Sensitive and Selective Quantification of Microcystin Toxins in Drinking Water By UHPLC-MS/MS
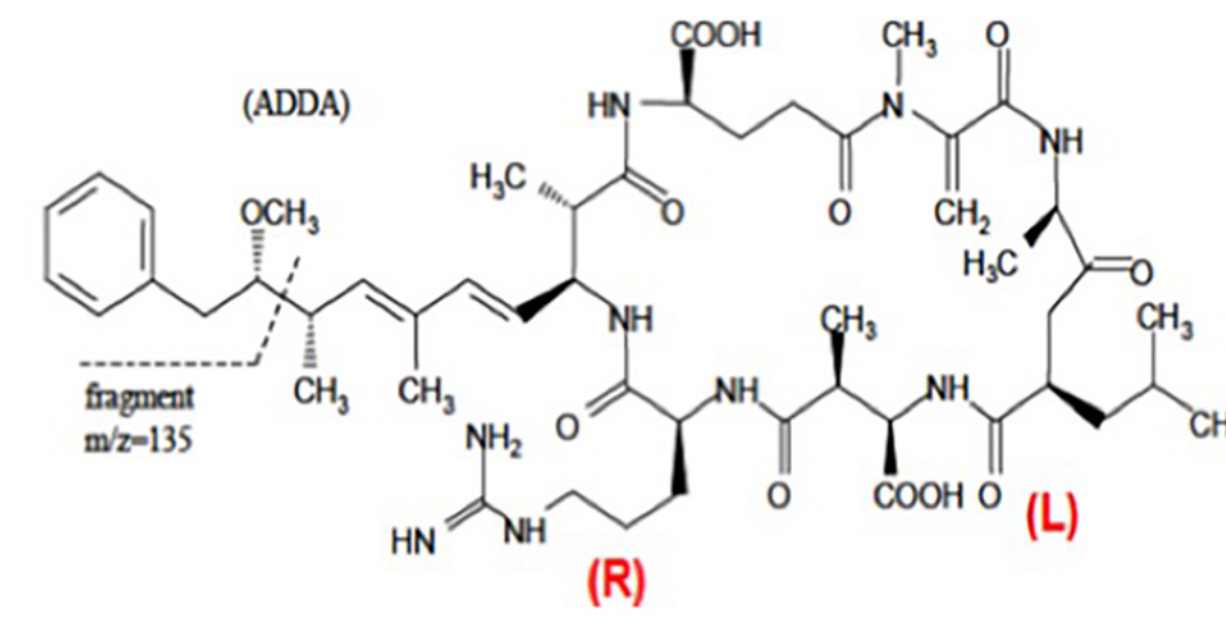
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[1]MAC-MOD Analytical

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.

2. Algae Blooms and Microcystins

http://www.aquarius-systems.com/Entries/View349/bluegreen_algae.aspx

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

(ADDA)

fragment m/z=135

(R) (L)

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

Over 80 known microcystin variants have been reported
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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: 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: 4500 V (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)
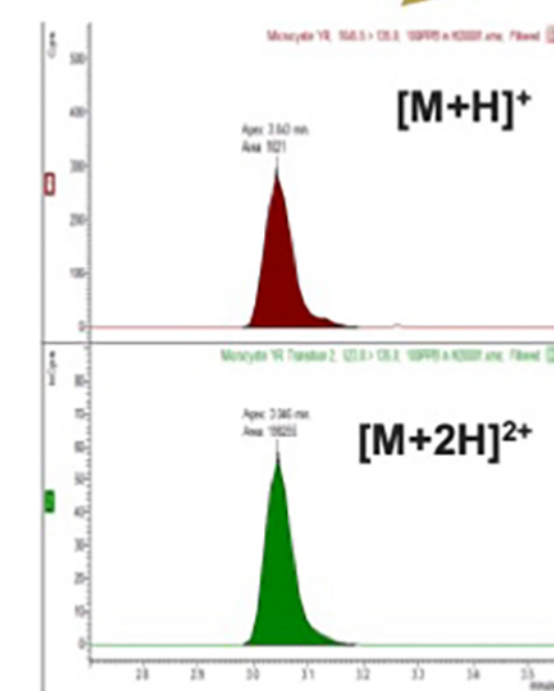MC YR (MW: 1045) transition: 523 → 135 (CE at 9V)

