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GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF TOXIC DIAMINES IN PERMANENT HAIR DYES

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SUMMARY

A rapid, simple and sensitive analytical method has been developed for the simultaneous determination of 1,4-diaminobenzene, 2,5-diaminotoluene and 2,4-diaminoanisole in permanent (oxidation type) hair dyes. The method utilizes an ethyl acetate extraction in the presence of NaCl followed by direct injection into a gas–liquid chromatograph equipped with a flame-ionization detector. The detection limits are 5 ng/μl each for 1,4-diaminobenzene and 2,5-diaminotoluene and 20 ng/μl for 2,4-diaminoanisole. The relative standard deviations at 5 times the detection limits are 5.6, 5.8 and 4.6% for these three compounds, respectively. Recovery of diamines from "spiked" dyes was generally found to be greater than 85%.

INTRODUCTION

Recently, the use of permanent hair dyes has been a subject matter of controversy[1–2]. These hair dyes also known as oxidation hair dyes consist usually of two ingredients which are mixed prior to use. One ingredient is an ammoniacal solution of a surface active agent in a hydroxylic solvent which contains oxidizable compounds (bases) such as arylamines, nitroarylamines, nitroaminophenols, aminophenols and polyphenols or their salts. Table I lists some important oxidizable bases and their abbreviations as used in the hair dye industry. The second ingredient is an oxidizing agent which is usually an aqueous solution of hydrogen peroxide. By mixing the two ingredients just before the application, oxidation takes place in the hair shaft thus permanently binding the developed shade. Some permanent hair dyes are commercially available in a package containing a single ingredient only which does not require mixing before application. Sodium sulfite is usually present in all permanent hair dyes as an antoxidant.

Several of the diaminic constituents present in the bases of these dyes have been found toxic and even carcinogenic by animal studies[2–6]. Some epidemiological studies suggest an increased risk of cancer among hairdressers and beauticians who are occupationally exposed to these chemicals[2,3]. Some of these chemicals are believed

TABLE I

PERMANENT HAIR DYE INTERMEDIATES, THEIR SYNONYMS AND ABBREVIATIONS

No.	*Hair dye intermediate*	*Synonym*	*Abbreviation*
1	1,4-Diaminobenzene	*p*-phenylenediamine	*p*-PDA
2	1,3-Diaminobenzene	*m*-phenylenediamine	*m*-PDA
3	2,5-Diaminotoluene	*p*-toluenediamine	2,5-TDA
4	2,3-Diaminotoluene	*o*-toluenediamine	2,3-TDA
5	2,4-Diaminotoluene	*m*-toluenediamine	2,4-TDA
6	2,4-Diaminoanisole	2,4-DAA or 4-methoxy-*m*-phenylenediamine	4-MMPD
7	*p*-Amino-diphenylamine	N-phenyl-*p*-phenylenediamine	N-Pen
8	2-Nitro-1,4-diaminobenzene	*o*-nitro-*p*-phenylenediamine	2-Ns
9	4-Nitro-1,2-diaminobenzene	*p*-nitro-*o*-phenylenediamine	2-No
10	4-Chloro-*p*-phenylenediamine		4-Clp
11	*p*-Aminophenol		*p*-AP
12	*m*-Aminophenol		*m*-AP
13	*o*-Aminophenol		*o*-AP
14	*p*-Nitro-*o*-aminophenol	2-amino-5-nitrophenol	2-A-5
15	*o*-Nitro-*p*-aminophenol	4-amino-2-nitrophenol	4-A-2
16	*p*-Nitro-*o*-aminophenol	2-amino-4-nitrophenol	2-A-4
17	Dinitroaminophenol	picramic acid	PIC
18	N-Phenyl-*p*-phenylenediamine		N-Phen
19	N,N-Dimethyl-*p*-phenylenediamine		N,N-DM
20	N,N-Diethyl-*p*-phenylenediamine		N,N-DE
21	Hydroquinone		Hy
22	Resorcinol		Res
23	α-Naphtol		α,N
24	Catachol		Cat
25	4,4′-Methylenebis	N,N-dimethylaniline	4,4′
26	*o*-Anisidine		*o*-An
27	*m*-Anisidine		*m*-An
28	4-Chloro-*m*-phenylenediamine		4-clm

