Analysis of Selected Microcystins in Drinking and Surface Water Using a Highly Sensitive Direct Injection Technique

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Overview

This application note describes a direct injection method using Liquid Chromatography (LC) coupled to tandem Mass Spectrometry (MS/MS) to analyze several microcystins, including MC-LR, in drinking and surface water. Time consuming and laborious extraction steps, i.e. SPE, are not required due to the high sensitivity and selectivity of the MS/MS detection using the AB SCIEX API 4000™ LC/MS/MS system. A limit of quantitation (LOQ) of 0.1 µg/L was achieved which is 10 times below the guideline value proposed by the World Health Organization (WHO).

Introduction

Microcystins (MC) are naturally occurring toxins produced by certain genera of cyanobacteria (Figure 1). Reports suggest that microcystins are hepatotoxic and they might also be tumor initiators.[1]

Traditionally MC were analyzed by HPLC with UV detection[2-3] but nowadays analytical methods are shifting more towards mass spectrometric detection.[4-5]

MC are cyclic peptides and the general structure is cyclo [-D-Ala-X-D-MeAsp-Y-Adda-D-Glu-Mdha-], where X and Y are variable L-amino acids, e.g. leucine (L), arginine (R), tyrosine (Y), tryptophan (W), and phenylalanine (F) as X, as well as arginine (R), alanine (A), and methionine (M) as Y (Figure 2). Due to the two variable amino acids and methylation/demethylation of the other amino acids, there is a large variety of microcystin compounds. More than 80 microcystins have been identified to date. In contrast to microcystins, nodularin (NOD) is a cyclic pentapeptide produced by Nodularia spumigena with the structure cyclo [-D-MeAsp-L-Arg-Adda-D-Glu-Mdhb-], where Mdhb stands for 2-(methylamino)- 2-dehydrobutyric acid.

The Microcystin MC-LR (Figure 2) is typically tested as a marker for cyanobacteria occurrence and is regulated by the WHO in the guidelines for drinking-water quality at a value of 1 µg/L.[7]

Figure 1. Bloom (left) and microscopic image (right) of Planktothrix rubescense

Experimental

Standard

Microcystin standards are available from Enzo Life Science International (http://www.enzolifescience.com).

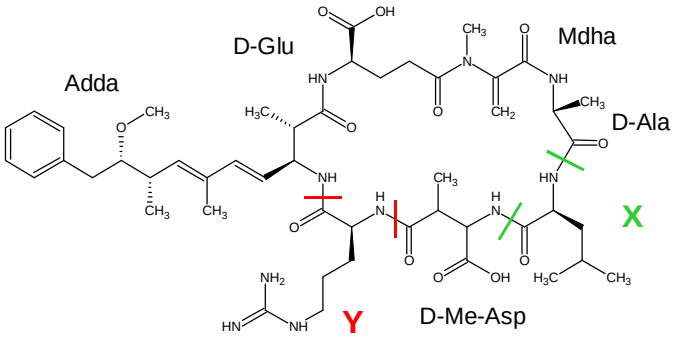


Figure 2. Structure of MC-LR where X is leucine and Y is arginine

LC

An Agilent 1100 LC system was used with a Phenomenex Phenomenex LUNA C18 3u (150x3 mm) column and a mobile phase of water and acetonitrile + 5 mM ammonium acetate + 0.1% formic acid (Table 1).

The injection volume was set to 25 to 100 µL.

Table 1. LC gradient

Time (min)	Flow (µL/min)	A (%)	B (%)
0	250	70	30
10	250	65	35
20	250	15	85
re-equilibration	250	70	30

MS/MS

An AB SCIEX API 4000™ LC/MS/MS system equipped with Turbo V™ source and Electrospray Ionization (ESI) probe was used for compound detection in positive polarity.

Multiple Reaction Monitoring (MRM) was used for its superior selectivity and sensitivity. Two MRM transitions for simultaneous quantitation and identification based on ion ratio calculation and compound dependent parameters were automatically optimized by direct infusion experiments and the 'Compound Optimization' tool in Analyst® software.

The full scan MS/MS spectrum is shown in Figure 3. Two product ions at m/z 135.0 amu (characteristic ADDA fragment for MC) and m/z 213.0 amu (Glu-Mdha) were measured.

