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

Alan P McKeown[1], Geoffrey Faden[2],

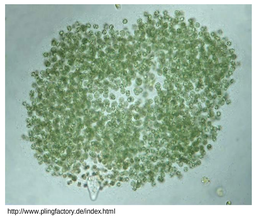
[1]Advanced Chromatography Technologies Ltd, 1 Berry Street, Aberdeen, Scotland, AB25 1HF UK [2]MACMOD Analytical Inc., 103 Commons Court, PO Box 587, Chadds Ford, PA 19317 USA

1. BACKGROUND

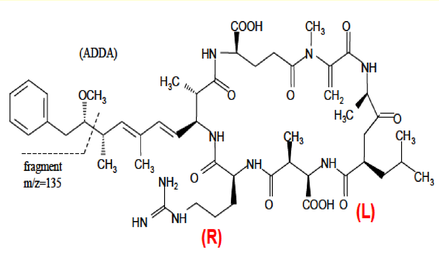
- Cyanobacteria / blue-green algae are common. Microcystins are cyclic heptapeptides produced by cyanobacteria and associated with human hepatotoxicity.
- Under nutrient-rich conditions, cyanobacteria can rapidly accumulate and form blooms.
- Due to their hepatotoxic nature, the WHO has established a provisional limit of 1µg/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 2µm C18 column.

2. ALGAE BLOOMS AND MICROCYSTINS

http://www.aquarius-systems.com/Entries/View/349/bluegreen_algae.aspx



http://www.plingfactory.de/index.html



Variants	R	L
Microcystin-LR	Leucine	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**

3. UHPLC AND MS CONDITIONS

Bruker Advance™ UHPLC

Column: ACE Excel 2µm C18, 100 x 2.1mm

Injection volume: 50µL
Flow rate: 0.4mL/min
Column temperature: 40°C
Mobile phase A: 0.1% formic acid (aq)
Mobile phase B: MeCN

Gradient conditions:

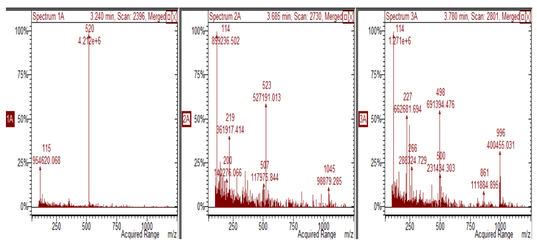
Time (mins)	%B
0	30
1.0	30
7.0	95
7.1	30
10.0	30

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: 4500V (Positive)
Active exhaust: On
Collision gas: Argon 1.5 mTorr
Optimized MRM transitions:

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

4. MS SCANS AND SENSITIVITY INVESTIGATIONS AT 100 PPB



m/z 520.0 [M+2H]$^{2+}$

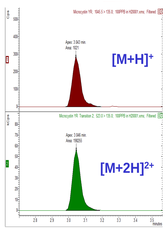
m/z 1,045.5[M+H]$^{+}$
m/z 523 [M+2H]$^{2+}$

m/z 996 [M+H]$^{+}$
m/z 498 [M+2H]$^{2+}$

Microcystin-YR:

x 194 signal sensitivity increase with

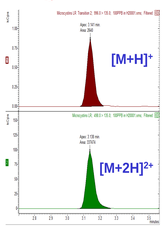
m/z 523 [M+2H]$^{2+}$



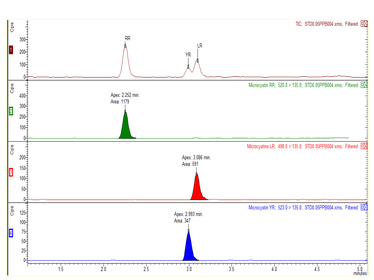
Microcystin-LR:

x 128 signal sensitivity increase with

m/z 498 [M+2H]$^{2+}$

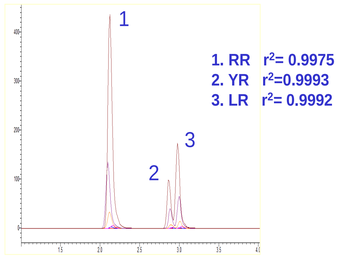


5. REPRODUCIBILITY AND CALIBRATION DATA

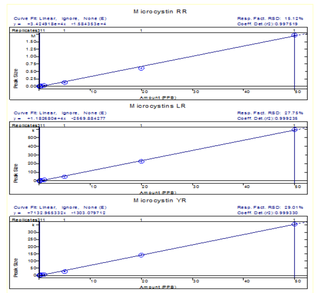


Area reproducibility at 0.05 ppb (n=7)

	MC RR	MC YR	MC LR
Area	1246	300	478
	1239	364	584
	1396	340	620
	1157	345	585
	1166	339	527
	1189	318	585
	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



1. RR r^2= 0.9975
2. YR r^2=0.9993
3. LR r^2= 0.9992



6. SUMMARY AND CONCLUSIONS

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