Determination of Fluoroquinolone Residues in Bovine Kidney Using SPE and LC/MS/MS

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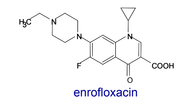
OVERVIEW

An LC/MS method is described for the trace quantification of nine fluoroquinolone (FLQ) residues (ciprofloxacin, enrofloxacin, sarafloxacin, enofloxacin, flumequine, norfloxacin, ofloxacin, lomefloxacin and danofloxacin) in bovine kidney. The drug residues were extracted from a 2 g kidney sample using an aqueous buffer and the sample extracts were cleaned and enriched using mixed-mode anion exchange solid phase extraction (Oasis® MAX) for sample clean-up and enrichment. All nine FLQ's can be resolved chromatographically using isocratic mobile phase conditions. Positive ion atmospheric pressure chemical ionization (APCI+) mass spectrometry was used to quantify and confirm the parent ion $[M+H]^+$ and daughter fragments for each analyte.

Fluoroquinolones are a synthetic class of antibiotics that are highly effective against gram-positive and gram-negative bacteria. This class of antibiotics was originally developed in 1962 for human use; however, in 1995 selected drugs from this family of antibiotics were approved for animal husbandry in North America. Now, there is concern because of reports that human diseases have developed antibacterial resistance to these drugs. Therefore, the FDA has recently banned the extralabel use of the fluoroquinolones in food-producing animals.

This presentation outlines a protocol for the effective clean-up and extraction of FLQ residues in bovine kidney and muscle. For regulatory purposes, the method was optimized for LC/MS/MS analysis to provide sensitivity and selectivity.

FLUOROQUINOLONE STRUCTURE



enrofloxacin

- Amphoteric behavior
  - pKa of acid functionality » 5
  - pKa of basic functionality » 8-9
- Can be extracted using ion-exchange sorbents
  - At pH below 6 using cation-exchange
  - at pH above 6 using anion-exchange

HPLC CONDITIONS

LC/MS/MS
Column: Atlantis™ dC_{18} 4.6 x 150 mm 5 µm
Mobile Phase: A: 0.2% NFPA* in water
B: Methanol
Gradient: Linear, 40% B to 80% B in 10 min.
Flow: 0.8 mL/min
Temperature: 30°C
Injection: 30-80 µL

LC-UV/Fluorescence/Single Quad MS
Column: Atlantis™ dC_{18} 4.6 x 150 mm 5 µm
Mobile Phase: 75% NFPA* (0.2%) in water
3% Methanol
22% Acetonitrile
Flow: 1.2 mL/min
Temperature: 30°C

* CF_3-CF_2-CF_2-CF_2-COOH
nonafluoropentanoic acid (NFPA)

MS CONDITIONS

Compound	MW	MRM	Cone (V)	Collsion Energy (eV)
flumequin	261	262→244	50	20
enoxacin	320	321→303	50	20
norfloxacin	319	320→302	50	23
Sarafloxacin	385	386→368	50	25
ofloxacin	361	362→344	50	20
Enrofloxacin	359	360→342	50	20
danofloxacin	357	358→340	50	25
lomefloxacin	351	352→334	50	20
ciprofloxacin	331	332→214	50	20

APCI+ or ESI+ can be used for FLQ analysis - in the absence of matrix, ESI+ may be somewhat more sensitive. APCI+ was chosen to minimize matrix suppression effects and the suppression effect of the ion-pairing reagent. Matrix effect was < 20% for all compounds.

LC SEPARATIONS

Isocratic Separation (Useful for UV or fluorescence)

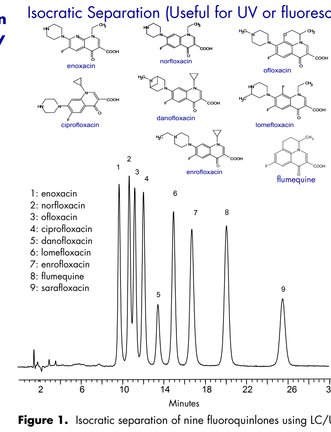
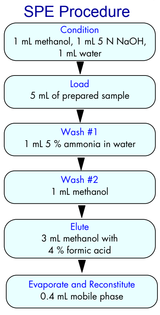


Figure 1. Isocratic separation of nine fluoroquinlones using LC/UV.

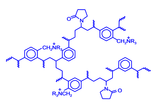
SPE CONDITIONS

Initial Tissue Extraction: Extract 2 g of kidney with 30 mL of 50 mM phosphate buffer (pH 7.4) centrifuge at 10 000 rpm for 10 minutes.

SPE Procedure



SPE
Oasis® MAX
(150 mg, 6 cc)



Considerations for Initial Tissue Extraction

Fluoroquinolone antibiotics can be extracted from tissue at low, high or neutral pH.

- At low pH, extraction into ethanol is preferred; higher matrix interference results from aqueous buffer extraction
- At high pH extraction is difficult using either ethanol or aqueous buffer; high amounts of matrix interference
- At neutral pH, extraction into aqueous buffer is preferred; higher matrix interference results from ethanol extraction

Neutral pH extraction into aqueous buffer was chosen for this study.

