Application Note: 464

Multi-class Antibiotic Screening of Honey Using Online Extraction with LC-MS/MS

Catherine Lafontaine, Yang Shi, Francois A. Espourteille, Thermo Fisher Scientific, Franklin, MA, USA

Key Words

- Aria TLX-1 System
- TSQ Quantum Ultra
- Food Testing
- TurboFlow Method

Introduction

Antibiotics are commonly used in bee hives to control bacterial disease in honey bees. Use of these antibiotics requires caution to prevent persistent residues from occurring in food-grade honey. If antibiotic residues are present in sufficient quantities, allergic reactions and bacterial resistance can develop.

Many countries now monitor antibiotic residues in honey. LC-MS/MS is currently a common analytical approach for the quantification of antibiotic contamination in honey. Sample preparation for LC-MS/MS analysis can be time and labor intensive, often involving pH modification, hydrolysis, liquid-liquid extraction, solid phase extraction, solvent evaporation, and pre-concentration. A quick, comprehensive, online screening liquid chromatography (LC) method using a Thermo Scientific Aria TLX system powered by Thermo Scientific TurboFlow technology has been developed here to monitor several classes of antibiotics.

Goal

To develop a broad, generic, automated LC-MS/MS method for screening multi-class antibiotics in honey.

Experimental

Method Information

Residues representative of several classes of antibiotics (macrolides, sulfonamides, aminoglycosides, and tetracyclines) were extracted from wildflower honey using buffer containing ethylenediaminetetraacetic acid (EDTA). The extract cleanup was accomplished using a TurboFlow™ method involving two TurboFlow columns placed in tandem, a mixed mode anion exchange column and a polar polymer-based column. Simple sugars were un-retained and moved to waste during the loading step while the analytes of interest were retained on the extraction column set. This was followed by organic elution to an end-capped silica-based mixed mode reversed phase analytical column (Thermo Scientific BETASIL Phenyl/Hexyl) and gradient elution to a Thermo Scientific TSQ Quantum Ultra triple stage quadrupole mass spectrometer with a Heated Electrospray Ionization (H-ESI) source operating in positive selective reaction monitoring (SRM) mode. The total LC-MS/MS method run time was less than 18 minutes. Positive SRM transitions and other MS parameters for individual analytes are shown in Table 1.

Sample Preparation

A McIlvaine/0.1 M EDTA buffer was used as a 1:1 w:v (gram weight honey: milliliter volume buffer) diluent for wildflower honey, the testing matrix in this study.[1] A stock solution was prepared for sulfapyridine, sulfathiazole, tilmicosin, tylosin, oxytetracycline, and erythromycin in 3:1 methanol:water at 100 µg/mL. Additionally, one was prepared for doxycycline, demeclocycline, streptomycin, and dihydrostreptomycin in water at 100 µg/mL. These stocks were each spiked into 1:1 honey:buffer matrix and used as a spiking stock to make a set of calibration standards and quality controls (QCs). All blanks, standards, and QCs were prepared and analyzed in polypropylene vials. Injection volumes were 0.050 mL.

Aria™ TLX-1: TurboFlow Method Parameters

TurboFlow Cyclone MAX and TurboFlow Cyclone-P columns (0.5 × 50 mm), in-tandem
BETASIL Phenyl/Hexyl column, 100 × 3 mm, 3 µm
Aria operating system 1.6.2 software

Loading Pump Mobile Phases

Mobile Phase A: 1.0% formic acid in water
Mobile Phase B: 0.1% formic acid in acetonitrile
Mobile Phase C: 10 mM ammonium acetate in water, pH 9
Mobile Phase D: 50 mM ammonium acetate in methanol with 0.1% formic acid

Elution Pump Mobile Phases

Mobile Phase A: 1 mM NFPA*, 0.5% formic acid, 0.04% TFA** in water
Mobile Phase B: 0.5% formic acid, 0.04% TFA in 1:1 methanol:acetonitrile

**NFPA is nonafluoropentanoic acid.*

***TFA is trifluoroacetic acid.*

Thermo SCIENTIFIC

TSQ Quantum Ultra™ Mass Spectrometer (MS) Parameters	
Ion Polarity:	Positive
Ionization Source:	H-ESI
Spray Voltage:	4000 V
Vaporizer Temperature:	400 °C
Capillary Temperature:	370 °C
Sheath Gas Pressure (N_2):	30 arb units
Auxiliary Gas Pressure (N_2):	60 arb units
Ion Sweep Gas Pressure (N_2):	0.0 arb units
Skimmer Offset:	5 V (for streptomycin), 0 V (for all others)
Collision Pressure:	1.2 mTorr
Chrom Filter Peak Width:	8.0 s
Scan Type:	SRM
Scan Time:	0.020 s
Scan Width:	0.100 *m/z*
Peak Width Q1 Da. (FWHM):	0.700
Peak Width Q3 Da. (FWHM):	0.700

Results and Discussion

Results were packaged using Thermo Scientific LCQUAN 2.5.6 data quantitation software and included subtraction of background due to the presence of a few endogenous analytes in the store-bought honey. Figure 1 shows a representative chromatogram of the 10 analytes at 100 ng/mL in 1:1 honey/buffer. Matrix-matched calibration standards showed linear response of two orders of magnitude ($r^2 > 0.99$) for all of the analytes investigated (Table 2). All %CVs (n=3) were less than 19% for the lower limit of quantifications (LLOQ) and less than 8% for all other points of the curves. Figure 2 shows an LCQUAN™ representative linear regression using oxytetracycline as an example. QC sample variability was determined by processing and analyzing three replicates of each of four QC samples (2, 50, 100, and 500 ng/mL). All % RSDs were lower than 7% (except for erythromycin which was below 15%). Data was not used for any QC level that fell below the analyte's determined LLOQ.

