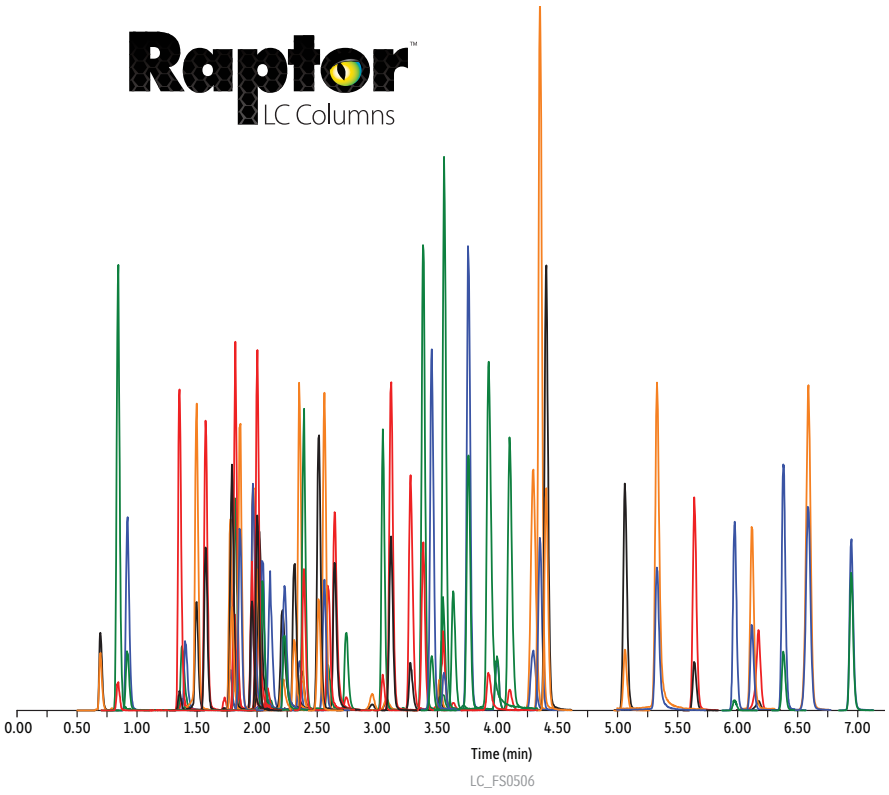
Featured Application: *Multiclass Veterinary Antibiotics on Raptor™ C18 by LC-MS/MS*

One Analysis, One Column, Less than 9 Minutes for Over 60 Multiclass Antibiotics

- Highly efficient peak separation and fast analysis times.
- Versatility and global applicability for antibiotic residue analysis—capable of individual class panel optimization for quantitation:
  - Macrolide, Lincosamide, and Streptogramin (Figure 1)
  - Amphenicol and Tetracycline (Figure 2)
  - Quinolone (Figure 3)
  - Penicillin, Cephalosporin, and Tetracycline (Figure 4)
  - Sulfonamide (Figure 5)

  (For Ionophore, use on Raptor™ Biphenyl. [Figure 6])

The use of antibiotics on food-producing animals is a public health and safety concern due to the potential of generating drug-resistant bacteria. Many countries in the European Union and Canada have banned the use of antibiotics for nontherapeutic purposes, and the United States is implementing a policy to reduce the use of medically important antibiotics for growth promotion. To regulate the proper use of veterinary antibiotics, the U.S. FDA has set maximum residue limits (MRL) for a variety of animal tissue and food products (21 CFR Part 556). A sensitive, efficient, and reliable analytical method for different classes of antibiotics is necessary to meet this regulation, and the Raptor™ C18 LC column is the ideal choice.



Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 35 °C; **Sample:** Diluent: Water; Conc.: 5–300 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0.00 min (10%), 4.50 min (35%), 7.00 min (55%), 7.01 min (10%); 9.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+/ESI-; Mode: Scheduled MRM; **Instrument:** UHPLC; **Notes:** 1. Positive and negative polarity data were collected simultaneously from a single injection. 2. Amphenicol compounds (chloramphenicol, thiamphenicol, and florfenicol) were detected with negative polarity. 3. The MRM was scheduled at +/- 20 to 30 seconds for each analyte. 4. Multiclass antibiotics include penicillin, cephalosporin, tetracycline, sulfonamide, macrolide, lincosamide, streptogramin, amphenicol, and quinolone. **The retention time for Tylosin is noted in the peak list; however, it was not included in the chromatogram.

