Analysis of Hair Dyes Using Agilent InfinityLab Poroshell HPH-C18 4 µm Columns

Application Note

Materials Testing and Research, Consumer Products

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Introduction

Hair dyes commonly contain aromatic compounds consisting of modified anilines and phenolics. Due to potentially harmful effects, the amounts of these compounds in hair dye 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 strongly polar with low volatility. A HPLC method was developed and modified on an Agilent InfinityLab Poroshell HPH-C18, 2.7 µm column with a basic mobile phase at a pH of 7.7 [2]. In this application note, the previously developed method was transferred to the InfinityLab Poroshell HPH-C18, 4 µm column.

InfinityLab Poroshell HPH-C18 columns are packed with superficially porous particles, and are part of the Agilent InfinityLab Poroshell 120 family. The column is designed to be stable when used with high pH mobile phases by integrating organic material into the porous outer silica layer of the Poroshell particles. Thus, the column resists dissolution under extreme high pH and high temperature conditions. The superficially porous 2.7 µm columns have almost the same efficiency as sub-2 µm totally porous particle columns. Therefore, these columns can be used to provide fast, high-resolution analyses, while generating lower backpressure. The InfinityLab Poroshell HPH-C18, 4 µm columns provide double the efficiency of 5 µm totally porous columns, and are scalable from InfinityLab Poroshell HPH-C18, 2 µm columns.

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Experimental

Materials and Methods

The Agilent 1290 Infinity LC included a binary pump, a thermostatic column compartment, a high performance autosampler, and a diode array detector. The following columns and SPE cartridges were used:

- Agilent InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-702)
- Agilent InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 4 µm (p/n 695970-702)
- Agilent InfinityLab Poroshell HPH-C18, 4.6 × 150 mm, 4 µm (p/n 693970-702)
- SPE cartridges: Agilent Bond Elut C18, 500 mg, 6 mL (p/n 12102052)

Compounds separated

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

To produce stock solutions, 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 and diluted to 0.3 mg/mL each with solvent A.

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

Sample preparation

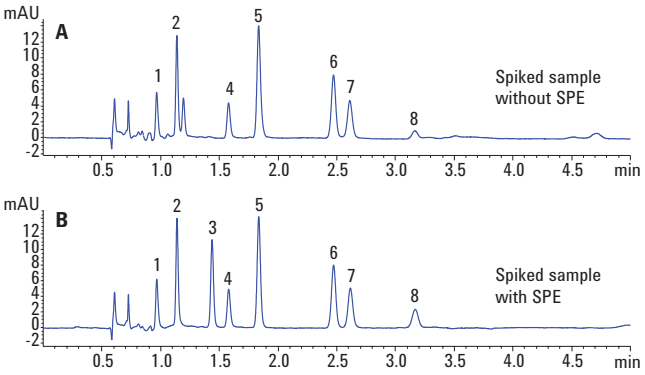
Hair dye gel (0.5 g) was weighed into a polypropylene centrifuge tube, then 10 mL of acetonitrile was added to the tube. The mixture was vortexed thoroughly for 1 minute, following which the sample solution was ready for the SPE cleanup.

SPE cartridges were preconditioned with 3 mL of acetonitrile. The sample solution was loaded onto a cartridge and passed through under gravity (about 1 mL/min). The first mL of eluted solvent was discarded, and the second mL of solvent eluted from the cartridge was collected. The collected sample was diluted to 1/10 with solvent B, and transferred to a 2 mL vial for analysis.

Results and Discussion

The analytes used for this application note are extremely unstable when exposed to air, therefore, it is recommended that the standards mixture and samples are freshly made and analyzed immediately. Hair dyes are primarily gel formulations containing many excipients including polymer compounds. These dyes are easily dissolved in the extraction solvents, and adhere in the column when they are injected for analyzing; the columns are easily clogged, and may even be killed quickly. To remove the polymer compounds in the sample matrix, a solid phase extraction (SPE) method was used in this method. The extraction procedure is described in the sample preparation section. Figure 1 shows the chromatograms produced. Compared to the spiked sample treated with SPE cleanup, the sample without SPE cleanup showed changes in several of the peaks. These changes demonstrated that most of the compounds were degraded more quickly in the sample matrix without SPE cleanup than with SPE treatment. As shown in Figure 1, peak 3 was not found, peak 8 was becoming smaller, and an extra peak eluting just after peak 2 was found in the sample without SPE cleanup.

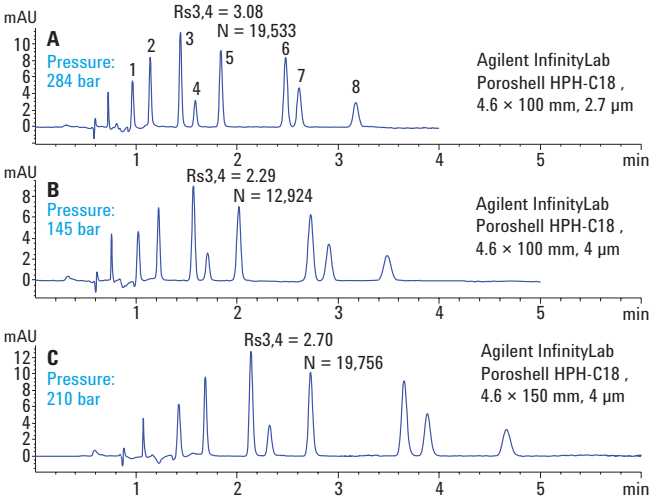
The original method was developed and modified on an InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm column. Figure 2 shows the method transferred from the 2.7 µm column to the 4 µm column, using 100 mm and 150 mm long columns. The same boning chemistry of the 4 µm column was scalable from the 2.7 µm column, and had the same selectivity as the 2.7 µm column. The 4 µm column had a much lower backpressure than the 2.7 µm column, and 70% of the efficiency of the 2.7 µm column. The method could easily be transferred to a long column with increased resolution and efficiency.



