Applicazione descritta: *Antibiotici veterinari multiclasse in LC-MS/MS sulla Raptor C18*

Una sola analisi, una sola colonna, meno di 9 minuti per oltre 60 antibiotici multiclasse

- Separazione dei picchi altamente efficiente e tempi di analisi rapidi.
- Applicabilità universale per l'analisi residuale degli antibiotici, con possibilità di ottimizzare l'analisi di singole classi di composti ai fini della quantificazione:

  - Macrolidi, lincosamidi, e streptogramine (Figura 1)
  - Amfenicoli e tetracicline (Figura 2)
  - Chinoloni (Figura 3)
  - Penicilline, cefalosporine e tetracicline (Figura 4)
  - Sulfamidici (Figura 5)

  (Per gli ionofori è stata utilizzata la Raptor bifenilica. Figura 6)

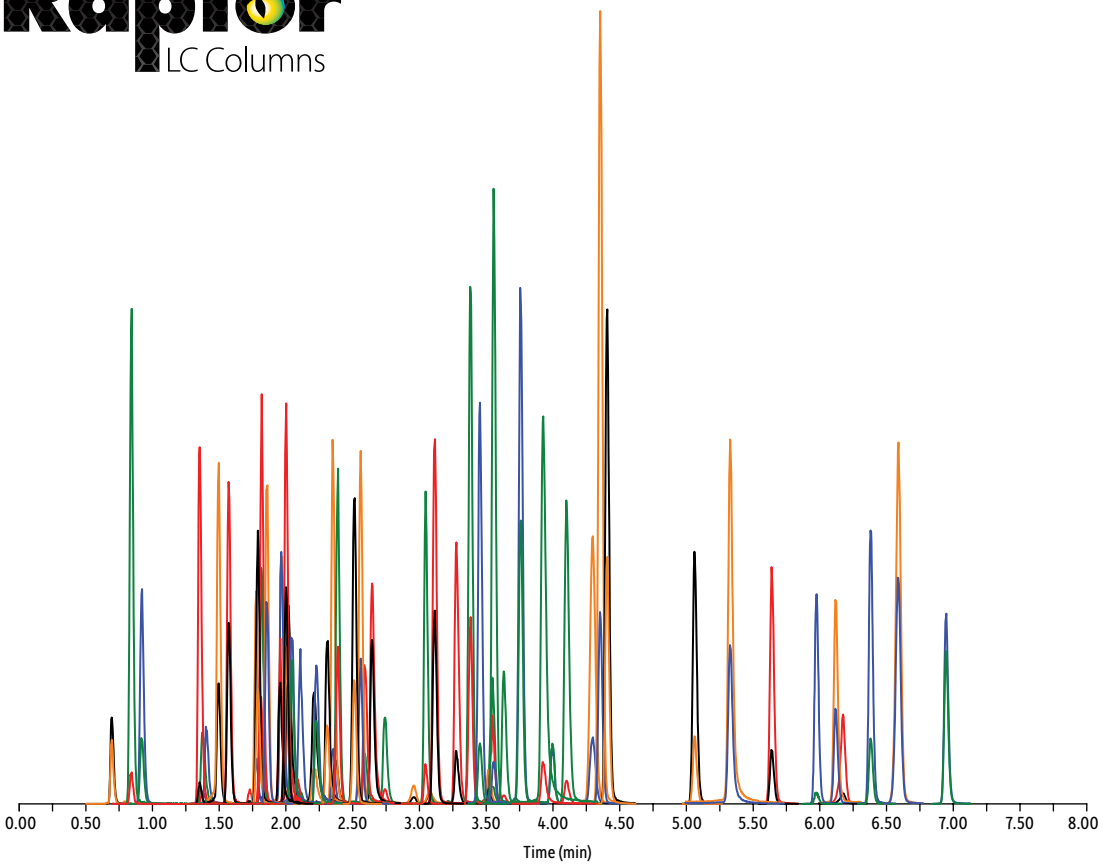
L'uso di antibiotici sugli animali destinati alla produzione alimentare è un problema di salute e sicurezza pubblica in quanto potrebbero svilupparsi batteri farmacoresistenti. Molti paesi dell'Unione europea e il Canada hanno vietato l'uso di questi farmaci per scopi non terapeutici, e gli Stati Uniti stanno implementando una politica volta a ridurre l'uso di antibiotici di rilievo clinico come promotori della crescita. Per regolamentare l'uso corretto degli antibiotici veterinari, la statunitense FDA ha fissato i limiti massimi dei residui (LMR) per diversi tessuti animali e prodotti alimentari (21 CFR Part 556). Per operare in conformità a queste disposizioni è necessario impiegare un metodo analitico sensibile, efficiente e affidabile per diverse classi di antibiotici, e la colonna LC Raptor C18 è la scelta ideale.

