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Determination of the Derivatives of Nitrofuran Metabolites in Marine Products by Ultra High Performance Liquid Chromatography / Triple Quadrupole Mass Spectrometry

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1. Introduction

This poster describes a method by ultra high performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) to analyze 4 derivatives of nitrofuran metabolites in marine products. Thanks for the high sensitivity and selectivity of UHPLC/MS/MS method, determination of these compounds was successful.
The limit of quantitation (LOQ) of AMOZ was 0.4 µg/kg, which is lower than the guideline value (0.5 µg/kg) proposed by the USFDA.
The derivatives of nitrofuran metabolites used in breeding of marine products are known to have a carcinogenic effect. Quantities of these compounds in marine products were regulated by the USFDA. This paper describes a UHPLC/ESI-MS/MS method to determine 4 derivatives of nitrofuran metabolites. The method is simple, rapid and highly sensitive and meets the requirements.

2. Methods and Materials

Sample Preparation

(1) Weighing 2.0 g of the sample in a 50 mL centrifuge tube,

(2) Add 10 mL of methanol - water (1:1) to the centrifuge tube, shaking for 10 minutes

(3) Centrifuge at 4000 rpm for 5 minutes.

(4) Discard the remaining solution in the centrifuge tube, add 10 mL of 0.2 mol/L hydrochloric acid, homogenize in a refiner.

(5) Add 100 µL of o-nitrobenzaldehyde and the centrifuge tube and vortexed for 30 seconds, shake for 30 minutes, reaction for 16 hours at 37°C incubator.

(6) Samples were removed by adding 2 mL of 0.3 mol/L potassium phosphate. The pH was adjusted at 7.4 using 2.0 mol/L sodium hydroxide.

(7) Extract the sample with 10 mL of ethyl acetate. Shake for 10 minutes, centrifuged at 10000 rpm for 10 minutes for collecting the ethyl acetate solution. Repeat this process twice.

(8) Drying of the ethyl acetate solution at 40°C with nitrogen. The residue was dissolved with 1.0 mL of 0.1% aqueous formic acid solution. Remove fat with n-hexane. Filtration of the lower water phase with a microporous membrane.

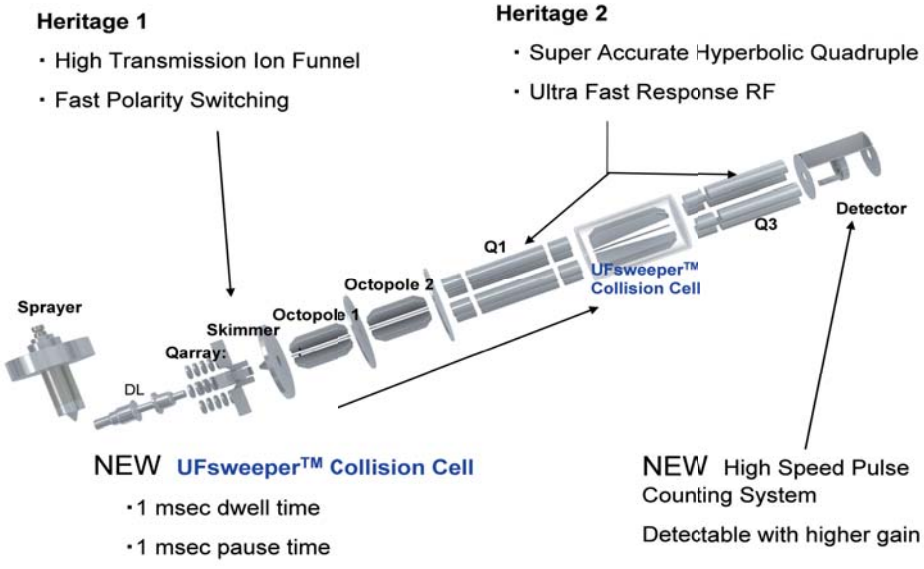
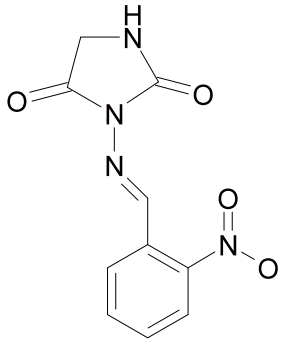
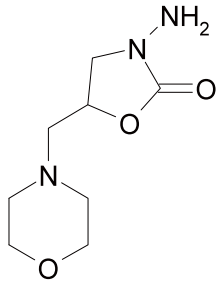


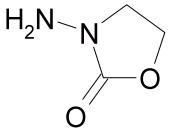
Fig. 1 Schematic diagram of LCMS-8030



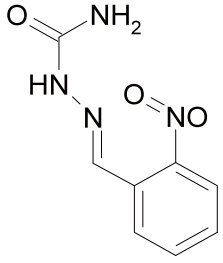
1-Amino-hydantoin (AHD)



3-Amino-5-morpholinomethyl-2-oxazolidinone (AMOZ)



3-Amino-2-oxazolidinone (AOZ)



Semicarbazide (SEM)

