Quantitation of Microcystins and Nodularin in Drinking Water Samples to Meet EPA Method 544 Requirements Using QSight LC/MS/MS

NEMC 2022 – Drinking Water Mollie Cyr PerkinElmer, Inc., Shelton, CT USA August 1st, 2022





Method Background

- •Cyanobacteria, aka blue-green algae, can produce high levels of cyanotoxins called microcystins and nodularin
- water
- 0.3ug/L for young children and infants 1.6ug/L for school aged children and adults •WHO recommends keeping microcystin levels below 1ug/L
- EPA Method 544 analyzes 6 microcystins and nodularin-R in 500 mL drinking water samples using SPE-LC/MS/MS analysis

The US EPA issued a health advisory for maximum levels of microcystins in drinking







Optimize LC and MS conditions for 6 microcystins and nodularin-R on PerkinElmer QSight LX50 UHPLC and QSight 210 triple quadrupole mass spectrometer



Analytes

Cyanotoxin	Acronyn
Microcystin-LA	MC-LA
Microcystin-LF	MC-LF
Microcystin-LR	MC-LR
Microcystin-LY	MC-LY
Microcystin-RR	MC-RR
Microcystin-YR	MC-YR
Nodularin-R	NOD
Ethylated Microcystin-LR (IS)	C ₂ D ₅ -MC-







EPA Method 544 LC Method

LC Conditions	EPA Method 544			
Column:	Phenomenex Kinetex C8, 2.6 µm, 2.1 x 100 mm			
Mobile Phase:	Mobile Phase A: 20 mM Ammonium Formate Mobile Phase B: Methanol			
	Time (min)	Flow (mL/min)	%A	%B
	Initial	0.3	90	10
	2.0	0.3	90	10
I C Gradient	16.0	0.3	20	80
	16.1	0.3	10	90
	22.0	0.3	10	90
	22.1	0.3	90	10
	26.0	0.3	90	10
Injection Volume:	10 µL			



QSight LX50 UHPLC Method

LC Conditions	PKI QSight LX50 UHPLC				
Column:	PerkinElmer Brownlee SPP C18, 2.7 µm, 2.1 x 100 mm				
Mobile Phase:	Mobile Phase A: 0.1% FA in water Mobile Phase B: 0.1% FA in acetonitrile				
	Time (min)	Flow (mL/min)	%A	%B	Curve
	Initial	0.6	95	5	
	4.0	0.6	95	5	Linear
LC Gradient:	9.0	0.6	5	95	Linear
	10.0	0.6	5	95	Linear
	10.1	0.6	95	5	Linear
	13.0	0.6	95	5	Linear
Column Oven Temp.:	50 °C				
Injection Volume:	10 µL				
Autosampler Temp.:	10 °C				





QSight 210 MS/MS Method

		An
•	Source parameters:	MC
		MC
	 Ionization mode: ESI - Positive 	MC
	 Drying gas: 120 	MC
	 HSID temperature: 250°C 	MC
	 Nebulizer gas: 350 	MC
	 Electrospray Voltage: 5100 V 	MC
	 Source temperature: 315°C 	MC
		N
		N
		C2D5-

alyte	Precursor Ion	Product Ion	CE	EV	CCL2	Qua /Qu
C-LR	498.0 [M+2H] ²⁺	135.0	-24	30	-65	Q
C-LR	498.0 [M+2H] ²⁺	482.3	-12	15	-80	C
C-LA	910.4 [M+H]+	135.0	-88	55	-180	Q
C-LA	910.4 [M+H]+	106.9	-114	30	-185	C
C-YR	523.4 [M+2H] ²⁺	135.0	-22	30	-85	Q
C-YR	523.4 [M+2H] ²⁺	91.1	-116	30	-145	C
C-LF	493.9 [M+2H] ²⁺	135.0	-50	15	-90	Q
C-LF	493.9 [M+2H] ²⁺	102.9	-94	30	-100	C
C-RR	519.9 [M+2H] ²⁺	135.0	-38	5	-130	Q
C-RR	519.9 [M+2H] ²⁺	103.0	-90	15	-140	C
C-LY	1002.4 [M+H]+	135.0	-82	20	-175	Q
C-LY	1002.4 [M+H]+	374.9	-46	20	-185	C
OD	825.4 [M+H]+	135.0	-74	10	-205	Q
OD	825.4 [M+H]+	103.0	-138	15	-195	C
MC-RR	515.0 [M+2H] ²⁺	103.1	-95	12	-110	





Calibration Curve

Analyte	Calibration Range - EPA Method 544 (ug/L)	Calibration QSight
MC-LF	10 - 400	3.12
MC-LR	10 - 400	3.12
MC-RR	4.7 - 187.5	3.12
MC-YR	10 - 400	3.12
NOD	4.9 - 195.7	3.12
MC-LA	25 - 1000	6.25
MC-LY	10 - 400	6.25

7-point calibration curve analyzed on QSight

on Range ht (ug/L)

- 25 200
- 25 200
- 25 200
- 25 200
- 25 200
- 5 400
- 5 400











Extracted Ion Chromatogram



100 ug/L sample



Sample Preparation Procedure



10 Footer text





QSight StayCleanTM HSID Technology

- Self-cleaning hot surface induced desolvation (HSID)
- lons and solvated charged species are entrained in a hot laminar flow of gas to be transported to the vacuum region
- Laminar flow gas shields ions and solvated species from striking the HSID walls, acting as a constant cleaning agent
- Hot laminar flow facilitates desolvation, increases ion transmission efficiency and reduces contamination
- Reduces background chemical noise
- Beneficial for drinking water applications, where samples can be dirty after SPE and reconstitution







