FOOD ANALYSIS

ANALYSIS OF SULFONAMIDES AND QUINOLONES IN SHRIMP USING THE AGILENT 6460 TRIPLE QUADRUPOLE LC/MS

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Solution Note

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Abstract

The objective of this analysis is to determine Quinolone and Sulphonamide antibiotics using the Agilent 6460 Triple Quadrupole LC/MS instrument.

This application note demonstrates the QuEChERS method for two classes of veterinary drugs – quinolones and sulfanilamides in Shrimp sample matrix. The veterinary drugs were quantified using the Agilent JetStream ionisation source.

Introduction

Sulfonamides and Quinolones are antibacterials, widely used in food producing animals for purposes of treatment and prevention of diseases. The excessive use of these drugs can result in the presence of drug residue in animal tissue, which contributes to the long-term health effects, including microbial antibiotic resistance. Thus, Regulatory agencies have defined maximum residue levels (MRL) for various food products to the safety of food, have been made to protect consumers' health.

Hardware Introduction

The Agilent 6460 Triple Quadrupole LC/MS system utilises the high ESI ion generation and focussing of Agilent's Jet Stream Technology.

Agilent Jet Stream (AJS) thermal gradient focussing technology helps to overcome the two issues of analyte retention in spray droplets due to insufficient desolvation and movement of droplets and desolved ions away from the sampling orifice, due to insufficient capture using only an electrostatic field. A precisely micro-machined sprayer surrounds the droplets in a sheath of superheated gas and creates flow dynamics which concentrate ions in a well-characterised thermal confinement zone for effective sampling by the MS system. The source uniformly supports flow rates from 20 µL/min up to 2 mL/min.

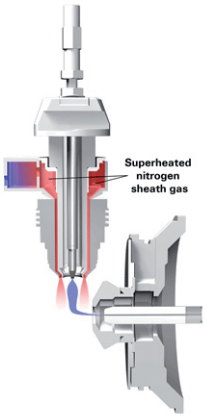


Figure 1.
Agilent Jet-Stream Technology

The quadrupoles used in the 6460 allow a mass range of 5 to 3,000 m/z with a scan speed of 12,500 Da/s. Pos/neg switching can be achieved in 30 ms and the minimum dwell time is 1 ms with no collision cell cross-talk.

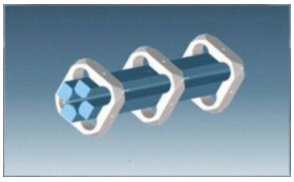


Figure 2.
Hyperbolic Quadrupole

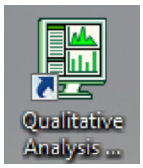
Agilent's MS systems can be coupled to a range of Agilent HPLC and UHPLC modules including the 1290 Infinity and 1260 systems, the 1290 Infinity HTC sampler for higher throughput analysis, as well as the 7000 Series Capillary Electrophoresis system.

Figure 3. From left: 1290 Infinity, 1260, 1290 Infinity HTC sampler and 7000 CE systems.

Software

MassHunter Acquisition software provides MRM, dMRM and tMRM acquisition together with product ion and neutral loss scans. 4,000 MRM per second using dynamic MRM is possible. Automated optimisation of the ion optics and mass calibration is performed using a proprietary reference solution.

MassHunter Qualitative Analysis software allows compound-centric evaluation of data sets including *Find compounds by MRM*, integration functionality for MS, UV and other signals, and spectral extraction.



MassHunter Quantitative Analysis software provides Batch-at-a-Glance views, full calibration capability and library matching of tMRM generated spectra. It enables automatic importing of MRMs and associated metadata (compound name, retention time and sample type). Multiple reporting styles and customised templates are available, together with linkage to LIMS.

Experimental

Analyses were performed using an Agilent 1290 Infinity UHPLC system coupled to an Agilent 6460 Triple Quadrupole LC/MS.

Calibration Standard and Spike Sample Preparation

A series of calibration solutions were prepared in shrimp matrix by spiking with antibiotic mix stock solution prepared in 50% Methanol. The concentration levels used for the matrix matched calibration standards are tabulated below.

Calibration level	Shrimp Matrix in g	Calibration concentration (in ppb)
1	2.0 ±0.05	2.5
2	2.0 ±0.05	5.0
3	2.0 ±0.05	10.0
4	2.0 ±0.05	20.0
5	2.0 ±0.05	30.0
6	2.0 ±0.05	50.0
7	2.0 ±0.05	100.0

Spiked samples were prepared at 10, 15 and 25 ppb levels for recovery studies.

Spiked samples	Shrimp Matrix in g	Calibration concentration (in ppb)
A	2.0 ±0.05	10
B	2.0 ±0.05	15
C	2.0 ±0.05	25

UHPLC Conditions

Mobile phase: 5 mM Ammonium formate + 0.1% Formic acid (A)
0.1% Formic acid in Acetonitrile (B)
Flow rate: 0.5 mL/min
Column: RRHD ZORBAX Eclipse Plus C18 (3.0 x 100 mm), 1.8 µm
Column Temp.: 30°C
Injection volume: 5 µL
Gradient program:

Time (min)	% of B
1.0	10
4.0	25
8.0	60
9.0	95
11.0	95
11.1	10

Post run time: 3.0 min

Sample Preparation

The matrix matched calibration solutions and spiked samples were processed using the QuEChERS methodology as specified below.

