Application Note 21518

A Rapid Screening Method for Sulfa Drugs Using an Advanced UHPLC Column and System

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Key Words

Vanquish, Hypersil GOLD aQ VANQUISH, sulfa drugs, UHPLC

Goal

To demonstrate the advantages of using the Thermo Scientific™ Hypersil GOLD™ aQ VANQUISH™ 1.9 µm column and Vanquish UHPLC system for the fast analysis of sulfur-containing drugs. In addition, highlight the built-in method scaling of Thermo Scientific™ Dionex™ Chromeleon™ software and high-pressure capabilities of the Hypersil GOLD VANQUISH columns that allow operation at higher flow rates, enabling the development of rapid analytical methods while maintaining high performance.

Introduction

Food safety is a growing field, in particular the analysis of animal products for drugs and drug residues. One common class of synthetic antibiotics is sulfonamide antimicrobial drugs that are widely used in veterinary medicine. This application note demonstrates the development of a rapid screening method for this compound class, exploiting the high-pressure capabilities of the Vanquish system and columns.

The Hypersil GOLD VANQUISH UHPLC column and Vanquish UHPLC systems were designed to achieve the best possible chromatographic performance. The Vanquish UHPLC system is optimized to reduce extra column band dispersion and system gradient delay volume and allow users to significantly improve the separation power in their analytical assays. The intelligent sample pre-compression prior to injection and extremely low pump pulsation result in outstanding flow stability. By exploiting the 1500 bar high-pressure capability of the Vanquish UHPLC system, the flow rate used with the Hypersil GOLD VANQUISH column can be increased, while maintaining peak capacity. This results in shorter method times and increased assay throughput.

The Hypersil GOLD VANQUISH range of HPLC columns were developed to give reproducible and reliable chromatography analysis with excellent peak shape. Based on highly pure silica, Hypersil GOLD VANQUISH columns provide very symmetrical peaks, even when analyzing compounds that give poor peak shape on traditional silica-based chemistries. For the Hypersil GOLD aQ VANQUISH columns, the media provides a stationary phase with polar embedded C18 selectivity and a predictable elution order and provides improved peak shape, increased peak capacity, and greater sensitivity, especially for trace compound analysis.

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Experimental

Consumables

- Hypersil GOLD aQ VANQUISH, 1.9 µm UHPLC column, 100 × 2.1 mm (P/N 25302-102130-V)
- LC-MS grade 18 MΩ·cm water from Thermo Scientific™ Barnstead™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ Optima™ UHPLC-MS grade acetonitrile (P/N A956-1)
- Fisher Scientific Optima LC-MS grade formic acid (P/N A117-50)
- Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit (P/N 60180-VT400)

Instrumentation

Analyses were performed using a Vanquish UHPLC System consisting of:

- System Base (P/N VH-S01-A)
- Binary Pump H (P/N VH-P10-A)
- Split Sampler HT (P/N VH-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Active Pre-heater (P/N 6732.0110)
- Diode Array Detector HL (P/N VH-D10-A)
- Thermo Scientific™ LightPipe™ flow cell, 10 mm (P/N 6083.0100)

Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 60180-VT-100)

Software

Thermo Scientific™ Dionex™ Chromeleon™ 7.2 SR2 MUa Chromatography Data System

Sample Preparation

Solutions of the twelve compounds shown in Table 2 were prepared by dissolving 10 mg in 10 mL of water/methanol (1:1, v/v) to produce 1 mg/mL primary solutions, apart from sulfaguanidine, sulfamerizine, sulfadoxin, and sulfamonomethoxine, which were prepared by dissolving 10 mg in 10 mL of water/methanol (3:7, v/v). Dilutions were then made with water/methanol (1:1, v/v) to produce 100 µg/mL working solutions.

Vial labeling was carried out by the Thermo Scientific Virtuoso Vial Identification System.

UHPLC Conditions	
UHPLC Column	Hypersil GOLD aQ VANQUISH, 1.9 µm, 100 × 2.1 mm
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in acetonitrile
Column Temperature	55 °C, still air with eluent pre-heating
Injection Volume:	1 µL
UV Detection	260 nm

A range of flow rates were used during the course of these experiments. A summary of the values used in these methods is shown below in Table 1.

Table 1. Gradient details.

Flow rate (mL/min)	0.5	1.0	1.6
%B	Time (min)		
5	0	0	0
30	4.667	2.333	1.458
30	5.000	2.500	1.563
5	5.067	2.533	1.583
5	6.667	3.333	2.083
Maximum Backpressure (bar)	440	913	1376

Results and Discussion

The results using a linear gradient with a 0.5 mL/min flow rate are shown in Figure 1.

