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Advantages of Ultra Performance Liquid Chromatography-High Resolution Mass Spectrometer for the Analysis of Cyanotoxins in Water for Human Consumption

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INTRODUCTION



RESULTS AND DISCUSSION

- ➤ LC-MS/MS method : reproducibility better than 17% and LODs were in the range of 0.003– 0.032 µg/L for all the analytes; a good linearity was achieved, with correlation coefficients in the range 0.9925 ≤ R² ≤ 0.9998.
- ➢ UPLC-QTOF method : recovery percentages above 85%, with relative standard deviations ≤16% and LODs between 0.001 and 0.047 µg/l for the intended purposes at the concentrations of interest; a good linearity was achieved, with correlation coefficients in the range 0.9902 ≤ R² ≤ 0.9999

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Microginin 527			
Anab B 47	Mar. Lind up	Anab B	
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Tag an		198,88	

of different classes (including 12 Microcystins, 5 Microginins, 2 Cyanopeptolins, and 2 Anabaenopeptins)



Figure 2. Structures of Microcystins, Anabaenopeptin B, Microginin 690, Cyanopeptolin 1041

EXPERIMENTAL METHOD

Drinking water samples were extracted and analyzed using a triple quadrupole and a High Resolution Q-TOF mass spectrometer and the results were compared.



EXTRACTION ON SPE CARBOGRAPH 4 CARTRIDGES:

Eluition: 1 mL CH₃OH + 6 mL CH₂Cl₂/CH₃OH (80:20 v/v) 10 mM TFA

Figure 5. MRM LC/MS/MS chromatogram resulting from analysis of standard solution containing 0.1μg/L of all the analytes and 1 μg/L of Nodularin (chromatogram left) and raw water sample spiked with 1 μg/L of Nodularin. It is reported in Blue colour the quantitation transition and in red the qualification transition for each analytes (chromatogram right)

837.462 0.0500Da

Recovery: 1 mL H₂O/C₂H₃N 70:30 v/v

LC-MS/MS METHOD

- API 3000 (Atmospheric Pressure Ionization)
- Source TIS (Turbo Ion Spray)
- TIS 5500 V (positive ionization mode)
- Temperature: 450 °C
- Curtain gas flow: 10 u.a.
- Nebulizer gas flow: 12 u.a.
- Turbo gas flow: 7 u.a.
- Alltima C18 column
- 50 μl injected

	Retention [min]	Flow [ul/min]	%B	%C	Curve	
1	0.000	200	15.0	0.0		
2	0.000	200	15.0	0.0	5	
3	14.000	200	65.0	0.0	5	
4	15.000	200	80.0	0.0	2	
5	17.000	200	100.0	0.0	5	Solvents
6	20.000	200	100.0	0.0	1	A1 VI 10 mM FA in Water
7	21.000	200	15.0	0.0	2	
8	27.000	200	15.0	0.0	1	B1 V 10 mM FA in ACN

UPLC-HRMS METHOD

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-0-

- Xevo G2 (Qtof)
- Scan Mass: 50 to 1200 Da
- Polarity: ES+
- Analyser Mode: Sensitivity
- Source Temperature (°C): 130
- Desolvation Temperature (°C): 500
- Desolvation Gas Flow (L/Hr): 1000.0
- Column Temperature (°C): 40
- Column: BEH C18 1.7 μm
- 10 µl injected

A1 -	ls 10 mM FA	in Water	•	
B1 -	10 mM FA	in ACN	•	
	Time (min)	Flow (mL/min)	%A	%
1	Initial	0.450	90.0	10.0
2	4.50	0.450	70.0	30.0
3	8.00	0.450	30.0	70.0
4	10.00	0.450	0.0	100.0
5	12.00	0.450	0.0	100.0