Conditions

Column:	Agilent InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-702)
Mobile phase:	Triethanolamine 10 mL/L in ultrapure water (adjusted to pH 7.7 with phosphoric acid): ACN (96:4)
Temperature:	30 °C
Flow rate:	1.5 mL/min
Injection volume:	5 µL
Detector:	UV, 280 nm

Figure 1. A comparison of the chromatograms produced between spiked sample with SPE cleanup treatment (B) and without SPE cleanup treatment (A) using Agilent InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm columns.



Conditions

Columns:	Agilent InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-702) Agilent InfinityLab Poroshell HPH-C18, 4.6 × 150 mm, 4 µm (p/n 693970-702)
Mobile phase:	Triethanolamine 10 mL/L in ultrapure water (adjust to pH 7.7 with phosphoric acid): ACN (96:4)
Temperature:	30 °C
Flow rate:	1.5 mL/min
Injection volume:	5 µL for 100 mm column, and 7.5 µL for 150 mm column
Detector:	UV, 280 nm

Figure 2. Method transferred from an Agilent InfinityLab Poroshell HPH-C18, 2.7 µm column to Agilent InfinityLab Poroshell HPH-C18, 4 µm columns.

The two samples marked A and B were purchased in a local shop. The spiked sample was produced by mixing 2.0 mL of stock solution into a polypropylene centrifuge tube with 0.5 g of sample and 8.0 mL of acetonitrile, and was treated according to the SPE procedure. Figure 3 and Figure 4 show the chromatograms of spiked and nonspiked sample. Using this method, both spiked samples showed good recoveries by SPE:

- In sample A, three target compounds were detected.
- In sample B, there was no target compound detected.

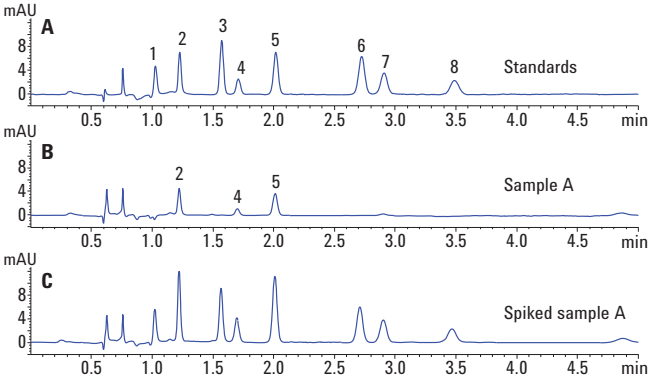


Figure 3. Overlay chromatograms of sample A and spiked sample using an Agilent InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 4 µm column.

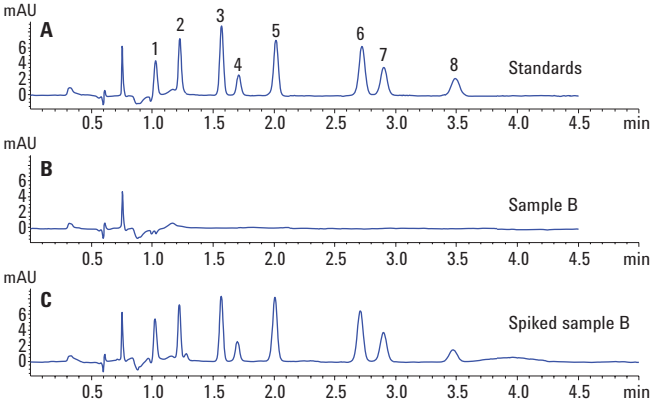


Figure 4. Overlay chromatograms of sample B and spiked sample by an Agilent InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 4 µm column.

Conclusions

The existing method using an Agilent InfinityLab Poroshell HPH-C18, 2.7 µm column could easily be transferred to 4 µm columns with similar selectivity. They are scalable from an InfinityLab Poroshell HPH-C18, 2.7 µm column. The sample preparation by SPE is an effective way to protect the column from contamination with dirty samples. In addition, the cleaned sample enhanced the stability of the analytes in the solution.

References

1. Zhu, H.; Yang, Y.; Zhang, W.; Zhu, Y. Determination of 22 components in hair dyes by high performance liquid chromatography. *Chinese Journal of Chromatography* **2008**, *26(5)*, 554-558.
2. Rongjie Fu, Qifu Lei. *Fast Analysis of Oxidative Hair Dyes at High pH with Poroshell HPH-C18 and other Phases*; Application note, Agilent Technologies, Inc. Publication number 5991-5263EN, **2014**.

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