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Simultaneous determination of 13 components in oxidative hair dyes by high-performance chromatography using an ion-pair reagent

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Synopsis

A reliable high-performance liquid chromatography (HPLC) method was developed for the simultaneous determination of 13 dye intermediates, including benzenediamines, aminophenols, benzenediols, naphthalenediol, and diaminopyridine, in oxidative hair dyes. Samples were extracted with 50% ethanol by adding sodium dithionite to prevent oxidation. The influences of buffer type, buffer pH, ion-pair reagent, and elution gradient were studied. A C_{18} column with aqueous compatibility and acetonitrile–citric acid mobile phase system (pH 2.6) with sodium 1-octanesulfonate as ion-pair reagent were selected for the separation of target compounds. Detection was performed by a diode array detector, (DAD) and two different wavelengths (280 and 331 nm) were used for quantification. Results showed that 13 dye intermediates got good separation within 25 min. The detection limits of these compounds were in the range of 0.2–2 mg/l. The calibration curves were linear within 2–500 mg/l with 0.999 as a typical correlation coefficient. The recoveries of target compounds in hair dyes ranged from 81.7% to 102.0% with four addition levels. The method described was validated by five different laboratories and successfully applied to the analysis of commercial oxidative hair dyes.

INTRODUCTION

Oxidative hair dye, the most widely used permanent dye, usually contains a mixture of aromatic compounds including benzenediamines, aminophenols, benzenediols, naphthalenediol, and diaminopyridine. Such substances are of great health concern due to their mutagenic or even carcinogenic activity (1,2). The use of these compounds in hair dyes is regulated by legislation in many countries. Therefore, a simple and rapid method for the determination of these dye intermediates in hair dyes is of utmost importance.

Several methods have been developed for the analysis of dye intermediates in hair dyes, including thin layer chromatography (TLC) (3,4), high-performance liquid chromatography (HPLC) (5–11), high performance liquid chromatography-mass

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spectrometry(HPLC-MS) (12), gas chromatography (GC) (13), gas chromatography-mass spectrometry (GC-MS) (14–16), capillary electrophoresis (CE) (17), and micellar electrokinetic chromatography (MEKC) (18,19). Usually, GC and CE have better separation ability than HPLC. However, GC has some restrictions for the determination of hydrophilic substances in hair dyes due to their high polarity and low volatility, and CE is not suitable for quantitative determination because of low reproducibility. Therefore, only HPLC is the most convenient method for the determination of hair dye intermediates, which has high polarity, low volatility, and thermostability. Several studies contribute to improving the amount of oxidative hair dyes analyzed in a single HPLC run by optimizing the separation parameters (7–10) or prolonging the analysis time (11). Up to now, the best result achieved (11) is the separation of 22 oxidative hair dyes in 50 min. Usually, a HPLC runs less than 30 min and allows the simultaneous determination of two to eight oxidative hair dyes (7–10). Therefore, the aim of this work was to develop a reliable HPLC method to analyze the most commonly used hair dye intermediates, such as benzenediamines, aminophenols, benzenediols, naphthalenediol, and diaminopyridine, with less time.

EXPERIMENTAL

CHEMICALS AND REAGENTS

Hydroquinone (99.5%), resorcinol (99.5%), *p*-aminophenol (99.0%), and phenol (99.5%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany); *o*-phenylenediamine (99.5%), 1,5-naphthalenediol (99%), and 3,4-diaminetoluene (99.8%) from ChemService (West Chester, PA); *N*,*N*-diethyl-*p*-phenylenediamine sulfate (97%) from IL (South San Francisco, CA); *m*-aminophenol (99%), 4-methylaminophenol sulfate (99%), *p*-phenylenediamine (99%), 2,5-diaminotoluene sulfate (99%), and 2,6-diaminopyridine (98%) from ACROS (Geel, Belgium); 1-octanesulfonic acid sodium salt monohydrate (97%) from J&KCHEMICA (Beijing, China). Water (Milli-Q Purification System, Millipore (Billerica, MA)), acetonitrile, and ethanol were of HPLC grade. Sodium dithionite and sodium sulfite were of AR grade.

Standard stock solutions of 13 dye intermediates each at a concentration of 2500 μg/ml was prepared in 50% (v/v) ethanol solution containing 0.1% sodium sulfite in brown bottles, and stored at −10°C. A calibration curve was prepared by injecting eight diluted solutions, obtained from the stock solution, in the concentration range of 2–500 μg/ml.

