Application Note: 411

Analyzing Phenolic Pollutants in Water Using U-HPLC

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Key Words

- Hypersil GOLD™ Columns
- Accela™ High Speed U-HPLC
- Surveyor Plus™ HPLC
- Phenols
- US EPA and EU Standards
- Water Pollutants

Overview

This study demonstrates analysis optimization by variation of column chemistry, and the viability of reducing stationary phase particle size to significantly increase analysis speed, while maintaining separation parameters and increasing sensitivity.

Introduction

Phenolic compounds are of particular environmental importance due to their relatively high toxicity at low levels and their presence in environmental waters and organic matter, following degradation of a range of industrial products such as pesticides and herbicides, as well as naturally occurring humic substances and tannins.

Previous studies[1,2] have shown that reversed-phase liquid chromatography (RP-LC) coupled to atmospheric pressure chemical ionization mass spectrometry (APCI-MS) can effectively separate and detect a range of phenolic compounds at low ppb levels, following various extraction methods. Such methods provide a realistic alternative to traditional analysis approaches using gas chromatography (GC), which involve lengthy sample preparation/analysis times and difficulty in derivatization of certain phenols.

In this study, the effect on the separation and analysis speed of a number of priority phenols cited within the U.S. Environmental Protection Agency (EPA) and European Union (EU) lists of priority pollutants[3] have been assessed by changing the chemistry and reducing the particle size of the stationary phase.

Materials and Methods

HPLC Columns

The effect of particle/column size variation on analysis speed and separation efficiency was studied using the following Hypersil GOLD columns (Thermo Fisher Scientific, Bellefonte, PA) and experimental conditions:

150 × 2.1 mm (5 µm particle size)
100 × 2.1 mm (3 µm particle size)
100 × 2.1 mm (1.9 µm particle size).
Mobile Phase: A) 0.1% Acetic Acid in Water
B) 0.1% Acetic Acid in Methanol.
Temperature: 60 °C
Detection: UV Diode array (270-320 nm),
Gradients, flow rates and injection volumes are listed in Table 1.
Phenols were prepared at a concentration of 5 ppm in Water:Methanol (95:5).

Stationary phase chemistry

The effect of stationary phase chemistry on the separation of five phenols (2-Chlorophenol, 4-Chlorophenol, 2-Nitrophenol, 4-Nitrophenol and 2,4-Dinitrophenol), using 1.9 µm particles, was studied using three column types (all 100 × 2.1 mm):

Hypersil GOLD
Hypersil GOLD aQ™ (polar endcapped C18)
Hypersil GOLD PFP (perfluorinated phenyl).
Analysis conditions were equivalent to those described within U-HPLC Method 1 (Table 1).

Instrumentation

A Thermo Scientfic Surveyor Plus HPLC system was used for 5 and 3 µm particle analyses, and a Thermo Scientific Accela U-HPLC system was used for 1.9 µm analyses.

Results

Effect of particle/column size on analysis speed and quality

The analysis times of eleven priority phenolic pollutants were significantly improved by reducing column dimensions from 150 to 100 mm and particle size from 5 µm to 3 µm. Further improvements were achieved by changing to 1.9 µm particles, using the Accela High Speed LC System.

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Typical chromatograms demonstrating improvements in analysis speed are provided in Figures 1 to 3.

Analysis time was further reduced by increasing the flow rate of the U-HPLC analysis to 1000 µL/min, without any adverse effects on resolution (Figure 3). This is illustrated in the Table inset in Figure 3, which indicates peak width and resolution values for all separations.