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
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

Consulta il cromatogramma e le condizioni a pagina 2

Raptor
LC Columns



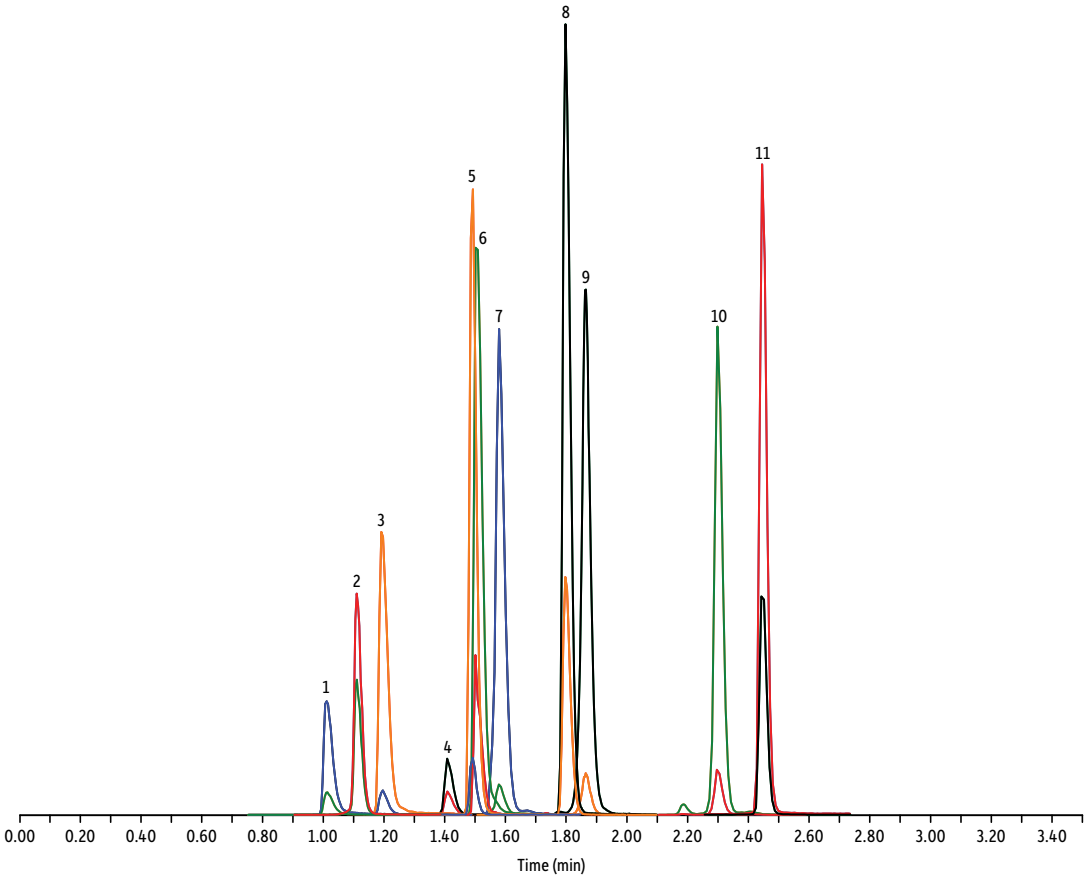
LC_FS0506

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.

