Application Note 21178

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

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Key Words

Vanquish, Accucore, beta blockers, beta antagonists, UHPLC, solid core, fused core, sotalol, pindolol, timolol, acebutolol, metoprolol, esmolol, celiprolol, oxprenolol, labetalol, propranolol, betaxolol, carvidilol, nebivolol, penbutalol

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 beta blockers. The advanced capabilities of the Vanquish UHPLC system allow the Accucore Vanquish columns to be operated at high flow rates that enable development of rapid analytical methods while maintaining high performance.

Introduction

Beta blockers (or beta antagonists) are a category of drugs used to treat a number of medical complaints, such as hypertension, angina, heart failure, and heart attacks. Beta blockers are designed to stop the functioning of a naturally occurring compound, noradrenaline, which is a chemical released in the body that can cause the arteries to narrow and the heart rate to increase.

The analysis of beta blockers provides a good demonstration of a multiple analyte method, as the similar structures of the compounds require an efficient separation to achieve good resolution. In addition, good peak shape for these basic analytes will highlight low secondary interactions. Creating screening methods for multiple analytes, such as the method described here for beta blockers, 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.

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 system 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.

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Experimental

Consumables and Apparatus

- Accucore Vanquish C18+, 1.5 µm UHPLC column, 100 × 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 MΩ water from Thermo Scientific™ Smart2Pure™ System (P/N 50129845)
- Fisher Scientific™ HPLC grade acetonitrile (P/N A/0626/17)
- Fisher Scientific analytical grade formic acid (P/N 10559570)
- Fisher Scientific ammonium formate (P/N A/5080/53)

Sample Preparation

Solutions of the fourteen 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 µg/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 × 2.1 mm
Mobile phase A	Ammonium formate, 20 mM, pH 3.0
Mobile phase B	Acetonitrile + 0.1% formic acid
Flow rate	500 µL/min
Column temp.	40 °C, still air with eluent pre-heating
Injection details	2 µL standard needle in loop
UV detection	270 nm

Table 1. LC gradient conditions.

Time (min)	% B
0.00	5
3.00	30
4.50	100
5.00	100
5.00	5

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 14 compounds within a 4.5 minute detection window (and a full method cycle time of six minutes) can be achieved (Figure 1). The performance of the Accucore Vanquish UHPLC column has ensured that even with the fast method time, good separation of critical pairs has been achieved with resolution > 2.00 for all pairs except celiprolol and oxprenolol at 1.53 (Table 2 and Figures 2 and 3). Replicate injections showed that the Accucore Vanquish UHPLC column produced stable and reproducible results (Table 2).

Using a 500 µL/min flow rate, the system pressure at the start of the gradient was 967 bar and rose to a maximum of 1024 bar during the gradient cycle. The Vanquish UHPLC system and column combination are able to routinely operate at these pressure conditions.

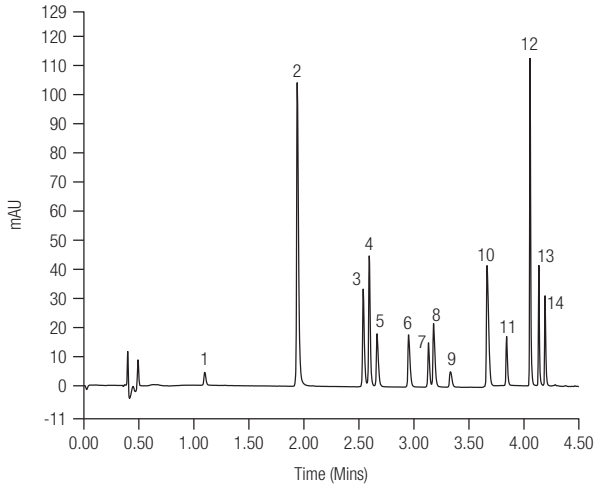

Figure 1. Chromatogram showing separation of 14 beta blockers within a 4.5 minute detection window.

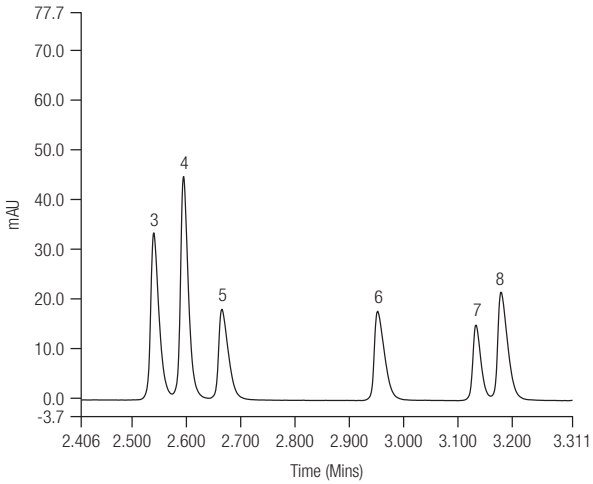

Figure 2. Chromatogram showing separation of timolol, acebutolol, celiprolol, and oxprenolol.

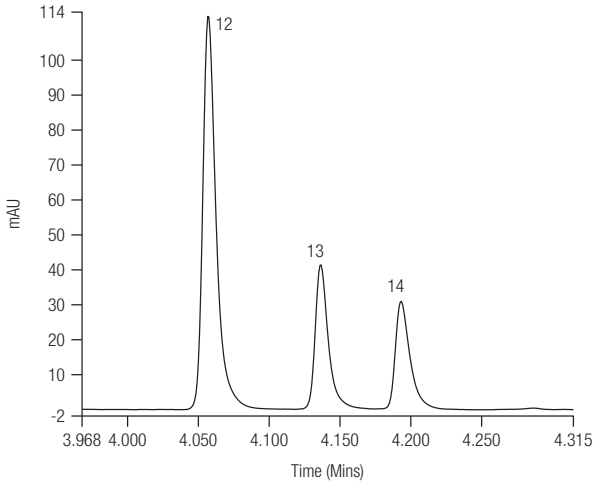

Figure 3. Chromatogram showing separation of carvidilol, nebivolol, and penbutalol.

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	Sotalol	1.10	27.18	0.10%
2	Pindolol	1.95	20.34	0.20%
3	Timolol	2.55	2.09	0.19%
4	Acebutolol	2.61	2.41	0.22%
5	Metoprolol	2.68	8.45	0.23%
6	Esmolol	2.96	5.88	0.23%
7	Celiprolol	3.14	1.53	0.18%
8	Oxprenolol	3.19	4.18	0.16%
9	Labetalol	3.34	8.72	0.14%
10	Propranolol	3.67	5.72	0.070%
11	Betaxolol	3.85	10.59	0.030%
12	Carvidilol	4.06	4.92	0.00%*
13	Nebivolol	4.14	3.37	0.0099%
14	Penbutalol	4.19	n.a.	0.00%*

* No retention time variation seen for replicate injections measured to three decimal places.

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 deliver the following:

- Rapid screening UHPLC method for 14 beta blockers
- Method time less than 6 minutes
- Excellent retention time reproducibility
- Resolution of critical pairs

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