Quantitation of Nitrofuran Metabolites in Shrimp and Poultry by LC/MS/MS Using the Agilent LC/MSD Trap XCT

Application

Food

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Abstract

An LC/MS/MS method was developed for the qualitative and quantitative measurement of nitrofuran metabolites in chicken and shrimp using the Agilent LC/MSD XCT Ion Trap. The limit of quantitation for all four nitrofurans investigated easily met the specified European Union Minimum Required Performance Level of 1 µg/kg and ranged from 0.125 µg/kg to 0.5 µg/kg.

Introduction

Nitrofuran antibiotics are widely used for the treatment of infectious diseases in cattle, pigs, shrimp, and poultry. In 1995 the four drugs Furazolidone, Furaltadone, Nitrofurantoin, and Nitrofurazone were banned by the European Union (EU) for their usage in food-producing animals. Due to their rapid metabolism nitrofuran parent substances are not suitable for monitoring and typically their metabolites are analyzed. A liquid chromatography /mass spectrometry/mass spectrometry (LC/MS/MS) method was developed for the sensitive, qualitative, and quantitative analysis of four derivatized nitrofuran metabolites found in shrimp and poultry. See Figure 1.

Experimental

All liquid chromatography/mass spectrometry (LC/MS) experiments were performed using an Agilent 1100 Series LC system coupled to a mass selective detector (MSD) Ion Trap XCT mass spectrometer. The Ion Trap was operated with an orthogonal electrospray source in positive ion mode using multiple reaction monitoring (MRM). A gradient method was used for chromatography. Deuterated NBA-AMOZ was used as the internal standard (ISTD) for NBA-AMOZ and deuterated NBA-AOZ was used as the ISTD for the other metabolites.

