Multiclass Veterinary Drug Residue Method for Aquaculture Products Using LC-MS/MS

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ABSTRACT

This methodology was developed for the quantitative and confirmatory determination of 42 different veterinary drug residues, from 10 different classes of drugs in aquaculture products. These drug classes include phenicols, beta lactams, fluoroquinolones, quinolones, sulfonamides, tetracyclines, macrolides, lincosamide, triphenyl methane dyes, and anthelmintics. The extraction procedure is based on the previously published LIB # 4615, which removes unwanted matrix components from the aquaculture tissue, while allowing for coverage of a broad range of residues. This extraction method, in combination with an optimized LC-MS/MS acquisition method using electrospray ionization in positive and negative ion mode, has provided accurate quantitative results. Method validation has been performed for shrimp, frog legs, barramundi, croaker, and cobia.

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INTRODUCTION

One of the fastest growing food industries across the world is aquaculture. This is partly due to the increasing world population, and because individuals are consuming a higher percentage of fish, crustaceans, and other aquatic species. Between 1961 and 2016, the average annual increase in global food fish consumption (3.2 percent) outpaced population growth (1.6 percent) (1). Furthermore, the aquaculture industry now accounts for 50% of the world's fish that is used for food (2). In 2012 the U.S. National Oceanic and Atmospheric Association (NOAA) reported that the United States alone consumed more than 4.5 billion pounds of seafood (2-5). Therefore, it is evident that the aquaculture industry is a thriving industry that has substantial economic impact globally.

Treatment and prevention of illnesses or diseases is of critical importance in aquaculture facilities. Administering antibiotics and veterinary drugs to aquaculture species is one of the primary methods for ensuring the health of aquaculture products. These drugs are usually readily available and efficient; however, the presence of veterinary drugs in foodstuff is of great interest to the United States, European Union, and many other countries. This is primarily because the presence of various drugs in foodstuff can lead to serious health risks to consumers. These include, but are not limited to aplastic anemia, bone marrow suppression, carcinogenic effects, mutagenic effects, Steven-Johnson syndrome, organ failure, and antibiotic resistance (5-12).

The U.S. Food and Drug Administration has an interest in expanding the number of veterinary drug residues determined to further ensure public health safety. However, most of the currently available analytical methods are not suitable for extraction and determination of several classes of drug residues, such as the more polar macrolides and extremely non-polar triphenyl methane dyes. Furthermore, some of these methods do not adequately remove many of the fats and phospholipids from several aquaculture matrices. This then leads to poor analyte recoveries, increased uncertainty, and inability to meet needed method detection limits (MDL).

In efforts to determine a wide variety of veterinary drugs, pass through solid-phase extraction (SPE) was used for extraction purposes. Furthermore, the use of HLB Prime SPE cartridges has been shown to be very effective for removal of fats and phospholipids. This technology was effectively utilized in LIB # 4615 for the screening of veterinary drug residues in fish, shrimp and eel using LC-HRMS (6). Slight modifications were made in the extraction procedure of LIB # 4615 to enhance quantitation.

This study illustrates the method development and validation of a multi-residue method for a variety of aquaculture matrices. The procedure can extract and determine 42 different veterinary drug residues from 10 different classes of drugs via LC-tandem MS (MS/MS) technology. Both positive and negative electrospray ionization were used for the analysis.

EXPERIMENTAL

Apparatus

a) *HPLC*– Agilent Technologies, 1260 series (Santa Clara, CA).

b) *Mass Spectrometer* (*MS)* – Agilent 6495 triple quadrupole mass spectrometer equipped with electrospray ionization.

c) *Chromatographic column* – Agilent Infinity Lab Poroshell 120 EC-C18, 150 x 2.1 mm, 2.7 µm

d) *Blender* – RobotCoupe (Ridgeland, MS)

e) *Solid-phase extraction cartridges* – Waters Oasis PRIME HLB 6cc (200 mg) (Milford, MA)

f) *15 mL disposable polypropylene centrifuge tubes with screw tight lids* – (Sarstedt, Newton, NC)

g) *50 mL disposable polypropylene centrifuge tubes with screw tight lids* – (Sarstedt)

h) *Multi-tube vortex shaker* – capable of holding 50 mL centrifuge tubes

i) *Refrigerated Microcentrifuge* – capable of ≥ 20,500 g.

j) *Refrigerated Centrifuge* – capable of ≥ 7,000 g.

k) *Mechanical shaker or multi-tube vortex mixer*

l) *Solvent evaporator:* Zymark Turbo Vap or equivalent

m) *2 mL Autosampler vials with inserts*– Agilent Technologies

n) *Autosampler vial caps* – Agilent Technologies

Reagents and Standards

a) *Acetonitrile* – LC-MS grade obtained from Fisher Scientific (Houston, TX)

b) *Methanol*– LC-MS grade obtained from Fisher Scientific

c) *Formic Acid* – LC-MS grade obtained from Fisher Scientific

d) *p-Toluenesulfonic acid monohydrate (p-TSA)* – Fisher Scientific

e) *Glacial acetic acid* – Fisher Scientific

f) *Water* – Millipore Milli-Q system (Burlington, MA)

g) *Enrofloxacin* – SPEX CertiPrep (Metuchen, NJ)

h) *Sarafloxacin hydrochloride hydrate* – SPEX CertiPrep

i) *Ciprofloxacin* – SPEX CertiPrep

j) *Danofloxacin* – SPEX CertiPrep

k) *Difloxacin hydrochloride* – SPEX CertiPrep

l) *Norfloxacin* – SPEX CertiPrep

m) *Nalidixic Acid* – SPEX CertiPrep

n) *Oxolinic Acid* – SPEX CertiPrep

o) *Flumequine* – SPEX CertiPrep

p) *Lincomycin hydrochloride* – SPEX CertiPrep

q) *Erythromycin* – SPEX CertiPrep

r) *Doxycycline hydrochloride* – SPEX CertiPrep

s) *Tetracycline* – SPEX CertiPrep

t) *Oxytetracycline* – SPEX CertiPrep

u) *Chlortetracycline* – SPEX CertiPrep

v) *Sulfamethazine* – SPEX CertiPrep

w) *Sulfamerazine* – SPEX CertiPrep

x) *Sulfadimethoxine* – SPEX CertiPrep

y) *Sulfadiazine* – SPEX CertiPrep

z) *Sulfachlorpyridazine* – SPEX CertiPrep

aa) *Sulfaquinoxaline* – SPEX CertiPrep

bb) *Sulfathiazole* – SPEX CertiPrep

cc) *Sulfacetamide* – SPEX CertiPrep

dd) *Sulfaethoxypyridazine* – SPEX CertiPrep

ee) *Sulfamethoxazole* – SPEX CertiPrep

ff) *Sulfamethoxypyridazine* – SPEX CertiPrep

gg) *Sulfapyridine* – SPEX CertiPrep

hh) *Sulfadoxine* – SPEX CertiPrep

ii) *Sulfamonomethoxine* – SPEX CertiPrep

jj) *Malachite Green* – SPEX CertiPrep

kk) *Leuco Malachite Green* – SPEX CertiPrep

ll) *Crystal Violet* – SPEX CertiPrep

mm) *Leuco Crystal Violet* – SPEX CertiPrep

nn) *Trimethoprim* – SPEX CertiPrep

oo) *Hydroxy Mebendazole* – SPEX CertiPrep

pp) *Mebendazole Amine* – SPEX CertiPrep

qq) *Mebendazole* – SPEX CertiPrep

rr) *Florfenicol Amine* – SPEX CertiPrep

ss) *Ampicillin* – SPEX CertiPrep

tt) *Amoxicillin* – SPEX CertiPrep

uu) *Cloxacillin* – SPEX CertiPrep

vv) *Thiamphenicol* – SPEX CertiPrep

ww) *Roxithromycin (ROX)*– Sigma Aldrich (St. Louis, MO)

xx) *Gentian Violet (GV d_6)* – Santa Cruz Biotechnology (Santa Cruz, CA)

yy) *Leuco Gentian Violet (LGV d_6)* – Santa Cruz Biotechnology

zz) *Malachite Green (MG d_5)* – Sigma Aldrich

aaa) *Leuco Malachite Green (LMG d_5)* – Sigma Aldrich

bbb) *Oxolinic Acid (OXO d_5)* – Sigma Aldrich

METHOD

Suggested Reagent and Standard Preparation:

**Black and blue markers often contain gentian violet; therefore, markers containing these colors should be avoided during standard preparation, and sample preparation and extractions. Furthermore, many of the drug residues are light sensitive and prolonged exposure to light should be avoided when possible.*

a. Extraction Solvent: 8 grams of p-TSA, and 80 mL of acetic acid diluted to 4.00 L with acetonitrile.

b. Stock Internal Standard Solutions (ISTD): Separate internal standard stock solutions were prepared in an appropriate organic solvent as follows:
(all internal stock standard solutions were stored at ≤ 0° Celsius, and are stable for six -months)

   i. 50.0 µg/mL for gentian violet d_6, leuco gentian violet d_6, malachite green d_5, and leuco malachite green d_5.
   ii. 200 µg/mL for roxithromycin, and oxolinic acid d_5.

c. Stock Standard Solutions for Calibration Standards: Prepare or purchase stock standards in methanol or other appropriate organic solvent at the following levels:
(all stock standard solutions were stored at ≤ 0° Celsius and are stable for one year)

   i. 400 µg/mL for the sulfonamides (sulfamerazine, sulfadiazine, sulfachlorpyridazine, sulfathiazole sulfaquinoxaline, sulfamethazine, sulfadimethoxine, sulfadoxine, sulfaethoxypyridazine, sulfamethoxypyridazine, sulfamethoxazole, sulfapyridine, sulfamonomethoxine, and sulfacetamide)
   ii. 40.0 µg/mL for the triphenyl methane dyes (gentian violet, leuco gentian violet, malachite green, and leuco malachite green)

iii. 2000 µg/mL for the tetracyclines (tetracycline, oxytetracycline, chlortetracycline, and doxycycline)
iv. 200 µg/mL for the anthelmintics (hydroxy mebendazole, mebendazole, and mebendazole amine)
v. 2000 µg/mL for florfenicol amine,
vi. 40.0 µg/mL for thiamphenicol
vii. 2000 µg/mL for the lincosamide (lincomycin)
viii. 400 µg/mL for trimethoprim
ix. 2000 µg/mL for the macrolide (erythromycin,)
x. 200 µg/mL for the fluoroquinolones (ciprofloxacin, enrofloxacin, sarafloxacin, difloxacin, norfloxacin, and danofloxacin)
xi. 400 µg/mL for the quinolones (oxolinic acid, nalidixic acid, and flumequine)
xii. 400 µg/mL for the beta lactams (ampicillin, amoxicillin, and cloxacillin)

d. Stock Standard Solutions for Initial Calibration Verifications (ICVs): A second set of stock solutions is prepared as the initial calibration verification (ICVs) solutions.

e. Intermediate Internal Standard Solution: Prepare an intermediate ISTD solution in methanol as described in Table 1.

Table 1: Preparation of an Intermediate ISTD Solution in Methanol

Internal Standard	Conc. of Stock Solution	Volume Used	Final Volume	Final Conc.
Roxithromycin	200 µg/mL	500 µL	25.0 mL	4.00 µg/mL
Gentian Violet d_6	50.0 µg/mL	200 µL	25.0 mL	0.400 µg/mL
Leuco Gentian Violet d_6	50.0 µg/mL	200 µL	25.0 mL	0.400 µg/mL
Malachite Green d_5	50.0 µg/mL	200 µL	25.0 mL	0.400 µg/mL
Leuco Malachite Green d_5	50.0 µg/mL	200 µL	25.0 mL	0.400 µg/mL
Oxolinic Acid d_5	200 µg/mL	500 µL	25.0 mL	4.00 µg/mL

These solutions are stored at ≤ -70°C and are stable for six-months.

f. Intermediate Analytical Calibration Standard Solution in methanol as described in Table 2.

Table 2: Intermediate Analytical Calibration Standard Solution in methanol

Analytical Standard	Conc. of Stock Solution	Volume Used	Final Volume	Final Conc.
Sulfamerazine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL

Analytical Standard	Conc. of Stock Solution	Volume Used	Final Volume	Final Conc.
Sulfadiazine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfachlorpyridazine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfathiazole	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfaquinoxaline	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfamethazine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfadimethoxine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfadoxine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfaethoxypyridazine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfamethoxypridazine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfamethoxazole	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfapyridine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfacetamide	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Sulfamonomethoxine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Ciprofloxacin	200 µg/mL	62.5 µL	25.0 mL	500 ng/mL
Norfloxacin	200 µg/mL	62.5 µL	25.0 mL	500 ng/mL
Sarafloxacin	200 µg/mL	62.5 µL	25.0 mL	500 ng/mL
Enrofloxacin	200 µg/mL	62.5 µL	25.0 mL	500 ng/mL
Difloxacin	200 µg/mL	62.5 µL	25.0 mL	500 ng/mL
Danofloxacin	200 µg/mL	62.5 µL	25.0 mL	500 ng/mL
Nalidixic Acid	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Oxolinic Acid	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Flumequine	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Hydroxy Mebendazole	200 µg/mL	62.5 µL	25.0 mL	500 ng/mL
Mebendazole Amine	200 µg/mL	62.5 µL	25.0 mL	500 ng/mL
Mebendazole	200 µg/mL	62.5 µL	25.0 mL	500 ng/mL
Erythromycin	2000 µg/mL	62.5 µL	25.0 mL	5.00 µg/mL

Analytical Standard	Conc. of Stock Solution	Volume Used	Final Volume	Final Conc.
Trimethoprim	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Lincomycin	2000 µg/mL	62.5 µL	25.0 mL	5.00 µg/mL
Doxycycline	2000 µg/mL	62.5 µL	25.0 mL	5.00 µg/mL
Tetracycline	2000 µg/mL	62.5 µL	25.0 mL	5.00 µg/mL
Oxytetracycline	2000 µg/mL	62.5 µL	25.0 mL	5.00 µg/mL
Chlortetracycline	2000 µg/mL	62.5 µL	25.0 mL	5.00 µg/mL
Thiamphenicol	40.0 µg/mL	62.5 µL	25.0 mL	100 ng/mL
Malachite Green	40.0 µg/mL	62.5 µL	25.0 mL	100 ng/mL
Leuco Malachite Green	40.0 µg/mL	62.5 µL	25.0 mL	100 ng/mL
Gentian Violet	40.0 µg/mL	62.5 µL	25.0 mL	100 ng/mL
Leuco Gentian Violet	40.0 µg/mL	62.5 µL	25.0 mL	100 ng/mL
Brilliant Green	40.0 µg/mL	62.5 µL	25.0 mL	100 ng/mL
Florfenicol Amine	2000 µg/mL	62.5 µL	25.0 mL	5.00 µg/mL
Amoxicillin	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Ampicillin	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL
Cloxacillin	400 µg/mL	62.5 µL	25.0 mL	1.00 µg/mL

These solutions are stored at ≤ -70˚C and are stable for six-months.

g. Intermediate Analytical ICV Standard Solutions can be prepared as shown in Table 2.

Sample Preparation and Extraction:

1. An appropriate amount of edible tissue (i.e. 100–150 grams) should be placed in a Robot-Coupe food processor with an adequate amount of dry ice. The components should be homogenized into a powder like form, with no apparent clumps of product. The homogenized product should be placed in a freezer or refrigerator for a minimum of 12 hours to allow the dry ice to sublime. Caution should be used when working with dry ice to ensure there is adequate ventilation in the room to prevent asphyxiation.

2. After the dry ice has sublimed, measure 4.00 grams (± 0.04 grams) of the ground tissue into a 50 mL centrifuge tube. Blank matrix matched tissue, without the compounds of interest, is used for quality control and calibration standards.

3. All internal standards, calibration standards and matrix spikes are fortified at the levels listed in Table 3 below. Please allow standards to reside in the matrix for 10–15 minutes prior to the addition of extraction solvent.

Table 3: Calibration standards and matrix spikes

Extracted Calibration Curve	Volume (µL) of Mixed Intermediate Standard	Fortification Level
Calibration Standard # 1	20.0	½ X
Calibration Standard # 2	40.0	X
Calibration Standard # 3	60.0	1 ½ X
Calibration Standard # 4	80.0	2 X
Calibration Standard # 5	200	5 X

**X is equal to the regulatory action level or the level of concern*

4. Add approximately 8 mL of extraction solvent to each vessel and shake/vortex for 10 minutes.

5. Centrifuge the tubes at a minimum of 7,000 RCF at 4ºC for 15 minutes.

6. Transfer 3.00 mL of the supernatant to an Oasis HLB PRIME (6cc, 200 mg) pass through SPE cartridge. Allow the sample to pass through the cartridge by gravity into an empty pre-weighed 15 mL centrifuge tube.

7. Evaporate the sample at 55ºC to a sample weight of 0.10–0.16 g. Do NOT allow the tubes to go dry. Reconstitute the sample with HPLC grade water (or equivalent) to a sample weight of 0.40 g (±0.03).

8. Sonicate the tubes for approximately 10 minutes, then vortex the samples for ~ 30 seconds, and transfer to a microcentrifuge tube.

9. Centrifuge the samples at a minimum of 20,500 RCF at 4ºC for 15 minutes. Transfer the supernatant to an autosampler vial for analysis.

Chromatography:

Table 4: HPLC Gradient

Time (min)	Flow (µl/min)	%Mobile Phase A	% Mobile Phase B
0.0	500	95	5
0.5	500	95	5
1.8	500	85	15

Time (min)	Flow (µl/min)	%Mobile Phase A	% Mobile Phase B
3.5	500	80	20
6.0	500	75	25
7.0	500	70	30
11.0	500	65	35
16	500	0	100
26	500	0	100

**A 4-minute post run was used to re-equilibrate the column.*

Mobile Phase A: LC/MS Grade 0.2% Formic Acid in water

Mobile Phase B: LC/MS Grade 0.2% Formic Acid in acetonitrile

Column: Agilent Infinity Lab Poroshell 120 EC-C18, 150 x 2.1 mm, 2.7 µm

Column Temperature: 40°C

Injection Volume: 5µL

Autosampler Temperature: 5°C

Mass Spectrometry using Electrospray Ionization (ESI):

The mass spectrometer was tuned and calibrated in positive and negative ion detection modes according to the manufacturer's instructions. The instrument was optimized by using flow injection analysis (50:50) 0.2% formic acid in water and 0.2% formic acid in acetonitrile, at an HPLC flow rate of 500 µL/min to optimize electronic voltages and gas flows. The triple quadrupole mass spectrometer was equipped with an electrospray ionization (ESI) source.