APPARATUS

HPLC system was composed of a Waters 2695 pump equipped with a liquid autosampler, and a Waters 2996 diode array detector.

Analysis was performed at 30°C on a ZORBAX SB-Aq C_{18} column (5 μm, 250 × 4.6 mm I.D., Agilent (Santa Clara, CA)) with a same packing precolumn (5 × 4.6 mm I.D.) through a gradient elution. The flow rate was 1.0 ml/min. Eluent A was a buffer solution (pH 2.6) containing 10 mmol/l citric acid and 10 mmol/l sodium 1-octanesulfonate. Eluent B was acetonitrile. Time program: 0–5 min, B 12%; 5.01–15 min, B 12–20%; 15.01–24 min, B 12%. Injection volume: 5 μl. Detection was performed by scanning from 190 to 400 nm. Quantitation was performed at 331 nm for 2,6-diaminopyridine and at 280 nm for all the other dye intermediates.

SAMPLE PREPARATION

About a 1.0 g sample was weighed and placed in a 25 ml calibrated flask, then 1 ml 1% sodium dithionite and 15 ml 50% (v/v) ethanol were added. The mixture was homogenized adequately by stirring before 15 min of the ultrasonic extraction process. Then the solution was diluted to volume with 50% (v/v) ethanol. About 1 ml aliquot of this solution was filtered through a 0.45 μm membrane filter and ready for HPLC analysis.

VALIDATION

To evaluate the accuracy and precision of the method, standard recovery test was performed on real sample A (containing 536.6 mg/kg resorcinol) and sample B (containing 3528 mg/kg *m*-aminophenol, 6618 mg/kg *p*-aminophenol, and 8727 mg/kg *p*-phenylenediamine), with four addition levels of 1 time, 2 times, 10 times, and 20 or 100 times the LOQ (limit of quantification) of target compounds. The method was also validated by five different laboratories with real sample determination and standard recovery test.

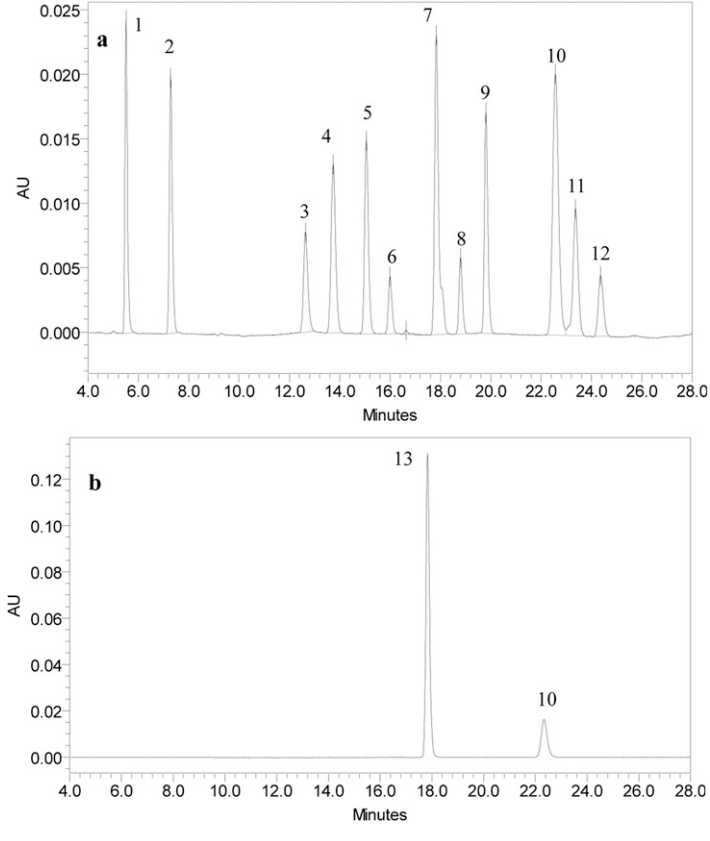

Figure 1. Chromatogram of dye intermediates at (a) 280 nm and (b) 331 nm. 1. Hydroquinone; 2. resorcinol; 3. *p*-aminophenol; 4. phenol; 5. *m*-aminophenol; 6. 4-methylaminophenol; 7. *o*-phenylenediamine; 8. *p*-phenylenediamine; 9. 2,5-diaminotoluene; 10. 1,5-naphthalenediol; 11. 3,4-diaminetoluene; 12. *N*,*N*-diethyl-*p*-phenylenediamine; 13. 2,6-diaminopyridine.

