Application Note 21497

Rapid Screening Method for Polycyclic Aromatic Hydrocarbons Using an Advanced UHPLC Column and System

Derek Hillbeck, Thermo Fisher Scientific, Runcorn, UK

Key Words
Hypersil GOLD VANQUISH, PAH, polycyclic aromatic hydrocarbons

Goal

To provide an application for the analysis of polycyclic aromatic hydrocarbons using the Thermo Scientific™ Hypersil GOLD™ VANQUISH™ 1.9 µm UHPLC column and the Vanquish UHPLC system, and demonstrate the opportunity for increased throughput by operating at higher flow rates and backpressures.

A group of sixteen PAHs is targeted by the United States Environmental Protection Agency (EPA Method 610) as priority pollutants. The separation of these PAHs is demonstrated in this application.

Introduction

The complementing technologies of the Hypersil GOLD VANQUISH UHPLC column and the Vanquish UHPLC system have been designed to achieve the best possible chromatographic performance. Exploiting the 1500 bar high pressure capability of the Vanquish UHPLC systems gives greater flexibility in choice of column length, allowing either short duration screening methods or longer duration confirmatory methods with increased peak resolution. The system is optimized to reduce extra column band dispersion and allow users to significantly improve the separation power in their analytical assays.

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.

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Experimental

Consumables

- Hypersil GOLD VANQUISH, 1.9 µm UHPLC column, 100 × 2.1 mm (P/N 25002-102130-V)
- Hypersil GOLD VANQUISH, 1.9 µm UHPLC column, 150 x 2.1 mm (P/N 25002-152130-V)
- Hypersil GOLD VANQUISH, 1.9 µm UHPLC column, 200 x 2.1 mm (P/N 25002-202130-V)
- C-MS grade 18 MΩ·cm water from Thermo Scientific™ Barnstead™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ Optima™ UHPLC-MS grade methanol (P/N A458-1)
- Fisher Scientific Optima UHPLC-MS grade acetonitrile (P/N A956-1)
- 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

PAH calibration mix purchased from a reputable supplier contained 10 µg/mL of each of the following components in acetonitrile: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*a*)anthracene, chrysene, benzo(b)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenzo(*a,h*)anthracene, benzo(*ghi*)perylene, indeno(1,2,3-*cd*)pyrene

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	Methanol/water (50:50 v/v)
Mobile Phase B	Acetonitrile
Flow Rate	500 µL/min
Column Temperature	40 °C for linear gradient, 30 °C for non-linear gradient, still air with eluent pre-heating
Injection Details	1 µL
UV Detection	254 nm

The gradient curve profile was investigated with either a multi-linear or a non-linear gradient section. Summary of the parameters used in these methods are shown below in Table 1 and 2.

Table 1. Initial multi-linear gradient details.

Time	%B	Curve
0	20	5
0.3	20	5
4.5	50	5
8	100	5
8.1	20	5
10	20	5

Table 2. Non-linear gradient details.

Time	%B	Curve
0	20	5
0.3	20	5
8	100	8
8.1	20	5
10	20	5

Results and Discussion

By exploiting the non-linear gradient capabilities of the Chromeleon software, in conjunction with the Vanquish UHPLC system and the Hypersil GOLD VANQUISH UHPLC column, it was demonstrated that an initial screening method for 16 compounds could be improved. The non-linear gradient increased resolution between the critical pair of acenaphthene (3) and fluorene (4), and dispersed the other components more evenly across the timescale.

