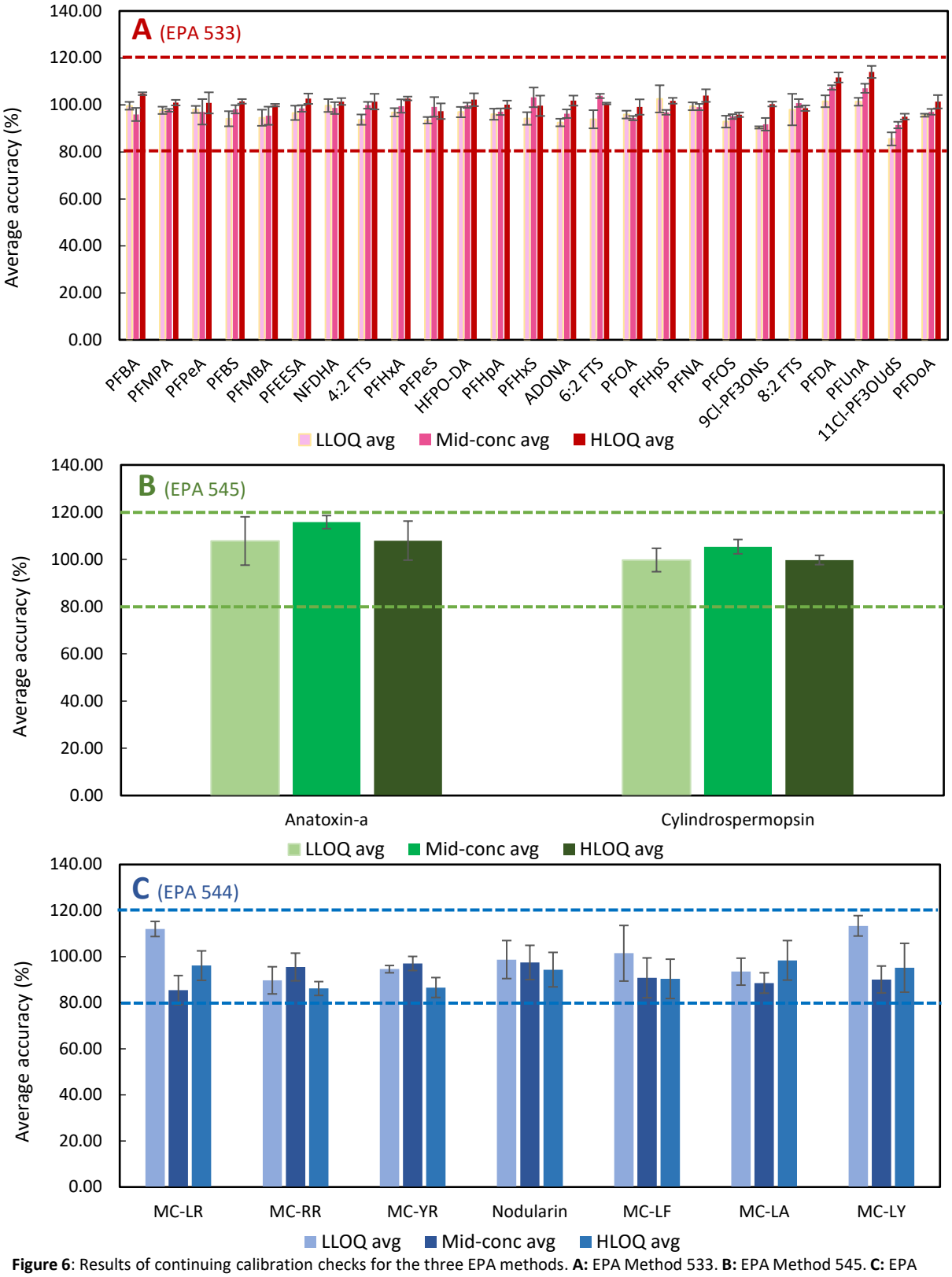

Figure 6: Results of continuing calibration checks for the three EPA methods. **A:** EPA Method 533. **B:** EPA Method 545. **C:** EPA Method 544.

Figure 7 shows chromatograms of two representative compounds: PFOA (a negative ion analyte from Method 533) and anatoxin-a (a positive ion analyte from Method 545). The comparison highlights their chromatographic profiles at the limit of quantitation (LOQ), both before and after method switching.

Chromatogram No. 1 represents one of the triplicate calibration injections, while chromatograms No. 2 through No. 4 show three subsequent calibration check injections. The consistent accuracy observed at the LOQ across all injections demonstrates the system's reliability and robustness, even after method switching.