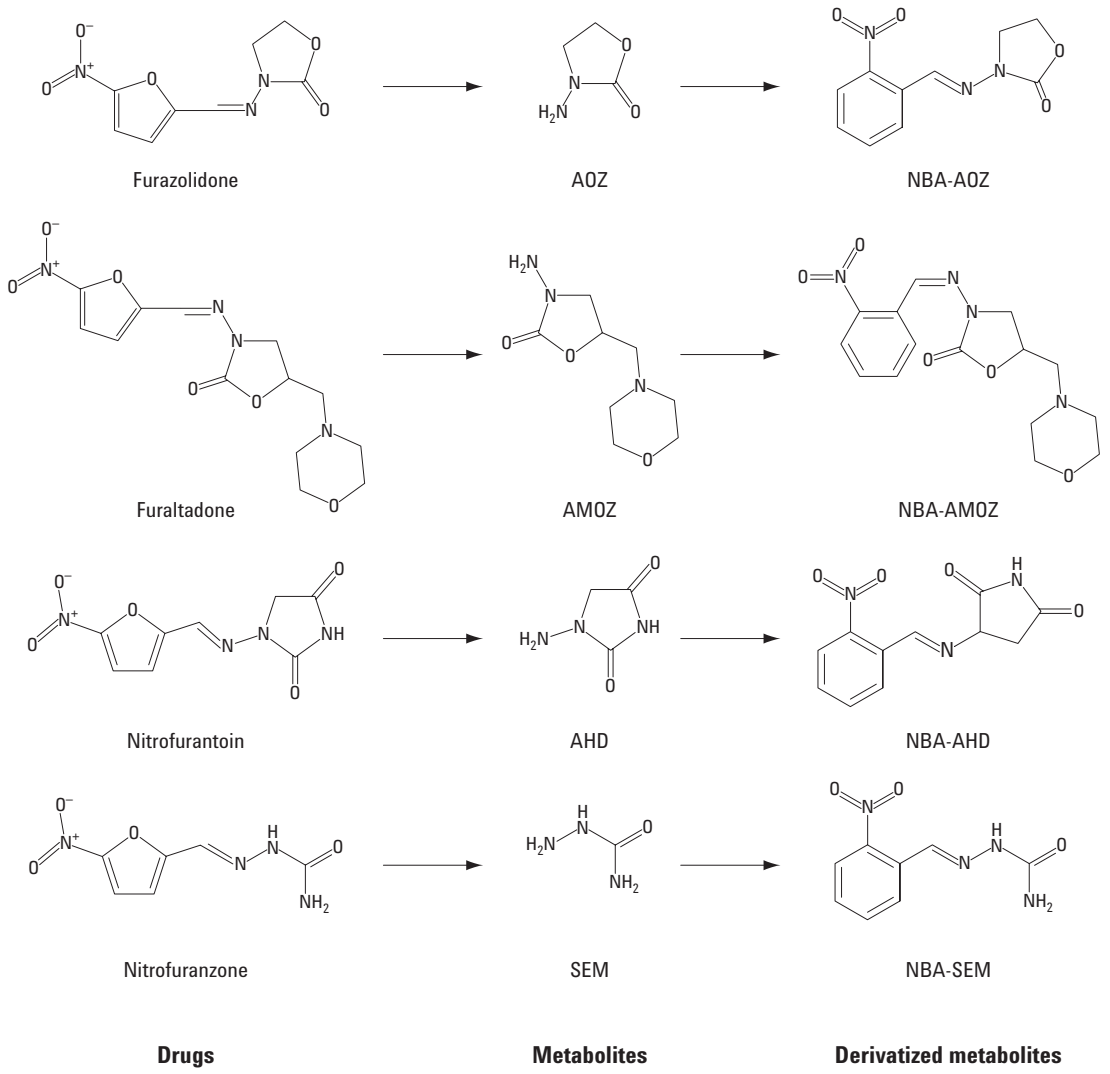

Figure 1. Chemical structures of nitrofurans, nitrofuran metabolites, and corresponding derivatives.

Sample Preparation

One (1) g of homogenized tissue from shrimp or poultry was mixed with 5 mL of a 0.2 M hydrochloric acid and 50 µL of 2-nitrobenzaldehyde (2-NBA, 100 mM in methanol) and incubated overnight at 37 °C. This is a protocol from the State Institute for Quality Control of Agricultural Products (RIKILT, The Netherlands). Using this protocol, tissue-bound residues of the nitrofurans with an intact side chain are released through acidic hydrolysis of the imine bond. The free amino groups of the corresponding metabolites are derivatized with 2-NBA to form an aromatic imine bond.

The sample was then neutralized with 500 µL of a 0.3 M Na_3PO_4 solution in water and adjusted to pH7 with 2 M NaOH solution. After the addition of 4-mL ethyl acetate, the sample was centrifuged and the organic layer was transferred to a clean tube. The sample was further extracted with a 4-mL aliquot of ethyl acetate, centrifuged, and the organic layer added to the first extract. After evaporation to near dryness, the sample was reconstituted in 500-µL solvent (50-mL acetonitrile, 80-mL water and 0.5-mL acetic acid) for subsequent LC/MS analysis.

Calibration standards were made by spiking known concentrations of all four underivatized analytes into shrimp and poultry prior to sample preparation.

Chemicals

Methanol	(Biosolve, 13683502)
Hydrochloric acid, HCL 32%	(Merck, 100319)
2-Nitrobenzaldehyde (2-NBA), $C_7H_5NO_3$	(Sigma, N6001)
Tri-sodiumphosphate-dodecahydrate, Na_3PO_4 12(H_2O)	(Merck, 106578)
Sodium hydroxide, NaOH	(Merck,106498)
Ethyl acetate, $CH_3COOC_2H_5$	(Biosolve 05402602)
Acetic acid, CH_3COOH	(Merck 10063)
Acetonitrile, CH_3CN	(Biosolve, 01203502)
Methanol-d, 99.5% D	(Aldrich, 15.193-9)

LC/MS/MS Method Details

HPLC:	Agilent 1100
Flow rate:	0.4 mL/min
Column:	Zorbax XDB-C18, 2.1 mm × 150 mm, 3.5 µm
Mobile phases:	A: Water + 0.1% acetic acid B: Acetonitrile + 0.1% acetic acid Gradient: 0–14 min: 10% A - 45% A; 14–16 min: 45% A - 90% A
Injection:	50 µL out of 500 µL

