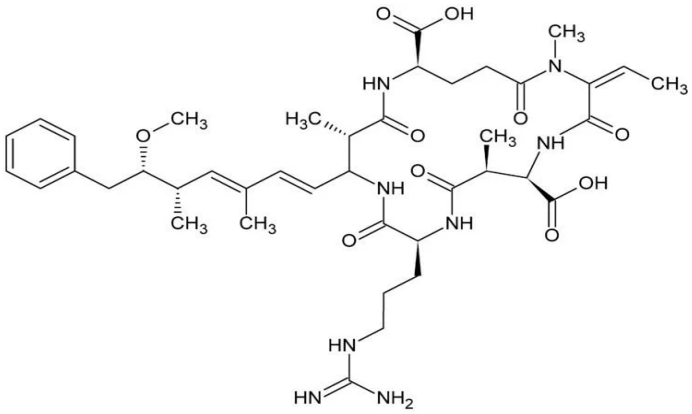
Determination of Microcystins and Nodularin in Drinking Water by SPE and LC-MS/MS Detection



UCT Part Numbers

ECHLD(150)6-P
ENVIRO-CLEAN® HL DVB
150 mg, 6 mL cartridge

VMFSTFR12
Large volume sample
transfer tubes

VMF016GL
16 position glass
block manifold

VMF02125
12 position large
volume collection rack

SLC-8100ID21-3UM
Selectra® C8 HPLC column
(100 × 2.1 mm, 3 µm)

SLC-8GDC21-3UM
Selectra® C8 guard cartridge
(10 × 2.1 mm, 3 µm)

Summary:

Microcystins are a group of hepatotoxins produced by freshwater cyanobacteria (also known as blue-green algae). Microcystins can be generated in large quantities during algae blooms and can pose a major risk to surface, ground and drinking water [1]. Approximately 100 different microcystins have been discovered so far, of which Microcystin-LR is the most common and one of the most potent congeners [2]. The Environmental Protection Agency (EPA) recently proposed the fourth Unregulated Contaminant Monitoring Rule (UCMR 4) which outlines the monitoring of 30 chemical contaminants, including microcystins. EPA Method 544 is used to identify a particular mycosystin congener when ELISA screening results for total microcystins is found to exceed the reporting limit (≥ 0.3 µg/L). EPA Method 544 uses solid-phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) for the analysis of six microcystins (LA, LF, LR, LY, RR, YR) and the related toxin, nodularin.

This application note outlines a SPE method for the determination of 6 microcystins and nodularin in drinking water according to EPA Method 544.

A 500 mL water sample is passed through a 6 mL SPE cartridge containing 150mg of divinylbenzene (DVB) based sorbent to extract the analytes. Microcystins and nodularin are retained on the sorbent and later eluted with a small quantity of organic solvent. The extract is then concentrated to dryness, reconstituted in 1 mL of methanol containing 10% water, and analyzed by LC-MS/MS equipped with a C8 HPLC column.

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Sample Pretreatment:

Preservation reagents, including 3.875g Trizma® pH 7 preset crystals (buffering reagent), 1g 2-chloroacetamide (antimicrobial), 50mg L-ascorbic acid (dechlorinating agent), and 0.175g EDTA trisodium salt (inhibits binding to metals), are added to each sample bottle (500 mL) prior to shipment to the field or prior to sample collection. Prior to SPE extraction, the water sample (500 mL) is fortified with surrogate standard and filtered. Both the filtrate and the filter are collected. The filter is placed in a solution of methanol containing 20% reagent water and held for at least one hour at -20°C to release the intracellular toxins from cyanobacteria cells captured on the filter. The liquid is drawn off the filter and added back to the 500-mL aqueous filtrate. For complete details of the sample pretreatment, see Section 11.3 in EPA Method 544 [3].

