Fast Analysis of Oxidative Hair Dyes at High pH with Poroshell HPH-C18 and Other Phases

Application Note

Materials Testing and Research, Consumer Products

Authors

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Abstract

A method was developed for separating eight polar components typically found in hair dyes on the Agilent Poroshell HPH-C18 column using an Agilent 1290 Infinity LC Selectivity of different Poroshell HPH-C18 bonding phases was also compared to other Agilent Poroshell 120 phases, including PFP and Phenyl-Hexyl. All eight compounds were separated within 4 minutes. Poroshell HPH-C18 was selected for the analysis because of its enhanced lifetime under high pH mobile phase.

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Introduction

Hair dyes are used by people all over the world. Common hair dyes contain aromatic compounds consisting of modified anilines and phenolics that may cause allergic reactions, and may even potentially cause cancer. Due to these potential harmful effects, the amounts of these compounds are restricted in many countries. Methods for the quantitative measurement of compounds in hair dyes include GC, GC/MS, LC, LC/MS, and so forth. HPLC methods are popular because these compounds are not thermally stable for GC analyses, and are also strongly polar with low volatility. Previous work described HPLC methods using Poroshell 120 EC-C18 and Bonus-RP with acetate buffer at a mid pH of 6.8 [1,2]. To retain basic compounds, a mobile phase with higher pH (normally 1 to 2 pH units higher than the pKa of the compounds) is used for separation.

Agilent Poroshell HPH-C18, 2.7 µm columns are packed with superficially porous materials based on the Poroshell 120 family. These columns have almost the same efficiency as sub-2 µm totally porous materials and can be used to provide similar fast and high-resolution analyses. The column is designed to be stable in high pH mobile phases by integrating organic into the particle surface, thus resisting dissolution under extreme high pH and high temperature conditions. In this application note, we used a Chinese industrial standard for cosmetics [3]. An isocratic method was developed on a Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm column at pH of 7.7.

Materials and methods

The Agilent 1290 Infinity LC included a binary pump, a thermostatic column compartment, a high performance auto-sampler, and a diode array detector. The columns used were Agilent Poroshell HPH-C18, Agilent Poroshell 120 PFP, and Agilent Poroshell 120 Phenyl-Hexyl.

The compounds separated were 1. *p*-phenylenediamine, 2. *p*-aminophenol, 3. hydroquinone, 4. 2,5-diaminotoluene, 5. *m*-aminophenol, 6. *o*-phenylenediamine, 7. resorcine, and 8. *p*-methylaminophenol.

Compounds 1, 2, 3, 5, 6, and 7 were prepared in solvent A at 3 mg/mL. Compounds 4 and 8 were prepared separately in solvent B, then mixed together and diluted to 0.3 mg/mL each with solvent A. One milliliter of the mixture was diluted to 10 mL with solvent B and filtered through a 0.20-µm filtration membrane before injection.

Solvent A was 0.1% Na_2SO_3 in ethanol, made by dissolving 0.5 g sodium sulfite (GR grade) in 25 mL ultrapure water followed by addition of 475 mL ethanol (HPLC grade). Solvent B was 0.1% Na_2SO_3 in water, made by dissolving 0.1 g sodium sulfite (GR grade) in 100 mL ultrapure water.

Results and Discussion

The recent practice of using superficially porous particle columns for fast HPLC analysis has shown that the columns provide similar efficiency to sub-2 µm columns but at 40 to 50% less backpressure, often below 400 bar. These results are achievable because superficially porous particles have a shorter diffusion path than totally porous particles and a much tighter particle size distribution. These two particle features mean the small, superficially porous particles generate high efficiency, similar to the efficiency of sub-2 µm columns.

The retention time of ionizable compounds is easily affected by mobile phase pH. Normally, 1 to 2 pH units lower than the pKa of acidic compounds or 1 to 2 pH units higher than the pKa of basic compounds are used to restrain ionization and hence increase retention on reversed-phase columns. Nevertheless, when using a high pH mobile phase for basic compound separation, especially with pH values above 8 or 9, silica-based columns will have a short lifetime due to dissolution of the silicaceous particles.

