Monitoring for Microcystins in Raw Water Supply Reservoirs Using the Agilent 6410 Triple Quadrupole LC/MS

Application Note

Environmental

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Abstract

A method for the determination of Microcystins (Microcystins-LR, -YR and -RR) in raw water supply reservoirs has been developed using the Agilent 6410 Triple Quadrupole LC/MS.

Introduction

Microcystins are toxins produced by cyanobacteria. Cyanobacteria, also known as blue-green algae, are organisms which have the characteristics of bacteria and algae. When conditions are favorable for growth in surface waters (warm, stable conditions are required), large populations known as algal blooms can occur. However, not all populations produce toxins. The Cyanobacteria may release toxins upon cell death or lysis. The toxins produced include hepatotoxins (Microcystins, Nodularins, and Cylidrospermopsin), neurotoxins (Anatoxin-a, Anatoxin-a(S), Saxitoxins), and dermatoxins (Lyngbyatoxin-a, Aphysiatoxins). When released, these toxins can persist for weeks to months. Microcystins present a health risk to humans and animals. Most are hepatotoxins (liver toxins) although they are also an irritant to the skin, eyes and throat.

Microcystins are listed on the United States Environmental Protection Agency's (USEPA) Contaminant Candidate Lists [1], and the World Health Organization (WHO) has proposed a provisional guideline of 1 µg/L for Microcystin-LR in treated drinking water [2]. In the UK, there are no regulations for Microcystin analysis. The Drinking Water Inspectorate (DWI) reviewed whether their analysis was necessary in the UK [3]. They concluded that while they do occur in raw water, their potential presence can be readily identified by only being present in significant concentrations when large blooms of cyanobacteria occur. In addition, they are readily removed by a number of water treatment processes.

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Data has shown that the health risks for drinking water associated with Microcystins from cyanobacteria in the UK are very low and most water companies in the UK successfully manage drinking water sources to minimize the numbers of cyanobacteria.

Various analytical techniques are used to analyze for Microcystins in water. These include biological assays such as enzyme-linked immunosorbent assays (ELISA) as well as Liquid Chromatography with Ultraviolet-Visible (LC/UV-Vis) and Mass Spectrometry (LC/MS) detection [1]. Of these techniques, LC/MS offers both specificity and good sensitivity, in particular the use of triple quadrupole LC/MS [4].

Wessex Water has developed a method for the determination of Microcystin-LR, -YR and -RR in water using the Agilent 6410 Triple Quadrupole LC/MS. It reports to 0.1 µg/L for Microcystin-LR, -YR and -RR in all waters that are tested. The bulk of the analyses are carried out on raw reservoir waters. However, when a significant bloom occurs, then both cell-bound and free Microcystins are looked for. As a precaution, treated water samples are also analysed to ensure that the water treatment works are effectively eliminating any possible contamination.

Experimental

Sample Preparation

Standards

DHI (Denmark) Covered by ISO 20179

LGC Mixed standard of Microcystin RR-YR-LR

Raw water samples for free Microcystin analysis

Raw water samples were filtered through GF/B (particle retention 1.0 µm) filter papers prior to solid phase extraction.

Intra-cellular samples for cell-bound Microcystin analysis

Samples were filtered and the filter paper was then boiled in methanol/water (70/30 v/v). This solution then requires either the methanol to be evaporated off or diluted prior to solid phase extraction.

Solid Phase Extraction (SPE)

Solid phase extraction was performed using 100 mg, 3 mL polystyrene-divinylbenzene (PS-DVB) SPE cartridges (Agilent Bond Elut LMS, p/n 12105024, or equivalent). The cartridges were conditioned with methanol (1 volume), followed by ultra pure water (1 volume) ensuring that the cartridges did not dry out. For the extraction, reservoirs were attached to the cartridges and 75 mL water sample spiked with Nodularin internal standard (1.0 µg/L) was slowly extracted under gravity or slight vacuum. A blank and a set of standards (0.02 µg/L, 0.1 µg/L, and 1.0 µg/L), prepared using a known filtered raw water, were spiked with internal standard and extracted along with the samples. After extraction, the cartridges were washed with 1 volume of 30% methanol and then dried using full vacuum for 3 minutes. The cartridges were then eluted with 1% formic acid in methanol (2x 0.5 mL), using vacuum to ensure complete elution. The extracts were then taken to dryness before being reconstituted in 150 µL of 0.1% acetic acid in 25% acetonitrile, vialed up and analyzed.

