Application Note 21496

Rapid Screening Method for Statins Using an Advanced UHPLC Column and System

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Key Words
Hypersil GOLD VANQUISH, statins, UHPLC, non-linear gradient

Goal

To provide an application for the analysis of statins using the Thermo Scientific™ Hypersil GOLD™ VANQUISH™ 1.9 µm UHPLC column and the Vanquish UHPLC system, exploiting the non-linear gradient capability of the system. Demonstrate the opportunity for increased throughput by operating at higher flow rates and backpressures.

Introduction

The Hypersil GOLD VANQUISH UHPLC column and Vanquish UHPLC system were designed to achieve the best possible chromatographic performance. The Vanquish system is optimized to reduce extra column band dispersion and allow users to significantly improve the separation power in their analytical assays. 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 UHPLC column can be increased while maintaining peak capacity, resulting in shorter method times and increased assay throughput.

The Hypersil GOLD VANQUISH range of UHPLC/HPLC columns was developed to give reproducible and reliable chromatography analysis with excellent peak shape. Based on highly pure silica, Hypersil GOLD VANQUISH UHPLC columns provide very symmetrical peaks, even when analyzing compounds that give notoriously poor peak shape on traditional silica-based chemistries. The Hypersil GOLD VANQUISH medium provides a stationary phase with C18 selectivity and a predictable elution order but can provide new capabilities such as improved peak shape, increased peak capacity, and greater sensitivity, especially for trace compound analysis.

The use of Hypersil GOLD VANQUISH UHPLC columns with the Vanquish UHPLC system has allowed for development of a fast multi-compound screening method.

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Experimental

Consumables

- Hypersil GOLD VANQUISH, 1.9 µm UHPLC column, 100 × 2.1 mm (P/N 25002-102130-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 nine compounds shown in Table 2 were prepared by dissolving 10 mg in 10 mL of methanol to produce 1 mg/mL primary solutions. Dilutions were then made with water to produce 100 µg/mL working solutions.

Vial labeling was supported by the Virtuoso Vial Identification System.

UHPLC Conditions	
UHPLC Column	Hypersil GOLD 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
Flow Rate	See Table 1
Gradient	See Table 1
Column Temperature	40 °C, still air with eluent pre-heating
Injection Volume	1 µL
UV Detection	240 nm

Various flow rates were used during the course of these experiments. In addition, the gradient curve profile was investigated with either a linear or a non-linear gradient section. Summary of the values used in these methods are shown below in Table 1.

Table 1. Gradient details.

Flow Rate (mL/min)			0.5	1.0	1.2	1.4
Gradient	%B	Curve	Time (min)			
	42	5	0	0	0	0
	80	5 or 8	3.900	1.950	1.625	1.393
	80	5	4.500	2.250	1.875	1.607
	42	5	4.600	2.300	1.917	1.643
	42	5	8.000	4.000	3.333	2.857
Maximum Backpressure (bar)			486	976	1153	1334

Results and Discussion

The results using a linear gradient with a 0.5 mL/min flow rate are shown in Figure 1 (lower chromatogram). Although resolution has been achieved for the majority of components, the highlighted peaks from fluvastatin and atorvastatin are not fully resolved. Adjustments to the gradient profile were made to gain better separation between the peaks. Chromeleon software allows the creation of non-linear gradient sections with either convex or concave curve profiles. In this case, it was found that a non-linear curve with a value of 8 and a full method time of 8 minutes produced results with good resolution between all the main analytes as shown in Figure 1 (upper chromatogram).