to be present in hair dyes in amounts varying from several hundredths of one percent to several percent[7]. Therefore, a need existed to develop a simple method for the routine determination of toxic diamines in bulk hair dyes. In the past such determinations were mostly made using insensitive and lengthy methods such as titration, spectrophotometry, paper and thin-layer chromatography[7–12]. These analytical methods were generally used for quality control work in the hair dyes industry. Gas chromatography (GC) has been used for separating and identifying diaminotoluenes[13–16] and *p*-phenylenediamines[17–20]. These GC methods were either very insensitive or needed time consuming derivatization procedures for analysis at lower concentration levels. Goldstein[9] used a direct gas chromatographic method to determine aromatic diamines in hair deys. He used an internal standard to determine *p*-phenylenediamine and *p*-toluenediamine in the milligram range.

In this paper, a simple, rapid and sensitive method has been developed to determine three diamines, namely, 1,4-diaminobenzene, 2,5-diaminotoluene and 2,4-diaminoanisole in bulk hair dyes. The minimum detection limit of the method is in the low nanogram range. Some examples of the bulk hair dye analyses using the developed method are presented.

EXPERIMENTAL*

Gas–liquid chromatography

A Varian Model 3700 gas chromatograph equipped with a flame-ionization detector (FID) and an auto sampler and interfaced to a Hewlett Packard Laboratory Data System was used for the investigation. The column used for sample separation and analyses was a 6 ft. × 1/4 in. × 2 mm I.D. coiled glass, packed with 10% Carbowax 20M + 2% KOH on 80–100 Chromosorb W. Operating conditions were as follows: injector temperature, 220 °C, detector temperature, 250 °C, column temperature, 210 °C (isothermal); nitrogen carrier gas flow-rate, 30 ml/min; injection volume, 1–2 μl; hydrogen flow-rate, 30 ml/min; air flow-rate, 290 ml/min.

Reagents and chemicals

Spectrograde organic solvents as obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.) Matheson, Coleman & Bell (East Rutherford, NJ, U.S.A.) and Burdick & Jackson Labs. (Muskegon, MI, U.S.A.), were used without further purification. Aromatic diamines were obtained from Pfaltz and Bauer (Stamford, CT, U.S.A.). 2,5-Diaminotoluene was supplied as sulfate salt rather than as a free amine. Standard solutions of the diamines were prepared in various solvents (methanol, ethanol, carbon disulfide, methylene chloride, ethyl acetate) by dissolving known amounts of the diamines in the solvent. 2,4-DAA and *p*-PDA were dissolved after 2 min of sonication, however, 2,5-TDA dissolved in these solvents only after addition of 1–2 drops of 1 *N* NaOH to the solution and a 30-sec shaking.

Based on the ease of solubility, GC–FID response and chromatographic interferences, ethyl acelate (Burdick & Jackson Labs.) was judged to be the best solvent and hence, was used throughout this investigation.

Samples of hair dyes as purchased from retail outlets were used for determining the diamines content.

Procedure

Standard solutions of the three diamines were prepared in ethyl acetate in approximately 1 μg/μl concentrations. Dilutions were made from these stock solutions as needed. The stock standard solutions were stored in the refrigerator until use. Seven permanent hair dyes (designated as A, B, C, D, E, F and G) were obtained from retail stores. Five of the dyes (A–E) had two distinct ingredients packaged separately; one base dye in a glass bottle and the oxidizer in a white plastic bottle. The two ingredients are supposed to be mixed together prior to application.

The general procedure for the bulk dye analysis was as follows: 1 ml of the dye was mixed with 10 ml of ethyl acetate in a 20 ml screw-cap scintillation vial and 1 g of sodium chloride was added to improve separation of the ethyl acetate layer. The vial was capped, shaken for about 30 sec and allowed to stand for 5 min. A clear ethyl acetate layer was formed in all cases except in case of some darker dyes when the organic layer appeared somewhat cloudy. However, 1–2-μl aliquot of the ethyl acetate layer in each case was injected directly into the gas chromatograph for analysis.