Table 1. LC and MS Instrument Conditions

LC conditions		
Analytical column	Bonus-RP 2.1 × 50 mm, 3.5 µm	
Column temperature	60 °C	
Injection volume	60 µL	
Mobile phase	A = 0.1% acetic acid in water	
	B = 0.1% acetic acid in acetonitrile	
Run time	12 minutes	
Flow rate	0.2 mL/min	
Gradient program	Time (minutes)	Gradient (% B)
	0	20
	1	20
	7	90
	9	20
MS conditions		
Acquisition parameters	ESI Positive	
Gas temperature	350 °C	
Gas flow	9 L/min nitrogen	
Nebuliser pressure	40 psi	
V_{Cap} voltage	4,000 V	

Table 2. Agilent 6410 Triple Quadrupole LC/MS Acquisition Parameters

Compound	Quantitation/ qualifier ion	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell (msec)	Fragmentor voltage (V)	Collision energy (V)	Polarity
Microcystin-LR, $[M+H]^+$	Quantitation	995.5	134.8	110	60	90	Positive
	Qualifier	995.5	212.9	110	60	70	Positive
Microcystin-YR, $[M+H]^+$	Quantitation	1045.5	135.1	110	300	90	Positive
	Qualifier	1045.5	212.6	110	300	80	Positive
Microcystin-RR, $[M+2H]^{2+}$	Quantitation	519.8	134.8	110	160	50	Positive
	Qualifier	519.8	102.9	110	160	70	Positive
Nodularin, $[M+H]^+$	Quantitation	825.4	134.8	110	190	70	Positive
Internal standard	Qualifier	825.4	162.9	110	190	50	Positive

Results and Discussion

Table 1 shows the instrument operating conditions. Table 2 shows the quantitation and qualifier ions, fragmentation voltages, and collision energies for each compound.

Microcystins are cyclic heptapeptides with seven amino acids. They are named for the various amino acids on the peptide structure. Microcystin-LR is named for the leucine (L) and arginine (R) amino acids, Microcystin-YR for the tyrosine (Y) and arginine (R) amino acids and Microcystin-RR for the 2 arginine (R) amino acids. Arginine has a free amino group which has a high proton affinity and as Microcystin-RR has two arginine groups it is, therefore, doubly charged. Figure 1 shows the structure of Microcystin-LR.