Sulfonamides and Quinolones in Shrimp

▼

Weigh 2 g homogenised Shrimp Sample into 50 mL centrifuge tube

▼

Spike with antibiotic standard mix as necessary and vortex for 60 s, add 10 mL of Water and vortex for 60 s

▼

Add 10 mL of 5% Formic acid in Acetonitrile, vortex for 60 s

▼

Add Bond Elute AOAC QuEChERS extraction kit (P/N: **5982-5755CH** or **5982-6755CH**) to each tube and cap

▼

Vortex at 2000 rpm for 60 s

▼

Centrifuge at 9000 rpm for 10 min at 10°C

▼

Transfer 6 mL of upper Acetonitrile layer to Bond Elute dispersive-SPE tube (P/N: **5982-4956CH**)

▼

Shake & Vortex for 60 s, then centrifuge at 9000 rpm for 10 min at 10°C

▼

Transfer 2 mL to extract tube, blow down @ 40° C with Nitrogen

▼

Reconstitute to 2 mL with 50:50 Methanol:Water

▼

Samples are ready for analysis

MS Conditions

Agilent JetStream ESI + mode
Gas Temp: 300°C
Gas Flow: 7 L/min
Nebuliser: ¡20 psi
Sheath Gas Temp: 400°C
Sheath Gas Flow: 11 L/min
Capillary: 2500 V
Nozzle Voltage: 0 V
Delta EMV: 300 V

