SHIMADZU

TP 120

Integrated LC-MS/MS Workflow for Simultaneous PFAS and Cyanotoxin Analysis: Evaluating Mobile Phase Acid Effects to Improve Accuracy and Robustness

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1. Introduction

- PFAS and cyanotoxins are priority drinking water contaminants regulated by the U.S. EPA, originating from industrial sources and harmful cyanobacterial blooms, respectively.
- EPA Methods 533 targets 25 PFAS in drinking water, including many shorter-chain compounds like ADONA, which are not covered by 537.1; Methods 544 and 545 target cyanotoxins like microcystins, nodularin, cylindrospermopsin, and anatoxin-a.
- A single Shimadzu LCMS-8060RX system with automatic method switching enables efficient, accurate analysis of PFAS and cyanotoxins, reducing instrument requirements and improving overall throughput.

2. Methods

Figure 1 illustrates the LC-MS system's method-switching capability, enabling seamless transitions between EPA Methods 533, 544, and 545 on a single platform. For EPA Method 533 (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.

To further minimize mobile phase cross-contamination when switching between PFAS and cyanotoxin analyses, a simple five-minute rinse—following the flow path indicated in Figure 2 - is sufficient to maintain system cleanliness and analytical reliability.

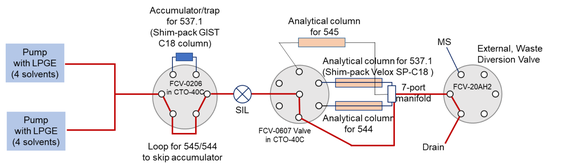

Figure 1: Flow diagram of the integrated LCMS-8060RX for multiple applications (red lines indicate the flow in the rinse step).

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 50 mm	Shim-pack GIST C18 column, 2 µm, 2.1 x 100 mm	Shim-pack Velox SP-C18 column, 2.7 µm, 2.1 x 100 mm
Delay Column	Shim-pack GIST C18 5 µm, 3.0 x 50 mm	Not applicable	Not applicable
Injection Volume	2 µL	20 µL	10 µL
Column Oven Temp.	45 °C	40 °C	40 °C
Mobile Phase	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: 0.2% Acetic Acid in Acetonitrile
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**

Figure 2 provides a detailed overview of the batch structure designed to evaluate the system's robustness and reliability during method switching. In total, the batch included 294 injections, covering null, solvent blank, rinse, and standard injections, with 54 hours of continuous operation.

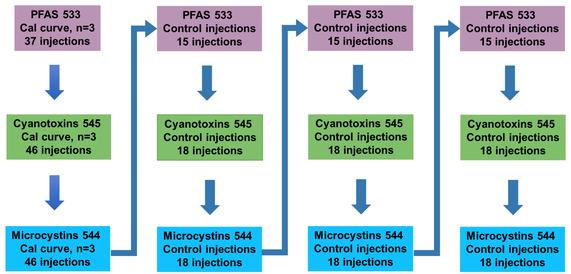

Figure 2: Batch structure designed to assess the performance and reliability of running three analytical methods on a single system.

3. Results

Calibration curves were successfully established for all the analytes in accordance with EPA Method 533, 545 and 544. All 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%.

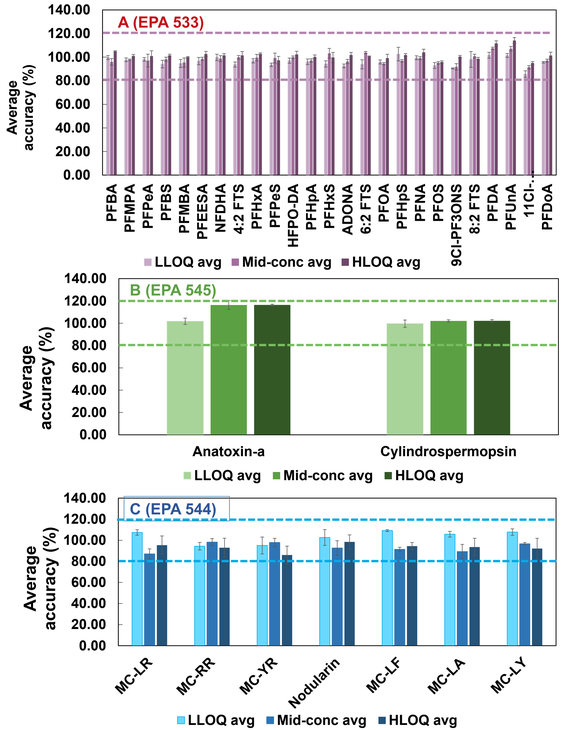

Figure 3: Results of continuing calibration checks for the three EPA methods.

Continuing calibration checks were performed after triplicate injections of Methods 533, 545, and 544 to evaluate rinse effectiveness and system consistency. Figure 3 shows that all analytes maintained 80–120% accuracy with %RSD below 15% at the LLOQ, mid, and HLOQ levels, confirming reliable performance and effective rinsing.

Acetic acid vs formic acid. Figure 4 shows chromatograms of PFBA and PFNA (5 ng/mL) before and after acid exposure. Prolonged formic acid exposure (>30 h) and overnight contamination of the PFAS analytical column caused substantial signal suppression for several PFAS compounds. In contrast, acetic acid showed no observable ion suppression regardless of exposure time. Significant signal loss of FOSE compounds in EPA Method 1633 was also observed when formic acid passed only through the LC lines without contacting the column, while no signal decrease was observed with acetic acid.

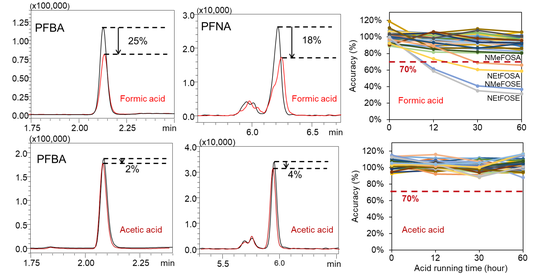

Figure 4: PFBA and PFNA chromatograms and PFAS accuracy after LC system exposure to formic acid or acetic acid mobile phases.

4. Conclusion

This study shows that PFAS and cyanotoxins can be accurately measured using one triple quadrupole mass spectrometer with automatic method switching. A quick five-minute rinse ensures clean transitions between methods, minimizing downtime and manual work. Replacing formic acid with acetic acid in cyanotoxins analysis could avoid potential ion suppression effect from formic acid.