The gas temperature, gas flow, sheath gas temperature, and sheath gas flow were set to 290°C, 20 L/min, 400°C, and 10 L/min respectively. Electrical voltages were optimized for the capillary voltage at +3000 volts/-3500 volts, nebulizer/nozzle voltage at +500 volts/-1500 volts, cell accelerator voltage of 5 volts, and fragmentor voltage of 380 volts. The high-pressure RF was set to +150 volts/-110 volts and the low-pressure RF was set to +75 volts/-60 volts. The collision energy and SRM transition information are listed in Table 5.

Table 5: Collision energy and SRM transition information

Name	Precursor ion m/z	Product ions m/z	Retention time (min)	CE (volts)	Polarity
Amoxicillin	366	**114**, 349.2	4.0	**25**, 5	Positive
Ampicillin	350.1	**106**, 160	5.1	**33**, 27	Positive
Brilliant Green	385.4	**341.2**, 241	16.2	**51**, 79	Positive
Ciprofloxacin	332.1	**231**, 288.4	5.6	**45**, 30	Positive
Cloxacillin	436	**160**, 276.8	14.5	**15**, 20	Positive
Chlortetracycline	479.1	**444.2** 462.1	7.0	**21**, 15	Positive
Danofloxacin	358.2	**314**, 283	5.8	**17**, 17	Positive
Doxycycline	445.1	**410.2**, 428.1	6.0	**25**, 17	Positive

Name	Precursor ion m/z	Product ions m/z	Retention time (min)	CE (volts)	Polarity
Difloxacin	400.2	**356.3**, 299	7.0	**17**, 25	Positive
Enrofloxacin	360.1	**342.1**, 245.1	6.1	**25**, 38	Positive
Erythromycin	734.8	**158.1**, 576.4	11.0	**39**, 15	Positive
Florfenicol Amine	248	**230**, 130	3.9	**17**, 35	Positive
Flumequine	262	**244.1**, 202	12.4	**27**, 40	Positive
Gentian Violet	372.2	**356.2**, 340.3	15.7	**55**, 73	Positive
Hydroxy Mebendazole	298.2	**266**, 220	7.1	**30**, 70	Positive
Leuco Gentian Violet	374.3	**358.3**, 238.2	6.5	**35**, 30	Positive
Lincomycin	407.2	**126.1**, 70.1	4.7	**37**, 80	Positive
Leuco Malachite Green	331.1	**239**, 223.1	12.0	**40**, 80	Positive
Mebendazole	296.1	**264**, 105	10.5	**35**, 50	Positive
Mebendazole Amine	238.2	**105.1**, 133.3	7.4	**40**, 47	Positive
Malachite Green	329	**313**, 208.1	14.8	**53**, 55	Positive
Nalidixic Acid	233	**187.1**, 215	11.6	**33**, 25	Positive
Norfloxacin	320.1	**302.2**, 276.1	5.4	**21**, 17	Positive
Oxytetracycline	461.2	**426.1**, 443.2	5.5	**17**, 9	Positive
Oxolinic Acid	262.1	**216**, 244	9.1	**41**, 40	Positive
Sulfacetamide	215	**92.1**, 108	4.4	**33**, 13	Positive
Sarafloxacin	386.1	**342.3**, 299.2	6.9	**16**, 25	Positive
Sulfachlorpyridazine	284.9	**92.1**, 108.1	7.4	**35**, 33	Positive
Sulfadimethoxine	310.9	**156**, 92	9.9	**27**, 41	Positive
Sulfadoxine	310.9	**92**, 156	8.2	**35**, 30	Positive
Sulfadiazine	251	**92.1**, 108	4.7	**20**, 20	Positive
Sulfaethoxypyridazine	295	**92**, 108	8.2	**41**, 39	Positive
Sulfamonomethoxine	281	**108**, 156	7.1	**31**, 25	Positive
Sulfamethoxypyridazine	280.9	**92**, 156.1	7.0	**37**, 23	Positive
Sulfamerazine	265	**108**, 92	5.4	**25**, 19	Positive
Sulfamethoxazole	254	**92.1**, 108	8.1	**30**, 35	Positive
Sulfamethazine	279	**92.1**, 124	6.1	**35**, 33	Positive
Sulfapyridine	250	**92**, 108.1	5.1	**35**, 30	Positive
Sulfaquinoxaline	300.9	**92.1**, 108	9.9	**41**, 23	Positive
Sulfathiazole	256	**92**, 156	5.0	**33**, 19	Positive
Thiamphenicol	354	**290**, 185	5.9	**10**, 25	Negative
Tetracycline	445.2	**409.9**, 153.9	5.5	**17**, 33	Positive
Trimethoprim	291	**230.2**, 123.1	5.3	**31**, 31	Positive
Leuco Malachite Green d_5	336.4	239	11.8	40	Positive
Leuco Gentian Violet d_6	380.2	364	6. 0	35	Positive
Malachite Green d_5	334	318	14.8	53	Positive
Oxolinic Acid d_5	267	249	9.1	40	Positive
Gentian Violet d_6	378.2	362	15.7	50	Positive
Roxithromycin	837.1	679	13.3	25	Positive

*Quantitation ions with corresponding collision energies are in bold.

Data Analysis: Some of the targeted drug residues included in this method made use of an internal standard to improve quantitation; however, due to cost or availability there were residues which did not use an internal standard for quantitation. The designated analyte/internal standard is listed below in Table 6 (* *No internal standard used for quantitation):*

Table 6: Designated analyte/internal standard

*Florfenicol Amine	* Amoxicillin	*Ampicillin
*Cloxacillin	*Hydroxymebendazole	*Mebendazole Amine
*Mebendazole	*Lincomycin	*Tetracycline
*Oxytetracycline	*Chlortetracycline	*Doxycycline
*Thiamphenicol	*Sulfacetamide	*Sulfadiazine
*Sulfathiazole	*Sulfapyridine	*Sulfamerazine
*Sulfamethazine	*Sulfamonomethoxine	*Sulfamethoxypyridazine
*Sulfadoxine	*Sulfachlorpyridazine	*Sulfadimethoxine
*Sulfaquinoxaline	*Sulfamethoxazole	*Sulfaethoxypyridazine
*Difloxacin	*Ciprofloxacin	*Danofloxacin
*Sarafloxacin	*Enrofloxacin	*Norfloxacin
Oxolinic Acid/OXO d_5	Flumequine/OXO d_5	Nalidixic Acid/OXO d_5
Gentian Violet/GV d_6	Leuco Gentian Violet/LGV d_6	Malachite Green/MG d_5
Leuco Malachite Green/LMG d_5	Erythromycin/ROX	*Trimethoprim

The calibration curves yielded a regression (R^2) of ≥0.99. For positive confirmation all product ions must be detected, the associated chromatographic peak must exhibit a retention time within 5% of the average retention time of the calibration standards, and the product ion ratios must be within 10% of the average product ion ratios obtained from the calibration standards (13).

Analysis of Reference Materials and Commercial Products. Reference materials were obtained from commercially available sources and were prepared as described in the sample preparation section. Samples were quantitated using matrix-matched extracted standards that were previously screened and determined to be free of the targeted residues. Additionally, an incurred residue was also analyzed in order to verify the method performance and accuracy (12).

Limits of Detection and Quantitation Studies: The method detection limits (MDL) and limits of quantitation (LOQ) for each analyte were determined on the basis of replicate (n =7). The MDL of each analyte was calculated by the multiplication of the standard deviation by the student's t value at the 99% confidence level (3.143), and the LOQ by multiplying the standard deviation by ten (13, 14).

RESULTS AND DISCUSSION

Method Optimization: Method optimization consisted of a 2-fold process. The first step was to develop an instrumental method to provide the needed sensitivity and chromatography for trace residue analysis in difficult matrices and capable of determining additional residues in the future. Subsequently, upon completion of

instrument optimization, evaluation of the extraction method used in LIB 4615 (6a) was evaluated to make certain it was usable for quantitative analysis.

Initial efforts focused on tuning and optimizing for each targeted compound with respect to response and peak shape. With a triple quadrupole instrument, optimization of collision energies for each analyte transition allows for more sensitive detection of targeted compounds as compared to the more universal acquisition parameters used for the wide-scope HRMS screening method (6b) originally coupled with the LIB 4615 extraction. Once optimal responses were achieved for each ion of interest, we began the difficult task of developing a chromatographic method to encompass numerous residues with vastly different chemical properties. Furthermore, there are targeted isobaric compounds in the method, and chromatographic resolution was essential for determination of these compounds.

During tuning and optimization, it was noticed that some residues had an increased response in positive ionization when transitioning from 0.1% to 0.2% formic acid mobile phase composition. Thus, all chromatographic gradient development was performed using a 0.2% formic acid in water solution, and a 0.2% formic acid in acetonitrile solution. Because the chromatographic method would need to resolve isobaric compounds and would contain a wide range in polarity of the residues determined, it was deemed that a fast-chromatographic gradient would not provide the best long-term results. It was also important to consider that many of the matrices analyzed could contain high contents of fats and lipids, which could potentially result in diminished detector response and poor chromatography. As a result, the chromatography method developed was a gradient that utilized a 16-minute gradient separation, followed by a 10-minute aggressive organic wash, and a 4-minute re-equilibration. The chromatography can be seen in Figure 1.

Once the instrument acquisition method was developed, all efforts focused on the extraction method. Study researchers felt that the extraction described in LIB 4615 could be used to provide accurate and reproducible quantitative results. This was primarily because of the specialized solid-phase extraction (SPE) cartridges used. Using pass through SPE, compounds with a wide range of polarities can be extracted simultaneously. Furthermore, these SPE cartridges are highly effective at removing fats and phospholipids.

Although the LIB 4615 extraction procedure is highly effective, it was designed for qualitative screening work using HRMS (6). So, as a result some minor modifications were needed to enhance quantitation. Some of these modifications include the use of internal standards to compensate for instrument fluctuations and varying extraction efficiencies. Sample size was increased from 2 to 4 grams of tissue. Another modification that was needed was the sample concentration process.

It was noted through prior experience of the researchers that several of the drug residues assayed in this method degrade rapidly when exposed to excessive or prolonged elevated temperatures. Therefore, when concentrating samples, it is imperative that temperatures not be excessive (55ºC) but be at an appropriate level that prolonged exposure to heat does not diminish recovery.

Another significant factor in the concentration process, is to prevent the samples from achieving complete dryness. This problem was observed during the initial method development process, and as a result several residues provided diminished response and some residues were not detected. It is critical that the samples not be concentrated to dryness; however, the ratio of aqueous and organic composition of the sample extract that is analyzed on the instrument must be very precise. If the organic ratio of the extract is too high, then the more polar compounds are poorly retained or suffered from poor chromatography when sample analyzed. However, if the organic ratio of the extract is too low, solubility issues arose with some of the dyes. Therefore, it was deemed necessary to evaporate the sample to a specified weight within a narrow tolerance, and then reconstitute the sample by weight to a specific volume for quantitative purposes.

Method Validation: Separate shrimp, frog, barramundi, croaker, and cobia validation recovery studies were performed. Validation was performed utilizing the U.S. Food and Drug Administration guidance for industry for the mass spectrometry confirmation and identification of animal drug residues and the FDA Foods Program guidelines for the validation of chemical methods 2nd edition (13, 14). Each individual matrix was verified on separate nonconsecutive days. The validation procedure consisted of a total of 65 matrix spikes, and 13 matrix blanks. Method accuracies and precision, using a matrix extracted calibration curve with internal standard correction for selected analytes, were acceptable for the fortified tissues (Tables 7-11).

All 65 assayed matrix spikes analyzed met the required confirmation criteria for all residues of interest. No false positives were observed in the 13 matrix blanks that were analyzed. In addition to the 65 assayed matrix spikes, residues of malachite green and leuco malachite green were confirmed in the incurred shrimp sample analyzed. Previous analysis of an incurred residue utilizing LIB 4562 found leuco malachite green at 5.77 ng/g, which was regarded as an estimated amount since it was slightly outside of the calibration curve. The result in the current study found leuco malachite green at 6.24 ng/g, which was within the validated uncertainty level of LIB 4562.

CONCLUSION:

A multi-class, multi-residue quantitative confirmatory LC-MS/MS method for multiple matrices was validated at the Arkansas Laboratory. The method can accurately quantitate 42 different drug residues from 10 different classes of drugs. The sample extraction and cleanup procedure is relatively simple and quick, all the while being extremely effective. The mobile phase gradient which was developed along with the use of pass through SPE technology allows for the method to be used for compounds of vastly different chemical properties. This makes this method a viable option for regulatory laboratories analyzing several different aquaculture matrices.

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Table 7: Quantitative Data for Shrimp.

* All Method Detection Limits (MDL) were calculated at the 99% confidence level (standard deviation times 3.143) from the quantitative results of the ½ X matrix spikes.

Compound	Level of Interest (1X) ng/g	% Recovery (% RSD)			*MDL ng/g
		½ X N=7	1 ½ X N=3	5X N=3	
Florfenicol Amine	50	102 (10)	113	99.4	7.70
Thiamphenicol	1	78.6 (10)	103	108	0.125
Lincomycin	50	88.8 (9)	94.5	85.8	6.55
Amoxicillin	10	95.3 (12)	93.6	97.7	1.87
Ampicillin	10	82.3 (10)	96.7	103	1.30
Cloxacillin	10	89.5 (6)	96.9	95.0	0.819
Hydroxy Mebendazole	5	89.0 (9)	100	102	0.603
Mebendazole Amine	5	106 (8)	90.1	90.8	0.660
Mebendazole	5	88.8 (20)	102	106	1.41
Tetracycline	50	80.3 (13)	113	122	8.43
Oxytetracycline	50	77.0 (10)	107	116	5.87
Doxycycline	50	87.8 (11)	100	101	7.32
Chlortetracycline	50	107 (10)	109	101	8.52
Erythromycin	50	76.9 (9)	65.0	63.2	5.47
Norfloxacin	5	96.8 (4)	81.8	85.5	0.345
Ciprofloxacin	5	111 (8)	91.2	92.0	0.695
Danofloxacin	5	118 (4)	107	97.7	0.428
Enrofloxacin	5	104 (5)	100	92.5	0.428
Sarafloxacin	5	94.5 (11)	118	113	0.811
Difloxacin	5	104 (6)	111	107	0.496
Flumequine	10	88.8 (5)	111	110	0.691
Oxolinic Acid	10	80.8 (6)	115	109	0.802
Nalidixic Acid	10	87.8 (6)	110	112	0.798
Gentian Violet	1	96.9 (8)	94.9	101	0.123
Leuco Gentian Violet	1	87.1 (10)	94.7	101	0.141
Malachite Green	1	95.2 (3)	102	108	0.0475
Leuco Malachite Green	1	92.0 (7)	101	114	0.0975
Trimethoprim	10	108 (7)	102	95.3	1.15
Sulfacetamide	10	104 (5)	98.9	89.3	0.770
Sulfadiazine	10	99.5 (9)	107	90.2	1.40

Sulfathiazole	10	100 (5)	102	89.0	0.730
Sulfapyridine	10	91.8 (6)	100	94.9	0.879
Sulfamerazine	10	93.0 (9)	97.5	99.8	1.29
Sulfamethazine	10	88.9 (5)	102	92.6	0.596
Sulfamethoxypyridazine	10	85.7 (9)	110	109	1.23
Sulfamonomethoxine	10	91.8 (10)	104	101	1.40
Sulfachlorpyridine	10	91.7 (7)	105	90.5	1.01
Sulfadoxine	10	86.2 (9)	103	102	1.29
Sulfamethoxazole	10	77.0 (8)	111	99.2	0.907
Sulfaethoxypyridazine	10	91.7 (6)	102	102	0.838
Sulfadimethoxine	10	87.1 (6)	106	116	0.889
Sulfaquinoxaline	10	76.4 (11)	107	109	1.35

Table 8: Quantitative Data for Frog.

Compound	**Level of Interest (1X)** ng/g	**% Recovery (% RSD)** ½ X N=7	1 ½ X N=3	5X N=3	***MDL** ng/g
Florfenicol Amine	50	108 (10)	114	113	8.11
Thiamphenicol	1	117 (9)	107	103	0.163
Lincomycin	50	109 (9)	109	105	8.05
Amoxicillin	10	120 (4)	120	124	0.852
Ampicillin	10	121 (7)	130	127	1.33
Cloxacillin	10	113 (8)	114	117	1.39
Hydroxy Mebendazole	5	111 (10)	119	116	0.848
Mebendazole Amine	5	106 (8)	124	100	0.688
Mebendazole	5	116 (11)	128	93.4	0.982
Tetracycline	50	132 (11)	140	136	11.1
Oxytetracycline	50	117 (9)	136	129	8.68
Doxycycline	50	128 (9)	128	125	8.78
Chlortetracycline	50	123 (6)	126	113	6.35
Erythromycin	50	93.8 (4)	79.3	76.0	3.23
Enrofloxacin	5	85.9 (14)	108	109	0.934
Sarafloxacin	5	100 (4)	95.2	88.8	0.338
Difloxacin	5	110 (6)	103	96.8	0.522
Norfloxacin	5	108 (5)	118	102	0.390

Ciprofloxacin	5	112 (9)	99.3	97.6	0.805
Danofloxacin	5	98.3 (16)	124	124	1.27
Nalidixic Acid	10	102 (8)	118	119	1.25
Flumequine	10	103 (12)	117	123	1.97
Oxolinic Acid	10	104 (11)	116	117	1.77
Gentian Violet	1	101 (12)	121	117	0.190
Leuco Gentian Violet	1	110 (15)	102	108	0.260
Malachite Green	1	88.6 (8)	110	112	0.117
Leuco Malachite Green	1	98.0 (8)	116	110	0.115
Trimethoprim	10	110 (10)	108	106	1.67
Sulfacetamide	10	124 (3)	117	111	0.678
Sulfadiazine	10	98.6 (7)	118	108	1.03
Sulfathiazole	10	120 (5)	102	96.3	0.908
Sulfapyridine	10	111 (8)	110	105	1.32
Sulfamerazine	10	99.4 (12)	104	92.2	1.93
Sulfamethazine	10	110 (7)	117	113	1.24
Sulfamethoxypyridazine	10	111 (10)	120	119	1.77
Sulfamonomethoxine	10	118 (11)	120	117	2.06
Sulfachlorpyridine	10	113 (10)	114	104	1.83
Sulfadoxine	10	103 (12)	116	106	2.05
Sulfamethoxazole	10	115 (9)	111	107	1.65
Sulfaethoxypyridazine	10	106 (10)	109	105	1.73
Sulfadimethoxine	10	112 (9)	109	105	1.61
Sulfaquinoxaline	10	116 (12)	112	107	2.14

Table 9: Quantitative Data for Croaker.