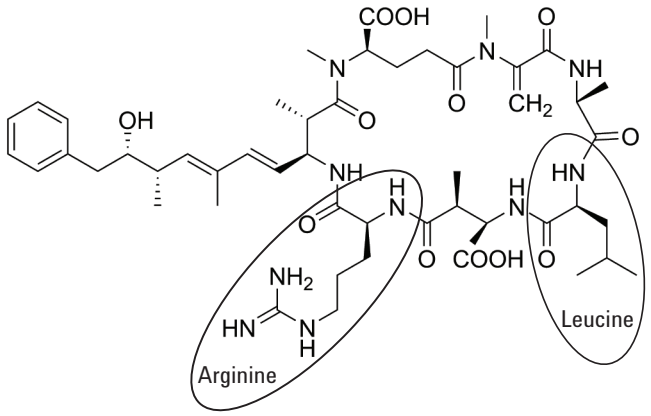
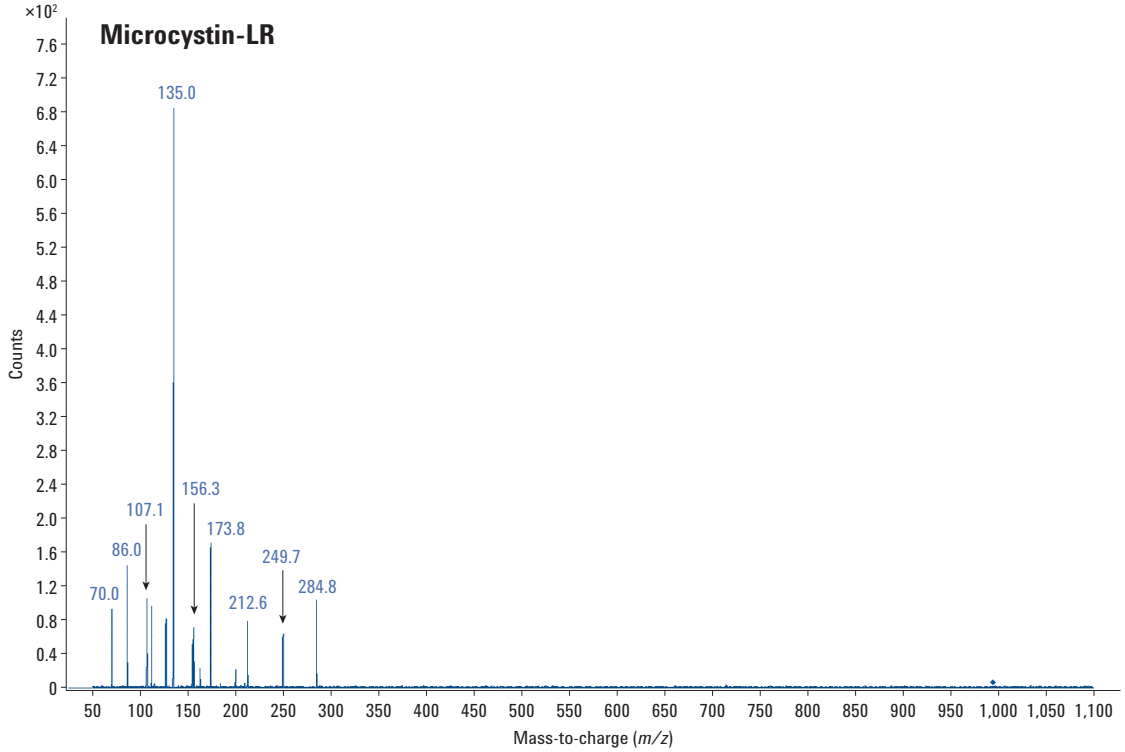


Figure 1. Structure of Microcystin-LR ($C_{49}H_{74}N_{10}O_{12}$, CAS: 101043-37-2).

Nodularin, a cyclic pentapeptide, was chosen as the internal standard. It is a good choice for the analysis of Microcystins in raw water reservoirs as it is structurally and chemically similar to the Microcystins yet only found in brackish waters. However, if brackish waters require analysis, Nodularin cannot be used.

Figure 2 shows the fragmentation patterns of the Microcystins and Nodularin.

Figure 3 shows chromatograms for Microcystins-LR and -YR. The first chromatogram demonstrates good chromatography for the 0.02 µg/L standard. The second chromatogram shows a water (raw water sample 1) where both Microcystin-LR and -YR are present. The third chromatogram shows a water (raw water sample 2) where Microcystin-LR is present, as well as an apparent Microcystin-YR. Although this is the correct transition for Microcystin -YR, it is not the correct retention time. This indicates that another Microcystin, which exhibits the same transition, is also present.



