

A High-Throughput Method for the Analysis of 22 Substituted Phenol Isomers Using a UHPLC Column and System Combination

Joanne Jones, Thermo Fisher Scientific, Runcorn, UK

Key Words

Vanquish, Hypersil GOLD, positional isomers, phenol, PFP

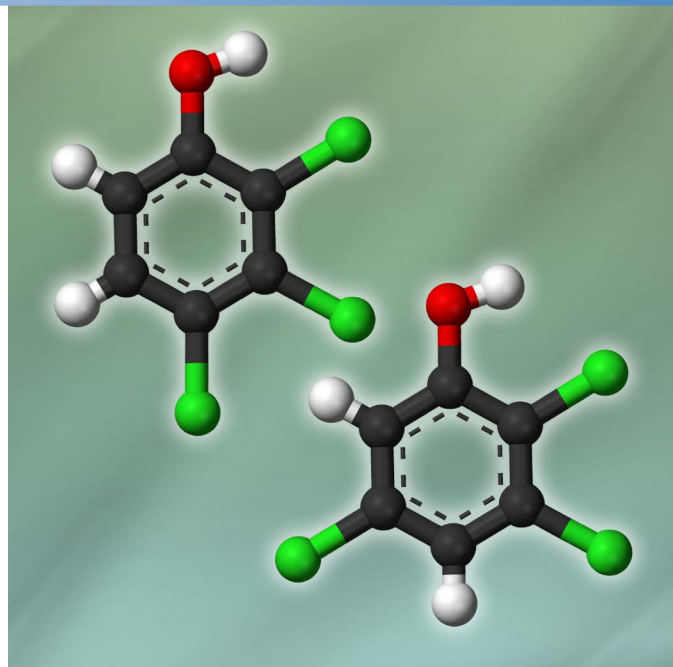
Goal

To demonstrate the advantages of using the Thermo Scientific™ Hypersil GOLD™ VANQUISH™ PFP 1.9 μm column and Vanquish UHPLC system for the analysis of 22 positional isomers.

Introduction

The complementing technologies of the Hypersil GOLD VANQUISH PFP UHPLC column and Vanquish UHPLC system allow for the best possible chromatographic performance. The low system gradient delay volume is ideal for high-throughput gradient separations. The system is optimized to reduce extra column band dispersion and allow users to significantly improve the separation power in their analytical assays. Sample pre-compression prior to injection and extremely low pump pulsation allows outstanding flow stability and improved column lifetime.

The Hypersil GOLD VANQUISH range of HPLC columns was developed to give reproducible and reliable chromatography analysis with excellent peak shape. Based on highly pure silica, Hypersil GOLD VANQUISH columns provide very symmetrical peaks, even when analyzing compounds that give poor peak shape on traditional silica-based chemistries. The introduction of fluorine groups into the Hypersil GOLD VANQUISH PFP (pentafluorophenyl) stationary phase causes significant changes in solute-stationary phase interactions. This can lead to extra retention and selectivity for positional isomers of halogenated compounds. Hypersil GOLD VANQUISH PFP columns are also well suited to the selective analysis of non-halogenated compounds, in particular polar compounds containing hydroxyl, carboxyl, nitro, or other polar groups. High selectivity is often most apparent when the functional groups are located on an aromatic or other rigid ring system.



The selectivity of the perfluorinated phase and the excellent reproducibility of both the UHPLC column and UHPLC system are demonstrated in the separation of 22 positional isomers.

Experimental

Consumables

- Hypersil GOLD VANQUISH PFP UHPLC column, 1.9 μm , 200 x 2.1 mm (P/N 25402-202130-V)
- LC-MS grade 18 M Ω -cm water from Thermo Scientific™ Barnstead™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ HPLC grade acetonitrile (P/N A/0626/17)
- Fisher Scientific™ Analytical grade formic acid (P/N 10559570)
- 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:

- Binary Pump H (P/N VH-P10-A)
- System Base (P/N VH-S01-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

Solutions of the 22 compounds shown in Figure 3 were prepared by dissolving 10 mg of compound in 10 mL of acetonitrile to produce 1 mg/mL primary solutions. Dilutions were then made with water to produce a 10 $\mu\text{g}/\text{mL}$ working solution.