SPE Procedure:

1. SPE Conditioning

a) Attach a large volume sample transfer tube **(VMFSTFR12)** to the top of each SPE cartridge **(ECHLD(150)6-P)** and position the cartridges onto the 16-position glass block manifold **(VMF016GL)**.
b) Insert the stainless steel end of the transfer tubes into a beaker of methanol (15 mL per sample) and slowly draw the methanol through the SPE sorbent, leaving a thin layer of methanol above the SPE frit.
c) Repeat the above step with DI water (15 mL per sample), leaving a water layer of about 1" above the frit.

2. Sample Extraction

a) Insert the stainless steel end of the transfer tubes directly into the sample bottles. Adjust the vacuum so that the flow rate is approximate 10-15 mL/min.

3. Wash Cartridge

a) Rinse the sample bottles with 10 mL DI water and draw the rinse through the transfer tubes and SPE cartridges.
b) Remove the large volume transfer tubes and rinse the SPE cartridges with another 5 mL DI water.
c) Dry the SPE cartridges under full vacuum (10-15 in. Hg) for 10 min.

4. Elution

a) Insert the 12 position large volume collection rack (VMF02125) containing 40-60 mL VOA vials into the SPE manifold. Attach the large volume sample transfer tubes back onto the SPE cartridges.
b) Rinse the sample bottles with 5 mL of methanol containing 10% water and elute the analytes from the cartridges by slowly pulling the 5 mL methanol solution through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion. After the solvent has passed through, apply full vacuum for 30 seconds so that all the elution solvent is collected.
c) Repeat the above step with an additional 5 mL of methanol containing 10% water.

5. Concentration

a) Remove the collection tubes from the manifold and concentrate the extracts to dryness under a gentle stream of nitrogen in a heated water bath (60°C).
b) Add 1 mL of methanol containing 10% water and vortex. Transfer the extract to an autosampler vial for LC-MS/MS analysis.

LC-MS/MS Parameters:

HPLC Parameters		
HPLC System: Thermo Scientific™ Dionex™ Ultimate™ 3000		
HPLC Column: UCT Selectra® C8, 100 x 2.1 mm, 3 µm		
Guard cartridge: UCT Selectra® C8, 10 x 2.1 mm, 3 µm		
Column temperature: 40 °C		
Flow rate: 0.3 mL/min		
Autosampler temperature: 10 °C		
Injection volume: 10 µL		
Gradient Program		
Time (min)	**A% (20 mM Ammonium Formate)**	**B% (MeOH)**
0.0	90	10
1.0	90	10
1.5	40	60
2.5	40	60
6.5	10	90
8.0	10	90
8.1	90	10
13.0	90	10

MS Parameters	
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem MS
Polarity	ESI +
Spray Voltage	4000 V
Vaporizer temperature	350 °C
Ion transfer capillary temperature	270 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	10 arbitrary units
Q1 and Q3 peak width (FWHM)	0.7 Da
Collision gas and pressure	Argon at 2 mTorr
Cycle time	1 sec
Acquisition method	EZ Method (scheduled SRM)

Retention Times and SRM Transitions							
Compound	**RT (min)**	**Precursor**	**Product 1**	**CE 1**	**Product 2**	**CE 2**	**S-Lens RF**
MC-YR	5.86	523.2	135.0	13	102.9	44	104
NOD	5.93	825.4	134.7	52	163.2	46	218
MC-RR	6.05	519.7	134.9	28	102.7	28	139
MC-LR	6.07	995.7	134.8	57	212.7	55	225
MC-LY	6.51	1002.4	134.6	42	212.9	43	184
MC-LA	6.53	910.4	776.2	17	162.7	36	200
MC-LF	7.36	986.4	134.7	43	212.8	42	194
C2D5-MC-LR	7.41	1028.6	134.6	60	246.1	57	266

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Results:

