



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

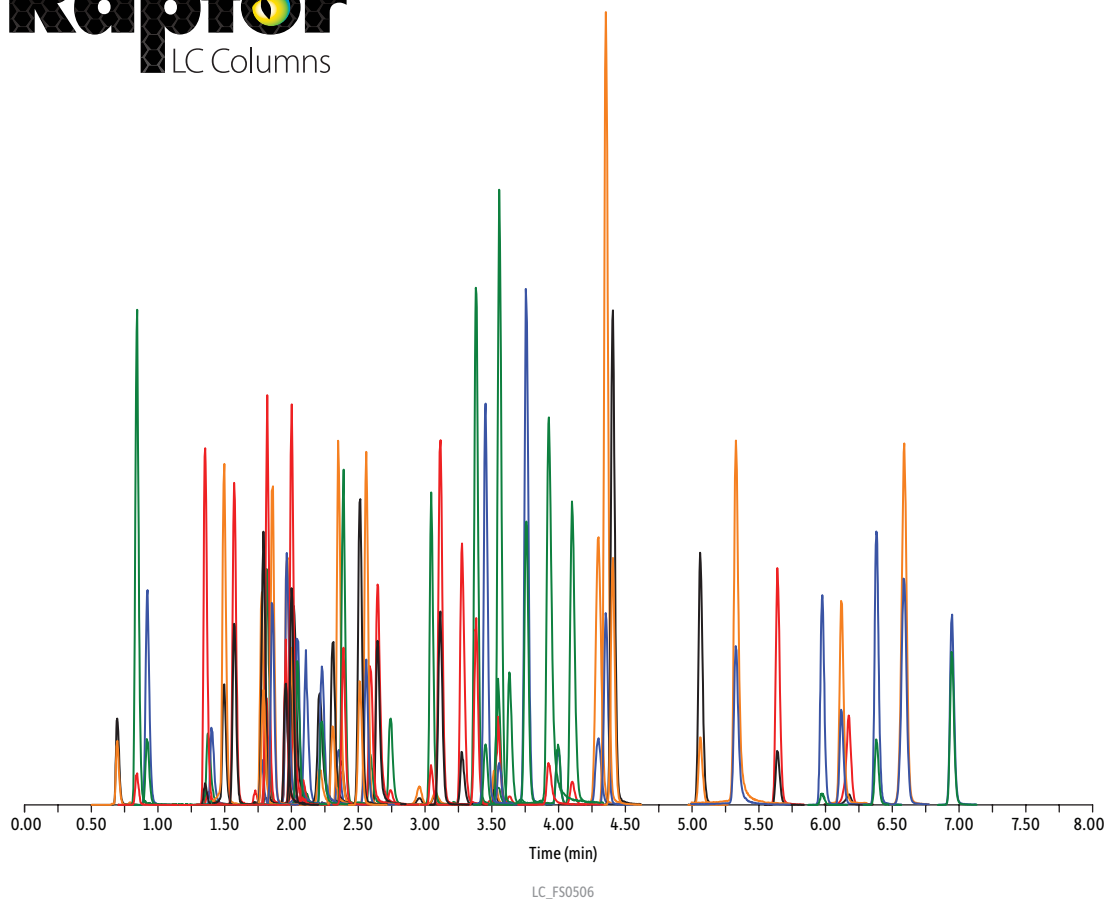
- Highly efficient peak separation and fast analysis times.
- Versatility and global applicability for antibiotic residue analysis—capable of individual class panel optimization for quantitation:
 - Macrolide, Lincosamide, and Streptogramin (Figure 1)
 - Amphenicol and Tetracycline (Figure 2)
 - Quinolone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