Poroshell HPH columns are designed to be stable up to pH 10 mobile phase, achieved by integrating organic into the silica surface, which resists degradation under high pH conditions. By using these columns, chromatographers can obtain the ultrahigh efficiency of these superficially porous particles and enhance column lifetime even at high pH and temperature. Poroshell 120 PFP columns are excellent choices for polar analytes and analytes with pi-characteristics due to their ability to undergo unique pi-pi interactions. Like the PFP phase, Poroshell 120 Phenyl-Hexyl columns are also suitable for analytes with pi-characteristics. PFP and Phenyl-Hexyl columns provide alternative phase chemistries for unique selectivity that is orthogonal to C18 chemistries.

The hair dyes included in the Chinese industrial standard for cosmetics are polar and aromatic compounds. In this application, we tried three different phases, shown in Figure 1. Under the same conditions, all eight compounds were baseline separated in only 3.5 minutes. The three different phases showed different selectivity. However, for a pH 7.7 mobile phase, we recommend Poroshell HPH-C18, which enhances column lifetime.

Conditions, Figure 1

Columns:	Agilent Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-702)
	Agilent Poroshell 120 PFP, 4.6 × 100 mm, 2.7 µm (p/n 695975-408)
	Agilent Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 2.7 µm (p/n 695975-312)
Mobile phase:	Triethanolamine 10 mL/L in ultrapure water (adjust to pH 7.7 with phosphoric acid):ACN (95:5)
Temperature:	30 °C
Flow rate:	1.5 mL/min for 4.6 mm id columns, 0.64 mL/min for 3.0 mm id column
Injection volume:	2 µL on 4.6 mm columns, 0.85 µL on 3.0 mm column
Detector:	UV, 280 nm

Peak ID, all figures

1. *p*-Phenylenediamine
2. *p*-Aminophenol
3. Hydroquinone
4. 2,5-Diaminotoluene
5. *m*-Aminophenol
6. *o*-Phenylenediamine
7. Resorcine
8. *p*-Methylaminophenol

