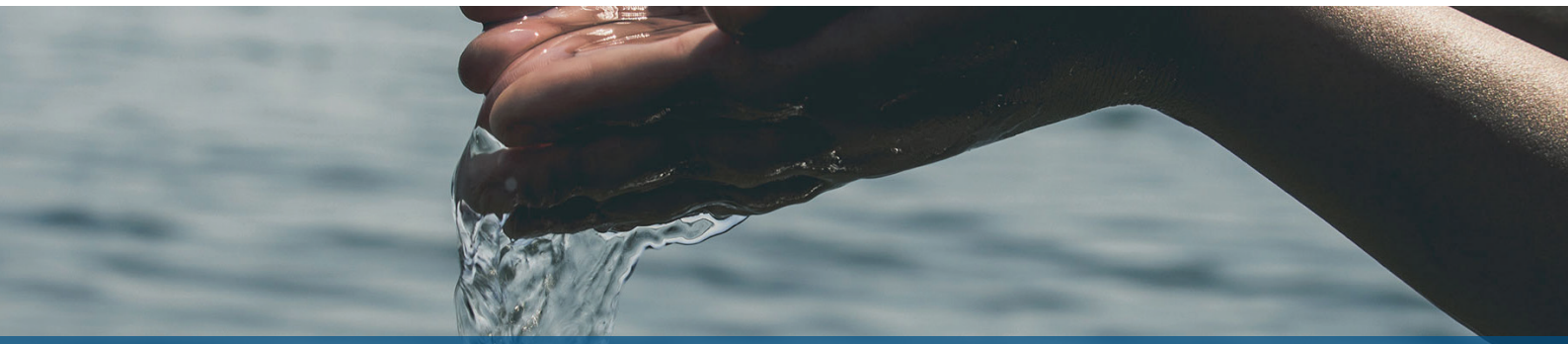


TECHNICAL DATASHEET

Analysis of Microcystin-LR (MC-LR) in Water

Overview

ALS Environmental can provide analytical testing for Microcystin-LR (MC-LR) in water to reporting limits of 5ng/L. MC-LR is a naturally occurring toxin produced by cyanobacteria that has been shown to possess toxic and carcinogenic properties and has been detected in drinking water supplies in the low parts-per-trillion (ng/L) range. In response to this issue ALS Environmental have developed, validated and UKAS accredited, a method capable of analysing for this compound at ultra-trace levels using state of the art instrumentation.

MC-LR Background

Microcystins are a group of naturally occurring toxins produced by various genera of cyanobacteria, including Microcystis, Anabaena, and Oscillatoria (Planktothrix). Cyanobacteria, commonly referred to as blue-green algae, are photosynthetic prokaryotes that occur naturally in surface waters. Nutrient rich, eutrophic, warm and low turbulent conditions in freshwater bodies typically promote the dominance of cyanobacteria within phytoplankton communities. Excessive proliferation of cyanobacteria leads to blooms that disrupt ecosystems, adversely affect the taste and odour of water, and increase water treatment costs (Figure 1). When a cyanobacterium dies, its cell wall degrades and the toxins are released in the water. Microcystins are extremely stable in water and withstand chemical breakdown such as hydrolysis or oxidation. At typical conditions in

the environment, the half-life of MC-LR is approximately 10 weeks.
Figure 1: Blue/green algae bloom

Microcystins are cyclic heptapeptides. They are composed of five common amino acids and pairs of L-amino acids as variants. The common ones are methyl aspartic acid, alanine, N-methyldehydro-alanine, glutamic acid, and a unique amino acid called Adda (3-amino-9-methoxy-2,6,8-trimethyl-10- phenyldeca-4,6 dienoic acid). The structural differences among the toxins are related to the remaining two L-amino acids. In MC-LR these variable residues are L-arganine and L-leucine (Figure 2).