Stationary phase chemistry

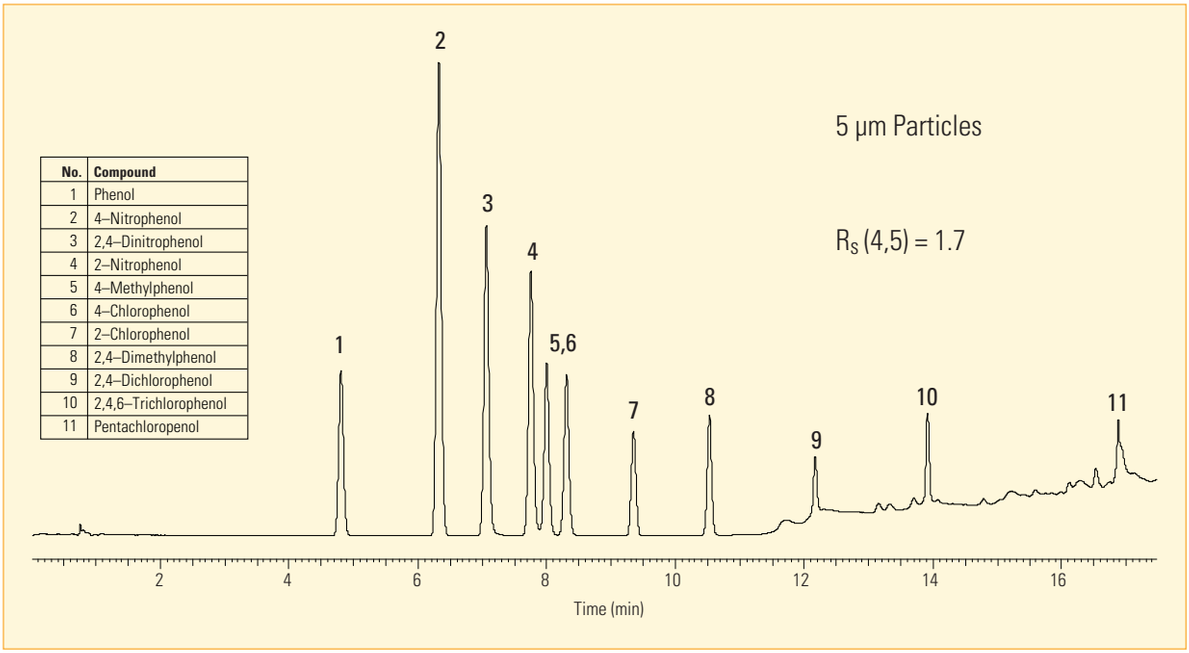
The Hypersil GOLD 1.9 µm phase produced the optimal overall separation of the chloro- and nitrophenols under the standard conditions used.

The Hypersil GOLD PFP (perfluorinated phenyl) phase showed superior selectivity between chlorophenol components, likely due to the unique selectivity enabled by the presence of fluorine in the stationary phase. However, the separation performance between the chloro- and nitrophenols was slightly reduced.

Chromatograms illustrating the effect on the separation of using different stationary phases are given in Figure 4, along with resolution values between 4- and 2-chlorophenol (R_S 6,7) and between 2-Nitro and 4-Chlorophenol (R_S 4,6).

Method A (Column 150x2.1 mm, 5 µm). Flow = 600 µL/min. Injection Volume = 5 µL.		**Method B** (Column 100x2.1 mm, 3 µm). Flow = 600 µL/min. Injection Volume = 1 µL.		**UHPLC Method 1** (Column 100x2.1 mm, 1.9 µm). Flow = 600 µL/min. Injection Volume = 1 µL.		**UHPLC Method 2** (Column 100x2.1 mm, 1.9 µm). Flow = 1000 µL/min. Injection Volume = 1 µL.	
Time (min)	**Eluent B (%)**	**Time (min)**	**Eluent B (%)**	**Time (min)**	**Eluent B (%)**	**Time (min)**	**Eluent B (%)**
0.0	5	0.0	5	0.0	5	0.0	5
1.5	5	1.0	5	1.0	5	0.6	5
19.5	95	13.0	95	13.0	95	7.8	95
21	95	14.0	95	14.0	95	8.4	95
21.01	5	14.01	5	14.01	5	8.41	5
22.5	5	15.0	5	15.0	5	9.0	5

Table 1: HPLC and U-HPLC gradients, flow rates, and injection volumes.



No.	Compound
1	Phenol
2	4–Nitrophenol
3	2,4–Dinitrophenol
4	2–Nitrophenol
5	4–Methylphenol
6	4–Chlorophenol
7	2–Chlorophenol
8	2,4–Dimethylphenol
9	2,4–Dichlorophenol
10	2,4,6–Trichlorophenol
11	Pentachloropenol

Figure 1: Separation of priority phenolic pollutants. using a 5 µm particle packed column.