RESULTS AND DISCUSSION

PRETREATMENT CONDITIONS

To allow for both alcohol-soluble and water-soluble dye intermediates, 50% ethanol solution was chosen as the extractant.

The impact of sodium sulfite and sodium dithionite as reductant on the extraction of easily oxidized dye intermediates was studied. Results showed that the recoveries of hydroquinone and 1,5-naphthalenediol were doubled by adding 1 ml 1% sodium dithionite compared to that by adding sodium sulfite; however, no difference was found for all the other dye intermediates studied. Therefore, sodium dithionite was chosen as the reductant.

HPLC CONDITIONS

The dye intermediates studied possess amino groups with two pK_a values in the range of 4.2–6.5 and 1.7–3.0, and/or hydroxy groups with the first and the second pK_a values in

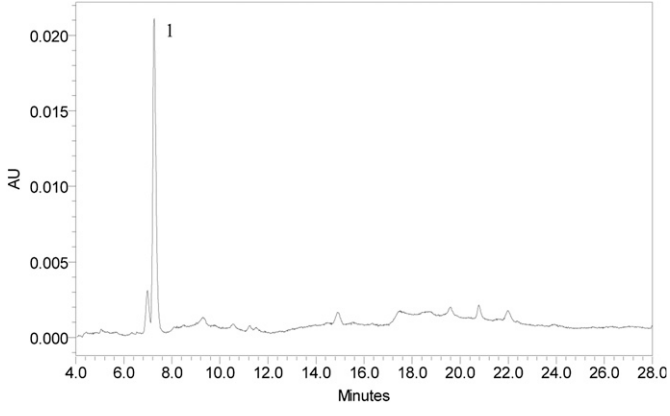

Figure 2. Chromatogram of hair dye A at 280 nm. 1. Resorcinol.

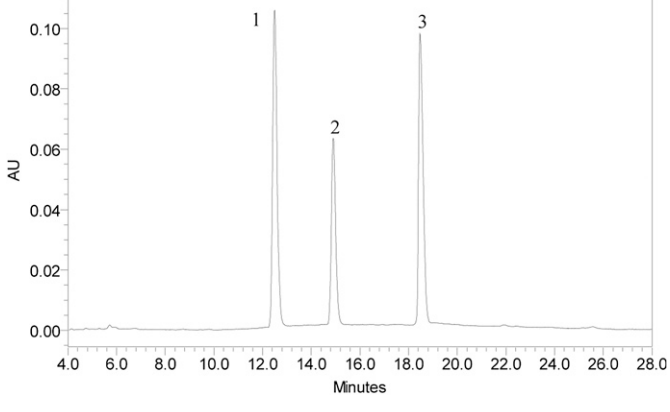

Figure 3. Chromatogram of hair dye B at 280 nm. 1. *p*-Aminophenol; 2. *m*-aminophenol; 3. *p*-phenylenediamine.

Table I
The Linear Ranges and Detection Limits of 13 Dye Intermediates Determined by HPLC

Compound	Linear regression equation	R^2	Detection limit (μg/ml)
Hydroquinone	Y = 1.02e + 004 X − 3.90e + 003	1	0.2
Resorcinol	Y = 8.18e + 003 X − 2.52e + 003	1	0.3
p-Aminophenol	Y = 5.79e + 003 X − 1.44e + 003	0.9999	0.6
Phenol	Y = 4.75e + 003 X − 2.81e + 003	0.9999	0.9
m-Aminophenol	Y = 7.47 e + 003 X − 1.51 e + 004	0.9998	0.6
4-Methylaminophenol	Y = 3.14e + 003 X − 4.85e + 003	0.9999	1
o-Phenylenediamine	Y = 1.04e + 004 X − 1.46e + 004	1	0.4
p-Phenylenediamine	Y = 3.70e + 003 X − 3.63e + 002	0.9999	0.8
2,5-Diaminotoluene	Y = 2.35e + 003 X + 2.31e + 003	0.9999	1
1,5-Naphthalenediol	Y = 1.39e + 004 X + 4.58e + 003	0.9999	0.4
3,4-Diaminetoluene	Y = 7.03e + 003 X − 2.59e + 004	0.9998	1
N,*N*-Diethyl-*p*-phenylenediamine	Y = 2.11e + 003 X − 1.15e + 003	0.9999	2
2,6-Diaminopyridine	Y = 6.32e + 004 X + 6.99e + 004	0.9999	0.1

