Fast Analysis of Hair Dyes Using an Agilent Poroshell 120 Bonus-RP Column by UHPLC and LC/MS/MS

Application Note

Consumer Products

Authors

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Abstract

UHPLC and LC/MS/MS methods were developed for separating components that are banned or restricted for use in hair dyes on an Agilent Poroshell 120 Bonus-RP column. Compared to alkyl-only phases, the Poroshell Bonus-RP column has enhanced retention and selectivity for acidic and polar compounds due to strong H-bonding and can be used in 100% aqueous mobile phase. The UHPLC method using an Agilent 1290 Infinity LC System separated 24 compounds, while 5 more compounds could be analyzed using an Agilent 6460 Triple Quadrupole LC/MS System. All the compounds were separated within 15 minutes. Two commercial samples of hair dyes were analyzed to test the UHPLC method with complex samples.

Introduction

Hair dyes are used by people all over the world. Commonly used hair dyes are composed of aromatic compounds containing modified aniline and phenolic compounds, which may cause allergic reactions and potentially even cause cancer. Due to these potentially harmful effects, the amounts of these compounds are banned or restricted in many countries according to their own regulations. Methods for the quantitative measurement of the compounds in hair dyes include GC, GC/MS, LC, LC/MS [1]. HPLC methods are popular for the quantitative analysis because the compounds analyzed are not thermally stable, and most are strongly polar with low volatility.

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A Poroshell 120 Bonus-RP, 2.7 µm column packed with superficially porous materials was used in the application. Poroshell 120 superficially porous columns have nearly the same efficiency as sub-2 µm totally porous columns and can, therefore, be used to provide similar fast and high resolution analyses. The Bonus-RP phases have a polar-embedded group inserted into the hydrophobic C14 alkyl chain that allows minimal interaction of polar samples with silanols, providing symmetrical peak shape for many analytes. The polar embedded group also helps to wet the hydrophobic chains and prevents phase collapse in highly aqueous mobile phase. Compared to alkyl-only phases, Bonus-RP has enhanced retention and selectivity for acidic and polar compounds due to strong H-bonding [2].

This application note describes a UHPLC method that was developed for separating 24 compounds in only 15 minutes. The LC/MS/MS method was used to identify more compounds because the compounds that could not be separated via HPLC, could be resolved by LC/MS/MS. A total of 29 compounds were analyzed using a 6460 Triple Quadrupole LC/MS System. Both methods were developed on a Poroshell 120 Bonus-RP, 3.0 × 100 mm, 2.7 µm column. Two commercially available samples were purchased and analyzed, and some dye compounds were detected and separated from other components in the hair dyes.

Experimental

The 1290 Infinity LC System includes a binary pump, a Thermostatic Column Compartment (TCC), a high performance autosampler, and a Diode Array Detector (DAD). The LC/MS/MS system includes a 1290 Infinity LC system and an Agilent 6460 Triple Quadrupole LC/MS System. The column used in the application is a Poroshell 120 Bonus-RP, 3.0 × 100 mm, 2.7 µm.

The stock solutions were prepared individually in methanol at 10 mg/mL and then mixed together and diluted down to an appropriate concentration of standard solution with 2 g/L sodium hydrogen sulfite solution. The standard solutions were then filtered through an Agilent 0.2 µm regenerated cellulose filter (part number 5064-8221). The filtered solutions were transferred to the autosampler vials for HPLC and LC/MS/MS analysis.

The 2 hair dye samples were purchased locally. Each sample (0.5 g) was extracted using 10 mL acetonitrile and then placed in an ultrasonic bath for 10 minutes. The solution was then filtered through a 0.2 µm regenerated cellulose filter, and the filtered solutions were transferred to the autosampler vials for HPLC analysis.

Table 1. The 29 hair dye compounds analyzed in this application.

