

Sensitive and Selective Quantification of Microcystin Toxins in Drinking Water By UHPLC-MS/MS

macmod

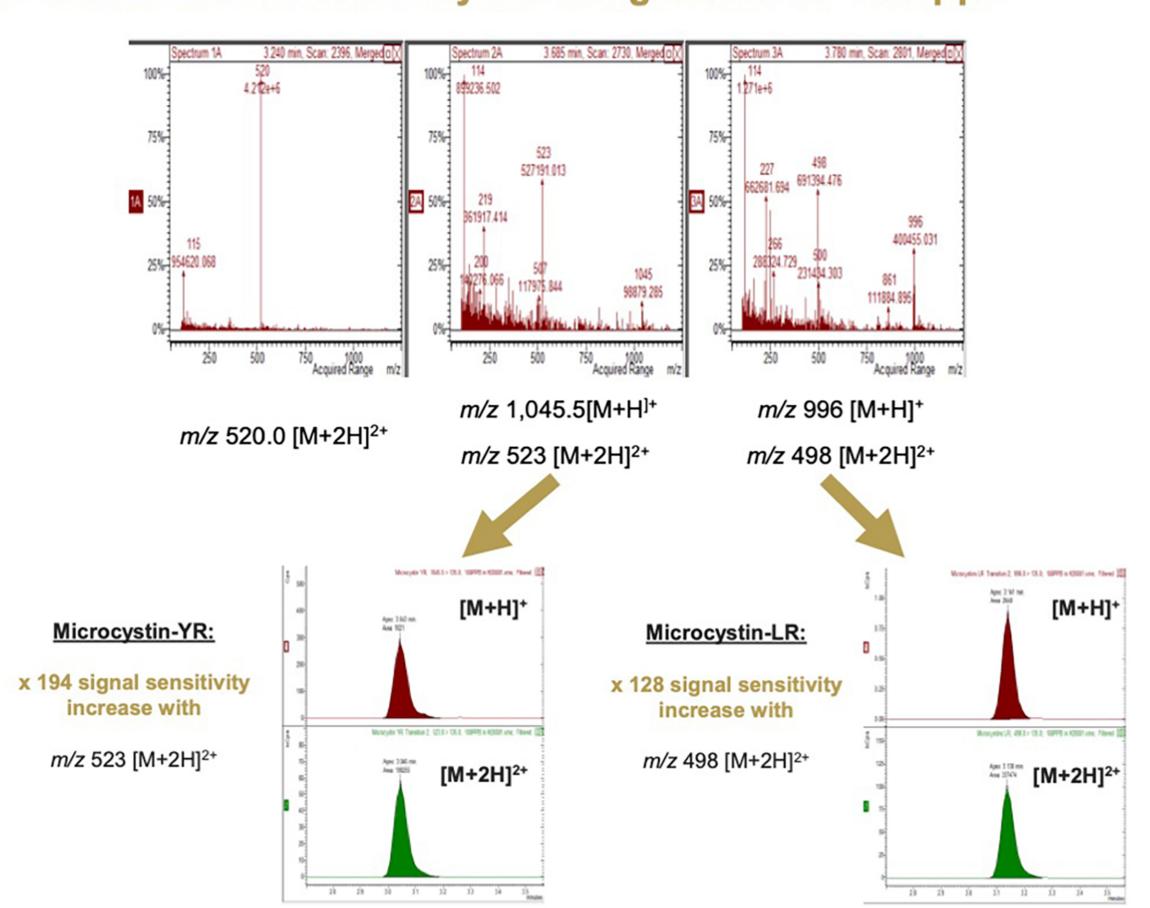
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¹MAC-MOD Analytical

1. Introduction

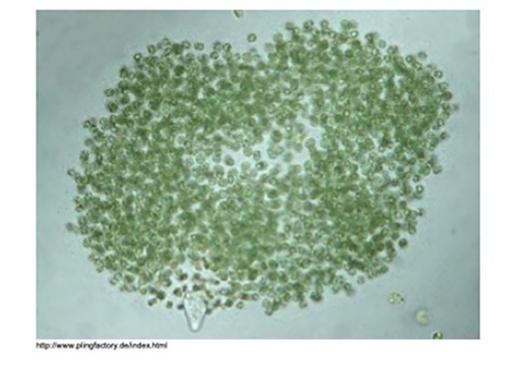
- Cyanobacteria / blue-green algae are common. Microcystins are cyclic heptapeptides produced by cyanobacteria and associated with human hepatotoxicity.
- Under nutrient-rich conditions, cyanobacteria can rapidly accumulate and form blooms.
- Due to their hepatotoxic nature, the WHO has established a provisional limit of 1mg/L in water supplies for total Microcystin-LR as a marker for cyanobacteria toxin levels.
- This poster describes a method for low ppb quantification of Microcystin-LR, Microcystin-RR and Microcystin-YR by UHPLC-MS/MS using an ACE Excel 2mm C18 UHPLC column.

