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LC–MS Determination of Fluoroquinolone Antibiotics in Beef Tissue Using the Atlantis® dC_{18} Column

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The Atlantis® dC_{18} column demonstrates selectivity and separation of nine fluoroquinolone antibiotics. Oasis® SPE products provide reproducible sample enrichment and cleanup.

This application note describes conditions for separation of nine fluoroquinolone antibiotic residues using Atlantis® dC18 HPLC columns. An isocratic separation is presented for analysis using fluorescence or UV, and a rapid gradient separation is presented for use with liquid chromatography (LC)–mass spectrometry (MS). A straightforward Oasis® MAX solid-phase extraction (SPE) protocol was applied for preparation of beef kidney samples prior to LC–MS analysis.

Experimental Conditions

Instruments: Alliance 2695 separations module equipped with column heater and sample chiller, Waters 2475 fluorescence detector, Waters 2487 dual wavelength UV detector, Waters/Micromass Quattro Micro™ mass spectrometer.

Sample Preparation and Tissue Extraction

A 2 g kidney sample was extracted with 30 mL of 50-mM sodium phophate buffer (pH 7.4), centrifuged, and the supernatant collected for SPE enrichment and clean-up.

SPE Procedure

1. Condition a 500 mg, 6 cc Oasis® MAX SPE cartridge with 1 mL methanol, 1 mL 5 N NaOH, and 1 mL water.
2. Load 5 mL of the collected kidney extract.
3. Wash the cartridge with 1 mL of 5% ammonia in water followed by 1 mL of methanol.
4. Elute with 5 mL of 4% formic acid in methanol. Evaporate solvent and reconstitute in 400 µL of mobile phase buffer.

LC Conditions

Isocratic Separation for Fluorescence or UV Detection

Column: Atlantis® dC_{18} 4.6 × 150 mm, 5 µm
Mobile phase A: 0.2% nonafluoropentanoic acid in water
Mobile phase B: acetonitrile
Mobile phase C: methanol
Flow rate: 1.2 mL/min
Isocratic composition: 75% A: 22% B: 3% C
Injection volume: 30–80 µL
Column temperature: 30 °C
Fluorescence detection: λ_{ex} = 278 nm λ_{em} = 445 nm

Gradient Separation for APCI MS

Column: Atlantis® dC_{18} 4.6 × 150 mm, 5 µm
Mobile phase A: 0.2% nonafluoropentanoic acid in water
Mobile phase B: methanol
Flow rate: 0.8 mL/min
Gradient composition: linear for 10 min from 40% B to 80% B
Injection volume: 30–80 µL
Column temperature: 30 °C

Monitored MS Transitions

Results and Discussion

Table I: Primary MS transitions for nine fluoroquinolone antibiotics

Compound	MW	MRM	Cone Voltage	Collision Energy
Flumequine	261	262 > 244	50	20
Enoxacin	320	321 > 303	50	20
Norfloxacin	319	320 > 250	50	23
Sarafloxacin	385	386 > 368	50	25
Ofloxaicn	361	362 > 344	50	20
Enrofloxacin	359	360 > 342	50	20
Danfloxacin	357	358 > 340	50	25
Lomefloxacin	351	352 > 334	50	20
Ciprofloxacin	331	332 > 214	50	20

Fluorescence detection has been a powerful regulatory tool for determination of these residues in a variety of samples matrices. Figure 1 shows an isocratic separation of nine fluoroquinolone antibiotics useful for fluorescence or UV detection.

LC–MS-MS provides greater sensitivity and selectivity for determination of these antibiotics. Figure 2 shows LC–MS-MS chromatograms obtained from a 10-ng/mL spiked bovine kidney sample. In APCI mode, the ion-pairing additive, nonafluoropentanoic acid, did not contribute to ion suppression. Table II shows recovery data obtained at 10 and 100 ng/g in spiked bovine kidney using LC–MS.

Conclusions

The Atlantis® dC_{18} analytical column provides suitable selectivity and retention for polar analytes in biological matrices. The stable sorbent is shown to be compatible with MS analysis and does not demonstrate column bleed.

