Analysis of nitrofuran metabolites by positive ion electrospray LC/MS/MS

Application Note

Environmental

Authors

Agilent Technologies, Inc.

Introduction

Nitrofuran antibiotics are veterinary drugs. The use of these in food production is already banned in the United States, Australia, Canada, Japan, Singapore, Bangladesh, and the European Union because of a possible increased cancer-risk through long-term consumption.

Trace amounts of nitrofurans contamination has been found in warm-water prawns, shrimp, and chicken. The contamination may occur from deliberate, direct misuse of the drugs as well as from contaminated feed.

The analyses of nitrofurans are based on the detection of the metabolites of the parent drugs. Since the parent drugs are very rapidly metabolized and the tissue-bound metabolites are detectable for several weeks after administration, the metabolites are a much better marker for the detection of the abuse of these antibiotics.

The metabolites of furazolidone, furaltadone, nitrofurantoine and nitrofurazone are 3-amino-2-oxazolidinone (AOZ), 5-methylmorpholino-3-amino-2-oxazolidinone (AMOZ), 1-aminohydantoin (AH) and semicarbazide (SC), respectively. Nitrobenzaldehyde, the most commonly used derivatizing reagent for these metabolites, forms a nitrophenyl derivative for LC/MS analyses.

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Instrumentation

- Agilent ProStar 430 AutoSampler
- Agilent ProStar 210 Isocratic Solvent Delivery Modules
- Agilent 1200L LC/MS equipped with ESI source

Materials and Reagents

- A 5 pg/µL standard mixture of 2-nitrobenzyladehyde derivative of AOZ, AMOZ, AH, and SC was kindly provided by Thai Unique, Thailand.
- All other chemicals are reagent grade or HPLC grade.

Conditions

Column : Agilent Polaris C18-A, 2 x 50 mm, 3 µm (Agilent Part Number: A2001050X020)

Mixer : 250 µL static mixer

Solvent A : water

Solvent B : methanol

LC Program :

Time (min:sec)	% A	% B	Flow (mL/min)
0:00	90	10	0.2
1:00	5	95	
2:00	5	95	
2:21	90	10	
6:00	90	10	0.2

Injection Volume : 40 µL

Sample Solvent : 10 mM ammonium acetate

MS Parameters

Collision Gas : 2.1 mTorr Argon

API Drying Gas : 20 psi at 300 °C

API Nebulizing Gas : 50 psi

Scan Time : 1 s

SIM Width : 0.7 amu total

Needle : -4400V

Shield : -300V

Capillary : -40V

Detector : 2000V

Results

The LC method used a 10 minute run cycle. It is clear from the Multiple Reaction Monitoring (MRM) data produced that the 1200L LC/MS system can qualitatively and quantitatively detect nitrofurans to the ultra trace levels required.

10 mM ammonium acetate was used in the sample solution to reduce formation of sodium adducts and to enhance the detection limit for the product ions.

The Limit of Detection (LOD) for SC, AOZ, AH, and AMOZ is about 60 fg/µL*, 10 fg/µL, 20 fg/µL, and 10 fg/µL in solution, respectively. The linearity of the detector response was excellent (Figure 1). The R^2 for 209>166, 236>134, 249>134 and 335>291 are 0.999, 0.998, 0.999 and 0.999, respectively. Typical chromatograms obtained are given in Figure 2.

