Fast Analysis of Hair Dyes Using an Agilent Poroshell 120 EC-C18 Column

Application Note

Cosmetics

Author

Rongjie Fu
Agilent Technologies (Shanghai) Co. Ltd.
412 Ying Lun Road
Pu Dong, Shanghai 200131
China

Abstract

A method was developed for separating 17 components typically used in hair dyes with the Agilent Poroshell 120 EC-C18 column using an Agilent 1200 Series SL LC System. All the compounds were separated within 10 minutes. Two commercial samples of hair dyes were analyzed to test the method with complex samples.

Introduction

Hair dyes are used by people all over the world. Commonly used hair dyes contain modified aromatic aniline and phenolic compounds that may cause allergic reactions or cancer. Due to these potentially harmful effects, the amounts of these compounds are restricted in many countries.

Methods for the quantitative measurement of these compounds in hair dyes include GC, GC/MS, LC and LC/MS [1]. HPLC methods are popular because they can analyze compounds that are not thermally stable, but are strongly polar with low volatility.

The Agilent Poroshell 120 EC-C18, 2.7 µm columns are packed with superficially porous materials. These columns have almost the same efficiency as that for sub-2-µm totally porous materials and provide similar fast and high resolution analyses. For this application, a gradient method was developed on an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm column. In this study, two commercial samples were analyzed and the dye compounds detected. Within 10 minutes, 17 compounds were well separated from other components in the hair dyes.

Experimental

The Agilent 1200 Series SL LC System includes a binary pump, a thermostatted column compartment (TCC), a high performance autosampler and a diode array detector (DAD). The column used in the application is an Agilent Poroshell 120 EC-C18 3.0 × 100 mm, 2.7 µm (p/n 695975-302).

The following compounds were separated: p-phenylenediamine, p-aminophenol, 3-aminophenol, 1,3-benzenediol, 2-methylresorcinol, 2-chloro-1,4-diamino-benzol-sulfat, 2-nitro-1,4-penylenediamine, 5-amino-o-cresol, 2-amino-5-methylphenol, 4-amino-3-nitophenol, o-phenylene-diamine, 6-hydroxyindole, 4,n,n-diethy-2-methyl-p-phenylene-diamine, 1,5-dihydroxy-naphthalin 2,7-dihydroxy-naphthalin, n-phenyle-1,4-phenylene-diamine, 1-naphthol, o-aminophenol.

Each compound was prepared in methanol at 10 mg/mL, mixed together, and diluted down to a level of 0.05 mg/mL each with 2 g/L sodium hydrogen sulfite solution.

The two samples were purchased locally and designated A and B. A 0.5-g amount of each sample was extracted using 10 mL of acetonitrile, then placed in an ultrasonic bath for 10 minutes. The solution was then filtered through a 0.2-µm regenerated cellulose filter. The filtered solution was transferred to an auto sampler vial for HPLC analysis.

Discussion

Chromatographers use sub-2-µm particle columns for fast HPLC analysis because they achieve excellent results. A UHPLC with a pressure limit of greater than 400 bar is often used for fast, high resolution analysis with sub-2-µm particle columns. However, Agilent Poroshell 120 columns have 2.7-µm superficially porous particles that provide similar efficiency to the sub-2-µm columns but at 40–50% less back pressure, at below 400 bar. This makes them suitable for HPLC instruments. These are achievable results because superficially porous particles have shorter diffusion paths and a much tighter particle size distribution than totally porous particles. These two particle features mean the small, superficially porous particles generate high efficiency, similar to the efficiency of sub-2-µm columns.

Figure 1 shows the separation of 17 potential hair dye standard components on an Agilent Poroshell 120 EC-C18 column in a 10-min gradient. Reasonable resolution is achieved between all the sample components as well as some minor impurities present in the sample. It is likely that the impurities were oxidative degradation products of the major compounds that were not sufficiently stabilized by the addition of the sodium hydrogen sulfite solution.