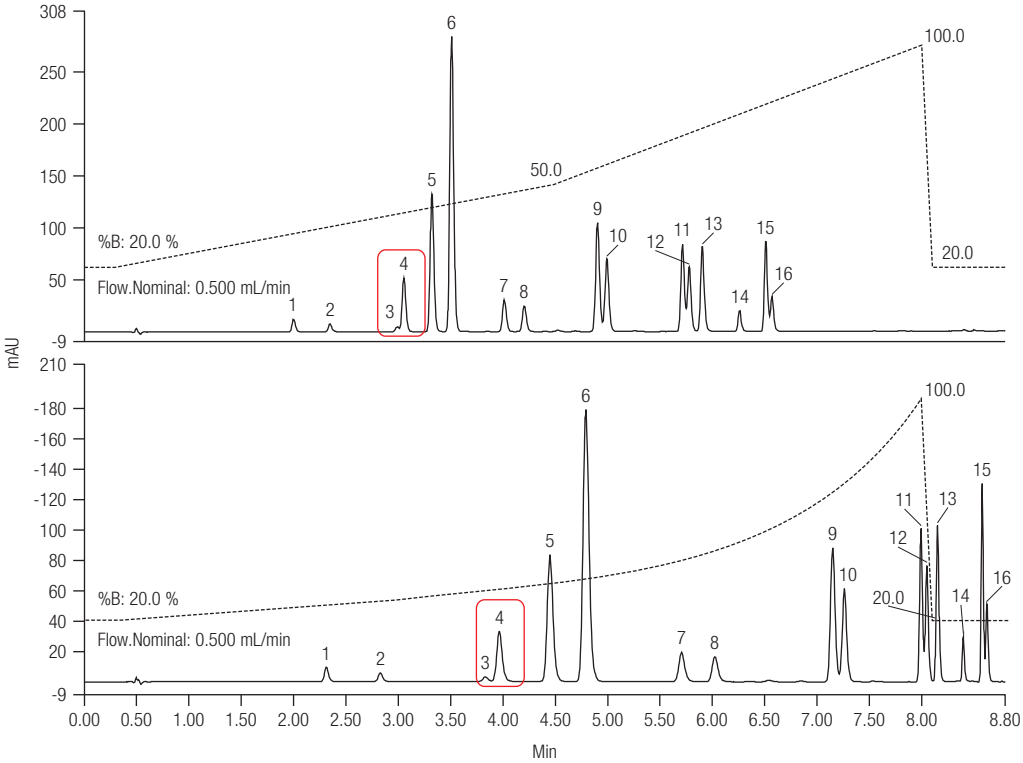

Figure 1. Chromatograms of PAH mixture using multi-linear (upper) and non-linear gradient (lower) profiles. Highlight shows critical pair of acenaphthene and fluorine.

Using a 500 µL/min flow rate, the maximum system pressure was 815 bar. The Vanquish UHPLC system and Hypersil GOLD VANQUISH UHPLC column are able to routinely operate at these pressure conditions, with the system and column capable of operating up to 1500 bar.

It was possible to improve peak resolution through the use of longer columns. Although the backpressure increased, it still fell within the operational limits of the system and column. The method parameters are easily scaled for the different column lengths by making use of the Chromeleon software in-built UHPLC method transfer tool.

Gradient times are shown in Table 3 and results are shown in Figure 2 and 3.

Table 3. Gradient times for three different column lengths.

Length (mm)	100	150	200
%B	Time (min)		
20	0.00	0.00	0.00
20	0.30	0.45	0.60
100	8.00	12.00	16.00
20	8.10	12.15	16.20
20	10.00	15.00	20.00

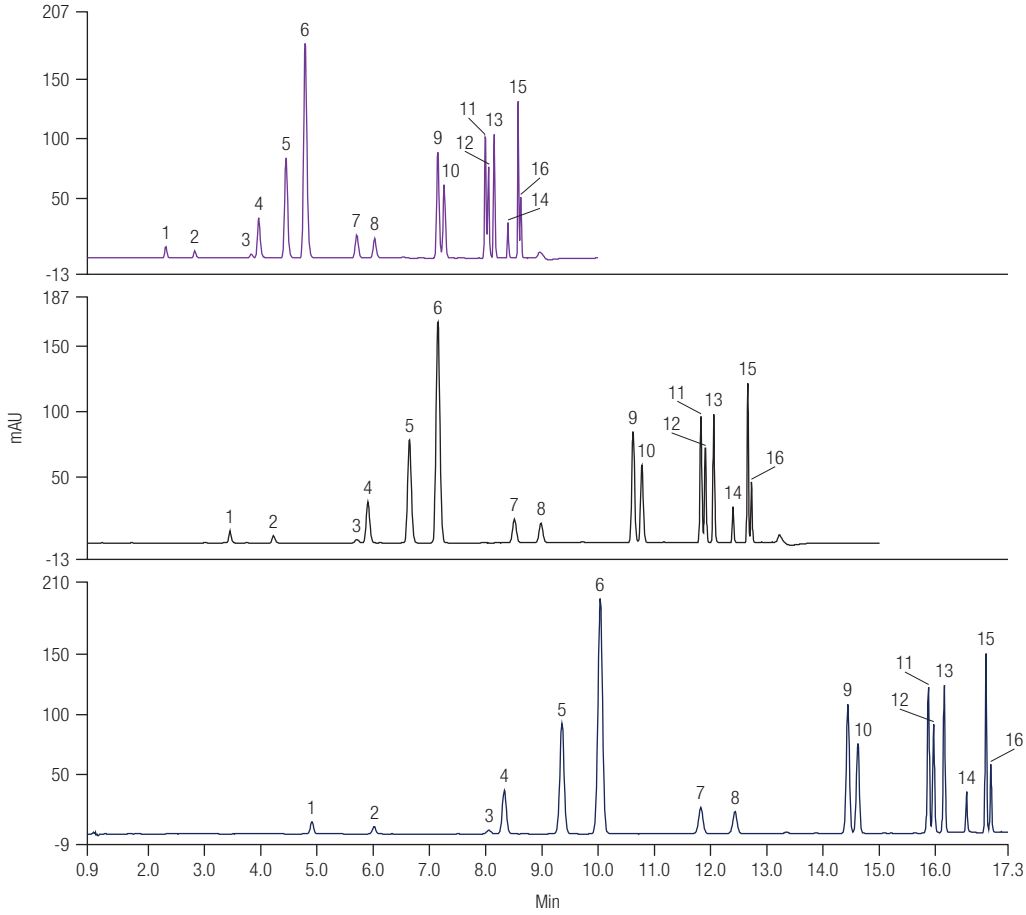

Figure 2. Comparison of PAH analysis on different length Hypersil GOLD VANQUISH UHPLC columns.

