APPLICATION BRIEF

Food/Nutraceutical

Analysis of Quinolones by UHPLC/ Fluorescence/SQ MS

Quinolones are structurally related antibiotics that are used in human and veterinary medicine. European Union (EU) has set tolerance levels for quinolones while FDA has banned the use of some fluoroquinolones in products of animal origin. We present a method to measure nine quinolones including some fluoroquinolones using UHPLC for separation followed by detection with a single quadrupole mass spectrometer and a fluorescence detector (Figures 1 and 2).

Experimental Conditions

Target Analytes: Norfloxacin, ofloxacin, ciprofloxacin, enoxacin, cinoxacin, nalidixic acid, pipemidic acid, enrofloxacin, flumequine

Standard Concentrations

Concentration of ciprofloxacin, nalidixic acid and flumequine were each at 2.5 µg/mL, while the remaining quinolones were at 4 µg/mL.

Liquid Chromatography Conditions

Pump Type:	PerkinElmer® Flexar™ FX-15
Column:	PerkinElmer Brownlee™ Supra™ C18 column (2.1 mm x 50 mm, 1.9 µm)
Mobile Phase:	A: water with 0.1% formic acid B: acetonitrile with 0.1% formic acid
Flow Rate:	0.8 mL/min with a post column split of 0.4 mL/min each into fluorescence and MS detectors

Injection Volume: 1 µL in partial fill mode

Gradient:	Time (min)	%A	%B
	0	90	10
	3	85	15
	2	80	20
	0.3	60	40
	0.7	60	40

Fluorescence Detector Conditions

Excitation:	275 nm
Emission:	400 nm

Mass Spectrometer Conditions

Ionization:	Ultraspray™ ESI – Positive mode
Scan Range:	200-400 m/z
Scan Rate:	400 u/sec
Capillary Exit Voltage:	100 V

Results

All nine quinolones were separated by UHPLC except for norfloxacin and ofloxacin which coeluted. Due to co-elution, norfloxacin and ofloxacin cannot be quantified individually by fluorescence detection. However, it is possible to quantitate the coeluting quinolones by mass spectrometry (Figure 3) since selected ions unique to the compounds can be monitored.

PerkinElmer®
For the Better

The sensitivity of MS detection can be improved by monitoring in SIM mode for the $[M+H]^+$ ion for each target analyte. Selectivity by MS detection can be further improved by monitoring for the product ion along with the precursor ion produced by front-end CID. A representative calibration curve is shown for enrofloxacin generated by monitoring in SIM mode for the $[M+H]^+$ ion and the fragment ion resulting from loss of CO_2 (Figure 4). Enrofloxacin can be quantitated in an unknown sample by monitoring, both for the precursor and product fragment which gives additional confirmation of the presence of the analyte in a given matrix.

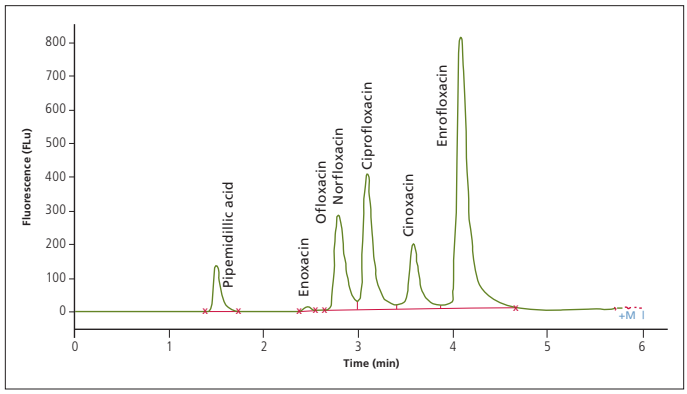

Figure 1. Separation and fluorescence detection of quinolones.

