


series	cap color	membrane	pore size	part #
eXtremelFV®		PVDF	0.2µm	85531

Analysis of Antibiotics in Honey by an Integrated On-Line Extraction UHPLC-MS/MS System

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Introduction

The most critical aspects of reliable food contamination analysis are the reduction of interferences from the sample matrix and analyte recovery. Traditionally, SPE, SLE, Liquid-Liquid, syringe filtration, and centrifugation have been used to reduce matrix interference prior to LC/MS analysis. However, these techniques are time consuming, adversely impact recovery, require expensive consumables, and use large amounts of solvent. Improved sample prep methods were developed using eXtremelFV for contaminant analysis of antibiotics honey.

Equipment

• UHLC Conditions

- Trap Column: YMC-Pack ODS-AQ, 10 µm, 10 mm x 3.0 mm I.D.
- Mobile Phase C: 0.1% Formic Acid in water
- Equilibration flow: 1000 µL (4.0 min)
- Loading Flow: 500 µL
- Analytical Column: YMC- UltraHT Pto C18 , 2 µm, 100 mm × 2.0 mm I.D.
- Column Temperature: 40 °C
- Injection Volume: 10 µL (100 µL Loop)
- Gradient
 - Mobile Phase A: 0.1% FA in water
 - Mobile Phase B: 100% MeOH

LC Gradient			
Time min.	Mobile Phase A(%)	Mobile Phase B(%)	Flow Rate µL/min.
0.0	80	20	200
0.2	80	20	200
4.0	0	100	200
6.0	0	100	200
6.1	80	20	200
8.0	80	20	200

• EVOQ Conditions

Source parameters	
Source:	HESI
Spray Voltage (+)	4000 V
Cone Gas Flow	20
Cone Temperature	350° C
Heated Probe Gas Flow	45
Heated Probe Temperature	400° C
Nebulizer Gas Flow	55
Exhaust Gas	On

Sample Preparation

- Weigh about 50mg of honey in the Thomson eXtremelFV (p/n 85531).
- Add MeOH/Water, 50/50, v/v make 100 mg/mL solution.
- Mix by pipet and depress the filter vial plunger, 0.2 µm PVDF completely to filter.
- Solution is ready for injection.

Results

Store bought honey samples analyzed by UHPLC-MS/MS ciprofloxacin, tetracycline, enrofloxacin and erythromycin were analyzed utilizing nine point calibration curves for the individual antibiotics, see Table 3. Simple sample prep was achieved using the Thomson eXtreme Filter Vial, 0.2um PVDF. Excellent linearity was achieved from 0.05ng/mL to 20ng/mL. The LOQ was determined to be < 0.5ng/g. Chromatograms at 0.05ng/mL of spiked honey show over lapping peaks that are resolved by mass for the ciprofloxacin, tetracycline, and enrofloxacin. While the erythromycin is nicely resolved by both LC and MS. In the chromatograms in fig.? 0.5ng antibiotics were spiked into 1.0g honey to yield a concentration of 0.05ng/mL.

Store bought honey from the US (3 different brands), Canada, China and India were analyzed for ciprofloxacin, tetracycline, enrofloxacin and erythromycin. Calculation is base on matrix calibration curve (=100/(detected amount/spiked amount).The recovery for iprofloxacin and erythromycin looks consistent across all levels. The enrofloxacin signal enhanced in matrix and tetracycline signal enhanced at low concentration. Results with an ND are < 0.05ng/mL.

Table 1. Antibiotics analyzed in this method.

Compound Name	Retention Time	Q1 First Mass	Q3 First Mass	Collision Energy	Quantifier Ions	Qual Mass
Ciprofloxacin	3.168	332.2	314	-18		314
			230.9	-34	230.90	
			245	-18		
Enrofloxacin	3.201	360.3	342	-18	342.00	
			286	-29		286
			316	-15		316
Tetracycline	3.169	445.2	410	-15	410.0	
			154	-24		154
			427.1	-9		427.1
Erythromycin	4.370	734	158.1	-27	158.1	
			576.3	-16		576.3

Table 2. Structures, Formula and LOQ for the Antibiotics used.

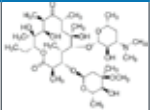
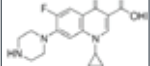
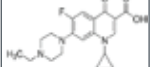
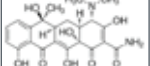
Name	Structure	Formula	Tolerance Limit (ug/kg, ppb)
Erythromycin		C37H67NO13	5
Ciprofloxacin		C17H18FN3O3	5
Enrofloxacin		C19H22FN3O3	5
Tetracycline		C22H24N2O8	5

Table 3. Calibration curve levels for antibiotics in honey.