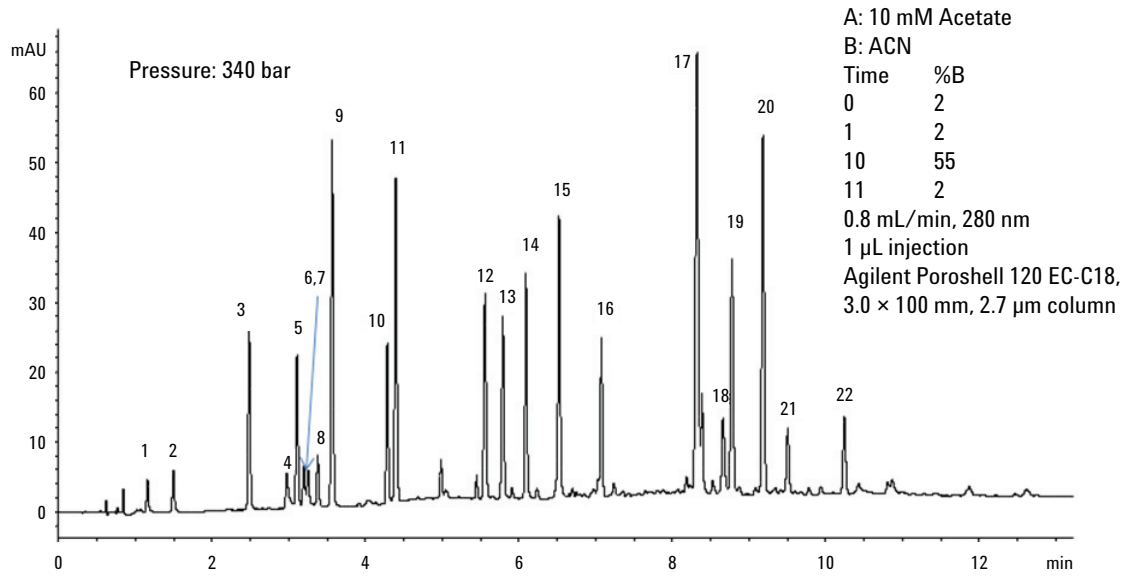

Figure 1. Standards chromatogram using Agilent Poroshell 120 EC-C18, 2.7 µm column. Standards: 1. p-phenylenediamine 2. p-aminophenol 3. 3-aminophenol 4. o-phenylenediamine 5. 1,3-benzenediol 6.unknown 7. 2-chloro-1,4-diamino-benzol-sulfat 8. 2-methylresorcinol 9. 2-nitro-1,4-penylenediamine 10. 5-amino-o-cresol 11. 4-amino-3-nitophenol 12. 6-hydroxyindole 13. 1,5-dihydroxy-naphthalin 14. 2,7-dihydroxy-naphthalin 15. unknown 16. unknown 17. n-phenyle-1,4-phenylene-diamine 18. 2-amino-5-methylphenol 19. 1-naphthol 20. o-aminophenol 21. unknown 22. unknown.

A 3.0 mm internal diameter column was used for this separation at a flow rate of 0.8 mL/min, which is almost twice the normal flow rate of the 0.4–0.5 mL/min typically used with this column ID. This produced a fast separation with no compromise in performance, due to the fast diffusion in the particles. Many may relate this to the flat region of the Van-Deemter curve at high flow rates – the "C" term. The Poroshell 120 2.7 µm particles have a Van-Deemter curve similar to columns with 1.8 µm particles. The performance of the Poroshell column does not decrease at high flow rate, compared to that of columns with 1.8 µm packing, but has lower pressure (340 bar), which is compatible with that of the 400-bar instruments. An example Van Deemter curve is shown in Figure 3.

Figure 2 shows an overlay of the chromatograms for Samples A and B and the standards. Only compound 12 (6-hydroxyindole) was found in Sample A and compounds 1 (p-phenylenediamine), 3 (3-aminophenol) and 5 (1,3-benzenediol) were detected in Sample B. Different components are used in hair dyes produced by different manufacturers. The amounts of detected compounds can be measured given the concentration of standards.

