Application Note 21498

# A High-Throughput Method for the Analysis of 19 Beta Blockers Using a UHPLC Column and System Combination

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## Key Words

Hypersil GOLD, VANQUISH, Beta Blockers, Beta Antagonists, UHPLC, atenolol, sotalol, carteolol, nadolol, pindolol, timolol, acebutolol, metoprolol, esmolol, celiprolol, oxprenolol, labetalol, bisoprolol, propranolol, alprenolol, betaxolol, carvidilol, nebivolol, penbutalol

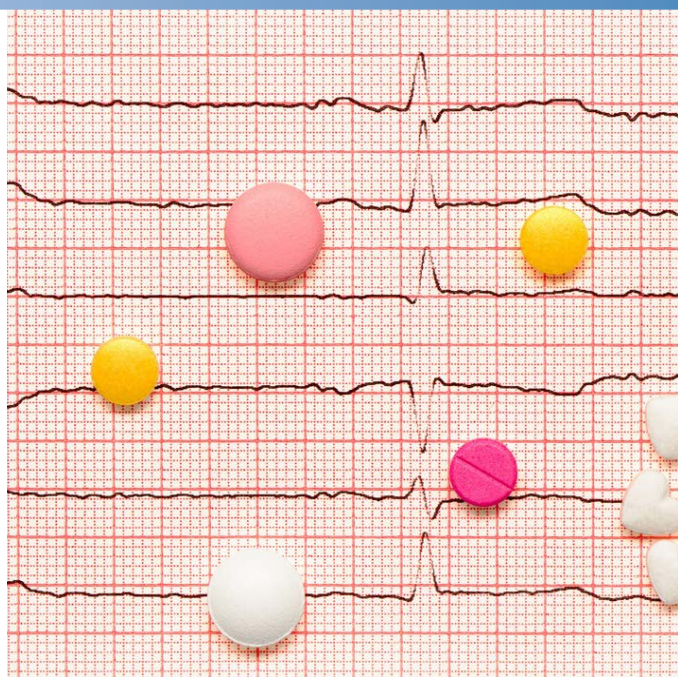
## Goal

To demonstrate the advantages of using the Thermo Scientific™ Hypersil GOLD™ VANQUISH™ 1.9 µm column and the Vanquish UHPLC system for the fast screening analysis of 19 beta blockers.

## Introduction

Creating screening methods for multiple analytes is more cost-effective than dedicated methods for fewer analytes. Reduced analysis times allow quicker release of data, reduced costs per assay, and overall greater sample throughput. The analysis of beta blockers provides a good demonstration of a multi-analyte method with structurally similar compounds that require an efficient separation to achieve good resolution.

The complementing technologies of the Hypersil GOLD VANQUISH UHPLC column and the Vanquish UHPLC system allow for the best possible chromatographic performance. 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 system is optimized to reduce extra column band dispersion and allow users to preserve the excellent peak efficiency provided by the column. The very fast acquisition rate, up to 200 Hz, allows the collection of sufficient data points, even for the narrowest peaks obtained in high-throughput application.



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 UHPLC 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.

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## Experimental

### Consumables

- Hypersil GOLD VANQUISH, 1.9 µm UHPLC column, 100 × 2.1 mm
- LC-MS grade 18 MΩ·cm water from Thermo Scientific™ Barnstead™ Smart2Pure™ system
- HPLC grade acetonitrile
- Analytical grade formic acid
- Ammonium formate
- Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit

### Instrumentation

Analyses were performed using a Vanquish UHPLC System consisting of:

- System Base
- Binary Pump H
- Split Sampler HT
- Column Compartment H
- Active Pre-heater
- Diode Array Detector HL
- Thermo Scientific™ LightPipe™ flow cell, 10 mm

Thermo Scientific™ Virtuoso™ Vial Identification System

### Software

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

### Sample Preparation

Solutions of the 19 compounds shown in Figure 3 were prepared by dissolving 10 mg amounts in 10 mL of methanol or water to produce 1 mg/mL primary solutions. Dilutions were then made with water to produce a 25 µg/mL working solution.

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 | Ammonium formate, 20 mM, pH 3.0 |
| Mobile Phase B | Acetonitrile + 0.1% formic acid |
| Flow Rate | See Table 1 |
| Column Temperature | 40 °C, still air with eluent pre-heating |
| Injection Details | 2 µL |
| UV Detection | 270 nm |
| Acquisition Rate | 100 Hz |
| Response Time | 0.04 s |

Table 1. LC gradient conditions.

| Flow Rate (mL/min) | 0.8 | | 1.7 | |
|---|---|---|---|---|
| Gradient | Time (min) | %B | Time (min) | %B |
| | 0 | 10 | 0 | 10 |
| | 1.50 | 10 | 0.71 | 10 |
| | 4.00 | 60 | 1.88 | 60 |
| | 4.01 | 10 | 1.89 | 10 |
| | 6.00 | 10 | 2.82 | 10 |
| Maximum Backpressure (bar) | 710 | | 1365 | |

## Results and Discussion

Full resolution of all components was achieved using a 0.8 mL/min flow rate within a 4 minute detection window. The maximum system pressure throughout the gradient was 710 bar (Figure 1). However, the Vanquish UHPLC system and the Hypersil GOLD VANQUISH UHPLC column combination are able to routinely operate at back pressures up to 1500 bar. Therefore, the method was adjusted to take advantage of the maximum operating conditions to ultimately increase throughput.