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Desacetyl cephapirin	0.70	150	382.03	111.92	124.21
2. Sulfanilamide	0.85	200	172.98	93.07	75.23
3. Amoxicillin	0.92	100	366.24	349.10	208.07
4. Cephapirin	1.36	50	424.17	292.08	124.14
5. Tildipirosin	1.38	200	734.59	561.45	204.15
6. Desfuroyl ceftiofur cysteine disulfide	1.40	300	549.16	183.02	126.00
7. Lincomycin	1.50	50	407.32	359.23	389.28
8. Sulfadiazine	1.57	20	251.18	156.04	92.08
9. Cefquinome	1.73	200	529.19	134.10	125.12
10. Ampicillin	1.78	50	350.19	106.07	160.06
11. Sulfathiazole	1.79	10	256.16	156.03	92.08
12. Marbofloxacin	1.81	10	363.20	72.11	320.10
13. Cefalexin	1.82	100	348.10	158.05	174.05
14. Sulfapyridine	1.86	10	250.13	156.10	92.08
15. Norfloxacin	1.96	20	320.23	276.20	233.13
16. Ofloxacin	1.98	10	362.21	318.20	261.15
17. Sulfamerazine	2.00	20	265.08	156.03	92.08
18. Cefalonium	2.01	100	459.16	337.03	123.10
19. Oxytetracycline	2.02	25	461.27	426.15	443.32
20. Ciprofloxacin	2.04	20	332.18	288.22	245.15
21. Cefacetrile	2.09	300	362.07	258.08	178.01
22. Tulathromycin A	2.11	100	806.65	577.42	420.31
23. Tetracycline	2.21	25	445.28	154.07	427.32
24. Danofloxacin	2.23	20	358.22	340.16	314.21
25. Enrofloxacin	2.32	10	360.29	316.22	245.13
26. Orbifloxacin	2.35	10	396.22	352.17	226.12
27. Thiamphenicol*	2.38	200	354.16	290.04	184.98
28. Sulfamethazine	2.39	10	279.23	186.08	124.08
29. Sulfamethizole	2.52	10	271.17	156.02	108.02
30. Sulfamethoxypyridazine	2.56	10	281.14	156.03	126.07

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
31. Sarafloxacin	2.59	10	386.20	342.20	368.15
32. Difloxacin	2.65	10	400.23	356.17	299.13
33. Cefazolin	2.75	100	455.10	323.06	295.09
34. Spiramycin	2.96	200	843.64	540.36	699.48
35. Pirlimycin	3.05	20	411.32	363.18	327.21
36. Chlortetracycline	3.08	25	479.27	154.07	371.06
37. Sulfachlorpyridazine	3.12	20	285.05	156.03	108.09
38. Gamithromycin	3.28	100	777.63	619.52	601.45
39. Sulfadoxine	3.39	10	311.17	156.03	108.09
40. Sulfamethoxazole	3.46	20	254.18	155.98	147.06
41. Cefoperazone	3.52	100	646.26	143.07	148.02
42. Florfenicol*	3.55	200	356.10	336.02	184.98
43. Sulfaethoxypyridazine	3.56	20	295.17	267.07	156.03
44. Tilmicosin	3.64	100	869.72	696.50	522.42
45. Sulfisoxazole	3.76	20	268.14	156.03	113.10
46. Oxolinic acid	3.94	5	262.10	244.06	215.96
47. Chloramphenicol*	4.00	200	321.16	151.99	257.04
48. Ceftiofur	4.11	50	524.14	241.08	125.24
49. Erythromycin	4.31	25	734.64	576.40	558.38
50. Sulfadimethoxine	4.36	10	311.17	156.09	108.09
51. Sulfaquinoxaline	4.42	20	301.18	156.04	108.02
52. Tylosin**	4.67	100	916.62	772.49	598.36
53. Penicillin G	5.07	100	335.18	176.07	160.07
54. Flumequine	5.34	5	262.15	244.11	202.03
55. Penicillin V	5.56	100	351.10	160.06	114.07
56. Oxacillin	5.99	100	402.15	160.05	114.06
57. Virginiamycin M1	6.13	50	526.43	508.31	355.10
58. Tylvalosin	6.19	50	1042.71	814.46	640.39
59. Cloxacillin	6.39	100	436.15	277.06	160.05
60. Nafcillin	6.60	25	415.19	199.09	171.06
61. Dicloxacillin	6.96	100	470.11	160.05	311.02