water–acetonitrile system. When the pH value was below 3.0, all substances got good peak shape, but the retention times of some basic dyes (such as benzenediamines) were too short to reach the baseline separation. When the pH value was between 4.0 and 5.0, some peaks tailed off slightly, but when the pH value was above 5.0, peak overlapping and broadening were obvious.

According to the formula: $pH = pK_a + \log([A^-]/[A])$, 99% of the compound will exist in one species when buffer pH value is above or below its pK_a of two units. Since most dye intermediates contain amino groups, low pH values contribute to reducing the interaction between amino groups and Si-OH and enable sharp chromatographic peak. At the optimum buffer pH below 3.0, substances containing amino groups exist as cationic species, while substance without amino groups but containing hydroxyl groups exist as neutral species; therefore, most substances obtained good chromatographic peak shape. To improve the separation of basic dyes below pH 3.0, sodium 1-octanesulfonate was added as ion-pair reagent to extend their retention times. Results showed that 13 kinds of dye intermediates achieved good separation within 25 min. The typical chromatograms of standards and two real samples (hair dyes A and B) are shown in Figures 1–3.

Online UV absorption spectra (Figure 4) of dye intermediates by DAD (diode array detector) can be used for initial qualitative analysis and reducing false-positive results. The target compounds have characteristic absorption bands in the range of 215–240 nm and 270–320 nm with the exception of 2,6-diaminopyridine, which has an absorption maximum at 331 nm.

LINEAR RANGE AND DETECTION LIMIT

Quantitative determination was performed on 13 dye intermediates. The calibration curves for these compounds were linear within 2–500 μg/ml with 0.999 as a typical correlation coefficient, and the detection limits ($S/N = 3$) were in the range of 0.2–2 μg/ml (Table I).