Peaks	tx (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion	Peaks	tx (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Desacetyl cephalirin	0.70	150	382.03	111.92	124.21	31. Sarafloxacin	2.59	10	386.20	342.20	368.15
2. Sulfanilamide	0.85	200	172.98	93.07	75.23	32. Difloxacin	2.65	10	400.23	356.17	299.13
3. Amoxicillin	0.92	100	366.24	349.10	208.07	33. Cefazolin	2.75	100	455.10	323.06	295.09
4. Cephalirin	1.36	50	424.17	292.08	124.14	34. Spiramycin	2.96	200	843.64	540.36	699.48
5. Tildipirosin	1.38	200	734.59	561.45	204.15	35. Pirlimycin	3.05	20	411.32	363.18	327.21
6. Desfuroyl ceftiofur cysteine disulfide	1.40	300	549.16	183.02	126.00	36. Chlortetracycline	3.08	25	479.27	154.07	371.06
7. Lincomycin	1.50	50	407.32	359.23	389.28	37. Sulfachlorpyridazine	3.12	20	285.05	156.03	108.09
8. Sulfadiazine	1.57	20	251.18	156.04	92.08	38. Gamithromycin	3.28	100	777.63	619.52	601.45
9. Cefquinome	1.73	200	529.19	134.10	125.12	39. Sulfadoxine	3.39	10	311.17	156.03	108.09
10. Ampicillin	1.78	50	350.19	106.07	160.06	40. Sulfamethoxazole	3.46	20	254.18	155.98	147.06
11. Sulfathiazole	1.79	10	256.16	156.03	92.08	41. Cefoperazone	3.52	100	646.26	143.07	148.02
12. Marbofloxacin	1.81	10	363.20	72.11	320.10	42. Florfenicol*	3.55	200	356.10	336.02	184.98
13. Cefalexin	1.82	100	348.10	158.05	174.05	43. Sulfaethoxypyridazine	3.56	20	295.17	267.07	156.03
14. Sulfapyridine	1.86	10	250.13	156.10	92.08	44. Tilmicosin	3.64	100	869.72	696.50	522.42
15. Norfloxacin	1.96	20	320.23	276.20	233.13	45. Sulfisoxazole	3.76	20	268.14	156.03	113.10
16. Ofloxacin	1.98	10	362.21	318.20	261.15	46. Oxolinic acid	3.94	5	262.10	244.06	215.96
17. Sulfamerazine	2.00	20	265.08	156.03	92.08	47. Chloramphenicol*	4.00	200	321.16	151.99	257.04
18. Cefalonium	2.01	100	459.16	337.03	123.10	48. Ceftiofur	4.11	50	524.14	241.08	125.24
19. Oxytetracycline	2.02	25	461.27	426.15	443.32	49. Erythromycin	4.31	25	734.64	576.40	558.38
20. Ciprofloxacin	2.04	20	332.18	288.22	245.15	50. Sulfadimethoxine	4.36	10	311.17	156.09	108.09
21. Cefacetrile	2.09	300	362.07	258.08	178.01	51. Sulfaquinolaxaline	4.42	20	301.18	156.04	108.02
22. Tulathromycin A	2.11	100	806.65	577.42	420.31	52. Tylosin**	4.67	100	916.62	772.49	598.36
23. Tetracycline	2.21	25	445.28	154.07	427.32	53. Penicillin G	5.07	100	335.18	176.07	160.07
24. Danofloxacin	2.23	20	358.22	340.16	314.21	54. Flumequine	5.34	5	262.15	244.11	202.03
25. Enrofloxacin	2.32	10	360.29	316.22	245.13	55. Penicillin V	5.56	100	351.10	160.06	114.07
26. Orbifloxacin	2.35	10	396.22	352.17	226.12	56. Oxacillin	5.99	100	402.15	160.05	114.06
27. Thiamphenicol*	2.38	200	354.16	290.04	184.98	57. Virginiamycin M1	6.13	50	526.43	508.31	355.10
28. Sulfamethazine	2.39	10	279.23	186.08	124.08	58. Tylosin	6.19	50	1042.71	814.46	640.39
29. Sulfamethizole	2.52	10	271.17	156.02	108.02	59. Cloxacillin	6.39	100	436.15	277.06	160.05
30. Sulfamethoxypropyridazine	2.56	10	281.14	156.03	126.07	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

