Rapid Screening Method for Veterinary Antibiotics Using an Advanced Solid Core UHPLC Column and UHPLC System with MS/MS Detection

Gordon Wilson and Derek Hillbeck, Thermo Fisher Scientific, Runcorn, UK

Application Note 21000

Key Words
Vanquish, Accucore, antibiotics, sulfonamides, penicillins, macrolides, screening

Abstract
This application shows the advantages of using the Thermo Scientific™ Accucore™ Vanquish™ C18 UHPLC 1.5 µm column and Vanquish UHPLC system for the analysis of thirty-six antibiotics.

Advanced capabilities of the Vanquish UHPLC system allow the Accucore Vanquish UHPLC columns to be operated at high flow rates that enable development of rapid analytical methods while maintaining performance. The need for chromatographic separation of isobaric compounds prior to MS detection is also highlighted.

Introduction
There is increasing demand to provide rapid and selective screening techniques for a wide range of antibiotic drugs. Their abuse as a feed supplement in food animals is of growing concern and is subject to regulations globally. Creating screening methods for multiple analytes is more cost effective than dedicated methods for fewer analytes. Reduced analysis times provide for quicker release of data, reduced costs per assay, and overall greater sample throughput.

Other methodologies exploit the high mass resolution capabilities of specialty mass spectrometers.Where these are not readily available, and where common precursor and product ions are present, there is a requirement for good chromatographic resolution, prior to MS detection.

Accucore Vanquish UHPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. This next-generation column features 1.5 µm solid core particles that are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a high coverage, robust phase. This coverage results in a significant reduction in secondary interactions and delivers highly efficient peaks. The tightly controlled 1.5 µm diameter of Accucore Vanquish particles, in combination with controlled manufacturing processes, results in a column that delivers the increased chromatographic performance required for rapid screening methods commonly used in food safety analysis.

The Accucore Vanquish UHPLC column and Vanquish UHPLC system were designed in combination to achieve the best possible chromatographic performance. The system is optimized to reduce extra column band dispersion and allow users to significantly improve the separation power in their analytical assays. By exploiting the 1500 bar high pressure capability of the Vanquish UHPLC system, flow rates can be increased while maintaining peak capacity, resulting in shorter method times and increased assay throughput.

Thermo SCIENTIFIC

Experimental Details

Consumables	Part Number
Accucore Vanquish C18, 1.5 µm UHPLC column, 100 × 2.1 mm	17101-102130
LC-MS grade 18 MΩ water from Thermo Scientific Smart2Pure™ system	50129845
Fisher Chemical™ LC-MS grade methanol	10653963
Fisher Chemical analytical grade formic acid	10559570
Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit	60180-VT400

Sample Preparation

Solutions of the 36 compounds shown were prepared by dissolving 1 mg amounts in 1 mL of water/methanol (1:1 v/v) to produce 1 mg/mL primary solutions. Where required, the diluents proportions were altered to reflect the solubility of the materials and ensure sample solubility. Serial dilutions were made with water/methanol (90:10 v/v) to produce a 100 ng/mL working solution. Vial labeling was supported by the Virtuoso Vial Identification System.

Instrumentation	Part Number
Vanquish UHPLC system consisting of:	
Binary pump H	VH-P10-A
Split sampler HT	VH-A10-A
Column compartment H	VH-C10-A
Thermo Scientific™ TSQ Vantage™ triple quadrupole mass spectrometer	
Virtuoso Vial Identification System	60180-VT-100

LC/MS Conditions

UHPLC column:	Accucore Vanquish C18, 1.5 µm, 100 × 2.1 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Flow rate:	400 µL/min
Column temperature:	(Forced air mode) with active eluent preheating set to 40 °C
Injection details:	2 µL
Gradient:	Refer to Table 1

Time (min)	% B
0.000	10
4.375	90
5.000	90
5.125	10
8.750	10

Table 1: LC gradient conditions

Compound detection was achieved by selected-reaction monitoring (SRM) experiments on a TSQ Vantage triple quadrupole mass spectrometer.
Source and tuning conditions are set out in Table 2 and compound specific parameters for the 36 different SRM transitions are shown in Table 3, together with the analyte retention time when using a 400 µL/min flow rate.

