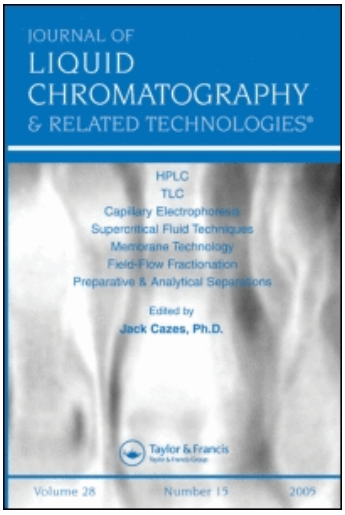

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QUANTITATIVE DETERMINATION OF COMMERCIAL OXIDATION HAIR DYES BY REVERSED-PHASE HPLC

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ABSTRACT

A simple and reliable RP-HPLC method for the separation and quantitative determination of *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, resorcinol, and 2,6-diaminopyridine in commercial oxidation hair dyes was developed. The separation was carried out on a μ-Bondapak C_{18} column with methanol-aqueous solution containing 0.1% triethylamine and 0.02 mol l^{-1} NH_4Ac (pH = 5.20) (10:90, vol./vol.) as the mobile phase. Without a complex extraction procedure, the method is sufficiently rapid and accurate for routine analyses. Two kinds of commercial hair dye samples were determined successfully.

INTRODUCTION

Hair dyeing represents an important aspect of cosmetology (1). Its interest has been growing due to the care taken in self-appearance by both women and men. There are three groups of hair dyes: temporary, semi-permanent, and permanent.

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The oxidation dyes based on *o*- or *p*-diamino derivatives and aminophenols as coupling agents in order to achieve a wider range of colors with more stability and better resistance to light and to outside agents, belong to the last group (2).

With different formulae, there exist many types of oxidation dyes. The hair dyes we determined, one type of oxidation dye, contain the following five active constituents: *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, resorcinol, and 2,6-diaminopyridine.

With paper chromatography (3), thin-layer chromatography (4), and gas chromatography (5), a lot of methods for analysing oxidation hair dyes are available in the literature, but in recent years, high performance liquid chromatography (HPLC) has been shown to be more powerful. Using octylammonium salicylate or octylammonium orthophosphate as the ion interaction reagent, a reversed-phase (RP) HPLC method was developed to determine *p*-phenylenediamine in commercial hair dyes (6), and using a polystyrene-divinylbenzene column, another RP-HPLC method was reported to separate the *ortho*, *meta*, and *para* isomers of aminophenol (7).

Besides, a systematic analysis of all the most common oxidation hair dyes (diamines, aminophenols, phenols, etc.) was proposed by Andrisano et al. using the binary mixtures of acetonitrile with a buffer solution (pH4.5) containing 1,8-diaminoctane and sodium heptansulfonate as the mobile phase (8). More recently, the simultaneous quantitative determination of resorcinol and 1-naphthol in hair color products, which used methanol-water (50:50, vol./vol.) as the mobile phase, was reported (9).

In this paper, we describe an RP-HPLC method suitable for the separation and quantitative determination of *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, resorcinol, and 2,6-diaminopyridine in commercial hair dyes, in which we used methanol-aqueous solution containing 0.1% triethylamine and 0.02 mol l^{-1} NH_4Ac (pH = 5.20) (10:90, vol./vol.) as the mobile phase and μ-Bondapak C_{18} column.

The method described here does not involve complex extraction procedure and it is sufficiently rapid and accurate for routine analyses of commercial hair dyes.

EXPERIMENTAL

Chemicals

Water for all applications was supplied by a Milli-Q II system (Millipore, Bedford, MA, USA). Methanol was of HPLC grade (Ludu Chemical Reagents Plant, Shanghai, China). Ethanol (Changzheng Chemical Plant, Hangzhou, China),

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triethylamine (Aldrich, Milwaukee, WI, USA), ammonium acetate (NH_4Ac, Shanghai Chemical Reagents Plant No 4, Shanghai, China), and acetic acid (HAc, Hangzhou Chemical Reagents Plant, Hangzhou, China) were of analytical grade. These five analyte standards and the two kinds of commercial hair dye samples (paste) were provided by Oushiman Cosmetics Factory (Zhejiang, China).

Chromatography

Analyses were performed using an LC-4A (Shimadzu, Kyoto, Japan) HPLC system equipped with a Model 481 (Waters, Milford, MA, USA) UV-detector and a C-R4A (Shimadzu) data system. The separation was carried out on a μ-Bondapak C_{18} column (Waters, 300 $\times$ 3.9 mm I.D.). Mobile phase was a mixture of methanol and aqueous solution (10:90, vol./vol.). The aqueous solution was prepared by dissolving 1.54 g NH_4Ac and 1 mL triethylamine in 1000 mL of water, and then adjusting the acidity to pH 5.20 with HAc.