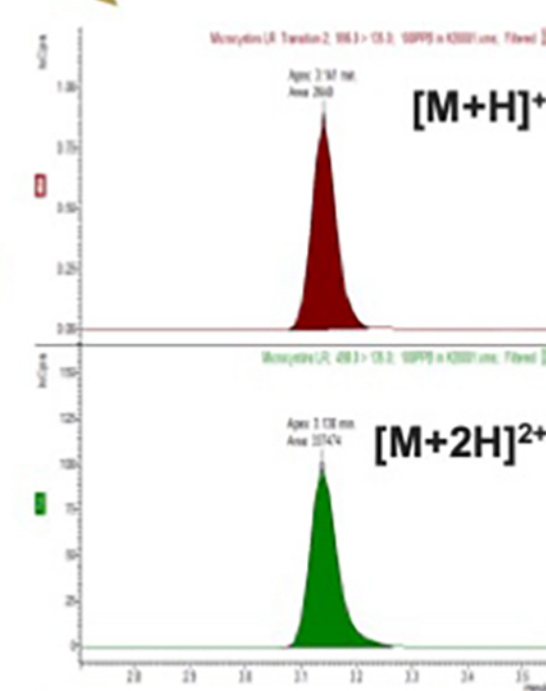## 4. MS Scans and Sensitivity Investigations at 100 ppb
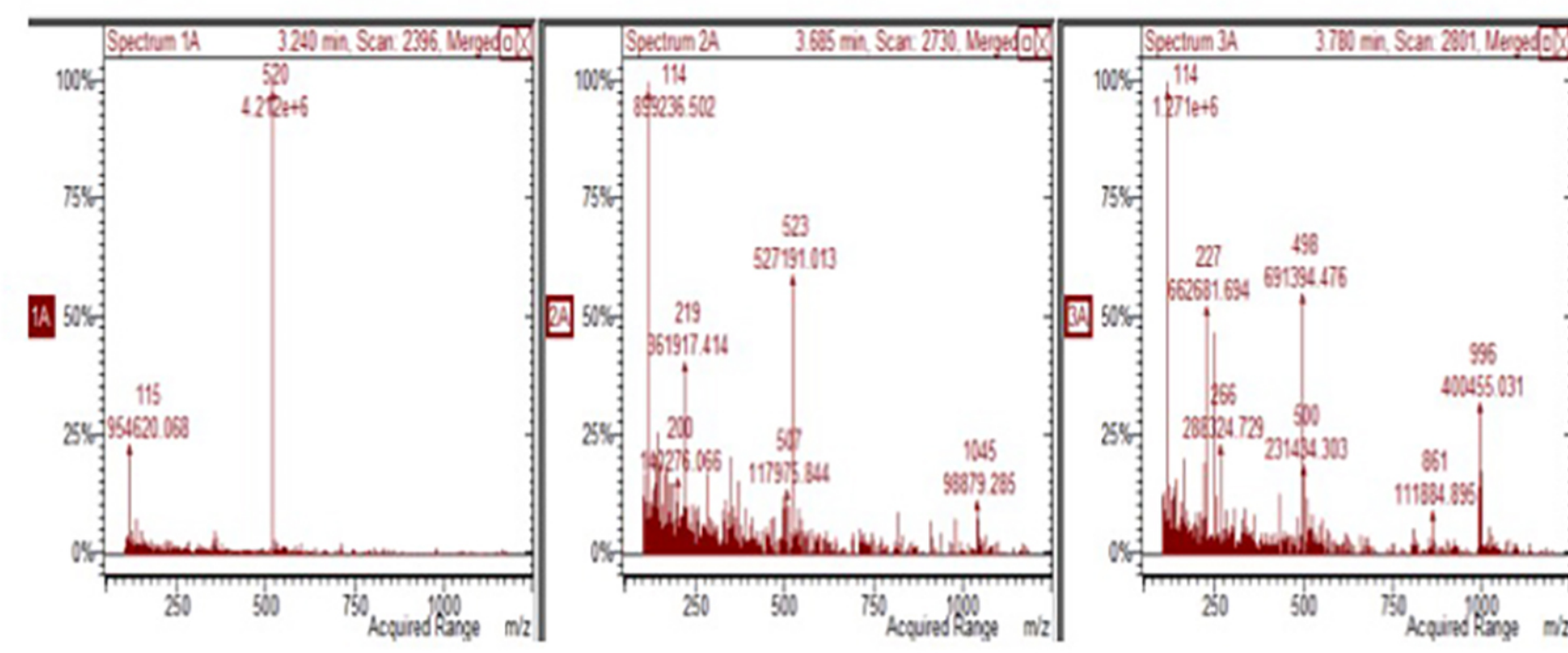
m/z 520.0 [M+2H]$^{2+}$

