

Method Development

- Past experience with ACN in Honey
- Past experience with fluoroquinolones in Fish Tissue

Current FDACS Confirmatory Method – LOD 2.5ppb

- Take 2.5 grams of sample and dilute with 5ml of DI water.
- Add 10ml of ACN, 200uL of Acetic Acid, and 2 grams of NaCL.
- Transfer the acetonitrile layer and concentrate to less than 1mL under Nitrogen.
- Dilute to 5mL with 16/84 acetonitrile/2% acetic acid in water.
- Inject on LC/MS/MS system

Instrument Conditions HPLC

- Column: Waters / 2.0x150, XTerra phenyl / 5 micron
- Flow Rate: 0.2 ml/min
- Mobile Phase: Gradient water / acetonitrile 5%-95% over 32 min
- Modifiers: 0.1% Formic Acid added to both the water and acetonitrile
- Injection Volume: 10 ul

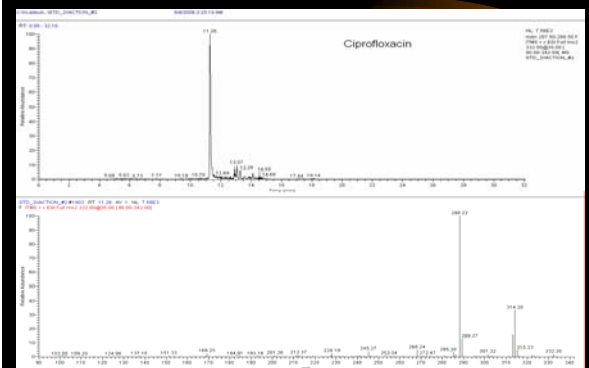
Instrument Conditions Mass Spectrometer

- Positive Mode Electrospray
- Capillary Temperature: 300 degrees Celsius
- Spray Voltage: 4 kV
- Collision Energy: 35.0
- Isolation Width: 3.0 m/z
- Scan Time: 1 microscan
- Max Injection Time: 200 m/s

Ions Monitored - MSMS

- 332.00 > 288.20 Ciprofloxacin
- 360.00 > 316.30 Enrofloxacin

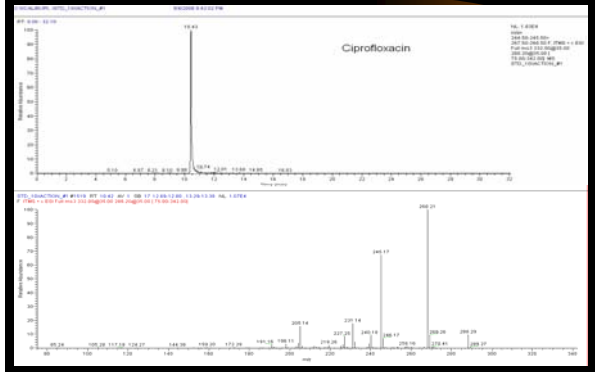
MSMS of Ciprofloxacin



Ions Monitored - MSMSMS

- Ciprofloxacin
332.00 > 288.20 > 268.00
332.00 > 288.20 > 245.00
- Enrofloxacin
360.00 > 316.30 > 245.00
360.00 > 316.30 > 288.00

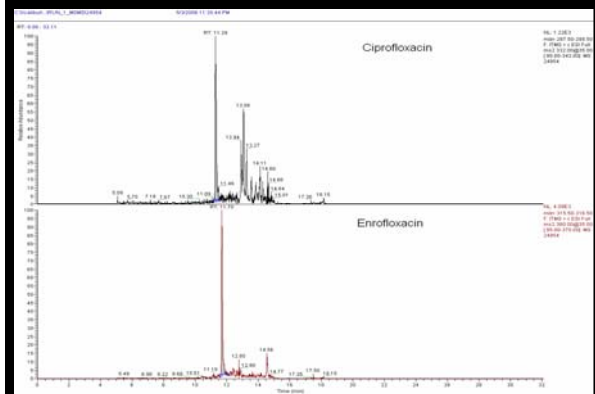
MSMSMS of Ciprofloxacin



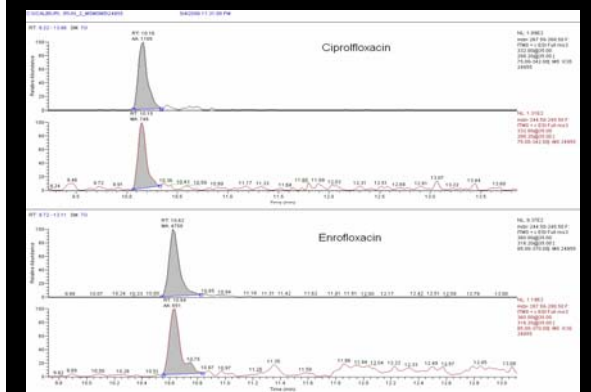
Method Validation Scheme for Confirmatory Analysis