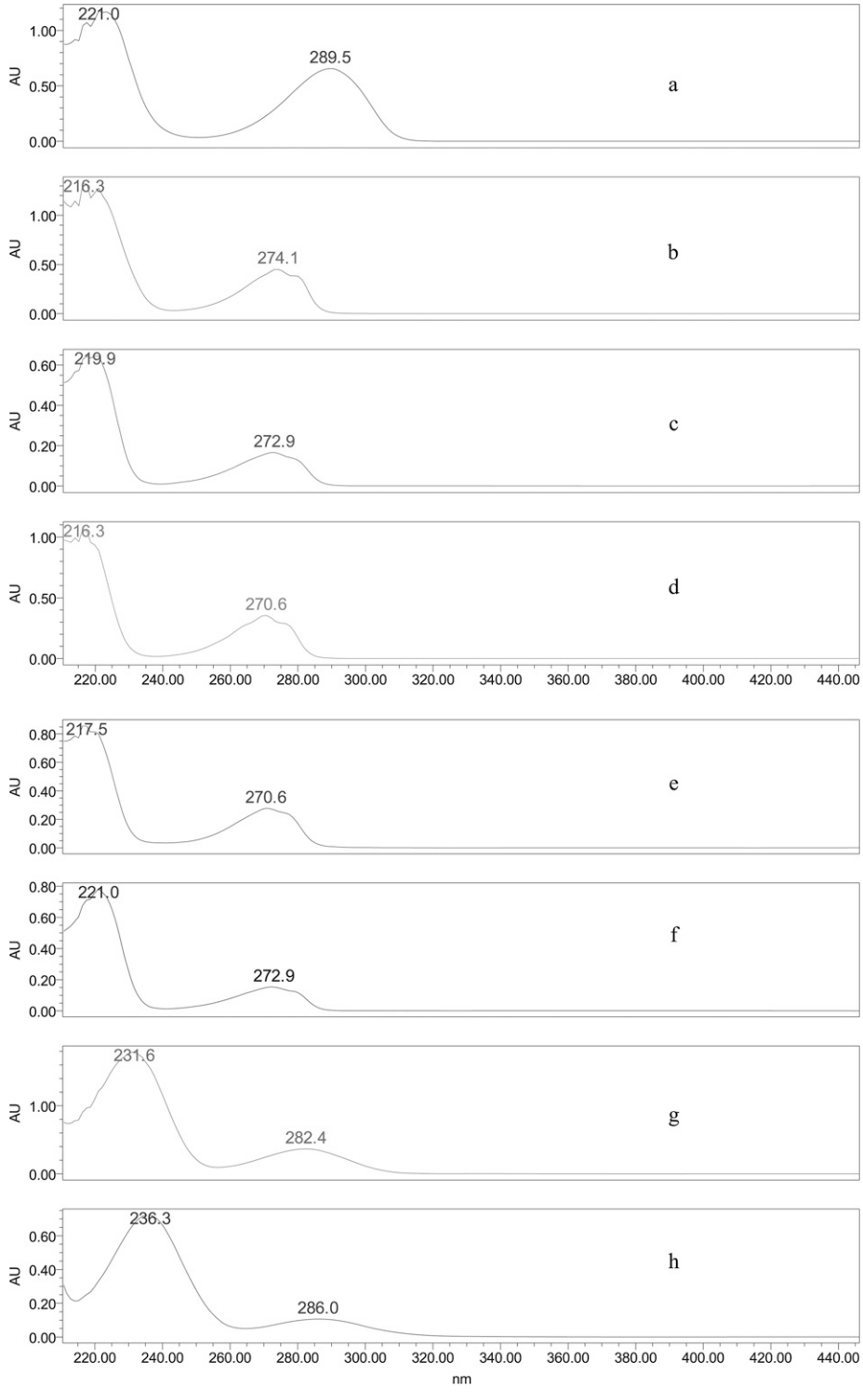

Figure 4. Continued

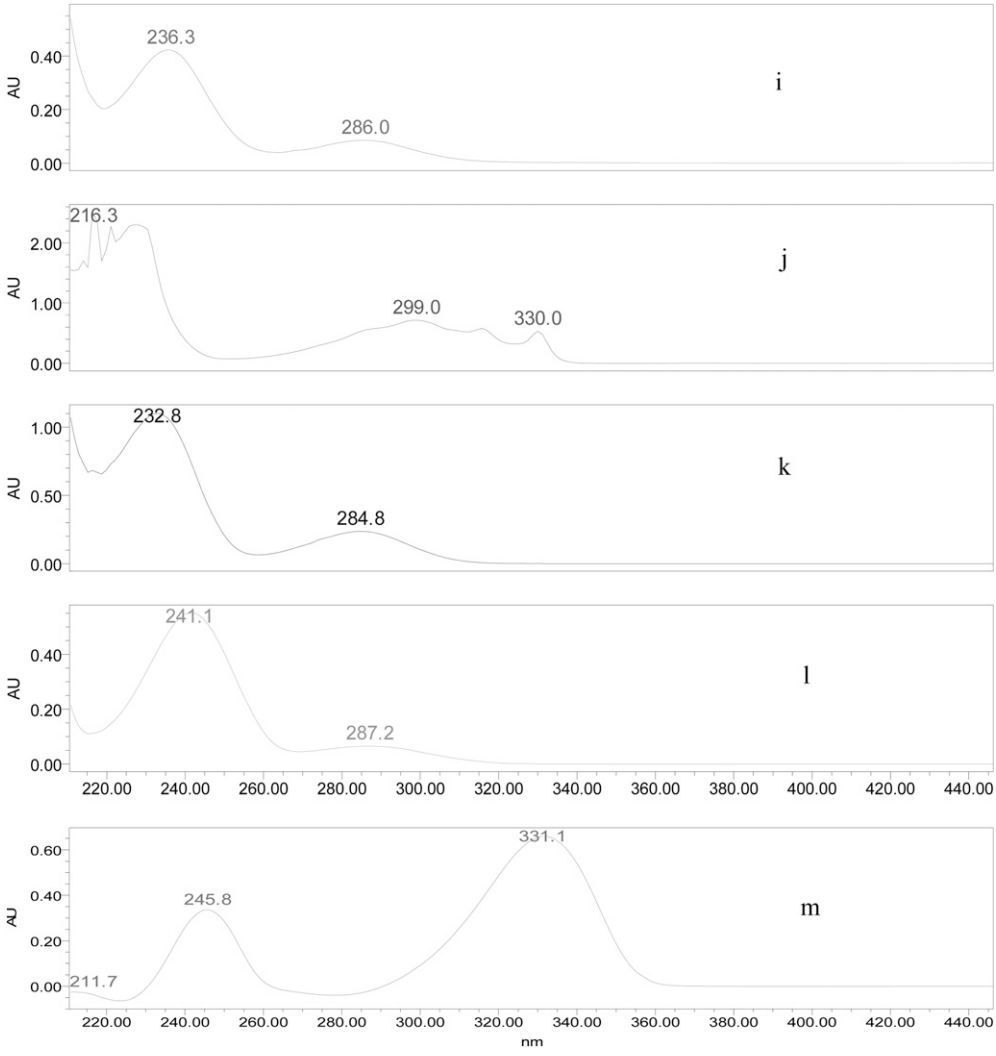

Figure 4. Online UV absorption spectra of dye intermediates. (a) Hydroquinone; (b) resorcinol; (c) *p*-aminophenol; (d) phenol; (e) *m*-aminophenol; (f) 4-methylaminophenol; (g) *o*-phenylenediamine; (h) *p*-phenylenediamine; (i) 2,5-diaminotoluene; (j) 1,5-naphthalenediol; (k) 3,4-diaminetoluene; (l) *N*,*N*-diethyl-*p*-phenylenediamine; (m) 2,6-diaminopyridine.

the range of 8.6–11.8 and 11.0–13.0, respectively (19). Therefore, the chromatographic resolution will be greatly influenced by adjusting the pH value of mobile phase. The effects of four mobile phase systems at different pH values, including water–acetonitrile, citric acid buffer–acetonitrile system (pH values were adjusted to 2.6, 3.0, and 3.5), sodium acetate buffer–acetonitrile system (pH values were adjusted to 4.0, 4.5, 5.0, and 5.5), and NaH_2PO_4-Na_2HPO_4 buffer–acetonitrile system (pH values were adjusted to 6.0, 6.5, 7.0, 7.5, and 8.0), on the separation efficiency were investigated. Unsatisfactory separation with poor peak shape and severe peak overlapping were observed by using pure

Table II
Recovery and Reproducibility of the Analysis of Commercial Permanent Hair Dyes by HPLC (n = 6)

		Hair dye A[a]			Hair dye B[b]		
Compound	Added (mg/kg)	Found (mg/kg)	Recovery (%)	C.V. (%)	Found (mg/kg)	Recovery (%)	C.V. (%)
