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LCMS™-8060RX High Performance Liquid Chromatograph Mass Spectrometer

Application News

One System, Multiple Solutions: Analysis of PFAS & Cyanotoxins in Water Adhering to EPA 537.1, 544, and 545

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User Benefits

- A single Shimadzu LCMS-8060RX triple quadrupole mass spectrometer can successfully quantitate both PFAS and cyanotoxins in water with automatic method switching.
- With only a five-minute rinsing time between methods, the system maintains high accuracy and sensitivity over an extended run time, even with multiple injections and method changes.
- This single system approach allows laboratories to respond swiftly to emergencies, like Harmful Algae Blooms, while minimizing disruption to routine PFAS testing and eliminating the need to invest in multiple instruments.

■ Background

Per- and Polyfluoroalkyl Substances (PFAS) are a group of synthetic chemicals extensively used in consumer products (e.g., food packaging materials and non-stick coatings) and industrial applications such as firefighting foams and polymer/plastics manufacturing. Their remarkable properties, including high stability and resistance to degradation, coupled with widespread usage, have led to their persistent accumulation in the environment. Consequently, regulatory and governmental organizations, including the US Environmental Protection Agency (EPA) and the European Chemicals Agency (ECHA) [1], are working to restrict their presence in the environment.

In the United States, EPA Method 537.1 is one of the approved methods by EPA for the analysis of PFAS in drinking water. It targets 18 compounds, utilizing solid-phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for detection at low ng/L concentrations [2]. EPA Method 533 is the only other EPA-approved method for PFAS analysis in drinking water. It follows a workflow similar to that of EPA Method 537.1 but expands the target list to include 25 PFAS compounds [3].

PFAS is not the only concern—other contaminants in drinking water can also pose significant health risks. Cyanobacteria, also referred to as blue-green algae, are photosynthetic organisms that thrive in both freshwater and marine ecosystems. The frequency and occurrence of Harmful Algae Blooms (HABs) have risen substantially over the years, primarily due to human-induced inputs of phosphorus and nitrogen into aquatic systems, fueling the global increase in harmful algal blooms. These organisms can significantly affect water quality by producing cyanotoxins such as cylindrospermopsin, anatoxin-a and microcystins. Exposure to these cyanotoxins can cause a range of adverse health effects in humans and animals, from mild skin irritations to severe illnesses [4].

The US EPA has established Methods 544 and 545 as analytical protocols for detecting cyanotoxins in drinking water and freshwater. EPA Method 544 is designed to detect microcystins and nodularin [5], while EPA Method 545 targets cylindrospermopsin and anatoxin-a [6]. Both methods employ liquid chromatography-tandem mass spectrometry (LC-MS/MS) for precise analysis.

Ideally, separate instruments should be dedicated to analyzing PFAS and cyanotoxins to minimize the risk of contamination and interference. However, with the growing demand for water testing and rapid turn-around-times during emergencies that may alter routine testing, a single system capable of handling multiple methods offers a more cost-effective solution. This application demonstrates the accurate and robust quantification of PFAS and cyanotoxins, performed in accordance with EPA Methods 537.1, 545, and 544, using a single Shimadzu LCMS-8060RX triple quadrupole mass spectrometer with automatic method switching.

■ Method

Standards preparation: EPA Method 537.1 was followed to analyze 25 PFAS in drinking water, including 18 target compounds, 4 surrogates, and 3 internal standards, all purchased from Wellington Laboratories. Calibration standards (0.5 – 50 µg/L) were prepared using methanol with 4% 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 used to quantify the PFAS and cyanotoxins in water. The front-end liquid chromatography (LC) system was configured with the Shimadzu LC-40 series, ensuring seamless automation, method switching, and high efficiency. Key modules include three degassers (one 3-channel and two 5-channel), two LC-40 solvent pumps equipped with low-pressure gradient (LPGE) modules, an autosampler, a system controller, a column oven with two column switching valves (one 6-port, 2-position valve and one 7-port, 6-position valve).

