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MP-205

Highly sensitive analysis of the related substance of ciguatoxins by the multiple reaction monitoring and electrospray ionization with LC/MS/MS

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1. Overview

The development of high sensitivity and selectivity analytical conditions for the determination of CTXs using MRM predicted on CID fragmentations by LC/MS/MS.

2. Introduction

Ciguatoxins (CTXs) are known as the seafood poisoning from fish around the tropical region. They have approximately 100 times stronger toxicity than puffer fish poison (Tetrodotoxin). Although one fish contains a very little amount of them each, many related substances among CTXs have been discovered. Thus, highly sensitive and selective analytical methods are strongly required for the determination. In previous reports, the sodium adducted ions have been used to determine by LC/MS/MS (ESI). In this study, we attempted to analyze five ciguatoxins (CTX1B, 52-epi-54-deoxyCTX1B, 51-hydroxyCTX3C, CTX3C, CTX4A) using multiple reaction monitoring. We designed the transition for MRM of each CTXs considering the product ions by collision-induced dissociation (CID) from protonated or ammonium adduct ions. We have developed the conditions in order to detect CTXs with high sensitivity and selectivity.

3. Methods and Materials

3-1. Standard Compounds

Five kinds of standard ciguatoxins were provided by Dr. Takeshi Yasumoto (Japan Food Research Laboratories). Figure 1 shows the structure of the five compounds. Ciguatoxin is a 3 nanometer long macromolecule consisting of 13 (ABCDEFGHIJKLM ring) cyclic ethers. CTX1B and 51-hydroxyCTX3C have hydroxyl residue in the M ring. CTX1B and 52-epi-54-deoxyCTX1B have hydroxyl residue in the A ring side. CTX1B has the most hydrophilic properties. CTX1B (43.3±1.3 ng), 52-epi-54-deoxyCTX1B (58.4±2.5 ng), 51-hydroxyCTX3C (45.3±7.2 ng), CTX3C (38.5±2.6 ng), and CTX4A (55.1±5.2 ng) standards were quantified by qNMR, respectively. Each stock solution was dissolved in 1 mL of methanol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The original 5 compounds mixture solution (approximate concentration: 10 ppb, each) was prepared from the five stock solutions in equal proportions followed by dilution with methanol to the using concentrations.

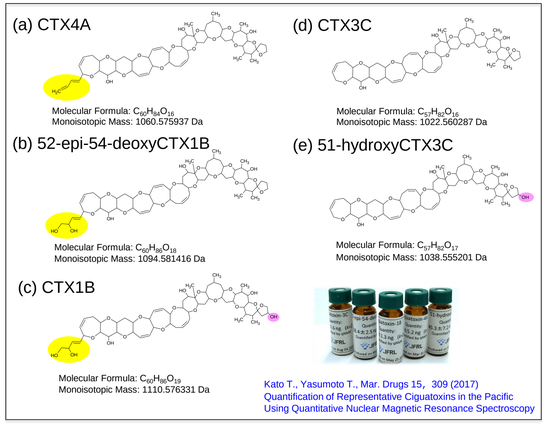

Figure 1 The structure of the five compounds.
(a) CTX4A, (b) 52-epi-54-deoxyCTX1B, (c) CTX1B, (d) CTX3C, (e) 51-hydroxyCTX3C.

3-2. Analytical conditions

The analysis was performed by a liquid chromatograph followed by a triple quadrupole tandem mass spectrometer, LCMS-8060 equipped with a Nexera™ X2 UHPLC system (Shimadzu Corporation, Kyoto, Japan). The chromatographic separation was performed by a Shim-pack Scepter™ C18-120 and a Shim-pack Velox™ C18 (Shimadzu Corporation).

■UHPLC conditions (Nexera™ X2 system)

Column	: Shim-pack Scepter ™ C18-120 (50 mm × 2.0 mm I.D., 1.9 µm)
Column	: Shim-pack Velox ™ C18 (50 mm × 2.1 mm I.D., 1.8 µm)
Mobile phase A	: 5 mmol/L ammonium formate aqueous solution containing 0.1% formic acid
Mobile phase B	: Methanol
Flow rate	: 0.4 mL/min
Time program	: B Conc. 60 % (0.0 min) →88 % (10.0 - 14.0 min) → 60 % (14.01 - 18.0 min)
Column temp.	: 40 ℃
Injection vol.	: 5 µL

■MS conditions (LCMS-8060 & LCMS-9030)

Ionization	: ESI, Positive MRM mode
IF voltage	: +4 KV
Interface temp.	: 300 ℃
DL temp.	: 190 ℃
Heat block temp.	: 400 ℃
Nebulizer gas	: 3 L /min
Heating gas	: 10 L/min
Drying gas	: 10 L/min
CID gas press.	: 270 KPa
MRM transition	: refer to Table 1

LCMS-8060 (TQ)

LCMS-9030 (Q-TOF)

Table 1. MRM transition list for the triple quadrupole tandem mass spectrometry.