*Acquired in negative ion mode

Figure 1: Macrolide, Lincosamide, and Streptogramin Antibiotics on Raptor™ C18 by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Tildipirosin	1.01	200	734.59	561.45	204.15
2. Lincomycin	1.11	50	407.32	359.23	389.28
3. Tulathromycin A	1.19	100	806.65	577.42	420.31
4. Spiramycin	1.41	200	843.64	540.36	699.48
5. Pirlymycin	1.49	20	411.32	363.18	327.21
6. Gamithromycin	1.50	100	777.63	619.52	601.45
7. Tilmicosin	1.58	100	869.72	696.50	522.42
8. Erythromycin	1.80	25	734.64	576.40	558.38
9. Tylosin	1.87	100	916.62	772.49	598.36
10. Tylvalosin	2.30	50	1042.71	814.46	640.39
11. Virginiamycin M1	2.45	50	526.43	508.31	355.10

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 20–200 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0.00 min (10%), 3.00 min (80%), 3.01 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC

LC_FS0502

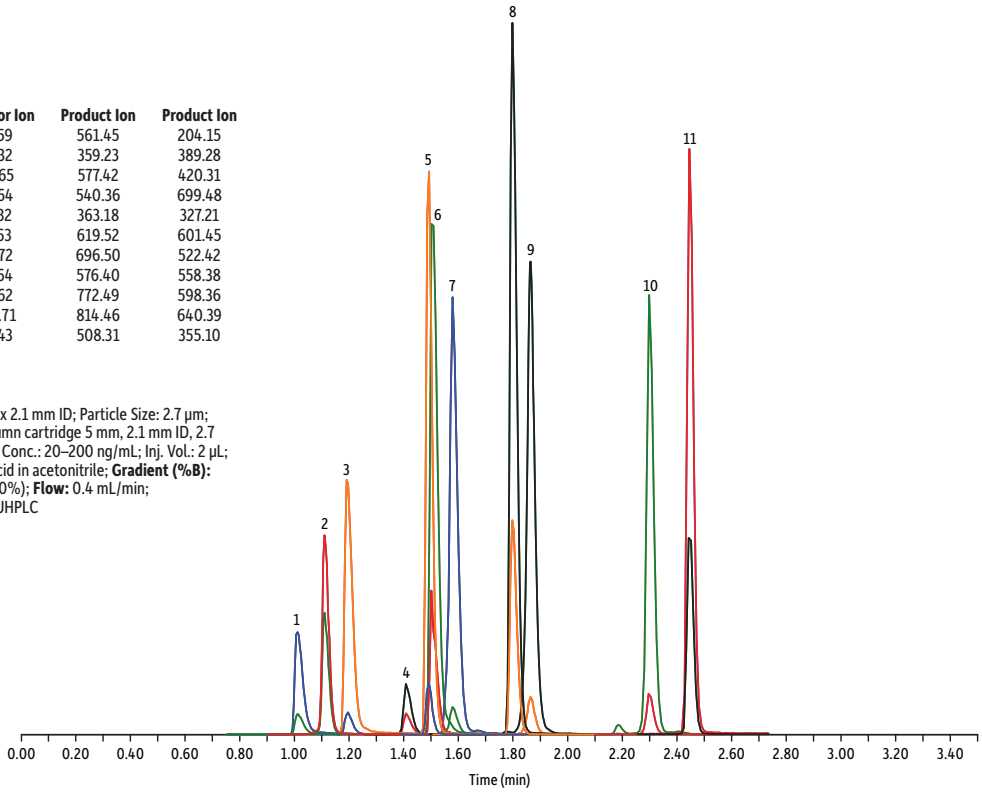


Figure 2: Amphenicol and Tetracycline Antibiotics on Raptor™ C18 by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Oxytetracycline	1.28	25	461.27	426.15	443.32
2. Tetracycline	1.34	25	445.28	154.07	427.32
3. Thiamphenicol*	1.48	200	354.16	290.04	184.98
4. Chlortetracycline	1.56	25	479.27	154.07	371.06
5. Florfenicol*	1.86	200	356.10	336.02	184.98
6. Chloramphenicol*	1.95	200	321.16	151.99	257.04

*Acquired in negative ion mode.