Compound	**Level of Interest (1X)** ng/g	**% Recovery (% RSD)** ½ X N=7	1 ½ X N=3	5X N=3	***MDL** ng/g
Florfenicol Amine	50	101 (5)	96.3	88.0	4.30
Thiamphenicol	1	100 (14)	100	105	0.219
Lincomycin	50	95.7 (9)	96.7	99.3	6.95
Amoxicillin	10	76.0 (7)	112	107	0.820
Ampicillin	10	83.7 (10)	108	109	1.31
Cloxacillin	10	92.7 (8)	101	98.5	1.18

Hydroxy Mebendazole	5	104 (7)	104	102	0.566
Mebendazole Amine	5	93.4 (10)	101	105	0.765
Mebendazole	5	101 (8)	106	104	0.631
Tetracycline	50	85.8 (9)	118	116	6.19
Oxytetracycline	50	106 (9)	111	101	7.85
Doxycycline	50	99.0 (10)	114	100	7.69
Chlortetracycline	50	107 (13)	117	111	11.2
Erythromycin	50	70.0 (9)	80.9	73.2	5.20
Ciprofloxacin	5	84.9 (18)	97.0	120	1.19
Danofloxacin	5	89.4 (10)	88.1	98.9	0.752
Norfloxacin	5	87.5 (13)	112	135	0.912
Enrofloxacin	5	90.2 (9)	90.8	101	0.643
Sarafloxacin	5	92.0 (8)	101	107	0.598
Difloxacin	5	90.1 (14)	103	99.3	0.948
Flumequine	10	71.5 (9)	104	103	1.00
Oxolinic Acid	10	81.9 (11)	109	110	1.45
Nalidixic Acid	10	77.3 (9)	110	101	1.08
Gentian Violet	1	80.9 (15)	97.1	104	0.191
Leuco Gentian Violet	1	78.9 (20)	87.3	81.4	0.249
Malachite Green	1	77.2 (9)	100	101	0.112
Leuco Malachite Green	1	89.4 (15)	101	92.8	0.207
Trimethoprim	10	95.0 (10)	104	107	1.55
Sulfacetamide	10	95.1 (8)	107	104	1.25
Sulfadiazine	10	93.6 (6)	95.9	87.9	0.876
Sulfathiazole	10	93.0 (6)	85.8	76.3	0.908
Sulfapyridine	10	101 (8)	84.7	78.7	1.25
Sulfamerazine	10	90.7 (11)	78.6	82.9	1.48
Sulfamethazine	10	81.2 (10)	103	104	1.32
Sulfamethoxypyridazine	10	95.8 (6)	101	90.9	0.979
Sulfamonomethoxine	10	91.8 (11)	112	101	1.65
Sulfachlorpyridine	10	94.1 (9)	94.9	91.1	1.34
Sulfadoxine	10	84.5 (6)	89.3	84.0	0.829
Sulfamethoxazole	10	96.1 (8)	95.1	97.3	1.19
Sulfaethoxypyridazine	10	104 (5)	98.7	92.8	0.855
Sulfadimethoxine	10	94.9 (7)	93.4	88.3	1.03

Sulfaquinoxaline	10	95.5 (5)	96.9	91.4	0.764

Table 10: Quantitative Data for Cobia.

Compound	**Level of Interest (1X) ng/g**	**% Recovery (% RSD)**			***MDL**
		½ X N=7	1 ½ X N=3	5X N=3	ng/g
Florfenicol Amine	50	123 (4)	102	90.4	3.87
Thiamphenicol	1	90.3 (6)	98.2	96.0	0.0915
Lincomycin	50	110 (6)	95.3	92.1	5.41
Ampicillin	10	130 (6)	110	103	1.21
Amoxicillin	10	132 (4)	109	105	0.769
Cloxacillin	10	97.0 (4)	98.2	102	0.626
Hydroxy Mebendazole	5	110 (8)	101	96.6	0.661
Mebendazole Amine	5	115 (5)	101	93.8	0.484
Mebendazole	5	129 (4)	104	88.8	0.447
Tetracycline	50	123 (8)	113	100	7.41
Oxytetracycline	50	123 (8)	121	105	7.75
Doxycycline	50	126 (4)	109	104	4.31
Chlortetracycline	50	118 (7)	121	103	6.28
Erythromycin	50	67.0 (9)	70.8	89.5	0.447
Norfloxacin	5	109 (11)	116	114	0.940
Enrofloxacin	5	121 (5)	107	101	0.511
Sarafloxacin	5	111 (5)	104	88.6	0.431
Difloxacin	5	117 (6)	103	101	0.592
Ciprofloxacin	5	116 (6)	99.7	110	0.518
Danofloxacin	5	124 (7)	105	96.0	0.666
Nalidixic Acid	10	90.9 (4)	99.3	101	0.655
Flumequine	10	83.4 (5)	97.8	100	0.723
Oxolinic Acid	10	82.9 (7)	91.7	92.1	0.928
Gentian Violet	1	79.1 (11)	103	107	0.134
Leuco Gentian Violet	1	109 (10)	95.8	80.5	0.183
Malachite Green	1	88.4 (8)	94.6	93.6	0.118
Leuco Malachite Green	1	113 (5)	100	98.0	0.0859
Trimethoprim	10	98.1 (11)	102	99.2	1.64
Sulfacetamide	10	102 (5)	107	98.2	0.747
Sulfadiazine	10	97.3 (11)	101	101	1.68

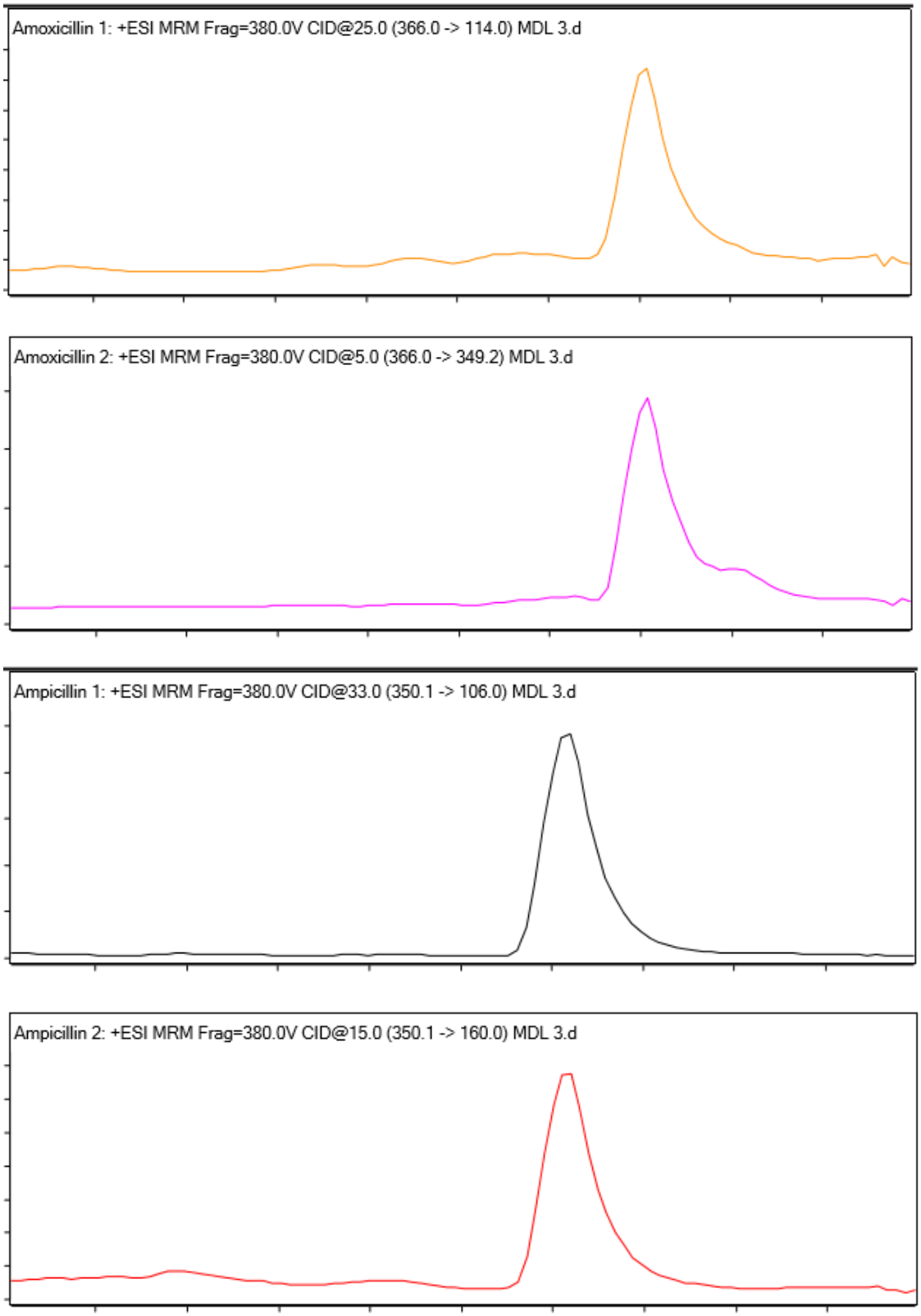
Sulfathiazole	10	91.5 (7)	92.7	93.3	1.08
Sulfapyridine	10	101 (6)	98.4	95.3	1.02
Sulfamerazine	10	94.7 (6)	113	99.0	0.933
Sulfamethazine	10	102 (5)	96.7	93.1	0.784
Sulfamethoxypyridazine	10	104 (7)	97.6	95.9	1.16
Sulfamonomethoxine	10	107 (6)	95.8	90.1	0.960
Sulfachlorpyridine	10	103 (6)	104	96.5	0.984
Sulfadoxine	10	102 (8)	103	96.9	1.29
Sulfamethoxazole	10	95.7 (7)	103	96.5	1.06
Sulfaethoxypyridazine	10	100 (4)	98.0	94.3	0.573
Sulfaquinoxaline	10	99.2 (6)	104	97.9	0.901
Sulfadimethoxine	10	101 (4)	107	97.0	0.684

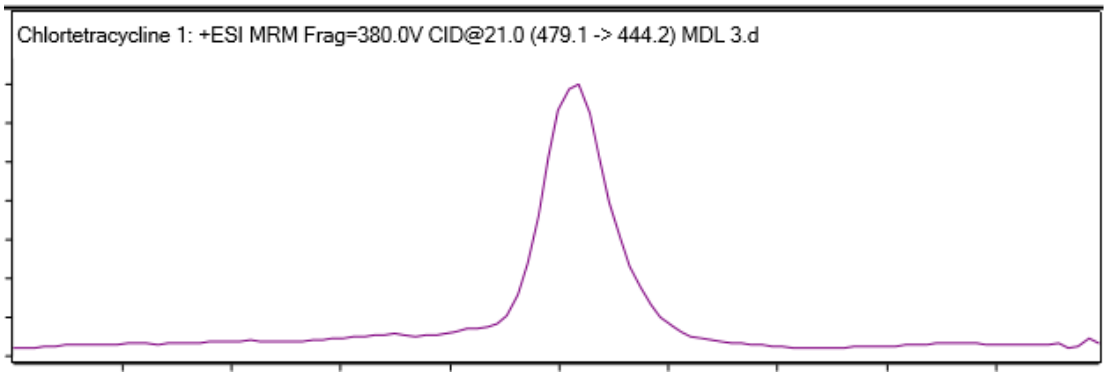
Table 11: Quantitative Data for Barramundi.

Compound	**Level of Interest (1X)** ng/g	**% Recovery (% RSD)** ½ X N=7	1 ½ X N=3	5X N=3	***MDL** ng/g
Florfenicol Amine	50	93.4 (8)	104	102	6.20
Thiamphenicol	1	115 (8)	101	105	0.147
Lincomycin	50	126 (7)	115	111	7.30
Amoxicillin	10	127 (7)	115	117	1.35
Ampicillin	10	134 (4)	119	126	0.952
Cloxacillin	10	123 (4)	112	114	0.870
Hydroxy Mebendazole	5	120 (5)	101	98.6	0.454
Mebendazole Amine	5	126 (7)	110	105	0.682
Mebendazole	5	131 (7)	117	109	0.723
Tetracycline	50	134 (8)	119	121	8.09
Oxytetracycline	50	136 (2)	127	132	2.19
Doxycycline	50	136 (1)	107	116	1.56
Chlortetracycline	50	133 (3)	111	118	3.47
Erythromycin	50	82.0 (10)	75.6	94.5	6.31
Norfloxacin	5	104 (9)	104	107	0.721
Ciprofloxacin	5	124 (5)	99.0	92.3	0.480
Danofloxacin	5	122 (6)	128	114	0.622
Enrofloxacin	5	101 (8)	94.3	84.1	0.688
Sarafloxacin	5	119 (2)	105	104	0.162

Difloxacin	5	135 (4)	102	100	0.479
Oxolinic Acid	10	95.2 (6)	103	108	0.869
Nalidixic Acid	10	101 (6)	113	126	0.955
Flumequine	10	103 (8)	112	120	1.24
Gentian Violet	1	107 (17)	108	119	0.289
Leuco Gentian Violet	1	107 (15)	114	113	0.255
Malachite Green	1	105 (6)	102	105	0.0938
Leuco Malachite Green	1	97.2 (9)	111	109	0.135
Trimethoprim	10	117 (4)	117	113	0.794
Sulfacetamide	10	113 (6)	96.8	94.2	1.11
Sulfadiazine	10	121 (6)	95.5	95.8	1.13
Sulfathiazole	10	112 (11)	100	92.8	1.96
Sulfapyridine	10	115 (6)	106	109	1.17
Sulfamerazine	10	110 (10)	100	109	1.78
Sulfamethazine	10	118 (7)	100	105	1.29
Sulfamethoxypyridazine	10	122 (4)	105	107	0.746
Sulfamonomethoxine	10	116 (5)	113	111	1.00
Sulfachlorpyridine	10	121 (4)	94.4	93.1	0.869
Sulfadoxine	10	122 (6)	109	108	1.23
Sulfamethoxazole	10	117 (6)	108	106	1.09
Sulfaethoxypyridazine	10	125 (6)	108	105	1.21
Sulfadimethoxine	10	123 (5)	121	119	1.05
Sulfaquinoxaline	10	118 (7)	116	112	1.33