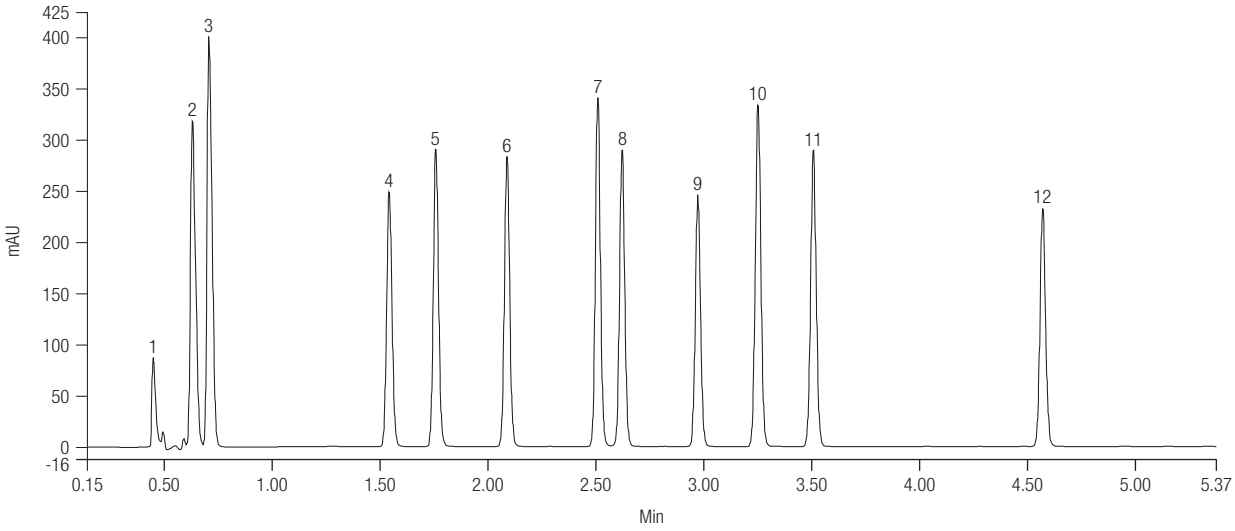

Figure 1. Separation with a linear gradient of twelve sulfa drugs.

Table 2. Analyte identification and elution order.

Peak Number	Analyte in Order of Elution
1	Sulfanilic acid
2	Sulfaguanidine
3	Sulfanilamide
4	Sulfadiazine
5	Sulfathiazole
6	Sulfamerazine
7	Sulfamethizole
8	Sulfadimidine
9	Sulfamonomethoxine
10	Sulfamethoxazole
11	Sulfadoxin
12	Sulfaquinoxaline

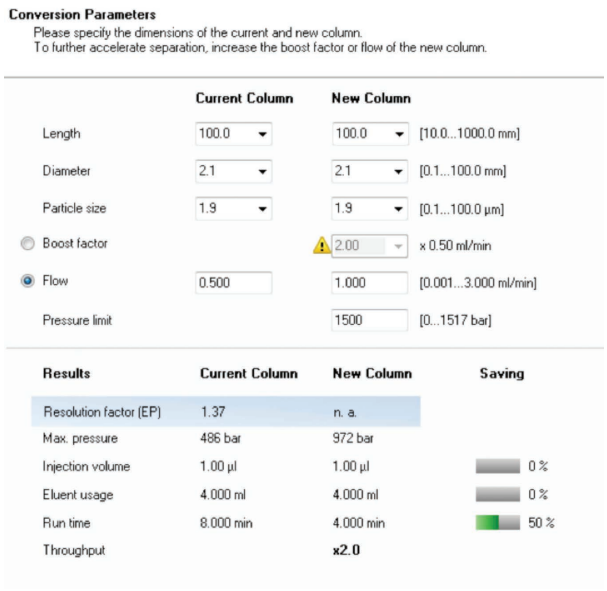

Figure 2. Screenshot of part of Chromeleon CDS UHPLC method transfer calculator.

Full resolution of all 12 compounds can be achieved in less than 7 minutes total run time, at a flow rate of 0.5 mL/min and with a backpressure of 440 bar. However, by exploiting the high-pressure capabilities of the Vanquish UHPLC system and Hypersil GOLD aQ VANQUISH column, the method time can be reduced by utilizing a higher flow rate.

Method scaling is quick and simple using the UHPLC speed up method transfer calculator built in to the Chromeleon CDS software (Figure 2). Using the current method as a starting point, changes to column dimensions or flow rate can be entered and the method is scaled automatically and made ready for immediate use.

