Degradation of algae and microcystin – Analysis of mircocystin by LC-MS/MS

Aim

In order to detect the degradation effect of microcystin when reacting with enzymes, the residual concentration of algal toxin mixed with enzymes was determined by High performance liquid chromatography (HPLC). HPLC has been widely used for the analysis of microcystin. The specific methods for detection of algae toxin by HPLC are as follows.

Materials

Microcystin-LR (MC-LR) 10μg/mL in methanol (Sigma-Aldrich, USA),

Microcystin-RR (MC-RR) 10μg/mL in methanol (Sigma-Aldrich, USA),

Phosphate-Buffered Saline (PBS) 10X, pH 7.4, RNase-free (Thermo Fisher, USA), enzymes developed from amphioxus intracellular digestion,

Methanol (HPLC grade) was purchased from Merck KGaA (Germany).

Procedure

Qualitative and quantitative analyses of MCs were performed on an Agilent 1200 HPLC system coupled with a triple quadrupole mass spectrometer in positive ion mode of electrospray ionization. A C18 column (250 mm x 2.1 mm i.d., 5 μm, 100 Å Alltima) was operated at 30 ℃ to separate five MC

compounds. The mobile phase solvents A and B were 0. 1% formic acid in water and methanol, respectively, and the flow rate was 0.400 mL/min. The optimized gradient elution for solvent B was as follows: 0-1 min, 40%, 1-16 min, 80%, and followed by 4 min for equilibrium with 40% solvent B. The injection volume was 5.00 μL and two microcystins were well separated and eluted within 13.0 min.

Direct infusion MS/MS was used to obtain the mass transitions (see Table 1) for multiple reaction monitoring(MRM) of MCs based on their fragmentation patterns.

Table 1
Mass spectrometry characteristics of the target microcystins determined by LC-MS/MS.

Compound	Mol wt.	Retention time(min)	Precursor ion	Product ion	DP[a]	CE[b]
Microcystin-RR	1038.2	2.14	520.1[M + 2H]$^{2+}$	135[c]/127[d]	110	40/65/85
Microcystin-LR	995.2	5.68	995.7[M + H]$^{+}$	135/213	160	95/80/70

[a] DP: decluster potential
[b] CE: collision energy voltages
[c] Quantitative ion
[d] Qualitative ion

Note

The following points should be paid attention to when using HPLC.

(1) Analytical methods of microcystins were established by using various standard samples to ensure baseline separation of microcystins (such as MC-LR, MC-RR) with similar retention time on C18 chromatographic column. In order to improve the accuracy of analytical results, the interference between different microcystins was eliminated to a certain extent in instrumental analysis.

(2) Avoid using plastic materials in sampling, pretreatment and analysis. Dissolved substances in plastics can interfere with the analysis and produce false positive results.

(3) In the pretreatment, a certain proportion of organic phase is needed in the solvent of microcystin resolving. Because microcystins are hydrophobic, they can be adsorbed into the filter membrane in the filtration process. Increasing the organic proportion of solvents can inhibit the adsorption on the filter membrane and improve the recovery rate.