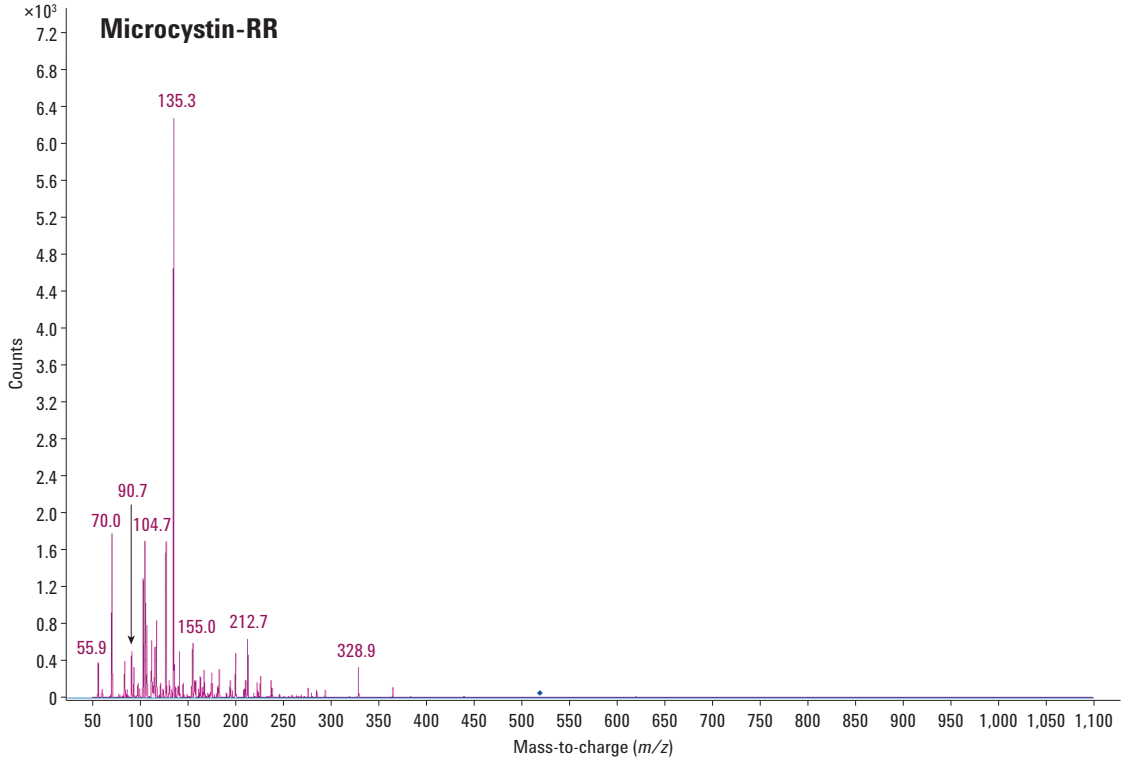
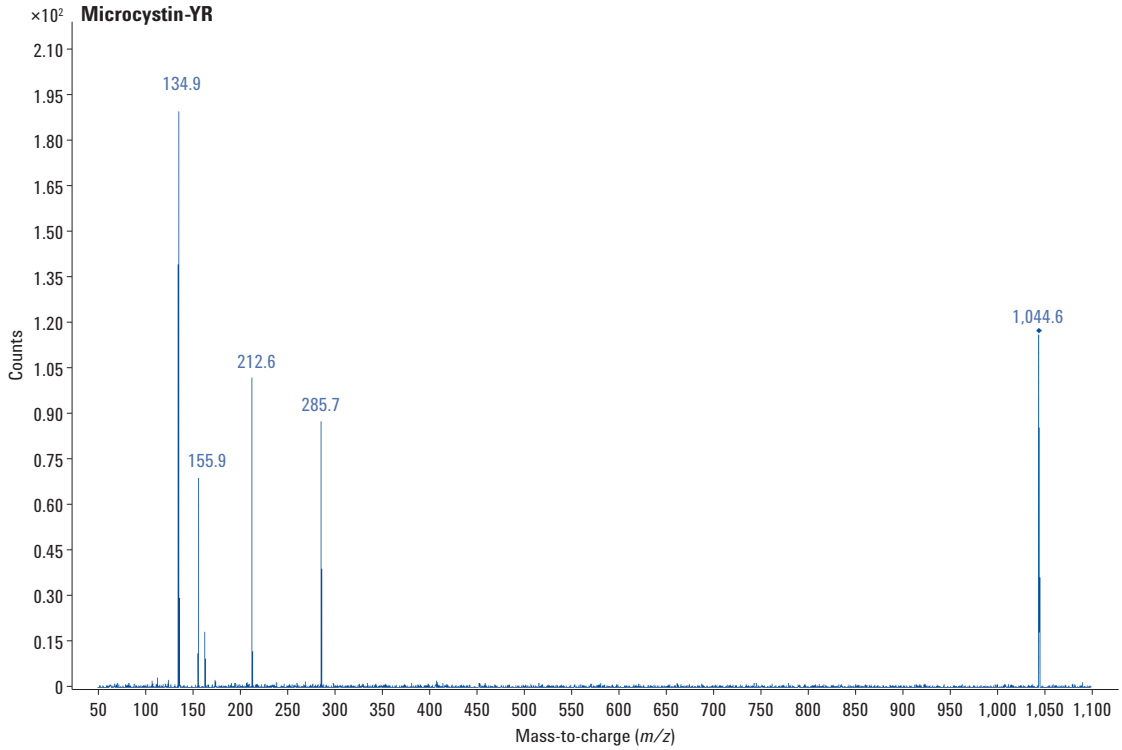