- Action Limit of 5ppb
- Detection Limit 2.5ppb
- 1 Reagent Blank
- 1 Matrix Blank
- 3 samples at 0.5 x AL
- 3 samples at 1.0 x AL
- 3 samples at 2.0 x AL
- 3 samples at 20 x AL

Standard in Honey Matrix at Limit of Detection



In-Matrix LOD Standard MS³



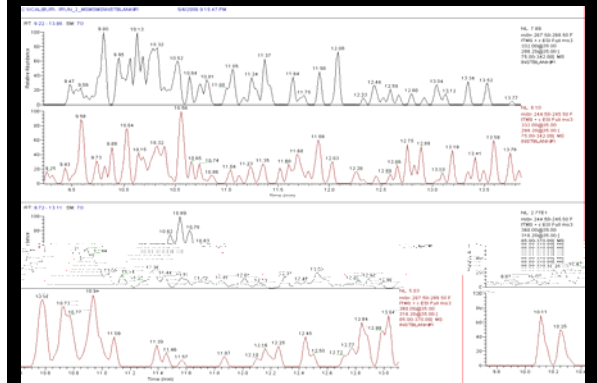
Method Validation Recoveries - Cipro

Sample #	Spike level ppb	% Recovery
24954	2.5	67
24955	2.5	71
24956	2.5	68
24957	5	82
24958	5	76
24959	5	80
24960	10	72
24961	10	71
24962	10	70
24963	100	73
24964	100	65
24965	100	62
Average Recovery		71

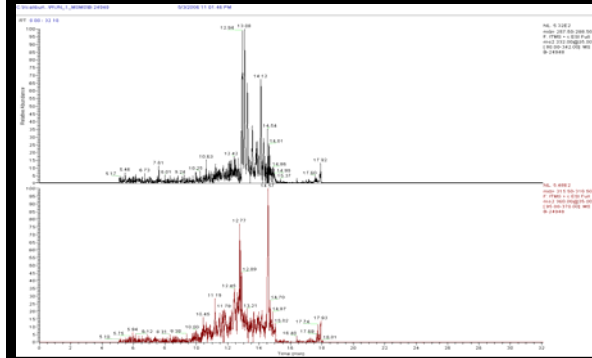
Method Validation Recoveries - Enro

Sample #	Spike level ppb	% Recovery
24954	2.5	98
24955	2.5	95
24956	2.5	92
24957	5	89
24958	5	95
24959	5	93
24960	10	95
24961	10	90
24962	10	95
24963	100	94
24964	100	92
24965	100	87
Average Recovery		93

