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Application News

No.C104

Liquid Chromatography Mass Spectrometry

Analysis of Diarrhetic Shellfish Toxin Using Triple Quadrupole LC/MS/MS (LCMS-8050)

The Japanese Ministry of Health, Labour and Welfare (JMHLW) specified in July, 1980 that the mouse bioassy (MBA) be used as the official method for diarrhetic shellfish toxin, and that the permissible exposure limit be 0.05 MU per gram of edible shellfish*). Shellfish in which the toxin exceeds this limit are prohibited from being sold at market according to the Japanese Food Sanitation Law Article 6, Item 2.

Due to significant technological advances since 1980, the sensitivity and accuracy obtained using the MBA method are significantly inferior compared to the high-precision, high-sensitivity possible using liquid chromatography mass spectrometry analytical instrumentation, which is currently used for this application. A complete transition to instrumental analysis for lipophilic marine biotoxins is scheduled to be implemented by January 2015 throughout the EU.

Based on this international trend, the JMHLW is currently considering migration to an instrumental analysis assay and setting new reference values to be used with instrumental analysis, in addition to the introduction of the Codex standard for okadaic acids (OA, Reference 1).

Table 1 CODEX Standard 292-2008

	Reference Value
OA Acids (OA and DTX group)	Permissible ingestion limit of 0.16 mg OA per kg of edible shellfish

Fig. 1 shows examples of LC/MS/MS high-sensitivity analysis of okadaic acid (OA), dinophysistoxin 1 (DTX1) and pectenotoxins (PTX1, 2, 6) and yessotoxin 1 (YTX1). Thus, it is possible to conduct high-sensitivity, high-separation analysis of each component.

Fig. 2 and Fig. 3 show MRM chromatograms of standard samples of OA and DTX1, respectively.

* The amount of toxin resulting in the death of two out of three mice following intraperitoneal administration of the equivalent of 20 g per edible shellfish.

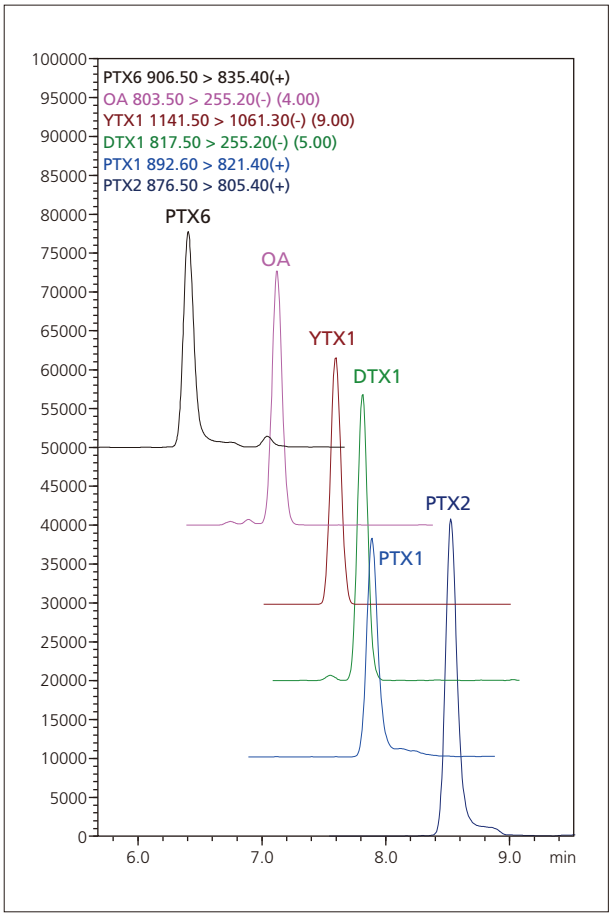

Fig. 1 MRM Chromatograms of Diarrhetic Shellfish Toxin (1 ng/mL)

