DIONEX

Application Brief 109

Rapid HPLC Separation of Multiclass Antibiotics in Food and Water

INTRODUCTION

The use of antibiotics on livestock, aquaculture, and bee husbandry helps maintain health and provides other benefits such as improved disease resistance, increased production, and in some cases, reduction in foodborne pathogens. However, antibiotic residues in foods can cause undesirable side effects such as idiosyncratic aplastic anemia, production of antibiotic-resistant bacteria, and the reduction of indigenous microbiota found in the human digestive tract. In addition, the wastes of these animals can lead to antibiotics being present in our water. Organizations responsible for food safety perform assays for the presence of allowed and banned antibiotics as part of their surveillance activities. This application brief demonstrates a reproducible Rapid Separation Liquid Chromatography (RSLC) method for the separation of polyketide (tetracycline and oxytetracycline), macrolide (tylosin), phenicol (chloramphenicol), nitrofuran (nitrofurantoin), and sulfonamide (sulfathiazole) classes of antibiotics with an MS-compatible mobile phase (Figure 1, Table 1).

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Column:	Acclaim RSLC Polar Advantage II, 2.2 µm, Analytical (2.1 × 150 mm)					
Mobile Phases:	A) 0.5% Formic acid in water					
	B) 0.5% Formic acid in acetonitrile					
Gradient Time (min):	0	2	4	4.1	5.9	6
%A	90	74	48	10	10	90
%B	10	26	52	90	90	10
Temperature:	30 °C (Autosampler: 5 °C)					
Flow Rate:	0.8 mL/min					
Inj. Volume:	10 µL					
Detection:	Absorbance, UV at 267 nm					

Peaks:		
	1. Sulfathiazole	10 µg/mL
	2. Oxytetracycline	10
	3. Tetracycline	10
	4. Nitrofurantoin	10
5 (a,b,c).	Tylosin and related products	10
	6. Chloramphenicol	10

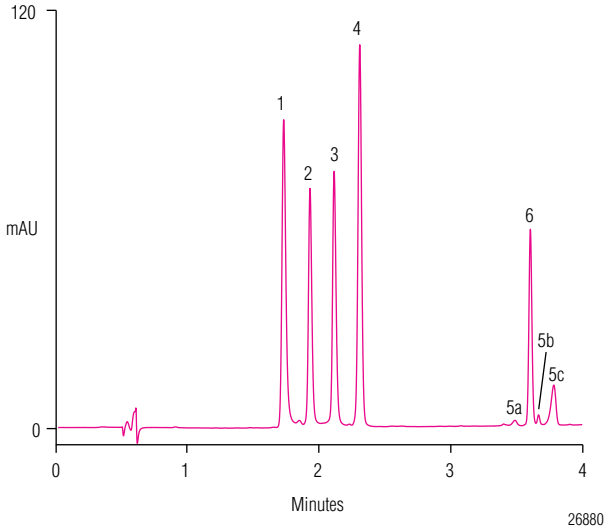

Figure 1. Separation of multiple classes of antibiotics using the Dionex RSLC system and UV-diode array detection at 267 nm.

CONDITIONS

System: UltiMate® 3000 RSLC consisting of HPG-3200 Binary pump, SRD-3200 solvent rack with degasser channels, WPS-3000TRS thermostatted in-line split-loop autosampler, TCC-3000RS themostatted column compartment, and DAD-3000RS diode array detector with semi-micro (2.5 µL, 7 mm) SS flow cell

Column: Acclaim® RSLC Polar Advantage II (2.1 x 150 mm packed with 2.2 µm particles, 120 Å average pore size), P/N 071401

Mobile Phases: A = 0.5% formic acid in water (pH 2.4)
B = 0.5% formic acid in acetonitrile

Gradient:

Time (min)	%A	%B
0.00	90.0	10.0
2.00	74.0	26.0
4.00	48.0	52.0
4.10	10.0	90.0
5.90	10.0	90.0
6.00	90.0	10.0

Maximum pressure – 685 bar (9900 psi)

Flow Rate: 0.80 mL/min

Inj. Volume: 10.0 µL

Temperatures: 30 ºC (column)
5 ºC (autosampler)

Detection: 267, 278, 287, 355, 367 nm (based on UV spectra of individual antibiotics)
Bandwidth – 11 nm each
Data collection rate – 25 Hz
Response time – 0.20 s

Table 1. Retention Time Precision for the Separation of Multiclass Antibiotics [a, b]

Peak	Antibiotic	RT (min)	RSD
1	Sulfathiazole	1.725	0.13
2	Oxytetracycline	1.925	0.11
3	Tetracycline	2.109	0.12
4	Nitrofurantoin	2.305	0.12
5a	Tylosin & Related Products	3.492	0.12
5b		3.673	0.11
5c		3.789	0.11
6	Chloramphenicol	3.610	0.12

[a] Acclaim RSLC Polar Advantage II column
[b] n = 10 replicates

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