Calibration Level	ng/g	ng/mL
1	0.5	0.05
2	1	0.1
3	2	0.2
4	5	0.5
5	10	1
6	20	2
7	50	5

Calibration Level	ng/g	ng/mL
8	100	10
9	200	20

Fig 1. 9 point calibration curves.

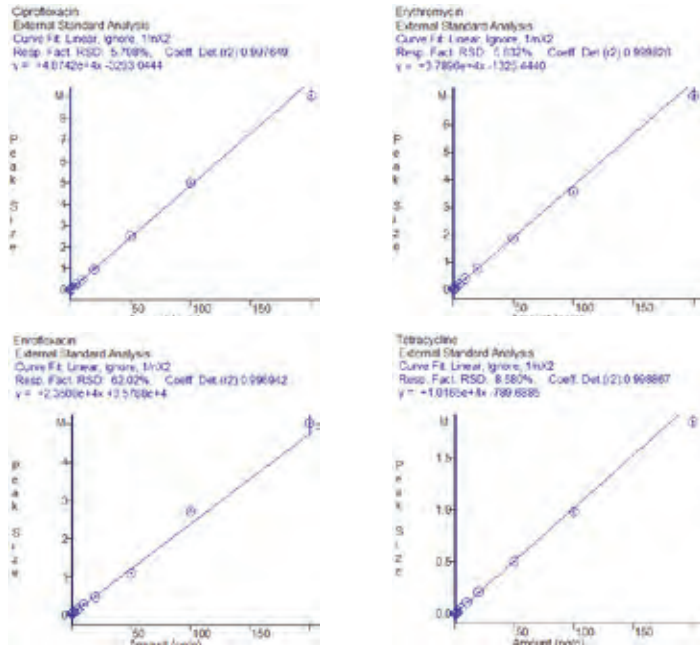


Fig 2. Chromatograms of antibiotics spiked into honey, USA-1.

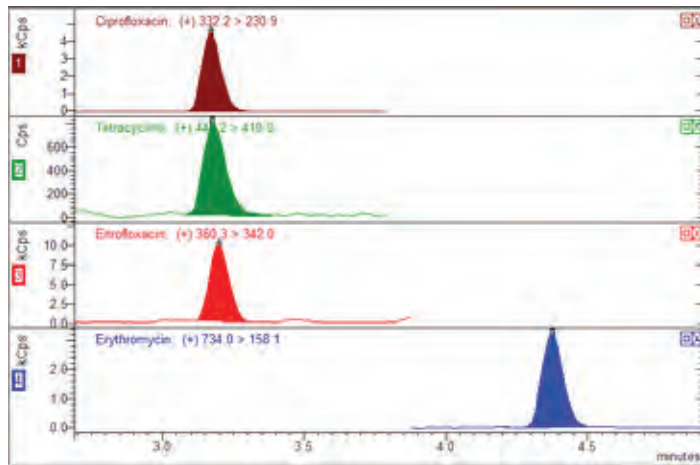


Table 4. Store bought honey from the US (3 different brands), Canada, China and India were analyzed for ciprofloxacin, tetracycline, enrofloxacin and erythromycin. ND is <0.5ng/g. test result based on calibration curve of antibiotics in honey. * Honey from China contains approximately 0.2ng/g Ciproloxacin

Antibiotics	Ciprofloxacin	Enrofloxacin	Erythromycin	Tetracycline
Honey Source	Concentration in ng/g			
USA-1	ND	ND	ND	ND
USA-2	ND	ND	ND	ND
USA-3	ND	ND	ND	ND
Canada	ND	ND	ND	ND
China	ND	ND	ND	ND
India	ND	ND	ND	3.8

Table 5. Recovery of antibiotics spiked into store bought honey, USA-1.

Recovery				
Standard (ng/g)	Ciprofloxacin	Enrofloxacin	Erythromycin	Tetracycline
0.5	109.6	-	85.5	191
1	121.1	-	85.7	278
2	114.2	6666.7	88.2	233
5	158.9	511.8	83.8	335
10	93.2	207.4	86.1	116
20	111.3	202.8	88.9	169
50	103.3	180.7	93.4	132
100	109.8	179.1	96.2	127
200	133.4	190.5	99.3	120

Conclusion

Bruker UHPLC combined with the EVOQ Elite Triple Quadrupole MS was used for identification and quantification of ciprofloxacin, tetracycline, enrofloxacin and erythromycin in store-bought honey utilizing the Thomson eXtremeIFV, 0.2um PVDF. Simple sample prep consisting of diluting the sample, filtering and injecting onto the UHPLC-MS/MS achieved LOQ of < 0.05ng/mL and LOD of 0.02ng/mL.