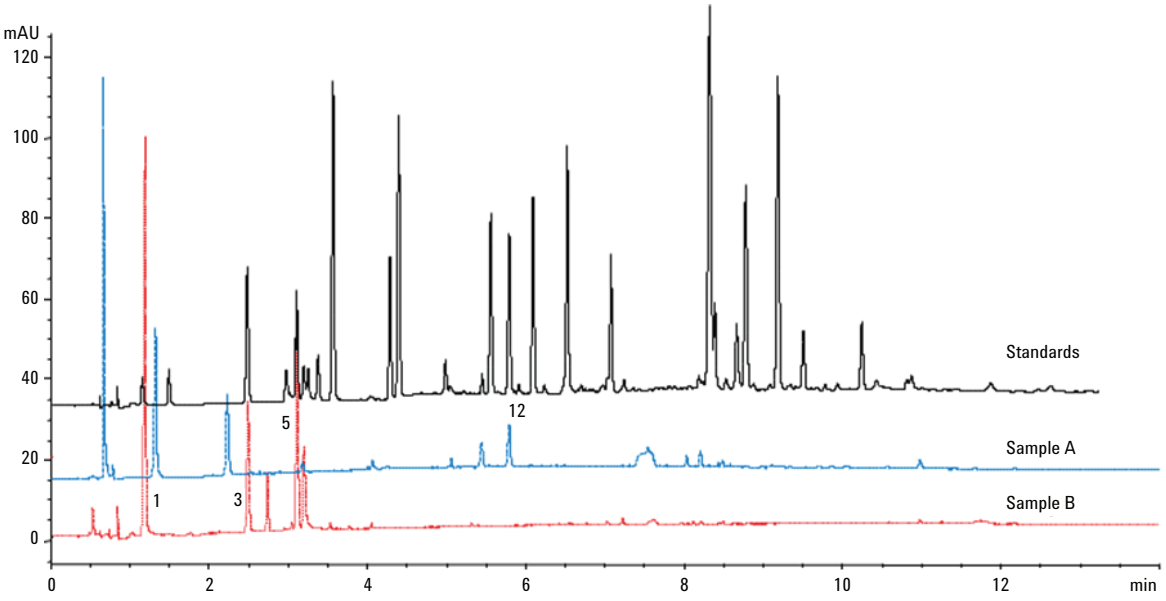

Figure 2. Chromatogram overlay for samples and standard components.

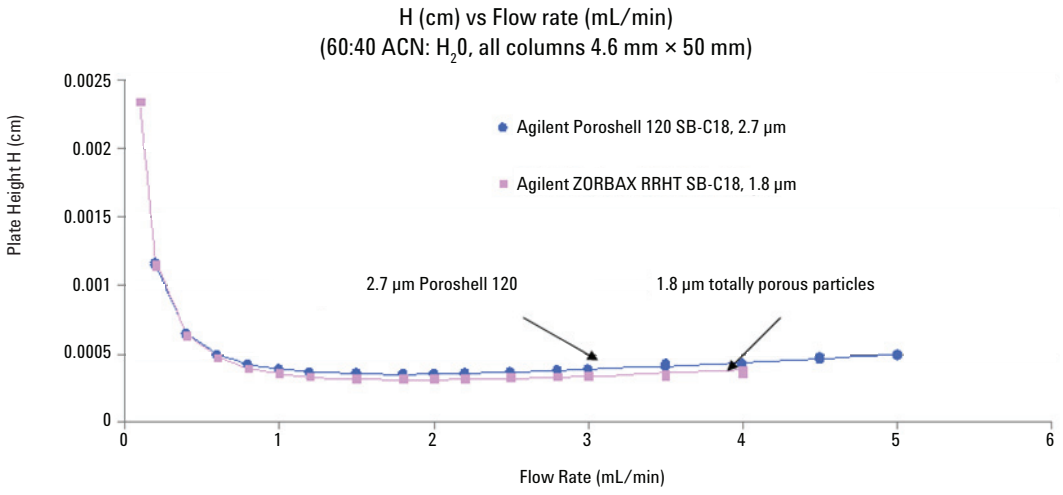

Figure 3. Van Deemter curve of Agilent Poroshell 120 column versus totally porous 1.8 µm particle columns.

Conclusion

The method developed for the separation of hair dye components on the new Agilent Poroshell 120 EC-C18 column provides good resolution and is suitable for fast screening of these compounds. The superficially porous 2.7 µm particle columns provide similar performance to the totally porous sub-2-µm columns but with lower pressure. Due to the low pressure, a 400-bar instrument can run this method. A higher flow rate allows faster separations on a UHPLC, up to the 600-bar pressure limit of the column.

Reference

1. "Determination of 22 components in hair dyes by high performance liquid chromatography," Chinese Journal of Chromatography, 26(5): 554-558.

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