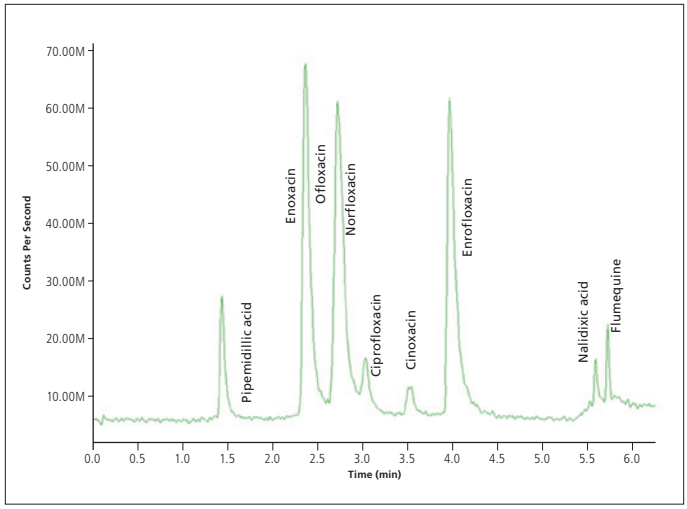

Figure 2. TIC of quinolones. Note the response of nalidixic acid and flumequine by MS detection was significantly higher compared to fluorescence detection of these analytes.

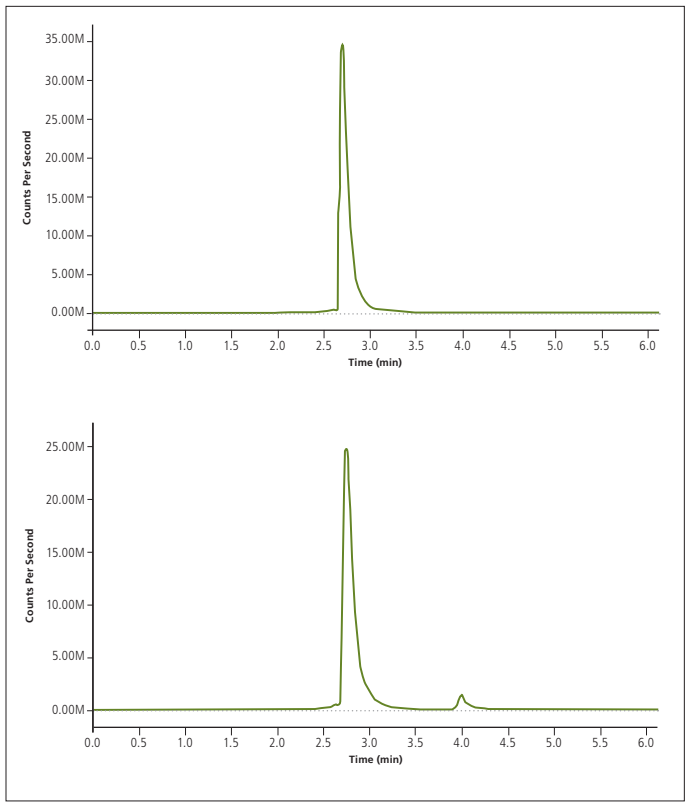

Figure 3. Extracted ion chromatograms (EICs) by MS detection of the coeluting compounds norfloxacin (320.1 m/z shown above) and ofloxacin (362.2 m/z shown below).

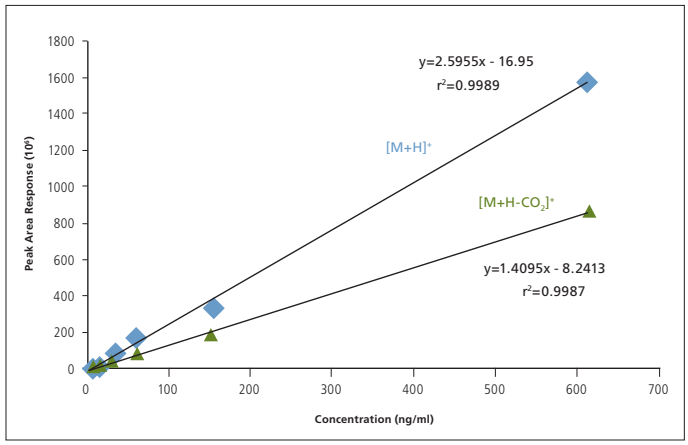

Figure 4. Calibration curve for enrofloxacin. Both $[M+H]^+$ ion (360.2) and fragment $[M+H\text{-}CO_2]^+$ (316.2) were monitored (dwell time of 150 ms each).

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

009557_14