Data Processing

Software:	Thermo Scientific™ Xcalibur™ 2.1 (MS control)
	Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System

Parameter	Setting
Ionization conditions	HESI
Polarity	Positive
Spray voltage (V)	5000
Vaporizer temperature (°C)	500
Sheath gas pressure (Arb)	75
Aux gas pressure (Arb)	20
Capillary temperature (°C)	380
Collision pressure (mTorr)	1.5
Scan time (s)	0.005
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7

Table 2: TSQ Vantage MS/MS experiment conditions

Standard	Precursor Ion	S-Lens (V)	Product Ion	Collision Energy (V)	Retention Time (min)
Amoxicillin	349	63	114	17	1.04
Sulfadiazine	251	57	92	25	1.28
Sulfathiazole	256	56	92	24	1.45
Sulfapyridine	250	71	92	27	1.55
Sulfamerazine	265	75	92	29	1.66
Cefquinome	265	52	134	14	1.73
Lincomycin	407	90	126	30	1.73
4-Epitetracycline	445	88	410	18	1.73
Marbofloxacin	363	78	72	21	1.74
Trimethoprim	291	84	230	22	1.75
Sulfamethazine	279	78	186	17	2.00
Ciprofloxacin	332	89	231	36	2.05
Tetracycline	445	84	410	17	2.08
Sulfamethoxypyridazine	281	78	92	29	2.10
Enrofloxacin	360	94	316	16	2.13
Danofloxacin	358	94	340	22	2.15
Ampicillin	350	65	106	13	2.20
Difloxacin	400	103	382	20	2.23
Chlortetracycline	479	97	462	18	2.27
Sarafloxacin	386	100	368	21	2.30
Sulfamethoxazole	254	58	156	15	2.31
Sulfadoxine	311	84	156	17	2.49
Spiramycin	438	72	174	20	2.80
Sulfadimethoxine	311	84	156	20	2.99
Sulfaquinoloxaline	301	69	92	29	3.13
Doxycycline	445	88	428	17	3.16
Tilmicosin	869	198	174	39	3.22
Clindamycin	425	89	126	28	3.26
Penicillin G	335	102	217	13	3.76
Erythromycin	734	112	158	27	3.80
Tylosin	916	159	174	32	3.83
Josamycin	828	154	174	29	4.13
Oxacillin	424	83	182	14	4.21
Cloxacillin	436	141	178	27	4.36
Nafcillin	415	87	199	14	4.54
Dicloxacillin	470	152	212	28	4.56

Table 3: Compound SRM transition details and retention time

Results

By exploiting the high pressure capabilities of the Vanquish UHPLC system in conjunction with the Accucore Vanquish UHPLC column and a simple binary gradient, it was demonstrated that a screening method for 36 compounds within a 5 minute detection window (and a full method cycle time of less than 9 minutes) can be achieved (Figures 1–4).

Using a 400 µL/min flow rate the system pressure at the start of the gradient was 840 bar, rising to a maximum of 1210 bar during the gradient cycle. The Vanquish UHPLC system is able to routinely operate at these pressure conditions.

