SHIMADZU

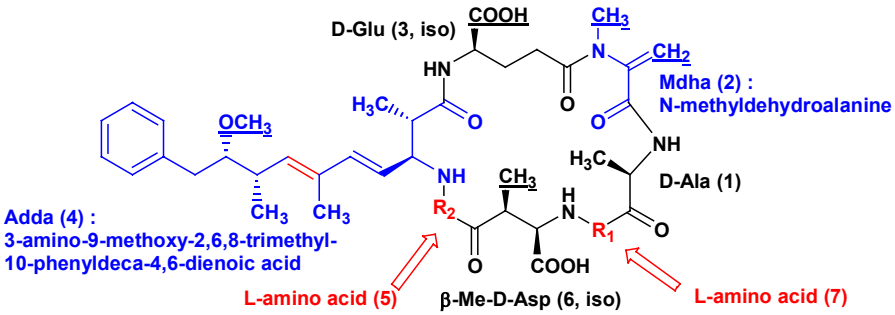
LC-MS Application Data Sheet No. 045

Analysis of Microcystins Using LC-MS

Microcystins (Fig. 1) are a liver toxin (carcinogen) produced by blue-green algae, which has increased at an abnormal rate in many water bodies due to eutrophication. In the Drinking Water Testing Method of Japan revised in 2001, HPLC and LC-MS are introduced as methods to analyze microcystins. In these methods, samples are concentrated by a factor of 500 using a solid-phase extraction cartridge, and then analyzed by HPLC or LC-MS.

The two methods, however, have different lower of limits quantitation—approx. 100 ng/L for HPLC and approx. 10 ng/L for LC-MS. The testing method also prescribes a precaution for the HPLC analysis: It says, "when analyzing raw water sample with UV detector (238 nm), an interference peak is observed near the retention time of microcystins". This low selectivity provents this HPLC method from detecting low concentrations of microcystins, thus sufficient sensitivity of the method could be not assured. In this example, microcystins (RR, YR, LR) were analyzed with LC-MS under the conditions prescribed in the Drinking Water Testing Method.

Figs.2 and 3 show selected ion monitoring (SIM) chromatograms at the concentration prescribed in provisional WHO guidelines (1000 ng/L) and the LC-MS determination limit (10 ng/L) obtained by the LC-MS method stipulated in the Drinking Water Testing Method. Fig.4 shows SIM and UV chromatograms for blank water obtained by the pretreatment procedure stipulated in the Drinking Water Testing Method.



Microcystin RR : R1= L-Arg (R), R2= L-Arg (R), MW 1037
Microcystin YR : R1= L-Tyr (Y), R2= L-Arg (R), MW 1044
Microcystin LR : R1= L-Leu (L), R2= L-Arg (R), MW 994