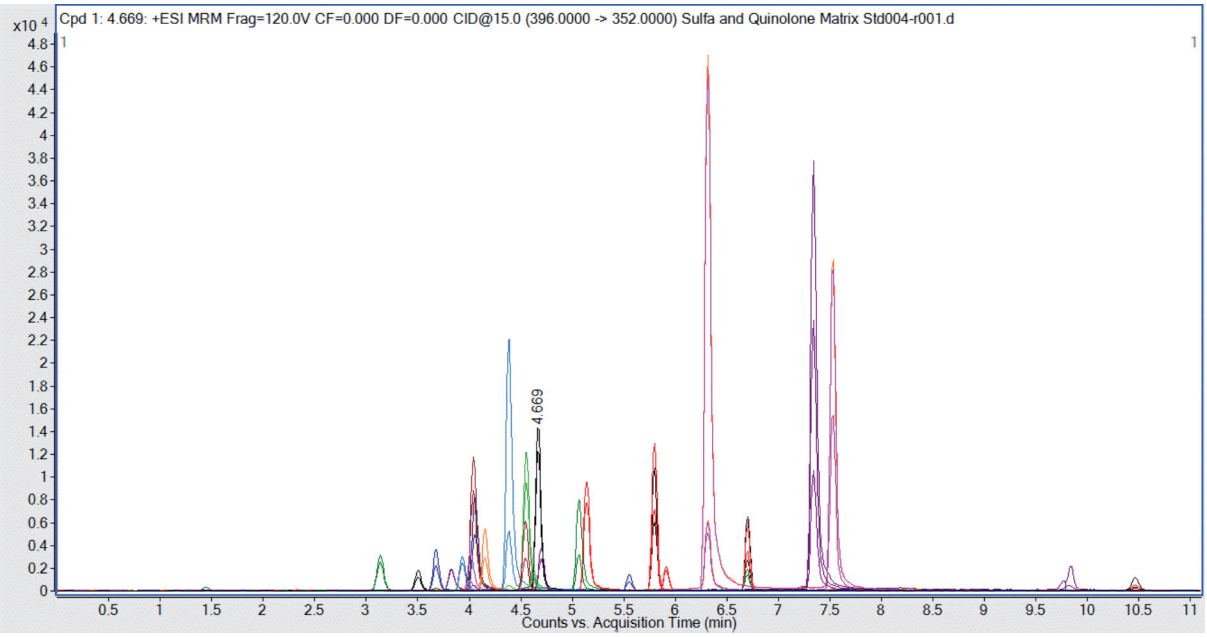
MRM transitions

Compound Name	ISTD?	Precursor	Ion MS1	Res Product	Ion MS2	Res Dwell	Fragmentor Collision	Energy Cell	Accelerator	Voltage Polarity
Ciprofloxacin	FALSE	332.1	Unit	314.1	Unit	10	130	18	7	Positive
Ciprofloxacin	FALSE	332.1	Unit	231	Unit	10	130	42	7	Positive
Danofloxacin	FALSE	358.2	Unit	340.1	Unit	10	140	22	7	Positive
Danofloxacin	FALSE	358.2	Unit	82	Unit	10	140	40	7	Positive
Enrofloxacin	FALSE	360	Unit	342.1	Unit	10	130	18	7	Positive
Enrofloxacin	FALSE	360	Unit	316.2	Unit	10	130	18	7	Positive
Fleroxacin	FALSE	370.1	Unit	326	Unit	10	110	18	7	Positive
Fleroxacin	FALSE	370.1	Unit	269	Unit	10	110	25	7	Positive
Flumequine	FALSE	262	Unit	244	Unit	10	80	13	7	Positive
Flumequine	FALSE	262	Unit	202	Unit	10	80	33	7	Positive
Levoflaxacin	FALSE	362.1	Unit	318	Unit	10	130	8	7	Positive
Levoflaxacin	FALSE	362.1	Unit	261	Unit	10	130	25	7	Positive
Marbofloxacin	FALSE	363	Unit	345.1	Unit	10	120	17	7	Positive
Marbofloxacin	FALSE	363	Unit	320.1	Unit	10	120	9	7	Positive
Nalidixic acid	FALSE	233	Unit	215	Unit	10	60	8	7	Positive
Nalidixic acid	FALSE	233	Unit	187	Unit	10	60	23	7	Positive
Norfloxacin	FALSE	320	Unit	302.1	Unit	10	140	17	7	Positive
Norfloxacin	FALSE	320	Unit	276.1	Unit	10	140	13	7	Positive
Orbifloxacin	FALSE	396	Unit	352	Unit	10	120	15	7	Positive
Orbifloxacin	FALSE	396	Unit	295	Unit	10	120	22	7	Positive
Oxalinic acid	FALSE	262.1	Unit	244	Unit	10	100	13	7	Positive
Oxalinic acid	FALSE	262.1	Unit	216	Unit	10	100	30	7	Positive
Sarafloxacin	FALSE	386.1	Unit	368.1	Unit	10	140	18	7	Positive
Sarafloxacin	FALSE	386.1	Unit	342.1	Unit	10	140	14	7	Positive
Sparfloxacin	FALSE	393.1	Unit	349	Unit	10	140	20	7	Positive
Sparfloxacin	FALSE	393.1	Unit	292	Unit	10	140	23	7	Positive
Sulfachloropyridazine	FALSE	285	Unit	156	Unit	10	100	10	7	Positive
Sulfachloropyridazine	FALSE	85	Unit	108	Unit	10	100	22	7	Positive
Sulfadiazine	FALSE	251.1	Unit	156	Unit	10	100	10	7	Positive
Sulfadiazine	FALSE	251.1	Unit	108	Unit	10	100	22	7	Positive
Sulfadimethoxine	FALSE	311.1	Unit	156	Unit	10	125	17	7	Positive
Sulfadimethoxine	FALSE	311.1	Unit	108	Unit	10	125	26	7	Positive
Sulfadoxine	FALSE	311.1	Unit	156	Unit	10	120	14	7	Positive
Sulfadoxine	FALSE	311.1	Unit	92	Unit	10	120	30	7	Positive
Sulfamerazine	FALSE	265.1	Unit	172	Unit	10	120	13	7	Positive
Sulfamerazine	FALSE	265.1	Unit	92	Unit	10	120	30	7	Positive
Sulfamethazine	FALSE	279.1	Unit	186	Unit	10	120	14	7	Positive
Sulfamethazine	FALSE	279.1	Unit	124	Unit	10	120	18	7	Positive
Sulfamethizole	FALSE	271	Unit	156	Unit	10	100	10	7	Positive
Sulfamethizole	FALSE	271	Unit	108	Unit	10	100	22	7	Positive
Sulfamethoxazole	FALSE	254.1	Unit	156	Unit	10	100	10	7	Positive
Sulfamethoxazole	FALSE	254.1	Unit	92	Unit	10	100	26	7	Positive
Sulfamethoxypyridazine	FALSE	281.1	Unit	156	Unit	10	125	14	7	Positive
Sulfamethoxypyridazine	FALSE	281.1	Unit	108	Unit	10	125	22	7	Positive
Sulfapyridine	FALSE	250.1	Unit	184	Unit	10	100	14	7	Positive
Sulfapyridine	FALSE	250.1	Unit	156	Unit	10	100	10	7	Positive
Sulfaquinoxaline	FALSE	301.1	Unit	156	Unit	10	110	11	7	Positive
Sulfaquinoxaline	FALSE	301.1	Unit	92U	Unit	10	110	29	7	Positive
Sulfathiazole	FALSE	256	Unit	156	Unit	10	100	9	7	Positive
Sulfathiazole	FALSE	256	Unit	108	Unit	10	100	21	7	Positive

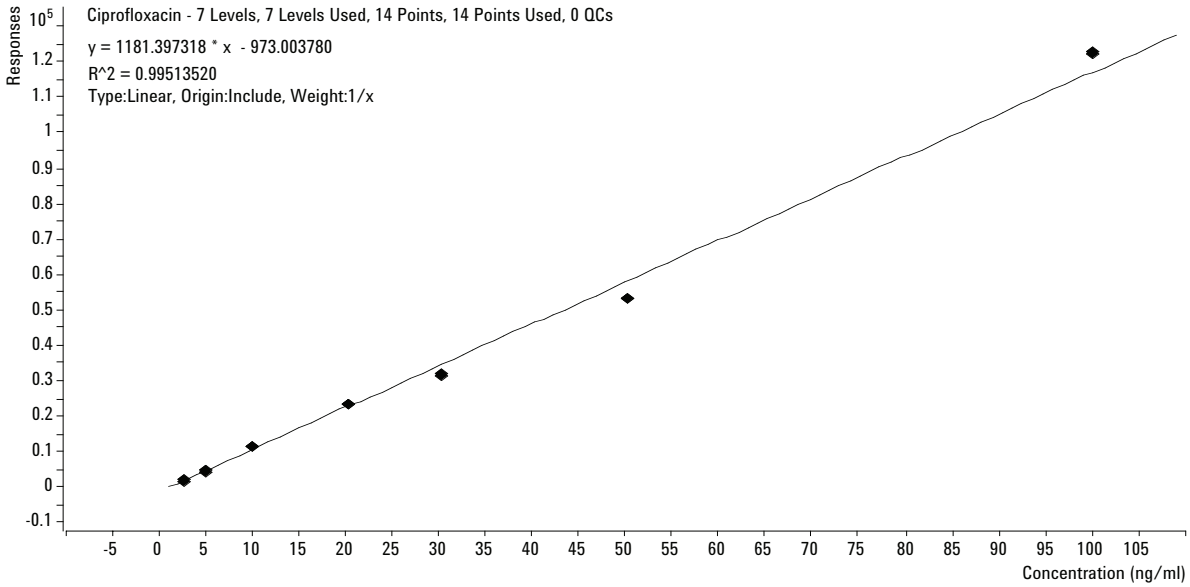
All the calibration standards were injected in duplicate and spiked samples were analysed in triplicate.