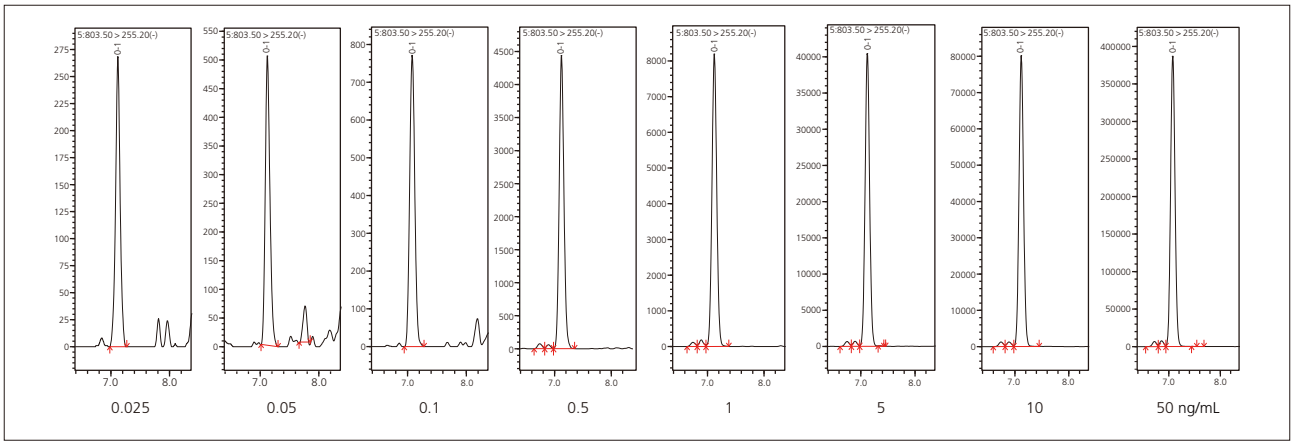

Fig. 2 MRM Chromatograms of Okadaic Acid (OA)

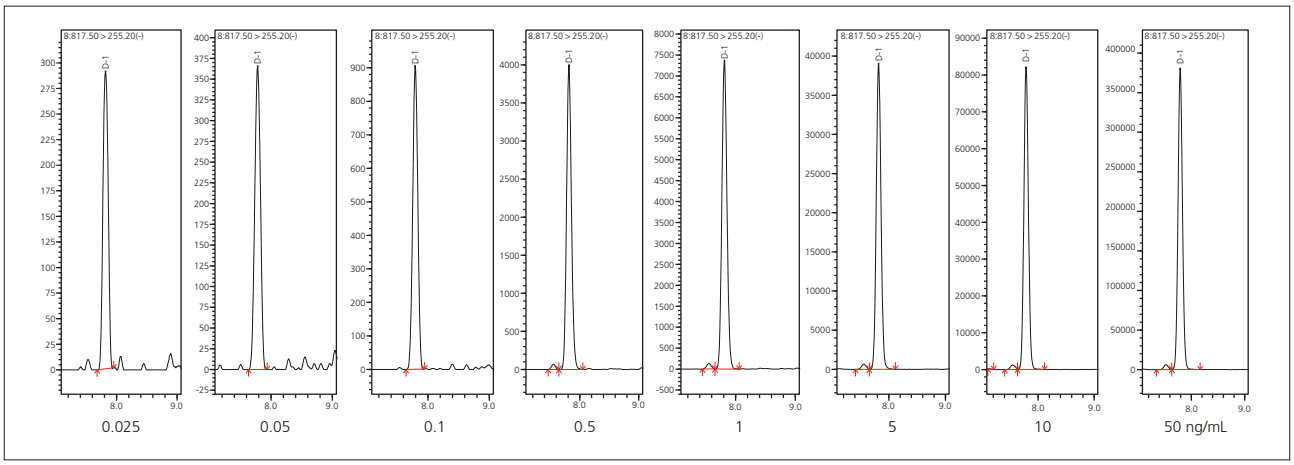

Fig. 3 MRM Chromatograms of Dinophysistoxin 1 (DTX1)

In addition, the calibration curves of OA and DTX1 are shown in Fig. 4. In both cases, the coefficient of determination R^2 was greater than 0.9999, indicating excellent linearity. Comparable linearity was also obtained for the other four substances.
Thus, instrumental analysis of shellfish by LC/MS/MS offers high sensitivity and accuracy, making it a highly effective analytical method. For this reason it is attracting attention as an alternative to the traditional MBA method.

