

### 1. Overview

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

### 2. Introduction

A method for the determination of nitrofurantoin 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 nitrofurantoin metabolite residues in aquatic products.

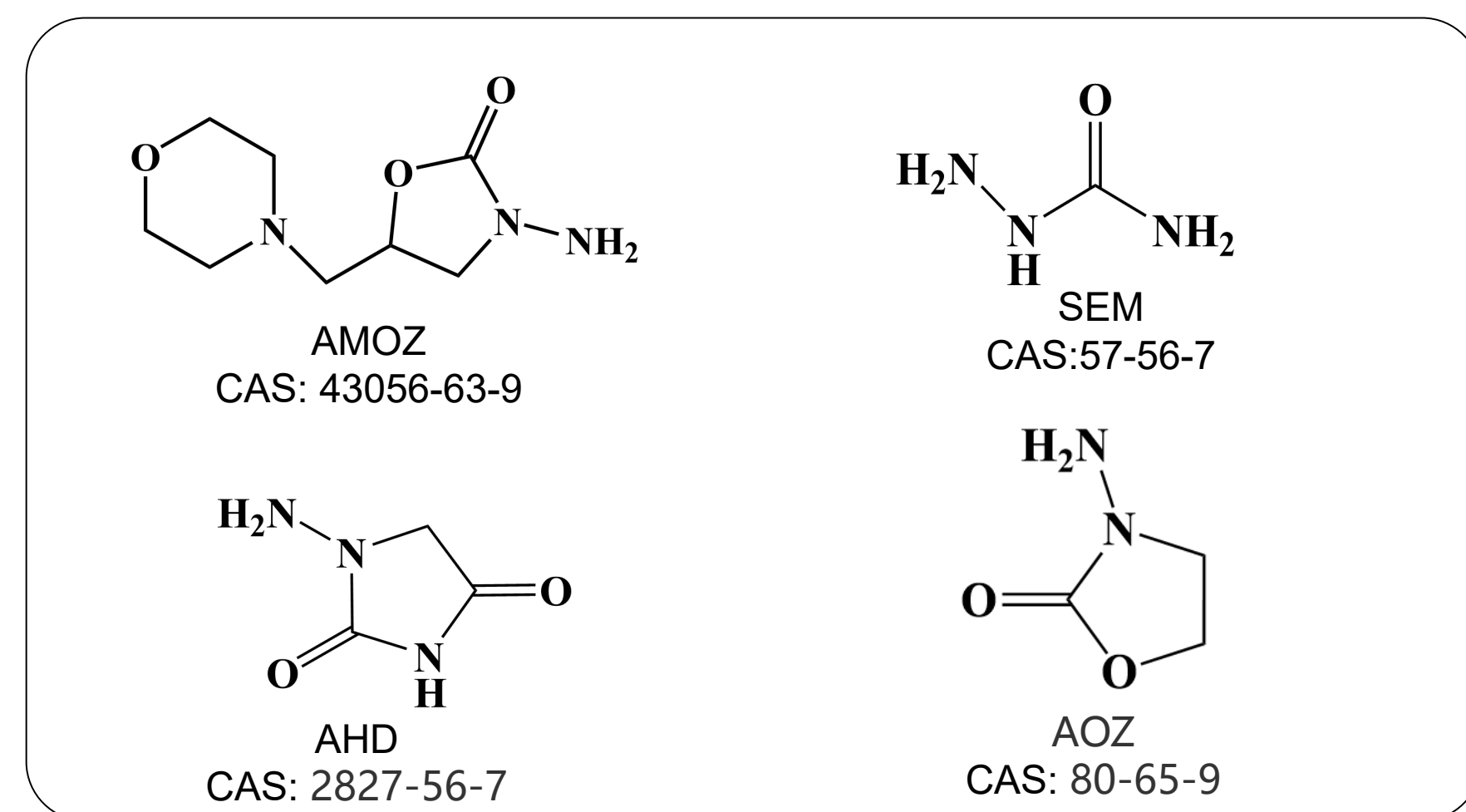


Figure 1 Structure of nitrofurantoin metabolite

### 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°C 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.



Figure 2 LCMS-8045 triple quadrupole mass spectrometer

High Speed Mass Spectrometer

- Ultra Fast Polarity Switching - 5 msec
- Ultra Fast MRM - Max. 555 transition /sec