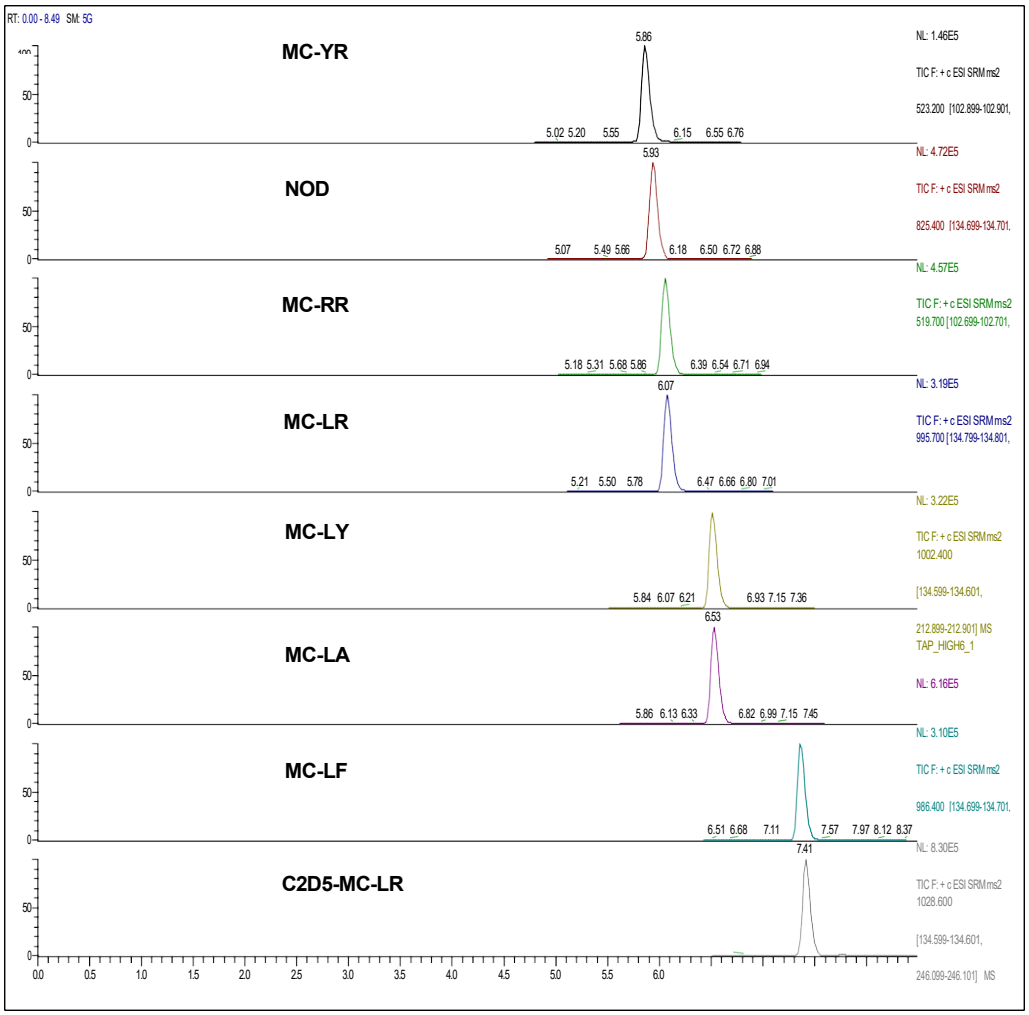

Figure 1: Chromatogram of a tap water sample fortified with the microcystins at 200 – 1000 ng/L.