To modify the method, the UHPLC speed-up method transfer calculator, built in to the Chromeleon software, was used. Increasing the flow rate to 1.7 mL/min does impact the resolution of two critical pairs (peaks 1/2 and 14/15). However, as a screening method, sufficient resolution to allow identification prior to confirmation analysis is demonstrated (Figures 2 and 3). The advantage is that by increasing the flow rate to 1.7 mL/min (and scaling the method appropriately), the detection window has now been halved from 4 to 2 minutes. Therefore, by exploiting the maximum operating conditions of both the UHPLC system and column, we can double throughput.

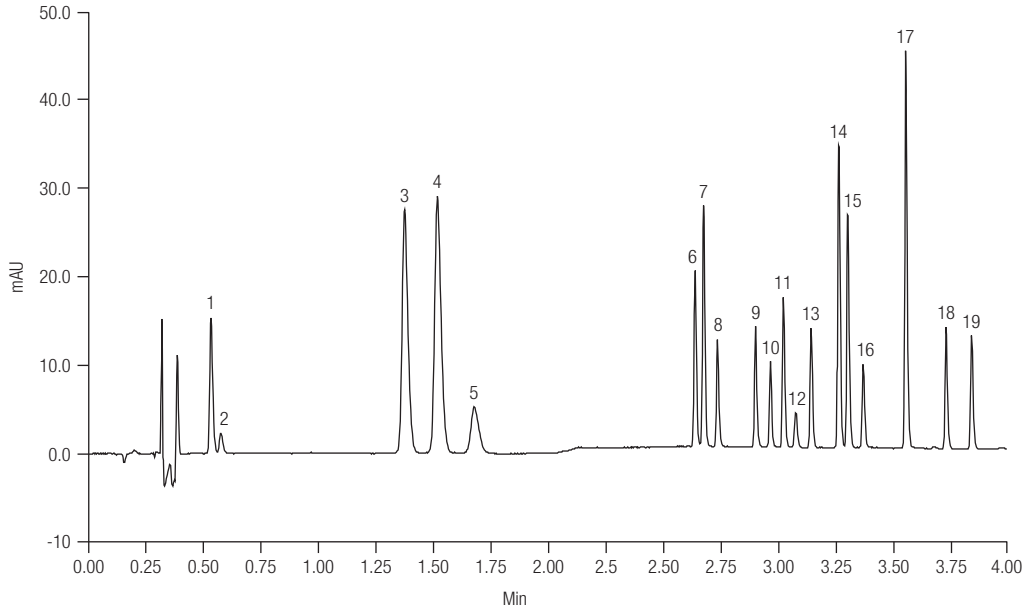


Figure 1. Chromatogram showing the separation of 19 beta blockers at a flow rate of 0.8 mL/min (710 bar back pressure), within a 4-minute detection window.

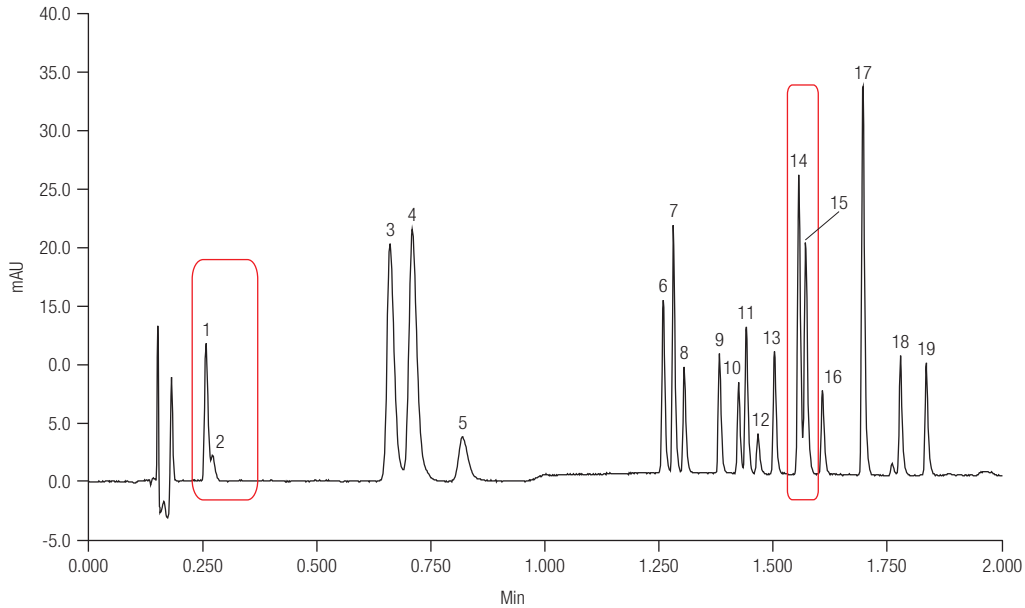


Figure 2. Chromatogram showing the separation of 19 beta blockers at a flow rate of 1.7 mL/min (back pressure 1365 bar), within a 2 minute detection window.