No.	Name	CAS
1	p-Phenylenediamine	106-50-3
2	2-Amino-3-hydroxypyridine	16867-03-1
3	m- Phenylenediamine	108-45-2
4	p- Aminophenol	123-30-8
5	2,6-Diaminopyridine	141-86-6
6	2,5-Diaminotoluene sulfate	615-50-9
7	o-Phenylenediamine	95-54-5
8	m-Aminophenol	591-27-5
9	o-Aminophenol	95-55-6
10	2-Chloro-1,4-phenylenediamine sulfate	61702-44-1
11	p-Methylaminophenol sulfate	1936-57-8
12	Hydroquinone	123-31-9
13	Resorcine	108-46-3
14	N,N-Diethyl-1,4-benzenediamine sulfate	6065-27-6
15	3,4-Diaminotoluene	496-72-0
16	1,4-Diamino-2-nitrobenzene	5307-14-2
17	5-Amino-o-cresol	2835-95-2
18	4-(N,N-Diethyl)-2-methyl-p-phenylenediamine monohydrochloride	2051-79-8
19	2-Methylresorcinol	608-25-3
20	6-Amino-m-cresol	2835-98-5
21	4-Nitro-o-phenylenediamine	99-56-9
22	5-Methyl-2-phenyl-1,2-dihydropyrazol-3-one	89-25-8
23	4-Amino-3-nitrophenol	610-81-1
24	6-Hydroxyindole	2380-86-1
25	4-Chlororesorcinol	95-88-5
26	2,7-Dihydroxynaphthalene	582-17-2
27	1,5-Dihydroxy naphthalene	83-56-7
28	4-Aminodiphenylamine	101-54-2
29	1-Naphthol	90-15-3

Results and Discussion

The superficially porous particles have nearly identical efficiency as sub-2 µm totally porous materials and can, therefore, be used to provide similar fast and high resolution analyses at lower pressure. The Bonus-RP phases have a polar-embedded group inserted into the hydrophobic C14 alkyl chain that allows minimum interaction of polar samples with silanols, providing symmetrical peak shape. Compared to alkyl-only phases, Bonus-RP has enhanced retention and selectivity for acidic and polar compounds due to strong H-bonding and can be used in 100% aqueous mobile phase. A previous application note developed a method using a Poroshell 120 EC-C18 separating 17 hair dye compounds [3]. However, to increase retention and separate more compounds, especially polar compounds, a gradient method starting from 100% aqueous phase on the Poroshell 120 Bonus-RP column was used for this application.

Figure 1 shows the separation of 24 potential hair dye standard components on 2 Poroshell 120 Bonus-RP columns in 15 minutes. This separation can be easily reproduced from column to column from different batches. Reasonable resolution is achieved between all the standard components. Due to the complexity of the compounds analyzed, it is difficult to optimize the pH of the mobile phase for all the compounds. In the application a mid pH mobile phase with 10 mM ammonium acetate was used to enhance retention of the compounds on the column because most compounds have amino groups that present neutral in a mid or high pH value mobile phase. These conditions were not optimal for all the components, but they were best for the majority of the components of the sample.

Figure 2 shows overlay chromatograms of sample 1, sample 2, and standards. Compounds vary in different samples. Compounds 2,5-diaminotoluene sulfate, 3-aminophenol, resorcine, and 5-amino-o-cresol were found in sample 1 and compounds p-phenylenediamine, 3-aminophenol, resorcine, and 2,7-dihydroxynaphthalene were detected in sample 2. The amounts of the detected compounds can be measured given the concentration of the standards.

