

# U-HPLC in the Analysis of Phenolic Pollutants in Water

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## Overview

An assessment of using U-HPLC for the analysis of phenolic pollutants in water is presented. This study demonstrates analysis optimisation by variation of column chemistry, and the viability of reducing stationary phase particle size to significantly increase analysis speed, whilst 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<sup>(1,2)</sup> have shown that reverse-phase liquid chromatography (RP-LC) coupled to atmospheric pressure chemical ionisation 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 tend to be hindered by lengthy sample preparation/analysis times and difficulty in derivatisation of certain phenols.

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

## Materials and Methods

### Effect of particle/column size on analysis speed and quality.

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

- 150 x 2.1 mm (5 µm particle size)
- 100 x 2.1 mm (3 µm particle size)
- 100 x 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), APCI-MS (negative ion mode).

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 analysis 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 x 2.1 mm):

- Hypersil GOLD
- Hypersil GOLD aQ™ (polar endcapped C18)
- Hypersil GOLD PFP (perfluorinated phenyl).

Analysis conditions were equivalent to those described within UHPLC Method 1 (Table 1).

### Instrumentation

A Surveyor™ HPLC system was used for 5 and 3 µm particle analyses and an Accela™ (Ultra High Pressure LC) system was used for 1.9 µm analyses (both Thermo Scientific, San Jose, CA).

TABLE 1. HPLC & U-HPLC gradients, flow rates and injection volumes

Method A (150x2.1mm, 5µm Column). Flow = 600 µl/min. Injection Volume = 5µl.		Method B (100x2.1mm, 3µm Column). Flow = 600 µl/min. Injection Volume = 1µl.		UHPLC Method 1 (100x2.1mm, 1.9µm Column). Flow = 600 µl/min. Injection Volume = 1µl.		UHPLC Method 2 (100x2.1mm, 1.9µm Column). Flow = 1000 µl/min. Injection Volume = 1µl.	
Time (Mins)	Eluent B (%)	Time (Mins)	Eluent B (%)	Time (Mins)	Eluent B (%)	Time (Mins)	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

## 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 ultra high pressure Accela system.

Typical chromatograms are provided in Figures 1 and 2, demonstrating improvements in analysis speed, whilst improving peak width and maintaining resolution (Table inset Figure 3).

FIGURE 1. Separation of Priority Phenolic Pollutants. Chromatographic effect of variation in column dimensions.

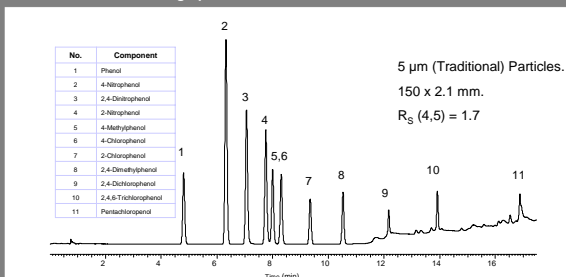
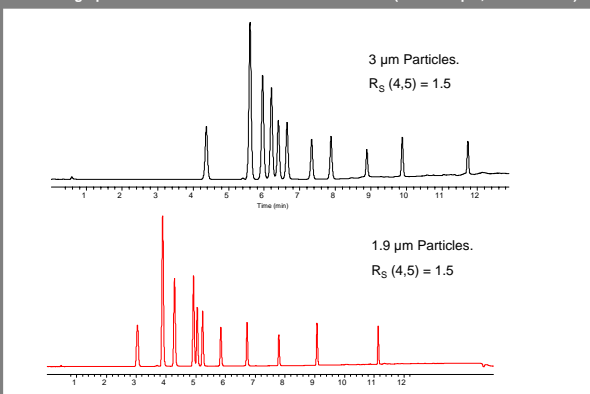


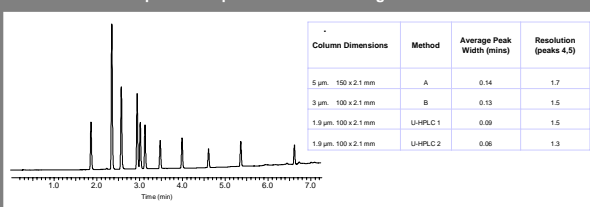
FIGURE 2.

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



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).

FIGURE 3. Increased Throughput using U-HPLC and 1.9 µm particles. Comparison of peak width at 10% height and resolution.



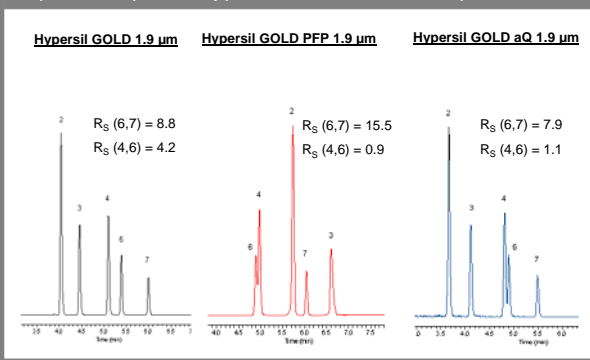
### Stationary phase chemistry

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 phase (perfluorinated phenyl) 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.

Example chromatograms are given in Figure 4, with resolution values between 4 and 2-chlorophenol ( $R_s$  6,7) and between 2-Nitro and 4-Chlorophenol ( $R_s$  4,6).

FIGURE 4. Comparison of 1.9 µm stationary phase chemistries for chloro & nitrophenols.



## Conclusions

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

• Increasing peak efficiency indicates that low level phenol analyses in environmental matrices described in previous studies,<sup>(1,2)</sup> 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. Silongier, M. Grasserbauer, D. Barcelo, J Chromatogr. A, 823 (1998) 231-239.
2. J. Martinez Vidal, A. Belmonte Vega, A. Garrido Frenich. Analytical & Bioanalytical Chem. 1. 379 (2004) 125-130.
3. EPA Method 625, Phenols, Environmental Protection Agency, Part VIII, 40 CFR Part 136, Washington DC, 1984.

## Additional Information

For additional information, please browse our chromatography Resource Centre which can be accessed from: <http://www.thermo.com/columns>

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