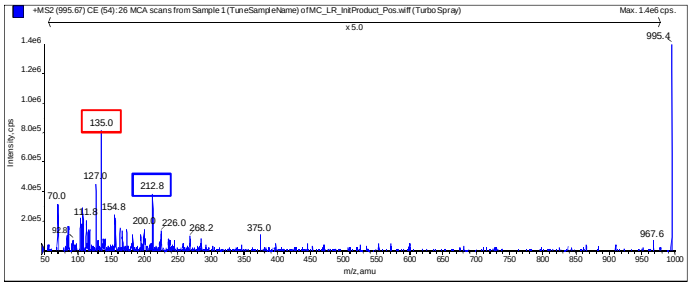
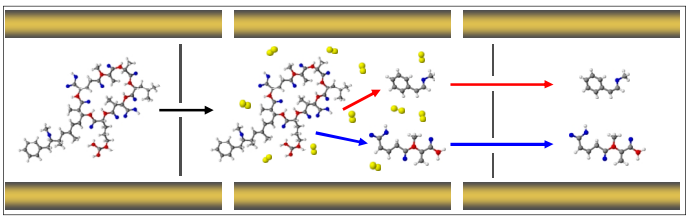


Figure 3. MS/MS spectrum of MC-LR with highlighted product ions used for quantitation



Transition	Q1 (amu)	Q3 (amu)	CE (V)
MRM 1	995.7	135.2	115
MRM 2	995.7	213.0	115

Figure 4. MRM transitions and Collision Energy (CE) to detect MC-LR

MRM transitions are shown in Figure 4.

Due to the thermal stability of MC-LR the nitrogen gas temperature to dry the eluent in the ion source was set to 650°C which evaporated the mobile phase completely yielding in enhanced sensitivity of the measurement.

Results and Discussion

The Turbo V™ ion source was designed and optimized (geometry, ceramic materials, orthogonal sprayers etc.) for highest sensitivity, reproducibility, robustness, and lowest carry-over.

The Signal-to-Noise values (S/N) of MC-LR, MC-RR, and MC-YR at a concentration of 0.1 µg/L were >10 (3 x standard deviation) resulting in a Limit of Detection (LOD) of 0.04 µg/L for MC-LR for example (Figure 4).

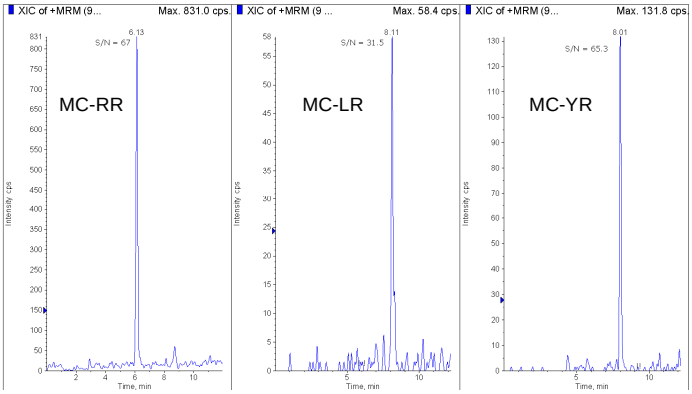


Figure 4. MRM chromatogram of MC-LR spiked drinking water at a concentration of 0.1 µg/L (injection volume of 25 µL)

Linearity was proven for MC-LR, MC-RR, and MC-YR standard solutions ranging from 0.1 µg/L to 100 µg/L.

The reproducibility of the developed method was tested by injecting spiked drinking water. The coefficients of variation were less than 4% (n=15) at all calibration levels.