Structural Class	Analyte	Precursor Ion	Product Ions
Sulfonamides	Sulfapyridine	250.1	156.0 (Q), 108.1 (C), 92.1 (C)
	Sulfathiazole	256.1	156.1 (Q), 92.0 (C), 108.1 (C)
Tetracyclines	Doxycycline	445.3	154.0 (Q), 428.5 (C)
	Oxytetracycline	461.2	426.4
	Demeclocycline	465.2	448.4 (Q), 430.4 (C)
Aminoglycosides	Streptomycin	582.3	263.0 (Q), 246.0 (C), 203.9 (C), 221.0 (C)
	Dihydrostreptomycin	584.3	262.9 (Q), 245.9 (C)
Macrolides	Erythromycin	734.5	576.2
	Tilmicosin	869.6	696.3
	Tylosin	916.5	772.3

NOTE: (Q)=Quantification Ion; (C)=Confirmation Ion.

Table 1: The 10 analytes and their positive SRM transition ions

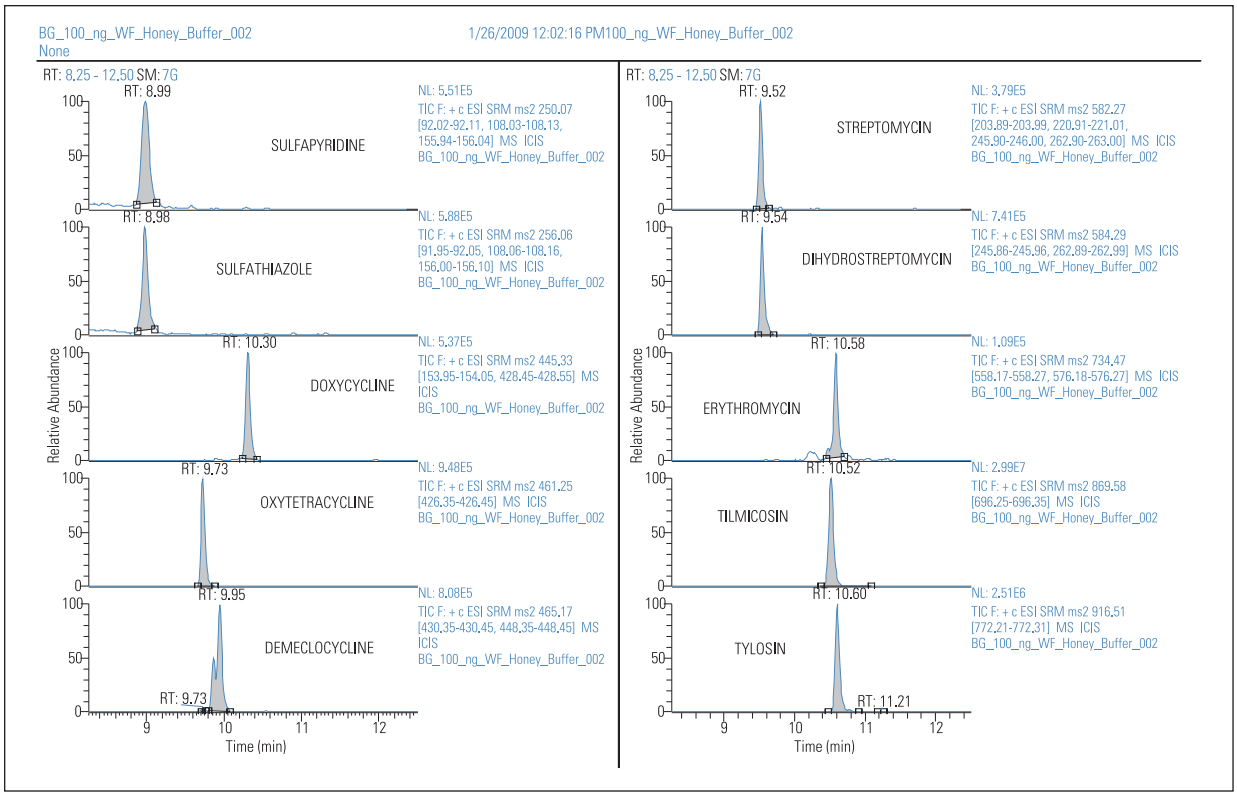

Figure 1: Example chromatogram of 100 ng/mL calibration standard in 1:1 honey/buffer