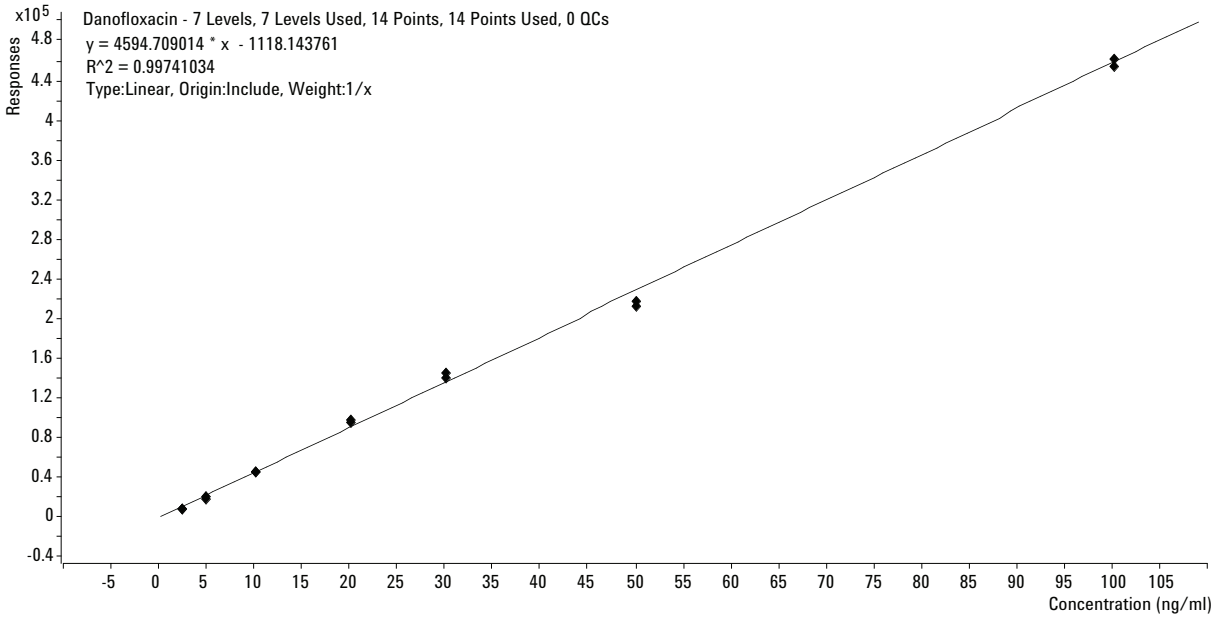
Results and Discussion

MRM Chromatogram



Reference Matrix Based Calibrations:
2.5 -100 ppb (1.25 – 50 pg on column)




Danofloxacin - 7 Levels, 7 Levels Used, 14 Points, 14 Points Used, 0 QCs
y = 4594.709014 * x - 1118.143761
R^2 = 0.99741034
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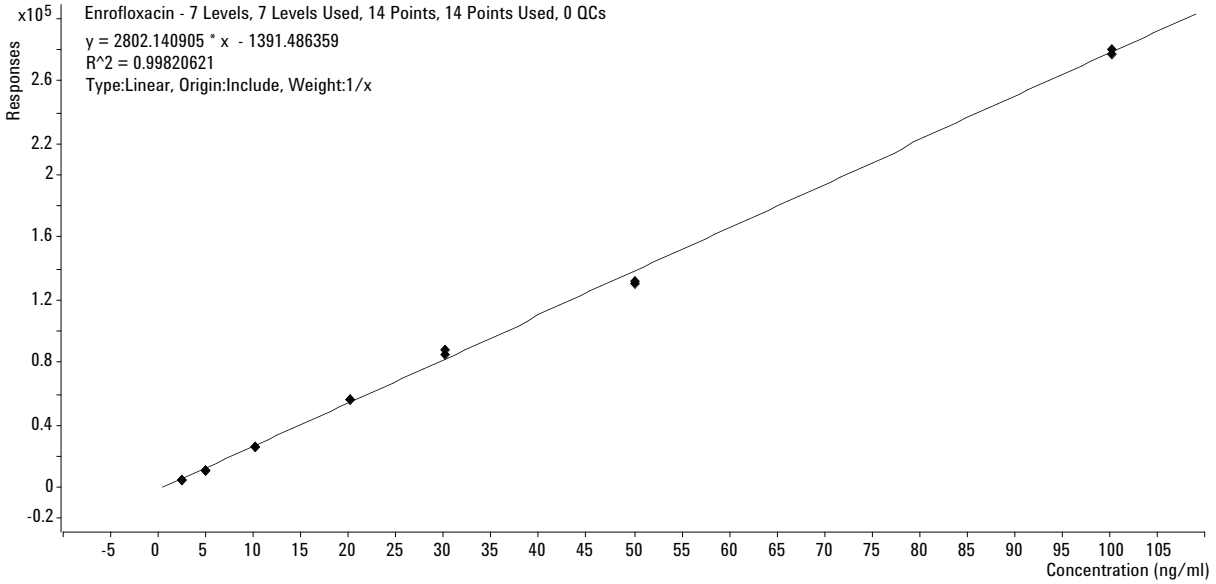
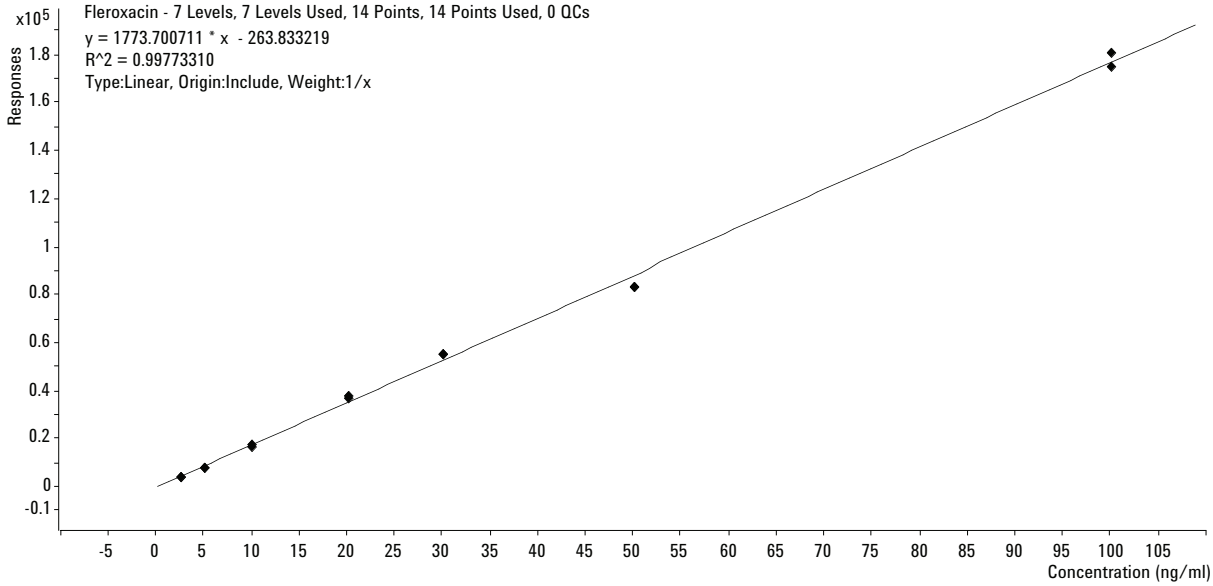
x10^5
Responses
Concentration (ng/ml)