The flow rate of mobile phase was 1.5 mL min^{-1}. The temperature of the column oven was set at 25°C. The wavelength of the UV-detector was set at 254 nm (but at 233 nm to determine *p*-aminophenol in commercial samples).

Preparation of Mixed Standard Solution

Appropriate amounts (20–30 mg) of five analyte standards, *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, resorcinol, and 2,6-diaminopyridine, were dissolved in ethanol and brought to a volume of 10 mL, respectively. Appropriate volumes of those five ethanol standard solutions were mixed and diluted with mobile phase to prepare mixed standard solutions and 5 μL (Hamilton microliter syringes, U6K, 25 μL) of the solution was injected into the HPLC system.

Preparation of Sample Solution

Appropriate amounts (0.1 g) of commercial hair dye samples (paste) were extracted ultrasonically with 5 mL of ethanol. The solution was filtered through a 0.45 μm disposable syringe filter and diluted to suitable concentration with mobile phase. The diluted solution was filtered again and passed through a disposable syringe SEP-PAK C_{18} precolumn (Waters, 10 $\times$ 13 mm) and then 5 μL of the solution was injected into the HPLC system.

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RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

Four of the five compounds, i.e., except resorcinol, have one or two amino groups ($-NH_2$). Amino groups will be protonated to $-NH_3^+$ in acid medium, which leads to increasing distribution in mobile phase. So, the acidity may be the main factor influencing the separation. Figure 1 shows the effects of acidity on separation.

From Figure 1, we can see that the retention times of all five compounds are reduced as acidity increases, but resorcinol is not influenced so significantly as the other four compounds in the acidity range of pH 4.0–7.0. What deserves to be mentioned, is the two intersections of the *m*-aminophenol and 2,6-diaminopyridine curves in Figure 1, indicating that the elution order of these two compounds may be reversed two times by changing the acidity of the mobile phase.

Retention behavior should be related to the extent of protonation. Based on the pKa values for those four amino-containing compounds, we can calculate the proportions of ionized and neutral forms of those four compounds at various pH values of the mobile phase. The calculated results are listed in Table 1.

From Table 1, we can see that the neutral form proportions of those four compounds increase as the pH values increase from 3.25 to 7.25, which results in the increase of retention times (see Fig. 1). For 2,6-diaminopyridine and *m*-aminophenol, compared with their neutral form proportions at pH 5.25 (15.09% and 2.63×10^{-3}%, respectively), the former reaches 94.68% at pH 7.25, while the latter only reaches 0.26%, so the retention of the former becomes stronger than that of the latter; on the other hand, the former still remains 0.17% at

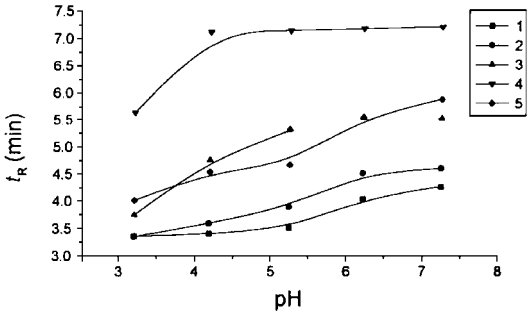

Figure 1. Effects of eluent acidity on the separation. **1.** *p*-phenylenediamine; **2.** *p*-aminophenol; **3.** *m*-aminophenol; **4.** resorcinol; **5.** 2,6-diaminopyridine. Conditions: μ-Bondapak C_{18} column; mobile phase: methanol-aqueous solution containing 0.04% triethylamine and 0.02 mol l^{-1} NH_4Ac (20:80, v/v); column temperature: 25°C; flow rate: 1 mL min^{-1}; injection volume: 5 μL; UV detector wavelength: 254 nm.

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Table 1. The Ionized and Neutral Forms Proportions (%) of Four Amino-Containing Compounds at Various pH Values

Compounds and Their Different Forms		pH 3.25	pH 5.25	pH 7.25
	+1	99.99997	99.997	99.7
m-Aminophenol	0	2.63×10^{-5}	2.63×10^{-3}	0.26
	−1	6.31×10^{-12}	6.31×10^{-8}	6.29×10^{-4}
	+2	5.32	4.77×10^{-2}	2.99×10^{-5}
2,6-Diaminopyridine	+1	94.52	84.86	5.32
	0	0.17	15.09	94.68
	+1	99.9994	99.94	94.67
p-Aminophenol	0	5.62×10^{-4}	5.62×10^{-2}	5.32
	−1	5.01×10^{-11}	5.01×10^{-7}	4.74×10^{-3}
	+2	52.26	0.95	8.60×10^{-4}
p-Phenylenediamine	+1	47.67	86.29	7.84
	0	7.05×10^{-2}	12.76	92.16

"+1", "+2" represent protonated forms, "0" represents neutral form, and "−1" represents negatively charged form.

pH 3.25, while the latter becomes very small (2.63×10^{-5}%), so the retention of the former also becomes stronger than that of the latter. These may explain the two intersections of *m*-aminophenol and 2,6-diaminopyridine curves in Figure 1.