Figure 1: Chromatograms from ½ X fortification spike from croaker

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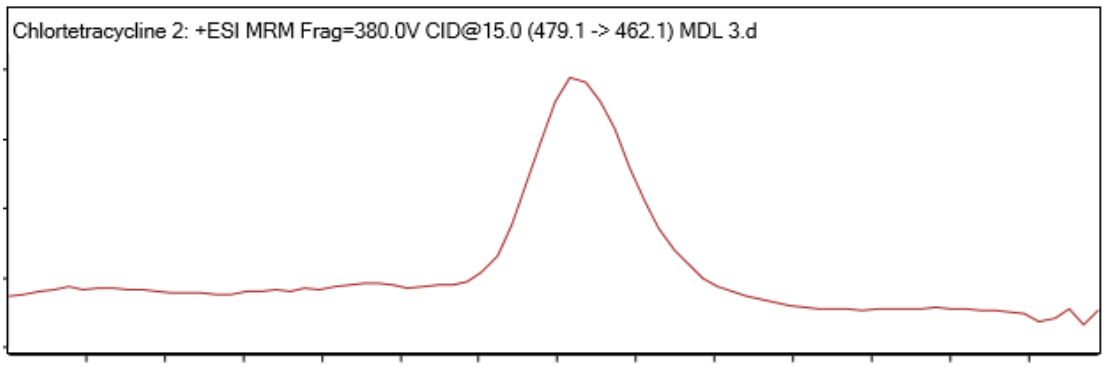
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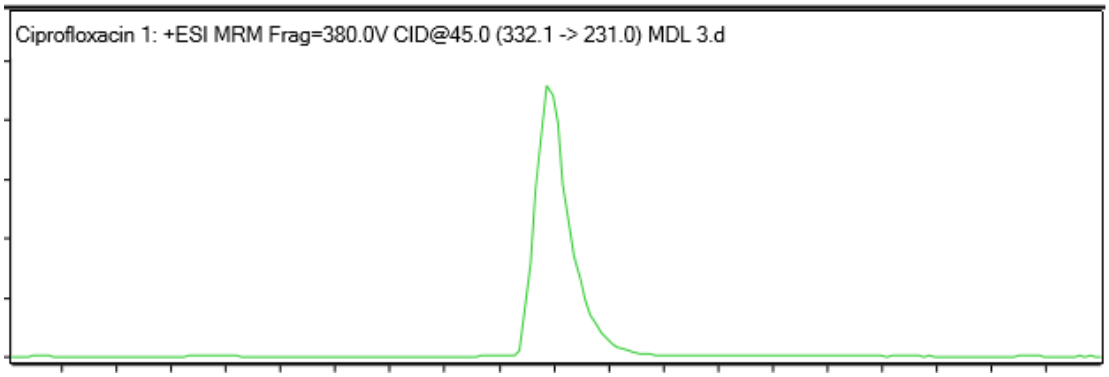
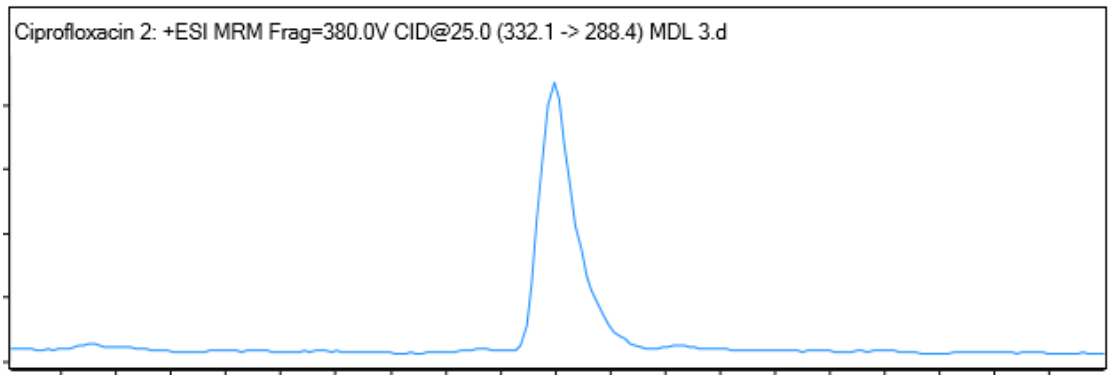
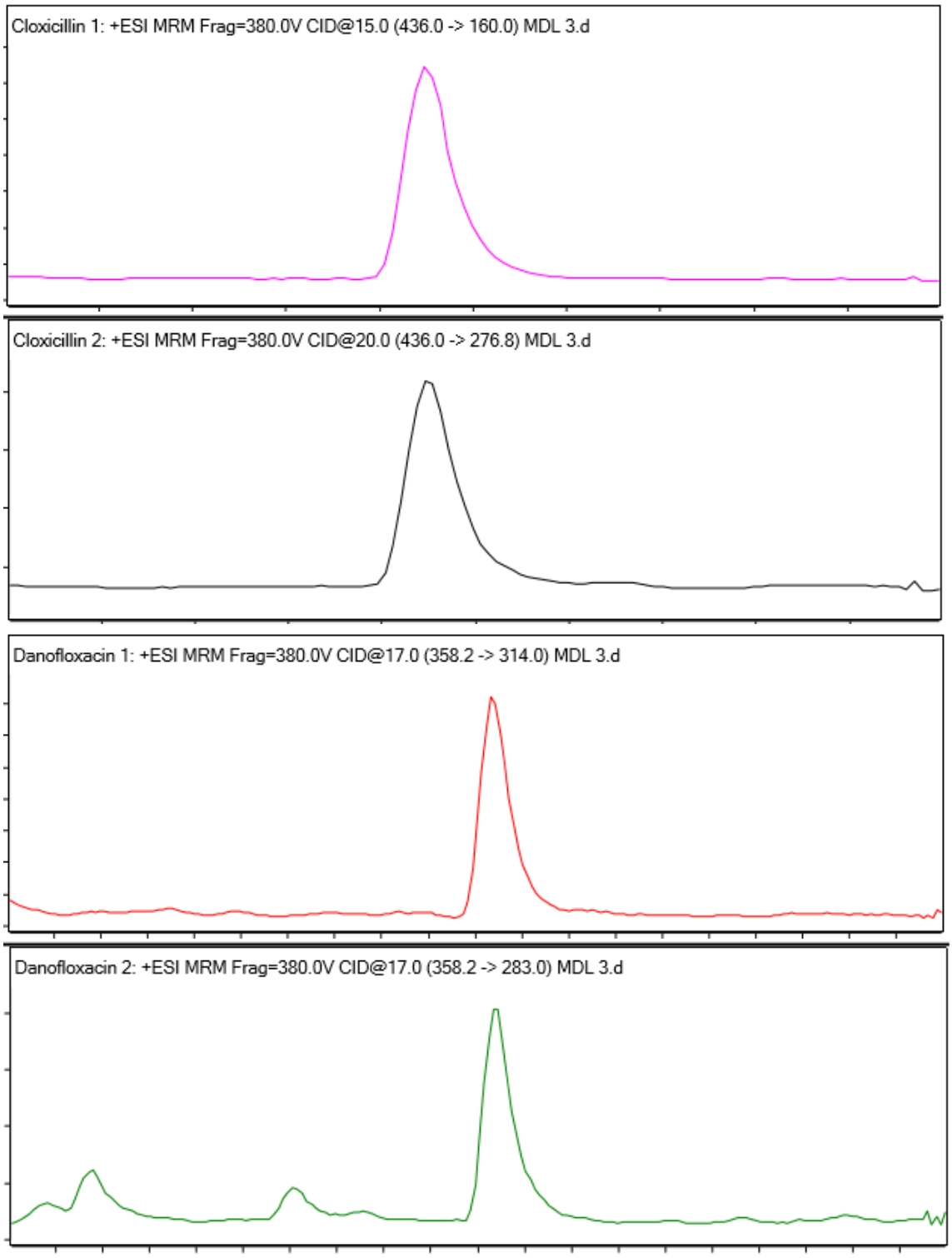
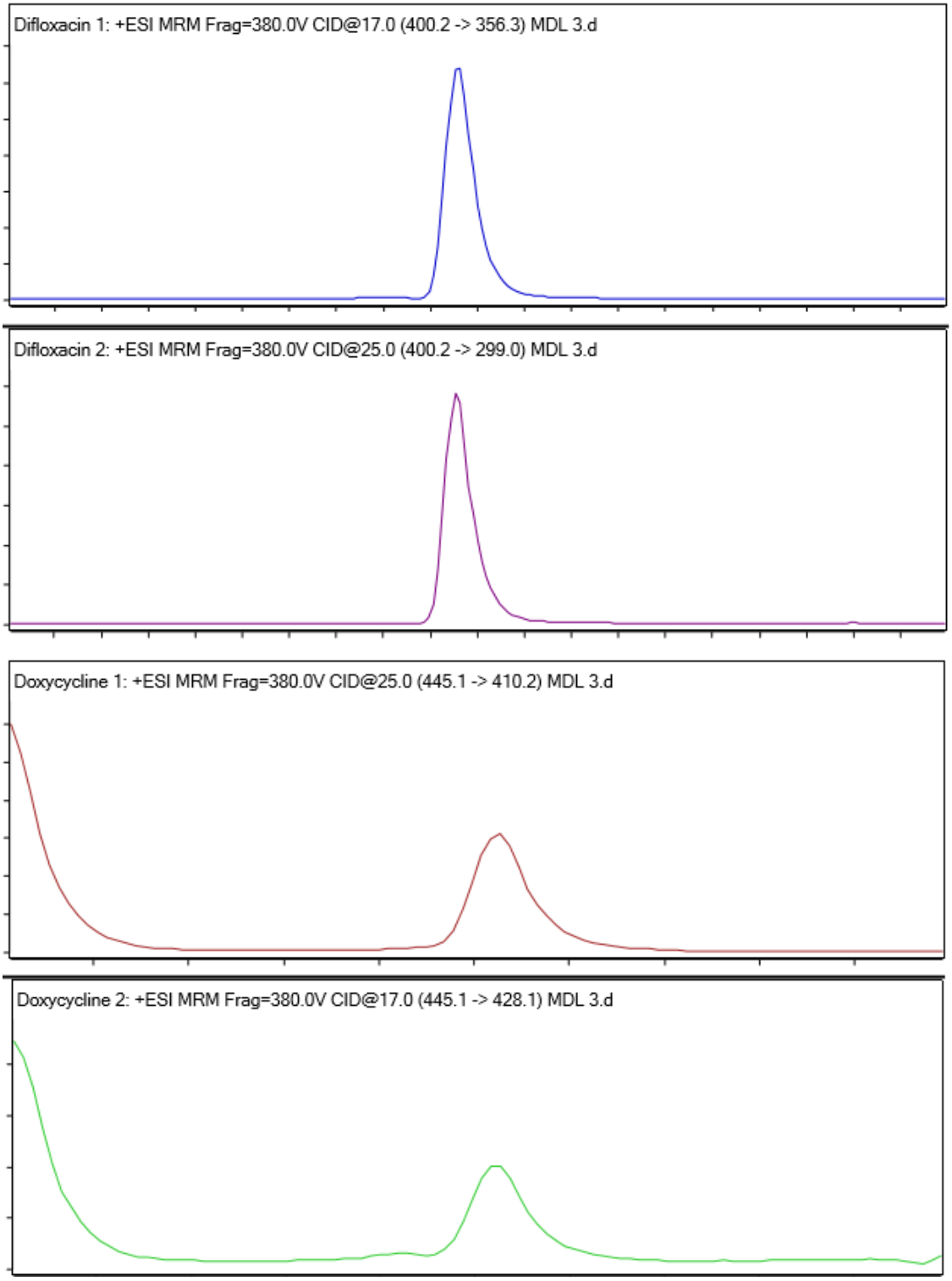
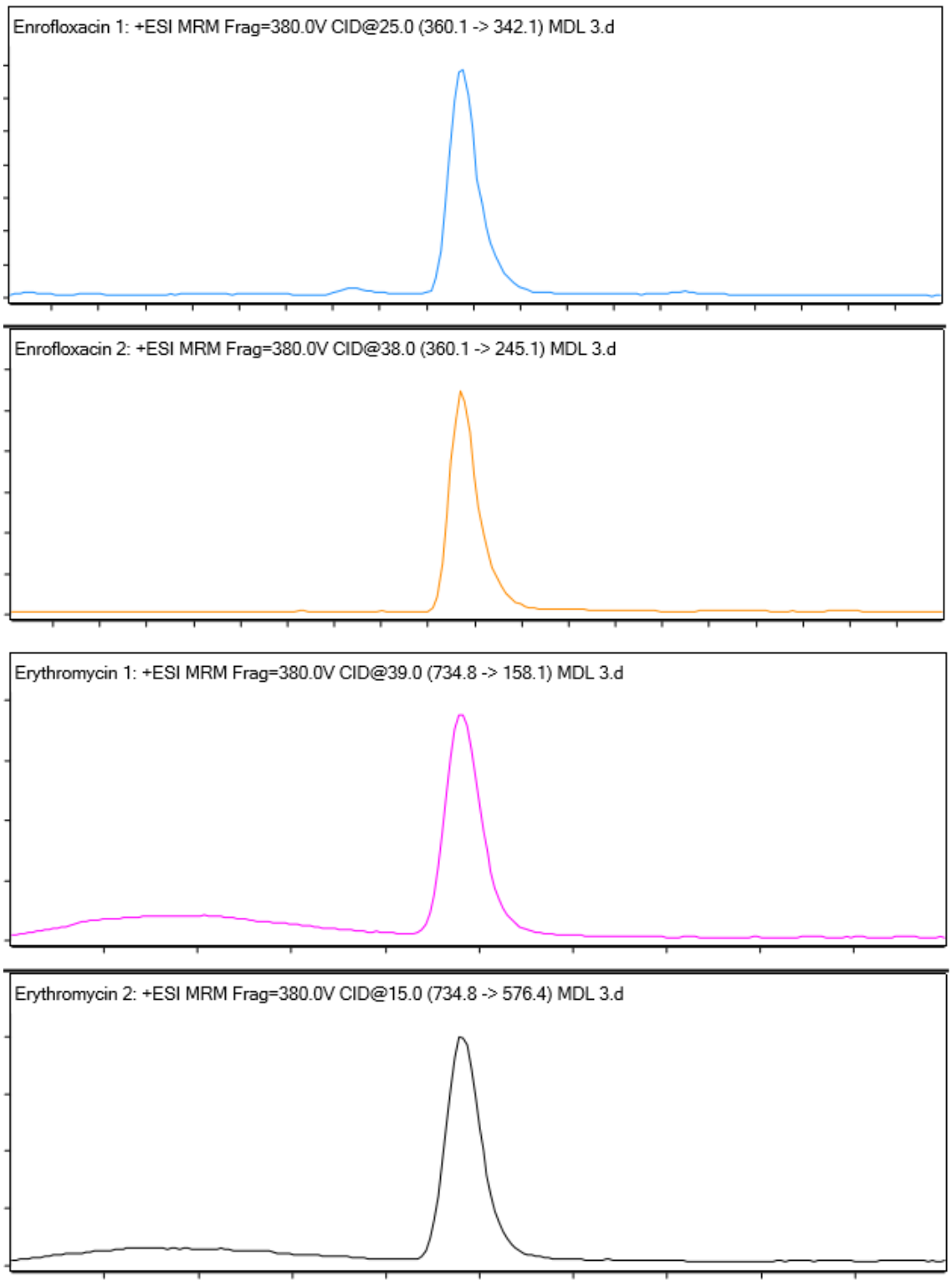
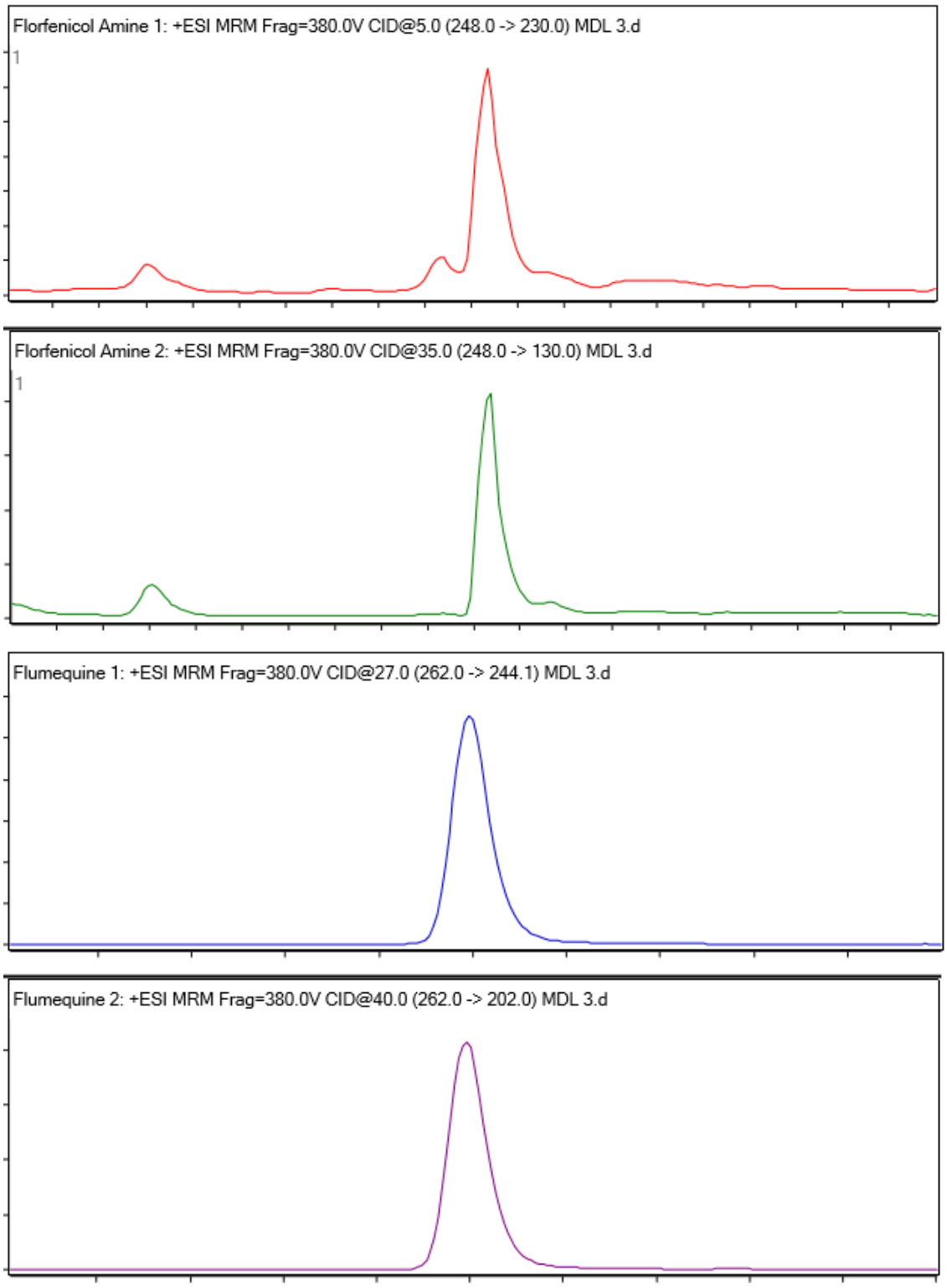