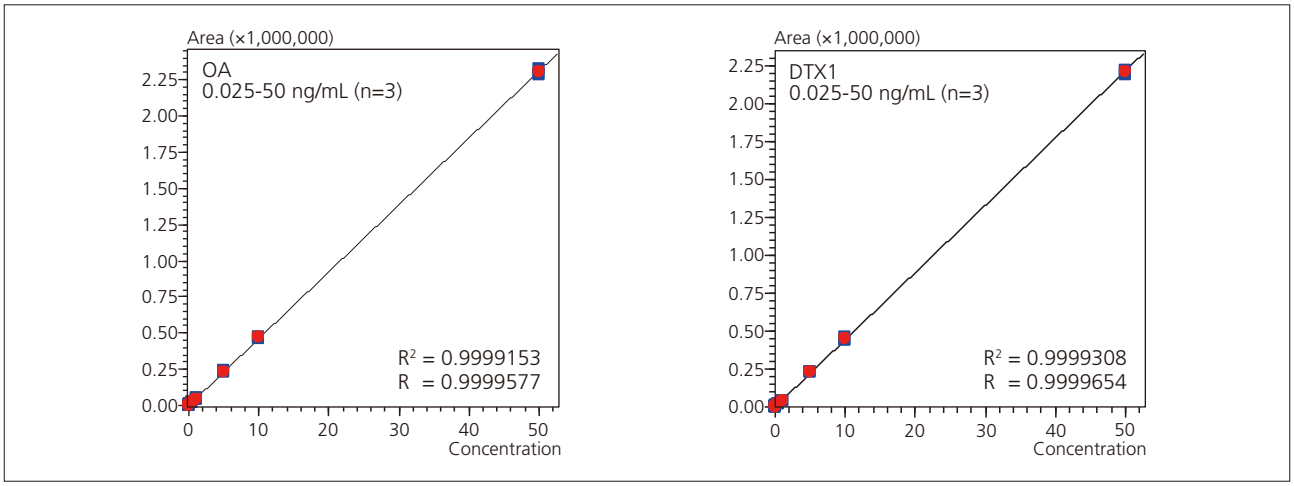

Fig. 4 Calibration Curves of OA and DTX1

Table 2 Analytical Conditions

Column	: InertSustain C8 (50 mm L. × 2.1 mm I.D., 3 µm)
Mobile Phases	: A 2 mmol/L Ammonium Formate – Water (pH adjusted to 8.5 with ammonia water)
	: B Methanol
Time Program	: 20 %B (0 min) – 100 %B (10 min) – 20 %B (10.01 min) – STOP (15 min)
Flowrate	: 0.2 mL/min
Column Temperature	: 40 ˚C
Injection Volume	: 10 µL
Probe Voltage	: +4.0 kV/-3.0 kV (ESI-positive / negative mode)
DL Temperature	: 200 ˚C
Block Heater Temperature	: 400 ˚C
Interface Temperature	: 350 ˚C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min
MRM Transition	: (+) PTX6 906.50 > 835.40, PTX1 892.60 > 821.40, PTX2 876.50 > 805.40
	: (-) OA 803.50 > 255.20, YTX1 1141.50 > 1061.30, DTX1 817.50 > 255.20

The diarrhetic shellfish toxin standards were provided courtesy of Dr. Toshiyuki Suzuki of the Japanese National Research Institute of Fisheries Science.

Reference 1: July, 2014, Food Safety Commission of Japan "Natural Poison Evaluation Report – Okadaic Acid Group Among Bivalves"
http://www.fsc.go.jp/fsciis/evaluationDocument/list?itemCategory=009

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