MS 1100 LCMSD XCT Ion Trap

Ionization mode:	Positive ESI
Nebulizer pressure:	45 psi
Drying gas flow:	12 L/min
Drying gas temperature:	350 °C
Skimmer:	20 V
Capillary exit:	55 V
Trap drive:	55
ICC:	On

MRM mode

4 Segments:	0–2.2 min, 2.2–7.4 min, 7.4–10.6 min, 10.6–13.9 min			
Segment 1:	Divert valve: to waste, no spectra			
Segment 2:	MS/MS of *m/z* 335,	Isolation width: 2.0,	Cut-off: 140;	Amplitude: 1.16
	MS/MS of *m/z* 340,	Isolation width: 2.0,	Cut-off: 140;	Amplitude: 1.16
Segment 3:	MS/MS of *m/z* 209,	Isolation width: 2.0,	Cut-off: 120;	Amplitude: 1.28
	MS/MS of *m/z* 249,	Isolation width: 2.0,	Cut-off: 100;	Amplitude: 1.25
Segment 4:	MS/MS of *m/z* 236,	Isolation width: 2.0,	Cut-off: 100;	Amplitude: 1.25
	MS/MS of *m/z* 240,	Isolation width: 2.0,	Cut-off: 100;	Amplitude: 1.25

Quantitation

NBA-AMOZ:	EIC of 261 + 291 (MS/MS of 335),	Ret. Time: 4.5 min
NBA-dAMOZ:	EIC of 266 + 296 (MS/MS of 340),	Ret. Time: 4.5 min
NBA-SEM:	EIC of 166 + 192 (MS/MS of 209),	Ret. Time: 9.9 min
NBA-AHD:	EIC of 134 (MS/MS of 249),	Ret. Time: 10.0 min
NBA-AOZ:	EIC of 134 (MS/MS of 236),	Ret. Time: 10.8 min
NBA-dAOZ:	EIC of 134 (MS/MS of 240),	Ret. Time: 10.8 min

Maximum accumulation time:	150 ms
Smart target:	100.000
Scan:	100–350

Results and Discussion

Very low limits of detection (LOD) are required for nitrofuran metabolites and the derivatization method increased the ionization efficiency, as well as improving the chromatographic separation. A liquid-liquid extraction procedure was used which resulted in a relatively high concentration factor to further improve LOD.

The ion trap mass spectrometer was operated in MRM mode. In this mode, only precursor ions are chosen and full-scan MS/MS-spectra of the corresponding analytes are acquired. These full scan-MS/MS spectra are then used for identification by comparing them with MS/MS-spectra stored in a library. No further qualifier ion has to be monitored. See Figure 2.