Hydroquinone	25	20.4	81.7	0.74	20.6	82.5	1.04
	50	41.5	83.1	0.83	42.8	85.4	3.01
	250	211.5	84.6	1.02	218.0	87.2	1.45
	2500	2095	83.8	1.47	2128	85.1	1.52
Resorcinol	25	–	–	–	21.2	84.8	1.51
	50	–	–	–	45.0	90.2	3.18
	250	807.5	97.8	2.15	222.8	89.1	1.39
	2500	3000	97.6	2.05	2318	92.7	1.12
o-Phenylenediamine	25	20.9	83.5	1.44	21.1	84.3	1.50
	50	43.8	87.6	0.94	44.0	88.0	3.69
	250	248.0	99.2	2.78	215.8	86.3	1.89
	2500	2470	98.8	2.60	2365	94.6	1.66
1,5-Naphthalenediol	25	20.7	82.6	1.18	20.8	83.2	1.02
	50	42.8	85.6	1.45	43.3	86.3	3.61
	250	217.0	86.8	0.89	219.0	87.6	1.68
	2500	2158	86.3	1.09	2188	87.5	1.87
2,6-Diaminopyridine	25	20.8	83.0	1.04	21.0	83.8	1.88
	50	43.8	87.7	1.85	44.5	88.8	3.51
	250	230.8	92.3	1.02	218.8	87.5	1.43
	2500	2495	99.8	1.65	2515	100.6	1.27
p-Aminophenol	125	106.5	85.3	2.48	–	–	–
	250	217.3	86.9	1.53	–	–	–
	1250	1168	93.3	2.41	–	–	–
	2500	2315	92.6	1.23	–	–	–
Phenol	125	118.0	94.4	2.56	121.3	97.0	1.54
	250	246.5	98.6	1.89	235.3	94.1	1.82
	1250	1213	97.0	1.88	1193	95.4	2.35
	2500	2430	97.2	1.58	2423	96.9	1.57
m-Aminophenol	125	106.3	85.0	1.92	–	–	–
	250	212.5	85.0	1.14	–	–	–
	1250	1178	94.1	1.83	–	–	–
	2500	2463	98.5	3.33	–	–	–
4-Methylaminophenol	125	108.3	86.6	3.09	107.5	85.9	3.09
	250	217.3	86.9	1.10	215.0	86.0	0.96
	1250	1093	87.3	2.43	1103	88.1	2.83
	2500	2158	86.3	1.84	2180	87.2	1.53
p-Phenylenediamine	125	107.8	86.3	3.05	–	–	–
	250	220.3	88.1	2.15	–	–	–
	1250	1175	93.9	1.90	–	–	–
	2500	2520	100.8	1.80	–	–	–