Event	Compound	ch	Precursor ion (m/z)	Product ion (m/z)	Collision Energy (V)	Event	Compound	ch	Precursor ion (m/z)	Product ion (m/z)	Collision Energy (V)	Event	Compound	ch	Precursor ion (m/z)	Product ion (m/z)	Collision Energy (V)
1	CTX1B	1	1111.6	149.1	-41	3	51-hydroxy-CTX3C	1	1039.6	149.1	-54	5	CTX4A	1	1061.6	155.1	-33
		2	1111.6	121.0	-54			2	1039.6	121.0	-44			2	1061.6	125.1	-42
		3	1128.6	149.1	-54			3	1056.6	149.1	-52			3	1078.6	155.1	-33
		4	1128.6	121.0	-40			4	1056.6	121.0	-46			4	1078.6	125.1	-48
		5	1128.6	1111.6	-19			5	1056.6	1039.6	-15			5	1078.6	1061.6	-18
		6	1128.6	1093.6	-21			6	1056.6	1021.6	-20			6	1078.6	1043.6	-19
2	52-epi-54-deoxyCTX1B	1	1095.6	155.1	-40	4	CTX3C	1	1023.6	155.1	-36						
		2	1095.6	125.1	-54			2	1023.6	125.1	-48						
		3	1112.6	155.1	-37			3	1040.6	155.1	-32						
		4	1112.6	125.1	-37			4	1040.6	125.1	-42						
		5	1112.6	1095.6	-20			5	1040.6	1023.6	-24						
		6	1112.6	1077.6	-22			6	1040.6	1005.6	-20						

4. Results

4-1. CID Spectrum of CTX3C

For example, the CID spectrum of CTX3C by LCMS-9030 (Q-TOF) is shown in Figure 2. The product ions were observed as *m/z* 155.10 and 125.10 which pertain to the specified structure (L-ring) in CTX3C in addition to the dehydrate-ion: $[M+H-H_2O]^+$ and $[M+H-2H_2O]^+$. Similar product ions were also detected in CTX4A, 52-epi-54-deoxyCTX1B. MRM transitions were optimized based on the two precursor ions ($[M+H]^+$ & $[M+NH^4]^+$) and four product ions including $[M+H]^+$, $[M+H-H_2O]^+$ (Table 1).

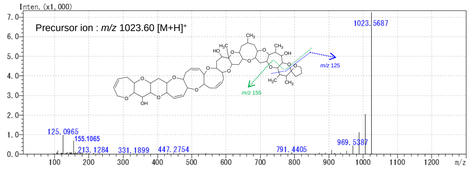

Figure 2 The CID spectrum of CTX3C (CE 0-100 V, ID off mode)

4-2. Separation Study by Two High Performance ODS Columns

Two different columns were evaluated on the same time program of mobile phase (Fig. 3, 4). The Sim-pack Scepter using an organo-silica hybrid substrate was characterized by strong retention and excellent separation (Initial pressure 42 MPa). On the other hand, the Sim-pack Velox column employs a core shell technology and is characterized by a low back pressure (Initial pressure 38 MPa) and a sharp peak shape. Peak height using the Velox column were approximately twice as high, except for 52-epi-54-deoxyCTX1B (Fig.5).

Shim-pack Scepter™

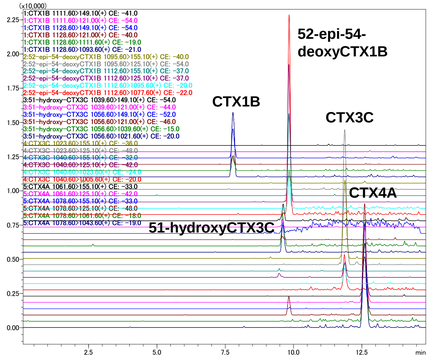

Figure 3 MRM Chromatograms by Shim-pack Scepter™ C18-120 (Conc. 10 ppb each)

Shim-pack Velox™

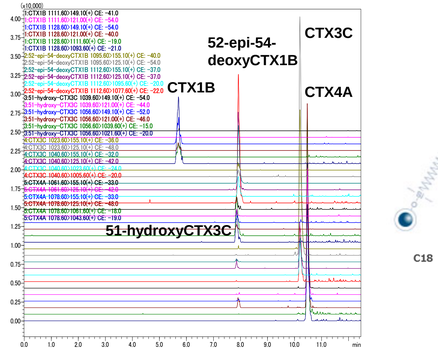

Figure 4 MRM Chromatograms by Shim-pack Velox™ C18 (Conc. 10 ppb each)