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 25–200 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B)** 0.00 min (10%), 3.00 min (80%), 3.01 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+/ESI-; Mode: MRM; **Instrument:** UHPLC; **Notes:** Tetracyclines and amphenicols were analyzed with ESI+ and ESI- mode, respectively.

LC_FS0504

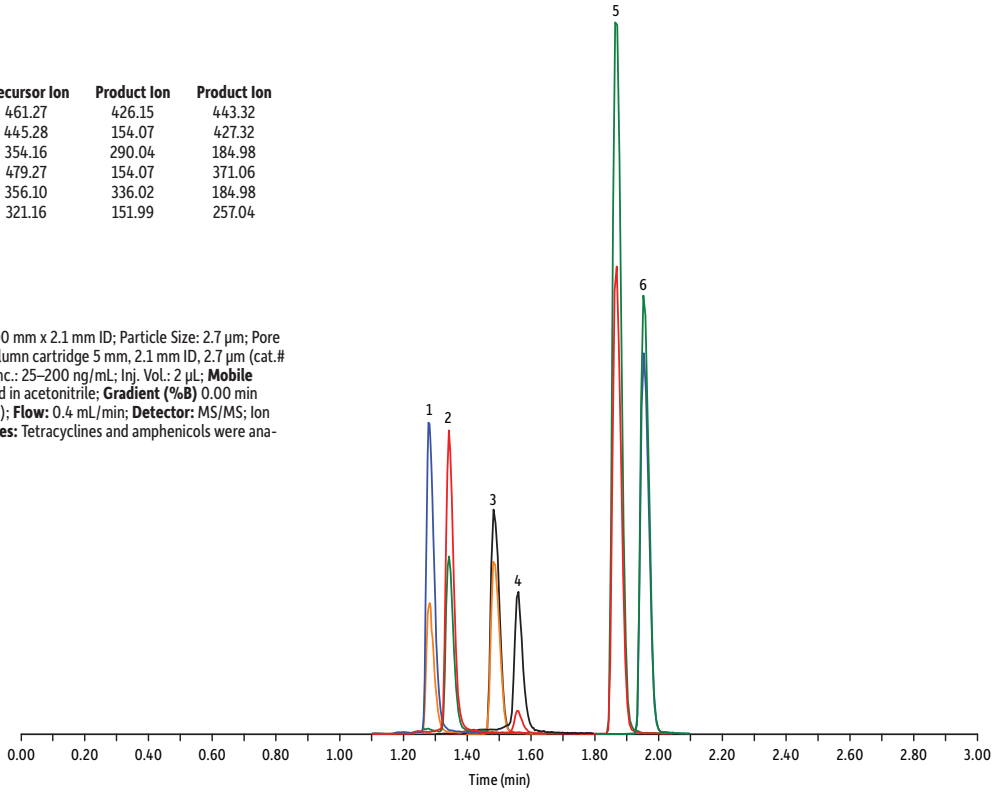


Figure 3: Quinolone Antibiotics on Raptor™ C18 by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Marbofloxacin	1.28	10	363.20	72.11	320.10
2. Norfloxacin	1.32	20	320.23	276.20	233.13
3. Ofloxacin	1.32	10	362.21	318.20	261.15
4. Ciprofloxacin	1.35	20	332.18	288.22	245.15
5. Danofloxacin	1.40	20	358.22	340.16	314.21
6. Enrofloxacin	1.44	10	360.29	316.22	245.13
7. Orbifloxacin	1.47	10	396.22	352.17	226.12
8. Sarafloxacin	1.55	10	386.20	342.20	368.15
9. Difloxacin	1.57	10	400.23	356.17	299.13
10. Oxolinic acid	2.23	5	262.10	244.06	215.96
11. Flumequine	2.78	5	262.15	244.11	202.03

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 5–20 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0.00 min (10%), 3.00 min (60%), 3.01 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC