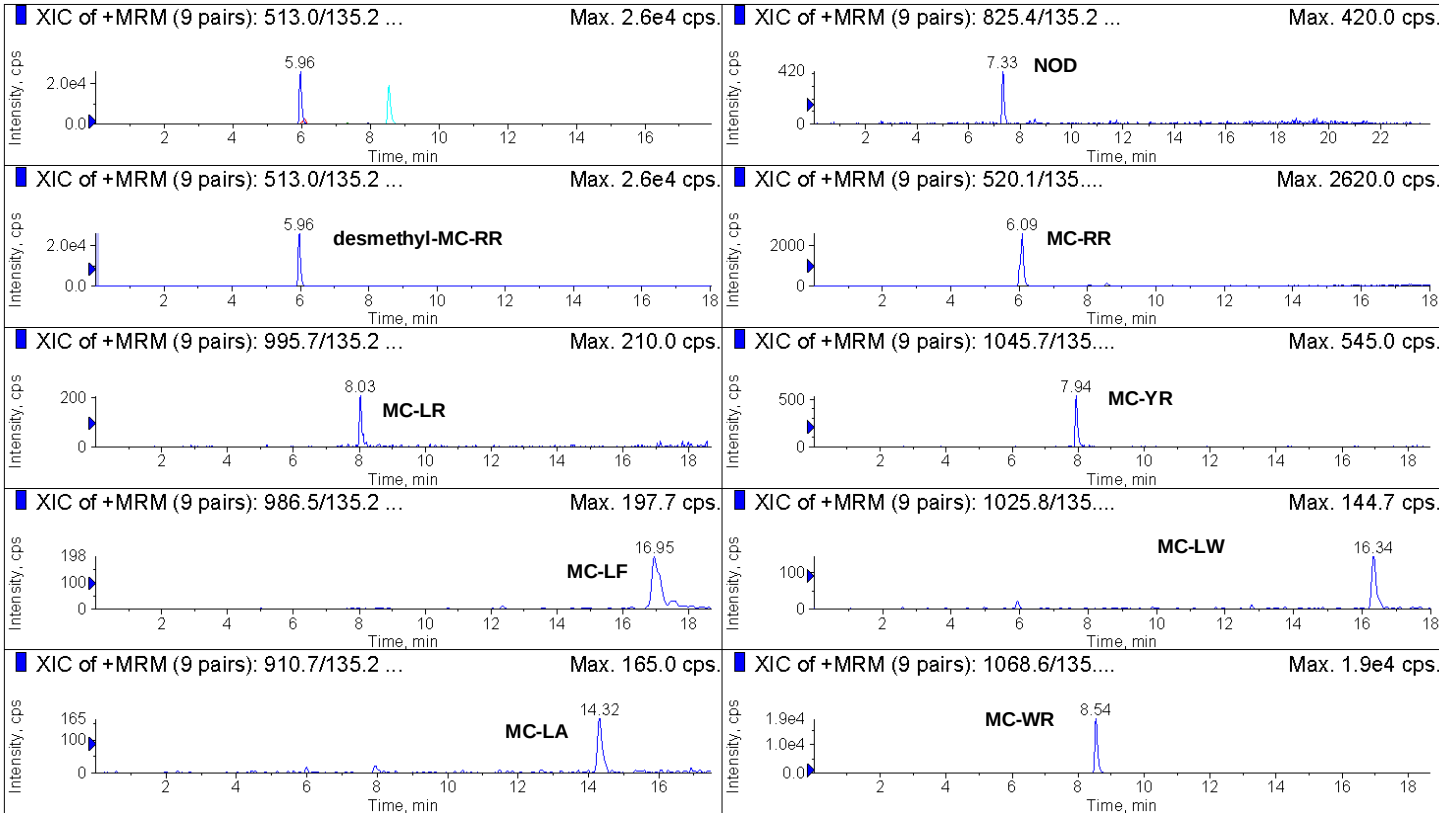


Figure 5. Chromatograms of various microcystins and nodularin (NOD), at a concentration of 1 µg/L, except desmethyl-MC-RR and MC-WR at 10 µg/L (injection volume of 25 µL)

In addition, the API 4000™ system is equipped with a Linear Accelerator (LINAC®) collision cell. The axial field gradient of the LINAC® collision cell accelerates product ions after fragmentation allowing fast MS/MS experiments without cross-talk and without loss in sensitivity, such as fast MRM using short dwell times for each transition.

This allows multi-target quantitation. The developed method can easily be extended to quantify other microcystins of interest. An example chromatogram for the quantitation of 9 microcystins, including MC-LR, MC-LF, MC-LA, MC-RR, MC-YR, MC-LW, MC-WR, desmethyl-MC-RR, and Nodularin is shown in Figure 5.

Summary

The AB SCIEX API 4000™ LC/MS/MS system offers sufficient sensitivity for the direct analysis of microcystins, including MC-LR, in drinking water with an LOQ of 0.1 µg/L. Time consuming and extensive sample clean-up and concentration is not required resulting in better reproducibility and accuracy.

The methodology is designed to also allow for the inclusion of other microcystinswater soluble cyanobacterial toxins such as anatoxins and cylindrospermopsin.

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