Development of Rapid Screening Methods Using a UHPLC Solid Core Column and System Combination

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Overview

This poster demonstrates how the high performance capabilities of a UHPLC solid core column and system combination can be utilized to achieve rapid screening methods for multiple compounds.

Fast gradient UHPLC methods were developed using a 1.5 µm solid core C18 column and high pressure UHPLC system.

Sub 10 minute method times were achieved while maintaining peak shape resolution and retention time stability.

Introduction

Creating screening methods for multiple analytes is more cost-effective than dedicated methods for fewer analytes. By combining multiple analytes in one method, analysis times can be reduced providing quicker release of data, reduced costs per assay and greater sample throughput. To successfully develop fast methods for suites of similar compounds a column and system combination is required which can generate highly efficient separations with good peak shape and excellent reproducibility even at high flow rates.

This poster demonstrates how fast screening methods can be developed using a solid core 1.5 µm column in combination with a next-generation UHPLC system.

Methods were developed for the separation and analysis of 14 beta blockers; six statins; and 16 PAHs.

Methods

Instrumentation

Analyses were performed using a Thermo Scientific™ Vanquish™ UHPLC system consisting of:
- System base
- Binary pump
- Split sampler HT
- Column compartment H
- Diode array detector HL

Column: Thermo Scientific™ Accucore™ Vanquish™ C18+, 1.5 µm UHPLC column, 100 x 2.1 mm

Data Analysis

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

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Statins

Mobile phase A: Ammonium acetate, 20 mM, pH 4.0
Mobile phase B: Acetonitrile + 0.1% acetic acid
Flow rate: 650 µL/min
Column temp: 40 °C, still air with eluent pre-heating
Injection details: 2 µL standard needle in loop
UV detection: 240 nm

Solutions of pravastatin(1), amlodapine(2), fluvastatin(3), atrovostatin(4), cervistatin(5) and simvastatin(5) 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.

Table 1. UHPLC Gradient conditions for statins separation.

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

Beta Blockers

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

Solutions of sotalol(1), pindolol(2), timolol(3), acebutolol(4), metoprolol(5), esmolol(6), celiprolol(7), oxprenolol(8) labetalol(9), propranolol(10), betaxolol(11), carvidilol(12), nebivolol(13) and penbutalol(14) 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.

Table 2. UHPLC gradient conditions for beta blockers separation.

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

PAH

Mobile phase A: Methanol/water (50:50 v/v)
Mobile phase B: Acetonitrile
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: 254 nm

PAH calibration mix (Sigma-Aldrich®) contained10 µg/mL of each of the following components in acetonitrile: naphthalene(1), acenaphthylene(2), acenaphthene(3), fluorene(4), phenanthrene(5), anthracene(6), fluoranthene(7), pyrene(8), benzo[*a*]anthracene(9), chrysene(10), benzo[*b*]fluoranthene(11), benzo[*k*]fluoranthene(12), benzo[*a*]pyrene(13), dibenzo[*a,h*]anthracene(14), benzo[*g,h,i*]perylene(15), and indeno[1,2,3-*cd*]pyrene(16)

Table 3. UHPLC gradient conditions for PAH separation.

Time (min)	% B
0.00	20
0.30	20
4.50	50
8.00	100
8.00	20

Results

Analysis time
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 the screening method for 14 beta blockers can be achieved within a 4.5 minute detection window (and a full method cycle time of 6 minutes) (Figure 1). Separation of six statins was achieved in 6 minutes (Figure 2) and 16 PAHs with a full method cycle of 10 minutes (Figure 3).

Figure 1. Chromatogram showing the separation of fourteen beta blockers.