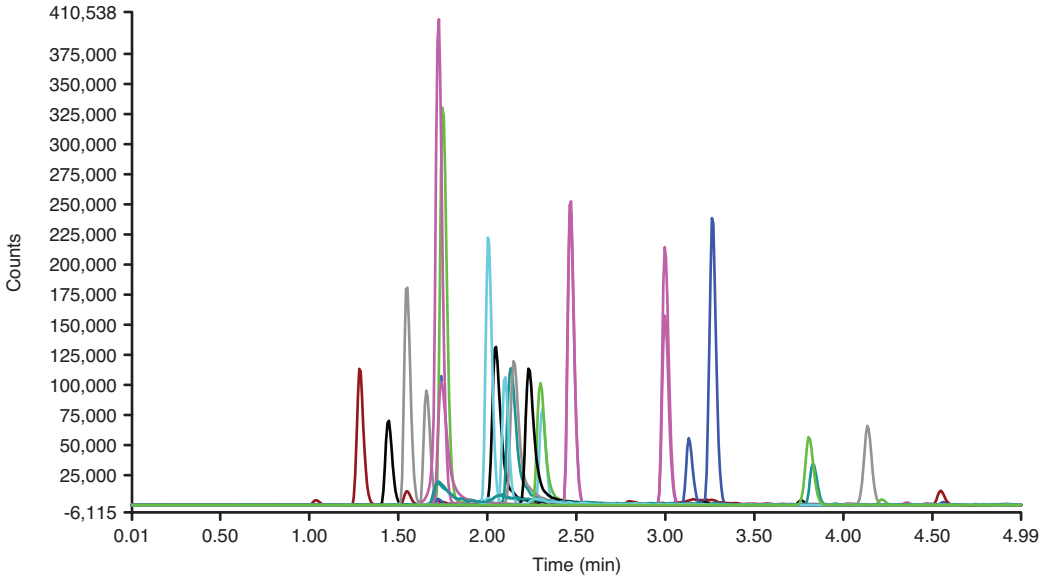

Figure 1: Overlaid selected-reaction monitoring chromatograms showing detection of 36 compounds within a 5 minute detection window

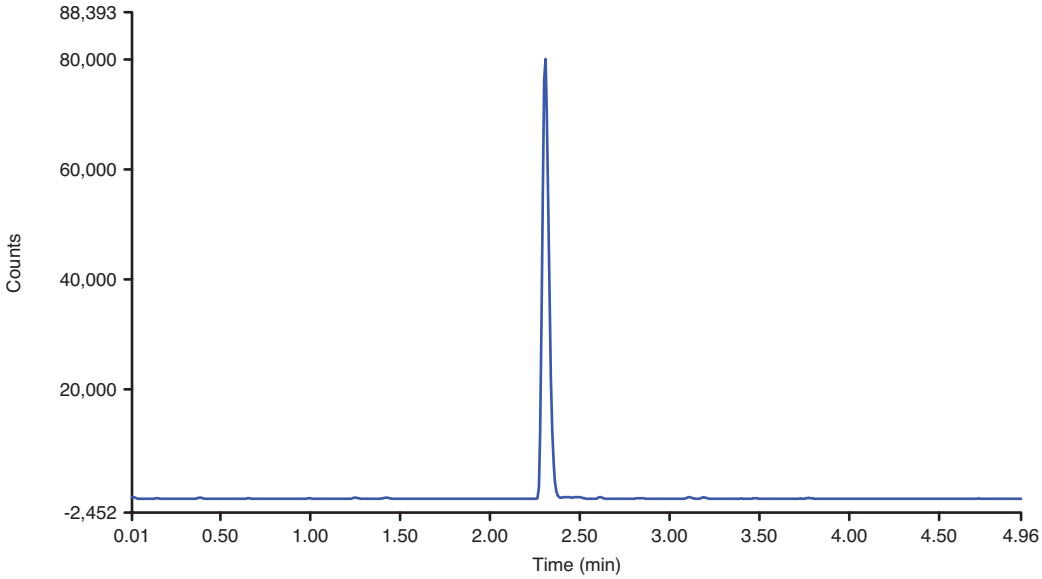

Figure 2: Selected-reaction monitoring chromatogram for sulfamethoxazole, representative of a sulfonamide antibiotic