### 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.

#	Items	Pattern1	Pattern2	Pattern3	Pattern4	Pattern5	Pattern6	Pattern7	Pattern8
1	Interface Voltage(kV)	1	1.5	2	3	4	5		
2	CID Gas(kPa)	210	240	270	300	330			
3	Nebulizing Gas Flow(L/min)	2	2.5	3					
4	Drying Gas Flow(L/min)	4	6	8					
5	Heating Gas Flow(L/min)	6	8	10	12				
6	Interface Temperature(C)	150	200	250	300	350			
7	DL Temperature(C)	150	200	250					
8	Heat Block Temperature(C)	200	250	300	350	400	450		

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 mm I.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 nitrofurantoin metabolite

Compound	Quantitative ion MRM transition	Qualitative ion MRM transition
AOZ	236.1>104.1	236.1 > 134.1
AOZ-D <sub>4</sub>	240.1>134.2	
AMOZ	335.0>291.2	335.0>262.1
AMOZ-D <sub>5</sub>	340.1>296.1	
AHD	249.1>134.0	249.1>104.0
AHD- <sup>13</sup> C <sub>3</sub>	252.1>134.0	
SEM	209.1>166.1	209.1>192.0
SEM- <sup>13</sup> C, <sup>15</sup> N <sub>2</sub>	212.1>168.1	