■ 3 ^R .				8.64	10.18.10.44 11	20 11.77 12.30	13.68	1.12e5
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
ANAB A			5.98					1: TOF MS ES+ 844,424 0 0500D+
100-			6.26	0.62	10.17 10.50	11 00 10 66	13.69	9.18e4
0.4	2.00	4.00	6003	8.00	10.00	12.00	14.00	
MICRO 704	2.00	1.00	0.00	0.00	10.00	12.00	14.00	1 TOP ME FOR
100-			5.34					705.353 0.0500Da
Nr.							13.68	3.76e5
0.4	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
CYP 1007	0.0000	1000	2010		0707080			1: TOF MS ES+
100			6.61			12.37	-	1007.52 0.0500Da
ől				8.63	10.27.10.45	11.79	13.68.13	89
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	1. 705 110 50
CYP 1041			7.1	18				1: TOP MS ES+ 1041.481 0.0500Da
100-			1	8.33			13.68	1.33e5
0.4	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
MICRO \$22 MITTO	ESTER	4.00	0.00	0.00	10.00	12.00	14.00	1: TOF MS ES+
100-2	COLLA	4.62						542 289 0.0500Da
18- 0-	2.50 2.65	1		8.74	10.40.10.65.1	10.92.11.73 12	.86	3.12e5
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
MICRO 527								1: TOF MS ES+
100		4.00			58 10.03 40.07	12.51	42.45	528.274 0.0500Da
0 0.41.0.60	2.33-2.543.10	4.66	5.56 6.22 6.98	8.25, 8.51	1021	11.84	13.851	4.02
1	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
MICRO 690 METY	LESTER		5.34					1: TOF MS ES+ 705 353 0 0500Da
38-			1				13.60	3.76e5
04,,,,,,	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
	2.00	4.00	0.00	0.00	10.00	12.00	14.00	1: TOF MS ES+
MICRO 690		4.56						691.337 0.0500Da
38.					10.72.10.9	8 11.68 12.48	13.69	1.98e5
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
MC-HilR			5933353					1: TOF MS ES+
1007			1	.38	11.05	11.60		1009.572 0.0500Da 1.28+5
ől		4.49		1 7.66 9.	179.76	11.0911.75	13.67	
1 110 2 -	2.00	4.00	6.00	8.00	10.00	12.00	14.00	1. 705 100 50
I MC-TYP			7.03	3				1. TOP MS ES+ 1059.551 0.0500Da
100			1		9.90 10.42	11.70.11.89		1.34e5
0.4	2.00	1.00	600	8.00	10.00	12.00	14 00	
MC-WR			0.00	0.00	10.00	12.00	14.00	1: TOF MS ES+
100-			2	7.46				1068.551 0.0500Da
20-				1	9.58 10.44	11.79 12.37	13.68	9.0864
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
I MC-LF								1: TOF MS ES+
199				8.73				2.17e5
0						12 26 12 44		
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	1. TOE NO FO
MC-LW				8.63				1025.534 0.0500Da
22-		4.49		1	10.54 11.06	11.61		2.59e5
0.4	2.00	4.00	6.00	8.00	10.00	12 00	14.00	
1 MARY			0.00	0.00	10.00		14.00	1: TOF MS ES+
100				8.33				1002.518 0.0500Da
2					9.42 11.48	12.28 12.58	13.59	2.1405
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
I MC-LA								1: TOF MS ES+
100				8.26	10.69			910.492 0.0500Da 1 92e5
õ.,					11	21	13,69	
C	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
MC-LR								1: TOF MS ES+
1001			7.1	5		0		995.556 0.0500Da 1.79±5
al.				7.63 9	.32 10.46 11	22		
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	4. 705 110 20
MC-DEM LR			75	2				1: TOF MS ES+ 981.54.0.0500Da
100			1		11.07			2 88#5

ő l						10.00		
ANAR A	2.00	4.00	6.00	8.00	10.00	12.00	14.00 1: TO	F MS ES
100-3			5.98				844.42	4 0.05000
3R-			6.26	7.93				3.41
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
MICRO 704							1: TO	F MS ES
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	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
CYP 1007				8.12 0			1: TO	F MS ES
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MICRO 527 METYL	ESTER						1: TO	F MS ES
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31	2.00	4.00	6.00	8.00	10.00	12.00	14.00	F 110 TO
MICRO 527				8.03	9 40.45	11.34	1: 10	4 0.0500
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MICRO 690 METVI	ESTER	4.00	0.00	0.00	10.00	12.00	1. TOP	MS ES
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32. 0.60 S	2.14.2.28 3.	4.70	5.30 5.47		and what	10.94, 11.82	13.69 13.87	2.28e
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Ins_Caprarola	_grezza_20-3	-10			9.76		1009.572	2 0.0500D
at 0.49 0.98	2.24 2,66	4.48	03 5.44	7.78 8.28 8.63 9	170 11.06	11.61 12.14	13.08 13.63	7.15e
0-	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
7_MS_Caprarola	_grezza_20-3	-18					1: TO	MS ES+
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	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
0.51	grezza_20-3	-18		814	10.54	12.15	1: 100	MS ES4 1 0.0500D
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0 test to the	2.00	4 00	6.00	8.00	10.00	12 00	14.00	alarite
7_MS_Caprarola	grezza 20-3	-18	2.00	0.04	10.00	12.00	1: TOP	MS ES
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and the	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
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	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
MC-LA							1: TOP	MS ES4
100 0.51 0.88	1,65 2,44	81 400	1 25 4 20 7.2	4 8.05 1	9.74 10.72	11.21 11.99	13.68	1.236
6 Laborer	-temperation	THIT WAY	125 6.30	-m Patter	and water	ATT THE WAY	13.87	
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
MC-LR							1: TC	F MS ES
100 0.51	41.84 2.72	01 405 470	4.81 6.28	7.15 8.35 8.64	9.42 10.70	-11.05.11.83	2.87 13.82 995.56	56 0.05000
ôl-hope	My Will	4.05 4.70	7.05	dentre where	and the particular	Wash trenet	14.5	1.10
	2.00	4.00	6.00	8.00	10.00	12.00	14.00	
MC-DEM LR		2000	28	7.18			1: 10	# MS ES 54 0 05000
100 0.51 0.92	198230	4.12. 4.36	12	18.04.8 20 8.6	2 10.05 10.61 11	10 11.62	1,13081381	8.85
				THE R P WE	10.000	C	the second second s	

1. TOF MS ES+ 837.462 0.0500Da 2.29e6



CONCLUSIONS

health.

Both methods have been proven to be robust, precise and accurate with recovery percentages above 85% and with relative standard deviations ≤17%, fit for the intended purposes at the concentrations of interest; a good resolution has been obtained with both methods. The performance and reliability of the method was proven to raw, treated and distributed water samples, with LODs 0.001 to 0.047 µg/L, at least 20-fold lower than the guideline value proposed by the WHO for drinking water (1.0 µg/L for microcystin-LR).

The advantages obtained by UPLC-HRMS/MS method are the shorter analysis times (16 minutes vs 27 minutes) and a lower injection volume (10 µl vs 50 µl).

Furthermore, this method allows the simultaneous identification of target and non-target compounds, allowing to detect the presence of other compounds potentially harmful to human