Vial labeling was carried out by the Virtuoso Vial Identification System.

UHPLC Conditions

UHPLC Column	Hypersil GOLD VANQUISH PFP, 1.9 μm , 200 x 2.1 mm
Mobile Phase A	Water + 0.1% formic acid
Mobile Phase B	Acetonitrile + 0.1% formic acid
Flow Rate	0.4 mL/min
Column Temperature	40 °C, still air with eluent pre-heating
Injection Volume	1 μL
UV Detection	270 nm

LC gradient conditions

Time (minutes)	%B
0	35
8	45
8.01	35
13	35

Results and Discussion

The analysis of 22 positional isomers was completed in approximately 8 minutes (Figure 1). The Hypersil GOLD VANQUISH PFP column demonstrated excellent selectivity between the positional isomers with good separation achieved between the structurally similar compounds. Exceptional peak shape was observed across all of the compounds analyzed. The average (n=100) asymmetry values across all of the fully resolved peaks is demonstrated in Figure 2. Also highlighted are the individual asymmetry values recorded over 100 injections of 2-bromophenol. The consistency of the measured asymmetry values demonstrates the superior performance and stability of the column.

The excellent retention time reproducibility is displayed in Figure 3. The Vanquish UHPLC system contains a new pumping technology that results in extremely low pump pulsation. Furthermore, the autosampler features the unique intelligent sample pre-compression, which compresses the sample to system pressure prior to injection. This results in a very stable flow delivery and, consequently, unparalleled retention time precision. In addition, the use of advanced peak detection algorithms and data wizards in the Chromeleon CDS software provide for rapid development of data processing methods for complex samples, which leads to consistent peak assignment even when peaks are not fully resolved.

One hundred replicate injections of the sample mixture showed that the Vanquish UHPLC system and Hypersil GOLD VANQUISH PFP column produced stable and reproducible results whilst maintaining excellent peak shape.

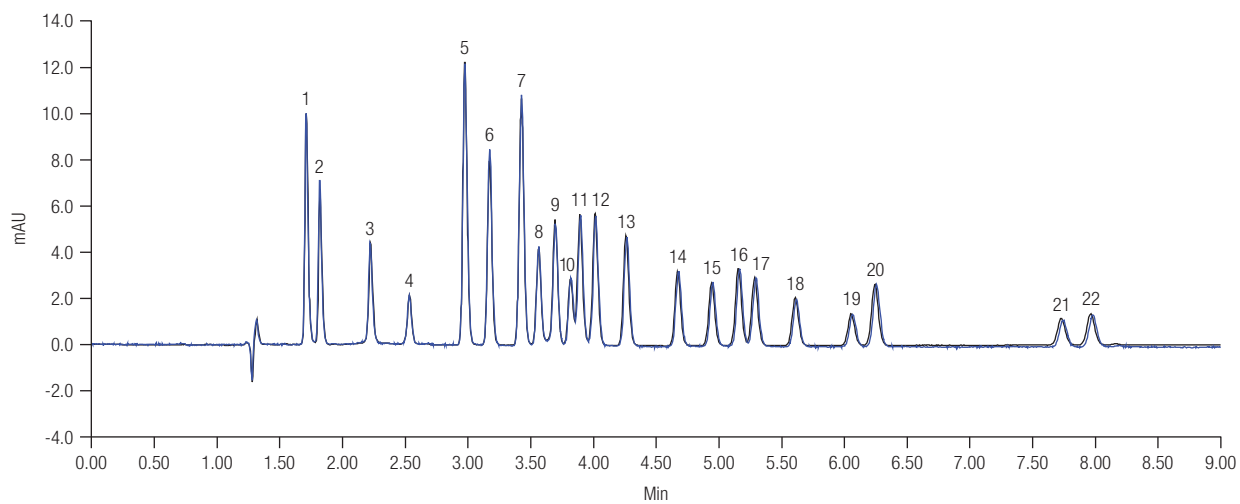


Figure 1. Overlaid chromatograms of injection 1 and injection 100 of 22 positional isomers.

