# Waters AOAC 2006

#### **INTRODUCTION/OVERVIEW**

In 2002 the MRL (maximum residue level) for the antibiotic enrofloxacin (Baytril) was 100 µg/kg in muscle and 200 µg/kg in liver for bovine or poultry. In 2005 the US FDA banned the use of the antibiotic enrofloxacin (Baytril) as a growth enhancer in poultry because such use may promote drug-resistant bacteria, such as Campylobacter, that can be harmful to humans. The EU, more aggressive in the regulation of antibiotic use for livestock growth enhancement, intends to ban all such use in 2006.

At HPLC 2003, we presented a poster on an SPE and LC-MS/MS method for determination of enrofloxacin and other fluoroquinolone antibiotics with LOQ of 10 µg/kg in beef kidney. That methodology was highly effective for determination of substances in tissue with MRL of 100  $\mu$ g/kg. However, in order to assure compliance with the ban on the use of enrofloxacin, an effective analytical procedure with LOQ below 1 µg/kg is required. This poster presents SPE and UPLC-MS conditions suitable for determination of enrofloxacin and its metabolite ciprofloxacin with 0.3 µg/kg LOQ in chicken muscle and liver. Fluoroquinolone antibiotics such as enrofloxacin are amphoteric substances with both acid and basic functionality. Therefore, both anion- and cation-exchange may be employed for sample preparation. Chicken tissue samples can be initially extracted using either acidified ethanol or pH 7.4 aqueous buffer. Recovery was about 70 % using aqueous buffer; recovery was about 85 % using ethanol/acetic acid. Optimized SPE protocols were developed for both of these types of tissue extracts. In each case, high analyte recovery was achieved, along with significant reduction of ion-suppression, by use of mixed-mode SPE protocols employing both anion and cation-exchange. Analysis was accomplished using UPLC coupled to ESI+/MS/MS.



#### **TISSUE EXTRACTION/SPE Ethanolic Acid Extraction**

A 1.5 gram sample is homogenized and extracted with 30 mL ethanol/acetic acid (99:1). After centrifugation, a 10 mL aliquot of the supernatant is collected for SPE enrichment and clean-up. For muscle samples, 10 mL of supernatant are diluted with 5 mL water prior to SPE; liver samples were not diluted. The SPE procedure involves initial mixed-mode cationexchange retention and cleanup (Oasis MCX, cartridge I), followed by further enrichment and cleanup using anionexchange (Sep-Pak Accell QMA, cartridge II)

#### Aqueous pH 7.4 Buffer Extraction

A 1.5 gram sample is homogenized and extracted with 30 mL of 50 mM sodium phosphate buffer (pH 7.4). After centrifugation, a 5 mL aliquot of the supernatant is collected for SPE enrichment and clean-up. The SPE procedure involves initial mixed-mode anion-exchange retention and cleanup (Oasis MAX, cartridge I), followed by further enrichment and cleanup using cation-exchange (Oasis MCX, cartridge II).

### **SPE PRODUCTS FOR THIS STUDY**



Mixed-mode Strong Cation-Exchange



Sep-Pak Accell™ Plus QMA Silica-Based Strong Anion-Exchange

### TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS

## UPLC-MS-MS DETERMINATION OF ENROFLOXACIN AND CIPROFLOXACIN IN CHICKEN TISSUE

### **SPE PROTOCOLS**



### **UPLC-MS ANALYSIS**



**Typical LC/MS Result** 

#### Liquid Chromatography Waters Acquity<sup>™</sup> UPLC<sup>™</sup>

Column: Acquity<sup>™</sup> BEH C18, 50 x 1 mm, (1.7 micron) Flow Rate: 0.12 mL/minute (approx. 7500 psi) Injection Volume: 10 µL Column Temperature: 30 °C Mobile Phase A: 1% formic acid in water Mobile Phase B: acetonitrile Gradient composition: Linear from 5 % B to 50 % B in 3 minutes



**Typical Calibration** 

#### Mass Spectrometry

Waters Quattro micro API electrospray positive mode

Compound	MRM	Cone (V)	Collision (eV)
Enrofloxacin	360 > 342	25	25
	360 > 316	25	20
Ciprofloxacin	332 > 314	25	23
	332 > 288	25	20



Mixed-mode Strong Anion-Exchange

KimVan Tran and Michael S. Young Waters Corporation, 34 Maple Street, Milford, MA 01757 USA

#### **Aqueous pH 7.4 Buffer Tissue Extraction**



#### RESULTS

- Enrofloxacin recovery averaged 74 % for tissue samples processed using ethanol/acid; ciprofloxacin recovery averaged 80 %
- Enrofloxacin recovery averaged 83 % for tissue samples processed using aqueous buffer; ciprofloxacin recovery averaged 78 %
- Typical precision for 6 replicates spiked at 2 ng/g was 10-15 % RSD
- LOQ is about 0.5 ng/g for enrofloxacin and 1.0 ng/ g for ciprofloxacin in chicken muscle or liver using either sample preparation protocol
- Ion-suppression was under 20 % using either SPE protocol

### CONCLUSIONS

- Straightforward SPE protocols were used to prepare chicken tissue samples for analysis using UPLC-MS-MS
- Tandem SPE using both cation and anion-exchange retention of the amphoteric fluoroquinolone antibiotics provides excellent sample enrichment and cleanup
- UPLC-MS analysis was performed in electrospray negative mode without ion-pairing mobile phase
- Lower detection limits and much faster analysis times were obtained compared with traditional HPLC