

AUTOMATED SOLID PHASE EXTRACTION OF MICROCYSTINS AND NODULARIN FOLLOWING EPA METHOD 544

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ABSTRACT

Microcystins and Nodularin are metabolites of cyanobacteria which thrive in warm and stagnant water sources during algae blooms. These compounds are extremely hepatotoxic causing serious liver damage with multiple health effects such as gastrointestinal issues, blistering of the mouth and pneumonia. It can accumulate in plants, roots, and agricultural soils through irrigation and in drinking water supplies due to their chemical stability and low molecular weight. Due to these reasons and seriousness of their health impact, a fast and reliable method of detection in water samples is required at the source of these contaminants.

INTRODUCTION

The Erie County Water Authority (ECWA) has made it their mission statement to provide clean and safe water to the public. With its own large algae blooms in Lake Erie in 2011, they understand the need for fast and effective detection of these analytes. In recent years with increasing amounts of eutrophication from agricultural off run, a fast and effective solid phase extraction (SPE) method is utilized for the quantification of Microcystins and Nodularin. The SPE-03 serves as an 8-channel extraction system for automated SPE complying with EPA method 544¹ to tackle the monitoring of Microcystin and Nodularin in water sources.

This application note validates the automation of Microcystin and Nodularin extraction by SPE with excellent recoveries, clean background, and low Minimum Reporting Levels (MRLs). ECWA's current monitoring focuses on Microcystin-LR, which is the most studied and toxic variant amongst the group of Microcystins, along with Nodularin.

MATERIALS

- PromoChrom SPE-03 with MOD-005 (PFAS configuration)
- Phenomenex Strata-X 33 μ M Polymeric Reversed Phase Cartridge, 150mg/6mL
- Reagents and standards following EPA Method 533
- LCMSMS

METHOD SUMMARY

SPE Method

Solvent 1 = Methanol, **Solvent 2** = H₂O, **Solvent 4** = 10% H₂O in Methanol, **W1** = Aqueous waste, **W2** = Organic waste



Table 1 – Microcystin and Nodularin extraction steps programmed on the SPE-03.

Action	Inlet 1	Flow	Volume	Description
Elute W2	Solvent 1	10 mL/min	15 mL	Condition cartridges with 15mL Methanol
Elute W1	Solvent 2	10 mL/min	15 mL	Condition cartridges with 15mL Water
Add Sample W1	Sample	15 mL/min	520 mL	Load the samples at 15mL/min
Clean	Solvent 2	20 mL/min	10 mL	Add 10mL Water to sample bottles
Wait	Manual Resume			¹ Pause to manually rinse bottles
Add Sample	Sample	15mL/min	15 mL	Pass rinsate through cartridges
Elute W1	Solvent 2	15mL/min	5 mL	Wash cartridges with 5mL Water
Air-Purge W1	Air	15mL/min	10 mL	Purge large water droplets out of cartridges
Blow N2	Time based		10 mins	Dry cartridges with nitrogen for 10 mins
Clean	Solvent 4	20mL/min	5mL	Add 5mL of 10% H2O in Methanol to sample bottles
Wait	Manual Resume			¹ Pause to manually rinse bottles
Collect 1	Sample	3mL/min	5 mL	Collect rinsate through the cartridges into fraction 1
Clean	Solvent 4	20mL/min	5 mL	Add 5mL of 10% H2O in Methanol to sample bottles
Wait	Manual Resume			¹ Pause to manually rinse bottles
Collect 1	Sample	3mL/min	10mL	Collect rinsate through the cartridges into fraction 1

1. ECWA uses an earlier SPE-03 model primarily dedicated to PFAS drinking water methods which performs automatic rinsing of plastic sample bottles mounted upside down (MOD-004). As MOD-004 is not compatible with glass bottles, they programmed the system to dispense the solvents and pause for manual rinsing of the sample bottles. Full automation of the method can since be achieved using the MOD-00P (Volume-Matrix Plus) configuration released in late 2020, which has the capability of rinsing any 50mL to 1L containers.