Figura 1: Macrolidi, lincosamidi e streptogramine in LC-MS/MS sulla Raptor C18



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

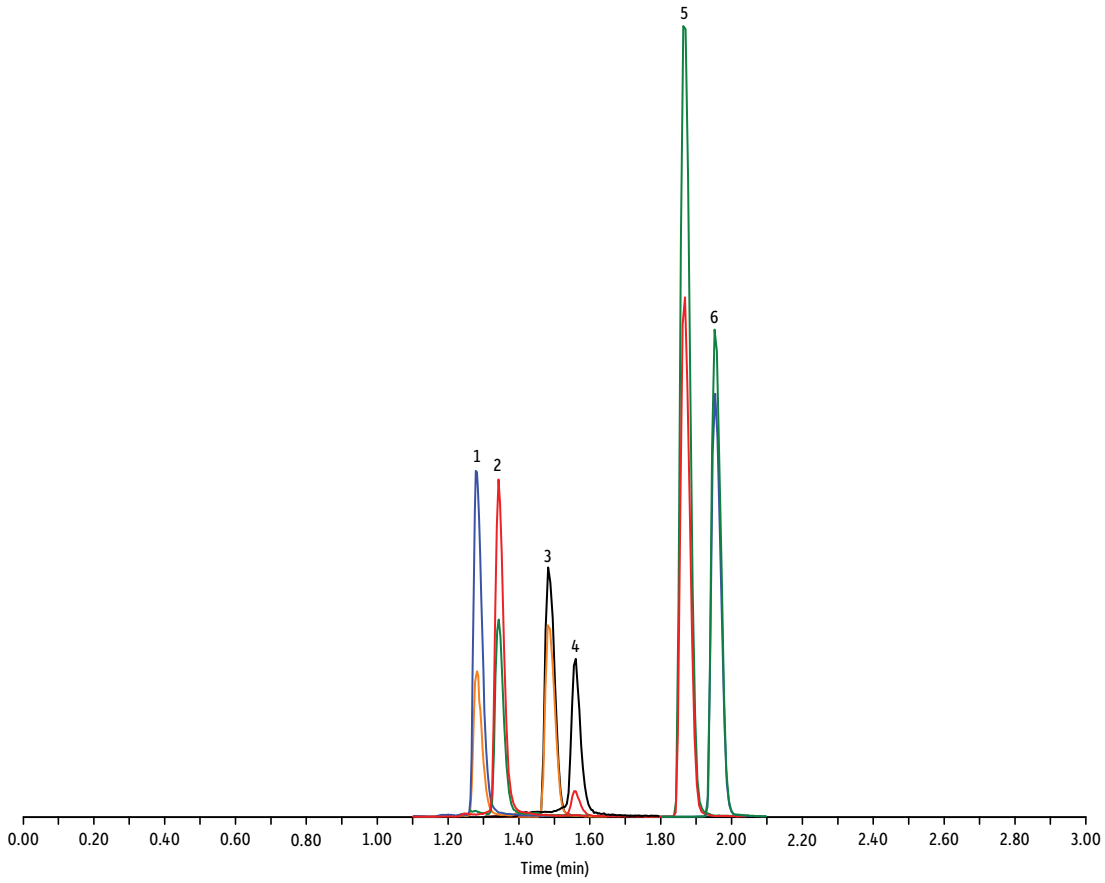
Sample
Diluent: Water
Conc.: 20–200 ng/mL
Inj. Vol.: 2 µL