Fig. 2 Analytes

UHPLC/MS/MS Analysis

The analyses were performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, a CTO-30A column oven, a DGU-30A$_3$ degasser, and an SIL-30AC autosampler. The separation was carried out on a Shim-pack XR-ODSIII column (150 mmL. × 2.0 mmi.d., 2.2 µm) with the column temperature at 40°C. The mobile phase consisted of (A) 0.02% formic acid water and (B) acetonitrile using a gradient elution of 40%B (0 min)-95%B (1.5 min)-40%B (1.51-4.0 min). The flow rate was 0.4 mL/min. A triple quadrupole mass spectrometer (Shimadzu LCMS-8030, Kyoto, Japan) was connected to the Shimadzu UHPLC instrument via an ESI interface. The MRM chromatograms were obtained in positive ion mode.

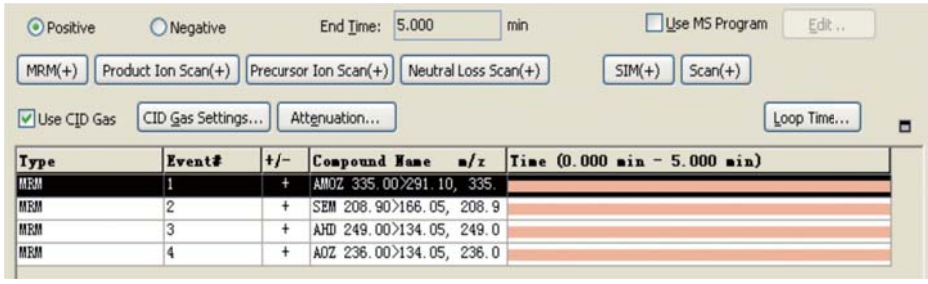
Analytical Conditions

UHPLC (Nexera system)

Column	: Shim-pack XR-ODS III column (150 mm × 2.0 mmID, 2.2 µm)
Mobile Phase A	: 0.02% formic acid water
Mobile Phase B	: acetonitrile
Gradient Program	: 40%B (0 min)-95%B (1.5 min)-40%B (1.51-4.0 min)
Flow Rate	: 0.4 mL/min
Column Temperature	: 40°C
Injection Volume	: 20 µL

MS/MS (LCMS-8030 triple quadrupole mass spectrometer)

Ionization	: ESI
Polarity	: Positive
Probe Voltage	: +4.5 kV (ESI-Positive mode)
Nebulizing Gas Flow	: 3.0 L/min
Drying Gas Pressure	: 20 L/min
DL Temperature	: 300°C
BH Temperature	: 500°C



MRM parameter

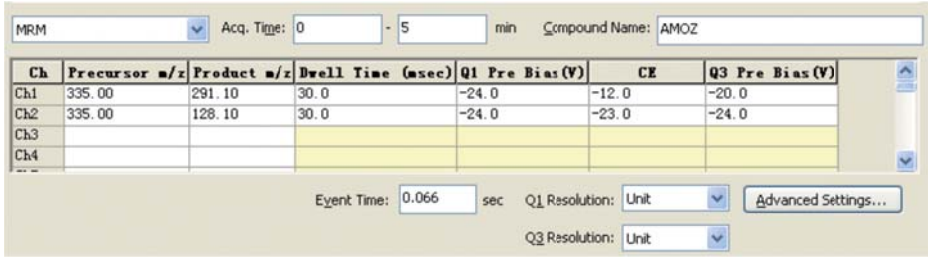


Fig. 3 User interface of MRM setting on LabSolutions software

Simple Quantitative Method

Based on the chromatograms obtained by injection of a fixed volume of individual reference standard solutions, the calibration curves of the 4 derivatives of nitrofuran metabolites were prepared. The MRM parameters are shown in Table 1.

Table 1 MRM mode parameters of 4 marine toxins

No.	Compound	Procursor Ion (*m/z*)	Product Ion (*m/z*)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	AOZ	236	134.10* 104.05	-18.0 -18.0	-12.0 -24.0	-29.0 -22.0
2	AMOZ	335	291.00* 262.00	-13.0 -18.0	-11.0 -17.0	-22.0 -19.0
3	AHD	249	134.00* 103.95	-13.0 -13.0	-13.0 -22.0	-15.0 -11.0
4	SEM	209	166.10* 192.00	-11.0 -11.0	-11.0 -12.0	-18.0 -14.0

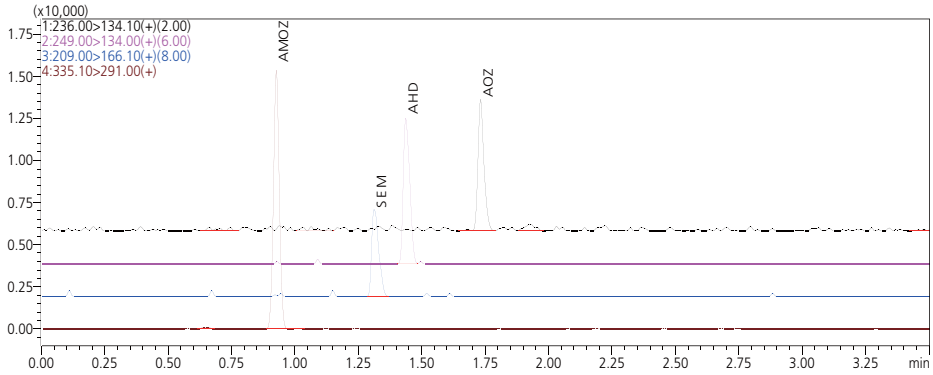


Fig. 4 MRM chromatograms of 4 derivatives of nitrofuran metabolites

Table 2 Repeatability of 4 marine toxins at different concentrations (n = 6)