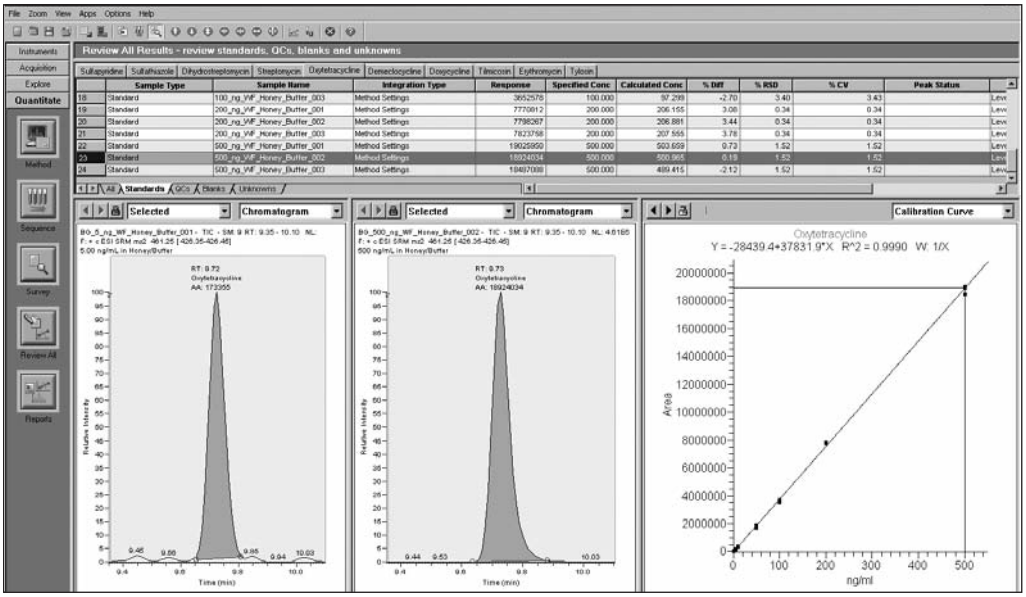

Figure 2: LCQUAN view of oxytetracycline calibration curve and LLOQ (left window) vs. ULOQ (right window) chromatograms

Analyte	R^2 (1/x weighting)	Dynamic Range* (ng/mL)**	Limit of Detection (ng/mL)	Percent Carryover (%)
Sulfapyridine	0.9980	50-500	10.0	8.95
Sulfathiazole	0.9988	50-500	10.0	5.46
Doxycycline	0.9990	10-500	5.0	10.80
Oxytetracycline	0.9990	5-500	2.0	11.70
Demeclocycline	0.9996	10-500	5.0	18.70
Streptomycin	0.9960	50-500	10.0	11.60
Dihydrostreptomycin	0.9980	50-500	10.0	6.47
Erythromycin	0.9877	50-500	10.0	1.16
Tilmicosin	0.9917	2-50	0.5	16.80
Tylosin	0.9958	10-100	5.0	13.70

**Based on analysis using 8 point standard curve (ng/mL): 0.500, 2.00, 5.00, 10.0, 50.0, 100, 200, & 500.*
***The level of carryover was included in the determination of dynamic range (kept to 20% or less).*

Table 2: Calibration curve statistics of the 10 analytes

Conclusion

During the honey quality monitoring process, it is always an analytical challenge to deal with a large number of antibiotics belonging to different classes. This often requires multiple LC-MS methods. In this study, a novel application was introduced using dual online TurboFlow extraction columns with different chemistries. The results reveal that this design facilitates the separation and quantification of all of the representative compounds in the complex honey matrix. Sample preparation time was minimal, requiring only the addition of a buffer to reduce sample viscosity. These factors enabled a broad screening for antibiotic contaminants to be performed quickly for a given sample, thus increasing sample throughput.

Additionally, multiplexing with an Aria TLX-4 system would further reduce total LC-MS/MS run time four-fold and enable screening of 12 samples per hour. Future work could involve screening a larger range of antibiotic and environmental contaminants and lowering detection limits for all analytes thus combining a screening method with accurate quantification.

References

1. *Qualitative Identification of Tetracyclines in Tissues,* CLG SOP No: CLG-TET2.01, Rev 01, p. 6, United States Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, 9/25/03.

View additional Thermo Scientific LC/MS application notes at: **www.thermo.com/appnotes**

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa-Other
+27 11 570 1840
Australia
+61 2 8844 9500
Austria
+43 1 333 50 34 0
Belgium
+32 2 482 30 30
Canada
+1 800 530 8447
China
+86 10 8419 3588
Denmark
+45 70 23 62 60
Europe-Other
+43 1 333 50 34 0
Finland/Norway/Sweden
+46 8 556 468 00
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 608 276 5659
Middle East
+43 1 333 50 34 0
Netherlands
+31 76 579 55 55
South Africa
+27 11 570 1840
Spain
+34 914 845 965
Switzerland
+41 61 716 77 00
UK
+44 1442 233555
USA
+1 800 532 4752

www.thermo.com

Thermo Fisher Scientific, San Jose, CA USA is ISO Certified.

AN63043_E 05/09M