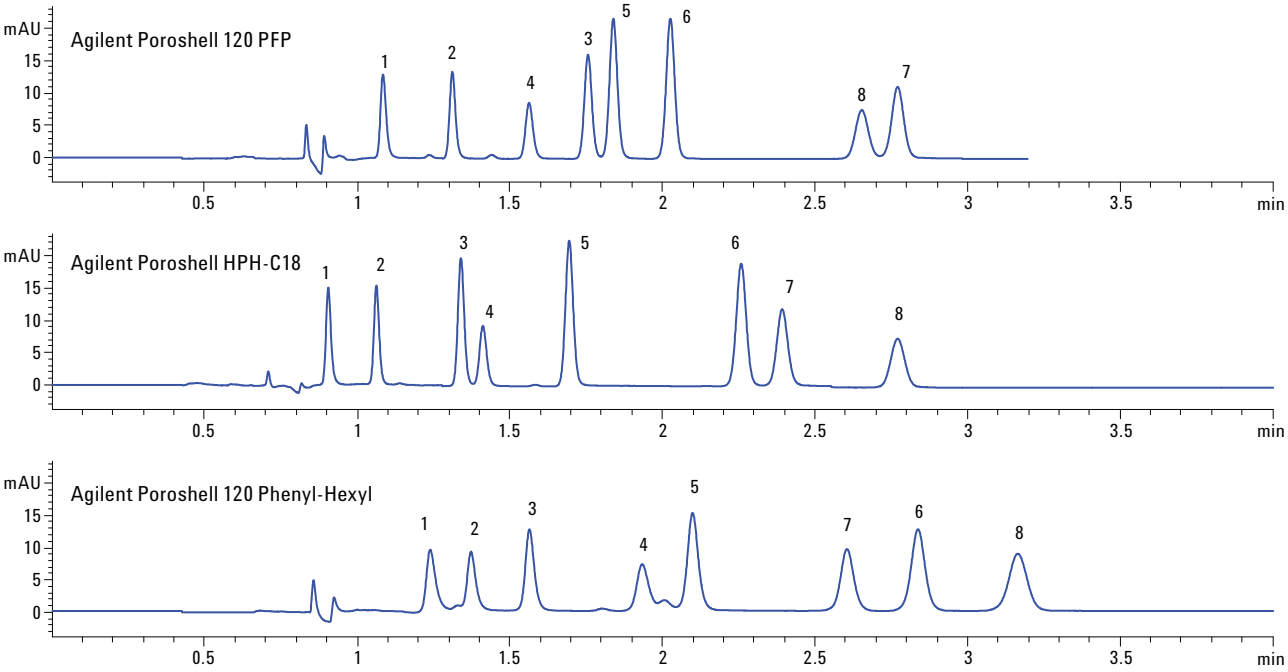

Figure 1. Selectivity comparison of different phases in the Agilent Poroshell 120 family.

Column temperature is an important parameter for method development, and is usually critical for resolution. The original column temperature was 20 °C, but compounds 3 and 4 were not resolved. We investigated four different temperatures, as shown in Figure 2. When increasing column temperature from 20 to 35 °C, the resolution of compounds 3 and 4 improved, while that of compounds 6 and 7 was reduced. A temperature of 30 °C was optimum for separating all compounds, and was suitable for this method.

Conditions, Figure 2

Columns:	Agilent Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-702)
Mobile phase:	Triethanolamine 10 mL/L in ultrapure water (adjust to pH 7.7 with phosphoric acid):ACN (95:5)
Temperature:	Shown in Figure 2
Flow rate:	1.5 mL/min
Injection volume:	2 µL
Detector:	UV, 280 nm

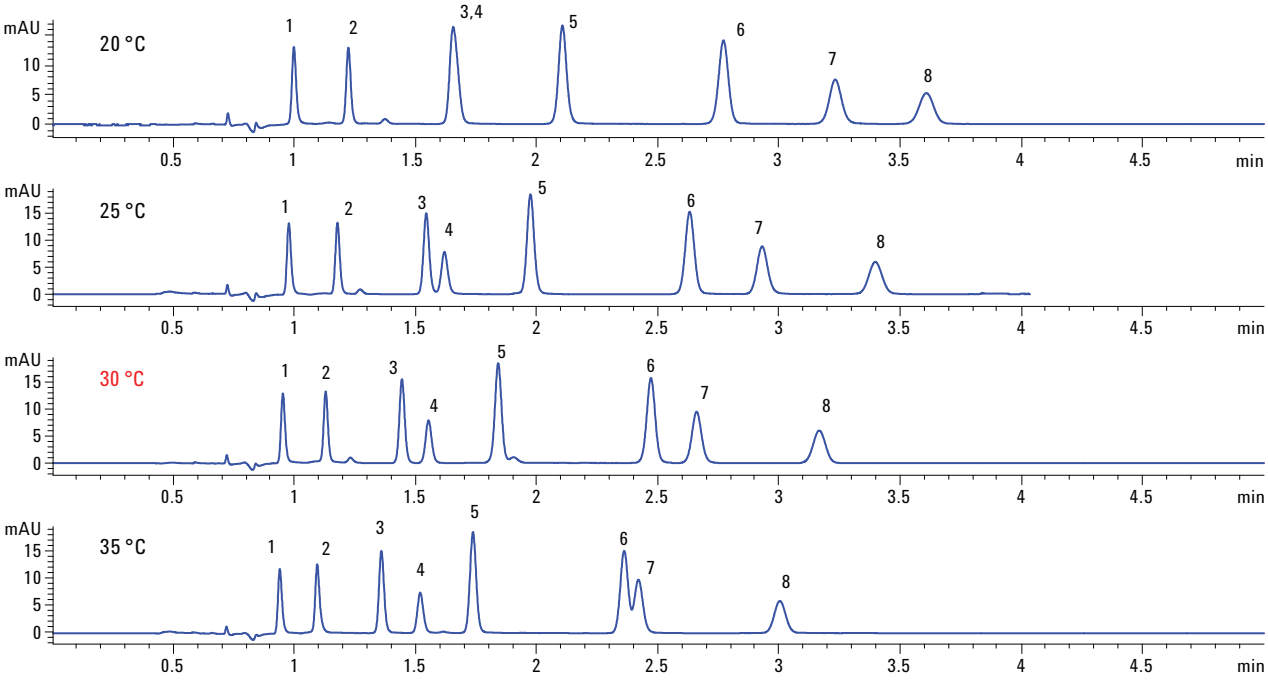

Figure 2. Optimizing separation using different column temperature on Agilent Poroshell HPH-C18.

