Food and Environmental

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Rapid determination of nitrofuran metabolite residues in aquatic products

Using the SCIEX Triple Quad™ 3500 LC-MS/MS System

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Nitrofuran drugs are widely used in the livestock, poultry and aquaculture industries because of their low price and effectiveness in treating enteritis, scabies, red fin disease, and ulcers caused by Escherichia coli or Salmonella. Due to the carcinogenic and teratogenic side effects of nitrofurans and their metabolites, individual countries have banned the use of nitrofurans in livestock, poultry, and aquatic animal foods, and have strictly enforce residue detection. Per Announcement No. 235 issued by the Ministry of Agriculture of the People's Republic of China on December 24, 2002 and Announcement No. 560 issued on October 28, 2005, nitrofurans should not be detectable in animal foods. Since the announcements were issued, the use of nitrofuran drugs in the feeding of animals has become illegal.

There are four common nitrofuran drugs: furazolidone, furantrone, nitrofurantoin, and furancillin. Because nitrofuran prototype drugs are rapidly metabolized in the organism, and their metabolites (AOZ, AMOZ, AHD, SEM) and proteins are quite stable, the detection of metabolites is often used to reflect the residual status of nitrofuran drugs.

Here, an accurate quantitative method for nitrofuran metabolites was developed on the SCIEX Triple Quad 3500 LC-MS/MS System. This method provides a simple and fast solution to the problem of nitrofuran compound residues in animal-derived foods.

Key features of method

- Highly sensitive method—a quantification limit of 0.05 μg/kg was achieved. This sensitivity is ~10 times better than required by the GB method (GB 21311-2007, Ministry of Agriculture 783-1-2006 and 781-4-2006 have a quantification limit of 0.5 μg/kg).
- High reproducibility of detection—less than 5.42% CV at quantification limit
- Method extraction recovery rate between 70%-120%, ensuring accuracy and reliability of actual sample detection
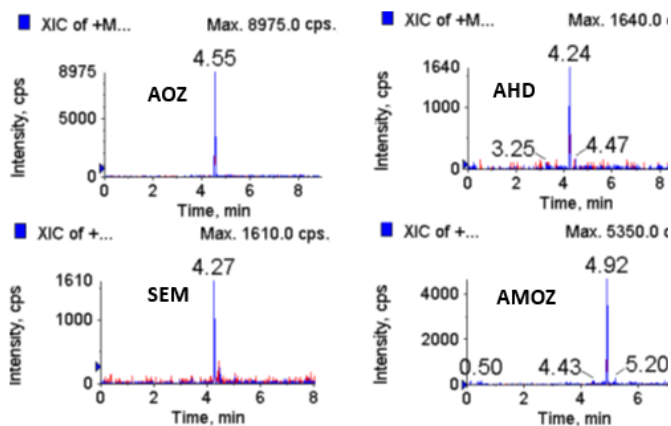
Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
1	SEM 1	0.0500	4 of 4	0.0500	0.0015	3.07
2	AOZ 1	0.0500	4 of 4	0.0500	0.0010	2.06
3	AHD 1	0.0500	4 of 4	0.0500	0.0027	5.42
4	AMOZ 1	0.0500	4 of 4	0.0500	0.0015	3.06

Figure 1. Typical chromatogram of nitrofuran metabolites. (Top) Signal for nitrofuran metabolites at a concentration of 0.05 μg/kg. (Bottom) High reproducibility was obtained with %CV < 5.42.

Methods

Sample preparation: Weigh 2g of homogenized sample in a 50 mL centrifuge tube. Add 0.05 mL of mixed isotope internal standard (100 ng/mL) and vortex for 30 seconds. Add 5 mL hydrochloric acid solution (0.2 mol/L) and 0.2 mL 2-nitrobenzaldehyde solution (0.05 mol/L), then vortex for 30 seconds and derivatize in a constant temperature water bath at 37 °C for 16 hours

Next, take the centrifuge tube to cool to room temperature. Add 3-5 mL of dipotassium hydrogen phosphate solution (1.0 mol/L) and adjust the pH to 7.0-7.5. Add 4 mL of ethyl acetate and vortex for 1 minute then centrifuge at 6,000 rpm for 5 minutes. Next, put 2 mL of the supernatant into a clean, 10 mL glass tube, then add 4 mL of ethyl acetate to the residue and vortex for 1 minute. Centrifuge at 6,000 rpm for 5 minutes. Take 3 mL of the supernatant and combine it into the above 10 mL glass tube. Combine the supernatant and blow dry with nitrogen at 40 °C.