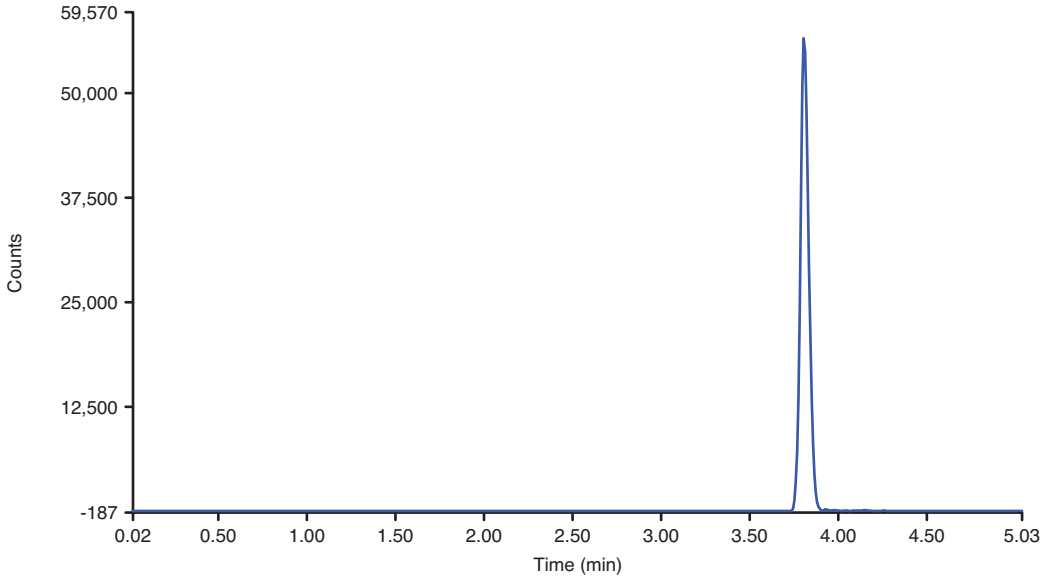

Figure 3: Selected-reaction monitoring chromatogram for erythromycin, representative of a macrolide antibiotic

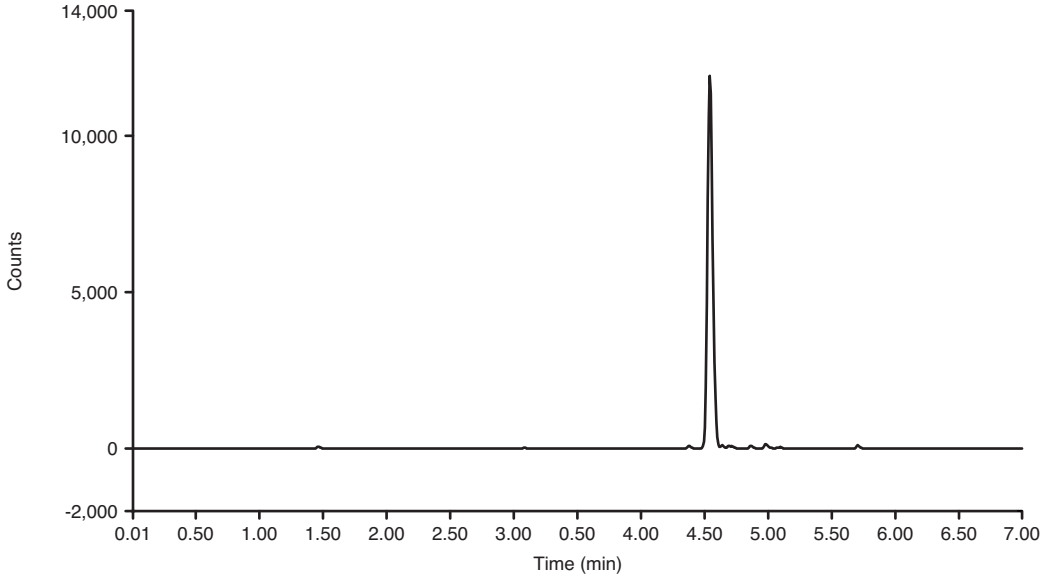

Figure 4: Selected-reaction monitoring chromatogram for nafcillin, representative of a penicillin antibiotic

When isobaric compounds are present in the screening portfolio it is not possible to rely solely on the mass resolution capability of the mass spectrometer, particularly as structurally similar compounds can have common precursor and product ions. In these cases the separation of the components using chromatography provides a clear advantage. The compounds of interest here, sulfadoxine and sulfadimethoxine, are structural isomers with the same molecular weight, product and precursor ions but slightly different structures.