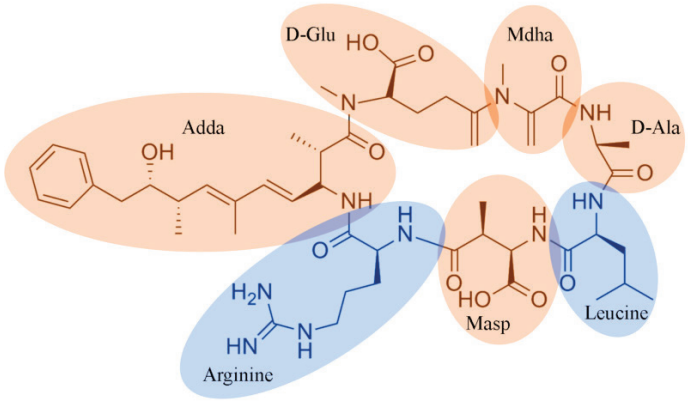
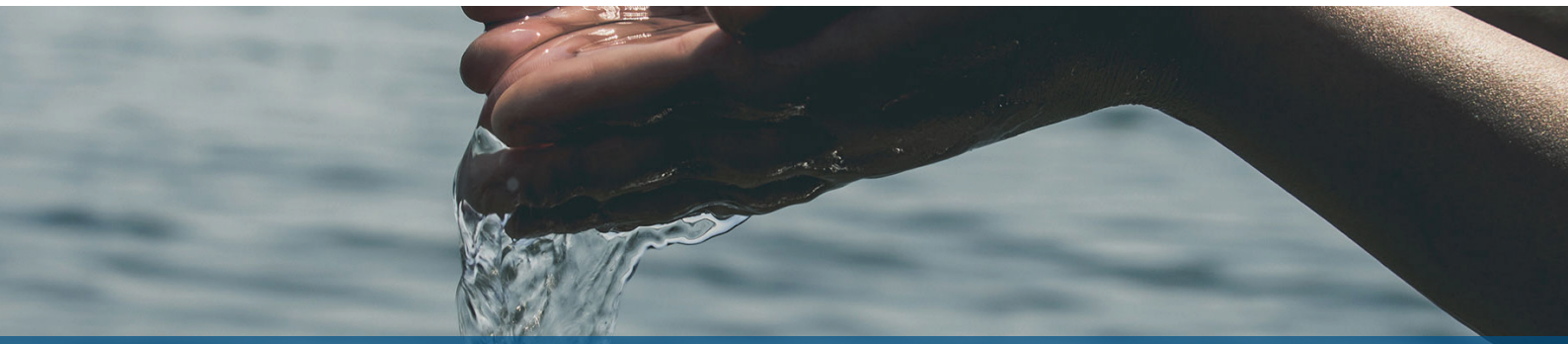


Figure 2: Structure of Microcystin-LR (C49H74N10012, CAS: 101043-37-2).

Over 80 structural variants are known, differentiated by the two variable L-amino acids as well as by chain modifications.

Microcystin-LR is one of the most prevalent and potent microcystins, it is designated as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC).

The need for a rapid, sensitive, and reliable analytical method for MC-LR has been emphasized by the awareness of toxic cyanobacteria as a human-health risk through drinking water.



TECHNICAL DATASHEET

Analysis of Microcystin-LR (MC-LR) in Water

MC-LR Regulatory Guidelines

Microcystins are listed on the United States Environmental Protection Agency's (USEPA) Contaminant Candidate Lists, and the World Health Organization (WHO) has proposed a provisional guideline of 1 µg/L for total microcystin-LR (free plus cell-bound) in treated drinking water. In the UK, there are no regulations for Microcystin analysis. The Drinking Water Inspectorate (DWI) reviewed whether their analysis was necessary in the UK. 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.

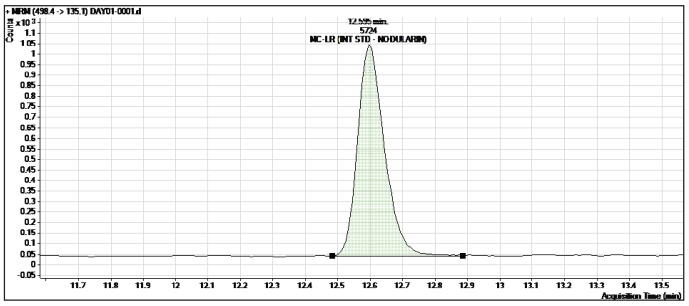
Analysis of MC-LR

Monitoring of water bodies for toxins is often difficult since cyanobacterial blooms may contain complex mixtures of microcystins and sometimes several classes of toxins. The two commonly used biochemical methods for detection of MC-LR are based on enzyme-linked immunoassays assays (ELISA) and protein phosphatase inhibition assays (PPIA). Although these methods have low limits of detection (LOD) (0.05–0.1 µg/L), they may lack detection specificity and lead to false-positive results due to the presence of similar structures to microcystins. In addition, detection methods using reversed-phase liquid chromatography (LC) coupled with ultra-violet detection (UV) have been developed. However, UV detection is susceptible to interferences from water matrices and requires sample cleanup and concentration to achieve desirable detection limits. Furthermore, UV based methods do not provide unequivocal identification of known microcystins. In order to meet these challenging requirements ALS have developed a direct aqueous injection on-line enrichment HPLCMS/ MS method for the determination of Microcystin-LR. It benefits from requiring no sample preparation. The use of this sensitive and selective instrumentation enables ALS to achieve a detection limit (LOD) of 5ng/L for MC-LR in treated and raw water. The range of application for this method is up to 1.250 µg/L.

Table 1: MC-LR Performance Summary for ALS Method WPC59.

Compound	CAS Number	Recovery from Water at 50ng/L	Limit of Detection (LOD)
Microcystin-LR (MC-LR)	01043-37-2	95.3%	5ng/L

Figure 3: Chromatogram of MC-LR calibration standard at 50ng/L.



General Sampling & Preservative Requirements

Bottle: 500ml brown glass bottle preserved with Ascorbic Acid.
Storage: Stored at 5°C.
Holding Time: Samples are stable for 21 days under these storage conditions.

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