Analysis of Nitrofurans in Honey

Using LC/MS/MS and an Ultra C18 Column

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- Sensitive detection of antibiotic metabolites in a complex matrix.
- Ultra C18 column assures the resolution needed for the LC/MS/MS method.
- Excellent peak shape at sub-ppb levels.

Nitrofurans are a class of veterinary antibiotics used to increase growth rate and prevent or treat disease in animals. Animals have been treated with antibiotics since the 1950s and, currently, about 45% of the antibiotics produced each year in the U.S. are administered to livestock. In Europe, this practice is illegal, because the inadvertent consumption of residual antibiotics in animal tissue, such as meat or liver, can lead to increased drug resistance or allergies in humans.

Nitrofurans have been detected not only in treated animals, but also in animal products, including honey. The low levels of these compounds and the complexity of honey as a matrix present challenges for the analysis of nitrofurans. In addition, nitrofurans are unstable and metabolize rapidly *in vivo*. Any analysis method for nitrofurans, therefore, must be able to separate and detect these metabolites. In the analysis of honey, it is of interest to quantify four nitrofurans: furazolidone, furaltadone, nitrofurazone, and nitrofurantoin, through their respective metabolites, 3-amino-2-oxazolidone (AOZ), 5-mofolinomethylmethyl-3-amino-2-oxazolidone (AMOZ), semicarbazide (SC) and 1-aminhydantoin (AHD). The method of choice for the analysis of nitrofuran and nitrofuran metabolites in honey is LC/MS/MS, with separation on a C18 column.

In this study, honey samples treated with the four nitrofuran metabolites were dissolved in water, then extracted with ethyl acetate. After centrifugation, the extract was evaporated and reconstituted in 125mM HCl, then derivatized with 2-nitrobenzaldehyde. After two liquid-liquid extractions with ethyl acetate, the extract was evaporated and reconstituted with mobile phase, filtered, and injected into the LC/MS/MS system. The column used for the analysis was a 100mm x 2.1mm, 3μm Ultra C18 column. For maximum sensitivity and specificity, a triple quadrupole analyzer was used, with electrospray ionization and selected reaction monitoring (SRM).

Results from the analysis of 0.3ppb nitrofuran metabolites in honey are shown in Figure 1. The Ultra C18 HPLC column is an excellent choice for this analysis. As a reliable general purpose column based on a high-purity, base-deactivated silica, its utility extends to other compounds that might be present in animal-derived matrixes, such as steroids and vitamins.

In analyses for nitrofuran antibiotics, an Ultra C18 HPLC column is an excellent choice, especially for analyzing trace levels of these compounds in a complex sample matrix.

Ultra C18 HPLC Column

For many other dimensions, refer to our catalog or visit our website. 9174312

100mm		
3μm Column, 2.1mm		cat. #

Figure 1 Nitrofuran metabolites in honey detected at 0.3ppb by LC/MS/MS, using an Ultra C18 column.

d5-AMOZ (3.7min.)
AMOZ (3.8min.)
AHD (5.46min.)
d4-AOZ (5.49min.)
AOZ (5.51min.)
SC (5.6min.)
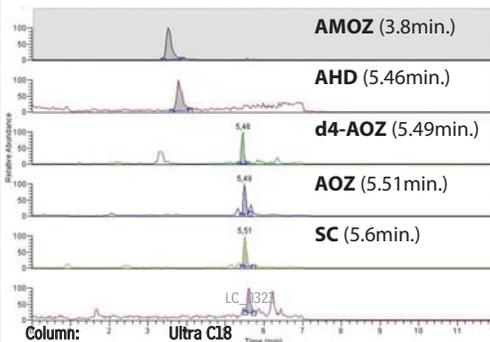
Column:	Ultra C18
Cat. #:	9174312
Dimensions:	100 x 2.1mm
Particle Size:	3μm
Pore Size:	100Å

Conditions:

Mobile phase: A: 0.05% formic acid in methanol
B: 0.05% formic acid − 5 mM NH_4 formate in water

Time (min)	%B
0	90
2.5	90
5	10
10	10
12	90
15	90

Sample:	0.3ppb each analyte
Flow:	200μL/min.
Temp.:	30°C
Det.:	MS/MS triple quadrupoles (Thermo Scientific Discovery)

Analyzer Parameters:

Ion source:	ESI (electrospray ionization)
Only segment:	15 min.
Polarity:	positive
Data type:	centroid
Scan mode:	SRM product
Scan width (m/z):	0.7
Scan time (s):	0.25
Peak width:	Q1: within 0.7
	Q2: 0.7
Collision gas pressure (mTorr):	1.5 (argon)
Divert valve:	active, with 3 positions
	Positions-1° 2 min., 2° 8 min., 3° 5 min.

Analyte	Prec. Ion	Prod. Ion	Collision E	Tube Lens
AOZ	236	134	12 V	120
AMOZ	335	291	10 V	100
SC	209	166	12 V	80
AHD	249	134	12 V	110

AMOZ = 3-amino-5-morpholinomethyl-2-oxazolidinone
AHD = 1-aminohydantoin hydrochloride
AOZ = 3-amino-2-oxazolidinone
SC = semicarbazide

Data courtesy of Dr. Alejandro Albornoz, EIDOMET SRL, Buenos Aires.