Ciprofloxacin 1: +ESI MRM Frag=380.0V CID@45.0 (332.1 -> 231.0) MDL 3.d

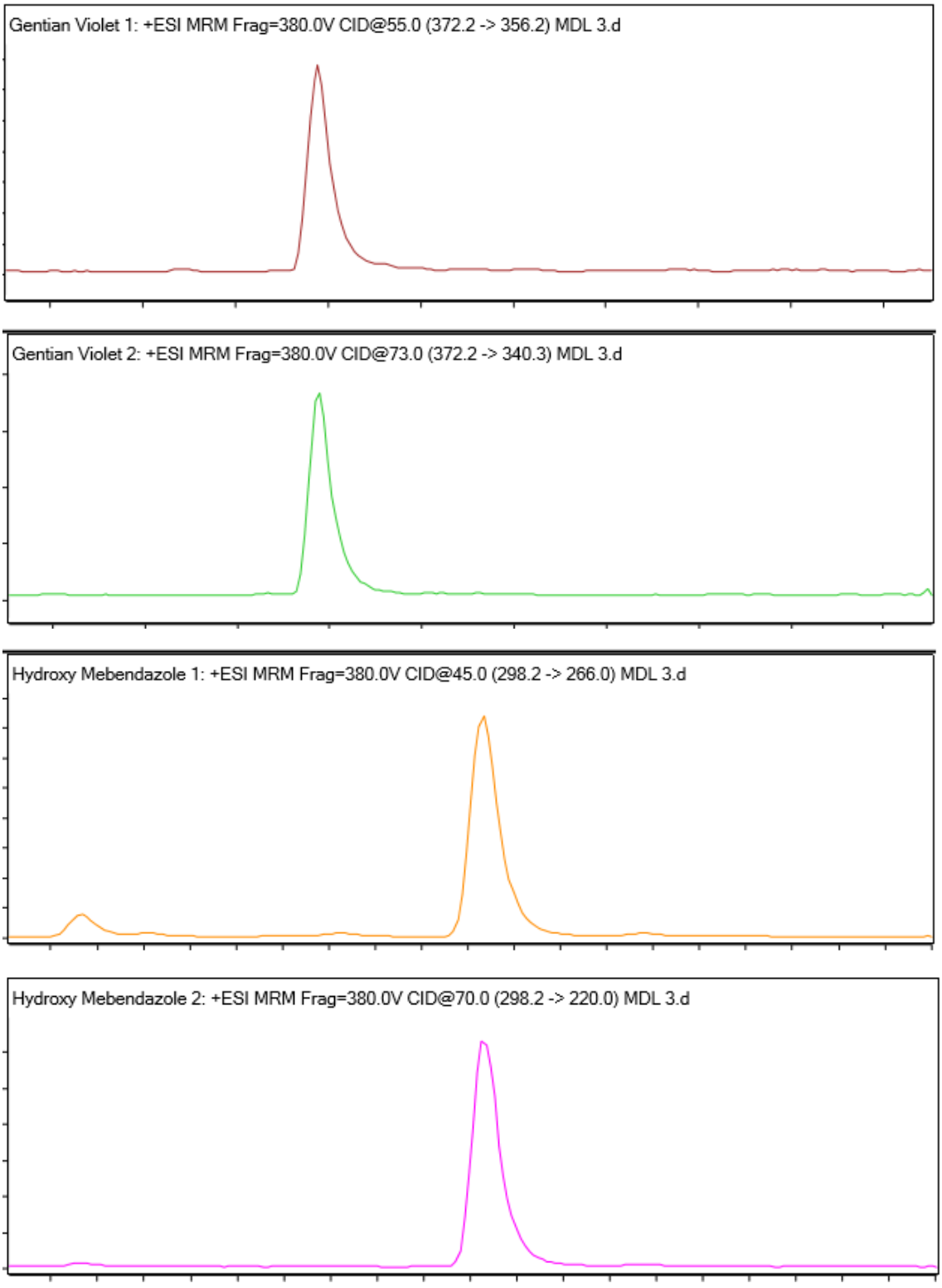

Ciprofloxacin 2: +ESI MRM Frag=380.0V CID@25.0 (332.1 -> 288.4) MDL 3.d

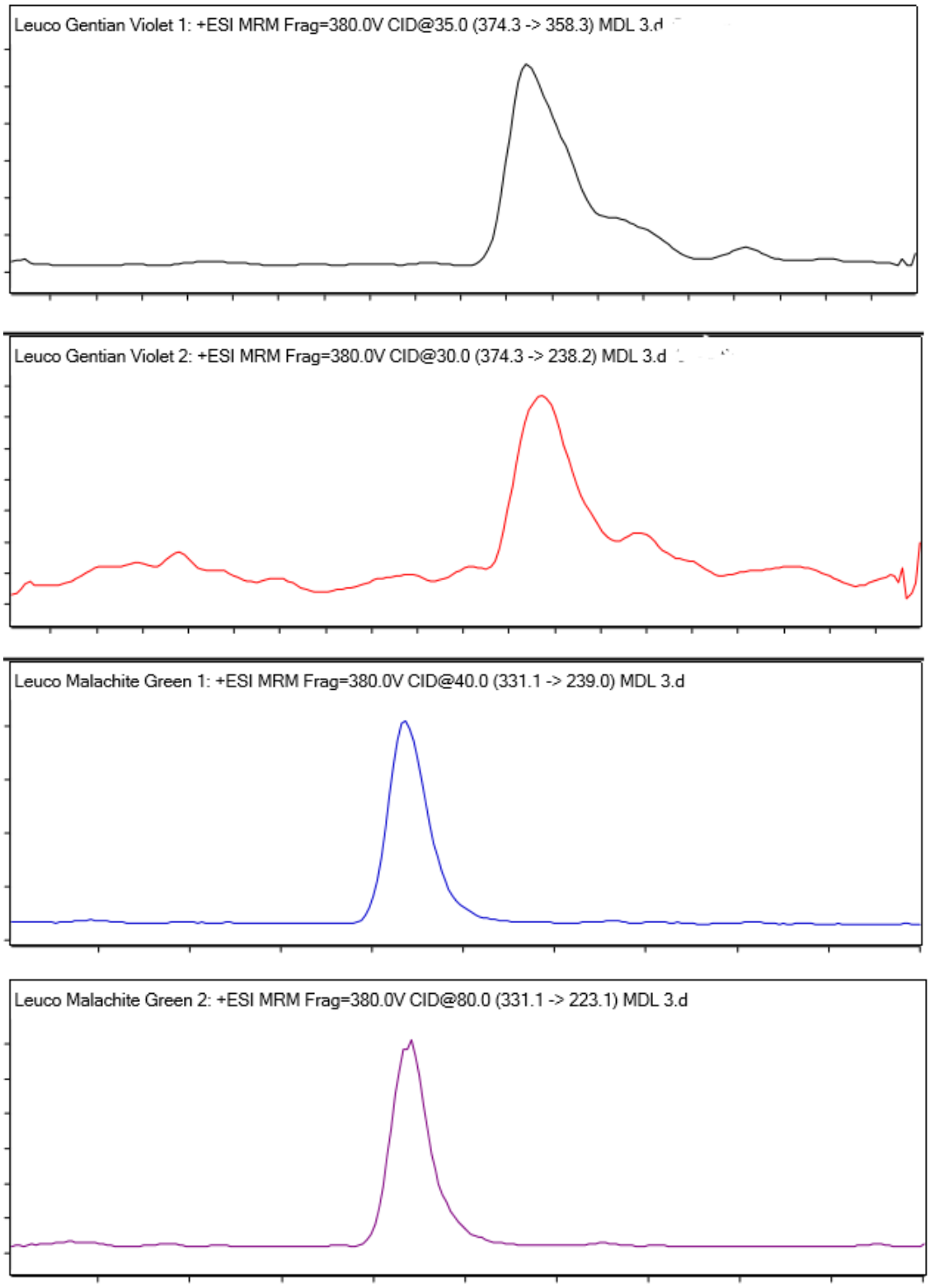

Cloxicillin 1: +ESI MRM Frag=380.0V CID@15.0 (436.0 -> 160.0) MDL 3.d
Cloxicillin 2: +ESI MRM Frag=380.0V CID@20.0 (436.0 -> 276.8) MDL 3.d
Danofloxacin 1: +ESI MRM Frag=380.0V CID@17.0 (358.2 -> 314.0) MDL 3.d
Danofloxacin 2: +ESI MRM Frag=380.0V CID@17.0 (358.2 -> 283.0) MDL 3.d

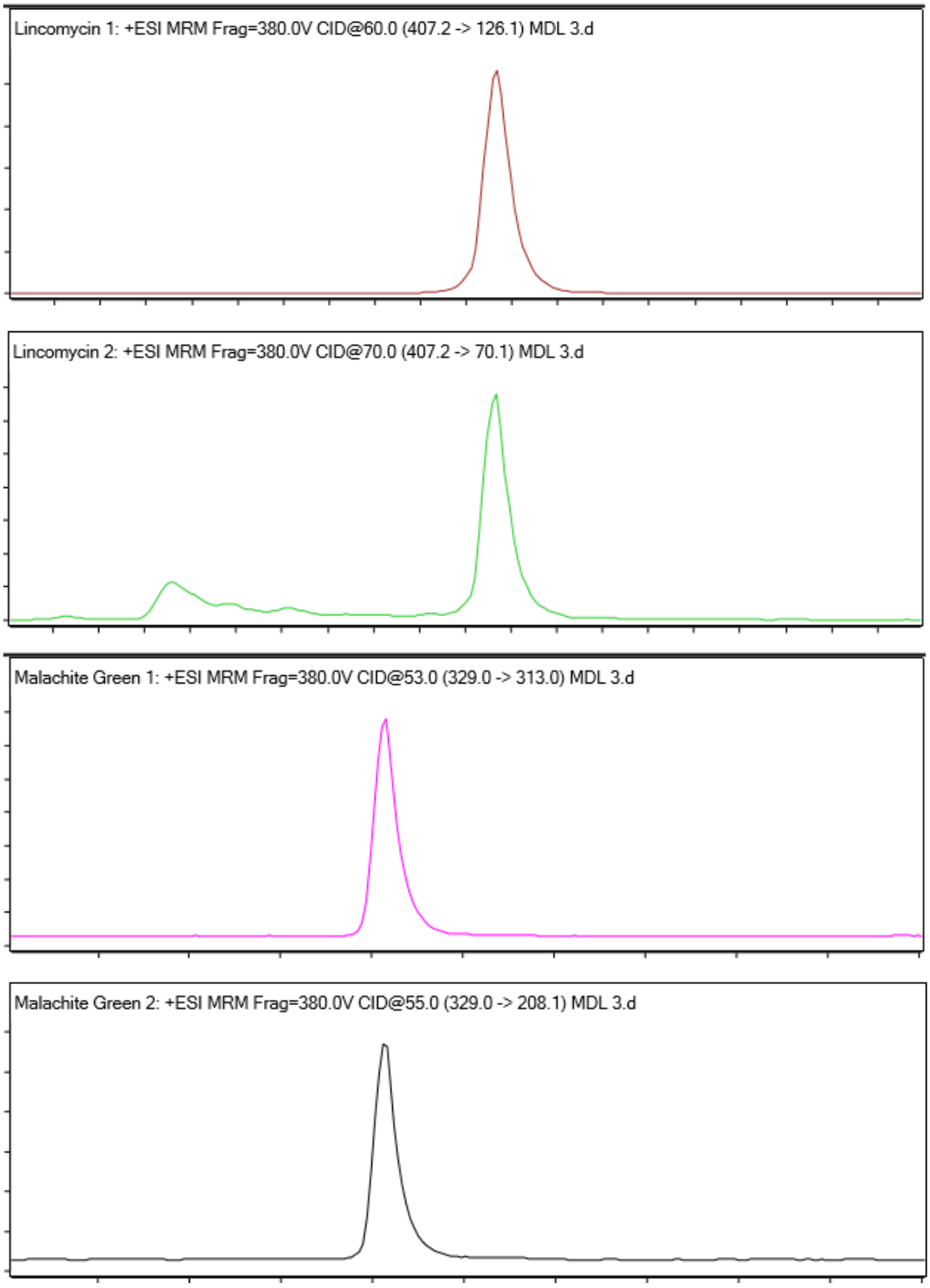

Difloxacin 1: +ESI MRM Frag=380.0V CID@17.0 (400.2 -> 356.3) MDL 3.d
Difloxacin 2: +ESI MRM Frag=380.0V CID@25.0 (400.2 -> 299.0) MDL 3.d
Doxycycline 1: +ESI MRM Frag=380.0V CID@25.0 (445.1 -> 410.2) MDL 3.d
Doxycycline 2: +ESI MRM Frag=380.0V CID@17.0 (445.1 -> 428.1) MDL 3.d

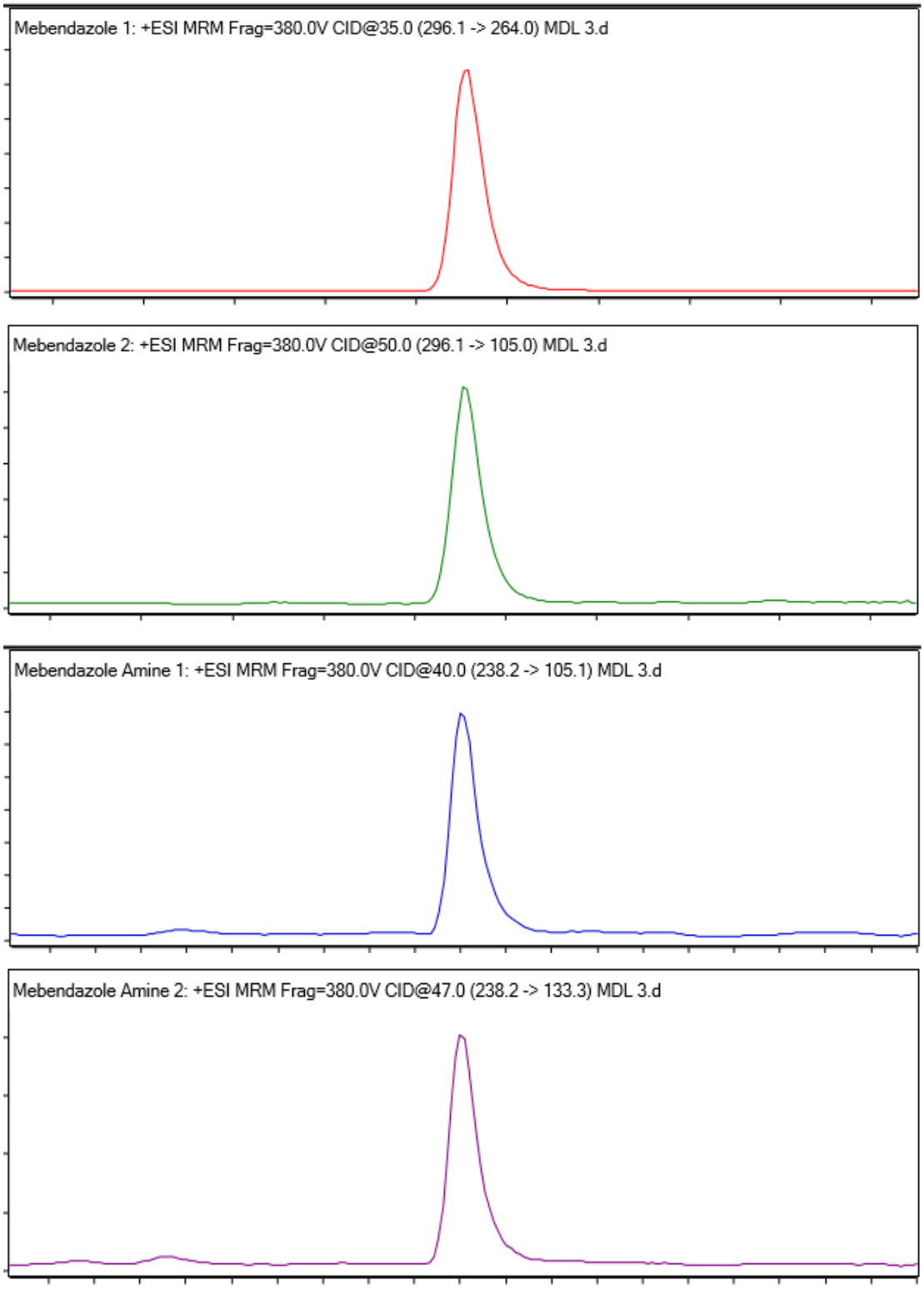

Enrofloxacin 1: +ESI MRM Frag=380.0V CID@25.0 (360.1 -> 342.1) MDL 3.d
Enrofloxacin 2: +ESI MRM Frag=380.0V CID@38.0 (360.1 -> 245.1) MDL 3.d
Erythromycin 1: +ESI MRM Frag=380.0V CID@39.0 (734.8 -> 158.1) MDL 3.d
Erythromycin 2: +ESI MRM Frag=380.0V CID@15.0 (734.8 -> 576.4) MDL 3.d

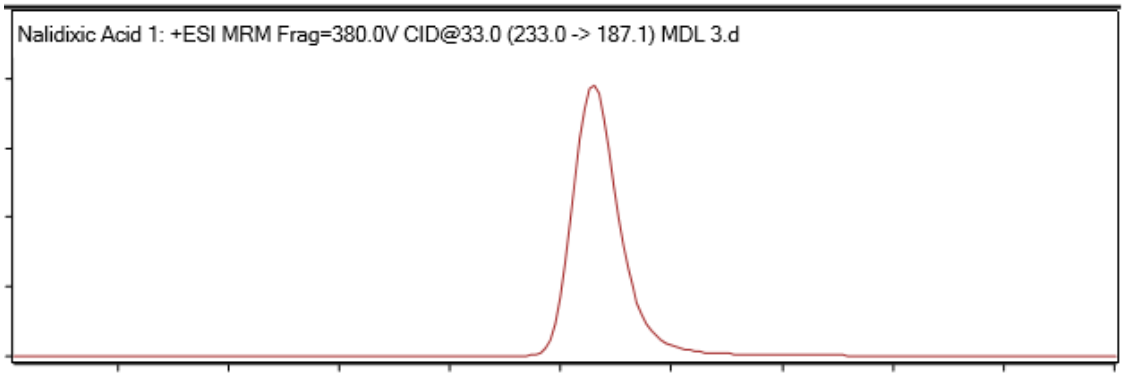

Florfenicol Amine 1: +ESI MRM Frag=380.0V CID@5.0 (248.0 -> 230.0) MDL 3.d
1
Florfenicol Amine 2: +ESI MRM Frag=380.0V CID@35.0 (248.0 -> 130.0) MDL 3.d
1
Flumequine 1: +ESI MRM Frag=380.0V CID@27.0 (262.0 -> 244.1) MDL 3.d
Flumequine 2: +ESI MRM Frag=380.0V CID@40.0 (262.0 -> 202.0) MDL 3.d

