On-line versus off-line solid phase extraction for the determination of Microcystins in Water by LC-MS/MS

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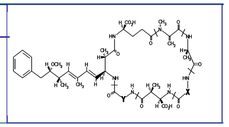
Overview

- Microcystins (MC) are natural toxins produced by Cyanobacteria in natural waters.
- Its toxicity has been demonstrated in both animals and human beings. The can produce extensive liver damage and promote liver tumor formation in subacute exposures.
- The World Health Organization (WHO) suggested a maximum daily intake concentration of MC of 1µg/L for adults. The European Water Framework Directive (2000/60/EC) has established a maximum permitted level of MC in drinking water at the same level suggested by the WHO, 1µg/L

Compound	X	Y	CAS Nr.	Nominal Mass
MC-LR	Leucin	Arginin	101043-37-2	994
MC-YR	Tryptophan	Arginin	1010-64-48-6	1044
MC-RR	Arginin	Arginin	111755-37-4	1037



Introduction

The present work describes the first fully automated method, based on on-line Solid Phase Extraction (SPE) – ultra fast liquid chromatography (UHPLC)-tandem mass spectrometry (MS/MS) for the determination of microcystins (MC-LR, -YR, -RR) in surface waters. Combination of both on-line SPE and UHPLC has been achieved by using special fused core™ chromatographic columns which allowed maintaining the high efficiencies obtained with UHPLC reducing the system backpressure. The analysis of MC was then performed in a total analysis time of 15 minutes. High recoveries, ranging from 99% to 115% were obtained and the focusing mode was used during SPE elution in order to focus analytes in the column head and to avoid peak broadening and high efficiencies were then achieved (N=34000-80000).The quality parameters of the method were established; limits of quantification (LOQ) were similar than those obtained with the off-line SPE ranging from 6 to 10 ng/L while lower run-to-run and day-to-day precisions were obtained in the range of 1.8-2.9% and 2.3-3.6% respectively.

Objectives

- Develop an on-line SPE-LC method coupled to tandem mass spectrometry for the determination of MC in water resources.
- Maintain or improve the benefits achieved with the existing off-line SPE UPLC-MS/MS in terms of resolution and sensitivity.
- Improve the method in terms of cost- and time effiency.
- Perform the XUHPLC-MS/MS coupling and validate the method.

Experimental conditions

Online SPE-LC (Symbiosis™ Pico, Spark Holland)

AUTOSAMPLER

Injection volume: 10 mL
Sample: Water (+5% Methanol pH=7)
Needle wash: 700 µL, 40% ACN, 0.2% FA

LC

Column: Ascentis Express C18 (Supelco, Sigma)
Column oven: 50 °C
Mobile phase: A) ACN, 0.1% FA B) Water, 0.1% FA

Gradient 1:

time (m:s)	flow (µL/min)	% A	% B
0.01	400	5	95
2.00	400	5	95
2.02	500	25	75
7.45	500	95	5
8.15	500	95	5
8.30	500	5	95
10.00	500	5	95

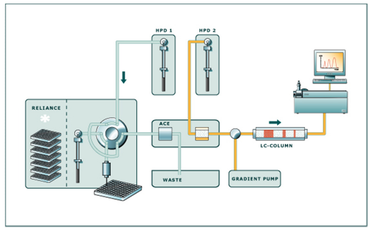
SPE extraction

Cartridge: HySphere C18-HD cartridge (10 x 2mm ID-7µm, Spark)

Conditioning solvents:
1 mL MeOH (5mL/min)
2 mL Water (5mL/min)

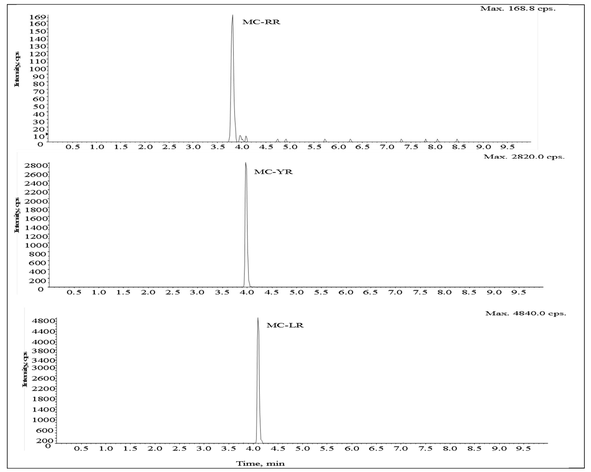
Sample loading:with Water
Washing:
1 mL MeOH:Water (20:80)