The extract is then blown down by N₂ in a water bath until dryness and reconstituted into 1mL of 10% H₂O in Methanol.

LC-MS/MS Conditions

Table 2 - LC-MS/MS Conditions.

Parameter	Value
LC-MS/MS	ThermoFisher Vanquish LC with Quantis MS/MS
LC Column	ThermoFisher Accucore C18, 2.6µm, 2.1 x 100mm
LC Column Conditions	Following EPA Method 544 ¹ Section 17 - Table 1
LC Gradient Program	Following EPA Method 544 ¹ Section 17 - Table 1

Table 3 - Target Ion m/z and Qualifier Ions.

Analytes	Retention Time [min]	Precursor Ion [m/z]	Product Ion [m/z]
Microcystin-LR (MC-LR)	13.12	995.5	135.1
Nodularin	12.76	825.4	135.1
C ₂ D ₅ -MC-LR (Surrogate)	16.07	1028.5	135.2

RESULTS

Background, Initial Demonstration of Precision and Accuracy

The background measurement from using a 500 mL Lab Reagent Blank (LRB) was 0.0002 µg/L for MC-LR and 0.00015 µg/L for nodularin. Compared to ECWA's MRL of 0.02 µg/L for MC-LR and 0.005 µg/L for Nodularin, the background is <1/10 of these levels, which are well below the method requirement of <1/3 of the MRL.

Initial demonstration of accuracy and precision was performed using 4 laboratory fortified blanks (LFB). Each LFB was spiked with MC-LR and Nodularin at 0.3 µg/L, and the Surrogate at 0.16 µg/L. The acceptance criteria are ±30% from the true value for the mean recovery and <30% for RSD. As seen below, the SPE-03 achieved excellent recoveries with low variance.

Table 4 - Precision and Accuracy Results of Microcystin-LR and Nodularin

Analytes	Mean Recovery	Acceptance	RSD	Acceptance
Microcystin-LR (MC-LR)	93.3%	70 - 130%	8.3%	<30%
Nodularin	98.1%	70 - 130%	8.4%	<30%

Minimal Reporting Limit

The MRL verification was performed by running 7 x 500mL spikes at the proposed MRL concentration of 0.02 µg/L for MC-LR and 0.005 µg/L for Nodularin. Surrogates were spiked at 0.16 µg/L. The acceptance criteria based on the Prediction Interval of Results (PIR) is Upper PIR ≤ 150% and Lower PIR ≥ 50%. Even at such trace levels, consistent recoveries were demonstrated for both compounds and satisfied the method requirements.

Table 5- MRL of Microcystin-LR and Nodularin

	MRL-1	MRL-2	MRL-3	MRL-4	MRL-5	MRL-6	MRL-7	Upper PIR	Lower PIR
MC-LR	70%	71%	78%	82%	84%	72%	77%	98.3%	54.2%
Nodularin	108%	106%	122%	108%	108%	102%	112%	134%	84.5%

CONCLUSIONS

PromoChrom's SPE-03 system offers a quick and effective solution for the extraction of Microcystins and Nodularin from water. Aside from achieving clean background and excellent recoveries, it provides high efficiency by simultaneously running up to 8 samples per system. ECWA diligently serves its community by performing a wide range of pollutant monitoring including PFAS and 1,4-Dioxane extracted on the same SPE-03 system.

RECOMMENDED SOLUTION

Part No.	Description	Notes
SPE-03	8-Channel SPE-03 System	Automated SPE System https://www.promochrom.com/spe-03
MOD-00P	Volume-Matrix Plus configuration	For automatic rinsing of up to 1L sample containers and handling of samples with particulates https://www.promochrom.com/spe-03
F-HC-30	High-Capacity Inline Filter	To enable the extraction of samples with particulates https://www.promochrom.com/inline-filters
F-T-M	Anti-clogging Tip	To handle large sample sediments https://www.promochrom.com/anti-clogging-tips

References

1. EPA Method 544
https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=306953

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