Pretreatment

1. Homogenize 10 g of sample in 100 mL of 0.12 M hydrochloric acid.
2. Take 1 mL aliquot and treat with 400 µL of 50 mM 2-nitrobenzaldehyde in dimethylsulfoxide.
3. Hydrolyze/derivatize the sample for 16 hours at 37 °C.
4. Adjust the sample to pH 7.4 with potassium hydrogen phosphate.
5. Centrifuge sample for 5 minutes at 8000 rpm.

SPE Procedure

Oasis® HLB 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 1 mL methanol
B. 1 mL water

↓

LOAD:
Approximatively 100 mL of sample

↓

WASH:
A. 2 mL water
B. 2 mL 30% methanol in water

↓

Dry for 20 minutes

↓

ELUTE:
3 mL methyl-t-butyl/methanol/formic acid (89:9:2, v/v/v)

Evaporate and reconstitute in 200 µL mobile phase

LC Conditions

Instrument:	Waters Alliance® HPLC 2695 System
Column:	XTerra® MS C_{18}, 2.1 x 100 mm, 3.5 µm
Flow rate:	0.2 mL/min
Mobile phase:	Isocratic 70% 20 mM ammonium formate pH 4, 30% acetonitrile
Injection volume:	20 µL
Column temperature:	30 °C

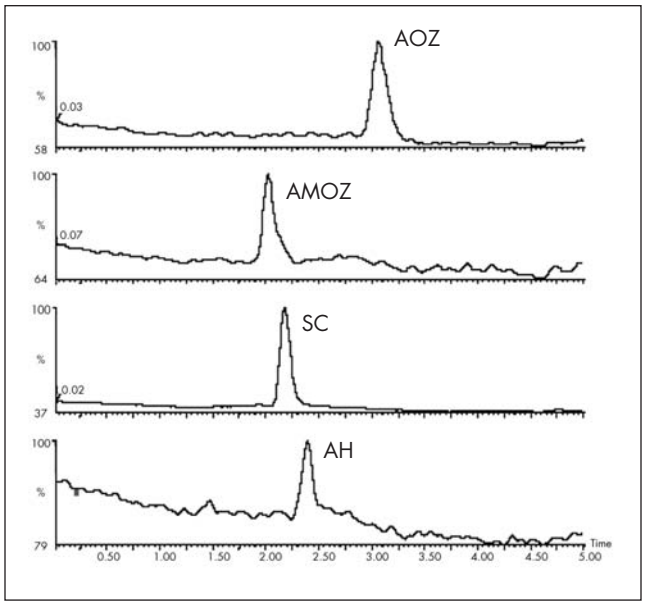
MS Conditions

Instrument:	Waters Quattro micro™ API
Ionization mode:	Positive electrospray (ESI+) Multiple reaction monitoring

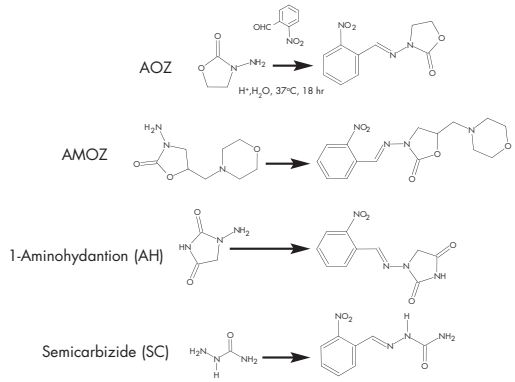
Analyte	MRM Transition
AOZ	236 → 134
AMOZ	335 → 291
SC	209 → 192
AH	249 → 178

MRM method parameters.

Results



Spiked chicken muscle (1 ng/g) metabolites as 2-nitrobenzaldehyde derivatives.



Ordering Information

Description	Part Number
Oasis HLB, 3 cc/60 mg, 30 µm, 100/box	WAT094226
XTerra MS C_{18}, 2.1 x 100 mm, 3.5 µm	186000404
Qsert™ Vials, LCGC Certified Combination Packs	186001126C

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