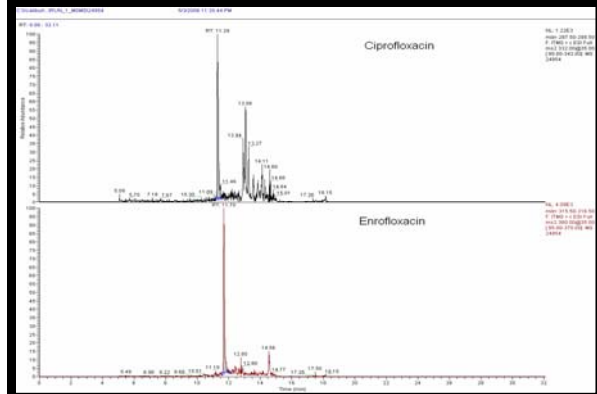
Instrument Blank



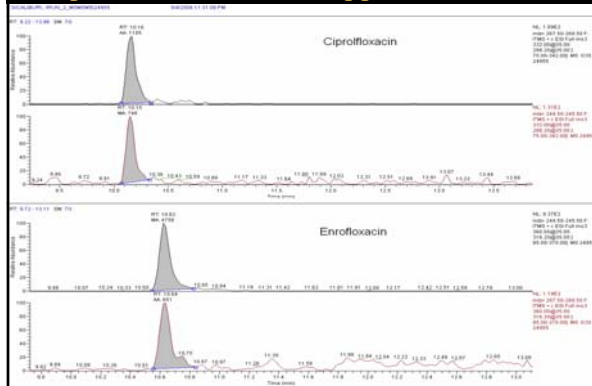
Blank Matrix MSMS



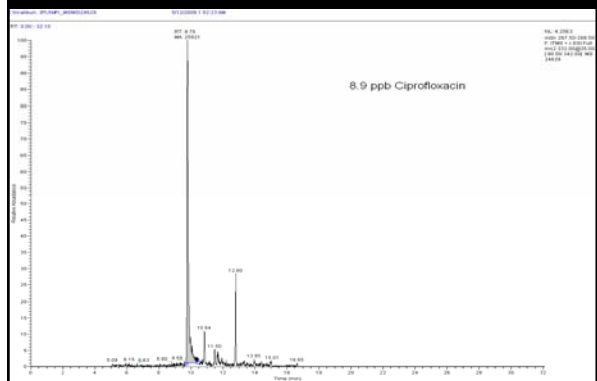
Spiked Residue at 2.5 ppb MSMS

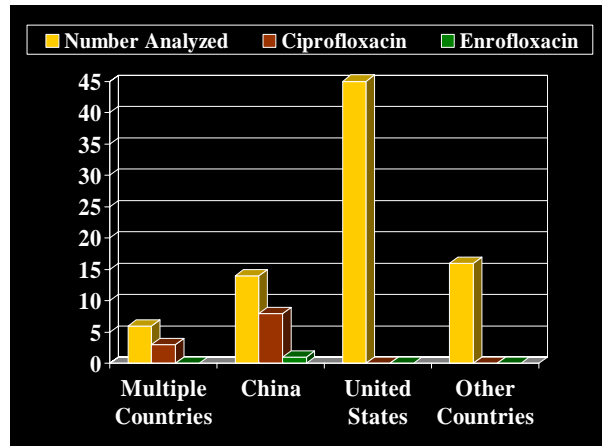
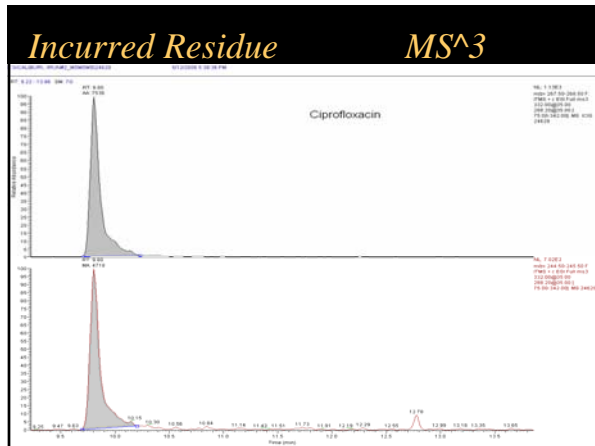


Spiked Residue at 2.5 ppb MS³



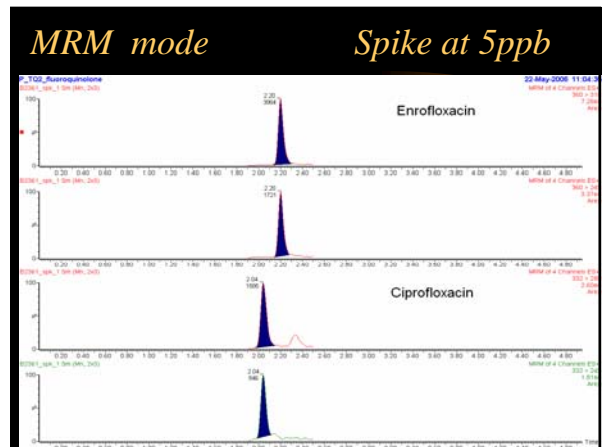
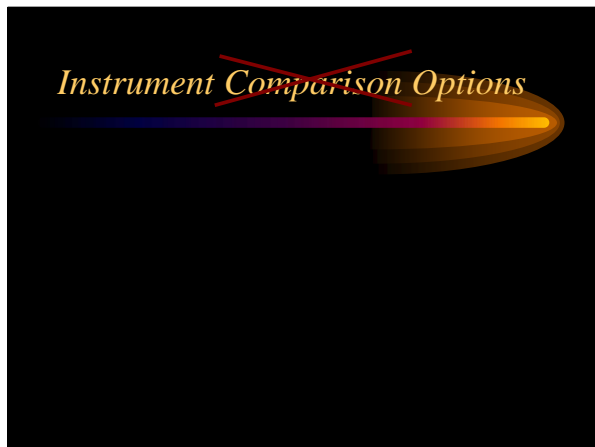
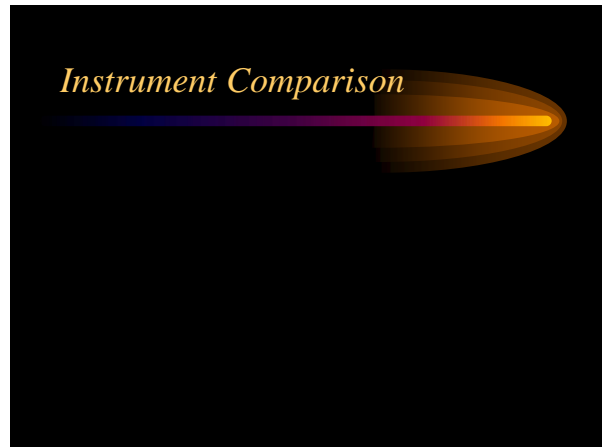
Incurred Residue MSMS





Results

	Number Analyzed	Ciprofloxacin	Enrofloxacin
Argentina	2	0	0
Austria	1	0	0
Brazil	1	0	0
Bulgaria	1	0	0
Canada	3	0	0
China	14	8	1
France	1	0	0
Multiple Countries	6	3	0
Taiwan (Republic of China)	1	0	0
Thailand	2	0	0
United States	45	0	0
Unknown	2	0	0
Vietnam	2	0	0
Total	81	11	1



Future Plans



- Continue to work with FDA
- Multiple Antibiotics
- Various Matrices

Questions???