Reconstitute the samples in 1 mL acetonitrile/water = 1/9 (v/v), perform LC-MS/MS analysis.

Chromatography: Separation was performed using the ExionLC™ System and a Phenomenex Kinetex C18 column (50×3.0 mm, 2.6 µm) and a flow rate of 0.4 mL/min. Column temperature of 40 °C and an injection volume of 20 µL.

Table 1. Chromatography.

Time (mins)	% A	% B
0.0	97	3
1.0	97	3
6.0	49	60
6.5	5	95
7.0	5	95
7.1	97	3
9.0	97	3

Mobile phase A: water (5 mM ammonium formate)
Mobile phase B: acetonitrile

Mass spectrometry: MS analysis was performed on the SCIEX Triple Quad 3500 System using an ESI source operated in positive ion mode.
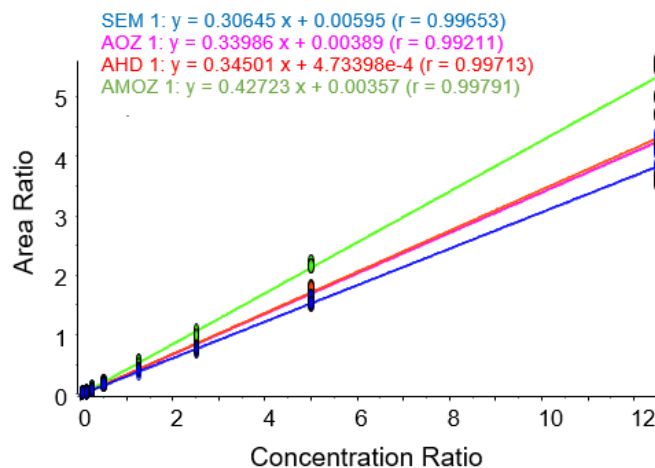
Ion source parameters were:

- IS voltage (ISV): 5500 V
- Curtain gas (CUR): 20 psi

- GS1: 50 psi
- GS2: 60 psi
- Source temperature TEM: 550 °C
- Collision gas CAD: 9 psi

Table 1. Nitrofuran metabolites and isotope internal standard mass spectrometry parameters.

Compound	Q1	Q3	DP	CE
AOZ	236.1	133.9	80	17
		103.9	80	31
AOZ-D_4	240.0	134.0	80	17
AHD	249.2	134.1	80	17
		104.1	80	27
AHD-$^{13}C_3$	252.0	134.1	80	17
SEM	209.2	166.2	80	14
		192.1	80	16
SEM-^{13}C-$^{15}N_2$	212.0	168.0	80	14
AMOZ	335.2	291.1	80	17
		262.2	90	23
AMOZ-D_5	340.0	296.0	80	17

SEM 1: y = 0.30645 x + 0.00595 (r = 0.99653)
AOZ 1: y = 0.33986 x + 0.00389 (r = 0.99211)
AHD 1: y = 0.34501 x + 4.73398e-4 (r = 0.99713)
AMOZ 1: y = 0.42723 x + 0.00357 (r = 0.99791)

Figure 2. Calibration curve of nitrofuran metabolites. Good linearity was obtained across the concentration range of 0.05-5.0 µg/kg (r > 0.99).

Table 3. Extraction and recovery rate of nitrofuran metabolites.

Compound	Extraction recovery rate	CV%
AOZ	98.9	3.6
AHD	101.7	1.9
SEM	94.5	1.5
AMOZ	101.2	4.2

Summary

Here, a rapid and accurate LC-MS/MS detection method for nitrofuran metabolites was established on the SCIEX Triple Quad 3500 System. The sensitivity of this method is 10 times better than the GB method. It fully meets the quantification limit requirements of GBT21311-2007, Ministry of Agriculture 783-1-2006 and 781-4-2006. And the method has a high extraction recovery rate, which can ensure the accuracy of real sample measurement.

Results

Chromatography was developed that enabled the separation of nitrofuran metabolites using a 9 min run time (Figure 1). Very good reproducibility of signal was observed at a concentration of 0.05 µg/kg. Concentration curves were generated across a concentration range of 0.05 to 5.0 µg/kg and demonstrated very good linearity (Figure 2) ensuring accurate quantification of samples at different concentration levels. Under the conditions of this method, the extraction recovery rate for the 4 compounds tested was between 94.5 and 101.7% (Table 3).

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