Mobile Phase
A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

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

Figura 2: Amfenicoli e tetracicline in LC-MS/MS sulla Raptor C18



LC_FS0504

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

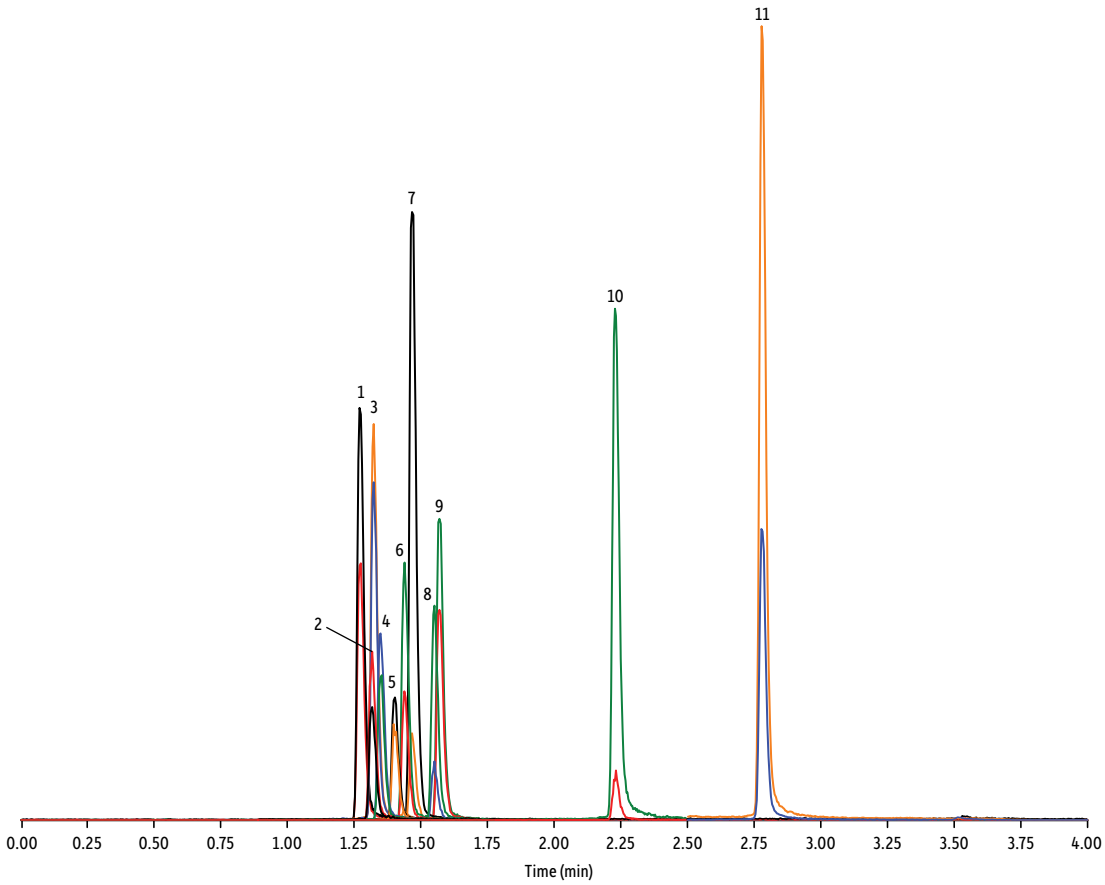
*Acquired in negative ion mode.

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

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.

Figura 3: Chinoloni in LC-MS/MS sulla Raptor C18



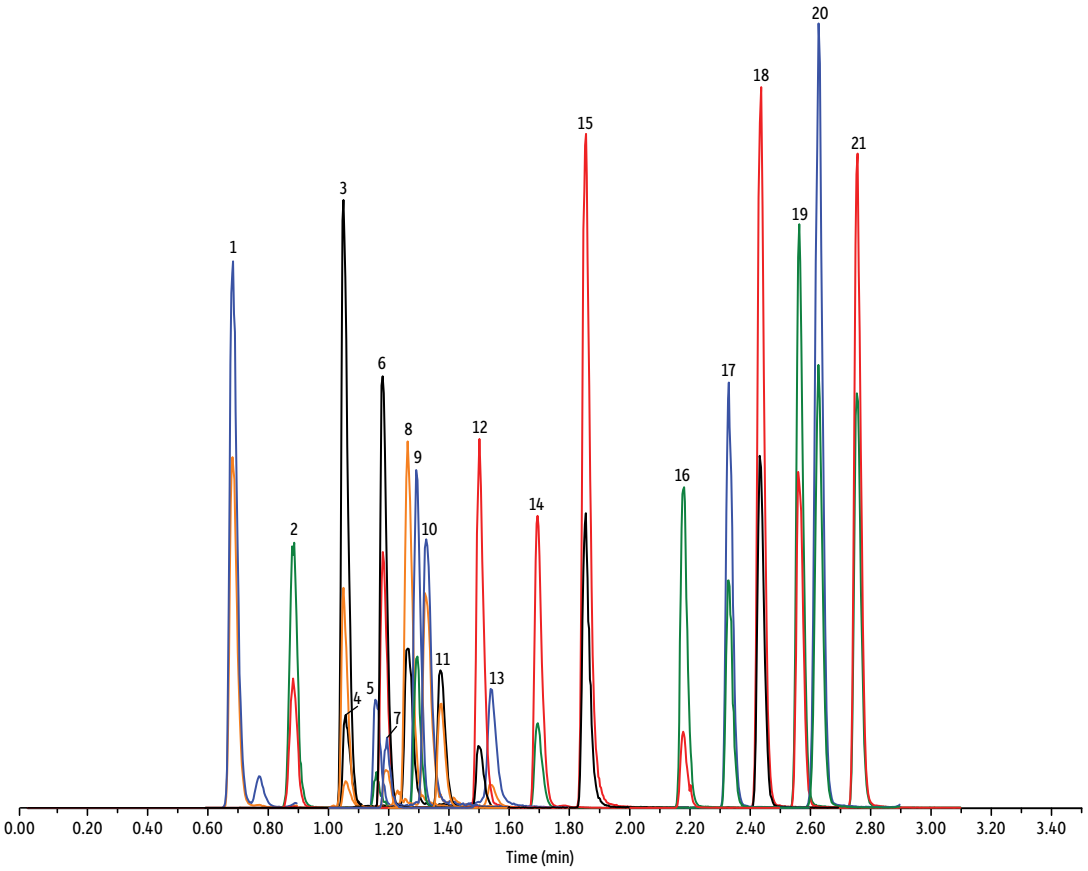
Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Marbofloxacin	1.28	10	363.20	72.11	320.10
2. Norfloxacin	1.32	20	320.23	276.20	233.13
3. Ofloxacin	1.32	10	362.21	318.20	261.15
4. Ciprofloxacin	1.35	20	332.18	288.22	245.15
5. Danofloxacin	1.40	20	358.22	340.16	314.21
6. Enrofloxacin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

