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# Determination of Constituents in Hair Dyes by Reversed-phase High-Performance Liquid Chromatography

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**Abstract:** A reversed-phase high-performance liquid chromatographic method for the separation and quantitation of 1,4-phenylenediamine, 4-aminophenol, 3-aminophenol, 1,3-benzendiol and 2,6-phenylenediamine in hair dyes was developed. The separation was carried out on a μ-Bondapak $C_{18}$ column with methanol-water containing 0.1% triethylamine and 0.02 mol/L $NH_4Ac$ (pH=5.20) (10:90, vol./vol.) as the mobile phase. Two real samples were analyzed.

**Keywords:** Hair dyes, 1,4- phenylenediamine, 4-aminophenol, 3-aminophenol, 1,3-benzendiol, 2,6-phenylenediamine, HPLC.

There are many kinds of hair dyes with different formulae. The kind we determined contains the following five constituents: 1,4-phenylenediamine (*p*-PDA), 4-aminophenol (*p*-APO), 3-aminophenol (*m*-APO), 1,3-benzendiol (*m*-BDO) and 2,6-diaminopyridine (DAP). With reversed-phase high-performance liquid chromatography (RP-HPLC), some other kinds of hair dyes had been determined[1,2], but not our kind. In this paper we describe a RP-HPLC method suitable for the separation and quantitative determination of all those five constituents in hair dyes.

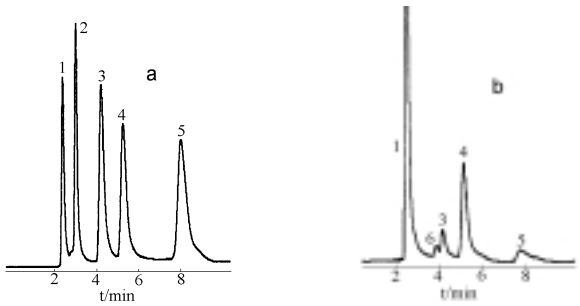
Analyses were performed using a Shimadzu LC-4A HPLC system equipped with a Waters M481 UV-detector and a Shimadzu C-R2A data system. Water for all applications was supplied by a Milli-Q Ⅱ system. Methanol was of HPLC grade. All other reagents were of analytical grade. The standards and real samples were provided by a cosmetics plant.

The chromatographic conditions were optimized. The effects of the acidity on the separation were investigated. The acidity may be the main factor influencing the separation because four of those five compounds, *i.e.*, except *m*-BDO, each has one or two amino group ($-NH_2$) and amino group will be protonated to $-NH_3^+$ in acid medium and followed the increasing distribution in mobile phase. In general, there are tailings in the chromatographic peaks for compounds with amino group. The reason is that amino group can interact with residual silanol groups on the stationary phase. We introduce triethylamine in the mobile phase to suppress that interaction and improve the tailed peak shape. The effects of methanol concentration in the mobile phase and the flow rate on separation were also investigated. **Figure 1** shows the chromatograms of those five compounds under the optimized conditions.

Calibration curves of all five compounds showed excellent linearity covering the ranges of tested concentration with correlation coefficients (r) in the range of 0.99963-0.99999. For *p*-PDA, *p*-APO, *m*-APO, *m*-BDO and DAP, the LOQs (limit of quantitation, S/N=8) were approximately 0.47, 0.54, 0.72, 3.41 and 0.32 μg/mL, and the average

recoveries were 93.58, 95.09, 93.25, 102.57 and 95.21% respectively.

**Figure 1** Chromatograms (a) Standards mixture; (b) Real sample




**1**. *p*-PDA; **2**. *p*-APO; **3**. DAP; **4**. *m*-APO; **5**. *m*-BDO; **6**. unknown
Conditions: $C_{18}$ column; mobile phase: methanol-water containing 0.1% triethylamine and 0.02 mol/L $NH_4Ac$ (pH=5.20) (10:90, vol./vol.); column temperature: 25℃; flow rate: 1.5mL/min; injection volumn: 5μL; detector wavelength: 254nm.

As the content of *p*-APO in the real sample is much lower than that of *p*-PDA and these two compounds are the most difficult separated pair, *p*-APO has no peak if the wavelength of the UV detector is set at 254 nm which is the detective wavelength of the other four compounds (see **Figure 1**). For this reason, we set the wavelength at 233 nm to determine *p*-APO and it was quantitated with standard addition method while the other four constituents were quantitated with calibration curve or external standard method.

A simple method of sample treatment without complex extraction procedure was proposed. About 0.1g of real sample was dissolved ultrasonically with 5ml of ethanol. The solution was filtered through a 0.45μm 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 $C_{18}$ pre-column (Waters, 10×13mm) before injected into the HPLC system in order to protect the column in the system. Two real samples were analyzed. The results are listed in **Table 1** and the chromatogram of sample $1^{\#}$ was illustrated in **Figure 1**.

In conclusion, this method is simple, accurate and reproducible and is suitable for quality testing in hair dye plants.

**Table 1** Content (%*) of those five analytes in two kinds of hair dye sample

| Sample | *p*-PDA | *p*-APO | *m*-APO | *m*-BDO | DAP |
|---|---|---|---|---|---|
| $1^{\#}$ | 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 |

* mean ± S.D. from 9 HPLC runs (3 times sampling and 3 times injections respectively)

## References

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