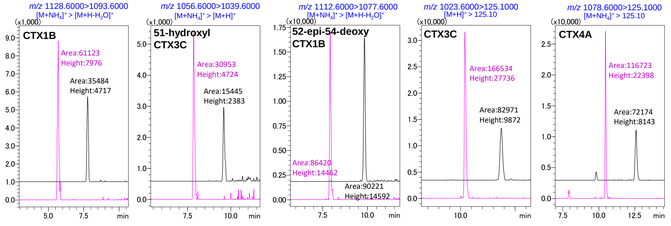

Figure 5 Comparison of the two columns for the major peaks of 10 ppb (Black: Scepter, Pink: Velox)

4-3. Quantitative Assessment

Quantitative evaluation test of the analysis using a Velox column was performed. The selected results of MRM transition having the best accuracy and area repeatability are showed below. The chromatogram at low concentrations (0.5 ppb) and calibration curve are shown in Fig. 6. The calibration curve was prepared from 0.1 ppb especially for CTX3C.

Table 2 shows accuracy and area repeatability (n=6). These results suggest that highly sensitive quantification is possible using a Velox column with the optimized condition.

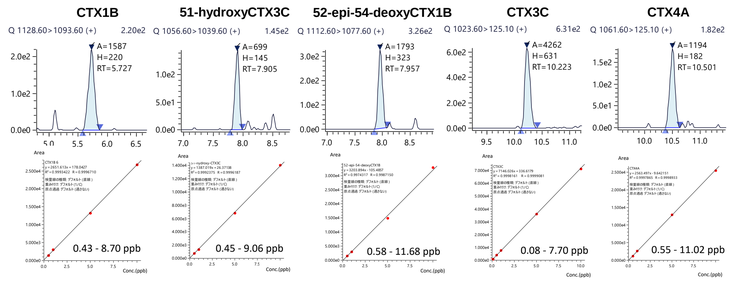

Figure 6 Chromatograms (0.5 ppb) and Calibration curves of CTXs

Table 2 The accuracy and area repeatability (%, n=6)

		Compound	CTX1B	51-hydroxy-CTX3C	52-epi-54-deoxyCTX1B	CTX3C	CTX4A
		m/z	1128.60 >1093.60	1056.60 >1039.60	1112.60 >1077.60	1023.60 >125.10	1061.60 >125.10
		Retention time (min)	5.73	7.89	7.96	10.23	10.50
		limit of quantitation (ppb)	0.43	0.45	0.58	0.08	0.55
Level	Conc. (ppb)	Correlation coefficient	0.9993422	0.9992375	0.9974317	0.9998161	0.9997865
1	0.1	Area %RSD (%)				18.46	
		Accuracy				94	
2	0.5	Area %RSD (%)	11.75	11.88	14.29	6.7	12.31
		Accuracy	94	107	106	102	96
3	1	Area %RSD (%)	5.91	18.60	13.35	5.82	14.48
		Accuracy	107	94	96	104	103
4	5	Area %RSD (%)	5.2	6.36	6.15	6.44	4.41
		Accuracy	99	98	94	100	101
5	10	Area %RSD (%)	2.24	6.83	5.91	1.19	4.13
		Accuracy	100	101	103	99	99

5. Conclusions

- ✓ Optimized the best MRM transitions for the five CTXs.
- ✓ A high-performance new ODS columns were evaluated.
- ✓ High-sensitivity simultaneous analysis of five CTXs was successful.
- ✓ Quantification of CTXs was made possible below 0.5 ppb concentration level.

6. Acknowledgements

We are grateful to Dr. Takeshi Yasumoto for providing the standard of CTXs.

7. Reference

- Yogi, K.; Oshiro, N.; Inafuku, Y.; Hirama, M.; Yasumoto, T. Detailed LC-MS/MS analysis of ciguatoxins revealing distinct regional and species characteristics in fish and causative alga from the Pacific. Anal. Chem. 2011, 83, 8886-8891.
- Yogi, K.; Oshiro, N.; Matsuda, S.; Sakugawa, S.; Yasumoto, T. Toxin profiles in fish implicated in ciguatera fish poisoning in Amani and Kakeroma islands, Kagoshima prefecture, Japan. Food Hyg. Saf. Sci. (Shokuhin Eiseigaku Zasshi) 2013, 54, 385-391.