PFAS contamination can originate from solvent containers and consumables used in the LC system. To minimize background interference, a delay column is implemented before the autosampler to separate background PFAS from target analytes.

Figure 2 illustrates the LC-MS system's method-switching capability, enabling seamless transitions between EPA Methods 537.1, 544, and 545 on a single platform. For EPA Method 537.1 (PFAS analysis), the flow path includes the delay column to remove background PFAS. In contrast, for EPA Methods 544 and 545 (cyanotoxin analysis), the delay column is bypassed to prevent unnecessary contaminations, ensuring accurate results. This automation enhances workflow efficiency and analytical flexibility.

Instrumentation parameters: For this application, we used the EPA 537.1 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 18 minutes by utilizing the Shim-pack Velox™ SP-C18 column (PN: 227-32003-02). 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 is 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 537.1 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 537.1		
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
8.10	0	100
13.00	0	100
13.10	95	5
18.00	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

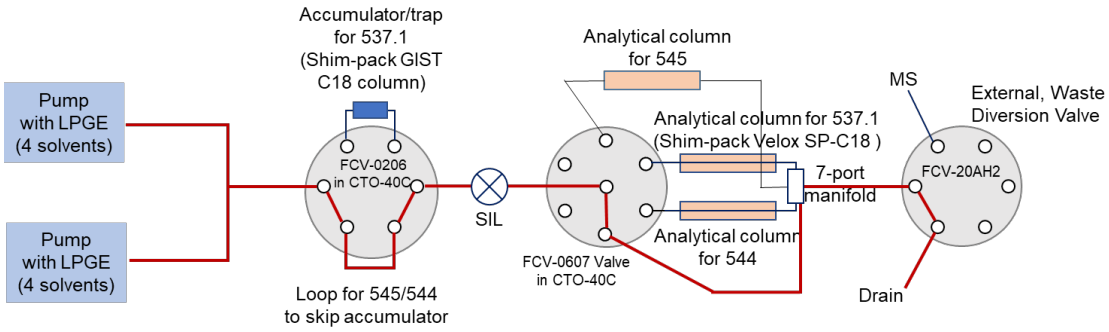

Figure 2: Flow diagram of the integrated LCMS-8060RX for multiple applications (red lines indicate the flow in the rinse step).

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

Parameter	EPA 537.1	EPA 545	EPA 544
Analytical Column	Shim-pack Velox SP-C18 column, 2.7 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 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	18 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 provides a detailed overview of the batch structure designed to evaluate the system's robustness and reliability during method switching. The experiment commenced with calibration injections for Method 537.1, where a triplicate calibration curve was generated to ensure accuracy and reproducibility. Following this, we conducted the same calibration procedure for Method 545 and Method 544, ensuring that each method was properly calibrated under consistent conditions.

After completing the calibration for all three methods, we performed a continuing calibration check for Method 537.1, followed by similar checks for Methods 545 and 544. This entire sequence was repeated twice more to thoroughly evaluate consistency and stability. In total, the batch included 294 injections, covering null, solvent blank, rinse, and standard injections, with 54 hours of continuous operation. This comprehensive dataset provides a thorough assessment of method-switching performance and system reliability.

