

# CYANOBACTERIAL TOXINS



**The Australian Water Quality Centre (AWQC) is dedicated to ensuring and responding to the public health requirements relating to the provision of water and wastewater services for communities in Australia and across the world.**

- Specialist water services
- Ensuring public health

Cyanobacteria, known also as blue green algae, are found in freshwater lakes, reservoirs, rivers, and marine waters. They are of the most concern to many health and water authorities. This is because several species of cyanobacteria can produce potent toxins, which have caused numerous wildlife and domestic animal deaths in many countries.

## **Toxin analyses**

Many techniques are available for determining cyanotoxins, e.g. enzyme linked immunosorbent assay (ELISA), protein phosphatase inhibition assay, capillary electrophoresis (CE), and high performance liquid chromatography (HPLC) coupled with various detection techniques such as photo diode array (PDA), fluorescence and mass spectrometry.

Of all the techniques, HPLC instrumental analysis is the most reliable and accurate. At AWQC, the Organic Chemistry Laboratory has developed many HPLC methods and provides a comprehensive analytical service for a wide range of toxins, including microcystins, nodularins, anatoxin-a, cylindrospermopsins and saxitoxins. We also evaluate other assays such as ELISA and protein phosphatase inhibition assay, and compare them with HPLC methods.

All our HPLC methods are designed to analyse both extracellular toxins (dissolved toxins) and intracellular toxins (toxins inside cyanobacterial cells). All methods are fully validated and many of them are NATA accredited.

### **Hepatotoxins - Microcystins and Nodularins**

Microcystins are cyclic peptides produced mostly by *Microcystis aeruginosa* and several other species. Of over 70 structural variants, about 20 are more frequently detected, in freshwater lakes, reservoirs, rivers and marine water. Nodularin, a similar cyclic peptide, is produced by *Nodularia spumigena*.

Microcystins and nodularin can be determined, simultaneously, by HPLC/DAD/MS. With a sample pre-concentration step, our NATA accredited method can report microcystin-RR, -YR and -LR to 0.05 µg/L, which is well below that in the Australian Drinking Water Guideline. The reporting limit for nodularin is also 0.05 µg/L. Intracellular and extracellular toxin are reported separately, and the total m-LR equivalent is calculated to comply with proposed guidelines.

### **Neurotoxins - Anatoxin-a and Saxitoxins**

Anatoxin-a: Anatoxin-a is neurotoxic alkaloid. Our fully validated HPLC/MS/MS method produces the most reliable and accurate results with a fast turn-around time and an absolute confirmation. The reporting limit is 0.1 µg/L.

Saxitoxins or paralytical shellfish poisons (PSPs): Saxitoxin, known also as PSP, is a neurotoxin naturally produced by certain species of marine dinoflagellates and cyanobacteria.

The PSP neurotoxins consist of 18 compounds with vastly different toxicities and structures and are most difficult to analyse. Several techniques can be used for their determination. However, there is not a method that gives reliable and accurate results, within the required reporting limits for surface water and drinking water, while, at the same time, gives a fast turn-around time.

The analytical method employed at the Organic Chemistry laboratory is the HPLC/post-column derivatisation fluorescence detection technique. The method requires three chromatographic runs and a high level of expertise in instrument operation and result interpretation. It is the only analytical method that can produce a complete separation and determination of saxitoxins at the required reporting limits. This validated method can report the individual toxins to 0.5 µg/L for both intracellular and extracellular toxins. STX equivalent also is calculated for determining the compliance with proposed guidelines.

### **Hepatotoxic Alkaloid Toxin - Cylindrospermopsin**

Cylindrospermopsin can be determined by HPLC/MS/MS method. Our fully validated method provides reliable and accurate results with a fast turn-around time and at the reporting limit of 0.1 µg/L.



### New LC/MS/MS toxin screening methods

1. LC/MS/MS — a screening method for four groups of toxins (total ten toxins)

This method analyses cylindrospermopsins, anatoxin-a, Nodularin, microcystin-RR, YR, LR, LA, LY, LF and LW. It is the fastest and most reliable method to eliminate four groups (ten toxins) with a single screening test.

2. LC/MS/MS — a screening method for thirteen STX analogues

This is the fastest and most reliable screening method for monitoring algal blooms in rivers and reservoirs. It screens thirteen STX analogues and provides details of toxin profile and earlier toxicity during toxic algal blooms.

More details can be provided by contacting AWQC.

### Sampling Requirement for Microcystins and Nodularin:

- Minimum 1 L water sample
- HDPE plastic or glass bottle
- Transport and store at 4°C

### Sampling Requirement for Saxitoxins:

- Minimum 500 mL water sample
- HDPE plastic or glass bottle
- Transport and store at 4°C

### Sampling Requirement for Anatoxin-a and Cylindrospermopsin:

- Minimum 100 mL water sample
- HDPE plastic or glass bottle
- Transport and store at 4°C