See chromatogram and conditions on page 2



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

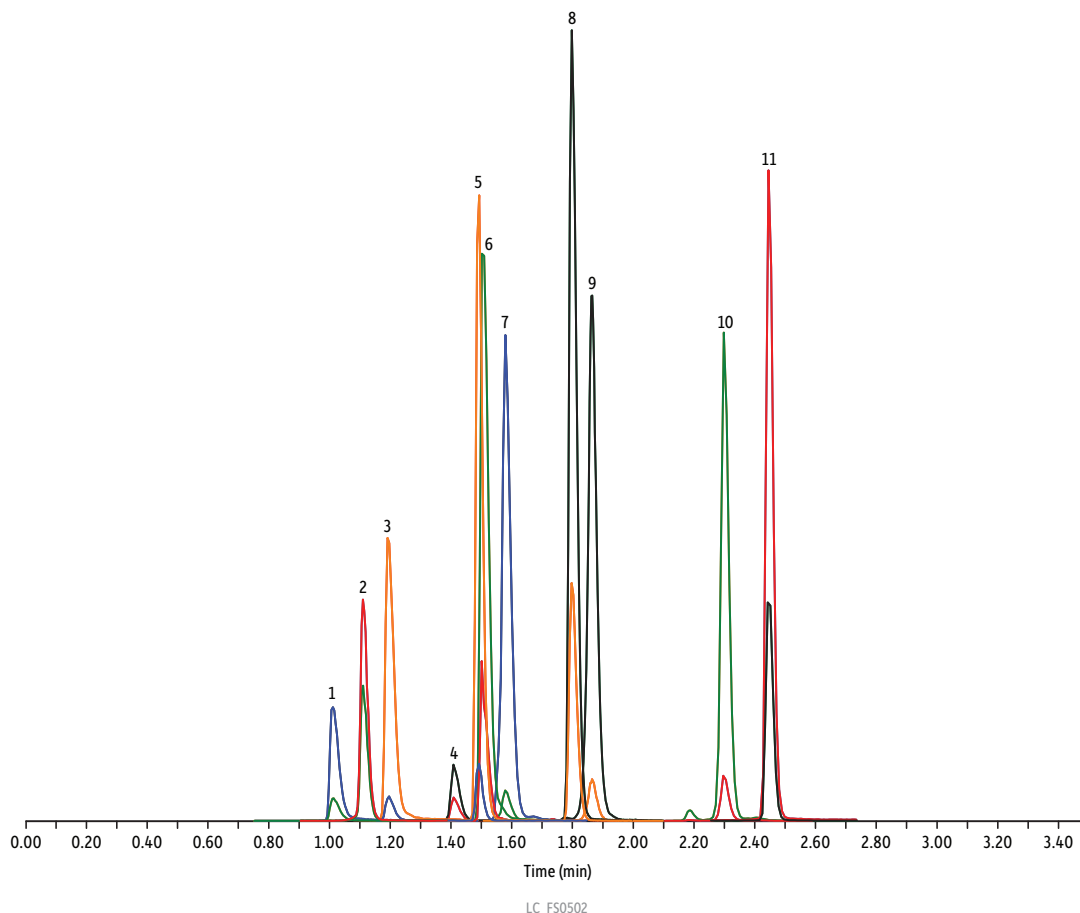
Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	90	10
4.50	0.4	65	35
7.00	0.4	45	55
7.01	0.4	90	10
9.00	0.4	90	10

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.

Want even better performance when analyzing metal-sensitive compounds? Check out Inert LC columns at www.restek.com/inert.