Figure 2. Fragmentation patterns for Microcystin-LR, -YR, -RR and Nodularin.



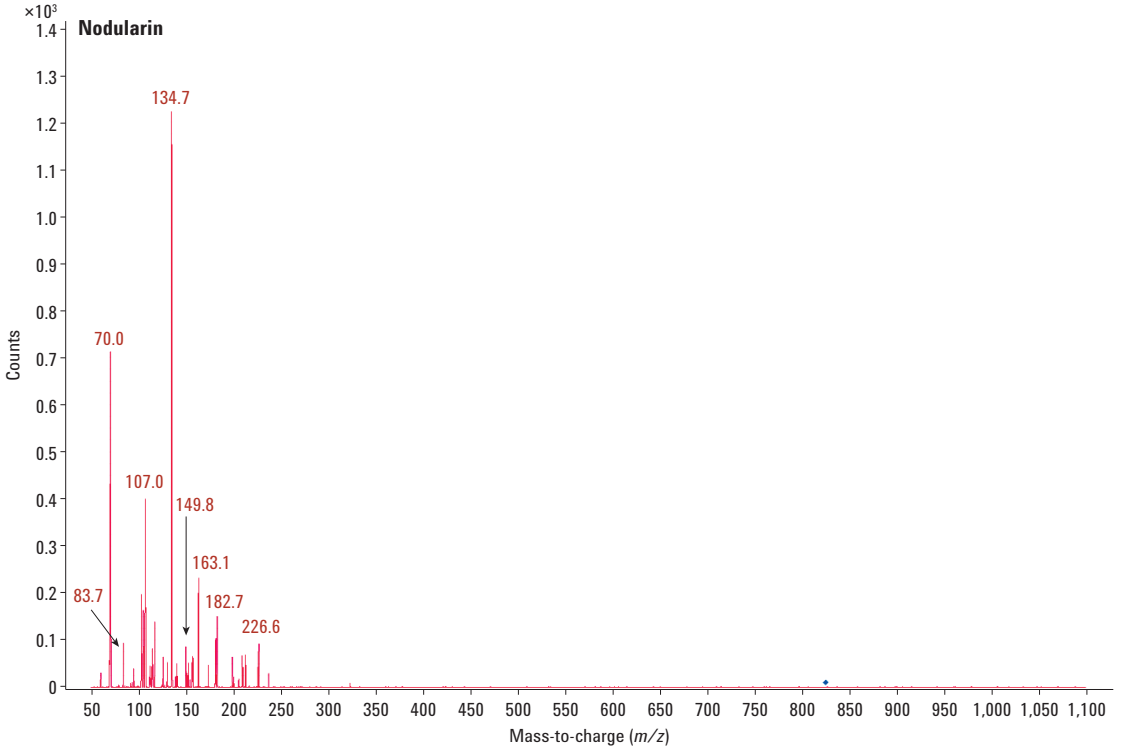


Figure 2. Fragmentation patterns for Microcystin-LR, -YR, -RR and Nodularin (continued).