Enrofloxacin - 7 Levels, 7 Levels Used, 14 Points, 14 Points Used, 0 QCs
y = 2802.140905 * x - 1391.486359
R^2 = 0.99820621
Type:Linear, Origin:Include, Weight:1/x
x10^5
Responses
Concentration (ng/ml)


Fleroxacin - 7 Levels, 7 Levels Used, 14 Points, 14 Points Used, 0 QCs
y = 1773.700711 * x - 263.833219
R^2 = 0.99773310
Type:Linear, Origin:Include, Weight:1/x
x10^5
Responses
Concentration (ng/ml)

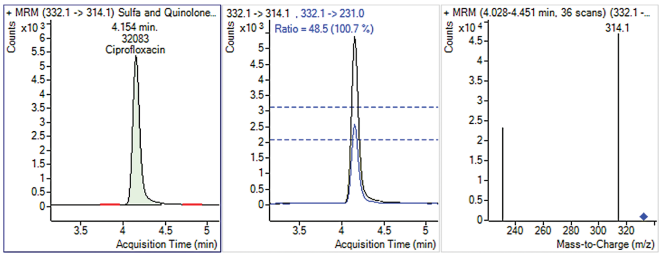
Summary of Recovery Results

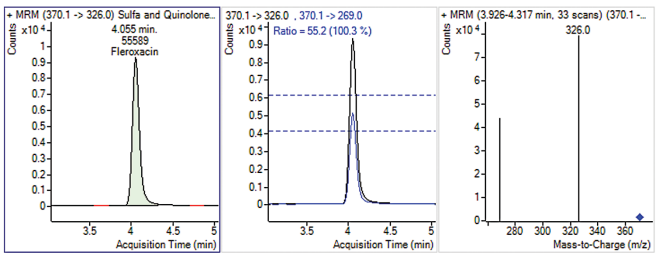
Analytes	10 ng/g fortified QC	15 ng/g fortified QC	25 ng/g fortified QC
Ciprofloxacin	11.33	15.91	26.97
Danofloxacin	10.65	16.19	27.87
Enrofloxacin	10.66	15.70	27.02
Fleroxacin	10.57	15.95	27.63
Flumequine	10.87	16.83	27.59
Levofloxacin	10.72	15.93	26.73
Marbofloxacin	11.21	16.25	26.44
Nalidixic acid	10.54	16.25	25.68
Norfloxacin	10.92	15.65	27.79
Orbifloxacin	10.65	15.65	24.78
Oxalinic acid	10.32	16.70	29.40
Sarafloxacin	10.50	15.76	26.43
Sparfloxacin	10.26	15.52	24.87
Sulfachloropyridazine	10.25	15.86	24.40
Sulfadiazine	10.01	15.20	24.49
Sulfadimethoxine	10.25	15.85	25.11
Sulfadoxine	9.77	14.87	23.69
Sulfamerazine	10.27	14.91	25.74
Sulfamethazine	9.95	15.27	26.37
Sulfamethizole	9.40	14.99	25.04
Sulfamethoxazole	10.07	14.76	23.94
Sulfapyridine	9.85	15.48	25.50
Sulfaquinoxaline	10.39	15.68	24.95
Sulfathiazole	9.80	14.89	23.42

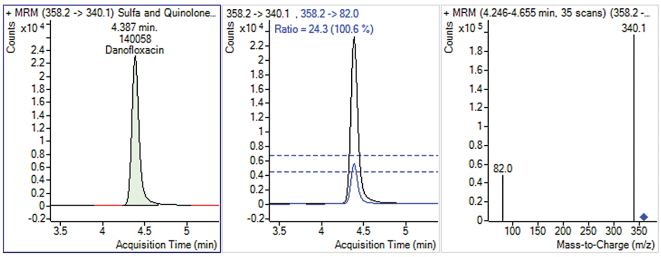
Conclusions

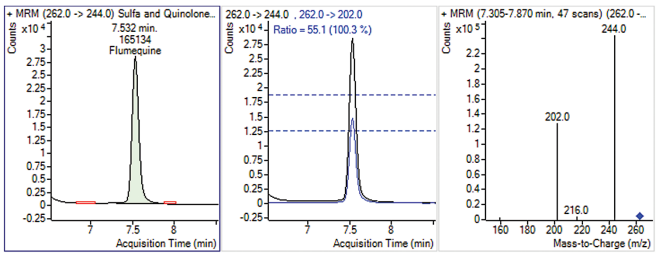
- The Agilent 6460 Triple Quadrupole LC/MS with JetStream ESI in positive mode was used for the analysis of Sulfonamides and Quinolones in Shrimp matrix.
- Matrix-matched calibration standards were used to compensate for matrix effects. The calibration range used was 2.5 - 100 ppb (1.25 - 50 pg on column concentration) for all twenty five Sulfonamides and Quinolones.
- The QuEChERS method was used for the sample clean-up process.
- The spiking studies were carried out at three concentration levels, with average recovery >98% for all compounds.