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

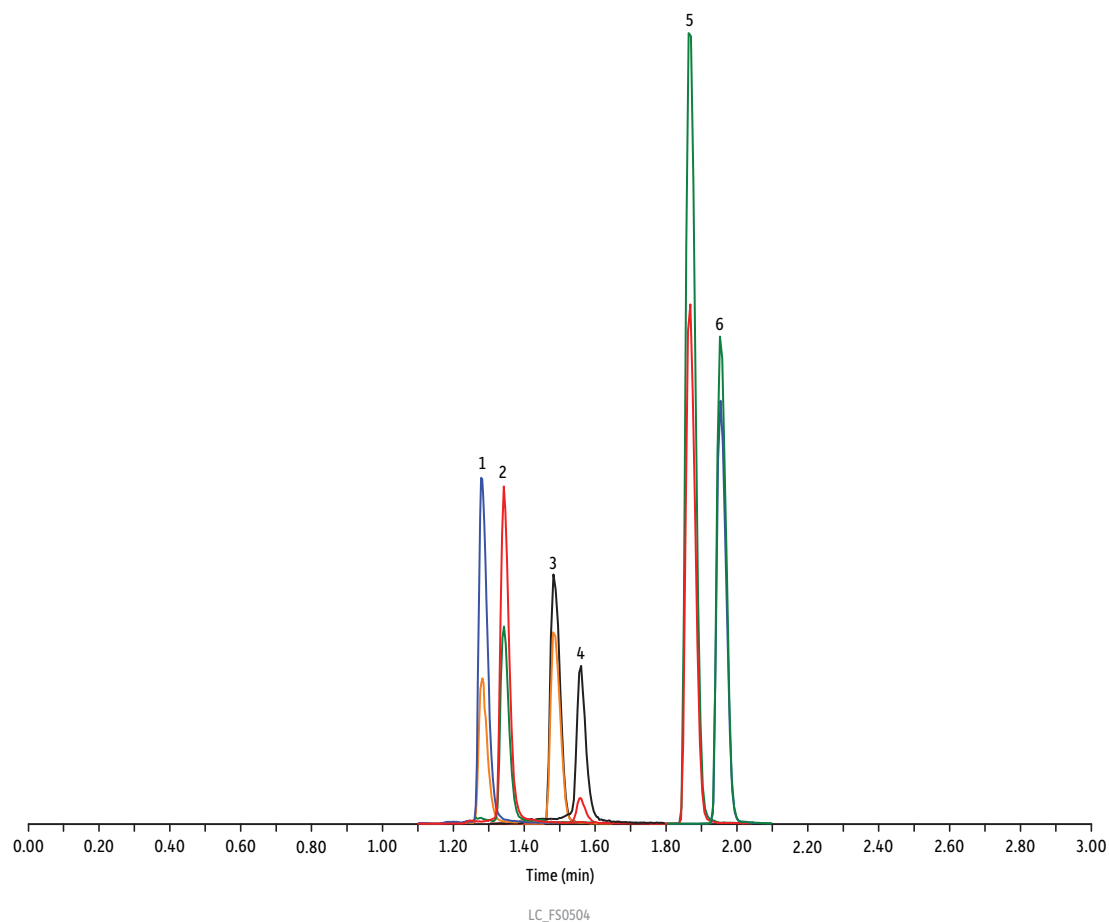


Peaks	ts (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion	Column
1. Tildipirosin	1.01	200	734.59	561.45	204.15	Raptor C18 (cat.# 9304A12)
2. Lincomycin	1.11	50	407.32	359.23	389.28	Dimensions: 100 mm x 2.1 mm ID
3. Tulathromycin A	1.19	100	806.65	577.42	420.31	Particle Size: 2.7 µm
4. Spiramycin	1.41	200	843.64	540.36	699.48	Pore Size: 90 Å
5. Pirlmycin	1.49	20	411.32	363.18	327.21	Guard Column: Raptor C18 EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252)
6. Gamithromycin	1.50	100	777.63	619.52	601.45	Temp.: 40 °C
7. Tilimicosin	1.58	100	869.72	696.50	522.42	Sample
8. Erythromycin	1.80	25	734.64	576.40	558.38	Diluent: Water
9. Tylosin	1.87	100	916.62	772.49	598.36	Conc.: 20–200 ng/mL
10. Tylvalosin	2.30	50	1042.71	814.46	640.39	Inj. Vol.: 2 µL
11. Virginiamycin M1	2.45	50	526.43	508.31	355.10	Mobile Phase
						A: 0.1% Formic acid in water
						B: 0.1% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	90	10
3.00	0.4	20	80
3.01	0.4	90	10
5.00	0.4	90	10

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC
Notes Want even better performance when analyzing metal-sensitive compounds? Check out Inert LC columns at www.restek.com/inert.

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



Peaks	tr (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.