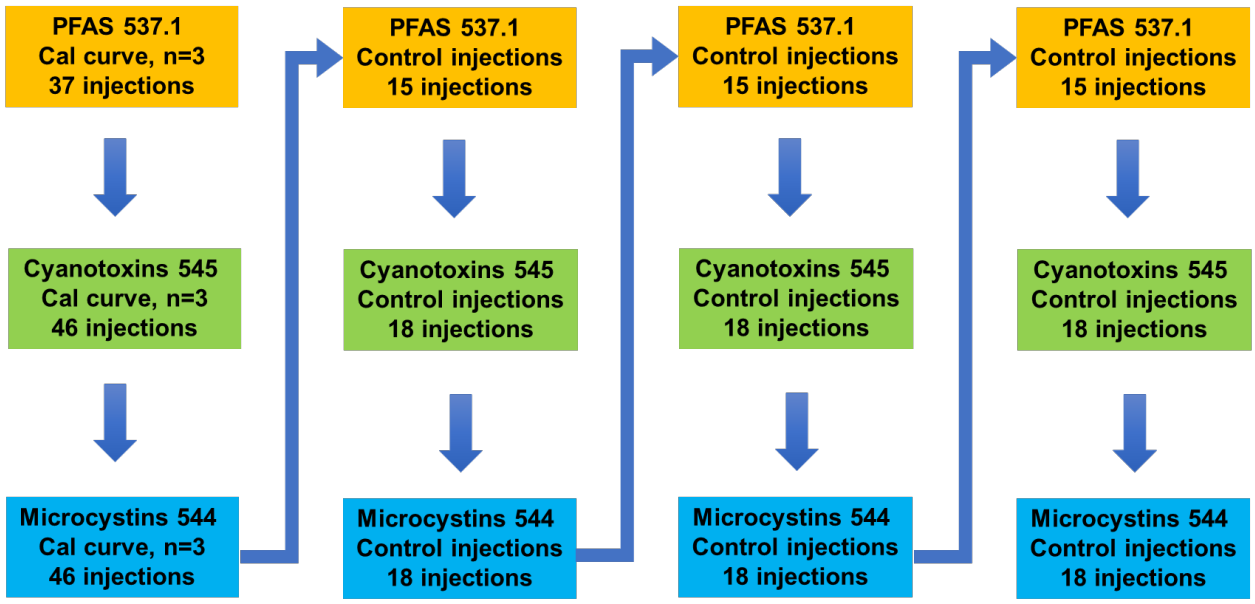

Figure 3: Batch structure designed to assess the performance and reliability of running three analytical methods on a single system.

■ Results and Discussion

Chromatographic separation: The combination of the Shim-pack Velox SP-C18 column and optimized gradient conditions enabled effective analyte retention and baseline separation of most of the 25 PFAS compounds and the internal standard within 18 minutes, as illustrated in **Figure 4A**. Despite overlapping signals—such as PFHxS with PFHpA and ADONA, PFOS with PFNA—the distinct MRM transitions allow for accurate identification and quantification using mass spectrometry.

Figure 4B and 4C present the chromatograms for EPA methods 545 and 544. Using an 8-minute gradient with the Shimadzu GIST C18 column, complete separation of cylindrospermopsin, anatoxin-a, and the two internal standards was achieved for EPA Method 545. For EPA Method 544, analytes were effectively retained, facilitating baseline separation of most of the seven cyanotoxins and the internal standard within 8 minutes. Although MC-LA and MC-LY signals overlap, their unique MRM transitions ensure precise identification and quantification through mass spectrometry.

