SHIMADZU

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Determination of nitrofuran metabolite residues in shrimp by LC-MS/MS

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1. Overview

In this paper, a method for the determination of nitrofuran metabolite residues in aquatic products by using LC-MS/MS was established,

2. Introduction

A method for the determination of nitrofuran metabolite residues in shrimp by using LC-MS/MS was established. The sample was hydrolyzed and derived under acidic conditions, after extraction and centrifugal purification, the triple quadrupole mass spectrometer LCMS-8045 was detected, and the internal standard was quantified. The four compounds had good linearities in the concentration range of 0.5 μg/L~20.0 μg/L, and the correlation coefficient r was above 0.999. The recoveries of spiked samples with concentrations of 1, 5 and 10 μg/kg were between 82.3~112.7%. This method can be used for accurate quantitative detection of nitrofuran metabolite residues in aquatic products.

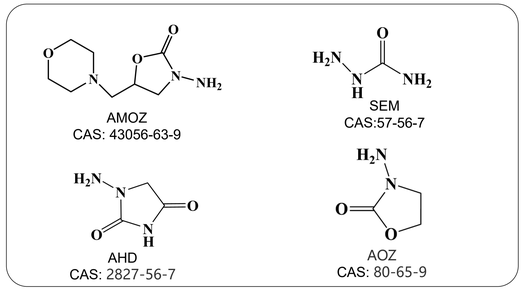

Figure 1 Structure of nitrofuran metabolite

3. Methods

Internal standard solution was added to 2 g of sample, vortexed and added 5 ml of hydrochloric acid solution and 0.15 mL of 2-nitrobenzaldehyde solution, vortexed and shake for 16 hours at 37℃ in dark. The solution was adjusted to pH 7.0~7.5 and added ethyl acetate. After vortex and centrifuge, the supernatant was dried by nitrogen. Then 1 mL of mobile phase was added to dissolve the residue, filtered by 0.22 μm membrane, and injected to LC-MS/MS.

High Speed Mass Spectrometer

Ultra Fast Polarity Switching
- 5 msec

Ultra Fast MRM
- Max. 555 transition /sec

Figure 2 LCMS-8045 triple quadrupole mass spectrometer

4. Result

4-1. Method development

Ion source parameters have an important influence on the response of compounds. Generally, there are factors such as nebulizing gas flow, dry gas flow, ion source voltage, ion source temperature, ESI probe position and other factors in the ion source are important for optimization. LabSolutions MRM Connect is a Shimadzu LC-MS/MS ion source parameter optimization software, through the design of experiments as shown in Figure 3, the automatic optimization process of ion source parameters can be realized.

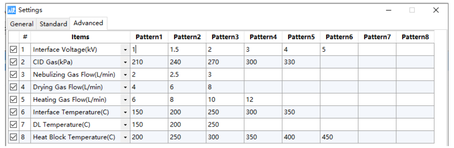

Figure 3 LabSolutions MRM Connect used for the design of experiments

UHPLC conditions (Nexera LC-40)

Column: Shim-pack GISS C18, （100 mm x 2.1 mmI.D., 1.9 μm）
Mobile phase A: 0.002 M Ammonium acetate B: methanol
Flow rate: 0.35 mL/min
Column temperature: 35°C
Elution mode: Gradient elution with an initial 10% B conc.
Time program: B conc.10%(0.5 min) -95%(0.51-4 min) - 95%(4.01-5.5 min)-10%(5.51-6 min)-10%(8 min)
Injection vol.: 10 μL

MS conditions (LCMS-8045)

Ionization: ESI, Positive MRM mode

Table 1. MRM transition of derivatives of nitrofuran metabolite

Compound	Quantitative ion MRM transition	Qualitative ion MRM transition
AOZ	236.1>104.1	236.1 > 134.1
AOZ-D_4	240.1>134.2	
AMOZ	335.0>291.2	335.0>262.1
AMOZ-D_5	340.1>296.1	
AHD	249.1>134.0	249.1>104.0
AHD-$^{13}C_3$	252.1>134.0	
SEM	209.1>166.1	209.1>192.0
SEM-$^{13}C,^{15}N_2$	212.1>168.1	

Figure 4 shows MRM chromatograms of the 4 derivatives of nitrofuran metabolite. It took 8 minutes per one LC-MS/MS analysis, including column rinsing, and excellent separation and highly sensitive detection were obtained.