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]$^{2+}$

[M+H]$^+$
[M+2H]$^{2+}$

Microcystin-LR:
x 128 signal sensitivity increase with
m/z 498 [M+2H]$^{2+}$

[M+H]$^+$
[M+2H]$^{2+}$

5. Reproducibility and Calibration Data
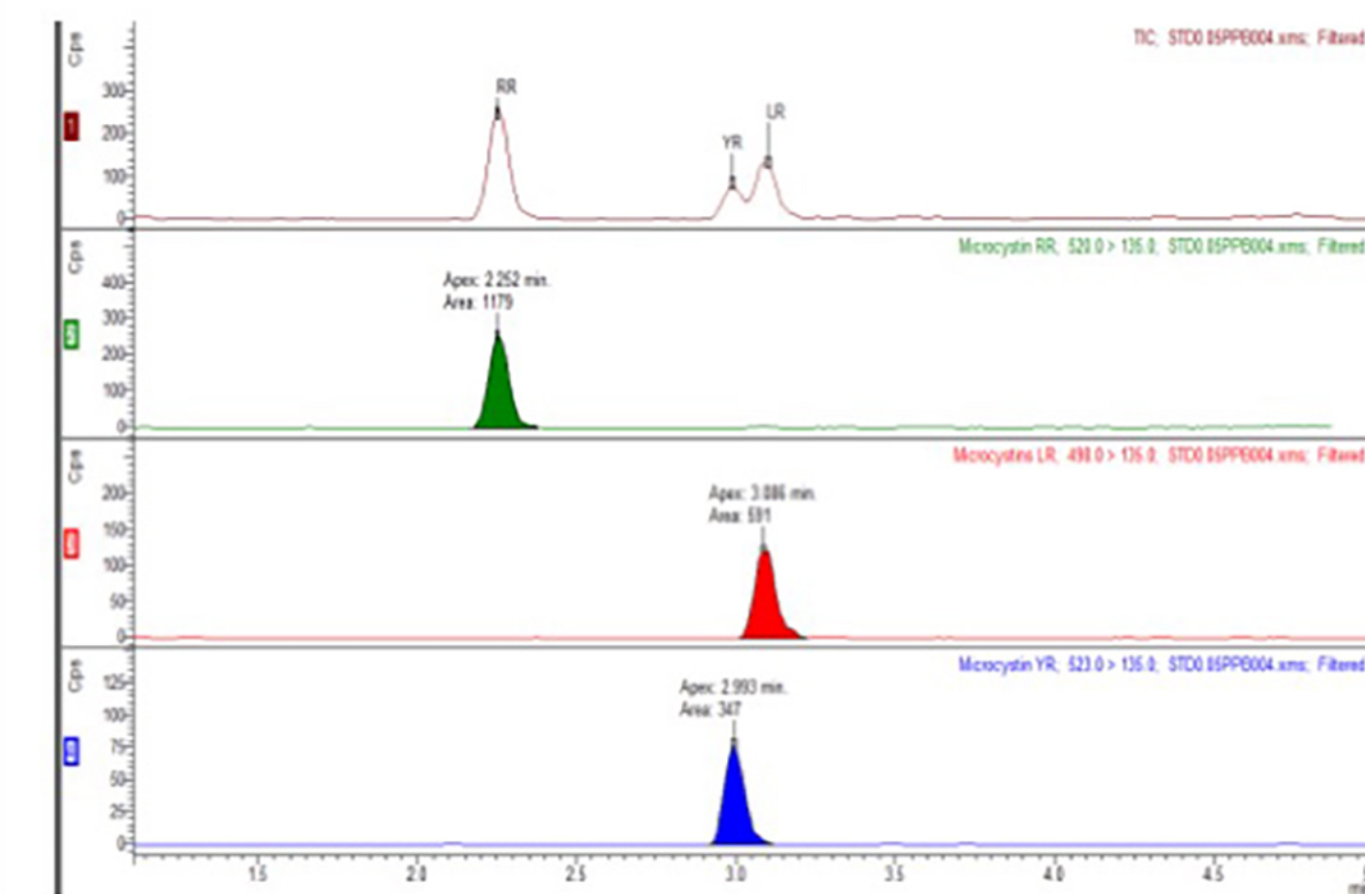
Peak area reproducibility at **0.05 ppb** (n=7)