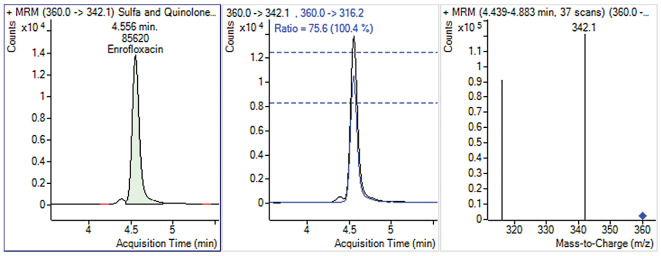
Appendix – MRM Chromatograms with Qualifier and Quantifier Ratio

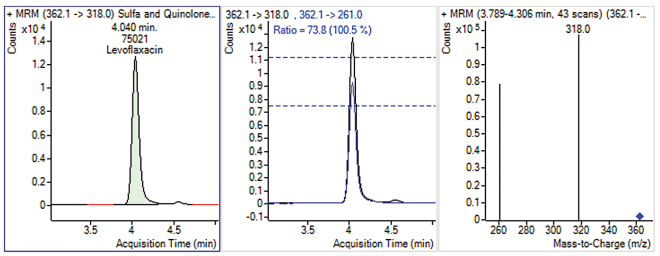


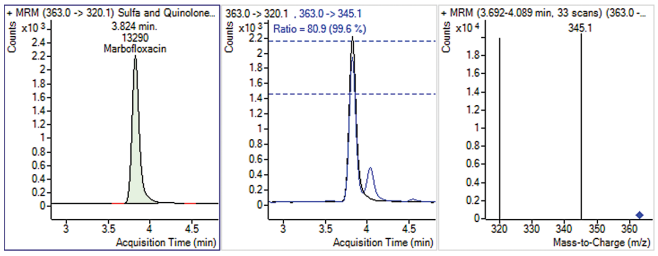


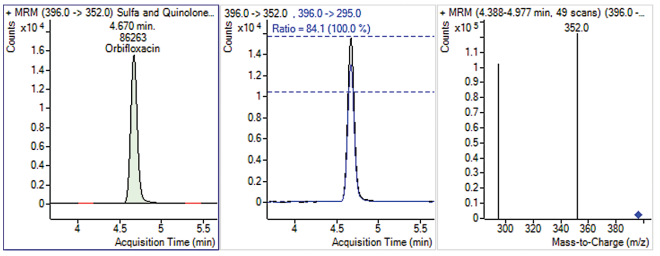


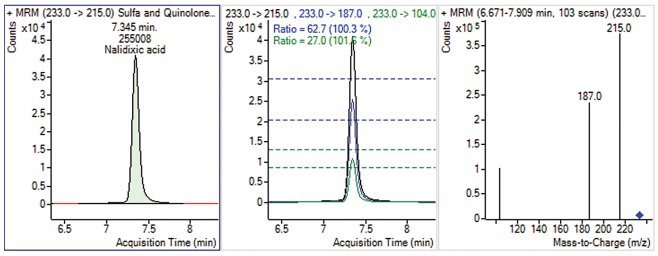


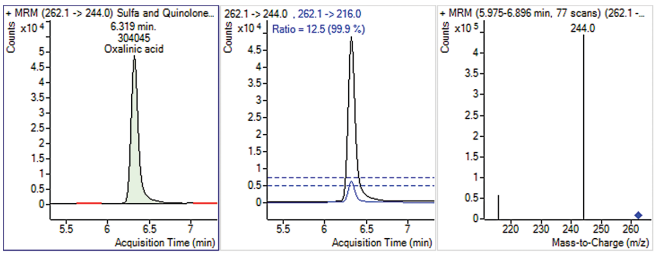


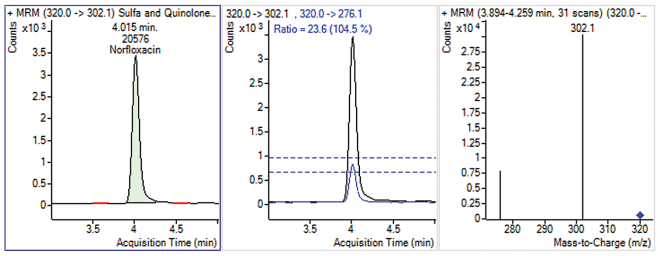


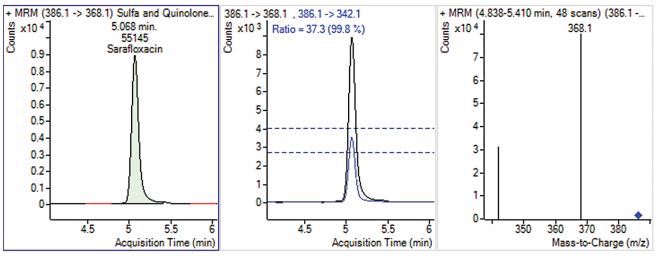


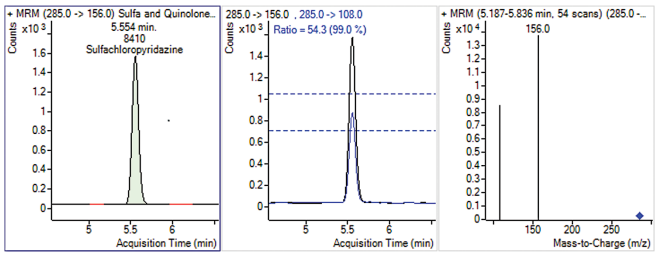


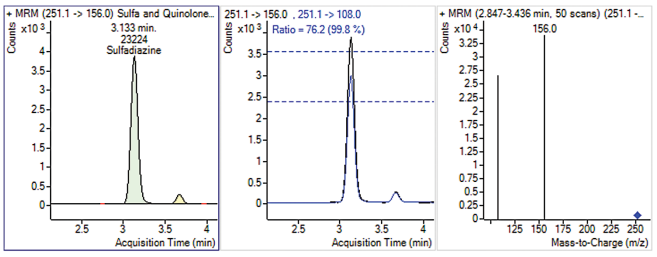


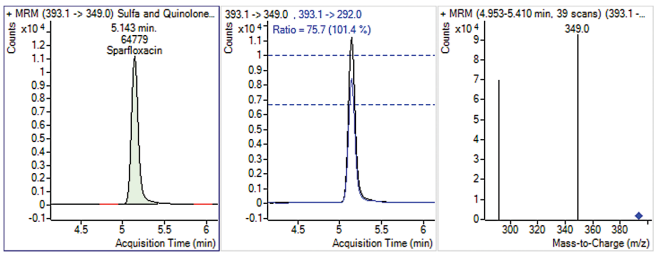


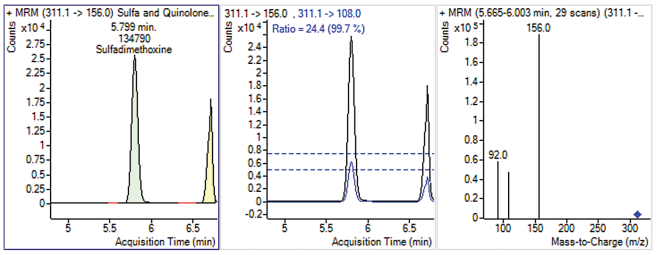


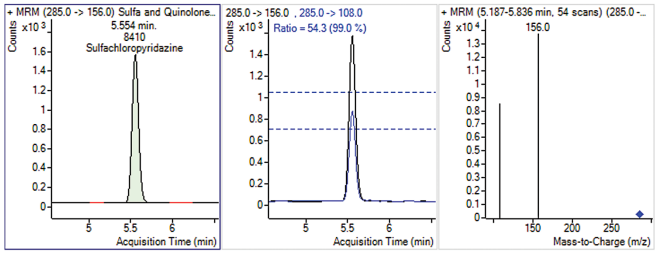


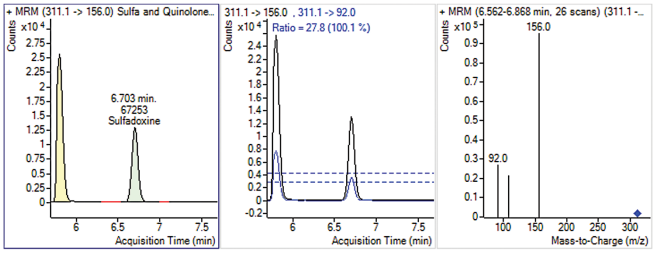

+ MRM (285.0 -> 156.0) Sulfa and Quinolone...
5.554 min.
8410
Sulfachloropyridazine
285.0 -> 156.0 , 285.0 -> 108.0
Ratio = 54.3 (99.0 %)
+ MRM (5.187-5.836 min, 54 scans) (285.0 -...