Fig. 1 Structures of microcystins RR, YR and LR

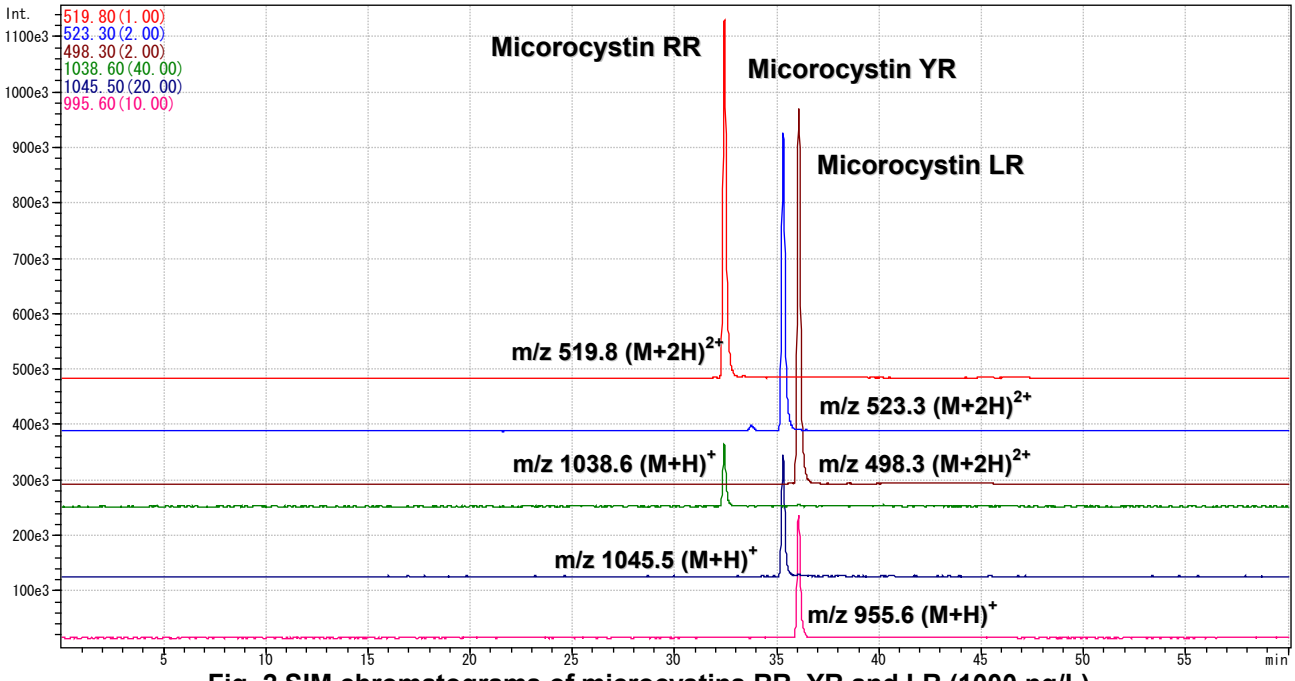


Fig. 2 SIM chromatograms of microcystins RR, YR and LR (1000 ng/L)

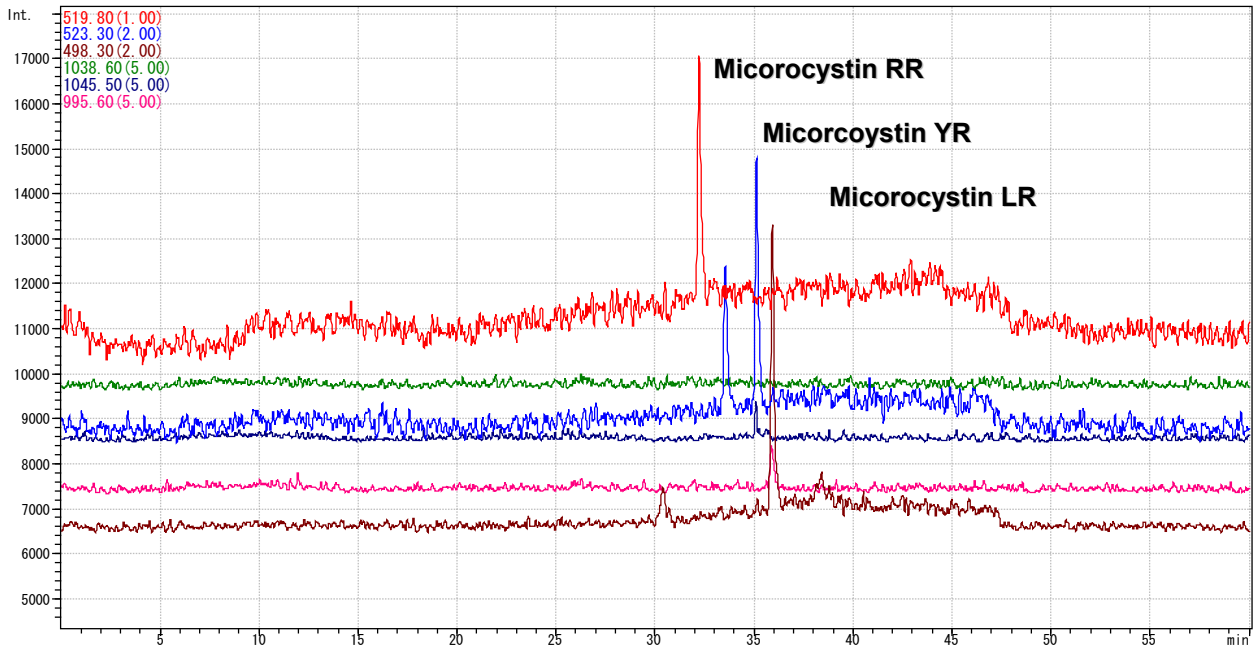


Fig. 3 SIM chromatograms of microcystins RR, YR and LR (10 ng/L)

