Determination of Microcystins in Blue-green Algae in Lake Water

Along with the recent progression of eutrophication in lakes and marshes, including Lake Biwa and Lake Kasumigaura, etc., the phenomenon known as “blue-green algae”, or “water bloom” has become a matter of public concern. Blue-green algae manifest themselves as a sudden occurrence of greenish powder which appears to have been blown onto the surface of lakes and ponds.

Some of the algae that make up these blooms produce toxins, affecting water quality. One of the typical varieties of these toxins is microcystins. Microcystins are hepatotoxins, and there are cases abroad in which domestic animals died after drinking water where such blue-green algae blooms were present.

The chemical structures of typical microcystins are shown in Fig. 1.1 They are cyclic peptides formed from 7 amino acids, and there are more than 50 variants depending on the types of amino acid bonds in the peptide. Among these, typical varieties found lakes in Japan include LR, YR and RR microcystins. Analysis of microcystins typically includes HPLC, TLC, LC/MS and ELISA methods. Here we introduce the most common method of analysis used, HPLC with UV detection.

Table 1 shows the analytical conditions and Fig. 2 shows a chromatogram of a blue-green algae sample. Separation of the compounds is conducted using the reversed phase mode. As shown in Fig. 3, the retention times of the various microcystins vary independently according to the pH of the buffer solution used as the mobile phase. Here, a buffer solution of pH 3.0 was best as the mobile phase for separation of the 3 types of microcystins. Detection was conducted by monitoring the UV absorption at 240 nm. Fig. 4 shows the UV spectra obtained from measurement of collected microcystins using a photodiode array UV-VIS detector.
The pretreatment procedure for the blue-green algae sample is shown in the Scheme 1. For extraction of the microcystins from the blue-green algae, here we used 5% acetic acid. Stirring and centrifuge separation was conducted 3 times, and the following cleanup was performed for the supernatant as well.

Cleanup was performed using the solid phase extraction method. Adsorption of the components was conducted by passing the above-mentioned extraction fluid through a commercial solid phase ODS extraction cartridge, and after washing once, elution was conducted using methanol. In the final elution operation, the most effective elution is achieved with just a small amount of solution, obtained by using as slow a flow rate as possible (1 to 2 mL/min).

Here we performed washing with a water/methanol = 9/1 solution and elution with 100% methanol, however, by increasing the methanol ratio in the wash solution (about 8/2), and adding water to the eluate (water/methanol = 1/9 to 2/8), the solvent front peak becomes smaller to obtain a cleaner chromatogram.

The sample was provided by Dr. Kaya of the Environmental Chemistry Division of the National Institute for Environmental Studies.

References:
1) K. Kaya : Environmental Chemistry,

NOTES:
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