As for *p*-phenylenediamine and *p*-aminophenol (their proportions of neutral form are 12.57% and 5.62×10^{-2}%, respectively, at pH 5.25), when pH value is 7.25, the former reaches 92.16% but the latter also reaches 5.32%, and when pH value is 3.25, both of them become very small (7.05×10^{-2}% and 5.62×10^{-4}%, respectively). These results may be the reasons why the elution order of *p*-phenylenediamine and *p*-aminophenol does not reverse at different pH values.

The best separation of *m*-aminophenol and 2,6-diaminopyridine is reached at about pH 5.20. On the other hand, the most difficult separated pair of those five analytes are *p*-phenylenediamine and *p*-aminophenol due to their similar structure, and the best acidity range for their separation is pH 5.0–6.0. Based on the above two facts, we selected pH 5.20 as the acidity of the mobile phase.

There are tailings in the chromatographic peaks for compounds with an amino group. The reason is that the amino group can interact with residual silanol groups on the stationary phase. Addition of amine modifiers into the mobile phase will improve the peak shape and resolution because the amine modifiers serve as competing bases for masking accessible surface silanol groups (10,11). We introduced triethylamine in the mobile phase and the tailed peaks are gradually improved as the concentration of triethylamine in aqueous solution of the mobile phase increasing from 0.02% to 0.1% (vol./vol.). But further increasing the

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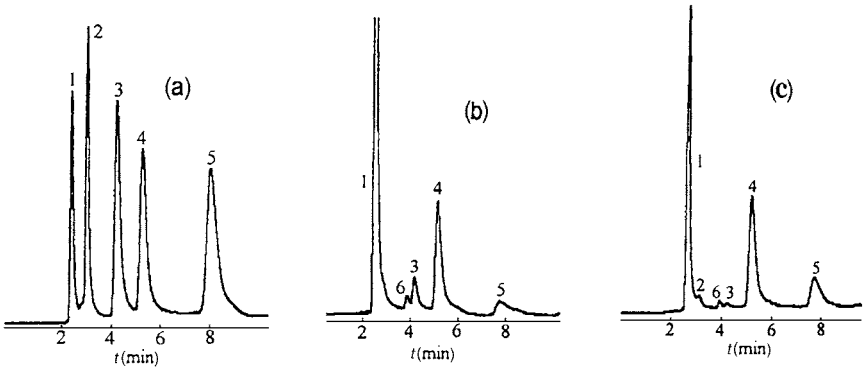

Figure 2. Chromatograms: (a) standards mixture; (b) and (c) commercial sample. **1.** *p*-phenylenediamine; **2.** *p*-aminophenol; **3.** 2,6-diaminopyridine; **4.** *m*-aminophenol; **5.** resorcinol; **6.** unknown. Conditions: μ-Bondapak C_{18} column; mobile phase: methanol-aqueous solution containing 0.1% triethylamine and 0.02 mol l^{-1} NH_4Ac (pH = 5.20) (10:90, v/v); column temperature: 25°C; flow rate: 1.5 mL min^{-1}; injection volume: 5 μL; UV detector wavelength: (a) and (b) 254 nm; (c) 233 nm.

triethylamine concentration does not result in any further improvement on the separation. Hence, the triethylamine concentration was maintained at 0.1%.

In order to stabilize the acidity of the mobile phase, we introduced NH_4Ac in the mobile phase and adjusted the acidity with HAc to form a buffer solution. We selected 0.02 mol l^{-1} as the concentration of NH_4Ac in aqueous solution of the mobile phase.

The effects of methanol concentration in the mobile phase and the flow rate of the mobile phase on separation were also investigated. As the ratio of methanol to aqueous solution decreased from 30:70 to 10:90 (vol./vol.), the retention times increased and the resolutions became better while the peaks became broadening. On the other hand, as the flow rate of the mobile phase increased from 1 mL min^{-1} to 1.5 mL min^{-1}, the retention times decreased, but the peaks became sharpened, resulting in weak influence on resolutions. We selected 10:90 (vol./vol.) as the ratio of methanol to aqueous solution in the mobile phase and 1.5 mL min^{-1} as the flow rate of the mobile phase.

Figure 2 shows the chromatograms of those five compounds under the optimized conditions.