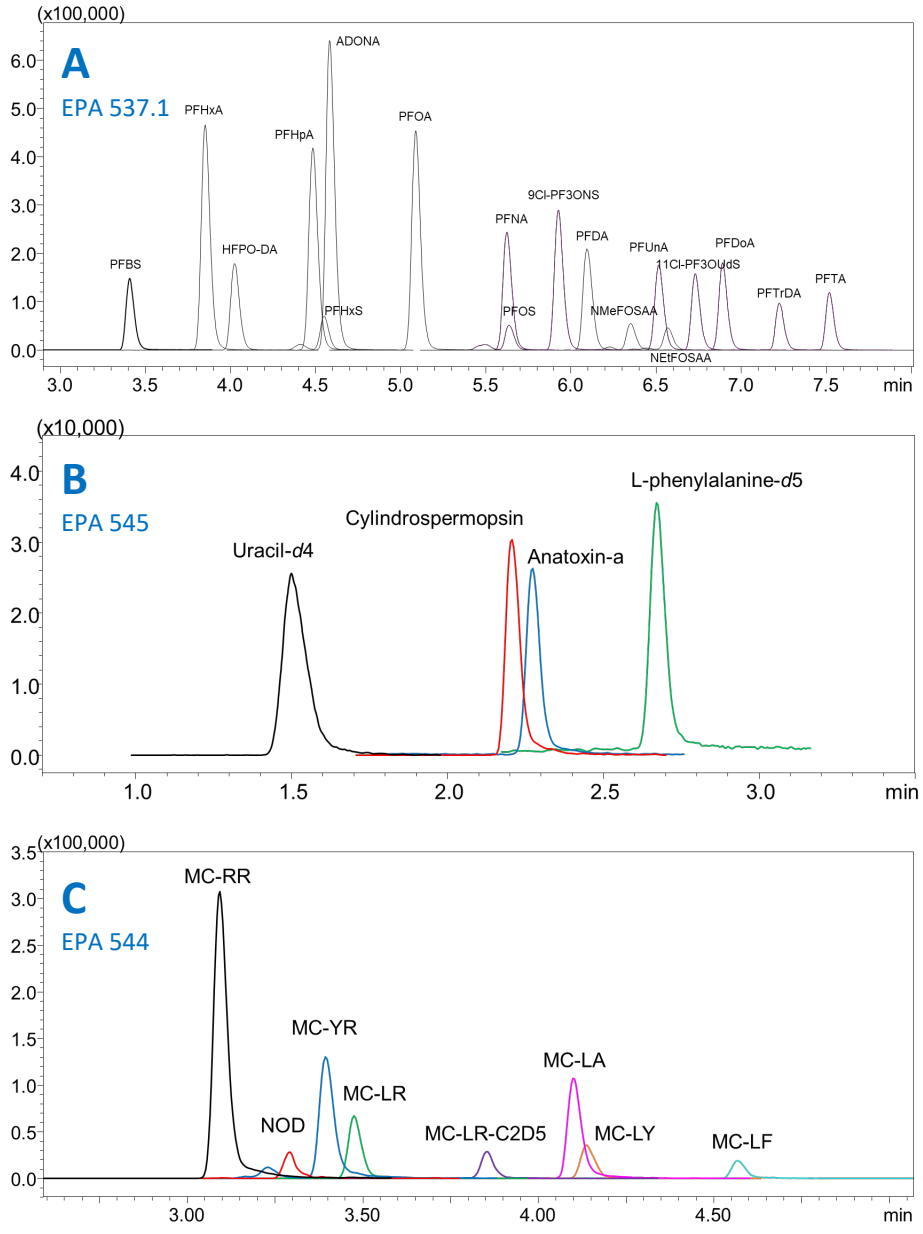

Figure 4: MRM quantifier ion mass chromatograms of the three EPA methods. **A**: EPA Method 537.1. **B**: EPA Method 545. **C**: EPA Method 544.

Calibrations: Linear calibration curves were successfully established for 18 PFAS compounds in the concentration range of 0.5 – 50 µg/L, in accordance with EPA Method 537.1. **Table 3** provides a summary of the linearity and accuracy obtained from triplicate calibration injections. Excellent linearity was observed, as evidenced by R^2 values exceeding 0.99 for all analytes across the full calibration range. This strong correlation demonstrates the reliability of the method for quantifying PFAS compounds with high precision. The accuracy of all injections fell within the range of 90 to 110%, indicating that the method consistently produced results within the expected limits. Furthermore, the relative standard deviation (%RSD) of the measured concentration at each calibration level was below 10% for all calibrators. This low variability highlights the robustness and reproducibility of the analytical system, ensuring that it can deliver precise and consistent measurements.

A linear calibration curve for anatoxin-a was established across a concentration range of 0.02 – 20 µg/L, while cylindrospermopsin demonstrated a calibration range of 0.005 – 10 µg/L. Both compounds exhibited excellent linearity, with R^2 values exceeding 0.997. The accuracy of all injections fell within 80% to 120%, and the %RSD of concentration measurements for all calibrator replicates remained below 15%, confirming the method's precision and reproducibility.

For microcystins and nodularin, either a linear or quadratic calibration curve was established, covering a broad concentration range of 0.5 – 500 µg/L. All seven analytes demonstrated R^2 values exceeding 0.99, confirming strong linearity across the calibration range. The accuracy of all injections remained within 80% – 120%, and %RSD values for all calibration levels were below 15%.