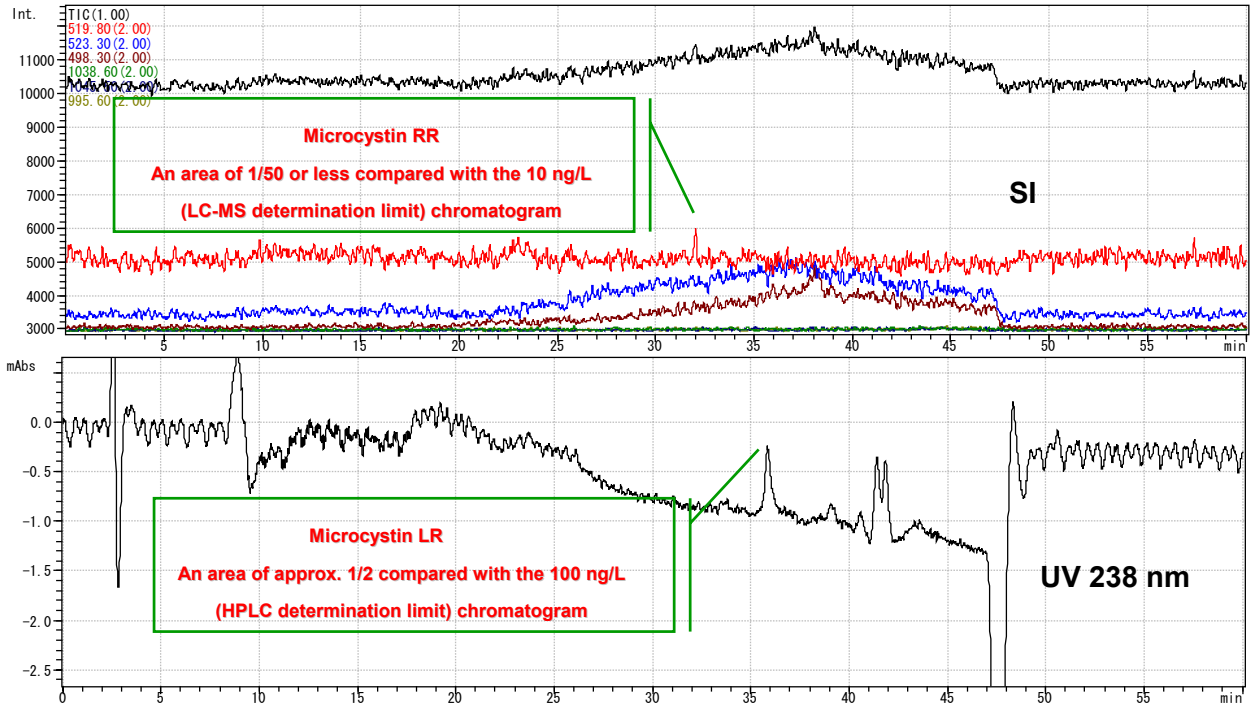


Fig. 4 SIM and UV chromatograms of blank according to the concentration procedure

Table 1 Analytical conditions for LC-MS

Column	: Shim-pack VP-ODS (2.0 mm I.D. x 150 mm)		
Mobile phase A	: 0.05% trifluoroacetic acid-water		
Mobile phase B	: acetonitrile		
Time program	: 10% B (0-15 min) -> 40% B (35-45 min)		
Flow rate	: 0.2 mL/min		
Injection volume	: 0.1 mL	**Column temperature**	: 40°C
Probe voltage	: +4.5kV (ESI-Positive mode)		
CDL temperature	: 200°C	**BH temperature**	: 200°C
Nebulizing gas flow	: 4.5 L/min		
CDL voltage	: +25 V		
Q-array DC voltage	: Scan-mode	**Q-array RF voltage**	: Scan-mode
SIM monitoring ions	: m/z 519.8, 1038.6, 523.3, 1045.6, 498.3, 955.6		

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