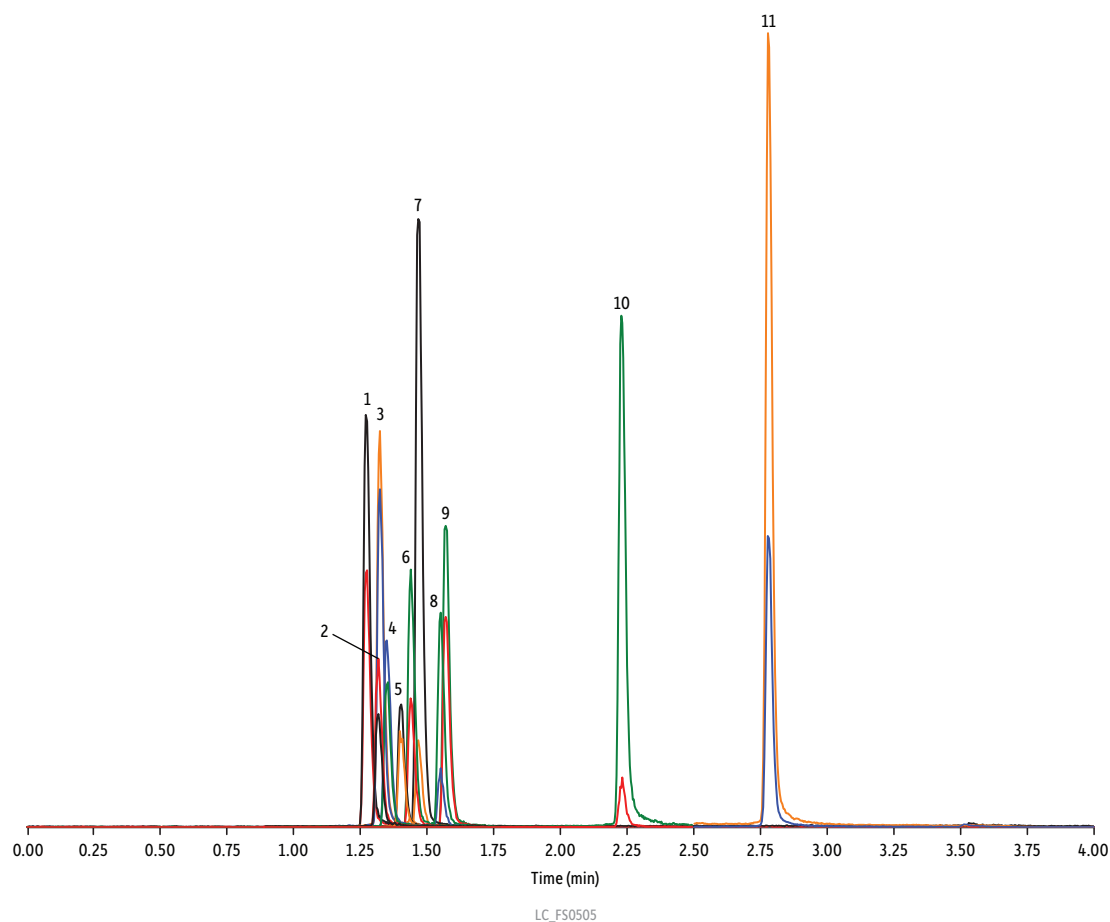
Column	Raptor C18 (cat.# 9304A12)
Dimensions:	100 mm x 2.1 mm ID
Particle Size:	2.7 µm
Pore Size:	90 Å
Guard Column:	Raptor C18 EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252)
Temp.:	40 °C
Sample	
Diluent:	Water
Conc.:	25–200 ng/mL
Inj. Vol.:	2 µL
Mobile Phase	
A:	0.1% Formic acid in water
B:	0.1% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	90	10
3.00	0.4	20	80
3.01	0.4	90	10
5.00	0.4	90	10

Detector	MS/MS
Ion Mode:	ESI+/ESI-
Mode:	MRM
Instrument	UHPLC
Notes	Tetracyclines and amphenicols were analyzed with ESI+ and ESI- mode, respectively.