These results affirm the suitability of the method for accurately quantifying PFAS and cyanotoxins across a wide range of concentrations. Representative calibration curves for key compounds analyzed in each method are shown 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 537.1							
PFBS	0.5 – 50	0.9999	92.6 – 101.8	9Cl-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	11Cl-PF3OUdS	0.5 – 50	0.9998	88.5 – 107.8
PFOA	0.5 – 50	0.9995	96.8 – 103.0	PFDoA	0.5 – 50	0.9998	96.7 – 103.6
PFOS	0.5 – 50	0.9996	92.0 – 102.8	PFTrDA	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				

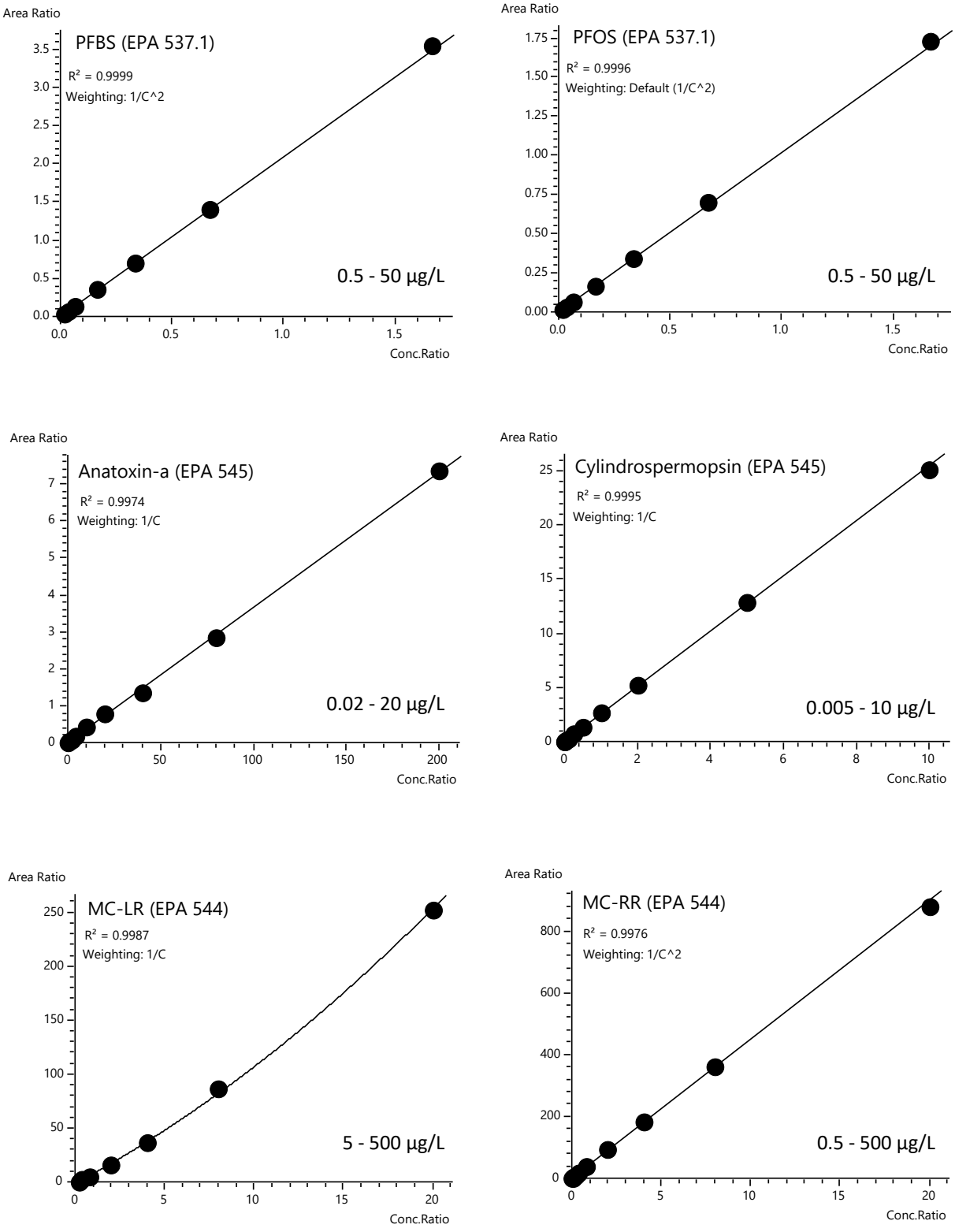

Figure 5: Calibration curves for representative compounds in the three EPA methods.