In this situation multiple peaks were observed but were able to be individually resolved (Figure 5). Without this chromatographic resolution the identification of these compounds in a screening set would be compromised.

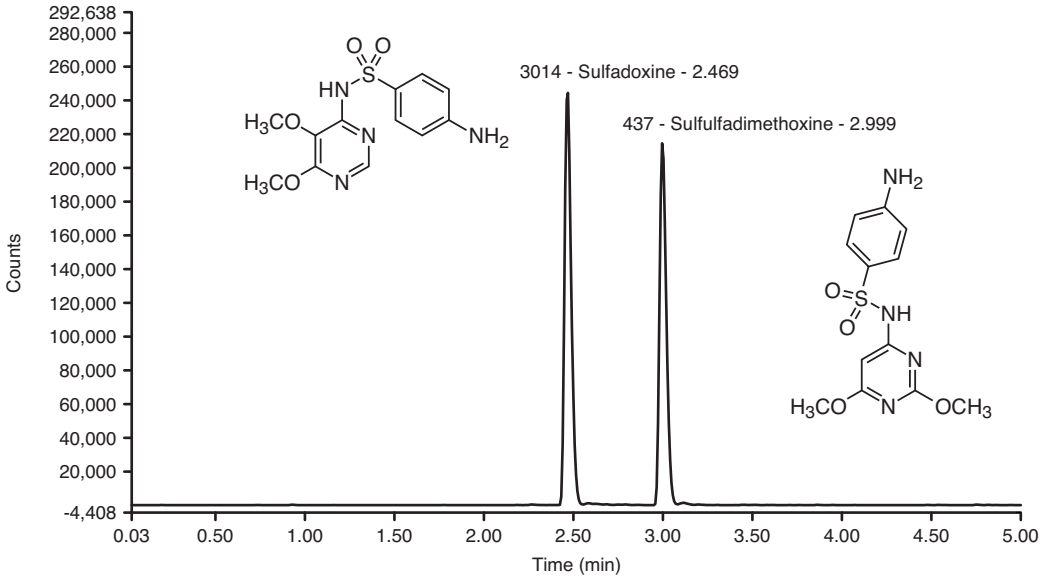

Figure 5: Selected-reaction monitoring chromatogram showing the chromatographic separation of structural isomers

Conclusion

This application note demonstrates the advantages of using the Accucore Vanquish C18 1.5 µm UHPLC column and Vanquish UHPLC system. This solution:

- Delivers a rapid screening UHPLC-MS method for 36 antibiotics
- Delivers a method time less than five minutes in duration
- Exploits the high pressure capabilities of the Vanquish UHPLC system
- Supports separation of isobaric compounds prior to MS detection

For research use only

thermoscientific.com/accucore

USA and Canada +1 800 332 3331
Australia 1300 735 292 (free call domestic)
China 800 810 5118 (free call domestic)
400 650 5118
France +33 (0)1 60 92 48 34
Germany +49 (0) 2423 9431 20 or 21
India +91 22 6742 9494
+91 27 1766 2352
Japan 0120 753 670 (free call domestic)
0120 753 671 fax
United Kingdom +44 (0) 1928 534 110
New Zealand 0800 933 966 (free call domestic)
Singapore +65 6289 1190
All Other Enquiries +44 (0) 1928 534 050

Technical Support
For advice and support, please visit our website:
www.thermoscientific.com/chromexpert

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

AN21000-EN 0814S