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SPE sample preparation for UPLC–MS determination of enrofloxacin (Baytril®) in chicken

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In 2005, the US FDA banned the use of the enrofloxacin (Baytril®) for growth enhancement in poultry production. Prior to this ruling, an LOQ of 10 µg/kg was appropriate for enrofloxacin residue methods. However, in light of the recent change in regulation, any detectable residue of enrofloxacin (or ciprofloxacin metabolite) can be considered evidence of inappropriate poultry farming practice. Consequently, much lower quantitation limits may be necessary.

The structure of enrofloxacin is presented in Figure 1. This compound is amphoteric with basic amine and acidic carboxylate functionalities. The amphoteric nature of this molecule allows both anion and cation-exchange to be considered for SPE enrichment and cleanup.

This application note describes SPE and UPLC–MS conditions suitable for the trace level determination of enrofloxacin and ciprofloxacin in chicken muscle or liver.

Sample preparation and tissue 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).

SPE procedure

Cartridge I

1. Condition cartridge I (Oasis® MCX, 6 cc, 150 mg) with 3 mL methanol, 3 mL water and 3 mL ethanol.
2. Load 10 mL sample.
3. Wash cartridge I with 3 mL of ethanol, 3 mL water and 3 mL methanol.

Cartridge II

1. Condition cartridge II (Sep-Pak® Accell™ QMA, 3 cc, 500 mg) with 3 mL 5% ammonia in methanol and 3 mL methanol.
2. Attach cartridge I atop cartridge II (6 cc cartridge on top, see Figure 2).
3. Elute the tandem cartridge assembly with 3 mL 5% ammonia in methanol. As the analytes are eluted from cartridge I, they are subsequently retained by anion-exchange on cartridge II. Therefore the eluate from cartridge I becomes the load for cartridge II.
4. Remove cartridge I.
5. Wash cartridge II with 3 mL ethanol.
6. Elute cartridge II with 3 mL methanol/formic acid (98:2).
7. Evaporate solvent and reconstitute in 150 µL of acetonitrile/water (15:85).

UPLC–MS-MS Analysis

Prepared samples were analyzed with an ACQUITY UPLC™ separations module interfaced to a Waters Quattro micro™ API mass-spectrometer operated in MRM mode. UPLC™ and MS conditions for this study are presented below. Data used in this study were collected using isocratic and gradient methods.

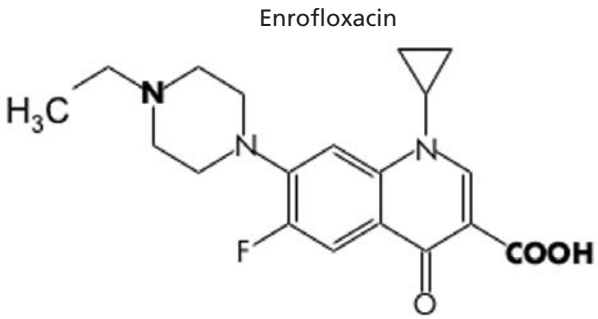

Figure 1: Enrofloxzacin chemical structure. Note that this compound is an amine (base) and a carboxylic acid.

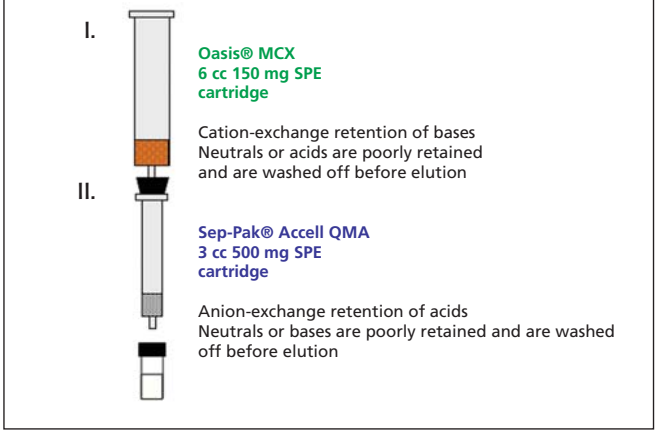

Figure 2: Tandem SPE cartridge setup.