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

Figura 4: Penicilline, cefalosporine, e tetracicline in LC-MS/MS sulla Raptor C18



LC_FS0500

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

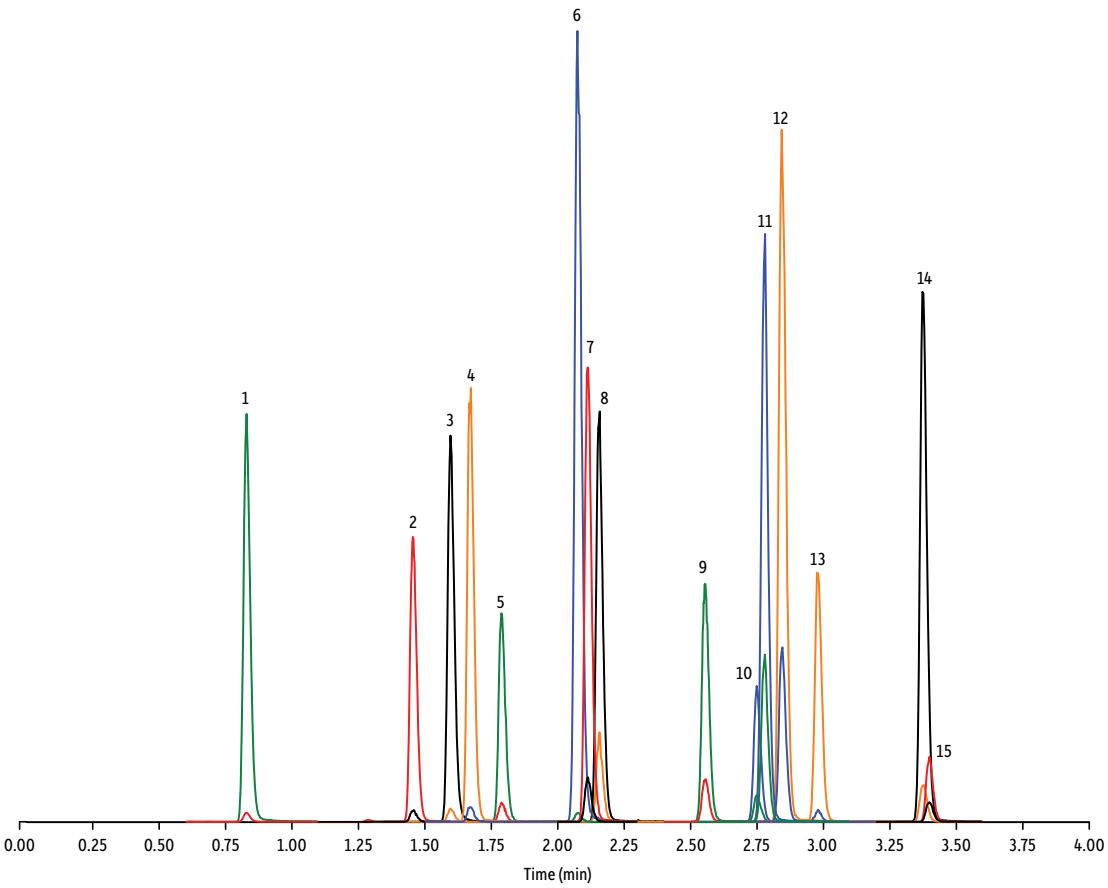
Sample
Diluent: Water
Conc.: 25–300 ng/mL
Inj. Vol.: 2 µL

