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- 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

The chemical structure shows a cyclic peptide backbone with several side chains. One side chain is a long chain containing a phenyl group and a carboxylic acid group. The structure is drawn in a circular fashion, with the backbone forming a ring and the side chains extending outwards.

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.

- 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 efficiency.
- Perform the XUHPLC-MS/MS coupling and validate the method.

ONLINE SPE-LC (SYMBIOSIS™ PICO, SPARK HOLLAND)

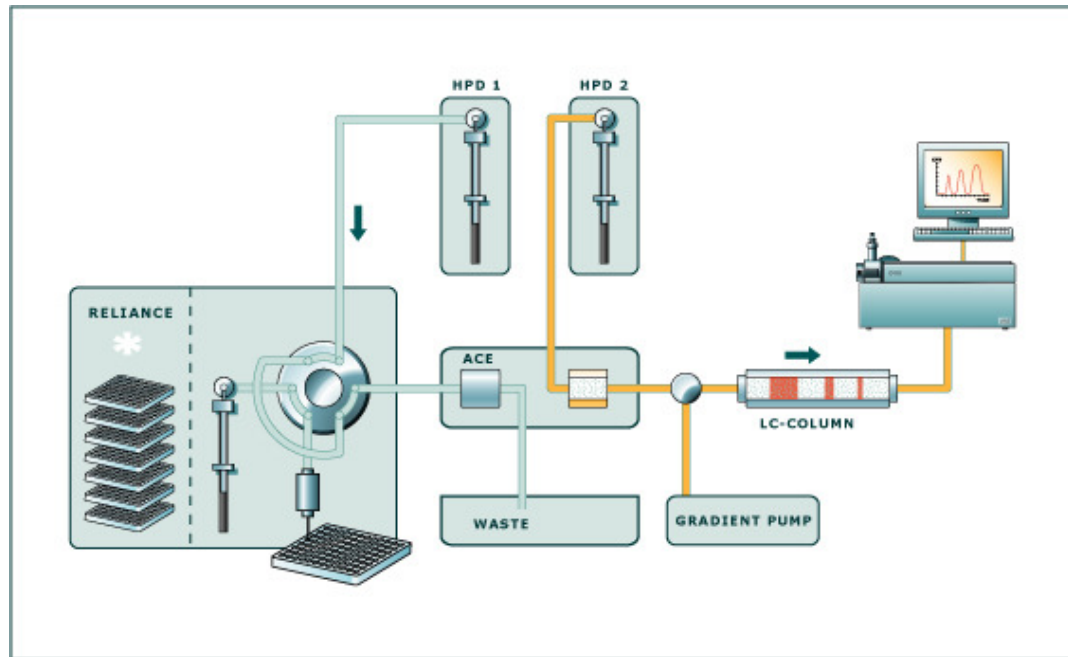
Injection volume: 10 µL
Sample: Water (+5% Methanol pH=7)
Needle wash: 700 µL, 40% ACN, 0.2% FA
LC
Column: Ascentis Express C18 (Supelco, Sigma)
Column oven: 50 °C
Mobile phase: A) ACN, 0.1% FA B) Water, 0.1% FA

This illustration shows a collection of laboratory equipment. On the left is a tall, teal-colored storage cabinet with multiple drawers and doors, some featuring handles and locks. In the center is a fume hood with a large glass front, a control panel with several knobs and buttons, and a digital display. To the right is a biosafety cabinet with a similar design, featuring a control panel with a digital display and a small window. The equipment is depicted in a clean, stylized manner with a light blue background.