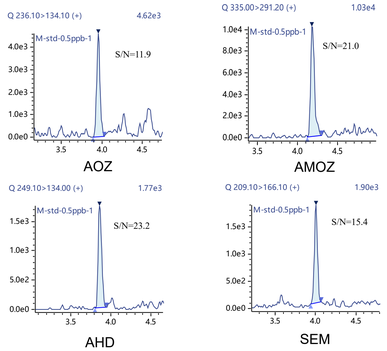

Figure 4 Mass Chromatgrams of derivatives of nitrofuran metabolite （concentration of each compound : 0.5 ppb）

The dilution series of these compounds were analyzed. The internal calibration was performed by plotting peak area ratios versus concentration ratios of nitrofuran metabolite residues and IS. All compounds were detected at ppb level with excellent linearity (Table 2, Figure 5).

Table 2 Linearities of 4 derivatives of nitrofuran metabolite

Compound	Calibration curve	Coefficient (r)	Detection limits (ng/mL)
AOZ	Y = (0.300989)X + (0.0584204)	0.9996	0.11
AMOZ	Y = (0.271546)X + (0.0570109)	0.9995	0.06
AHD	Y = (0.222288)X + (0.0396839)	0.9993	0.06
SEM	Y = (0.359552)X + (0.0547542)	0.9995	0.09

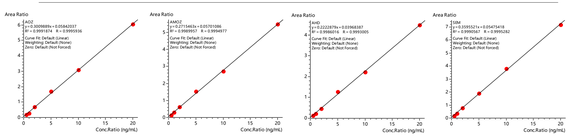

Figure 5 Calibration curves of nitrofuran metabolite derivatives

Nitrofuran metabolite derivatives at concentrations of 0.5, 2 μg/L and 20 μg/L were determined consecutively for 6 times to investigate the precision of the instrument. The relative standard deviation of the retention time and peak areas in different concentrations of samples was in the range of 0.08%~0.13% and 0.89%~9.28%, respectively, and the instrument precision was good.

Table 3. Repeatability results of 4 nitrofuran metabolite derivatives (n=6)

Compound	RSD%（0.5 μg/L）		RSD%（2 μg/L）		RSD%（20 μg/L）	
	R.T	Area	R.T	Area	R.T	Area
AOZ	0.19	5.77	0.09	3.75	0.08	1.90
AMOZ	0.11	6.04	0.09	4.11	0.08	0.89
AHD	0.10	9.28	0.08	4.40	0.08	1.99
SEM	0.13	4.06	0.08	2.82	0.08	1.07

Spiked recoveries were detected as follows: Mixed standard working solution was added to 2 g of blank shrimp, making the spiked concentrations at 1, 5 and 10 μg/kg, and the spiked recoveries of four nitrofuran metabolites was determined according to the analysis conditions, and the spiked recovery of four nitrofuran metabolites was determined in parallel for 3 times. The recoveries of the four compounds was between 82.3~112.7%, and the specific results are shown in Table 4.

Table 4. Recovery results of 4 nitrofuran metabolite derivatives (n=3)

Compound	Spiked at 1 μg/kg		Spiked at 5 μg/kg		Spiked at 10 μg/kg	
	Rec.%	RSD%	Rec.%	RSD%	Rec.%	RSD%
AOZ	103.0	8.43	92.9	2.63	87.2	7.61
AMOZ	101.9	3.91	112.7	2.48	94.0	1.34
AHD	92.8	4.26	105.0	8.53	86.8	6.53
SEM	82.3	8.50	84.9	5.51	86.4	6.13

4-2. Analysis of real samples

Analysis of 3 kinds of shrimp samples from the supermarket, no nitrofuran metabolites were detected.

5. Conclusions

In this paper, a method for the determination of residues of nitrofuran metabolites in shrimps by using LC-MS/MS was established. After the sample was hydrolyzed and derived under acidic conditions, extracted by ethyl acetate, after cleaning by high-speed centrifugation, LCMS-8045 was used for detection. The four nitrofuran metabolites had good linearities in the concentration range of 0.5 μg/L~20.0 μg/L, and the correlation coefficient r was above 0.999. The recovery rate of spiked samples with concentrations of 1, 5 and 10 μg/kg was between 82.3~112.7%. This method was sensitive, rapid and accurate, and can be used for the accurate detection of nitrofuran metabolite residues in aquatic products.