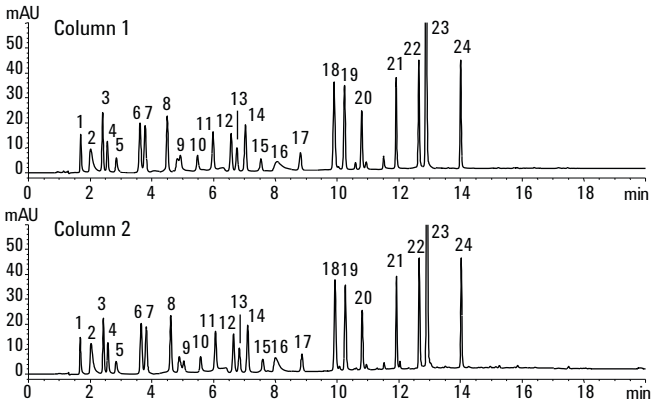

Figure 1. Standards chromatograms on 2 batches of Agilent Poroshell 120 Bonus-RP, 2.7-µm columns on an Agilent 1290 Infinity LC System.

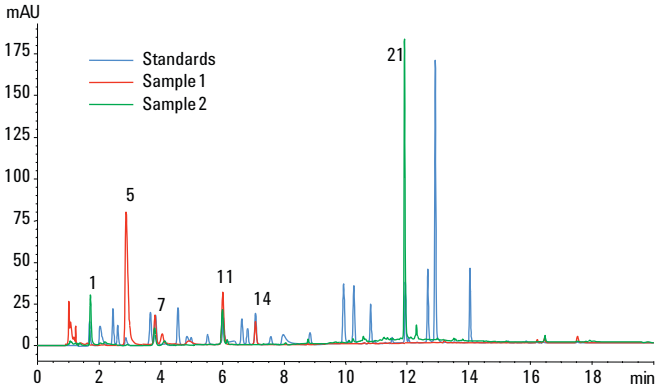

Figure 2. Overlay chromatograms of samples and standard components using an Agilent Poroshell 120 Bonus-RP, 2.7-µm column.

LC conditions

Instrument:	Agilent 1290 Infinity LC System(installed with 1290 inline filter after injector valve, p/n 5067-4638)
Column:	Agilent Poroshell 120 Bonus-RP, 3.0 × 100 mm, 2.7 µm (p/n: 695968-301)
Mobile phase:	A, 10 mM acetate; B, ACN
Gradient:	0 min 0% B 2 min 0% B 8 min 20% B 15 min 70% B 18 min 80% B
Stop time:	20 min, post run 3 min
Flow rate:	0.5 mL/min
Injection:	1 µL
Detector:	UV 280 nm

Standards:

1. p-Phenylenediamine
2. 2-Amino-3-hydroxypyridine
3. m-Phenylenediamine
4. 4-Aminophenol
5. 2,5-Diaminotoluene sulfate
6. o-Phenylenediamine
7. 3-Aminophenol
8. Hydroquinone
9. 2-Chloro-1,4-phenylenediamine sulfate
10. 4-Methylaminophenol sulfate
11. Resorcine
12. 3,4-Diaminotoluene
13. 1,4-Diamino-2-nitrobenzene
14. 5-Amino-o-cresol
15. 2-Methylresorcinol
16. 6-Amino-m-cresol
17. 4-Nitro-o-phenylenediamine
18. 4-Amino-3-nitrophenol
19. 6-Hydroxyindole
20. 4-Chlororesorcinol
21. 2,7-Dihydroxynaphthalene
22. 1,5-Dihydroxy naphthalene
23. 4-Aminodiphenylamine
24. 1-Naphthol

The above LC method could be easily transferred to an LC/MS/MS method because the mobile phase using ammonium acetate is MS friendly. While isomers, such as m-Aminophenol and o-Aminophenol, could not be resolved with LC, these could be identified by LC/MS/MS due to the different product ions. The same for the isomer pairs of hydroquinone and resorcine. Multiple Reaction Monitor (MRM) chromatograms of respective 29 hair dye components are shown in Figure 3, and the optimized conditions for MRM are listed in Table 2.