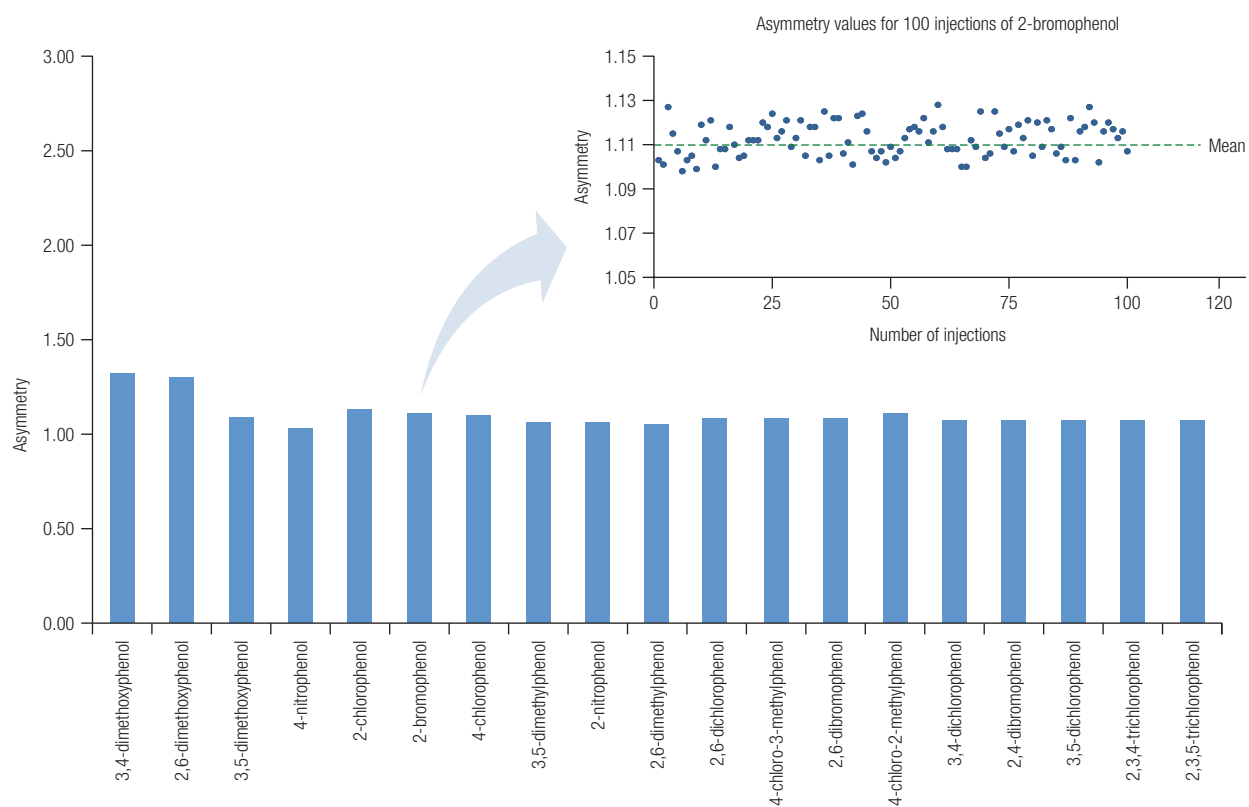


Figure 2. The average asymmetry for each fully resolved compound (n=100) and the individual asymmetry values for 2-bromophenol.