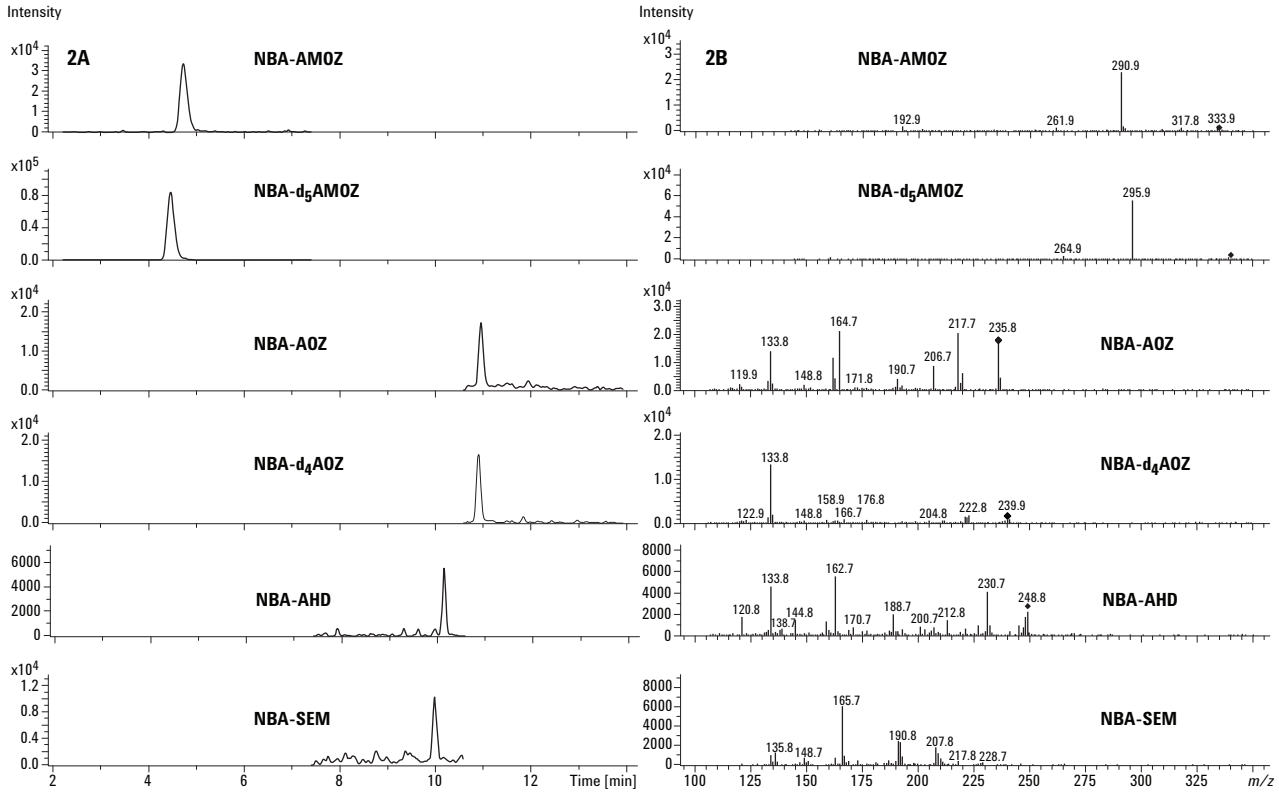

Figure 2. Representative chromatograms (2A) and MS/MS spectra (2B) for all analytes plus ISTDs (1 µg/kg).

Quantitation is performed by selecting one or more product ions to create extracted ion chromatograms for each analyte and ISTD. The product ions used for quantitation were selected for best signal-to-noise (S/N) ratio post-acquisition. See Figures 3 and 4.

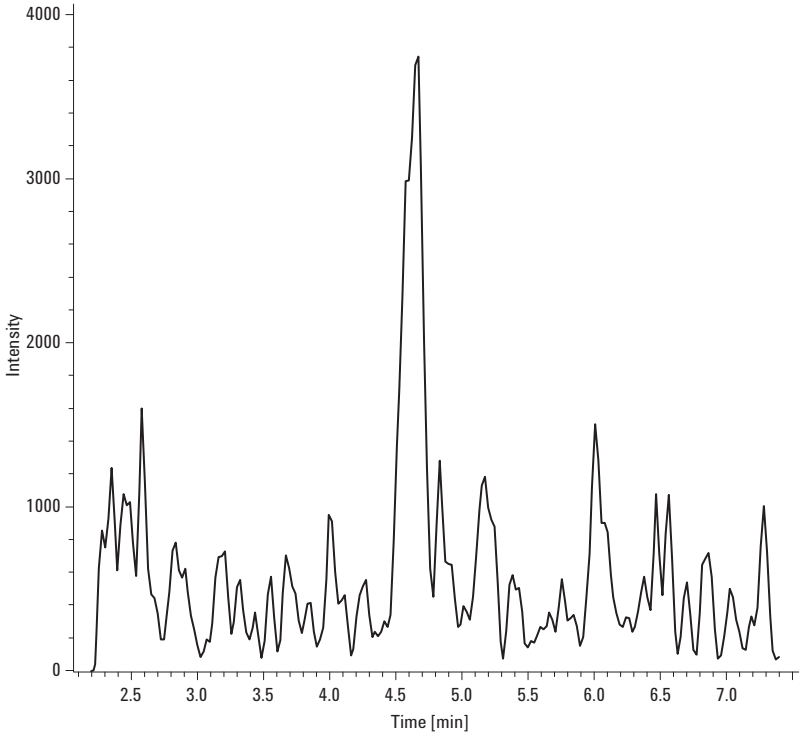

Figure 3. Limit of quantitation (LOQ) for NBA-AMOZ, 0.125 µg/kg in shrimp matrix.