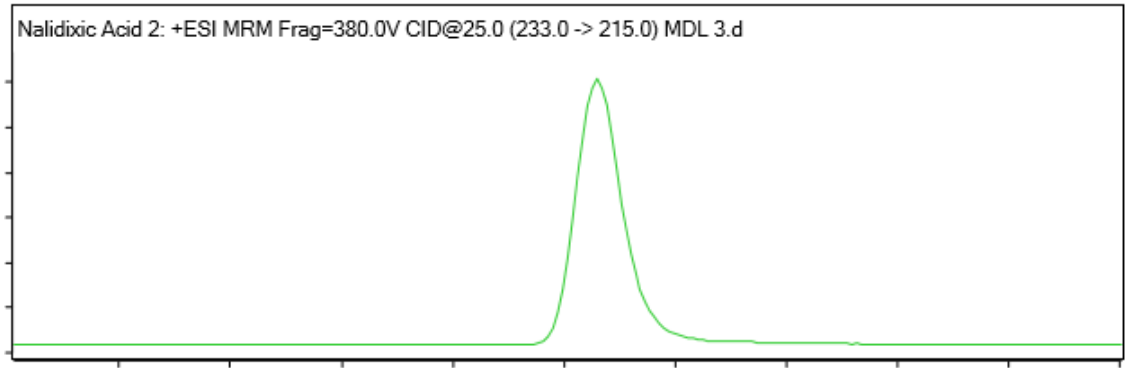

Gentian Violet 1: +ESI MRM Frag=380.0V CID@55.0 (372.2 -> 356.2) MDL 3.d
Gentian Violet 2: +ESI MRM Frag=380.0V CID@73.0 (372.2 -> 340.3) MDL 3.d
Hydroxy Mebendazole 1: +ESI MRM Frag=380.0V CID@45.0 (298.2 -> 266.0) MDL 3.d
Hydroxy Mebendazole 2: +ESI MRM Frag=380.0V CID@70.0 (298.2 -> 220.0) MDL 3.d

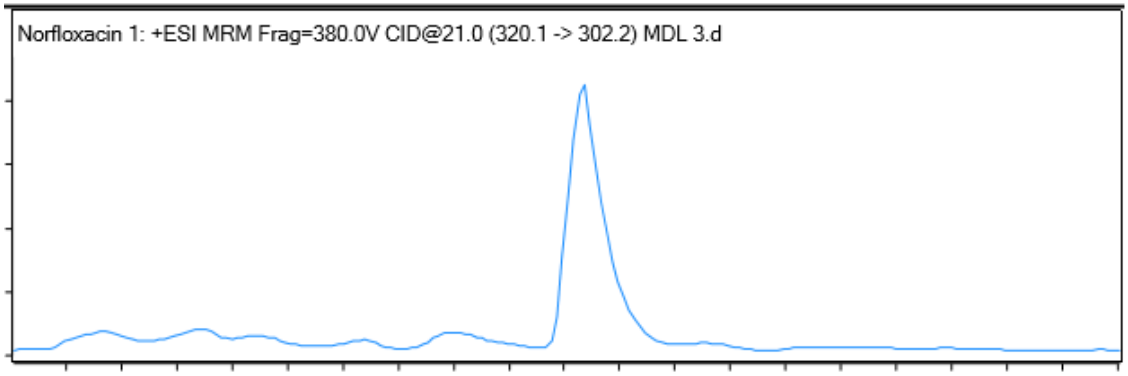

Leuco Gentian Violet 1: +ESI MRM Frag=380.0V CID@35.0 (374.3 -> 358.3) MDL 3.d
Leuco Gentian Violet 2: +ESI MRM Frag=380.0V CID@30.0 (374.3 -> 238.2) MDL 3.d
Leuco Malachite Green 1: +ESI MRM Frag=380.0V CID@40.0 (331.1 -> 239.0) MDL 3.d
Leuco Malachite Green 2: +ESI MRM Frag=380.0V CID@80.0 (331.1 -> 223.1) MDL 3.d

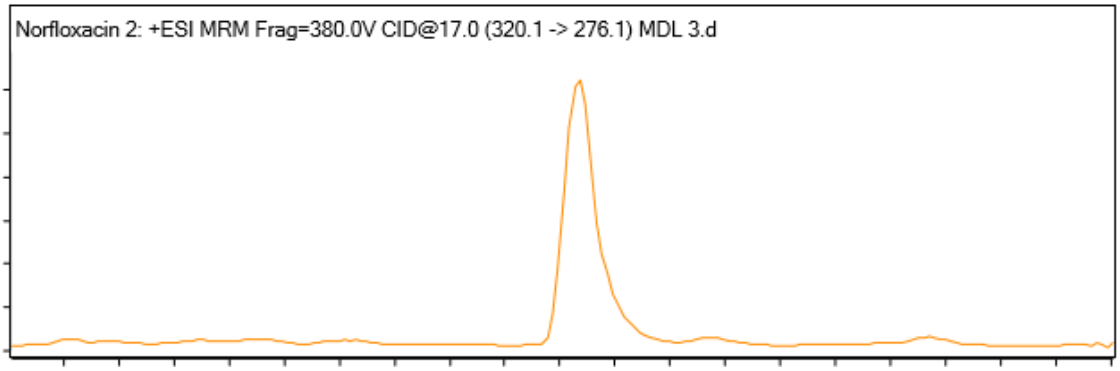

Lincomycin 1: +ESI MRM Frag=380.0V CID@60.0 (407.2 -> 126.1) MDL 3.d
Lincomycin 2: +ESI MRM Frag=380.0V CID@70.0 (407.2 -> 70.1) MDL 3.d
Malachite Green 1: +ESI MRM Frag=380.0V CID@53.0 (329.0 -> 313.0) MDL 3.d
Malachite Green 2: +ESI MRM Frag=380.0V CID@55.0 (329.0 -> 208.1) MDL 3.d

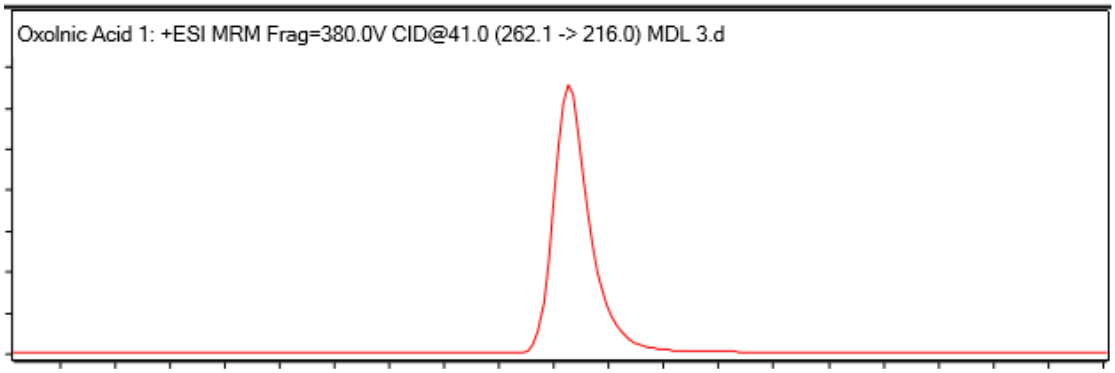

Mebendazole 1: +ESI MRM Frag=380.0V CID@35.0 (296.1 -> 264.0) MDL 3.d
Mebendazole 2: +ESI MRM Frag=380.0V CID@50.0 (296.1 -> 105.0) MDL 3.d
Mebendazole Amine 1: +ESI MRM Frag=380.0V CID@40.0 (238.2 -> 105.1) MDL 3.d
Mebendazole Amine 2: +ESI MRM Frag=380.0V CID@47.0 (238.2 -> 133.3) MDL 3.d