Focusing (Elution):
HPD syringe: MeOH 200 µL 100 µL/min
Gradient pump: Mobile phase (A:B; 5:95) 400µL/min



MS : AB/Sciex API 3200 Qtrap MS/MS

	RT (min)	Q1	DP(V)	Q3	CE(V)	Q3	CE(V)
MC-RR	3.46	995	116	135	87	105	127
MC-YR	3.85	1045	146	135	99	105	129
MC-LR	3.97	1038	131	135	103	127	117
Nodularin (I.S.)	3.68	825	96	103	127		



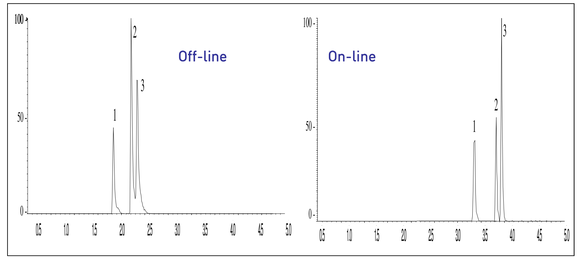
Method Performance

	LOD (ng/L)	LOQ (ng/L)	Run-to-run (%RSD)*	Day-to-day (%RSD)*	Linearity
MC-RR	1.2	10	2.9	3.6	0.995
MC-YR	0.4	6	1.8	2.3	0.997
MC-LR	0.8	8	2.2	2.3	0.998

- Satisfactory LODs were obtained in the low ng/L level.
- Excellent linearity's were achieved for each compound.

Off-line SPE VS On-line SPE

	Off-line	On-line
Sample volume	100 mL	10 mL
SPE Solvent volume	12 mL	1.2 mL
Evaporation	N_2	-
Reconstitution volume	6 mL	-
Total Analysis time	2 hours	15 minutes



Off-line SPE VS On-line SPE

	SPE-(Off-line) UPLC- MS/MS				SPE-(On-line) LC-MS/MS			
	Rt (min)	Rs	N	Asym.	Rt (min)	Rs	N	Asym.
MC-RR	1.83	-	3513 0	0.8	3.46	-	33965	1.1
MC-YR	2.35	4.0	21114	1.0	3.85	1.8	79985	1.3
MC-LR	2.46	0.6	23260	1.1	3.97	0.8	52755	1.1

Discussion and Conclusion

- The benefits of both on-line SPE system and UHPLC technologies (coupled to tandem MS) have been successfully combined for the analysis of microcystins in waters.
- The combination of both techniques was performed by using a fused core particle column which allowed to obtain high efficiencies and reduced backpressures.
- The three MC were separated in less than 4 minutes without losing resolution.
- Focusing mode during the SPE elution step avoided peak broadening and the high efficiencies achieved with the UHPLC were maintained
- Under these conditions the total analysis time was reduced to 15 minutes yielding to a reduction in both time and solvent consumption without losing efficiency.
- High recoveries (99-115%) and low LOQs (6-10 ng/L) were obtained for all the compounds.

Off-line SPE routine:

For the off-line SPE method, a Waters Acquity ultra-performance liquid chromatograph (UPLC®) system, equipped with a quaternary pump system (Milford, MA, USA) using an Acquity BEH C18 column (5.0 cm × 2.1 mm i.d., 1.7 µm particle size) (Waters Corp., Milford, MA, USA) was used.

Waters Oasis® HLB Cartridges were rinsed with 10 mL of methanol and 10 mL of Milli-Q water, samples were percolated through the cartridge at a flow rate of 10 mL/min. The SPE cartridge was washed with 8 mL of a 20% methanol aqueous solution and dried with nitrogen gas for 10 minutes. Analytes were eluted from the cartridge using 6 mL of methanol and the extracts were stored at -20 ºC. Prior to injection, the extract was evaporated to dryness at 40 ºC under a stream of nitrogen in a TurboVap LV evaporator from Zymark (Zymark, Hopkinton, MA). Samples were reconstituted in 200 µL of an aqueous solution with a 5% of methanol. The final extracts were filtered through 0.2 µm before injection

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