Calibration and Precision

The calibration graph was obtained by plotting peak height against concentrations (seven data points). They showed excellent linearity covering the tried

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Table 2. Calibration Data for Five Analytes

Analytes	Concentration Range (μg mL^{-1})	Regression Equation[a]	r
p-Phenylenediamine	5.134 – 192.525	$Y = -0.067 + 0.590\ X$	0.99990
p-Aminophenol	2.783 – 111.320	$Y = 0.004 + 0.121\ X$	0.99991
m-Aminophenol	6.188 – 92.820	$Y = -0.006 + 0.235\ X$	0.99990
Resorcinol	4.104 – 102.600	$Y = -0.031 + 0.057\ X$	0.99995
2,6-Diaminopyridine	0.633 – 79.150	$Y = -0.018 + 0.570\ X$	0.99963

Conditions: μ-Bondapak C_{18} column; mobile phase: methanol-aqueous solution containing 0.1% triethylamine and 0.02 mol l^{-1} NH_4Ac (pH = 5.20) (10:90, v/v); column temperature: 25°; flow rate: 1.5 mL min^{-1}; injection volume: 5 ul; UV detector wavelength: 254 nm.

[a] Y: peak height (mV); X: concentration (μg mL^{-1}).

concentrations (Table 2), with correlation coefficients (r) in the range of 0.99963–0.99999.

Precision was evaluated by performing five replicate analyses of all five analyte standards. For *p*-phenylenediamine (53.80 μg mL^{-1}), *p*-aminophenol (33.14 μg mL^{-1}), *m*-aminophenol (91.04 μg mL^{-1}), resorcinol (39.35 μg mL^{-1}), and 2,6-diaminopyridine (7.32 μg mL^{-1}), the RSDs were 0.47, 1.07, 0.32, 1.34, and 0.72%, respectively, suggesting that the method has excellent precision.

Quantitation Limits and Recoveries

The quantitation limit was measured as the concentration corresponding to a S/N of 8:1. According to this rule, the values for *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, resorcinol, and 2,6-diaminopyridine were approximately 0.47, 0.54, 0.72, 3.41, and 0.32 μg mL^{-1}, respectively.

The recoveries were checked through spiking three different known amounts of five analyte standards in commercial hair dye samples and the contents were re-quantitated. The average values for *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, resorcinol, and 2,6-diaminopyridine were 93.58, 95.09, 93.25, 102.57, and 95.21%, respectively.

Determination of Commercial Hair Dye Samples

As the content of *p*-aminophenol in commercial hair dye samples is much lower than that of *p*-phenylenediamine, and these two compounds are the most difficult separated pair, *p*-aminophenol has no peak if the wavelength of the

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Table 3. Content[a] (%) of Five Analytes in Two Kinds of Commercial Samples

Sample	*p*-Phenylenediamine	*p*-Aminophenol	*m*-Aminophenol	Resorcinol	2,6-Diaminopyridine
1	2.84 ± 0.05	0.26 ± 0.02	1.71 ± 0.04	1.11 ± 0.03	0.11 ± 0.005
2	2.71 ± 0.04	0.28 ± 0.02	0.25 ± 0.01	0.81 ± 0.03	0.10 ± 0.005

Conditions were identical with Table 2 except that the UV detector wavelength was set at 233 nm for *p*-aminophenol.

[a]Mean ± SD from 9 HPLC runs (3 times sampling and 3 times injections respectively).

UV detector is set at 254 nm which is the detective wavelength of other four compounds (see Fig. 2b). We found that at 233 nm, which is the maximum absorption wavelength of *p*-aminophenol, the UV absorption of *p*-aminophenol is larger than that of *p*-phenylenediamine. So we set the wavelength at 233 nm to determine *p*-aminophenol in commercial hair dye samples (see Fig. 2c); *p*-aminophenol was quantitated with the standard addition method, while the other four constituents were determined with a calibration curve or the external standard method.

A simple method of sample treatment without a complex extraction procedure was proposed, as described in the section of preparation of sample solution. Besides the analytes, the composition of commercial hair dyes (paste) is complex. In order to protect the column in the system, the sample solution was passed through a disposable syringe SEP-PAK C_{18} precolumn before being injected into the HPLC system. Two kinds of commercial hair dye samples of the same type were determined.

The results are listed in Table 3, and the chromatogram of sample 1 is illustrated in Figure 2b and c. The SDs from 9 HPLC runs are also listed in Table 3. The RSDs were 1.76, 7.69, 2.34, 2.70, and 4.55% (sample 1), 1.48, 7.14, 4.00, 3.70, and 5.00% (sample 2) for *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, resorcinol, and 2,6-diaminopyridine, respectively, indicating that the method is also precise for commercial hair dye sample determination.

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