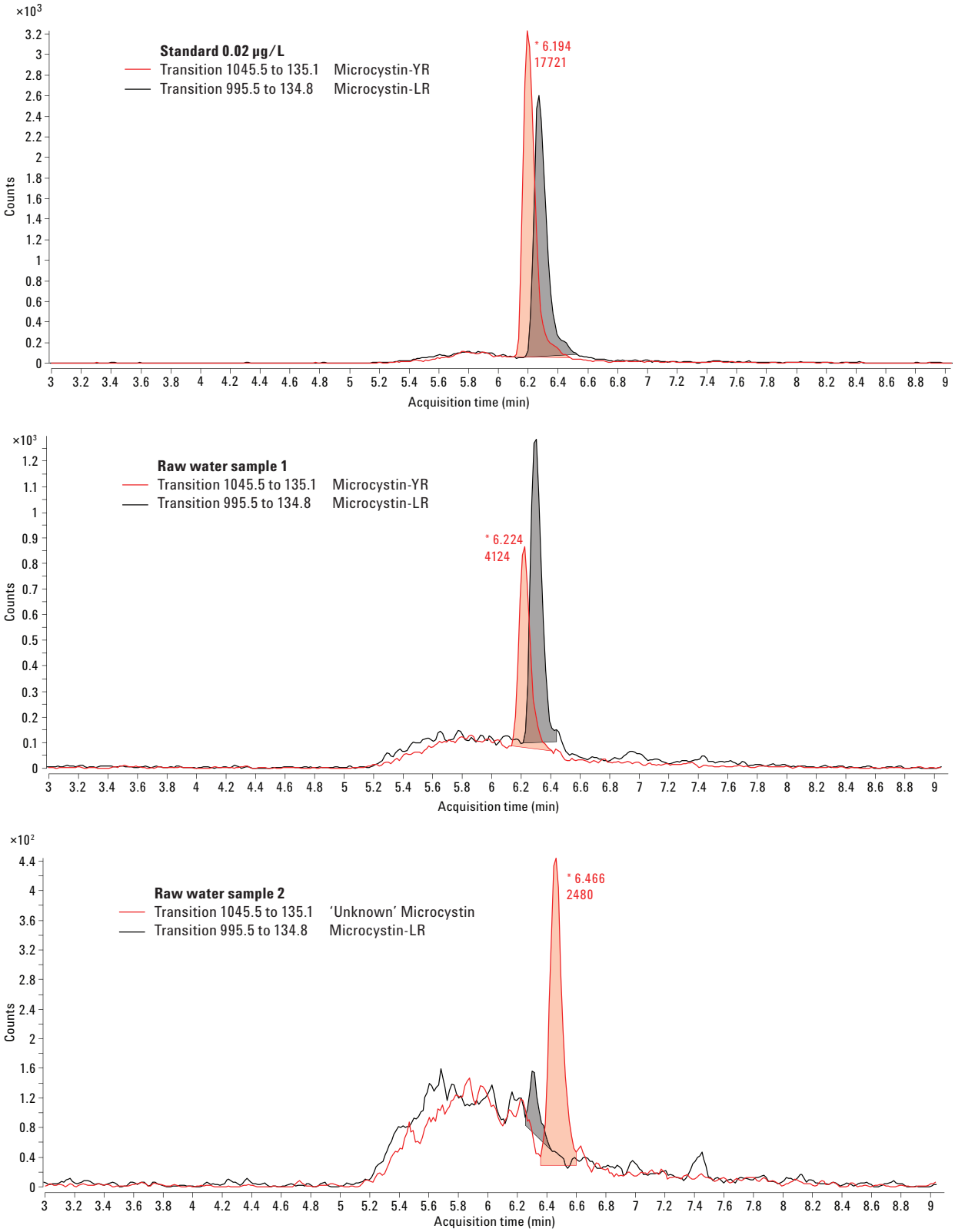


Figure 3. Chromatograms of 0.02 µg/L standard and two raw water samples.

The calibration range for each Microcystin was 0.02 µg/L to 1 µg/L and an analytical quality control (AQC) at 0.5 µg/L was analyzed. Figure 4 shows a typical calibration graph for Microcystin-LR. As the method was generally for raw water samples only, there was no requirement for it to be validated. The limits of detection (LODs) appear to be lower than 0.005 µg/L for all three Microcystins, although a reporting limit of 0.1 µg/L was used.