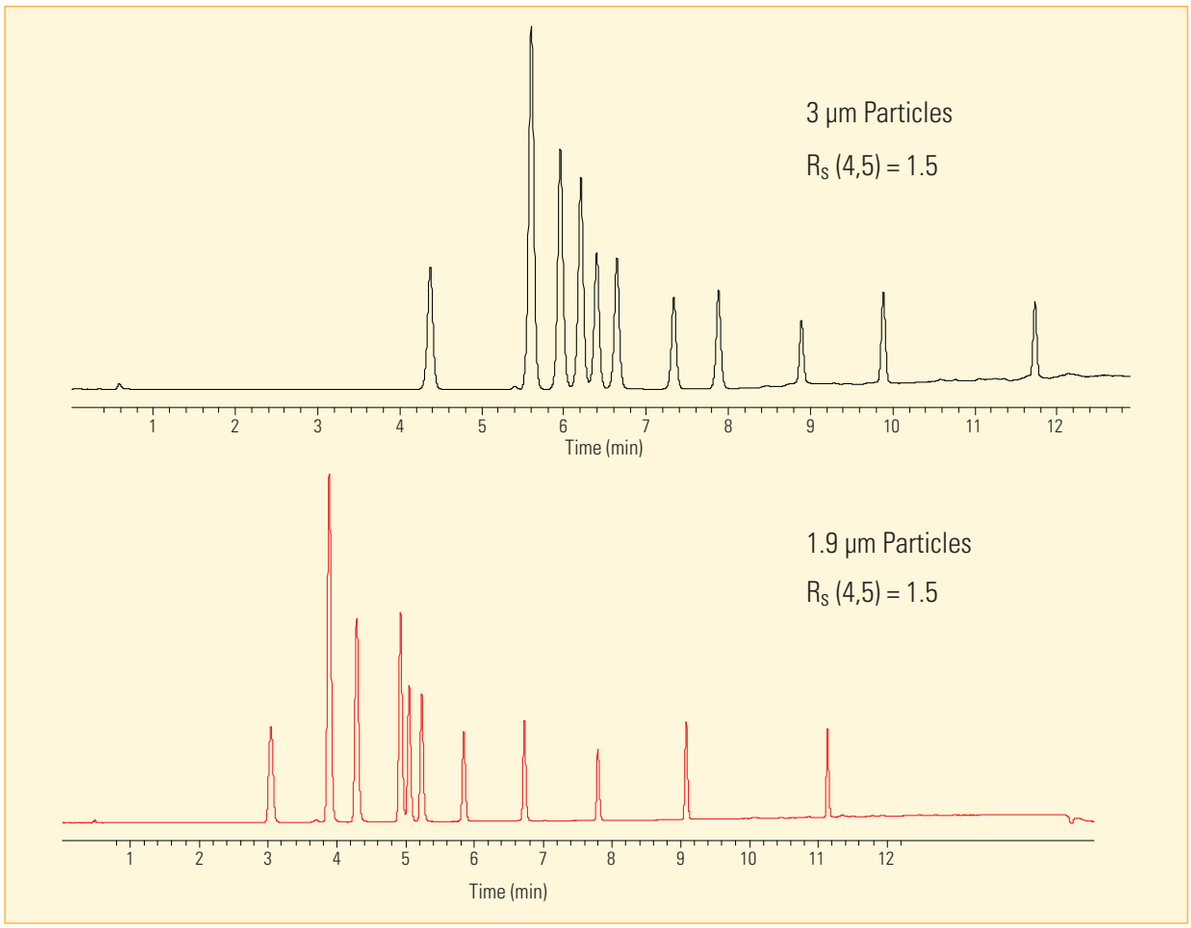
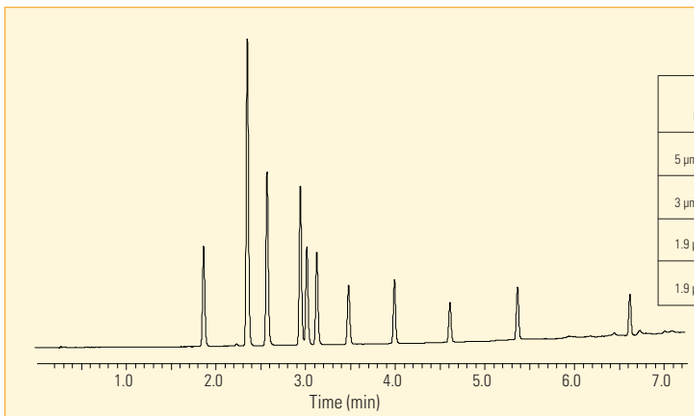


Figure 2: Chromatographic effect of variation in column dimensions (3 and 1.9 µm, 100 x 2.1 mm).