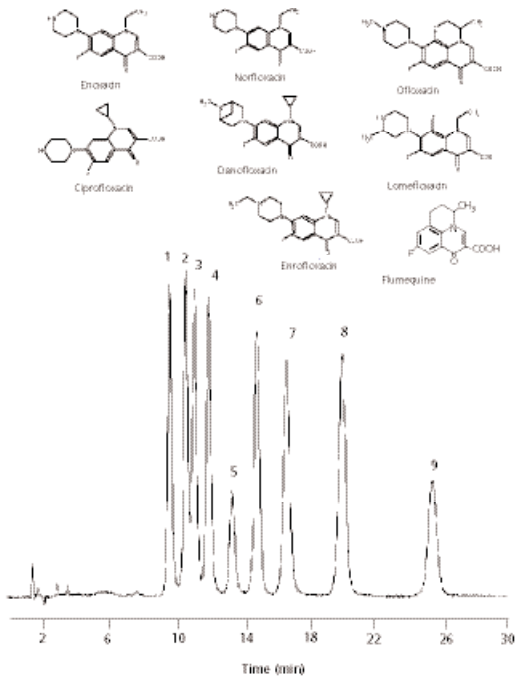

Figure 1: An LC–UV chromatogram demonstrating the isocratic separation of nine fluoroquinolone antibiotics at a standard tissue equivalent of 1 μg/g. Peaks: 1 = enoxacin; 2 = norfloxacin; 3 = ofloxacin; 4 = ciprofloxacin; 5 = danofloxacin; 6 = lomefloxacin; 7 = enrofloxacin; 8 = flumequine; 9 = sarafloxacin.

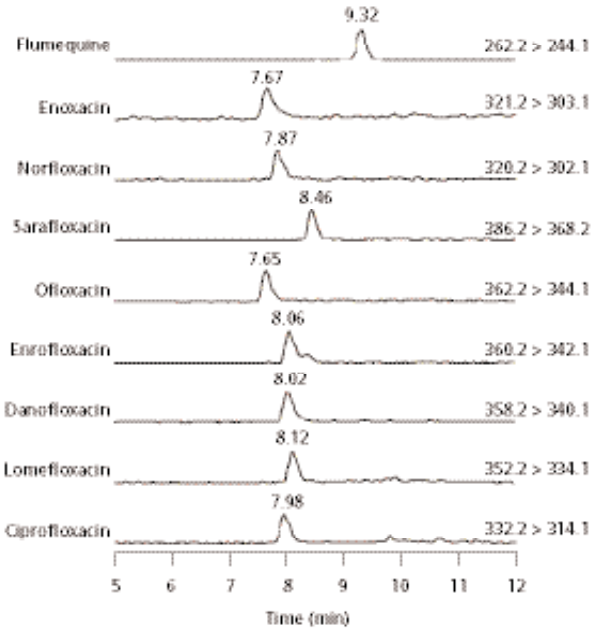

Figure 2: An LC–MS chromatogram demonstrating the gradient separation of nine fluoroquinolone antibiotics spiked into bovine kidney at a standard equivalent of 10 ng/g. Table II summarizes the recovery data obtained at this level.

Table II: Recovery data of spiked bovine kidney at 10 and 100 ng/g. The relative standard deviation at each level is bracketed; five replicates per level

	Analyte				
Level (ng/g)	Lomefloxacin	Danofloxacin	Ofloxacin	Flumaquine	Norfloxacin
10	76.9 [6.5]	75.5 [7.7]	85.3 [6.2]	75.0 [13.5]	70.2 [8.2]
100	87.8 [5.3]	77.5 [7.3]	91.9 [7.7]	68.0 [6.8]	75.7 [5.9]

	Analyte			
Level (ng/g)	Enoxacin	Ciprofloxacin	Enrofloxacin	Sarafloxacin
10	69.4 [11.0]	70.2 [11.3]	73.3 [21.3]	71.9 [10.9]
100	78.7 [7.6]	80.5 [4.7]	108.4 [5.6]	82.0 [5.5]

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