No.	Compound	RSD% (1 µg/L)		RSD% (10 µg/L)		RSD% (50 µg/L)	
		R.T. (min)	Area	R.T. (min)	Area	R.T. (min)	Area
1	AOZ	0.13	3.84	0.15	2.36	0.09	2.16
2	AMOZ	0.11	4.24	0.09	2.13	0.07	1.69
3	AHD	0.14	3.56	0.27	3.49	0.12	2.24
4	SEM	0.27	4.76	0.28	4.66	0.12	1.68

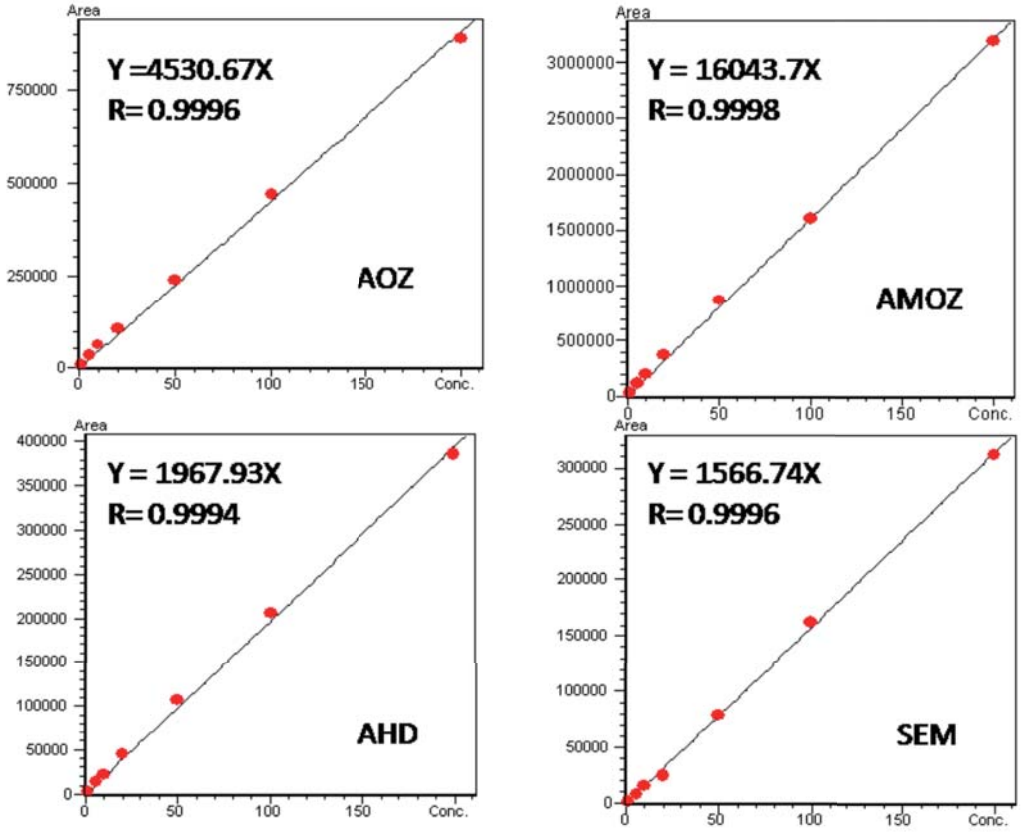


Fig. 5 Calibration curve of 4 derivatives of nitrofuran metabolites

3. Results and Discussion

The MRM chromatograms in the positive ion modes of 4 derivatives of nitrofuran metabolites are shown in Fig. 4. Fig. 5 shows the calibration curves of 4 derivatives of nitrofuran metabolites. Linearity was demonstrated in the range of 1.0 to 200 µg/L for AOZ, AMOZ, AHD and SEM, with correlation coefficients greater than 0.999. The repeatability of those compounds in different concentration were investigated, and the RSDs of peak area were less than 4.8%, and RSDs of retention time were less than 0.3%, as shown in Table 3.

4. Conclusions

A LC/MS/MS method has been developed for quantitative analysis of 4 derivatives of nitrofuran metabolites in marine products were detected by Shimadzu Nexera UHPLC and LCMS-8030 triple quadrupole mass spectrometer. All of them were separated in 4 minutes, and analyzed in positive mode. The calibration curves were linear well between peak area of the selected MRM transitions and the concentration of target compounds with the correlation coefficient over 0.999. This method was established for fast quantitative determination of 4 derivatives of nitrofuran metabolites.

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