Column Dimensions	Method	Average Peak Width (mins)	Resolution (peaks 4,5)
5 µm. 150 x 2.1 mm	A	0.14	1.7
3 µm. 100 x 2.1 mm	B	0.13	1.5
1.9 µm. 100 x 2.1 mm	U-HPLC 1	0.09	1.5
1.9 µm. 100 x 2.1 mm	U-HPLC 2	0.06	1.3

Figure 3: Increased throughput using U-HPLC and 1.9 µm particles. Comparison of peak width (at 10% height) and resolution.

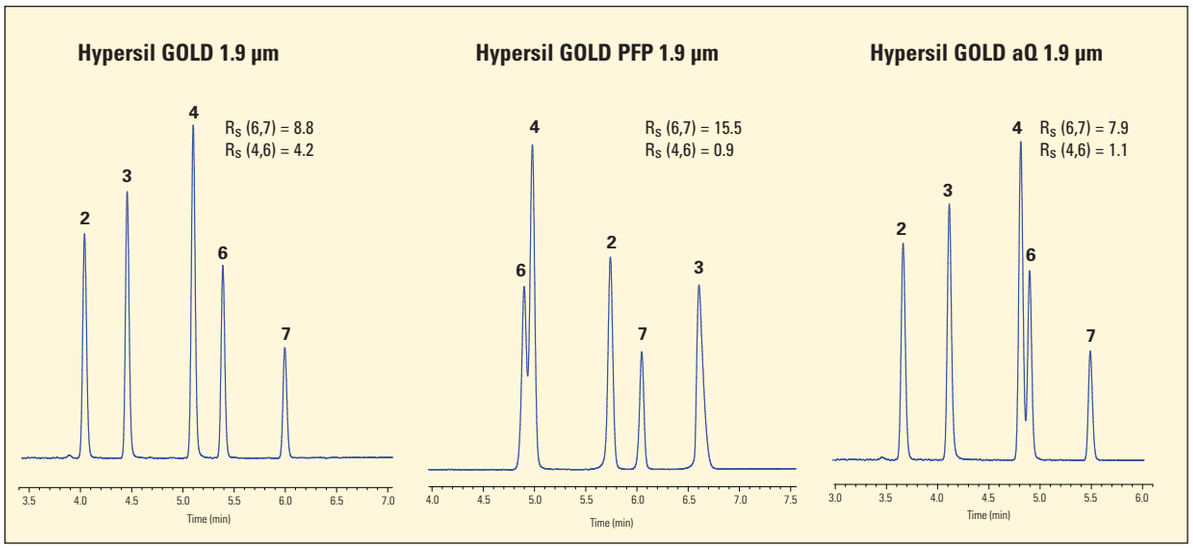


Figure 4: Comparison of 1.9 µm stationary phase chemistries for the separation of chloro- and nitrophenols.

Conclusions

A number of priority phenols can be successfully separated in shorter analysis times by transferring to U-HPLC methods using Hypersil GOLD 1.9 µm particle columns, without losing any significant resolution.

The increased peak efficiency observed for 1.9 µm particle packed columns indicates that low level phenol analyses in environmental matrices described in previous studies,[1,2] would be further enhanced with increased sensitivity.

Different column chemistries create important differences in selectivity for method development purposes, which may aid studies involving, for example, the separation of halophenols using a Hypersil GOLD PFP phase.

References

[1] M.C. Alonso, D. Puig, I. Silonger, M. Grasserbauer, D. Barcelo, *J Chromatogr. A*, 823 (**1998**) 231-239.

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[3] EPA Method 625, Phenols, Environmental Protection Agency, Part VIII, 40 CFR Part 136, Washington DC, 1984.

Additional Information

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