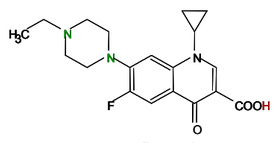
ADVANCED SPE PROTOCOLS FOR UPLC-MS-MS DETERMINATION OF ENROFLOXACIN AND CIPROFLOXACIN IN CHICKEN TISSUE AT SUB PPB LEVELS

Michael S. Young, Kimvan Tran, and Kevin M. Jenkins
Waters Corporation. Milford. MA USA
michael_s_young@waters.com

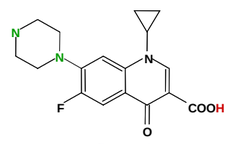
INTRODUCTION/OVERVIEW

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 µg/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.



- Amphoteric Compounds
  - pKa of acid functionality » 5
  - pKa of basic functionality » 8-10
- Can be extracted using ion-exchange sorbents
  - at pH below 6 using cation-exchange

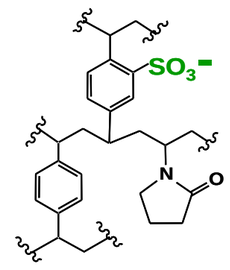


TISSUE EXTRACTION/SPE

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 cation-exchange retention and cleanup (Oasis® MCX, cartridge I), followed by further enrichment and cleanup using anion-exchange (Sep-Pak Accell® QMA, cartridge II).

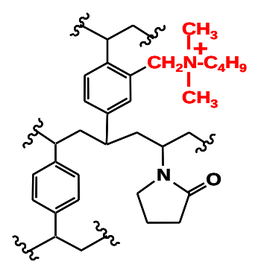
Aqueous pH 7.4 Buffer Extraction
A 1.5 gram sample is homogenized and extracted with 30 mL of 50 mM sodium phosphate buffer (pH 7.4). After centrifugation, a 5 mL aliquot of the supernatant is collected for SPE enrichment and clean-up. The SPE procedure involves initial mixed-mode anion-exchange retention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

Oasis® MCX



Mixed-mode
Strong Cation-Exchange

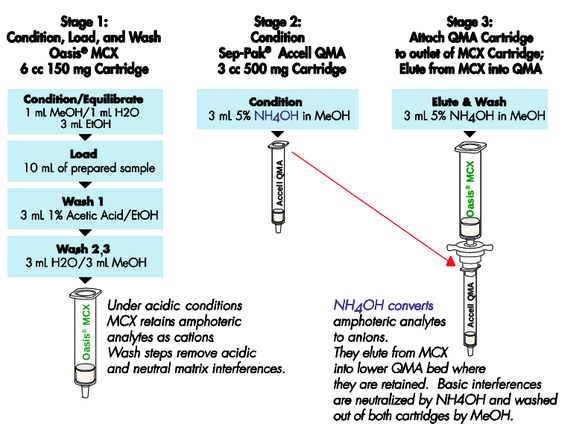
Oasis® MAX



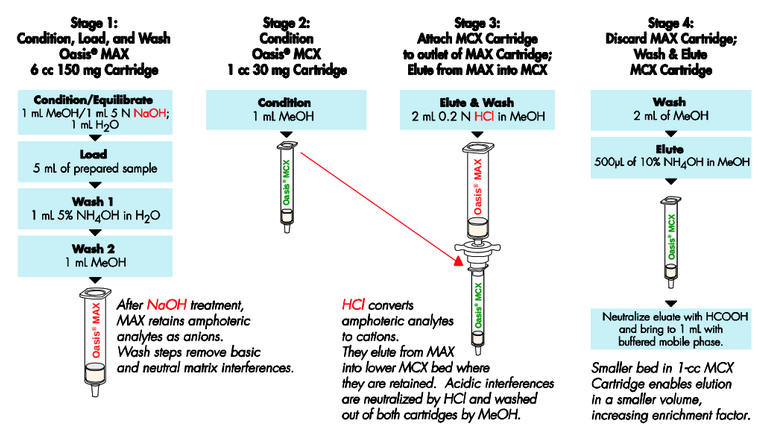
Mixed-mode
Strong Anton-Exchange

SPE PROTOCOLS

Ethanol/Acid Tissue Extraction



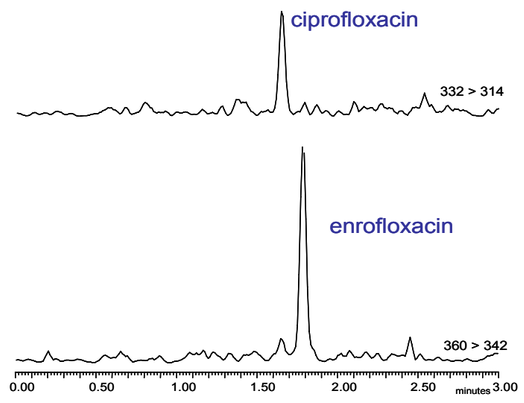
Aqueous pH 7.4 Buffer Tissue Extraction



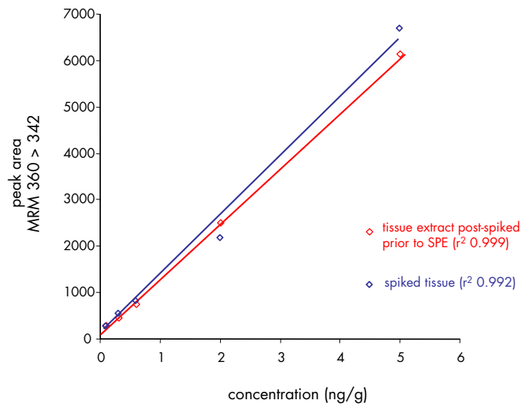
RESULTS

- 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 0.6 ng/g for enrofloxacin and 1.0 ng/g for ciprofloxacin in chicken muscle or liver.

UPLC-MS ANALYSIS



Typical LC/MS Result
Spiked Chicken Muscle, 2 ng/g



Typical Calibration Curve

Ion-suppression was under 20 % using either SPE protocol

Liquid Chromatography
Waters Acquity™ UPLC™

Column: ACQUITY UPLC™ 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

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

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

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