Minimum Reporting Level

- The minimum reporting level (MRL) is the minimum concentration that can be reported as a quantitated value for a method analyte in a sample after analysis
 - Fortify, extract, and analyze 7 laboratory fortified blanks (LFBs) at proposed MRL concentration

Analyte
MC-RR
Nodularin
MC-YR
MC-LR
MC-LA
MC-LY
MC-LF

MRL on QSight LC/MS/MS (ng/L)
24
20
16
16
80
80
60



Minimum Reporting Level Calculations

- Calculate mean and standard deviation of the 7 replicates Determine half range for prediction interval of results (HR_{PIR}) using equation: 0
 - - $HR_{PIR} = 3.963s$
 - where s = standard deviation and 3.963 = a constant value
- Confirm the upper and lower limits for the Prediction Interval of Result (PIR = Mean +/- HR_{PIR}) meet the upper recovery limit (<150%) and the lower limit recovery (>50%)



Minimum Reporting Level Data

Analyte	Fortified Concentration (ng/L)	Mean Result	STDDEV	HR _{PIR}	Upper PIR	Lower PIR
MC-RR	24	20	2	8	115	52
Nodularin	20	17	1	4	109	64
MC-YR	16	15	2	7	137	52
MC-LR	16	14	1	3	109	67
MC-LA	80	88	7	26	143	77
MC-LY	80	83	9	36	148	60
MC-LF	60	64	2	9	122	92

Upper PIR must be <150% Lower PIR must be >50%



Accuracy and Precision

- Initial demonstration of accuracy and precision
 - Fortify, extract, and analyze 4 LFBs 0

Analyte	Fortified Concentration (ng/L)
MC-RR	300
Nodularin	300
MC-YR	300
MC-LR	300
MC-LA	300
MC-LY	300
MC-LF	300

- 0
- 0 of the true value

For demonstration of precision, the %RSD of replicate analyses must be less than 30% For demonstration of accuracy, the average recovery of replicate values must be +/-30%





Demonstration of Accuracy





Accuracy of QSight LC/MS method



Demonstration of Precision



%RSD between replicates



EPA Method 544 QC Requirements

- Ongoing QC requirements when analyzing field samples
 - Laboratory reagent blank every 20 samples 0
 - 0 field samples, and at the end of the batch
 - 0 batch to batch
 - Surrogate recovery calculation to assess method performance 0
 - Quality control samples 0



Continuing calibration checks are analyzed at the beginning of each batch, after every 10

Laboratory fortified blank, rotated between low, medium, and high concentrations from



Field Sample Batch Analysis

500 mL amber glass bottles

- QC samples:
 - Laboratory fortified blank 0
 - Continuing calibration check samples 0
 - Laboratory reagent blank 0

Drinking water samples from Meriden, CT and Shelton, CT were collected in



Laboratory Fortified Blank

- Fortified 2 times above MRL
 - Result must be within +/-50% of the fortified concentration

	LFB, fortified Concentration	
Analyte	(ng/L)	LFB Results (ng/l
MC-RR	48	31
NOD	40	32
MC-YR	32	24
MC-LR	32	31
MC-LA	160	135
MC-LY	160	196
MC-LF	120	118



Low Level Continuing Calibration Check

- Low level CCC fortified at MRL
 - Result must be within +/- 50% of fortified concentration

	CCC Low Fortified	CCC results
Analyte	Concentration (ng/L)	(ng/L)
MC-RR	24	19
NOD	20	25
MC-YR	16	11
MC-LR	16	10
MC-LA	80	69
MC-LY	80	102
MC-LF	60	49

Mid Level Continuing Calibration Check

- Mid level CCC
 - Must be within +/- 30% of fortified concentration

Analyte	CCC Mid Fortified Concentration (ng/L)	CCC results (ng/L)
MC-RR	80	58
NOD	80	83
MC-YR	80	79
MC-LR	80	89
MC-LA	160	155
MC-LY	160	142
MC-LF	80	86

Unknown Drinking Water Sample Results

500 mL samples were collected, extracted, and analyzed •

Analyte	Meriden, CT Drinking Water Result (ng/L)	Shelton, CT Drinking Water Result (ng/L)
MC-RR	<mrl< td=""><td><mrl< td=""></mrl<></td></mrl<>	<mrl< td=""></mrl<>
NOD	<mrl< td=""><td><mrl< td=""></mrl<></td></mrl<>	<mrl< td=""></mrl<>
MC-YR	<mrl< td=""><td><mrl< td=""></mrl<></td></mrl<>	<mrl< td=""></mrl<>
MC-LR	<mrl< td=""><td><mrl< td=""></mrl<></td></mrl<>	<mrl< td=""></mrl<>
MC-LA	<mrl< td=""><td><mrl< td=""></mrl<></td></mrl<>	<mrl< td=""></mrl<>
MC-LY	<mrl< td=""><td><mrl< td=""></mrl<></td></mrl<>	<mrl< td=""></mrl<>
MC-LF	<mrl< td=""><td><mrl< td=""></mrl<></td></mrl<>	<mrl< td=""></mrl<>

Summary

- mass spectrometer is fit for purpose for EPA Method 544
- Reduce runtime by 50%, from 26 minutes to 13 minutes
- method suitability
- For more information, check out our application note

Demonstrate PerkinElmer's Qsight LX50 UHPLC and QSight 210 triple quadrupole

Minimum reporting levels were established to be 16-80 ng/L for each cyanotoxin

Field drinking water samples were analyzed alongside QC samples to demonstrate

Thank you for your time

Mollie Cyr Application Scientist

Avinash Dalmia Sr. Application Scientist Feng Qin Product Manager