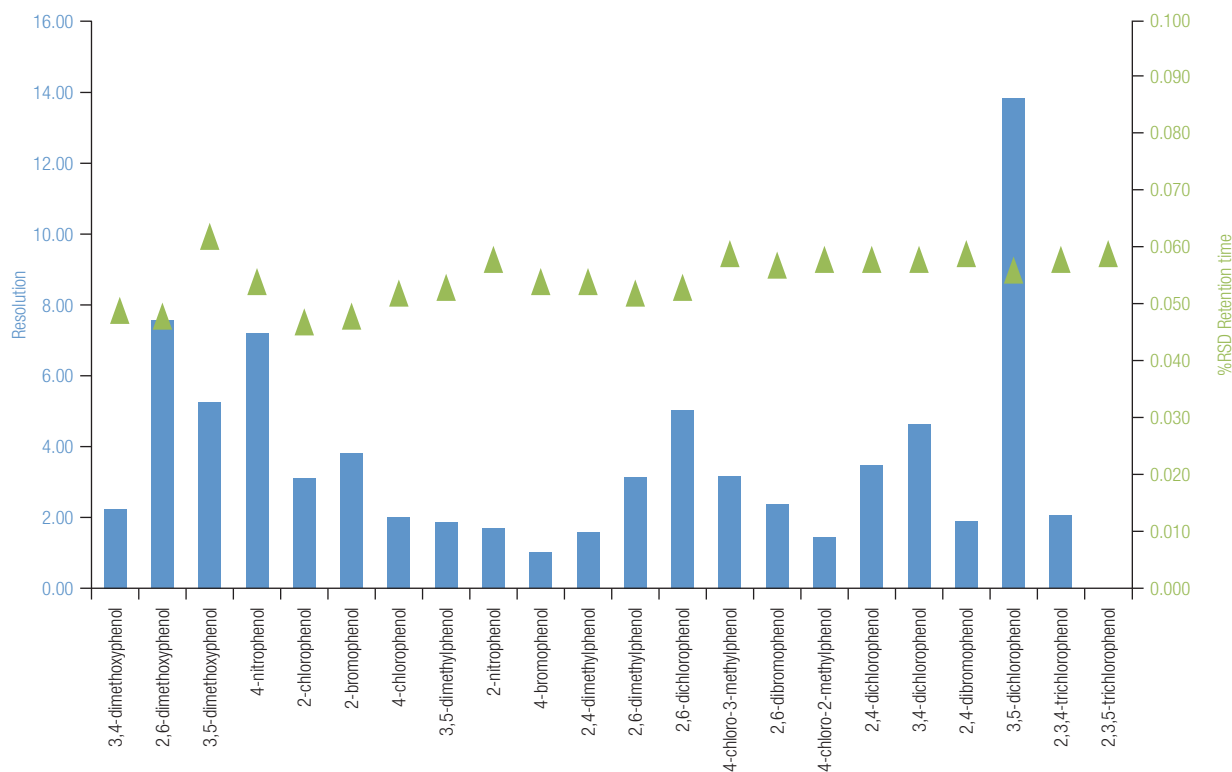


Figure 3. Peak identification, resolution, and retention time variability for 100 replicate injections.

Conclusion

This application demonstrates the advantages of using the Hypersil GOLD VANQUISH PFP 1.9 μm UHPLC column in conjunction with the Vanquish UHPLC system. The performance of the Hypersil GOLD VANQUISH PFP column coupled with the sample pre-compression and extremely low pulsation pump of the Vanquish UHPLC system, together with Chromeleon software delivers:

- UHPLC separation of 22 positional isomers
- Excellent and consistent peak shape
- Exceptional retention time reproducibility
- Consistent data processing

Useful Links

AppsLab Library

The eWorkflow and the Chromeleon Backup (cmbx) file can be downloaded at AppsLab Library:

www.thermofisher.com/appslab