Continuing calibration check: Continuing calibration checks were performed after triplicate calibration injections for each method, starting with Method 537.1, 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 mobile phase contamination and maintaining system performance across different analytical methods.

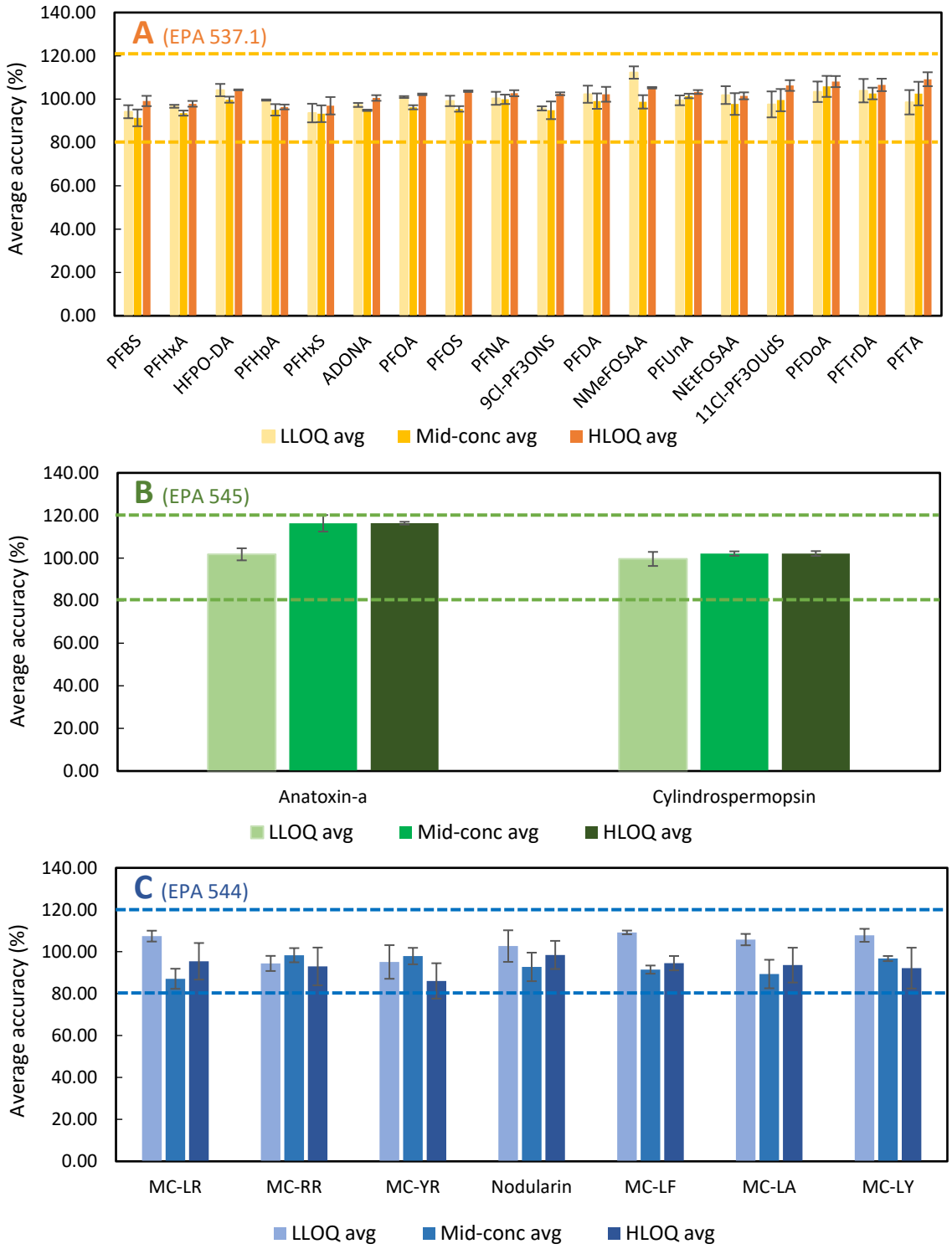

Figure 6: Results of continuing calibration checks for the three EPA methods. **A:** EPA Method 537.1. **B:** EPA Method 545. **C:** EPA Method 544.

Figure 7 displays chromatograms of two representative compounds: PFBS (a negative ion analyte from Method 537.1) and cylindrospermopsin (a positive ion analyte from Method 545). The comparison illustrates their chromatographic profiles at the limit of quantitation (LOQ) before and after method switching.