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



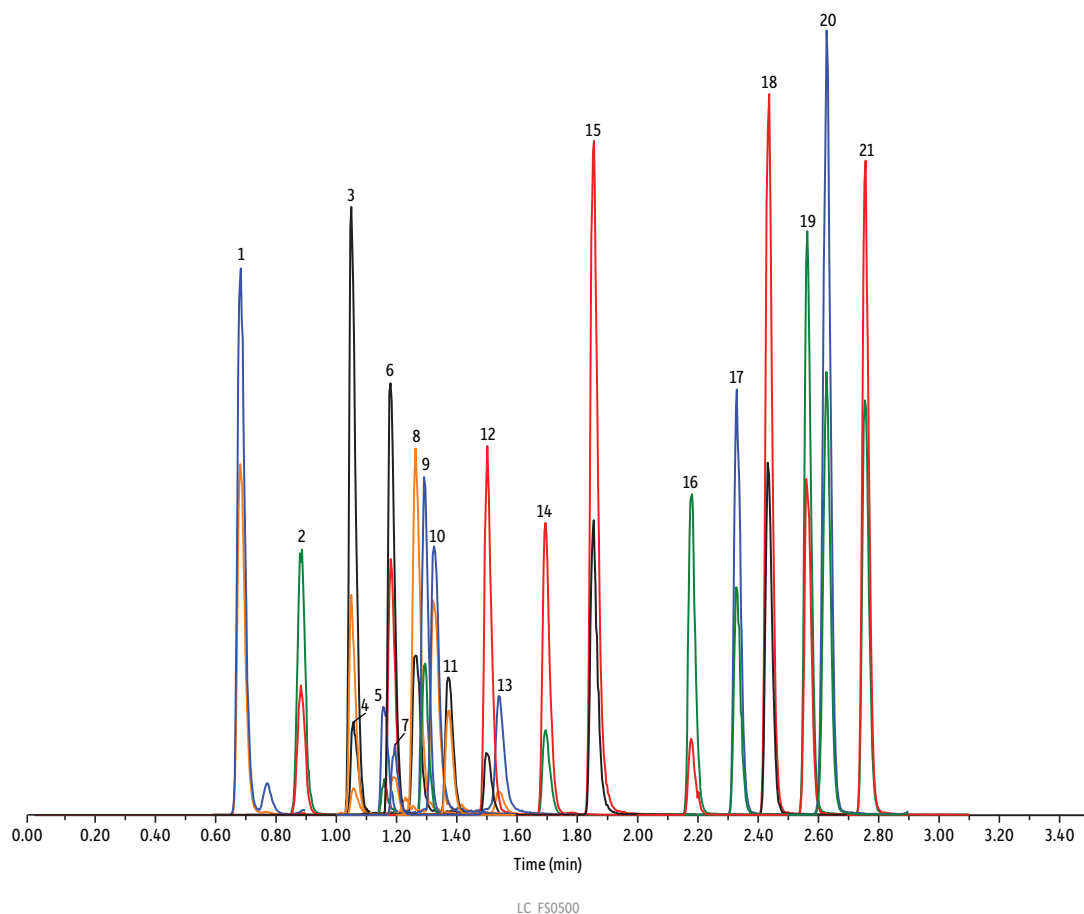
Peaks	tr (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Marbofloxacin	1.28	10	363.20	72.11	320.10
2. Norfloxacin	1.32	20	320.23	276.20	233.13
3. Ofloxacin	1.32	10	362.21	318.20	261.15
4. Ciprofloxacin	1.35	20	332.18	288.22	245.15
5. Danofloxacin	1.40	20	358.22	340.16	314.21
6. Enrofloxacin	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

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	90	10
3.00	0.4	40	60
3.01	0.4	90	10
5.00	0.4	90	10

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC
Notes Want even better performance when analyzing metal-sensitive compounds? Check out Inert LC columns at www.restek.com/inert.