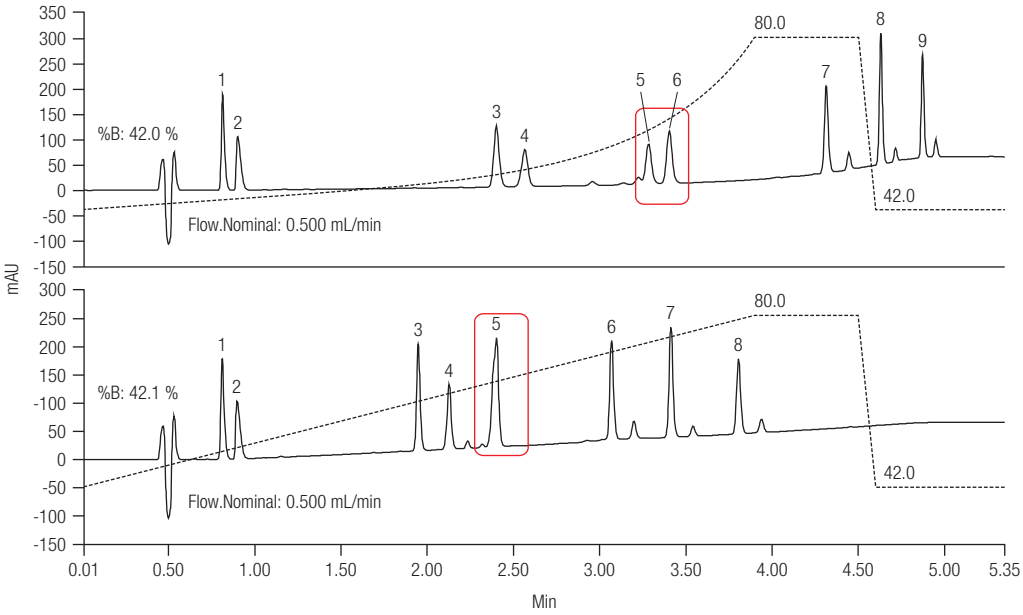

Figure 1. Comparison between separation with a linear gradient (lower chromatogram) and non-linear gradient (upper chromatogram).

The high pressure capabilities of the Vanquish UHPLC system and Hypersil GOLD VANQUISH UHPLC column allow the use of higher flow rates and scaling of the method to increase throughput.

Method scaling is quick and simple using the UHPLC speed-up method transfer calculator built-in to the Chromeleon software. Using a current method as a starting point, changes to column dimensions or flow rate can be entered (Figure 2). The method is scaled automatically and made ready for immediate use. As it can be seen in Figure 3 and Table 2, peak resolution was not largely affected by the increased flow rate.

Conversion Parameters

Please specify the dimensions of the current and new column.
To further accelerate separation, increase the boost factor or flow of the new column.

	Current Column	New Column	
Length	100.0	100.0	[10.0...1000.0 mm]
Diameter	2.1	2.1	[0.1...100.0 mm]
Particle size	1.9	1.9	[0.1...100.0 µm]
Boost factor		2.00	x 0.50 ml/min
Flow	0.500	1.000	[0.001...3.000 ml/min]
Pressure limit		1500	[0...1517 bar]

Results	Current Column	New Column	Saving
Resolution factor (EP)	1.37	n. a.	
Max. pressure	486 bar	972 bar	
Injection volume	1.00 µl	1.00 µl	0 %
Eluent usage	4.000 ml	4.000 ml	0 %
Run time	8.000 min	4.000 min	50 %
Throughput		x2.0	