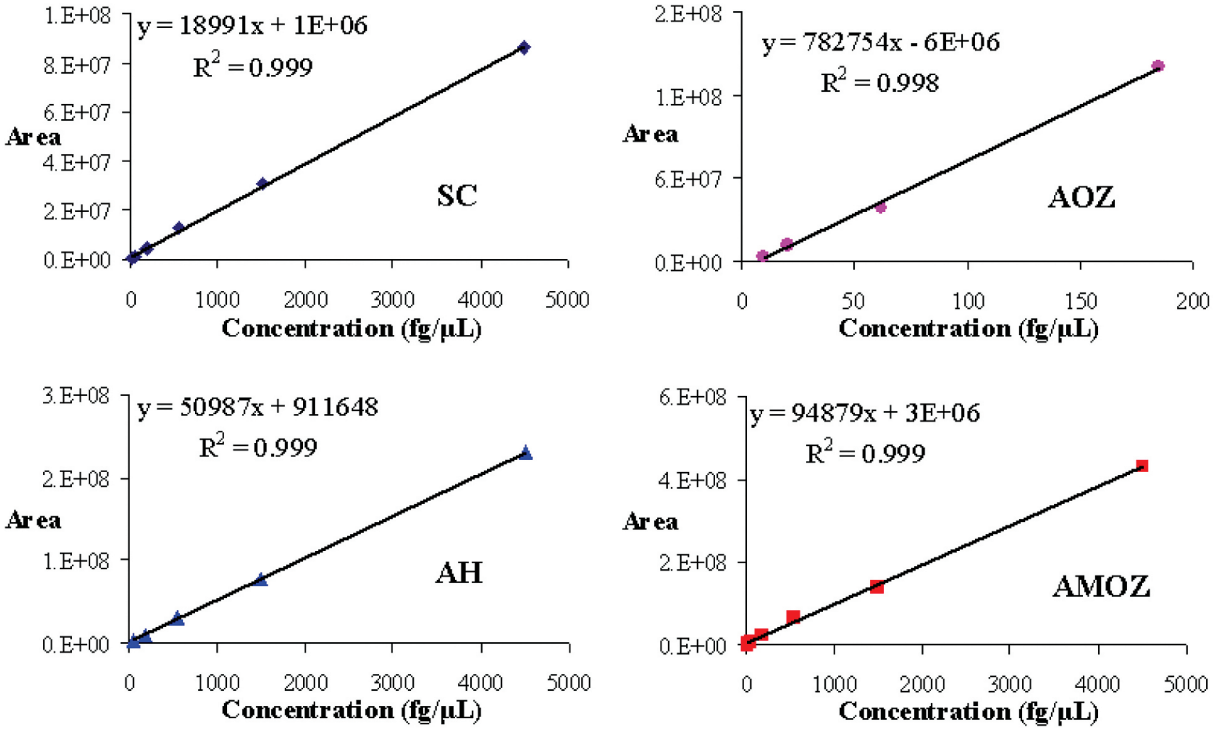

Figure 1. Standard Calibration Curves

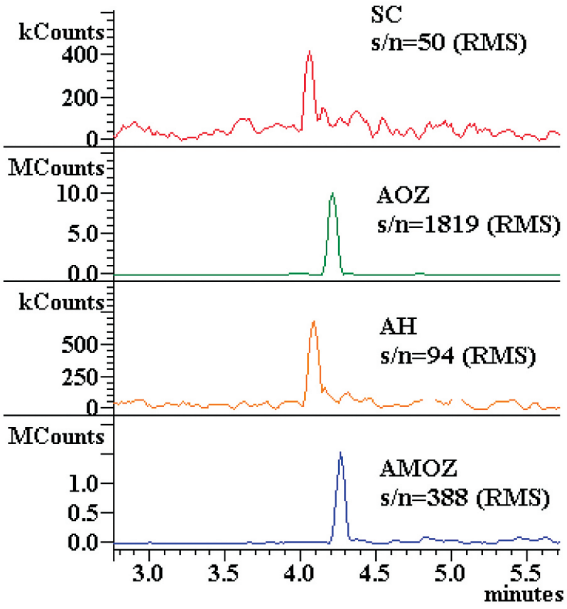

Figure 2. MRM Chromatograms

Conclusion

The Agilent 1200L LC/MS/MS system demonstrates excellent performance for the analyses of nitrofurans metabolites and is able to assist in the detection of potentially harmful substances in food imports/exports to improve food safety throughout the world.

* pg/µL=ppb and fg/µL=ppt

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