	MC RR	MC YR	MC LR
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
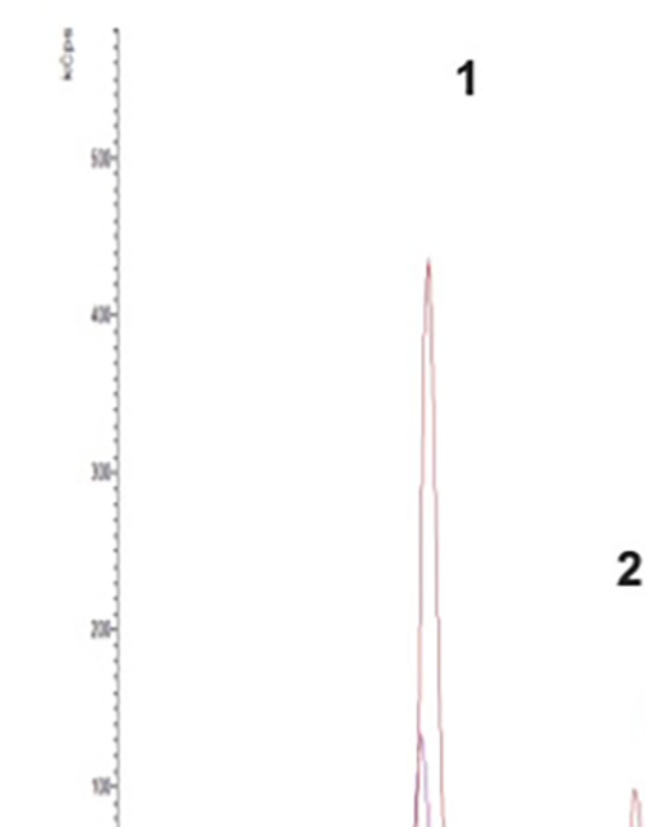
1. RR $r^2 = 0.9975$
2. YR $r^2 = 0.9993$
3. LR $r^2 = 0.9992$
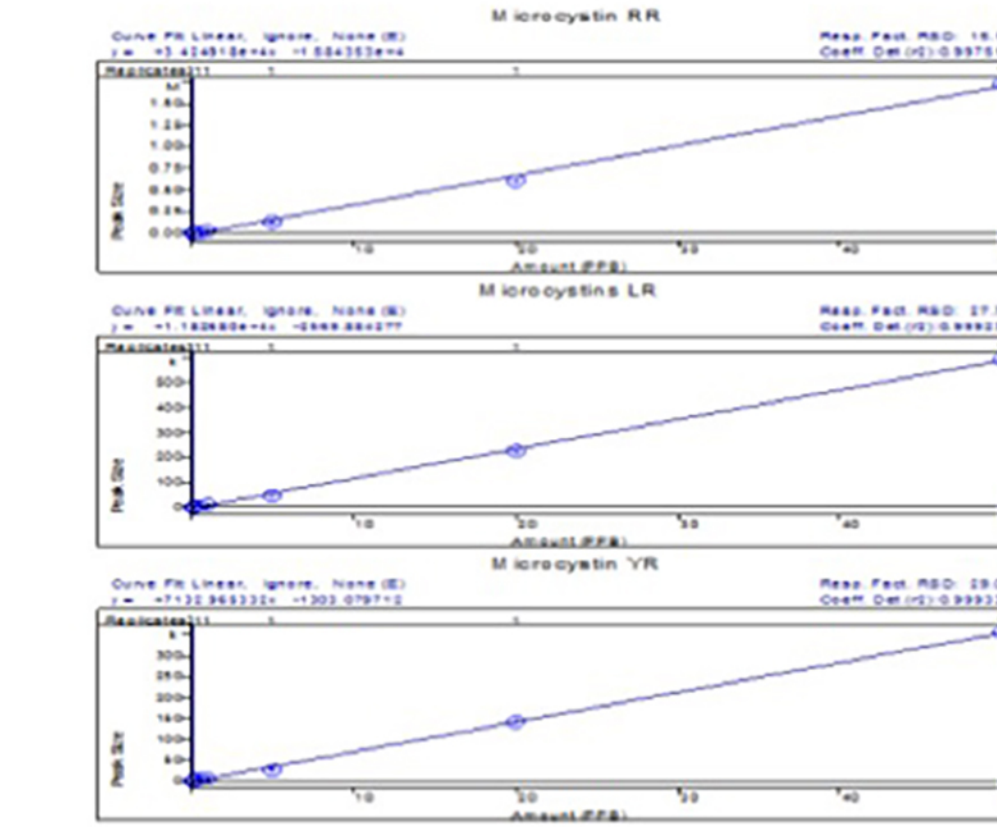
5. Conclusions

- 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.