LC_FS0505

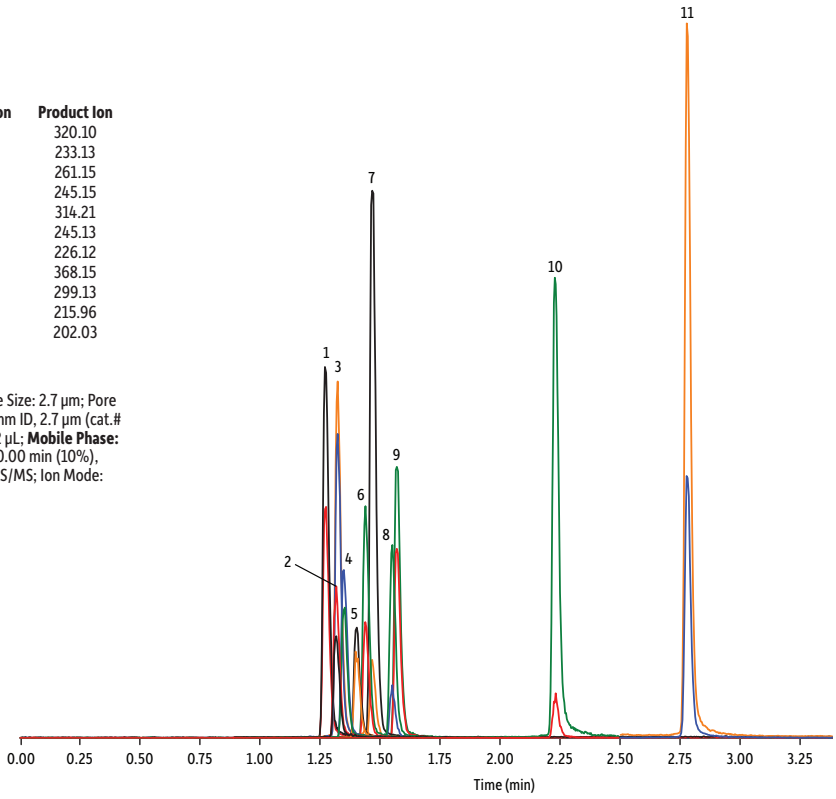


Figure 4: Penicillin, Cephalosporin, and Tetracycline Antibiotics on Raptor ™ C18 by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Desacetyl cephapirin	0.68	150	382.03	111.92	124.21
2. Amoxicillin	0.89	100	366.24	349.10	208.07
3. Cephapirin	1.05	50	424.17	292.08	124.14
4. Desfuroyl ceftiofur cysteine disulfide	1.06	300	549.16	183.02	126.00
5. Cefquinome	1.16	200	529.19	134.10	125.12
6. Ampicillin	1.18	50	350.19	106.07	160.06
7. Cefalexin	1.19	100	348.10	158.05	174.05
8. Oxytetracycline	1.26	50	461.27	426.15	443.32
9. Cefalonium	1.29	100	459.16	337.03	123.10
10. Tetracycline	1.32	50	445.28	154.07	427.32
11. Cefacetrile	1.37	300	362.07	258.08	178.01
12. Cefazolin	1.50	100	455.10	323.06	295.09
13. Chlortetracycline	1.54	50	479.27	154.07	371.06
14. Cefoperazone	1.69	100	646.26	143.07	148.02
15. Ceftiofur	1.85	50	524.14	241.08	125.24
16. Penicillin G	2.18	100	335.18	176.07	160.07
17. Penicillin V	2.33	100	351.10	160.06	114.07
18. Oxacillin	2.44	100	402.15	160.05	114.06
19. Cloxacillin	2.56	100	436.15	277.06	160.05
20. Nafcillin	2.63	25	415.19	199.09	171.06
21. Dicloxacillin	2.76	100	470.11	160.05	311.02

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 25–300 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0.00 min (10%), 3.00 min (80%), 3.01 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC.