(*Continued*)

Table II
Continued

Compound	Added (mg/kg)	Hair dye A[a] Found (mg/kg)	Recovery (%)	C.V. (%)	Hair dye B[b] Found (mg/kg)	Recovery (%)	C.V. (%)
2,5-Diaminotoluene	125	105.3	84.2	2.22	112.5	90.1	2.32
	250	217.3	86.9	1.08	218.5	87.4	1.53
	1250	1185	94.8	2.04	1198	95.7	2.66
	2500	2540	101.6	1.42	2533	101.3	1.04
3,4-Diaminetoluene	125	117.5	93.9	1.87	117.8	94.2	1.77
	250	244.0	97.6	2.64	245.0	98.0	2.17
	1250	1153	92.3	2.40	1248	99.9	2.23
	2500	2290	91.6	1.63	2525	101.0	0.80
N,*N*-diethyl-*p*-phenylenediamine	250	238.0	95.2	1.98	247.5	99.0	1.91
	500	477.5	95.6	2.54	490.0	97.8	2.32
	2500	2550	102.0	0.90	2253	90.1	1.26

[a]Hair dye A contained 536.6 mg/kg resorcinol.
[b]Hair dye B contained 3528 mg/kg *m*-aminophenol, 6618 mg/kg *p*-aminophenol, and 8727 mg/kg *p*-phenylenediamine.

ACCURACY AND RECOVERY

The results of the standard recovery test (Table II) showed that the average recoveries were in the range of 81.7–102.0% with four addition levels of two original commercial samples, and the RSDs (relative standard deviation) were about 0.9–3.5%. The precision data obtained from the evaluation of the results of a collaborative trial involving five laboratories and three replicates with hair dye A (containing 536.6 mg/kg resorcinol) and hair dye B (containing 3528 mg/kg *m*-aminophenol, 6618 mg/kg *p*-aminophenol, and 8727 mg/kg *p*-phenylenediamine) were shown in Table III.

CONCLUSIONS

A sensitive and accurate analytical method for the determination of 13 dye intermediates in commercial hair dyes was developed. The introduction of sodium dithionite as a reductant

Table III
The Repeatability (*r*) and Reproducibility (*R*) of This Method (mg/kg) (20)

Sample	Compound	m[a]	S_r[b]	S_R[c]	r[d]	R[e]
Hair dye A	Resorcinol	553	10	12	29	34
Hair dye B	*m*-Aminophenol	3525	66	68	183	189
	p-Aminophenol	6631	60	90	167	252
	p-Phenylenediamine	8636	78	78	217	217

[a]m is the general average.
[b]S_r is the repeatability standard deviation.
[c]S_R is the reproducibility standard deviation.
[d]r is calculated according to ISO 5725-2:1994.
[e]R is calculated according to ISO 5725-2:1994.

during the extraction process effectively prevented the deterioration of dye intermediates and increased the recovery. Good separation of 13 dye intermediates was achieved within 25 min using acetonitrile–citric acid mobile phase system (pH 2.6) with sodium 1-octanesulfonate as an ion-pair reagent. The good linearity, high recovery, excellent sensitivity, and precision recommended the use of the proposed methodology for the quantification of dye intermediates in all commercially available hair dye products.

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