Mobile phase composition is easy to investigate by changing the organic percentage. When reducing acetonitrile composition, resolution improves for all eight compounds and several degraded compounds. An appropriate mobile phase composition should be chosen in line with the real sample matrix. Here, we only ran a standard mixture, shown in Figure 3.

Conditions, Figure 3

Columns:	Agilent Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-702)
Mobile phase:	Triethanolamine 10 mL/L in ultrapure water (adjust to pH 7.7 with phosphoric acid):ACN
Temperature:	30 °C
Flow rate:	1.5 mL/min
Injection volume:	2 µL
Detector:	UV, 280 nm

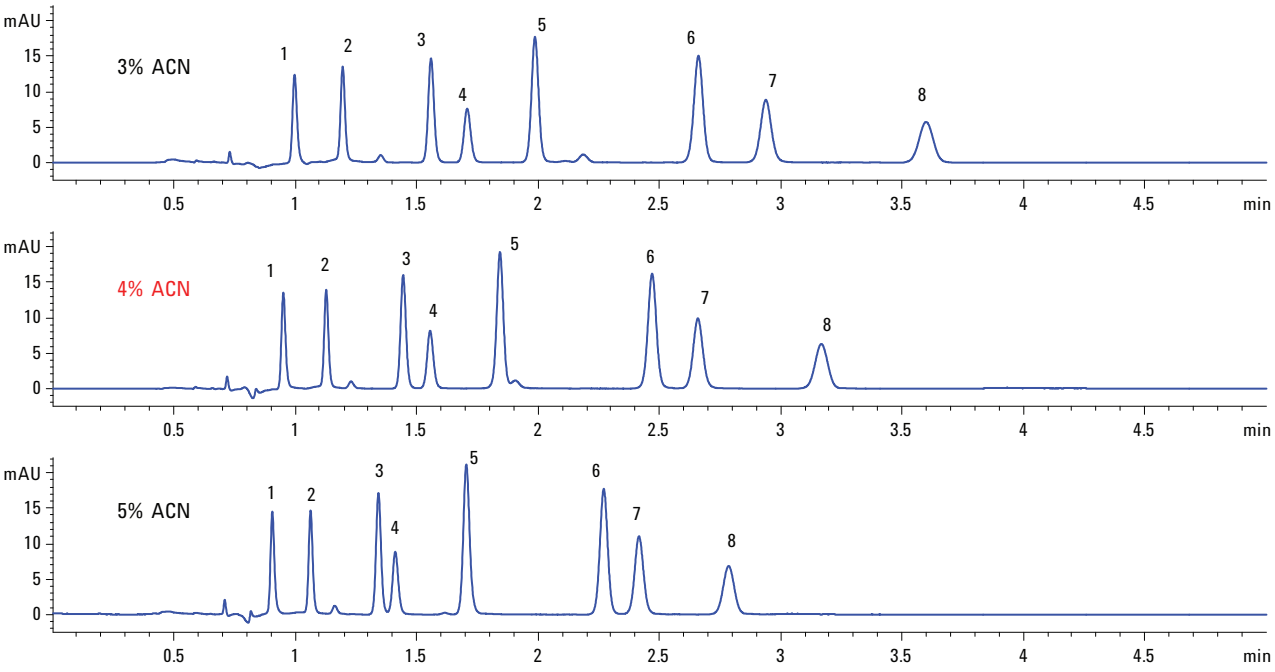

Figure 3. Separation comparison under different organic solvent composition on Agilent Poroshell HPH-C18.