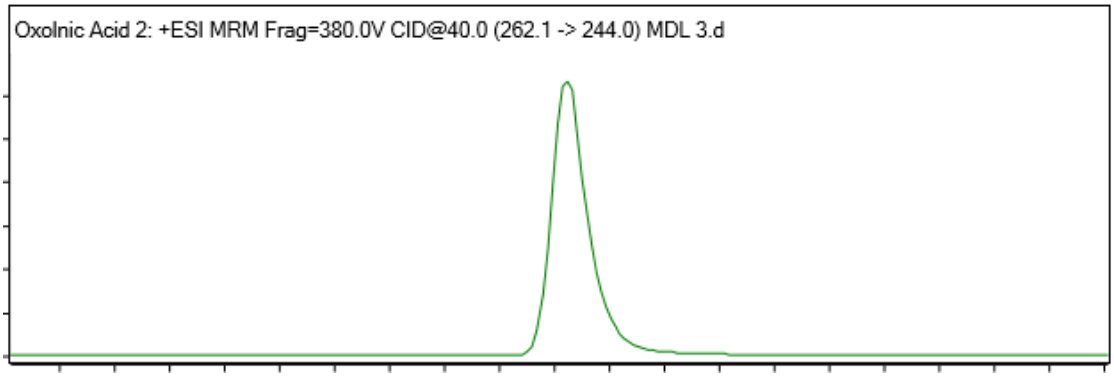

Nalidixic Acid 1: +ESI MRM Frag=380.0V CID@33.0 (233.0 -> 187.1) MDL 3.d

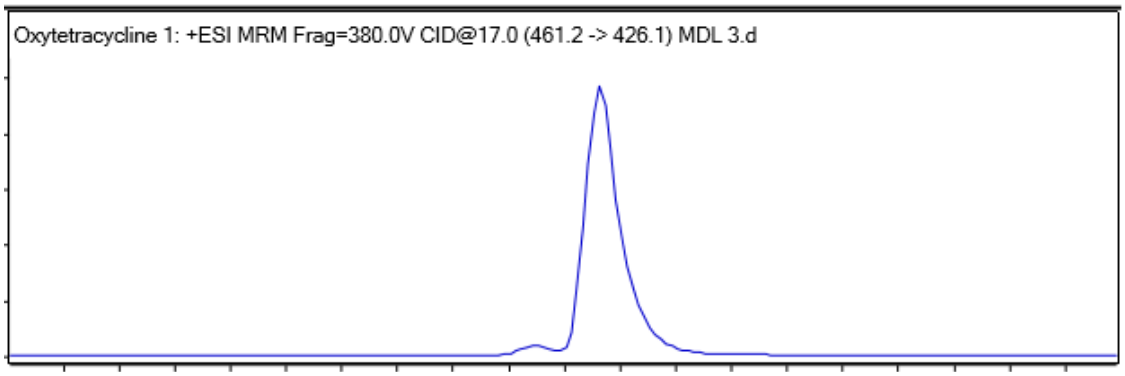

Nalidixic Acid 2: +ESI MRM Frag=380.0V CID@25.0 (233.0 -> 215.0) MDL 3.d

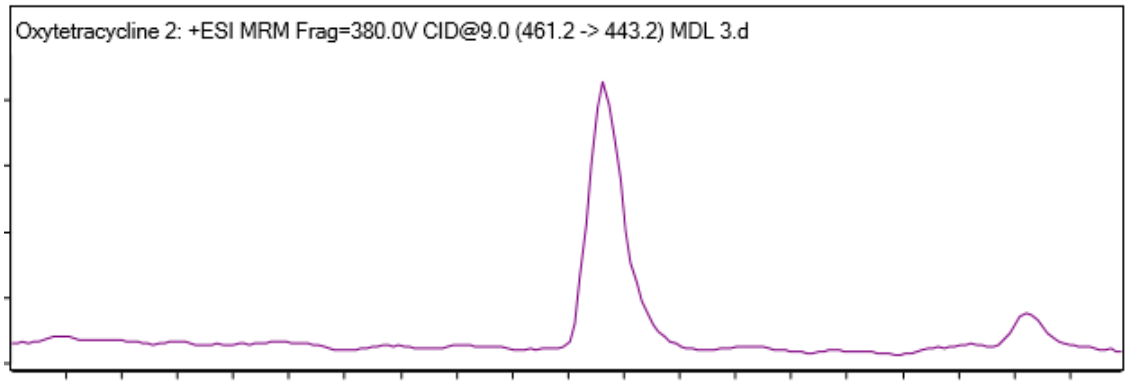

Norfloxacin 1: +ESI MRM Frag=380.0V CID@21.0 (320.1 -> 302.2) MDL 3.d

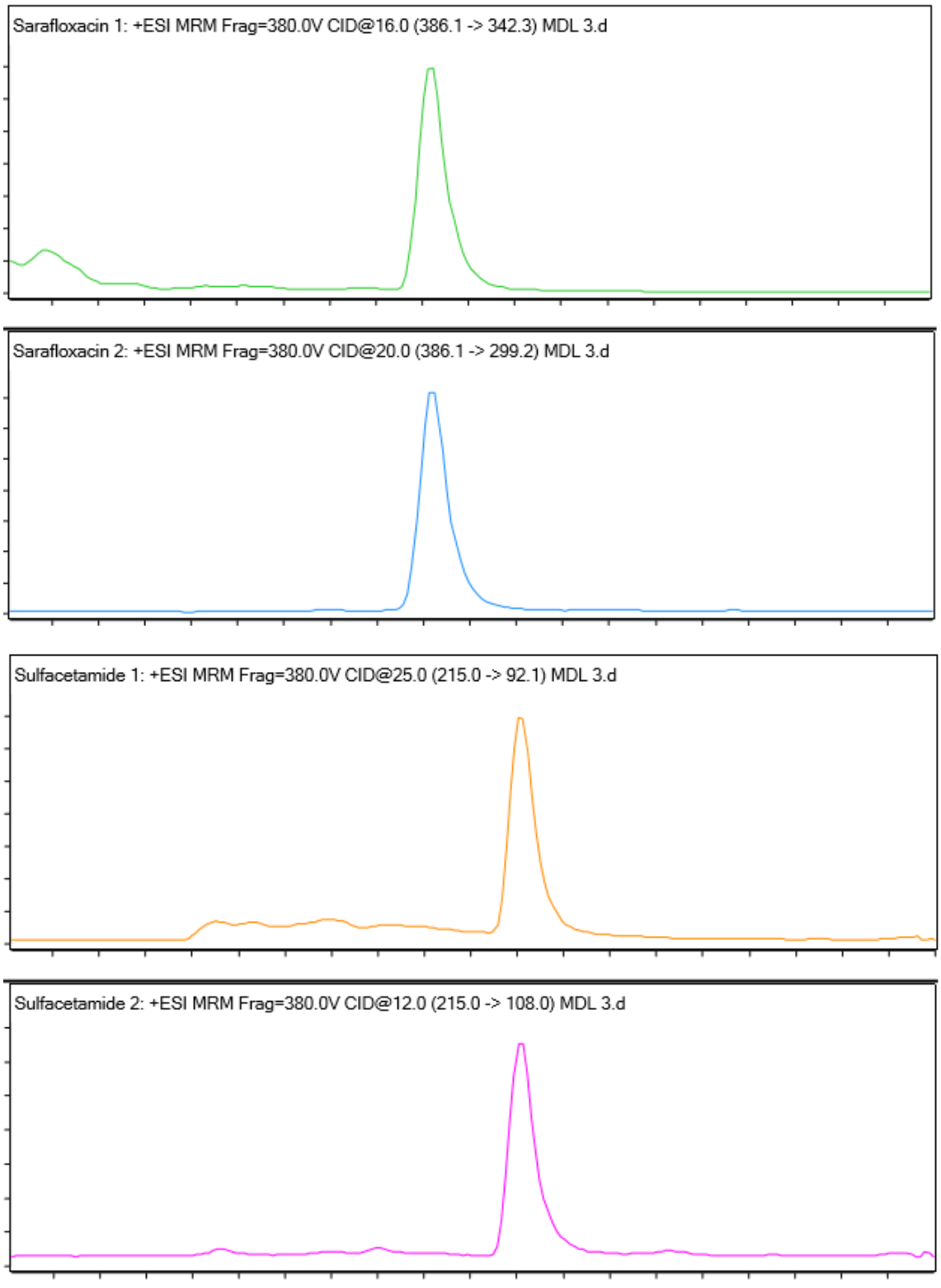

Norfloxacin 2: +ESI MRM Frag=380.0V CID@17.0 (320.1 -> 276.1) MDL 3.d

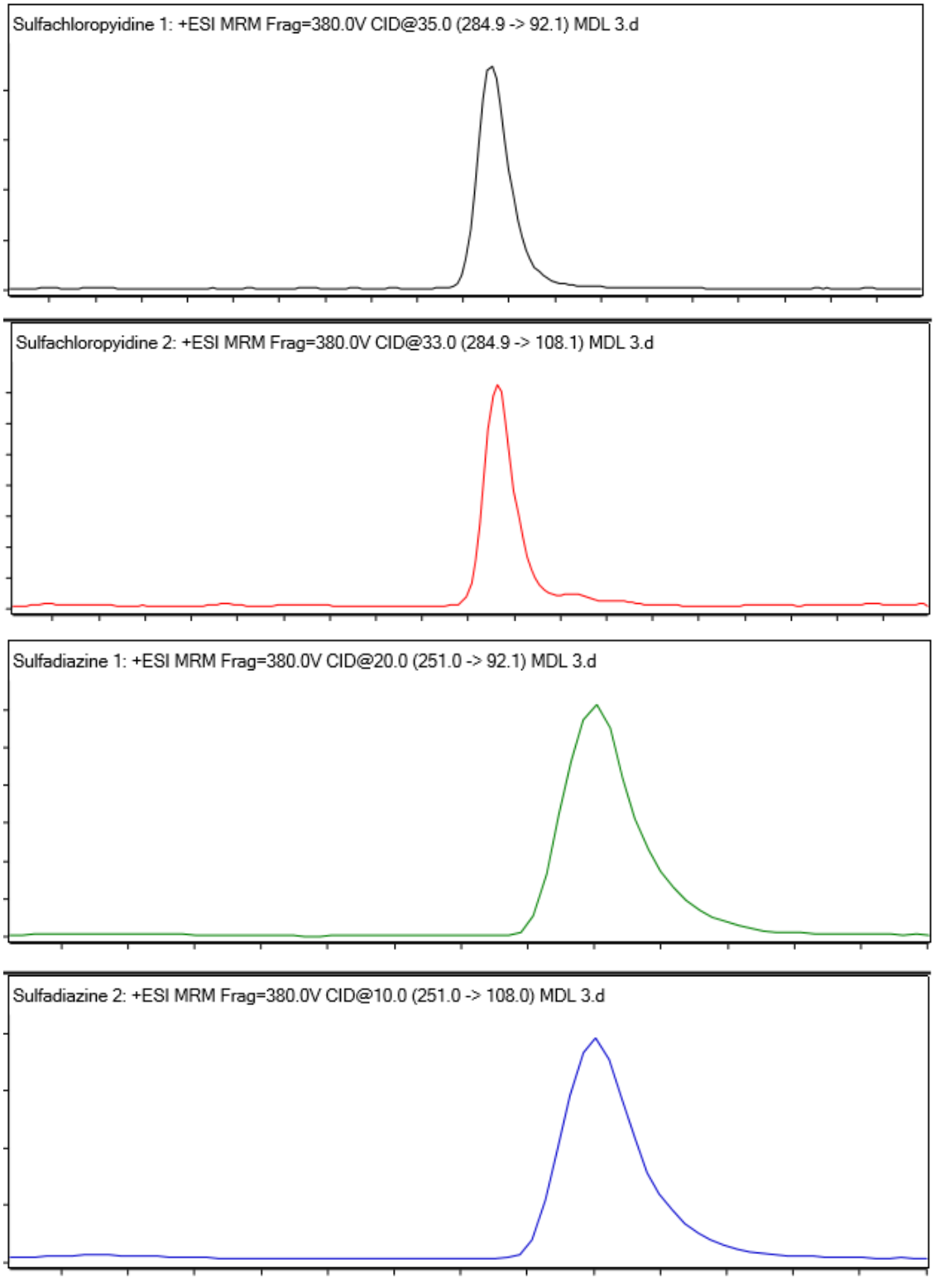

Oxolnic Acid 1: +ESI MRM Frag=380.0V CID@41.0 (262.1 -> 216.0) MDL 3.d

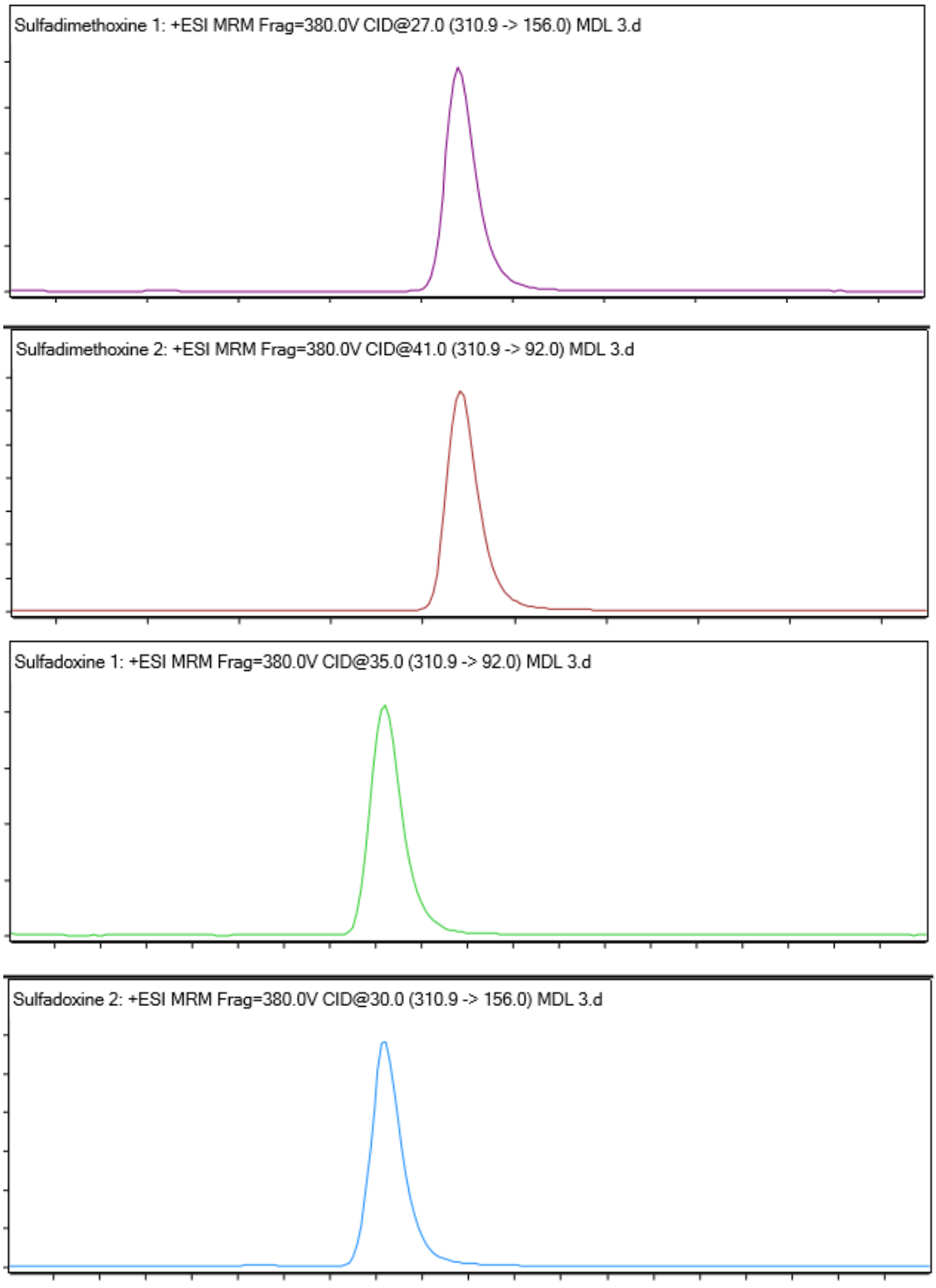

Oxolnic Acid 2: +ESI MRM Frag=380.0V CID@40.0 (262.1 -> 244.0) MDL 3.d

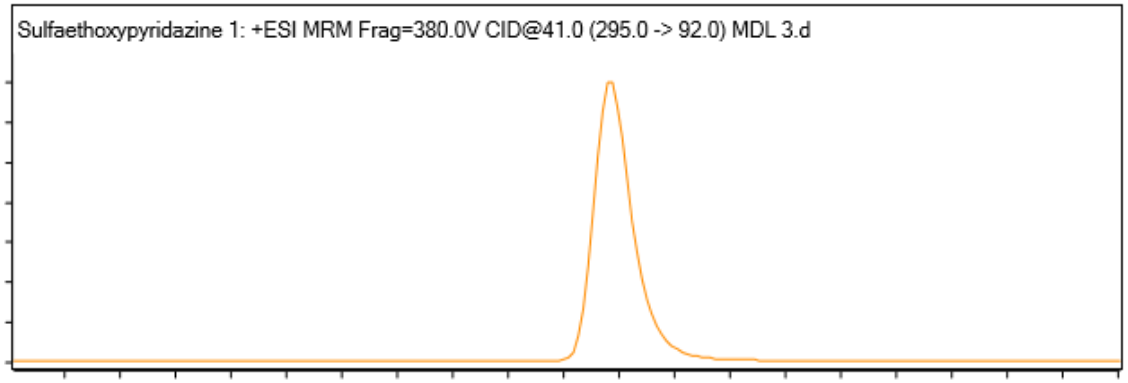

Oxytetracycline 1: +ESI MRM Frag=380.0V CID@17.0 (461.2 -> 426.1) MDL 3.d

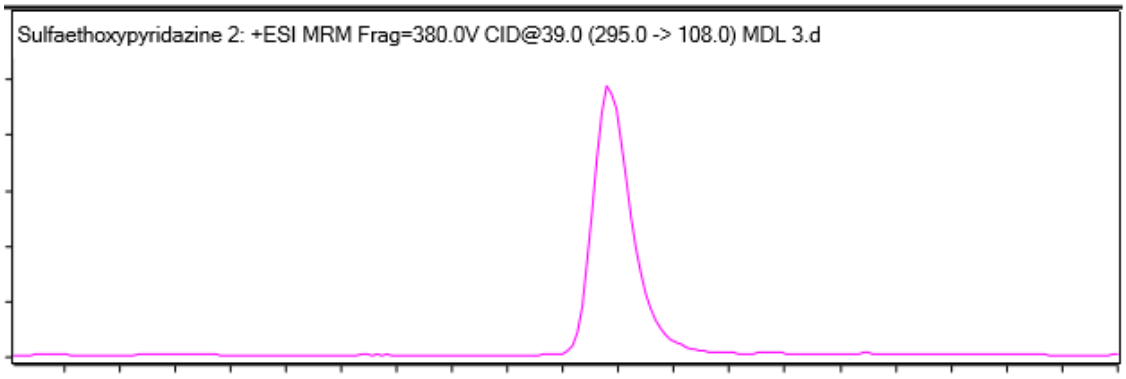

Oxytetracycline 2: +ESI MRM Frag=380.0V CID@9.0 (461.2 -> 443.2) MDL 3.d

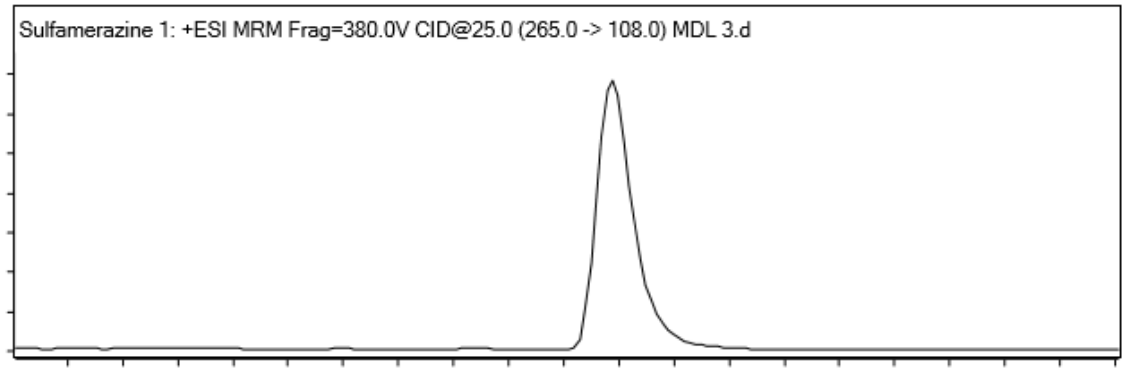

Sarafloxacin 1: +ESI MRM Frag=380.0V CID@16.0 (386.1 -> 342.3) MDL 3.d
Sarafloxacin 2: +ESI MRM Frag=380.0V CID@20.0 (386.1 -> 299.2) MDL 3.d
Sulfacetamide 1: +ESI MRM Frag=380.0V CID@25.0 (215.0 -> 92.1) MDL 3.d
Sulfacetamide 2: +ESI MRM Frag=380.0V CID@12.0 (215.0 -> 108.0) MDL 3.d

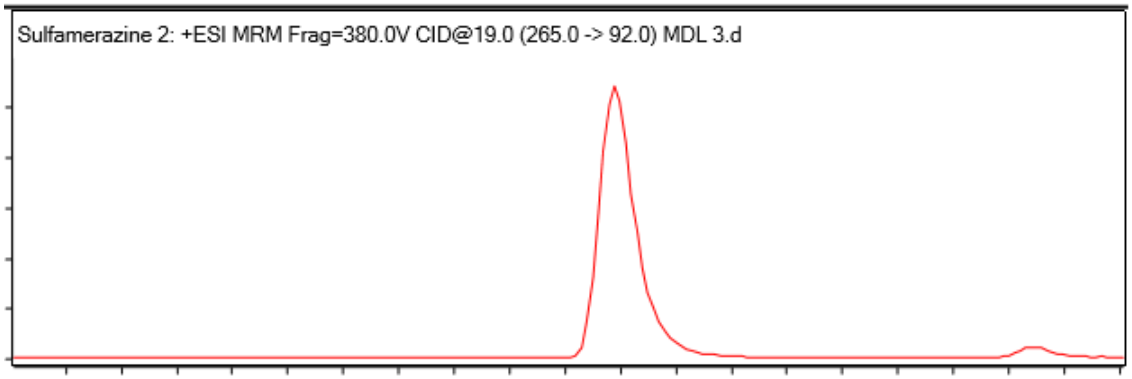

Sulfachloropyidine 1: +ESI MRM Frag=380.0V CID@35.0 (284.9 -> 92.1) MDL 3.d
Sulfachloropyidine 2: +ESI MRM Frag=380.0V CID@33.0 (284.9 -> 108.1) MDL 3.d
Sulfadiazine 1: +ESI MRM Frag=380.0V CID@20.0 (251.0 -> 92.1) MDL 3.d
Sulfadiazine 2: +ESI MRM Frag=380.0V CID@10.0 (251.0 -> 108.0) MDL 3.d

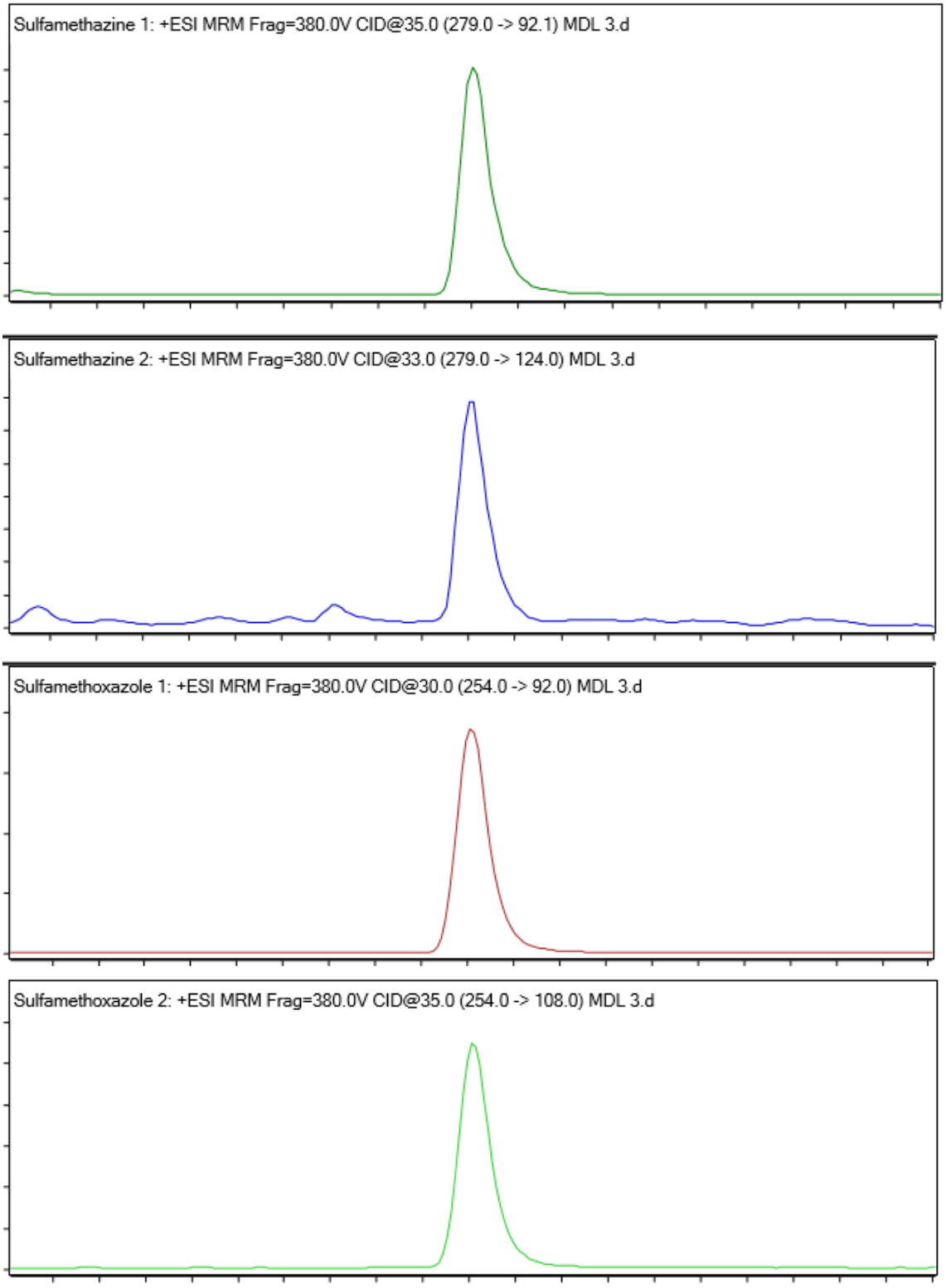

Sulfadimethoxine 1: +ESI MRM Frag=380.0V CID@27.0 (310.9 -> 156.0) MDL 3.d
Sulfadimethoxine 2: +ESI MRM Frag=380.0V CID@41.0 (310.9 -> 92.0) MDL 3.d
Sulfadoxine 1: +ESI MRM Frag=380.0V CID@35.0 (310.9 -> 92.0) MDL 3.d
Sulfadoxine 2: +ESI MRM Frag=380.0V CID@30.0 (310.9 -> 156.0) MDL 3.d