Mobile Phase
A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

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

Figura 5: Sulfamidici in LC-MS/MS sulla Raptor C18



LC_FS0501

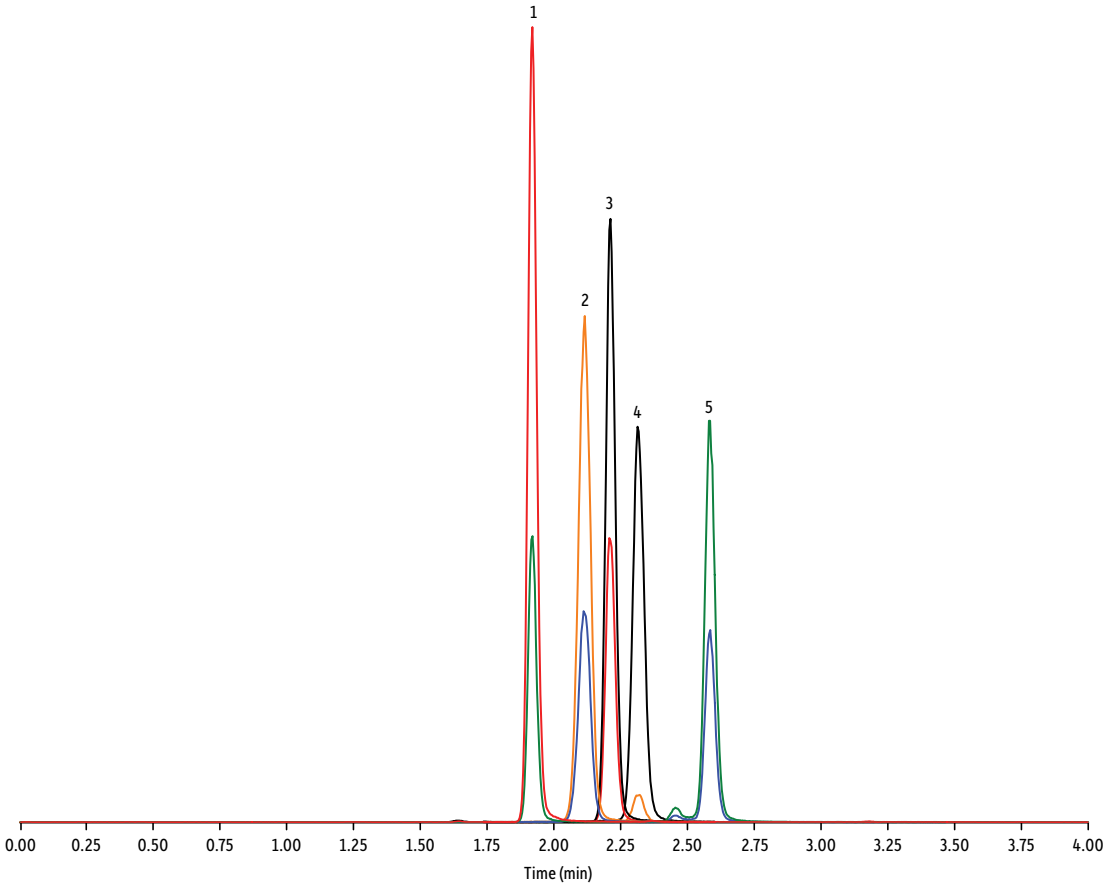
Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Sulfanilamide	0.83	200	172.98	93.07	75.23
2. Sulfadiazine	1.45	20	251.18	156.04	92.08
3. Sulfathiazole	1.60	10	256.16	156.03	92.08
4. Sulfapyridine	1.67	10	250.13	156.10	92.08
5. Sulfamerazine	1.79	20	265.08	156.03	92.08
6. Sulfamethazine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

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

Figura 6: Ionofori in LC-MS/MS sulla Raptor C18



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

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

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