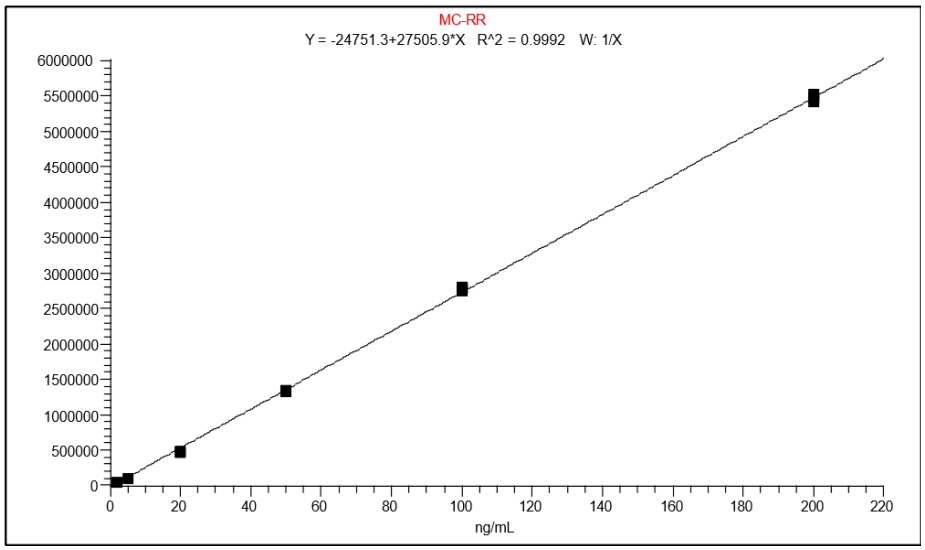

Figure 2: MC-RR Calibration Curve

Accuracy and Precision in Fortified Reagent Water

Analyte	Fortified Conc. (ng/L)	Mean Recovery (%)	RSD (%) (n = 4)	Fortified Conc. (ng/L)	Mean Recovery (%)	RSD (%) (n = 4)
MC-YR	100	**95.7**	5.2	1000	**100.1**	6.9
NOD	20	**95.3**	4.4	200	**104.0**	8.3
MC-RR	20	**98.8**	5.3	200	**106.0**	11.8
MC-LR	40	**96.6**	2.5	400	**108.5**	5.7
MC-LY	40	**91.6**	2.9	400	**104.3**	1.8
MC-LA	100	**91.5**	4.4	1000	**106.3**	1.9
MC-LF	40	**90.9**	3.7	400	**102.8**	1.8
C2D5-MC-LR	250	**93.9**	2.7	250	**98.8**	6.3

Accuracy and Precision in Fortified Tap Water*

Analyte	Fortified Conc. (ng/L)	Mean Recovery (%)	RSD (%) (n = 4)	Fortified Conc. (ng/L)	Mean Recovery (%)	RSD (%) (n = 4)
MC-YR	100	**101.3**	3.7	1000	**96.2**	3.7
NOD	20	**105.6**	1.5	200	**99.6**	2.4
MC-RR	20	**106.0**	2.1	200	**99.1**	2.2
MC-LR	40	**103.5**	2.7	400	**99.7**	2.4
MC-LY	40	**103.2**	2.4	400	**98.7**	2.6
MC-LA	100	**102.7**	3.0	1000	**99.5**	2.8
MC-LF	40	**101.9**	3.1	400	**98.1**	4.4
C2D5-MC-LR	250	**97.9**	5.7	250	**92.3**	3.5

** Results were quantified against matrix-matched calibration standards to compensate for any matrix effects.*

Conclusion:

This application note outlines a simple and efficient SPE method for the determination of microcystins and nodularin in drinking water according to EPA Method 544. Excellent recoveries (ranging from 90.9 to 108.5%) and RSD values (≤ 11.8%) were achieved in both reagent and tap water using UCT's Enviro-Clean® HL DVB SPE cartridge. LC-MS/MS analysis was conducted in 13 min (including re-equilibration) using a Selectra® C8 HPLC column, which is half the run time outlined in EPA Method 544.

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References:

[1] https://iaspub.epa.gov/tdb/pages/contaminant/contaminantOverview.do?contaminantId=-1336577584

[2] https://www.epa.gov/sites/production/files/2015-06/documents/microcystins-report-2015.pdf

[3] J. A. Shoemaker, D. R. Tettenhorst, and A. de la Cruz. Method 544. Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. Environmental Protection Agency, Washington, DC, 2015

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