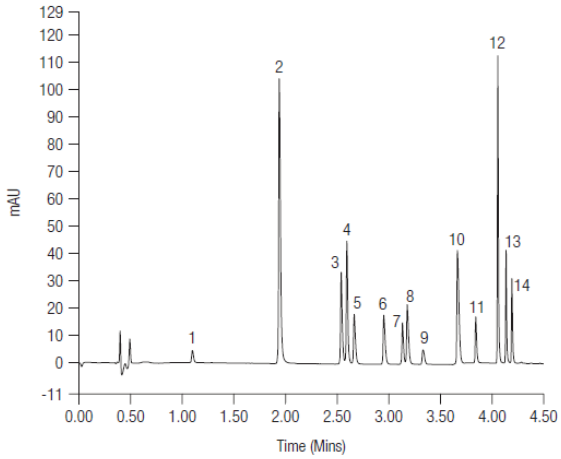


Figure 2. Chromatogram showing the separation of six statins.

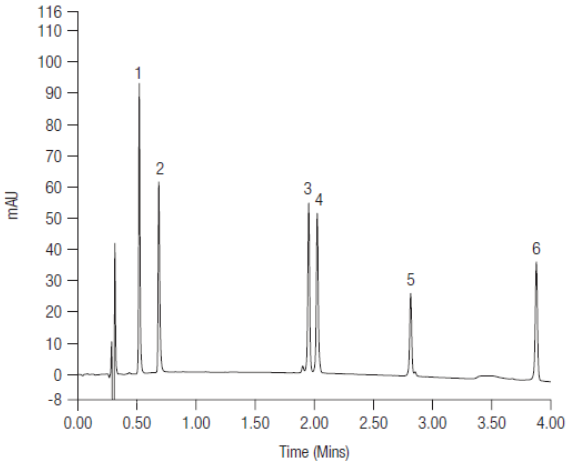
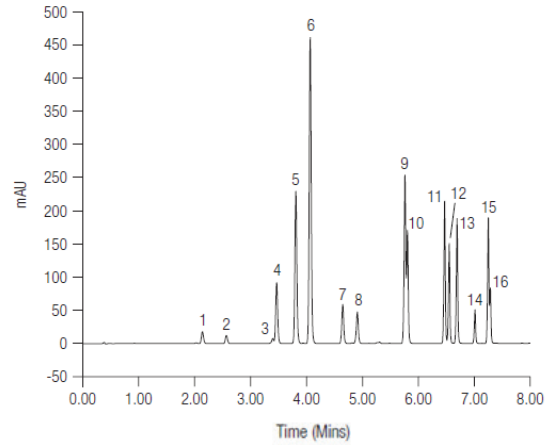


Figure 3. Chromatogram showing the separation of 16 PAHs.



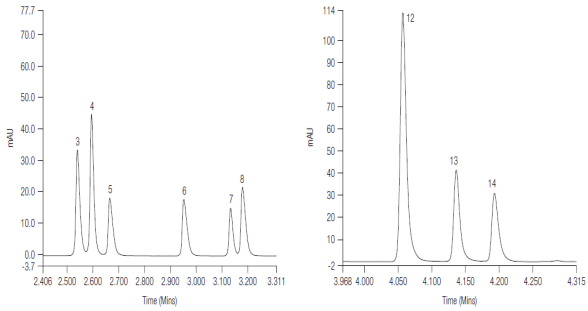
Retention time stability

Replicate injections showed that the UHPLC column produced stable and reproducible results for all three methods with variation not higher than 0.25% for all compounds in all three methods.

Resolution

The performance of the 1.5 µm UHPLC column has ensured that even with the fast method time, good separation of critical pairs has been achieved. For the beta blockers method resolution of > 2.00 for all pairs was achieved except celiprolol and oxprenolol at 1.53 (Figure 4). For the statins analysis, seperation of the critical pair fluvastatin and atrovostatin was achieved with a resolution of 2.54. The PAH method achieved seperation of all components except for the critical pairs chrysene/benzo[*a*]anthracene and indeno[1,2,3-*cd*]pyrene/benzo[*g,h,i*]perylene however, as a screening method, sufficient resolution to allow identification prior to conformation analysis is demonstrated.

Figure 4. Chromatogram showing the resolution of critical pairs for the beta blockers method.



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:

- Screening UHPLC methods for multiple compounds
- Significantly reduced method times
- Excellent retention time reproducibility
- Resolution of critical pairs

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