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# 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 eXtreme|FV® for contaminant analysis of antibiotics honey.

## Equipment

- UHPLC 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
- EVOQ Conditions

## Sample Preparation

- Weigh about 50mg of honey in the Thomson eXtreme|FV® (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|FV®, 0.2µm 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. 1., 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.** 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 |
| 8 | 100 | 10 |
| 9 | 200 | 20 |

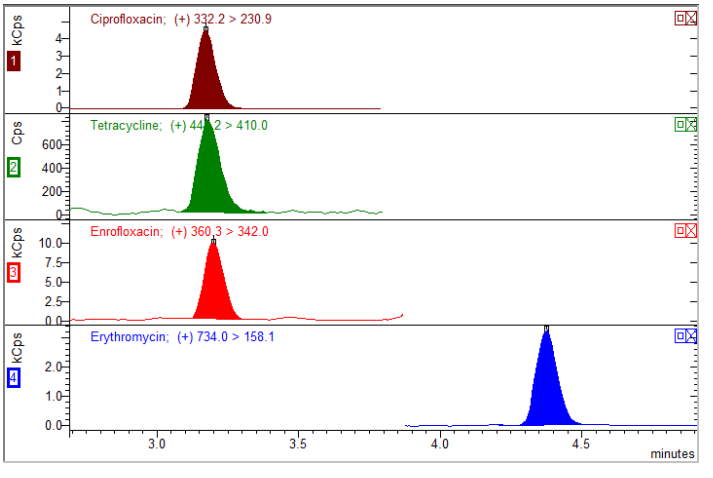


**Fig 1.** Chromatograms of antibiotics spiked into honey, USA-1.

## 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 eXtreme|FV®, 0.2µm 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.