The first chromatogram (No. 1) represents one of the triplicate calibration injections, while chromatograms No. 2 through No. 4 correspond to three subsequent calibration checks. The results clearly demonstrate that accuracy at the LOQ remains stable and consistent throughout the batch, confirming the system's reliability and robustness despite of method switching.

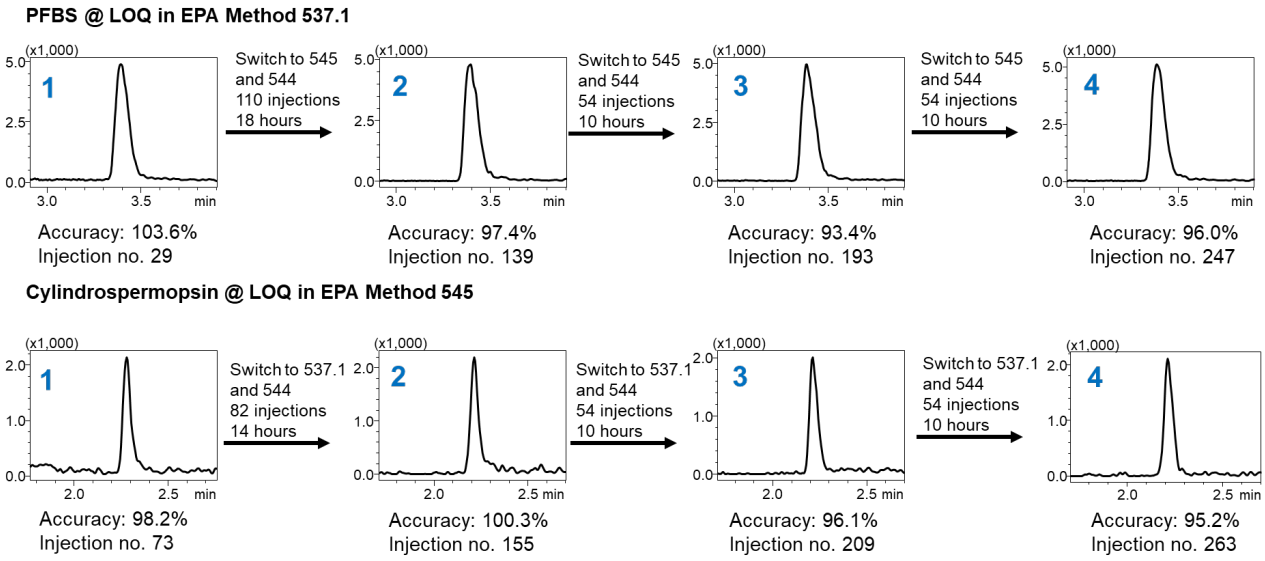

Figure 7: Mass chromatogram and quantitative accuracy of representative compounds before and after switching methods.

■ Conclusion

This study demonstrates the successful quantification of PFAS (EPA 537.1) 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. By leveraging this streamlined approach, we achieved precise and reliable measurements while maintaining high accuracy and sensitivity across extended analytical runs, even with 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

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