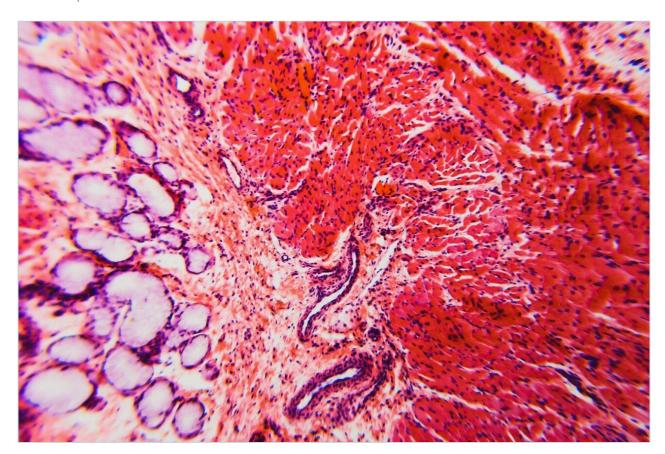
Waters™

Application Note

Nitrofurans in Tissues

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application describes the methods to determine Nitrofurans in food producing animal tissues.

Introduction

The United States Food and Drug Administration (US FDA) banned Nitrofuran drugs are banned in food producing animals because they pose a public health risk. The rule went into effect as a result of evidence that the drugs may induce carcinogenic residues in animal tissues.

Experimental

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 μ L 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

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

MS Conditions

MS System: Waters Quattro micro API

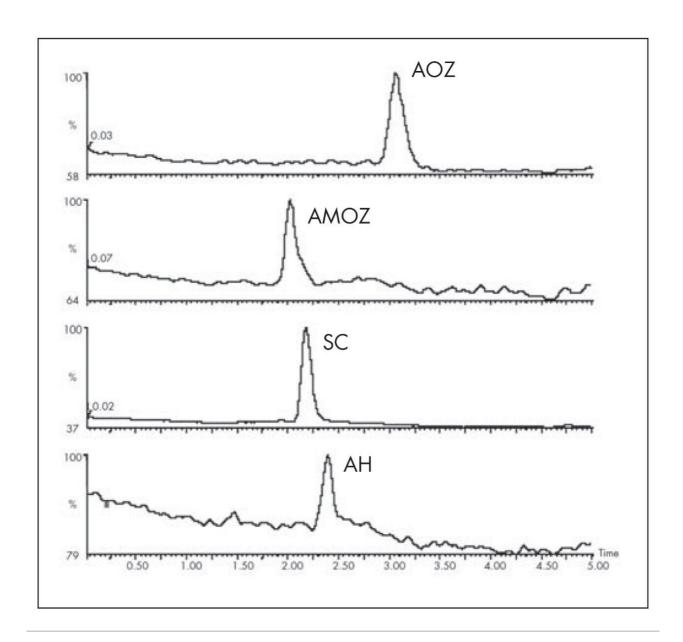
Ionization mode: Positive electrospray (ESI⁺)

Multiple reaction monitoring

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

MRM method parameters.

Results and Discussion



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

AOZ
$$AOZ$$

$$H_{2}NH_{2}$$

$$H_{2}NH_{2}O, 37^{\circ}C, 18 \text{ hr}$$

$$1-Aminohydantion (AH)$$

$$H_{2}NH_{2}O, 37^{\circ}C, 18 \text{ hr}$$

$$NO_{2}NH_{2}O, 37^{\circ}C, 18 \text{ hr}$$

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Alliance HPLC System https://www.waters.com/534293

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