As well as analyzing raw water supply reservoirs, intracellular analysis is also performed when required. This is where the cyanobacterial cells are lysed and the contents analyzed for cell-bound Microcystins. This is usually done when algal blooms occur or surface scums are formed and can indicate whether possible poisoning of a reservoir is likely to occur when these bloom populations die. Treated water samples are also analyzed to ensure that the water treatment works are effectively eliminating any possible contamination and if necessary, water is not abstracted.

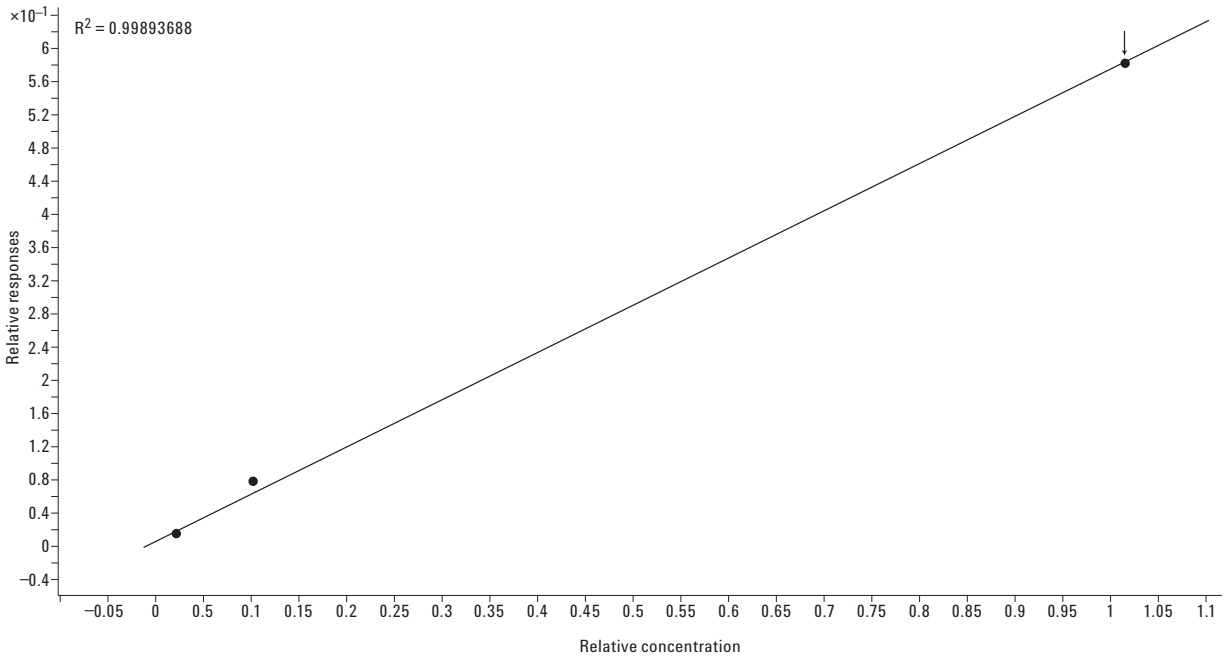


Figure 4. Typical calibration graph for Microcystin-LR.

Conclusions

A method has been developed for the determination of Microcystins (Microcystins-LR, -YR and -RR) in raw water supply reservoirs using the Agilent 6410 Triple Quadrupole LC/MS. This method demonstrates good sensitivity in raw waters and is capable of detecting these compounds at levels lower than 0.005 µg/L.

Acknowledgements

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References

1. United States Environmental Protection Agency, Office of Water, Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems, EPA-810F11001, July 2012.
2. World Health Organisation, Cyanobacterial Toxins: Microcystin-LR in Drinking Water; WHO/SDE/WSH/03.04/57, Geneva, Switzerland, 2003.
3. D. Maycock, J. Fawell, G. Merrington and C. Watts, Review of England and Wales Monitoring Data for Which a National or International Standard Has Been Set, DWI0855, March 2008.
4. A. Mekebri, G.J. Blondina, D.B. Crane, Journal of Chromatography A, 1216 (2009) 3147.

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