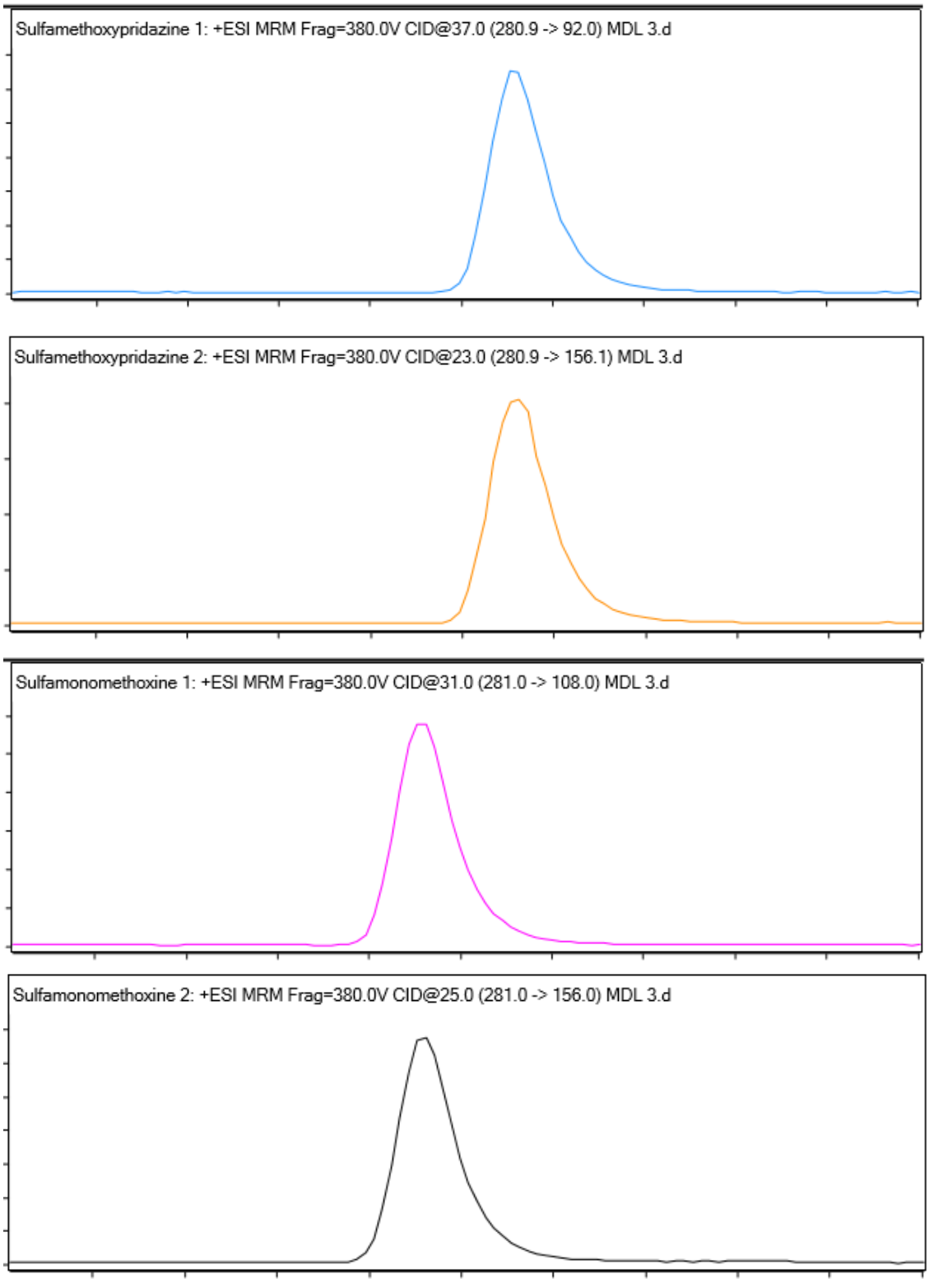

Sulfaethoxypyridazine 1: +ESI MRM Frag=380.0V CID@41.0 (295.0 -> 92.0) MDL 3.d

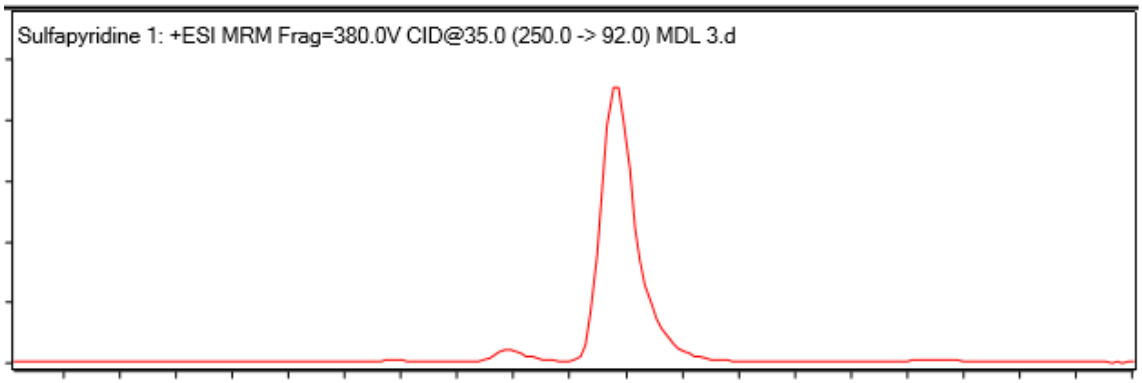

Sulfaethoxypyridazine 2: +ESI MRM Frag=380.0V CID@39.0 (295.0 -> 108.0) MDL 3.d

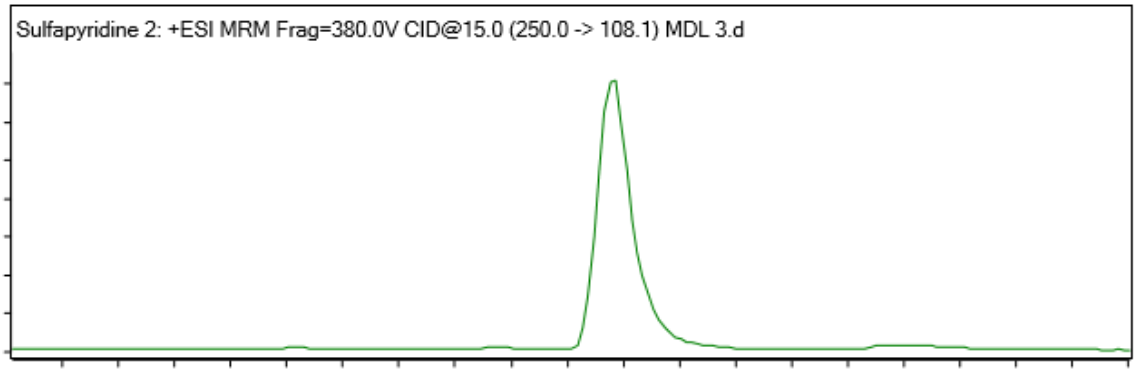

Sulfamerazine 1: +ESI MRM Frag=380.0V CID@25.0 (265.0 -> 108.0) MDL 3.d

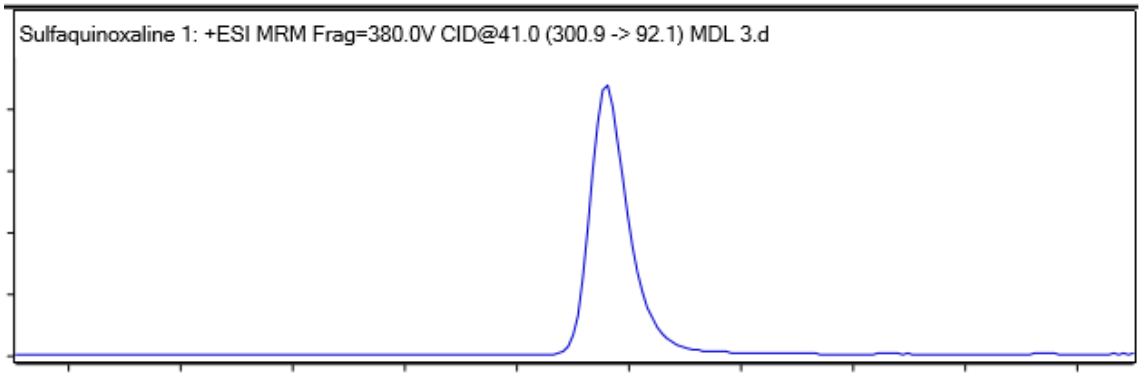

Sulfamerazine 2: +ESI MRM Frag=380.0V CID@19.0 (265.0 -> 92.0) MDL 3.d

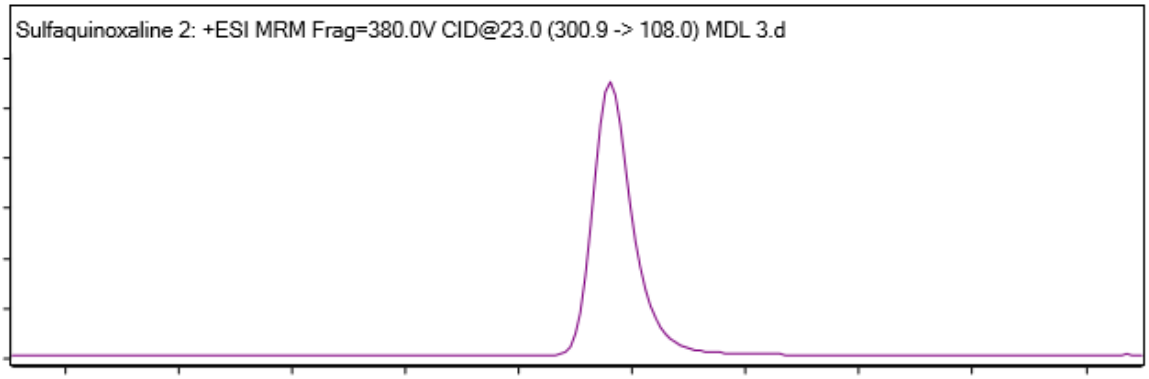

Sulfamethazine 1: +ESI MRM Frag=380.0V CID@35.0 (279.0 -> 92.1) MDL 3.d
Sulfamethazine 2: +ESI MRM Frag=380.0V CID@33.0 (279.0 -> 124.0) MDL 3.d
Sulfamethoxazole 1: +ESI MRM Frag=380.0V CID@30.0 (254.0 -> 92.0) MDL 3.d
Sulfamethoxazole 2: +ESI MRM Frag=380.0V CID@35.0 (254.0 -> 108.0) MDL 3.d

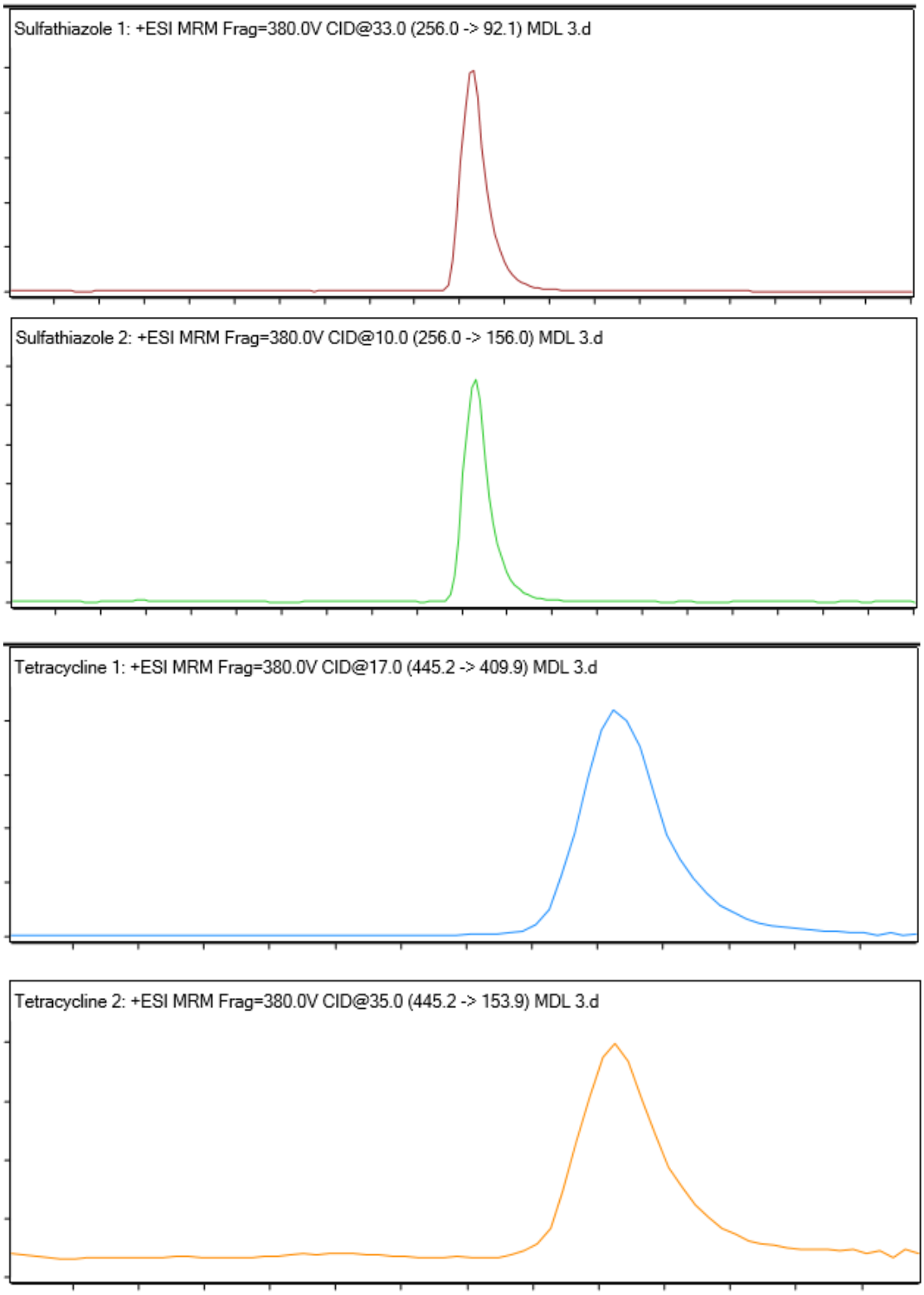

Sulfamethoxypridazine 1: +ESI MRM Frag=380.0V CID@37.0 (280.9 -> 92.0) MDL 3.d
Sulfamethoxypridazine 2: +ESI MRM Frag=380.0V CID@23.0 (280.9 -> 156.1) MDL 3.d
Sulfamonomethoxine 1: +ESI MRM Frag=380.0V CID@31.0 (281.0 -> 108.0) MDL 3.d
Sulfamonomethoxine 2: +ESI MRM Frag=380.0V CID@25.0 (281.0 -> 156.0) MDL 3.d

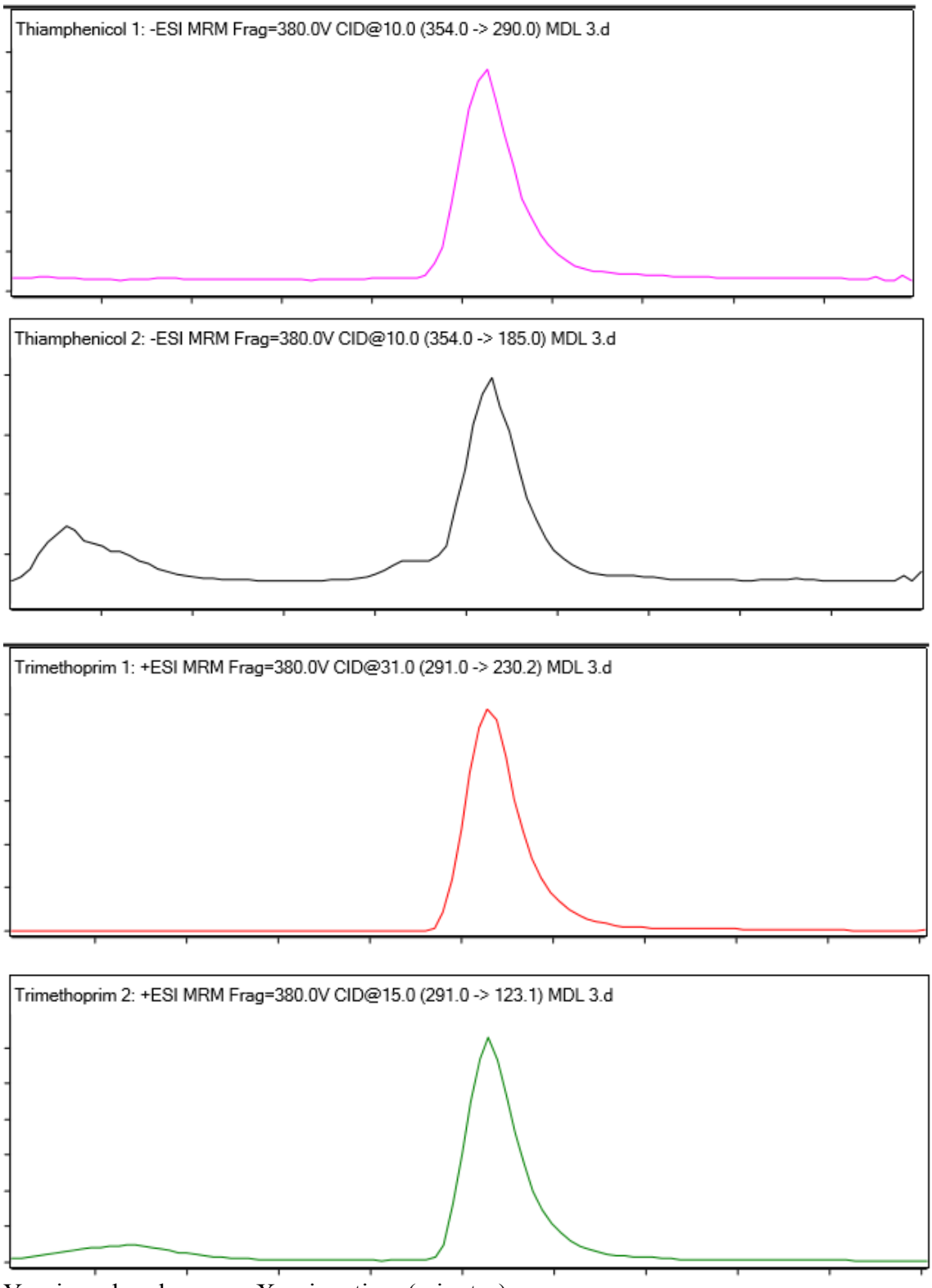

Sulfapyridine 1: +ESI MRM Frag=380.0V CID@35.0 (250.0 -> 92.0) MDL 3.d


Sulfapyridine 2: +ESI MRM Frag=380.0V CID@15.0 (250.0 -> 108.1) MDL 3.d


Sulfaquinoxaline 1: +ESI MRM Frag=380.0V CID@41.0 (300.9 -> 92.1) MDL 3.d


Sulfaquinoxaline 2: +ESI MRM Frag=380.0V CID@23.0 (300.9 -> 108.0) MDL 3.d


Sulfathiazole 1: +ESI MRM Frag=380.0V CID@33.0 (256.0 -> 92.1) MDL 3.d
Sulfathiazole 2: +ESI MRM Frag=380.0V CID@10.0 (256.0 -> 156.0) MDL 3.d
Tetracycline 1: +ESI MRM Frag=380.0V CID@17.0 (445.2 -> 409.9) MDL 3.d
Tetracycline 2: +ESI MRM Frag=380.0V CID@35.0 (445.2 -> 153.9) MDL 3.d



Y-axis = abundance vs. X-axis = time (minutes)