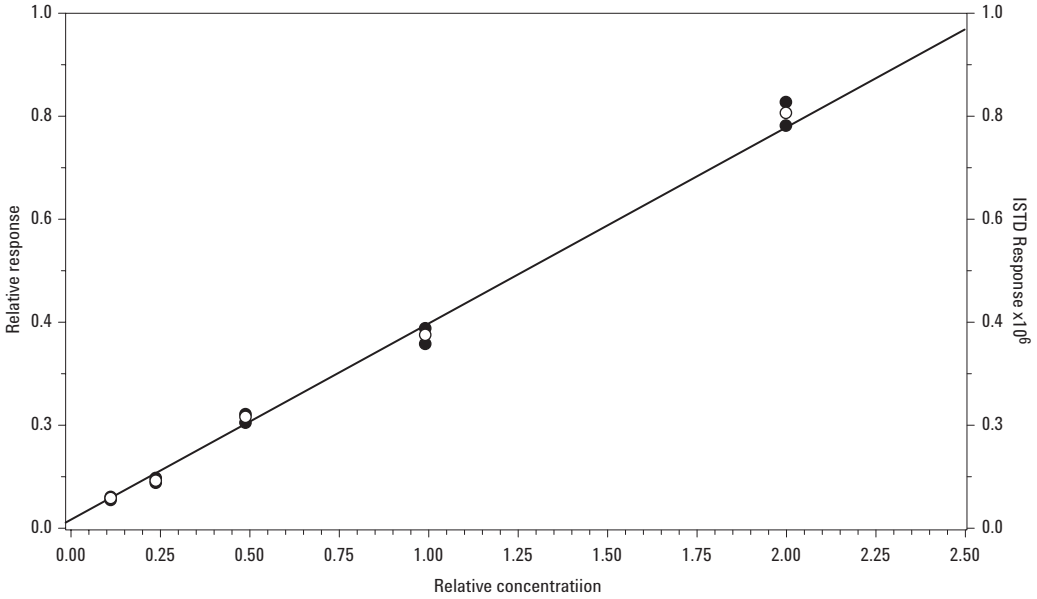

Figure 4. Calibration curve for NBA-AMOZ, 0.125 µg/kg – 2 µg/kg poultry matrix, three replicates.

NBA-AMOZ and NBA-SEM were quantified using the sum of two product ions, while NBA-AHD and NBA-AOZ were quantified using one product ion.

The European Union has set a Minimum Required Performance Level (MRPL) of 1 µg/kg for nitrofuran metabolites. These detection limits are easily reached using this method with LOQs ranging from 0.125 µg/kg for NBA-AMOZ to 0.25 µg/kg for NBA-AOZ and NBA-SEM, and 0.5 µg/kg for NBA-ADH. See Figure 5.