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

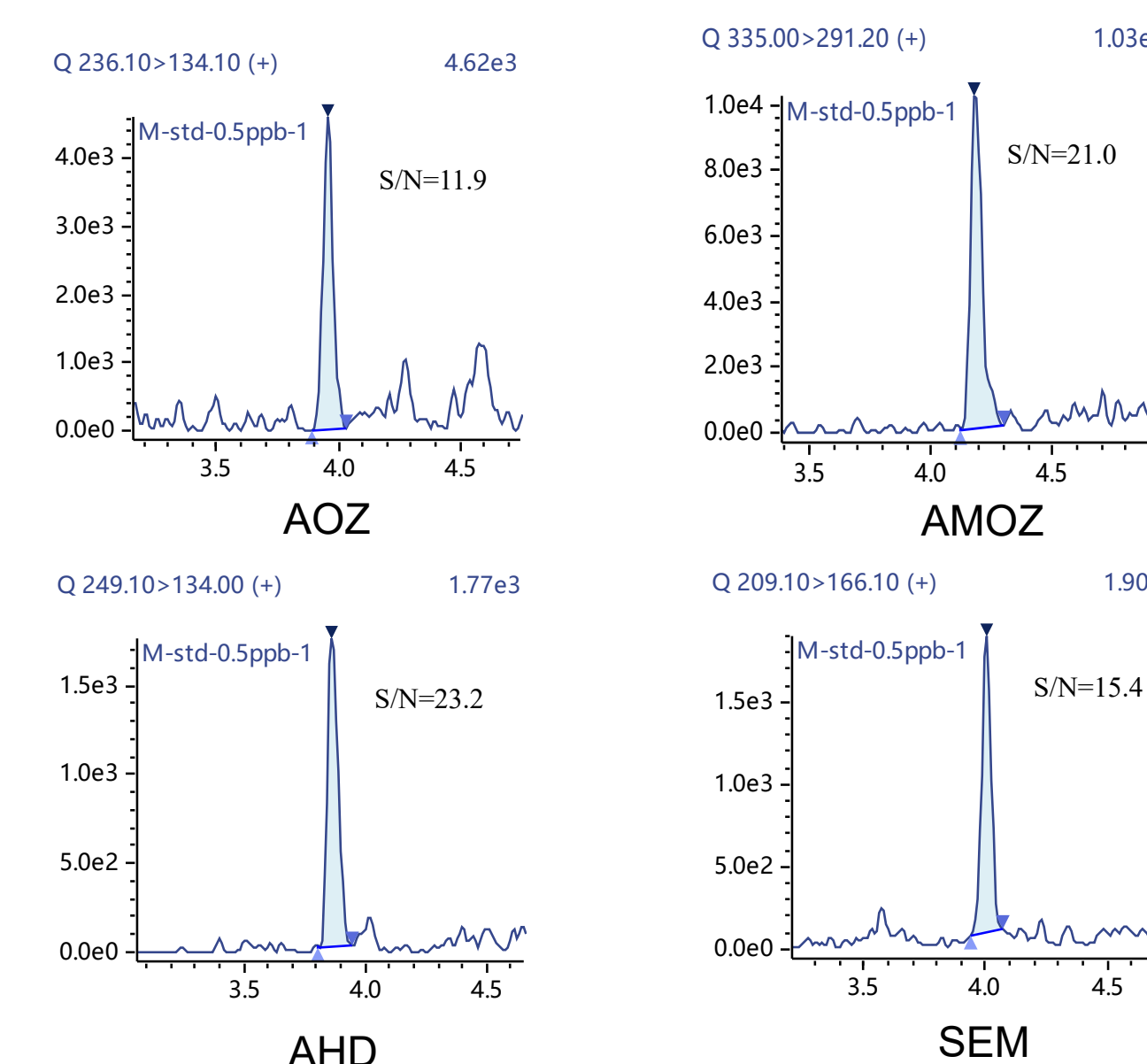


Figure 4 Mass Chromatograms of derivatives of nitrofurantoin metabolite (concentration of each compound : 0.5 ppb)

The dilution series of these compounds were analyzed. The internal calibration was performed by plotting peak area ratios versus concentration ratios of nitrofurantoin 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 nitrofurantoin 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

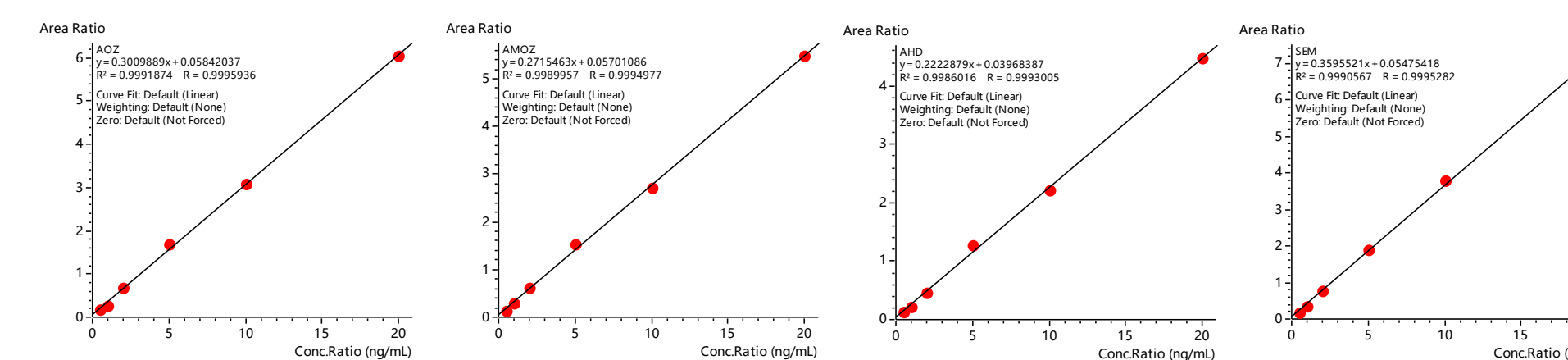


Figure 5 Calibration curves of nitrofurantoin metabolite derivatives

Nitrofurantoin 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 nitrofurantoin 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 nitrofurantoin metabolites was determined according to the analysis conditions, and the spiked recovery of four nitrofurantoin 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 nitrofurantoin 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 nitrofurantoin metabolites were detected.

### 5. Conclusions

In this paper, a method for the determination of residues of nitrofurantoin 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 nitrofurantoin 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 nitrofurantoin metabolite residues in aquatic products.