Gradient Separation (Useful for LC/MS)

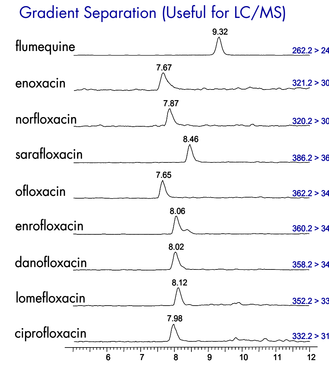


Figure 2. Gradient separation of nine fluoroquinlones using LC/MS/MS.

RESULTS

Table 1. Recovery data for nine fluoroquinolones using LC/MS/MS.

Analyte	5 ppb			10 ppb			25 ppb			50 ppb			75 ppb			100 ppb		
	Recovery (%)	STD Dev	RSD (%)	Recovery (%)	STD Dev	RSD (%)	Recovery (%)	STD Dev	RSD (%)	Recovery (%)	STD Dev	RSD (%)	Recovery (%)	STD Dev	RSD (%)	Recovery (%)	STD Dev	RSD (%)
Cipro	81.3	11.5	14.1	70.2	8.0	11.3	62.7	3.6	5.7	64.0	5.0	7.8	72.0	5.4	7.4	80.5	3.8	4.7
Lome	68.3	17.3	25.4	76.9	5.0	6.5	81.6	6.0	7.3	70.9	6.4	9.0	84.3	5.3	6.3	87.8	4.7	5.3
Dano	86.2	16.8	16.5	75.5	5.8	7.7	75.9	4.2	5.5	66.6	5.0	7.5	76.4	7.0	9.1	77.5	5.6	7.3
Enro	79.2	11.6	14.6	73.3	15.6	21.3	82.4	3.0	3.6	70.6	5.4	7.7	85.6	5.7	6.7	108.4	6.1	5.6
Oflo	77.0	7.6	9.8	85.3	5.3	6.2	77.1	5.8	7.5	72.7	5.1	7.0	81.5	5.7	6.9	91.9	7.1	7.7
Flum	70.9	12.0	17.0	75.0	10.1	13.5	75.2	7.2	9.6	65.9	7.4	11.2	67.4	4.9	7.3	68.0	4.6	6.8
Sara	87.0	11.6	13.3	71.9	7.8	10.9	91.1	4.0	4.3	69.6	4.7	6.7	82.8	4.3	5.2	82.0	4.5	5.5
Nor	70.1	12.0	17.1	70.2	5.8	8.2	61.8	4.6	7.5	62.9	4.9	7.9	67.2	5.6	8.3	75.7	4.5	5.9
Eno	63.9	7.6	12.0	69.4	7.7	11.0	59.3	6.0	10.1	63.0	6.2	9.9	67.5	6.9	10.3	78.7	6.0	7.6

FUTURE CONSIDERATIONS

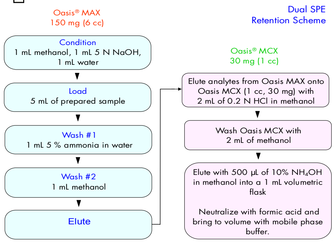
Optimization of SPE for LC/Fluorescence

Oasis® MAX
150 mg 6cc
SPE Cartridge

Retains anionic species by ion-exchange. Cationic and neutral species are bound by a reversed phased mechanism.

Oasis® MCX
30 mg 1cc
SPE Cartridge

Only cationic species are retained. All neutral and acidic species pass through.



A dual cartridge SPE clean-up improves sample cleanliness and lowers detection limits. The following two chromatograms show the improved clean-up.

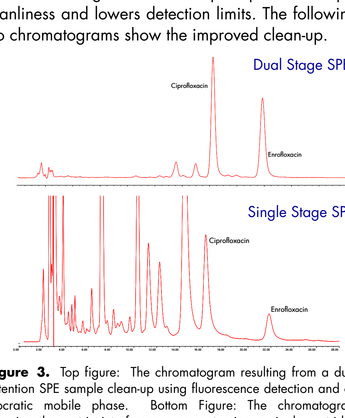


Figure 3. Top figure: The chromatogram resulting from a dual retention SPE sample clean-up using fluorescence detection and an isocratic mobile phase. Bottom Figure: The chromatogram showing the matrix interferences present using a single cartridge SPE clean-up. Both samples are from a 100 ppb spiked bovine kidney sample.

CONCLUSIONS

- Improved SPE protocol for bovine kidney
  - Reproducible 70% recovery
  - LOQ below 10 µg/kg
  - Fast, straightforward method
- Improved HPLC separation
  - NFPA allows for near baseline separation for LC or LC/UV
  - NFPA gives good separation from matrix for LC/MS/MS
- Detection independent method
  - Allows for MS/MS confirmation
  - Ideal for fluorescence detection