4. MS Scans and Sensitivity Investigations at 100 ppb



2. Algae Blooms and Microcystins





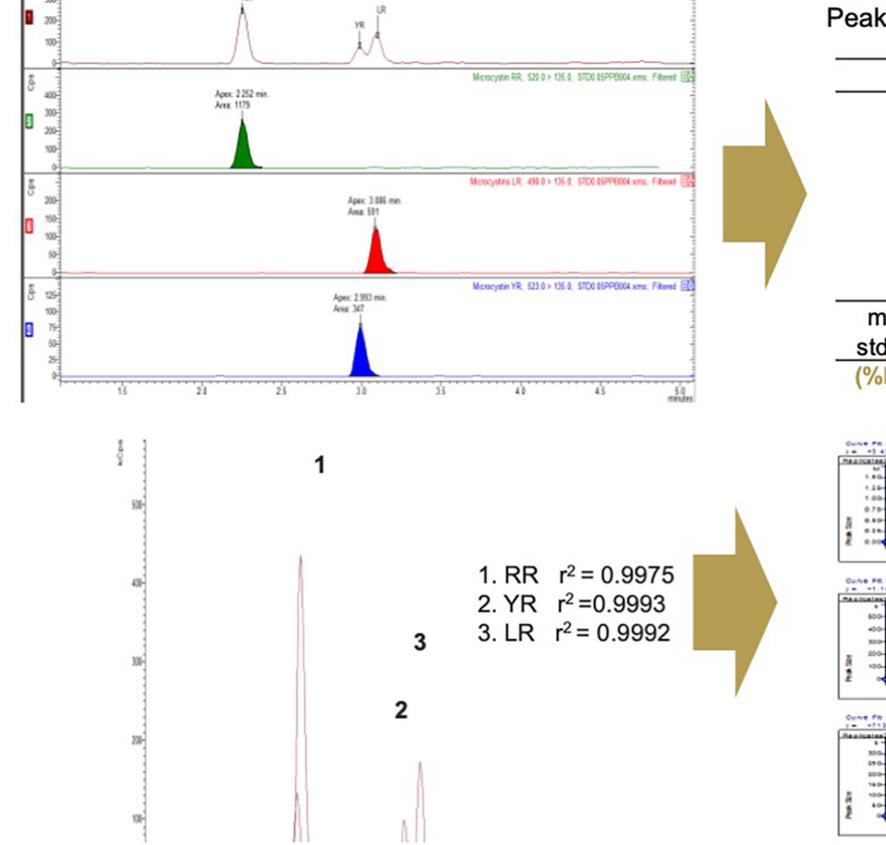
	(ADDA)	HN COOH CH ₃ O NH CH ₂ H ₃ C O
fragment m/z=135	CH ₃	CH ₃ NH ₂ O COOH O (L)

Variants	R	L
Microcystin-LR	Leuicine	Arginine
Microcystin-RR	Arginine	Arginine
Microcystin-YR	Tyrosine	Arginine

Over 80 known microcystin variants have been reported

Int. J. Environ. Res. Public Health 2012, 9

5. Reproducibility and Calibration Data



Peak area reproducibility at 0.05 ppb (n=7)

MCRR MCYR MCLR

1	1246	300	478
2	1239	364	584
3	1396	340	620
4	1157	345	585
5	1166	339	527
6	1189	318	585
7	1147	384	487
mean	1220.00	341.43	552.29
std dev.	86.78	27.71	55.00
(%RSD)	7.11	8.12	9.96
Curve FR Linear, Ignore, h y= +3.424)18e-4s -1.884 Magicales111 1 M 1.404 1.26- 1.004		ne ne	eff Del (42) 0.997619
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Day of the series of the serie	10 To Amount ##	S LR	40 Fact Ratio 17,76%
Dune FE Linear, ignore, h	10 10 Market ## Micro cystin 10 10 10 10 10 10 10 10 10 10 10 10 10 1	50 Se	40 Fact Ratio 17,76%
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3. UHPLC and MS Conditions

Bruker Advance™ UHPLC

Column: ACE Excel 2mm C18, 100 x 2.1 mm Part Number: EXL-101-1002U

Injection volume: 50 µL
Flow rate: 0.4 mL/min
Column temperature: 40 °C

Mobile phase A: 0.1% formic acid (aq)

Mobile phase B: MeC

Gradient conditions:

Time (mins)	%E				
0	30				
1.0	30				
7.0	95				
7.1	30				
10.0	30				

Bruker EVOQ™

VIP Heated-ESI Temp: 350 °C
Heated gas: 50 units
Nebulizer gas: 50 units
Cone gas temp: 200 °C
Cone gas: 15 units

Spray voltage: 4500 V (Positive)

Active exhaust: On

Collision gas: Argon 1.5 mTorr

Optimized MRM transitions:

MC RR (MW: 1038) transition: $520 \rightarrow 135$ (CE at 24V) MC LR (MW: 995) transition: $498 \rightarrow 135$ (CE at 11V) MC YR (MW: 1045) transition: $523 \rightarrow 135$ (CE at 9V)



5. Conclusions

- A new method was established using an ACE Excel 2μm C18 UHPLC column and Bruker Advance / EVOQ UHPLC-MS/MS instrumentation.
- The method was found to be reproducible and sensitive at very low levels (0.05 ppb) for microcystins in water.
- Rapid separation and elution of Microcystin-LR, Microcystin-RR and Microcystin-RR was achieved with an excellent linear range of 0.05 ppb – 50 ppb.
- MS optimization work indicated significantly enhanced sensitivity was possible using doubly charged ions.

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