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



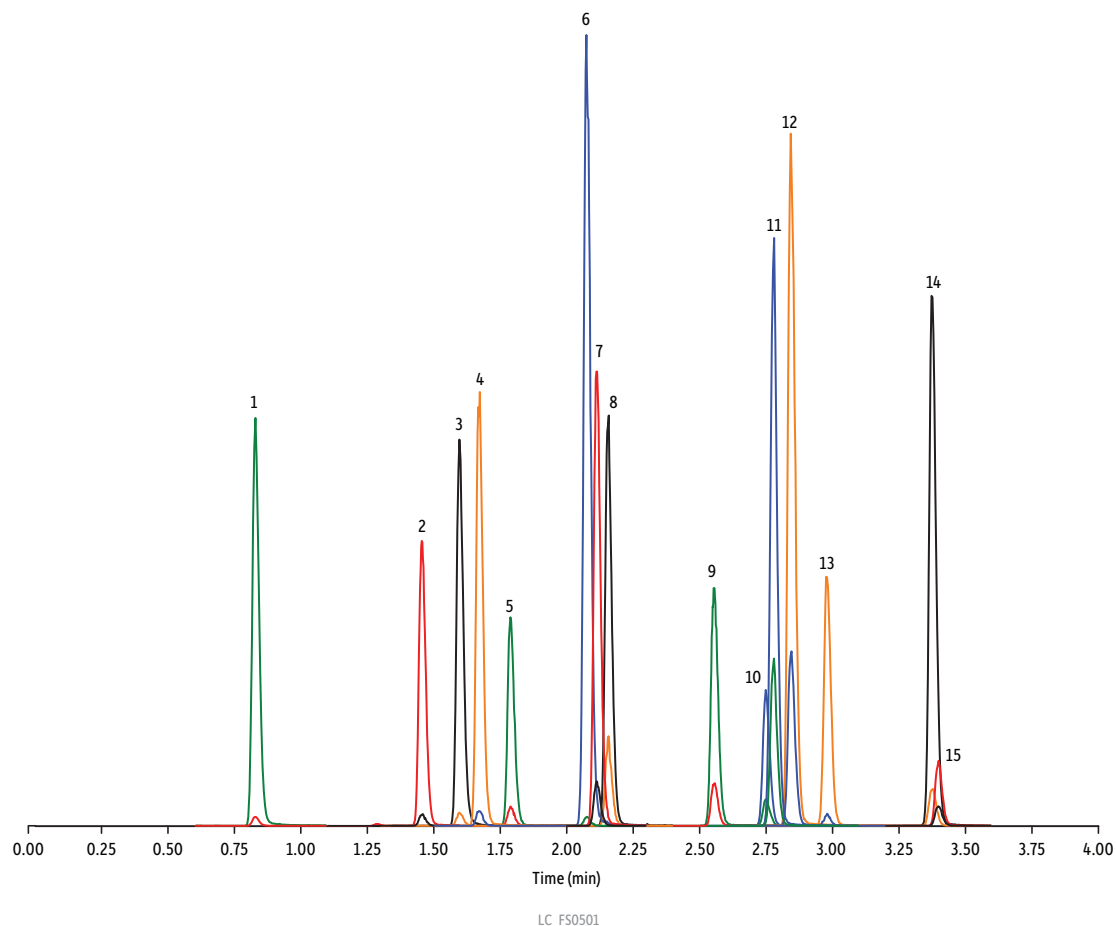
Peaks	ts (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Desacetyl cephalirin	0.68	150	382.03	111.92	124.21
2. Amoxicillin	0.89	100	366.24	349.10	208.07
3. Cephalirin	1.05	50	424.17	292.08	124.14
4. Desfuroyl ceftiofur cysteine disulfide	1.06	300	549.16	183.02	126.00
5. Cefquinome	1.16	200	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
Dimensions: Raptor C18 (cat.# 9304A12)
Particle Size: 100 mm x 2.1 mm ID
Pore Size: 2.7 µm
Guard Column: 90 Å
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

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	90	10
3.00	0.4	20	80
3.01	0.4	90	10
5.00	0.4	90	10

Detector
MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument Notes: UHPLC
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Figure 5: Sulfonamide Antibiotics on Raptor C18 by LC-MS/MS



