Analysis of Microcystin by GC/MS

■ Introduction

Microcystin is a liver toxin produced by blue-green algae, which occur in large quantities due to eutrophication in lakes and marshes. The toxicity, expressed in terms of the median lethal intraperitoneal dose (LD/50) in mice, is about 100μg/kg. Efforts are being made to develop technology to control this generation of the toxic blue algae and ways to

neutralize such toxic substance. However, it is difficult to control all water sources, and ensuring the safe supply of drinking water is an important issue. This Application News introduces an example of analyzing 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB), a decomposition product of microcystin.

■ Outline of Analysis

Generally, microcystins are extracted from the water sample, the conjugate double bonds are decomposed by oxidization using potassium permanganate / sodium periodate, the 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) generated is methylated and then quantitated using GC/MS. The converted equivalent quantities of microcystin-L and R are determined. MMPB-d3 is used as an internal standard substance.

Fig.1 Microcystin-LR

Fig.2 MMPB Derivation from Microcystin

Table 1 Analytical conditions for GC/MS

[GC]

 $\label{eq:column} \text{Column} \qquad :DB\text{-}1(30m\!\!\times\!\!0.25mm \text{ I.D. df=}0.5\mu\text{m})$

Column Temp :80°C(1min)-5°C/min-250°C

Carrier Gas :He 64.5kPa Injection Mode :Splitless Injection Volume :1µL Sampling Time :1min [MS]

Interface Temp :250°C

Ionized Method:CI(Isobutane)100kPa

 <SCAN MODE>
 <SIM MODE>

 Scan Range
 :m/z 100-300
 :m/z 223, 226

 Interval Time
 :0.5sec
 :0.2sec

■ Analysis with the El Scan Mode

The MMPB chromatogram obtained by the scan mode EI (electron ionization) method is shown below. (Refer to Table 1 for analytical conditions.)

In the mass spectrum obtained by the EI method, molecular ions cannot be obtained and the mass number of the strong peak is small, possibly

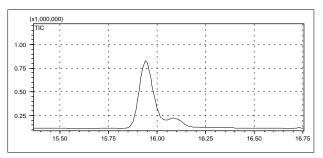


Fig.3 MMPB Total Ion Chromatogram by EI method

overlapping with impurity peaks. Furthermore, MMPB and MMPB-d3 (internal standard substance) generate extremely similar mass spectra, making separation quite difficult. Therefore the CI (chemical ionization) method was attempted.

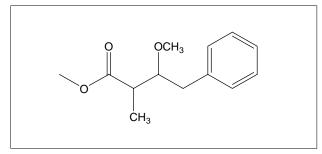


Fig.4 MMPB Structure

■ Analysis with the CI SIM Mode

An example of analyzing MMPB and MMPB-d3 using the SIM method is shown at right.

Isobutane was used as a reaction gas. The target ions were the quasi-molecular ion $(MH)^+$ at m/z = 223 for MMPB, and m/z = 226 for MMPB-d3. (Refer to Table 1 for analytical conditions.)

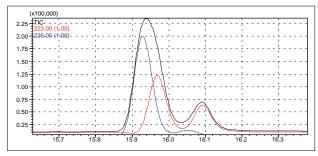


Fig.5 SIM Chromatograms with CI method

■ Calibration Curve

The calibration curve was created by preparing MMPB solutions of three different concentrations (24ppb, 120ppb and 600ppb). (SIM mode analysis; See Table 1 for analytical conditions.) A calibration curve with

Area ratio
7.0
6.0
5.0
4.0
2.0
1.0
0.0
2.5
5.0 Concentration ratio

Fig.6 Calibration Curve

good linearity was obtained. 100ppb of MMPB-d3 was added to each solution as the internal standard substance.

Compound Name: MMPB m/z: 223 f(x)=1.168354*x+0.000000Correlation coefficient (R) =0.999972 Contribution ratio (R²)=0.999943 Mean RF: 1.16 RFSD: 0.03 RFRSD: 2.48 Calibration curve: Linear Zero intercept: Yes Weighting: None Internal standard method Concentration ratio Mean area ratio 0.240 0.28 2 1.200 1.36

7.02

3

6.000