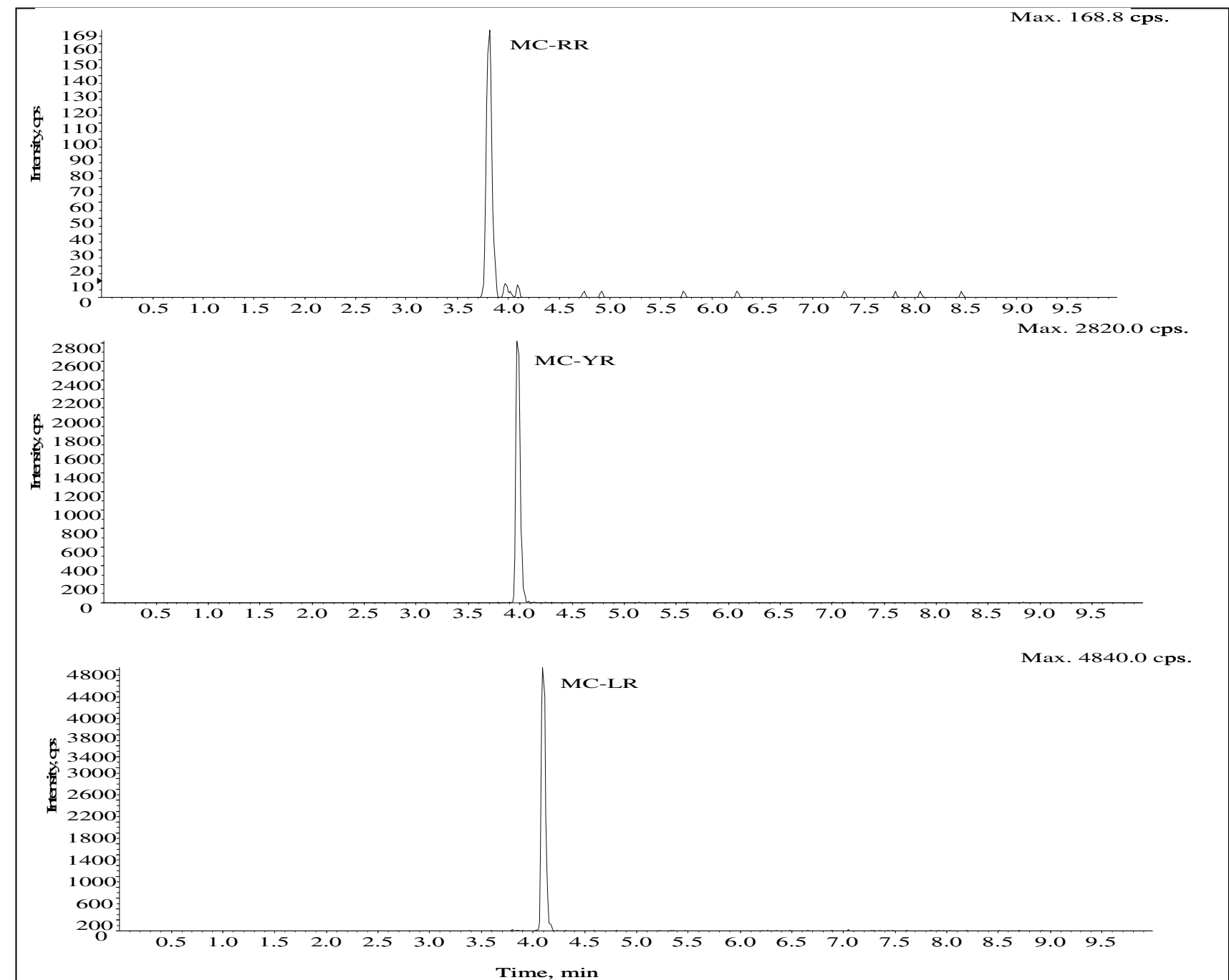
Cartridge: HySphere C18-HD cartridge
(10 x 2mm ID-7µm, Spark)

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

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



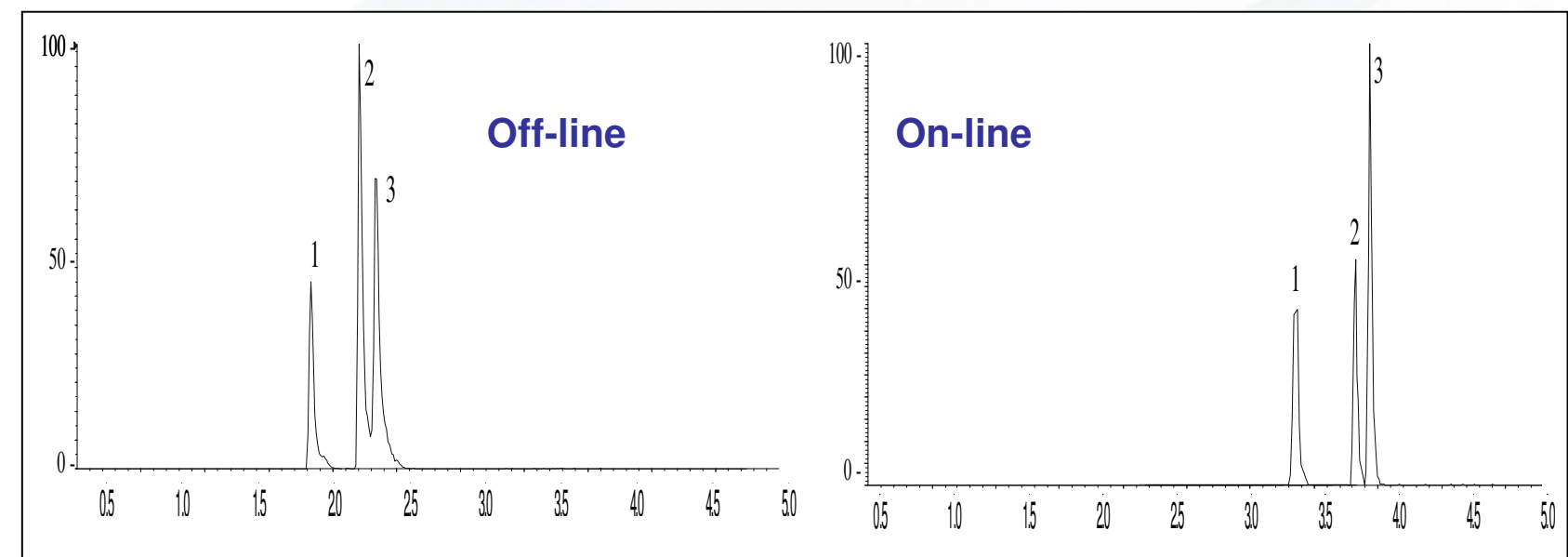
MS : AB/Sciex API 3200 Qtrap MS/MS								
	RT (min)	Q1	DP(V)	Q3	CE(V)	Q3	CE(V)	
MC-RR	3.46	995	116		135	87	105	127
MC-YR	3.85	1045	146		135	99	105	129
MC-LR	3.97	1038	131		135	103	127	117
Nodularin (I.S.)	3.68	825	96		103	127		



	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

- ## 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



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

- 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.

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 °C. Prior to injection, the extract was evaporated to dryness at 40 °C 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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