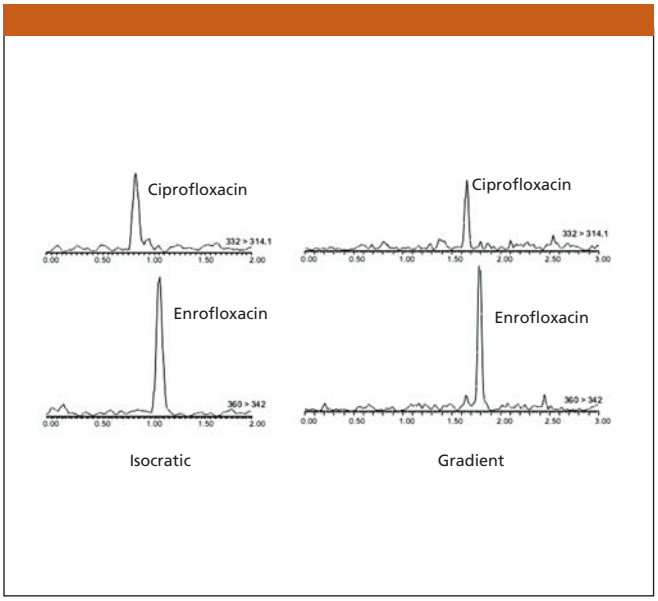

Figure 3: Typical UPLC–MS-MS chromatograms obtained from chicken muscle spiked at 2 µg/kg, isocratic conditions.

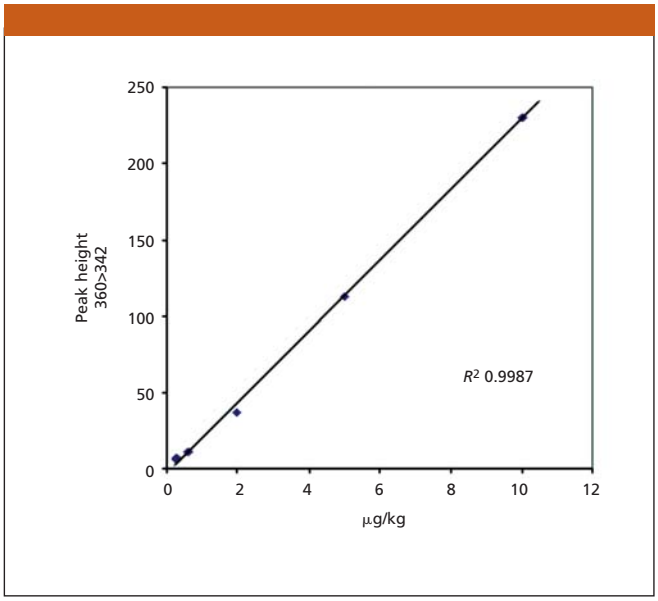

Figure 4: Typical UPLC calibration curve for enrofloxacin in chicken muscle.

Both separation modes gave comparable results.

UPLC Conditions

Column: ACQUITY UPLC™ BEH C18, 1.7 µm, 1.0 × 50 mm
Mobile Phase A: 1% formic acid in water
Mobile Phase B: acetonitrile
Flow Rate: 0.12 mL/minute (approx. 7500 psi)
Injection Volume: 10 µL
Column Temperature: 30 °C

Isocratic separation

Isocratic composition: A: 85%; B: 15%

Gradient separation

Gradient composition:

Linear from 5 % B to 50 % B in 3 minutes
hold at 50% B (3.5 minutes)
linear from 50% B back to 5 % B (4 minutes)
equilibrate at 5% B (5 minutes)

Mass Spectrometry Conditions

Table I. Monitored MS transitions.

Compound	MW	MRM	Cone Voltage	Collision Energy
Enrofloxacin	359	360 > 342	25	25
		360 > 316	25	20
Ciprofloxacin	351	332 > 314	25	23
		332 > 288	25	20

Results and Discussion

UPLC–MS-MS provides great sensitivity and selectivity for determination of these antibiotics in chicken tissue. Figure 3 shows chromatograms obtained from a 2.0 µg/kg spiked chicken muscle sample. Figure 4 shows typical calibration data obtained from spiked chicken muscle samples; results for chicken liver were similar. Recovery averaged 75 % measured by comparison of results from chicken samples spiked before and after sample preparation. Precision measured for six replicate samples spiked at 2 µg/kg was 12 %.

Based on these data, the LOQ is about 0.6 µg/kg for enrofloxacin and 1.0 µg/kg for ciprofloxacin in chicken muscle or liver.

Conclusions

Straightforward SPE protocols were used to prepare chicken tissue samples for analysis using UPLC–MS-MS. Compared with prior methods, much lower quantitation limits were obtained for determination of enrofloxacin and ciprofloxacin in chicken. Two important factors made these results possible. First, tandem SPE using both cation and anion-exchange retention of the amphoteric fluoroquinolone antibiotics provided improved sample enrichment and cleanup. Second, the UPLC–MS system employed for this study gave outstanding chromatographic performance with lower detection limits and much faster analysis times compared with traditional HPLC.

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