

METHOD STATEMENT



Determinand:

Determination of Microcystin-LR

Matrix:

Sample Type: treated and raw water.

Principle of Method:

The method is applicable to the analysis of extracellular microcystin-LR only. Intracellular microcystin-LR can be recovered following ultrasonic disruption of algal cells. The method is a large volume direct aqueous injection on-line solid phase extraction procedure. Samples are analysed by high performance liquid chromatography using a triple quadrupole mass spectrometer as a detector. The sample is injected by large volume injection (LVI) onto an enrichment column. The enrichment column is back flushed on to the analytical column. The organic compounds are separated and then identified and quantified with mass spectrometric detection (MSD) in dynamic reaction monitoring (DMRM) mode. Quantitation is based on an internal standardisation procedure.

NOTE: The method was validated by spiking a range of water matrices with microcystin-LR, i.e. extracellular toxin and not intracellular. In practice, treated waters having been filtered during the treatment process are likely to contain only extracellular microcystin and hence do not require any form of pre-preparation. Treated water samples are analyzed to ensure that the water treatment works are effectively eliminating any possible contamination and if necessary, water is not abstracted. In order to determine the total amount of microcystin-LR within a sample, samples should be disrupted by ultrasonic treatment prior to determination by the above method. 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.

Sampling and Sample Preparation:

Sampling, samples should be collected in 500mL coloured glass bottles with PTFE lined screw caps and contain 0.9mL of 10mg/L sodium thiosulfate.

Storage – samples should be analysed as soon as possible after collection. When this is not possible, they should be stored under refrigeration at 1-5°C in the dark, until analysis can begin. The maximum permissible storage time prior to analysis is given below:

Determinand	Maximum period of analyte stability prior to any extraction step (days)	Maximum period of analyte stability after to any extraction step (days)	Data is quoted from BS EN ISO 5667-3: 2018 ["ISO"] or ALS in-house data ["ALS IHD"]
Microcystin-LR	21	N/A	ALS IHD

Interferences

HPLC-MS/MS is an extremely selective technique and interferences should only be encountered very rarely. Any interfering compounds would have to display the identical MRM transition at the same retention time; this is extremely unlikely in potable water samples. However, any compound, which passes through the extraction procedure, and has a similar liquid chromatographic retention time and mass spectrometric properties to the compound of interest, will cause interference. Samples containing high humic or fulvic loading have been demonstrated to not cause significant ion suppression for the compounds.

Performance of Method:

Range of Application:

LOQ - 1.500 µg L⁻¹

Limit of Quantification:

24 µg L⁻¹

METHOD STATEMENT



Bias, Recoveries of Compounds and Uncertainty of measurement: Instrument 1, Q3

<u>Determinand</u>	<u>Direct Standards</u>				<u>Elvington Treated Water (Hard Hardness)</u>	
	<u>Low Standard</u>		<u>High Standard</u>		<u>PCV Spike</u>	
	<u>Bias</u>	<u>RSD</u>	<u>Bias</u>	<u>RSD</u>	<u>Recovery</u>	<u>RSD</u>
Microcystin-LR	-3.43%	6.70%	-0.82%	4.37%	101.25%	5.39%

Instrument 2, Q6

<u>Determinand</u>	<u>Direct Standards</u>				<u>Elvington Treated Water (Hard Hardness)</u>	
	<u>Low Standard</u>		<u>High Standard</u>		<u>PCV Spike</u>	
	<u>Bias</u>	<u>RSD</u>	<u>Bias</u>	<u>RSD</u>	<u>Recovery</u>	<u>RSD</u>
Microcystin-LR	0.98%	4.47%	0.73%	3.46%	95.84%	4.78%

References:

Monitoring of Microcystins in Raw Water Supply Reservoirs Using the Agilent 6410 Triple Quadrupole LC/MS, Agilent Technologies Application Note 5991-2057EN.

LC-MS/MS Analysis of Microcystins in Drinking Water on EVOQ Elite, Buker Application Note #704424.

Analysis of Selected Microcystins in Drinking and Surface Water Using a Highly Sensitive Direct Injection Technique, ABSciex, Publication number: 1830410-01.

Analysis of Microcystins Using LC-MS, Shimadzu LC-MS Application Data Sheet No.45.

Analysis of Microcystins Using LC-MS (No.2), Shimadzu LC-MS Application Data Sheet No.49.

Determination of Microcystin-LR in Drinking Water Using UPLC Tandem Spectrometry-Matrix Effects and Measurement, W Li, J Duan, C Niu, N Qiang and D Mulcahy, Journal of Chromatographic Science, Vol.49, pg 665-670, October 2011

Agilent 6410 QQQ LC/MS Techniques and Operation, Agilent Technologies Course Number R1893A, Student Manuals Volumes 1 and 2.

Agilent 1200 Series, Reference Manuals

Agilent 6410 Triple Quad LC/MS, Maintenance and Familiarization Guides.