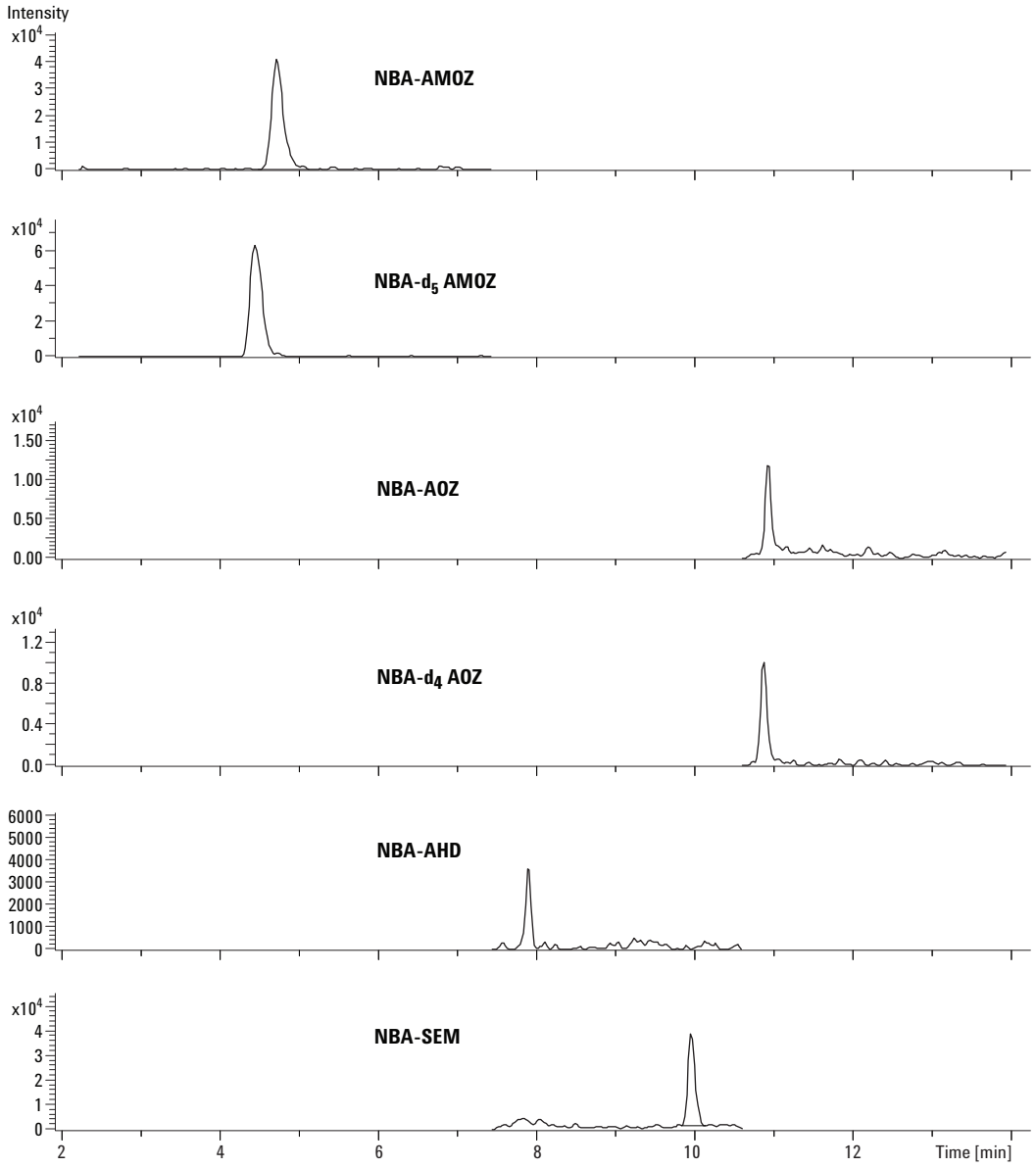

Figure 5. Representative chromatogram of a positive shrimp sample at a level of 0.25 µg/kg.

Linearity of the method was evaluated up to twice the MRPL (2 µg/kg) and showed a linear weighted regression (1/×) with coefficients of correlation of 0.99 or better. Intraday relative standard deviations (RSDs) were below 10% for all analytes at all concentrations. See Table 1.

Table 1. Method Reproducibility and Accuracy for the Four Target Derivatized Metabolites

	NBA-SEM		NBA-AOZ		NBA-AMOZ		NBA-AHD	
Standard (µg/kg)	SD %	Accuracy % (average)	SD %	Accuracy % (average)	SD %	Accuracy % (average)	SD %	Accuracy % (average)
0.125	3.77	98.81	2.29	98.94	4.34	101.10		
0.25	2.26	102.72	2.52	101.76	5.87	95.55	6.05	98.31
0.5	3.40	100.72	3.35	100.58	6.21	105.19	4.59	103.87
1	3.56	96.62	3.01	101.90	5.46	99.11	6.53	100.22
2	2.94	101.12	2.13	96.82	5.77	99.05	6.97	97.60

All calibration curves linear weighted 1/x n = 6

Conclusions

An LC/MS/MS method was developed for the qualitative and quantitative measurement of nitrofuran metabolites in chicken and shrimp using the Agilent XCT Ion Trap. The LOQ for all four nitrofurans investigated easily met the specified EU MRPL of 1 µg/kg and ranged from 0.125 µg/kg to 0.5 µg/kg.

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