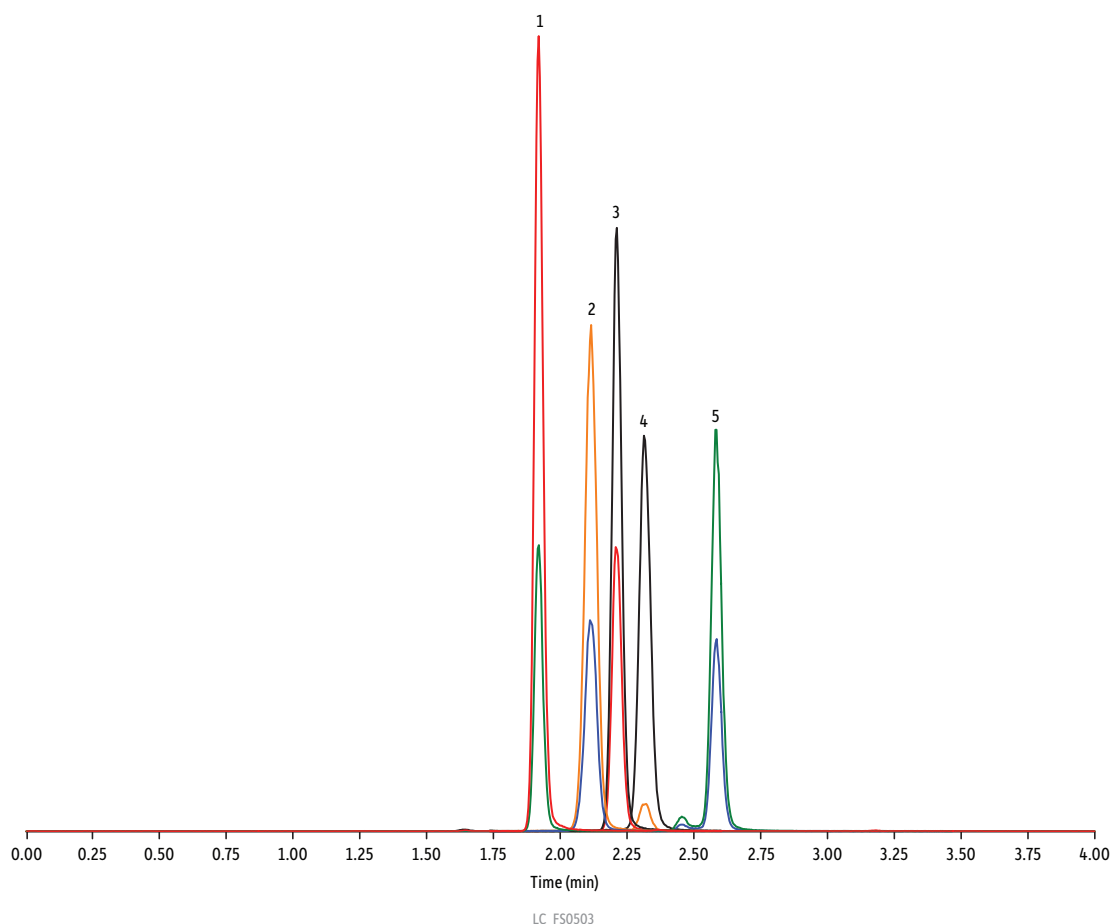
Peaks	tr (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Sulfanilamide	0.83	200	172.98	93.07	75.23
2. Sulfadiazine	1.45	20	251.18	156.04	92.08
3. Sulfathiazole	1.60	10	256.16	156.03	92.08
4. Sulfapyridine	1.67	10	250.13	156.10	92.08
5. Sulfamerazine	1.79	20	265.08	156.03	92.08
6. Sulfamethazine	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

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	90	10
3.50	0.4	60	40
3.51	0.4	90	10
5.00	0.4	90	10

Detector MS/MS
 Ion Mode: ESI+
 Mode: MRM
 Instrument: UHPLC

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



Peaks	tr (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
Dimensions:
Particle Size:
Pore Size:
Guard Column:
Temp.:
Sample
Diluent:
Conc.:
Inj. Vol.:
Mobile Phase
A:
B:

Raptor Biphenyl (cat.# 9309A12)
100 mm x 2.1 mm ID
2.7 µm
90 Å
Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
40 °C

Water:methanol (10:90)
100 ng/mL
2 µL

0.5% Formic acid in water
0.5% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	35	65
3.00	0.5	25	75
3.01	0.5	35	65
5.00	0.5	35	65

Detector
Ion Mode:
Mode:
Instrument
Notes

MS/MS
ESI+
MRM
UHPLC
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