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

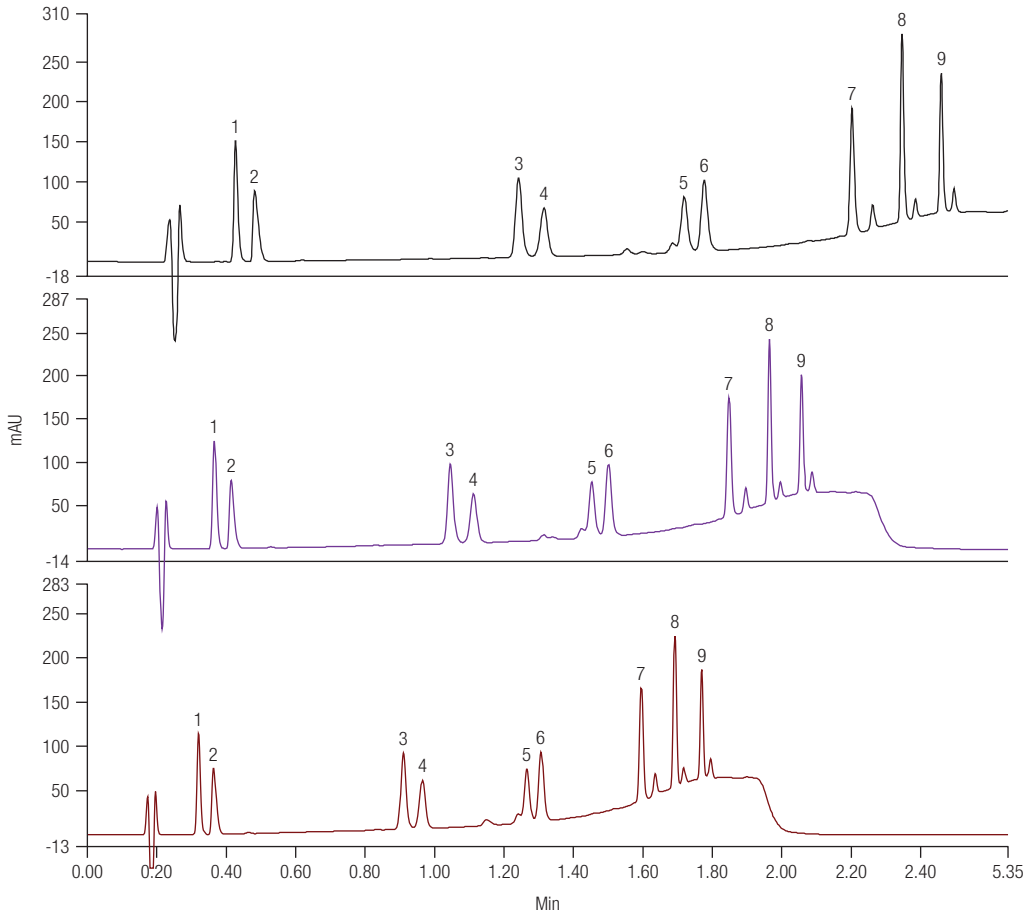

Figure 3. Chromatograms achieved at various flow rates by keeping the gradient slope constant.

Upper: 1.0 mL/min, 976 bar backpressure
Middle: 1.2 mL/min, 1153 bar backpressure
Lower: 1.4 mL/min, 1334 bar backpressure

Table 2. Resolution values for different analysis times.

	Flow (mL/min)	1.00	1.20	1.40
Peak	**Analyte in Order of Elution**	**Resolution (USP)**		
1	Pravastatin	n.a.	n.a.	n.a.
2	Amlodipine	2.28	2.06	2.11
3	Ezetimibe	24.31	21.89	20.98
4	Cerivastatin	2.01	2.03	1.81
5	Fluvastatin	10.87	10.18	9.87
6	Atorvastatin	1.69	1.58	1.41
7	Mevastatin	15.28	13.34	12.20
8	Lovastatin	7.34	5.88	5.32
9	Simvastatin	6.77	5.12	4.68

Replicate injections of the mix using the 1.2 mL/min method showed that the Vanquish UHPLC system and Hypersil GOLD VANQUISH UHPLC column produced stable and reproducible results (Table 3). The Vanquish UHPLC system features a binary pump with extremely low pulsation ripple and an outstanding gradient precision, even for challenging non-linear gradient profiles. In addition, the Vanquish UHPLC system pre-compresses the sample prior to the injection, which results in a highly stable flow delivery. Thanks to these benefits, the Vanquish UHPLC system is capable of providing unmatched retention time precision.

Table 3. Peak identification, retention time variability, and resolution for twelve replicate injections using the 1.2 mL/min non-linear curve method.

Peak	Analyte	Average Retention Time (min)	Retention Time RSD (%)	Average Resolution (USP)
1	Pravastatin	0.362	0.20	-
2	Amlodipine	0.409	0.18	2.24
3	Ezetimibe	1.044	0.09	23.44
4	Cerivastatin	1.111	0.11	2.07
5	Fluvastatin	1.454	0.07	10.86
6	Atorvastatin	1.502	0.06	1.69
7	Mevastatin	1.848	0.02	14.48
8	Lovastatin	1.964	0.02	6.92
9	Simvastatin	2.056	0.02	6.40

Conclusion

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

- Rapid screening UHPLC method for statins
- Resolution of critical pair achieved
- Method time of less than three minutes at maximum operating pressure
- Excellent retention time reproducibility

Useful Links

AppsLab Library

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

For Research Use Only. Not for use in diagnostic procedures

www.thermofisher.com/LC-columns

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