To achieve reliable HPLC results, column-to-column reproducibility is very important. Figure 4 shows chromatograms from different batches of Poroshell HPH-C18. The retention time and resolution of the eight compounds demonstrate excellent reproducibility.

Conditions, Figure 4

Columns:	Agilent Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-702)
Mobile phase:	Triethanolamine 10 mL/L in ultrapure water (adjust topH 7.7 with phosphoric acid):ACN (96:4)
Temperature:	30 °C
Flow rate:	1.5 mL/min
Injection volume:	2 µL
Detector:	UV, 280 nm

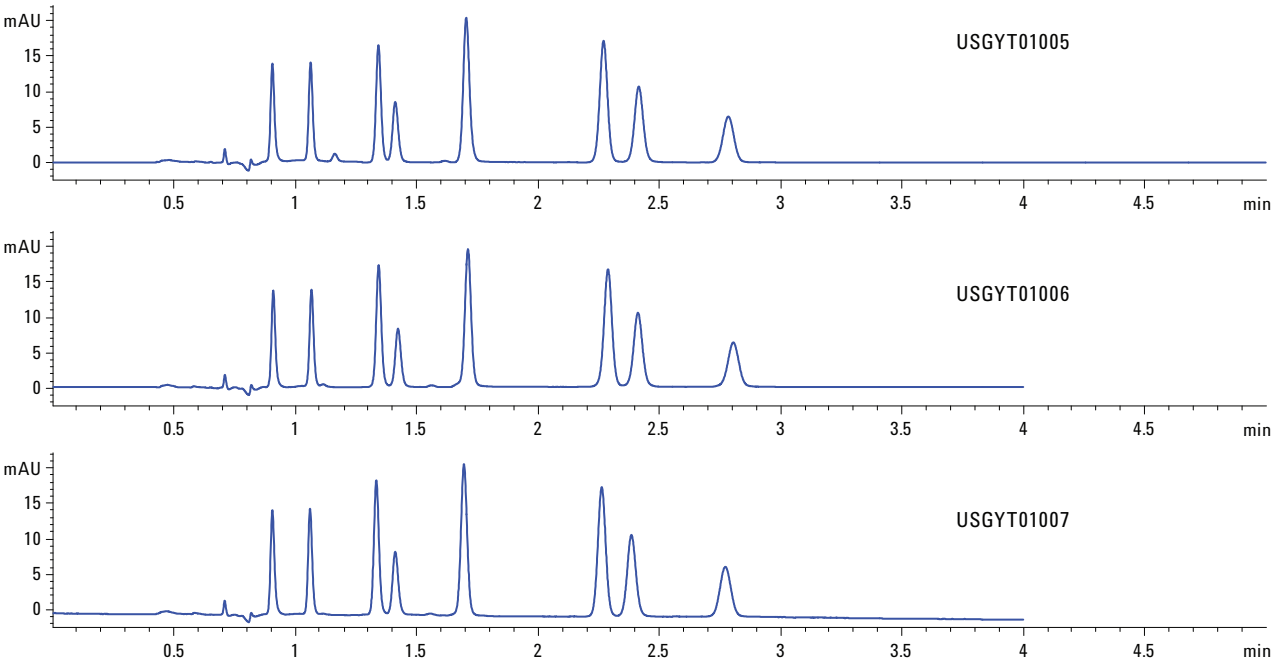

Figure 4. Reproducibility on three different serial numbers of Agilent Poroshell HPH-C18 columns.

Conclusions

The method developed using an Agilent Poroshell HPH-C18, PFP, and Phenyl-Hexyl easily and quickly separates eight hair dye components. All the columns provide good resolution, and are suitable for fast screening of these compounds. However, considering column lifetime, the Poroshell HPH-C18 column provides more reliable results under a basic mobile phase.

The Poroshell HPH-C18 phase is based on superficially porous 2.7 µm particles. The columns provide similar performance to totally porous sub-2 µm columns but with lower pressure. Due to the low pressure, the method could be run on a traditional 400 bar instrument and on a UHPLC up to the 600 bar pressure limit of the column, for ultrafast analysis. Poroshell HPH-C18 columns have excellent reproducibility.

References

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