

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

Ken Meadows, Thermo Fisher Scientific, Runcorn, UK

Key Words

Vanquish, Accucore, statins, pravastatin, amlodapine, fluvastatin, atrovostatin, cervistatin, simvastatin, solid core, fused core, UHPLC

Goal

To demonstrate the advantages of using the Thermo Scientific™ Accucore™ Vanquish™ C18+, 1.5 µm column and Thermo Scientific Vanquish UHPLC system for the fast analysis of statins. The advanced capabilities of the Vanquish UHPLC system allow the Accucore Vanquish UHPLC columns to be operated at higher flow rates that enable development of rapid analytical methods while maintaining high performance.

Introduction

Statins (or HMG-CoA reductase inhibitors) are a class of drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the body. Statins have been found to prevent cardiovascular disease and mortality in those who are at high risk. The use of Accucore Vanquish columns with the Vanquish UHPLC system has allowed for development of a fast multi compound screening method for six commonly prescribed statins.

Accucore Vanquish C18+ 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.

The Accucore Vanquish UHPLC column and Vanquish UHPLC systems were designed in combination to provide 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. By exploiting the 1500 bar high pressure capability of the



Vanquish UHPLC system, the flow rate used with the Accucore Vanquish UHPLC column can be increased while maintaining peak capacity, resulting in shorter method times and increased assay throughput.

Experimental

Consumables and Apparatus

- Accucore Vanquish C18+, 1.5 μm UHPLC column, 100 \times 2.1 mm (P/N 27101-102130)
- Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 60180-VT-100)
- Thermo Scientific™ Virtuoso™ 9 mm wide opening screw thread vial, 2 mL, clear glass vial with V-patch and red PTFE/white silicone/red PTFE septum (P/N 60180-VT400)
- LC-MS grade 18 M Ω water from Thermo Scientific™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ HPLC grade acetonitrile (P/N A/0626/17)
- Fisher Scientific ammonium acetate (P/N A/3440/53)
- Fisher Scientific acetic acid (P/N A113-50)

Sample Preparation

Solutions of the six compounds shown in Table 1 were prepared by dissolving 10 mg amounts in 10 mL of methanol to produce 1 mg/mL primary solutions. Dilutions were then made with water to produce 100 $\mu\text{g}/\text{mL}$ working solutions.

Vial labeling was supported by the Thermo Scientific™ Virtuoso™ vial identification system.

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)
- Diode array detector HL (P/N VH-D10-A)

UHPLC Conditions

UHPLC column	Accucore Vanquish C18+, 1.5 μm , 100 \times 2.1 mm
Mobile phase A	Ammonium acetate, 20 mM, pH 4.0
Mobile phase B	Acetonitrile + 0.1% acetic acid
Flow rate	650 $\mu\text{L}/\text{min}$
Column temp.	40 $^{\circ}\text{C}$, still air with eluent pre-heating
Injection details	2 μL standard needle in loop
UV detection	240 nm

Table 1. LC gradient conditions.

Time (min)	% B
0.00	40
3.90	70
3.90	40
5.00	40

Software

The Thermo Scientific™ Dionex™ Chromeleon™ 7.2 SR2 Chromatography Data System was used for data acquisition and analysis.

Results and Discussion

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 six compounds within a four minute detection window (and a full method cycle time of five minutes) can be achieved. Replicate injections of the mix showed that the Accucore Vanquish UHPLC column produced stable and reproducible results (Figure 1 and Table 2). Resolution of the critical pair fluvastatin and atrovostatin was achieved with a resolution of 2.54.

Using a 650 $\mu\text{L}/\text{min}$ flow rate, the maximum system pressure during the gradient was 1258 bar. The Vanquish UHPLC system and column combination are able to routinely operate at these pressure conditions.

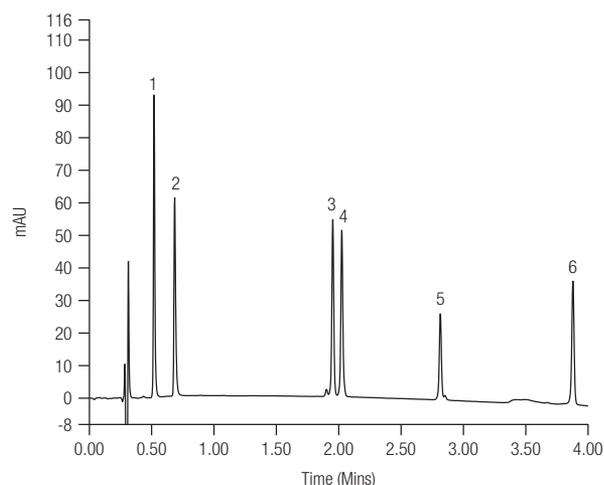


Figure 1. Chromatogram showing separation of statins within a 4 minute detection window.

Table 2. Peak identification, resolution, and retention time variability.

Peak Number	Peak Name	Retention Time (min)	Resolution (USP)	% RSD of Retention Time (n=6)
1	Pravastatin	0.520	7.52	0.16%
2	Amlodapine	0.685	48.31	0.12%
3	Fluvastatin	1.953	2.54	0.028%
4	Atrovostatin	2.025	26.79	0.040%
5	Cervistatin	2.815	32.77	0.029%
6	Simvastatin	3.878	n.a.	0.021%

Conclusion

The performance of the Accucore Vanquish C18+ 1.5 µm UHPLC column coupled with the low internal volume and advanced capabilities of the Vanquish UHPLC system delivers the following:

- Rapid screening UHPLC method for statins
- Method time of five minutes
- Excellent retention time reproducibility
- Resolution of critical pair

For Research Use Only

thermoscientific.com/Accucore

©2015 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

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)
Korea +82 2 3420 8600
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