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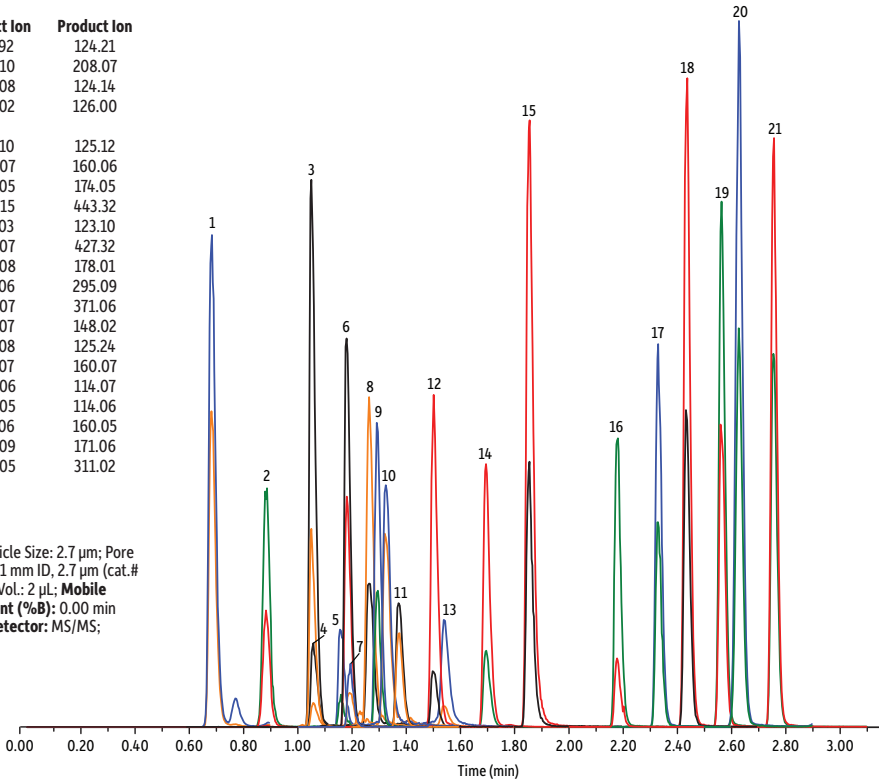


Figure 5: Sulfonamide Antibiotics on Raptor™ C18 by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Sulfanilamide	0.83	200	172.98	93.07	75.23
2. Sulfadiazine	1.45	20	251.18	156.04	92.08
3. Sulfathiazole	1.60	10	256.16	156.03	92.08
4. Sulfapyridine	1.67	10	250.13	156.10	92.08
5. Sulfamerazine	1.79	20	265.08	156.03	92.08
6. Sulfamethazine	2.07	10	279.23	186.08	124.08
7. Sulfamethizole	2.11	10	271.17	156.02	108.02
8. Sulfamethoxypyridazine	2.16	10	281.14	156.03	126.07
9. Sulfachlorpyridazine	2.55	20	285.05	156.03	108.09
10. Sulfadoxine	2.75	10	311.17	156.03	108.09
11. Sulfamethoxazole	2.78	20	254.18	155.98	147.06
12. Sulfaethoxypyridazine	2.84	20	295.17	267.07	156.03
13. Sulfisoxazole	2.98	20	268.14	156.03	113.10
14. Sulfadimethoxine	3.37	10	311.17	156.09	108.09
15. Sulfaquinoxaline	3.40	20	301.18	156.04	108.02

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 10–200 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B)** 0.00 min (10%), 3.50 min (40%), 3.51 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC

LC_FS0501

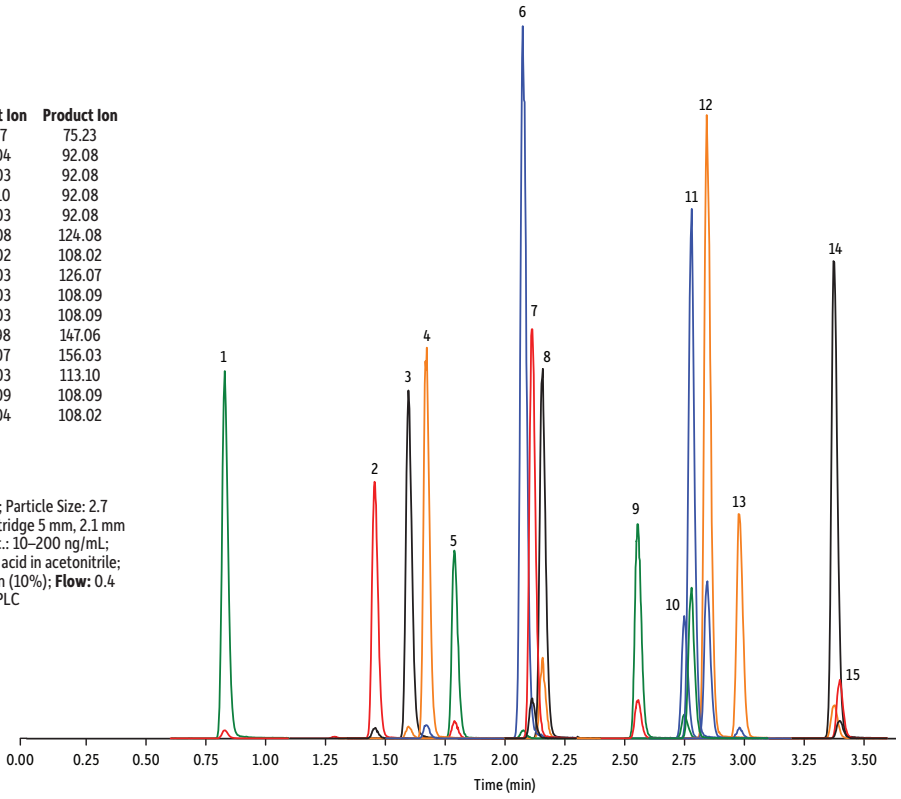
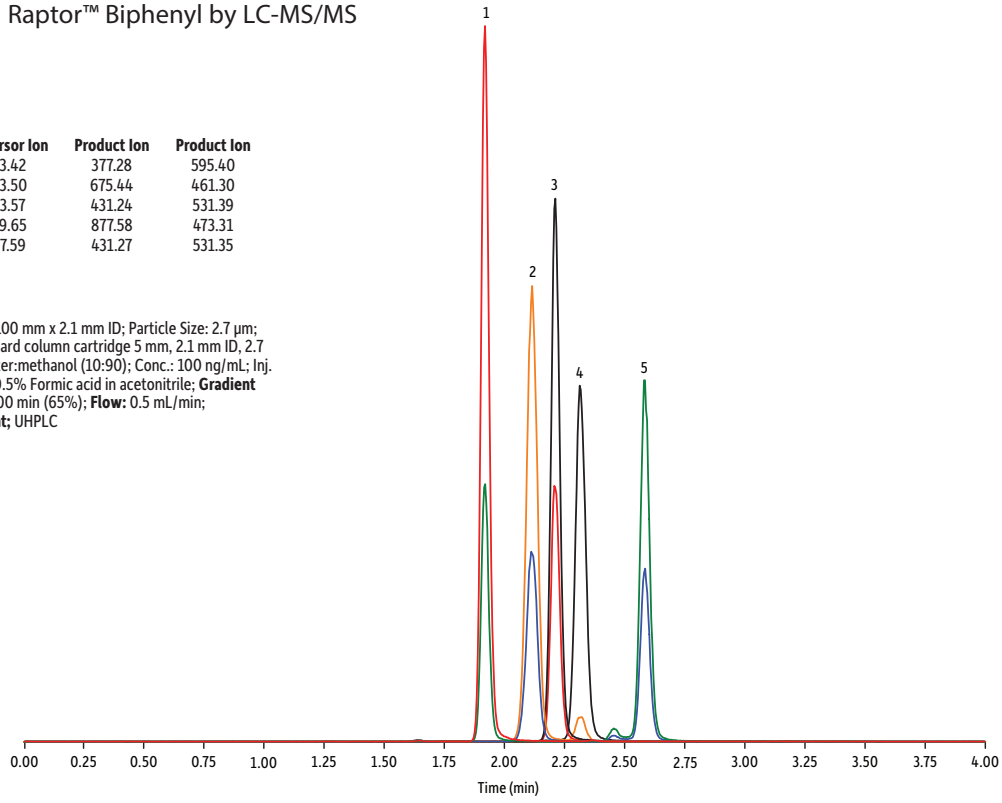


Figure 6: Ionophore Antibiotics on Raptor™ Biphenyl by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Lasalocid A	1.92	100	613.42	377.28	595.40
2. Monensin	2.12	100	693.50	675.44	461.30
3. Salinomycin	2.19	100	773.57	431.24	531.39
4. Maduramicin	2.30	100	939.65	877.58	473.31
5. Narasin	2.58	100	787.59	431.27	531.35

Column: Raptor™ Biphenyl (cat.# 9309A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252); Temp.: 40 °C; **Sample:** Diluent: Water:methanol (10:90); Conc.: 100 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.5% Formic acid in water; B: 0.5% Formic acid in acetonitrile; **Gradient (%B)** 0.00 min (65%), 3.00 min (75%), 3.01 min (65%), 5.00 min (65%); **Flow:** 0.5 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument;** UHPLC

LC_FS0503



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