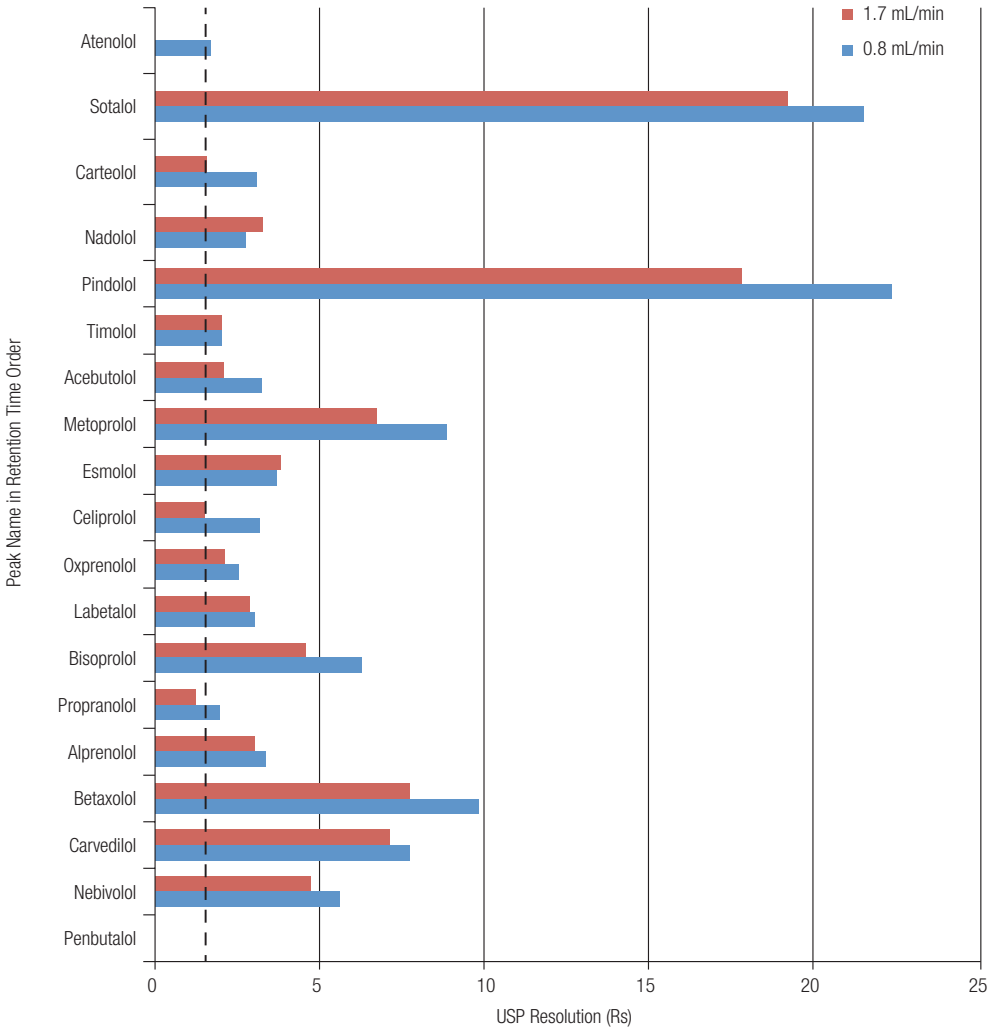


Figure 3. Peak identification and resolution with baseline resolution indicator (Rs=1.5) for 19 beta blockers.

## Conclusion

By exploiting the high pressure capabilities of the Vanquish UHPLC system, in conjunction with the Hypersil GOLD VANQUISH UHPLC column and a simple binary gradient, it was demonstrated that a screening method for 19 compounds within a 2 minute detection window (and a full method cycle time of 2.8 minutes) can be achieved.

This application demonstrates the advantages of using the Hypersil GOLD VANQUISH 1.9 µm UHPLC column and the Vanquish UHPLC system. The performance of the Hypersil GOLD VANQUISH UHPLC column coupled with the low internal volume and fast acquisition of the Diode Array Detector deliver the following:

- Full resolution UHPLC method for 19 beta blockers in a method time < 4 minutes
- Rapid screening UHPLC method for 19 beta blockers in a method time < 2.8 minutes

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## Useful Links

**AppsLab Library**

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

**www.thermofisher.com/LC-columns**



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