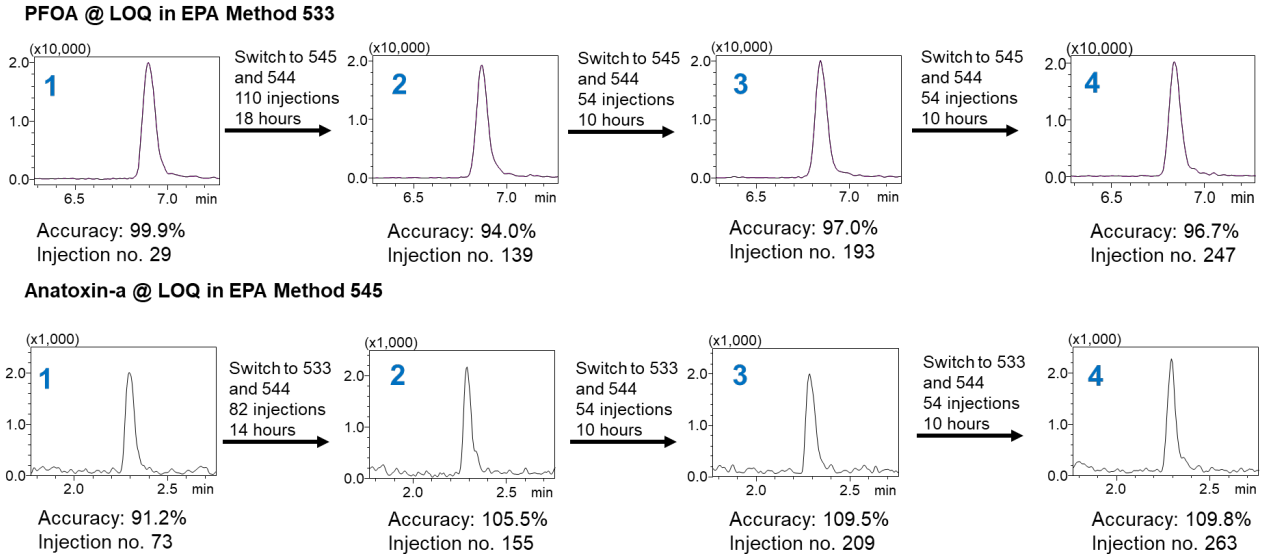

Figure 7: Mass chromatogram and quantitative accuracy of representative compounds before and after switching methods.

■ Conclusion

This study demonstrates the successful quantification of PFAS (EPA 533) and cyanotoxins—including microcystins and nodularin (EPA 544), as well as cylindrospermopsin and anatoxin-a (EPA 545)—using a single triple quadrupole mass spectrometer with automatic method switching. This streamlined approach enabled precise and reliable measurements while maintaining high accuracy and sensitivity throughout extended analytical runs, despite multiple injections and frequent method transitions.

A key advantage of this approach is the efficient switching between analytical methods with only a five-minute rinse between methods. This ensures complete removal of residual analytes and prevents mobile phase contamination, allowing smooth transitions between PFAS and cyanotoxin analysis without compromising data quality. The rapid automatic rinsing process eliminates the need for extensive manual intervention, reducing instrument downtime and increasing overall laboratory productivity.

By consolidating multiple analytical methods into a single instrument, this approach minimizes the need for separate systems, thereby reducing both capital investment and maintenance costs. This single system enables laboratories with the capability to respond quickly to emergencies, such as HABs, without major disruption of their routine testing, for PFAS and potentially other organic contaminants. Furthermore, automation significantly enhances operational efficiency by minimizing manual labor requirements, improving reproducibility, and optimizing high-throughput sample analysis. This method provides a cost-effective and reliable solution for laboratories performing environmental monitoring and regulatory compliance testing.

■ References

1. Progress update on the per- and polyfluoroalkyl substances (PFAS) restriction process [Online]
https://echa.europa.eu/documents/10162/67348133/pfas_status_update_report_en.pdf
2. EPA Method 537.1: Determination of Selected Per- and Polyflourinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) [Online]
https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=343042&Lab=NERL
3. EPA Method 533: Determination of Per- And Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry [Online]
https://www.epa.gov/sites/default/files/2019-12/documents/method-533-815b19020.pdf
4. EPA Drinking Water Health Advisories for Cyanotoxins [Online] https://www.epa.gov/habs/epa-drinking-water-health-advisories-cyanotoxins
5. EPA Method 544: Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). [Online]
https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=306953
6. EPA Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS). [Online]
https://www.epa.gov/sites/default/files/2017-10/documents/epa_815-r-15-009_method_545.pdf
7. One System, Multiple Solutions: Analysis of PFAS & Cyanotoxins in Water adhering to EPA 537.1, 544, and 545 [Online]
https://www.shimadzu.com/an/apl/25350/index.html

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