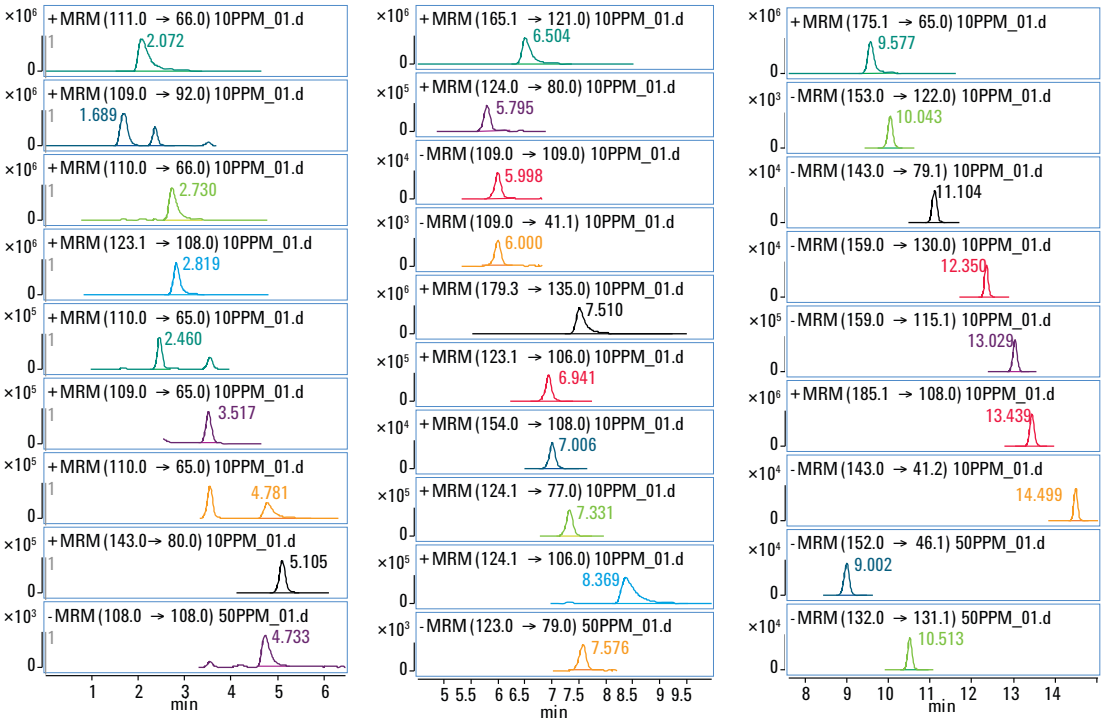

Figure 3. MRM chromatograms of respective 29 hair dye components using an Agilent Poroshell 120 Bonus-RP, 2.7-µm column.

Table 2. MRM conditions for the 29 components analyzed.