* Mention of a specific product or company does not constitute endorsement by the National Institute for Occupational Safety and Health.

All organic layers after extraction appeared to darken gradually on standing. Hence, the analyses were performed as soon as possible (usually within 12 h) after extraction.

Some of the dyes themselves gradually changed to darker shades with time upon opening the bottle. Sometimes these had to be replaced by fresh bottles of original shades if the analysis was not completed within a few days after opening.

The pH of the dyes was monitored by an electronic pH meter and 6 *N* NaOH solution was used to control it.

RESULTS AND DISCUSSION

Fig. 1 illustrates a gas–liquid chromatographic separation of 1,4-diaminobenzene (*p*-PDA), 2,5-diaminotoluene (2,5-TDA) and 2,4-diaminoanisole (2,4-DAA) on a Carbowax 20M–KOH column. Excellent resolution with negligible peak tailing was achieved even at low levels of concentrations (in the low nanogram range) for the three compounds. The minimum detectable limits on a reasonably noiseless baseline were 5 ng each for *p*-PDA and 2,5-TDA and 20 ng for 2,4-DAA. These minimum

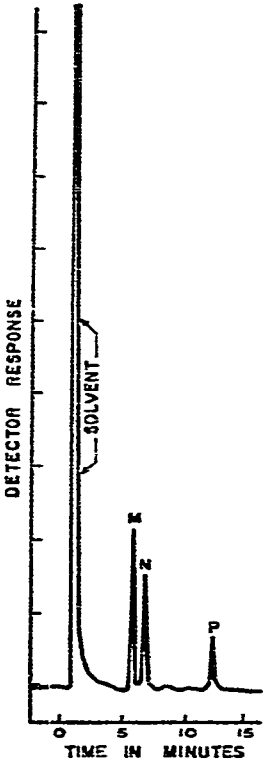

Fig. 1. A typical gas–liquid chromatographic separation of low nanogram concentrations of M, 1,4-diaminobenzene (*p*-PDA); N, 2,5-diaminotoluene (2,5-TDA) and P, 2,4-diaminoanisole (2,4-DAA) on 10% Carbowax 20 M–2% KOH coiled glass column at 210 °C isothermal conditions. Column: 6 ft. × 1/4 in. × 2 mm I.D.; nitrogen flow-rate: 30 ml/min.

detection levels could be decreased 5–10 fold but with increased baseline noise. The differing FID response factor was observed to be the main reason affecting the detection of 2,4-DAA. The precision of the determination of the above three diamines are given in Table II. The standard solutions for this investigation consisted of 26 ng/μl each of *p*-PDA and 2,5-TDA and 100 ng/μl of 2,4-DAA. The runs in the table were made on various days within a 2-week period. The standard mixture was stored in a refrigerator when not in use.

TABLE II

PRECISION OF THE GAS–LIQUID CHROMATOGRAPHIC DETERMINATIONS FOR 1,4-DIAMINOBENZENE (*p*-PDA), 2,5-DIAMINOTOLUENE (2,5-TDA) AND 2,4-DIAMINOANISOLE (2,4-DAA) ON CARBOWAX 20 M–KOH COLUMN AT 210 °C

Diamine	*Retention time (min)*	*Computer area count*	*R.S.D. (8 runs) (%)*
p-PDA (26 ng/μl)	7.07	7295	5.6
	7.04	8064	
	7.06	7350	
	7.10	8100	
	6.97	6874	
	6.99	7640	
	6.99	7825	
2,5-TDA (26 ng/μl)	8.40	6423	5.8
	8.36	6500	
	8.39	6548	
	8.42	6700	
	8.43	6948	
	8.40	7134	
	8.28	5941	
	8.30	6332	
2,4-DAA (100 ng/μl)	15.86	4196	4.6
	15.79	4348	
	15.84	4487	
	15.90	4213	
	15.90	4242	
	15.88	4152	
	15.67	3942	
	15.68	3907	

Two typical chromatograms as obtained by the injection of hair dye extracts are shown in Fig. 2a and b. Although the chromatograms appear to be complicated, no interferences were found to impede the determinations of either of the three diamines under consideration. 2,4-DAA showed up as a shoulder on a large peak (×) but the computer integration was adequate to quantify this small 2,4-DAA separation.