Upper trace: 100 mm column – max backpressure 815 bar
Middle trace: 150 mm column – max backpressure 1115 bar
Lower trace: 200 mm column – max backpressure 1370 bar

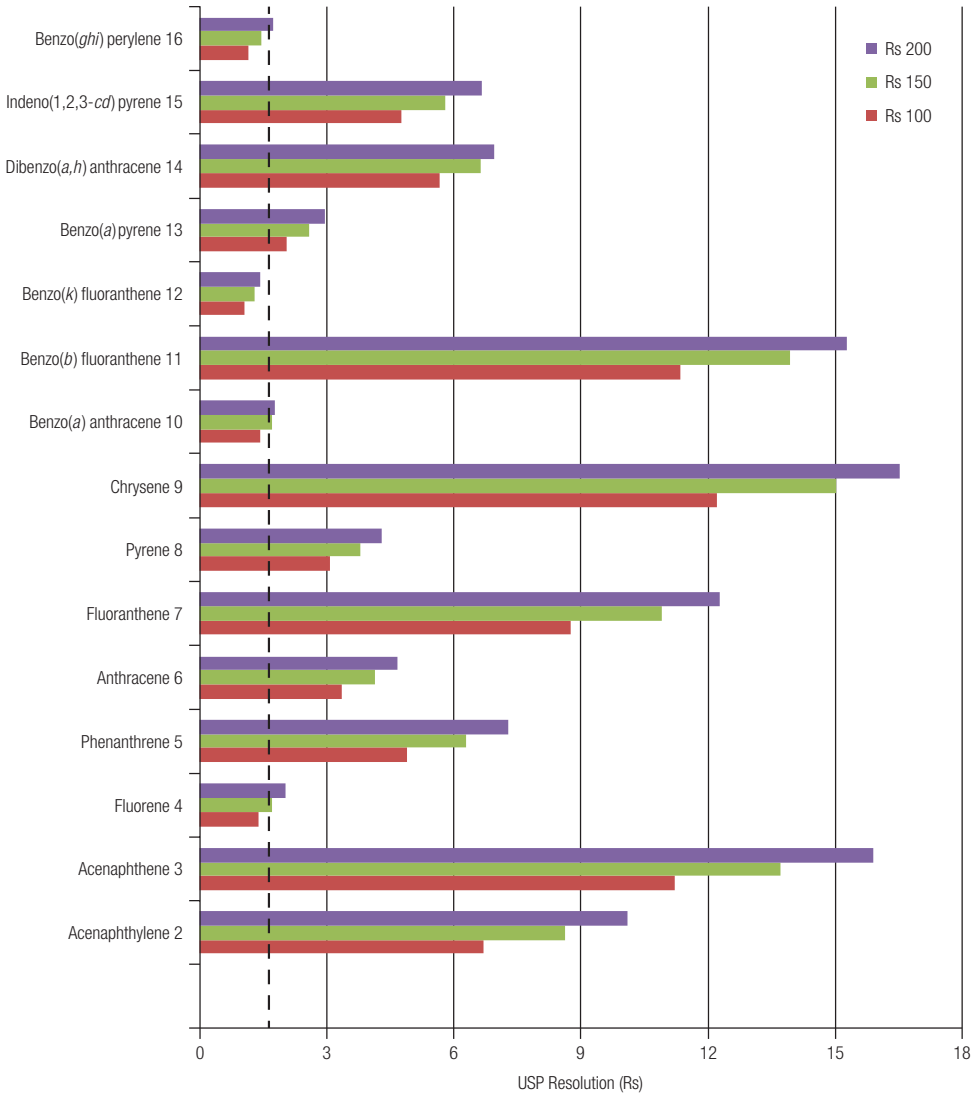

Figure 3. Resolution values between analyte peak and preceding peak for three column lengths. Dashed line indicates 1.5 value required for baseline separation.

Conclusion

This application demonstrates the advantages of using the Hypersil GOLD VANQUISH 1.9 µm UHPLC column and the Vanquish UHPLC system for the separation of PAHs. The performance of the Hypersil GOLD VANQUISH UHPLC column and the low internal volume and ultra-high pressure capabilities of the Vanquish UHPLC system, together with Chromeleon method scaling tools deliver:

- Rapid screening UHPLC method for 16 PAHs in 10 minutes
- Market-leading capability to operate at pressures above 1350 bar
- Freedom to increase column length for improved resolution
- Enhanced resolution for critical pairs using non-linear gradients

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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