No.	Name	Product Ions				Retention time (min)	Polarity
1	p-Phenylenediamine	109 → 92	(12 V);	109 → 65	(22 V)	1.706	Positive
2	2-Amino-3-hydroxypyridine	111 → 66	(25 V);	111 → 94	(25 V)	2.069	Positive
3	m- Phenylenediamine	109 → 92	(12 V);	109 → 65	(22 V)	2.362	Positive
4	p- Aminophenol	110 → 65	(20 V);	110 → 93	(13 V)	2.465	Positive
5	2,6-Diaminopyridine	110 → 93	(21 V);	110 → 66	(23 V)	2.748	Positive
6	2,5-Diaminotoluene sulfate	123.1 → 108	(15 V);	123.1 → 77	(22 V)	2.813	Positive
7	o-Phenylenediamine	109 → 92	(12 V);	109 → 65	(23 V)	3.511	Positive
8	m-Aminophenol	110 → 65	(20 V);	110 → 93	(13 V)	4.731	Positive
9	o-Aminophenol	108 → 108	(0 V);	108 → 107	(22 V)	4.733	Negative
10	2-Chloro-1,4-phenylenediamine sulfate	143 → 108	(16 V);	143 → 80	(25 V)	5.107	Positive
11	p-Methylaminophenol sulfate	124 → 109	(14 V);	124 → 80	(35 V)	5.792	Positive
12	Hydroquinone	109 → 109	(0 V);	109 → 108	(15 V)	6.008	Negative
13	Resorcine	109 → 65	(17 V);	109 → 41.1	(23 V)	6.010	Negative
14	N,N-Diethyl-1,4-benzenediamine sulfate	165.1 → 136	(11 V);	165.1 → 121	(20 V)	6.456	Positive
15	3,4-Diaminotoluene	123.1 → 106	(12 V);	123.1 → 79	(20 V)	6.947	Positive
16	1,4-Diamino-2-nitrobenzene	154 → 108	(16 V);	154 → 91	(20 V)	7.012	Positive
17	5-Amino-o-cresol	124.1 → 77	(19 V);	124.1 → 109	(16 V)	7.329	Positive
18	4-(N,N-Diethyl)-2-methyl-p-phenylenediamine monohydrochloride	179.3 → 150	(11 V);	179.3 → 135	(22 V)	7.477	Positive
19	2-Methylresorcinol	123 → 79	(12 V);	123 → 55	(18 V)	7.573	Negative
20	6-Amino-m-cresol	124.1 → 106	(12 V);	124.1 → 79	(16 V)	8.271	Positive
21	4-Nitro-o-phenylenediamine	152 → 46.1	(23 V);	152 → 105	(14 V)	9.008	Negative
22	5-Methyl-2-phenyl-1,2-dihydropyrazol-3-one	175.1 → 65	(25 V);	175.1 → 133	(20 V)	9.581	Positive
23	4-Amino-3-nitrophenol	153 → 122	(11 V);	153 → 123.1	(19 V)	10.042	Negative
24	6-Hydroxyindole	132 → 131.1	(20 V);	132 → 104.1	(15 V)	10.513	Negative
25	4-Chlororesorcinol	143 → 107	(12 V);	143 → 79.1	(17 V)	11.105	Negative
26	2,7-Dihydroxynaphthalene	159 → 130	(30 V);	159 → 102	(30 V)	12.359	Negative
27	1,5-Dihydroxy naphthalene	159 → 115.1	(22 V);	159 → 131.1	(22 V)	13.025	Negative
28	4-Aminodiphenylamine	185.1 → 108	(18 V);	185.1 → 93	(22 V)	13.425	Positive
29	1-Naphthol	143 → 115.1	(45 V);	143 → 41.2	(45 V)	14.499	Negative

LC conditions

Instrument:	Agilent 1290 Infinity LC System (installed with 1290 inline filter after injector valve, p/n: 5067-4638)	
Column:	Agilent Poroshell 120 Bonus-RP, 3.0 × 100 mm, 2.7 µm (p/n 695968-301)	
Mobile phase:	A, 10 mM acetate; B, ACN	
Gradient:	0 min	0% B
	2 min	0% B
	8 min	20% B
	15 min	70% B
	18 min	80% B
Stop time:	20 min, post run 3 min	
Flow rate:	0.5 mL/min	
Injection:	1 µL	

MS conditions

Gas temp:	325 °C
Gas flow:	10 L/min
Nebulizer:	50 psi
Sheath gas temp:	350 °C
Sheath gas flow:	11 L/min
Capillary:	positive 4,000 v; negative 3,500 v

Conclusion

The method was developed for the separation of hair dye components using the Poroshell 120 Bonus-RP column. The column gives a good selectivity for these compounds and provides good resolutions. The method developed on 1290 Infinity LC Syste is suitable for fast screening and quantitative analysis of these compounds. The LC/MS/MS method enables co-eluted compounds be identified and quantified using MRM and is suitable for low level concentration analysis in complex sample matrices.

Reference

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

2. Anne E. Mack,"Fast screening methods for steroids by HPLC with Agilent Poroshell 120 columns", Application Note, Agilent Technologies, Inc, Publication Number 5991-0245EN.

3. Rongjie Fu, "Fast Analysis of Hair Dyes Using an Agilent Poroshell 120 EC-C18 Column", Application Note, Agilent Technologies, Inc, Publication Number 5990-6501EN.

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