156.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)

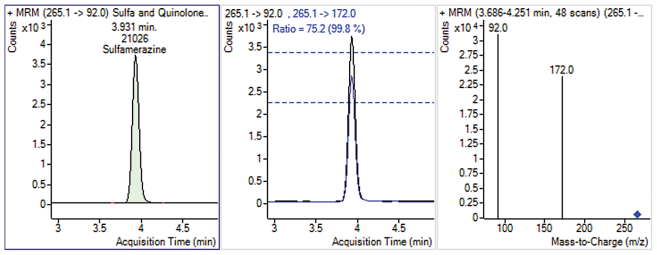

+ MRM (251.1 -> 156.0) Sulfa and Quinolone...
3.133 min.
23224
Sulfadiazine
251.1 -> 156.0 , 251.1 -> 108.0
Ratio = 76.2 (99.8 %)
+ MRM (2.847-3.436 min, 50 scans) (251.1 -...
156.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)

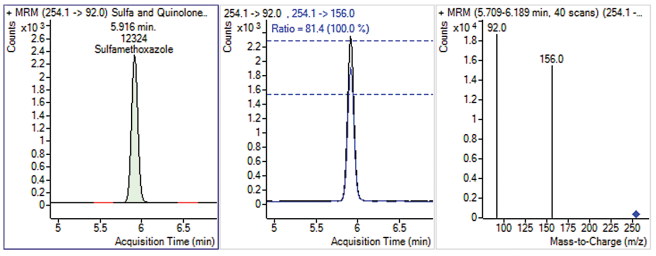

+ MRM (393.1 -> 349.0) Sulfa and Quinolone...
5.143 min.
64779
Sparfloxacin
393.1 -> 349.0 , 393.1 -> 292.0
Ratio = 75.7 (101.4 %)
+ MRM (4.953-5.410 min, 39 scans) (393.1 -...
349.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)

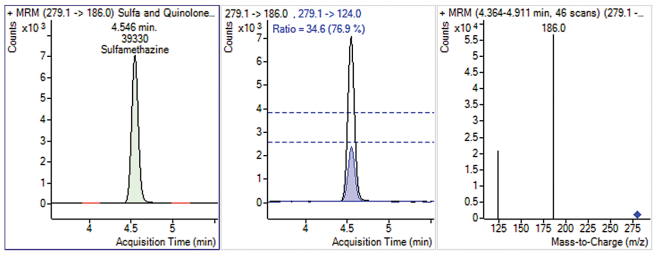

+ MRM (311.1 -> 156.0) Sulfa and Quinolone...
5.799 min.
134790
Sulfadimethoxine
311.1 -> 156.0 , 311.1 -> 108.0
Ratio = 24.4 (99.7 %)
+ MRM (5.665-6.003 min, 29 scans) (311.1 -...
156.0
92.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)