By increasing the flow rate to 1.6 mL/min, the method time can be reduced to approximately 2 minutes, while still maintaining baseline resolution (Figures 3 and 4). The system backpressure at this flow rate is 1376 bar, which is within the specification of both the UHPLC column and system.

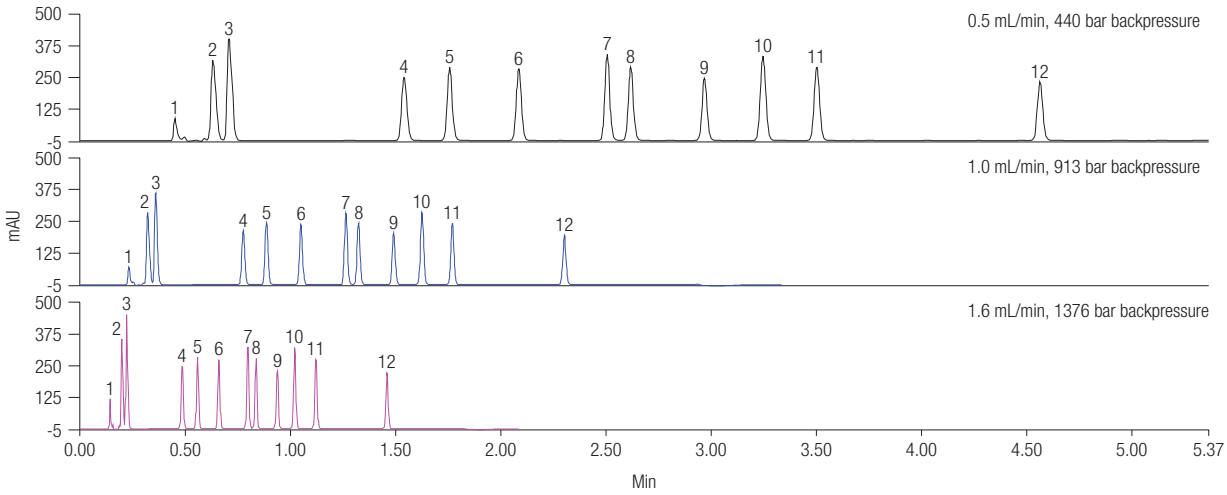

Figure 3. Chromatograms achieved at various flow rates with constant gradient volume.

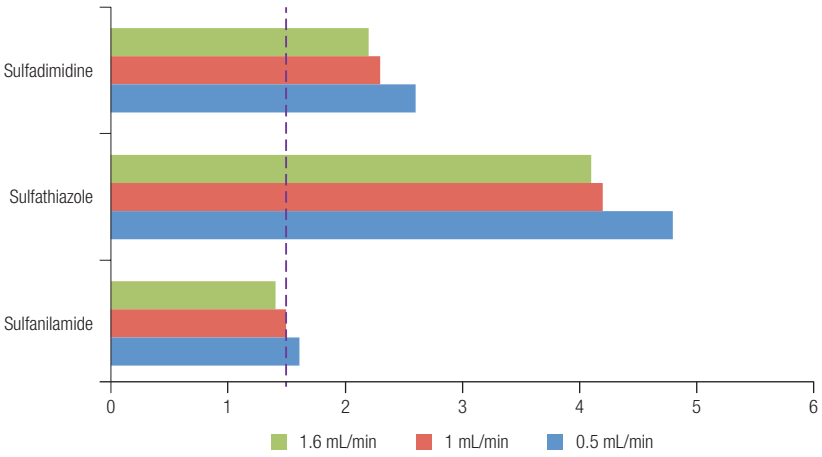

Figure 4. Resolution values of three critical pairs demonstrating minimal reduction in resolution with increased flow rate. Baseline resolution values greater than or equal to 1.5 are maintained.

Conclusion

This application demonstrates the advantages of using the Hypersil GOLD aQ VANQUISH 1.9 µm UHPLC column in conjunction with the Vanquish UHPLC system and Chromeleon CDS software. The performance of the Hypersil GOLD aQ VANQUISH column, coupled with the low internal volume and advanced capabilities of the Vanquish UHPLC system, delivers:

- A rapid screening UHPLC method for sulfa drugs
- Baseline resolution of critical pairs
- A method run time of less than 2.1 minutes

Useful Links

AppsLab Library

The eWorkflow and the Chromeleon Backup (cmbx) file can be downloaded at AppsLab Library:
www.thermofisher.com/appslab

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