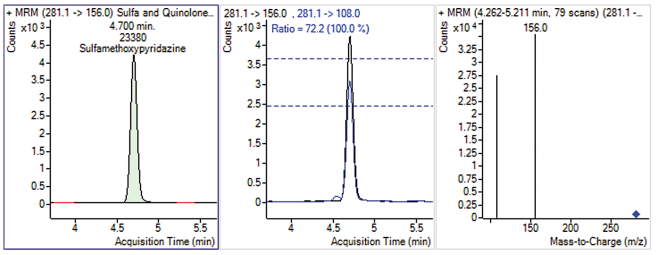

+ MRM (285.0 -> 156.0) Sulfa and Quinolone...
5.554 min.
8410
Sulfachloropyridazine
285.0 -> 156.0 , 285.0 -> 108.0
Ratio = 54.3 (99.0 %)
+ MRM (5.187-5.836 min, 54 scans) (285.0 -...
156.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)

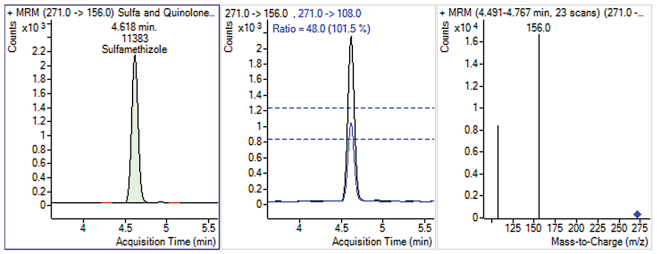

+ MRM (311.1 -> 156.0) Sulfa and Quinolone...
6.703 min.
67253
Sulfadoxine
311.1 -> 156.0 , 311.1 -> 92.0
Ratio = 27.8 (100.1 %)
+ MRM (6.562-6.868 min, 26 scans) (311.1 -...
156.0
92.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)

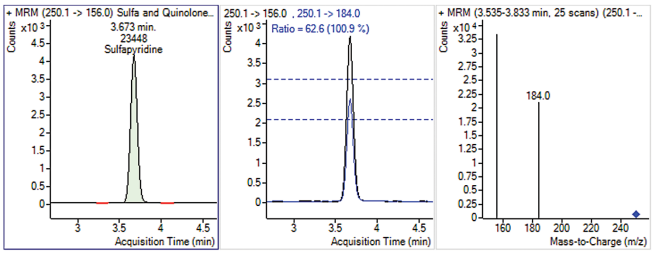

+ MRM (265.1 -> 92.0) Sulfa and Quinolone...
3.931 min.
21026
Sulfamerazine
265.1 -> 92.0 , 265.1 -> 172.0
Ratio = 75.2 (99.8 %)
+ MRM (3.686-4.251 min, 48 scans) (265.1 -...
92.0
172.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)

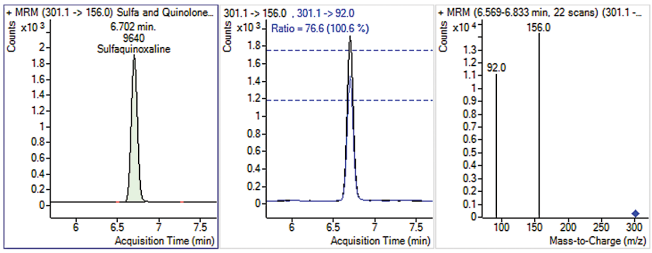

+ MRM (254.1 -> 92.0) Sulfa and Quinolone...
5.916 min.
12324
Sulfamethoxazole
254.1 -> 92.0 , 254.1 -> 156.0
Ratio = 81.4 (100.0 %)
+ MRM (5.709-6.189 min, 40 scans) (254.1 -...
92.0
156.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)

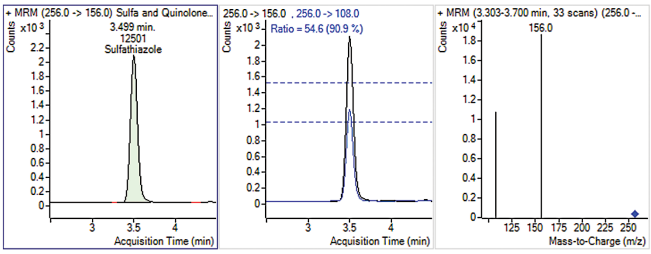

+ MRM (279.1 -> 186.0) Sulfa and Quinolone...
4.546 min.
39330
Sulfamethazine
279.1 -> 186.0 , 279.1 -> 124.0
Ratio = 34.6 (76.9 %)
+ MRM (4.364-4.911 min, 46 scans) (279.1 -...
186.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)


+ MRM (281.1 -> 156.0) Sulfa and Quinolone...
4.700 min.
23380
Sulfamethoxypyridazine
281.1 -> 156.0 , 281.1 -> 108.0
Ratio = 72.2 (100.0 %)
+ MRM (4.262-5.211 min, 79 scans) (281.1 -...
156.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)


+ MRM (271.0 -> 156.0) Sulfa and Quinolone...
4.618 min.
11393
Sulfamethizole
271.0 -> 156.0 , 271.0 -> 108.0
Ratio = 48.0 (101.5 %)
+ MRM (4.491-4.767 min, 23 scans) (271.0 -...
156.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)


+ MRM (250.1 -> 156.0) Sulfa and Quinolone...
3.673 min.
23448
Sulfapyridine
250.1 -> 156.0 , 250.1 -> 184.0
Ratio = 62.6 (100.9 %)
+ MRM (3.535-3.833 min, 25 scans) (250.1 -...
184.0
Counts
Acquisition Time (min)
Mass-to-Charge (m/z)





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