No attempt was made to identify the big artifact peak. This artifact peak was present on the chromatograms of all the investigated hair dyes and it might be one of the dye ingredients or their reaction product. Besides the computer matching of the retention times, the presence of the three diamines under investigation were also confirmed by GC–mass spectrometry (MS). The prominent mass spectrometric ions for *p*-PDA were at *m/e* 108, *m/e* 80 and *m/e* 81; for 2,5-TDA were at *m/e* 122,

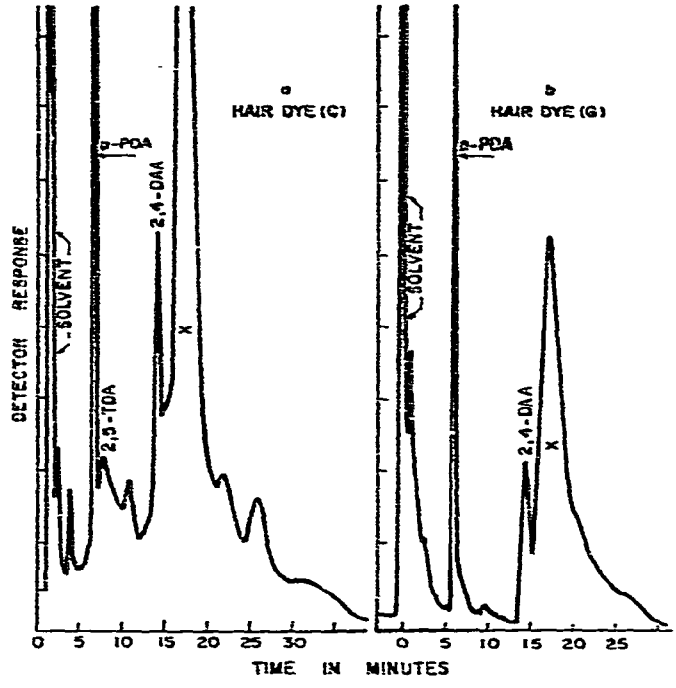

Fig. 2. Gas–liquid chromatograms as obtained from the extractions of two permanent hair dyes: C (a) and G (b). Temperature: 210 °C isothermal; column: 6 ft. × 1/4 in. × 2 mm I.D. coiled glass packed with 10% Carbowax 20 M–2% KOH on Chromosorb W AW (80–100 mesh), nitrogen flow-rate; 30 ml/min.

m/e 121 and *m/e* 94 and for 2,4-DAA were at *m/e* 138, *m/e* 123 and *m/e* 89. The fragmentations were consistent with those of the diaminic peaks from the dye extractions. The authenticity of the determinations were checked frequently by running standards and by GC–MS investigations.

The results for the three diamines as determined from the investigations of 7 permanent hair dyes are given in Table III. The table also shows the precision of the results as observed during these determinations; the RSD values in the table are based on 3 to 4 replicate determinations for each dye and are found higher than those of standards. This relatively low precision is probably associated with the more complex chromatograms in the case of the dye extractions.

Spiking

In order to confirm the validity of the analysis, 1 ml of each dye was spiked with each diamine in the concentration range of 3 to 10 mg/ml and analyzed in the usual way. The recovery in each case was between 85 and 109%. Duplicate (sometimes triplicate) runs were made for each spiking. In case of the dyes which has the presence of one or more of the diamines during the analysis, the recovery calculations were based on the difference between the pre-determined amount of the analyte present and amount added. When the spikings were done with a low concentration of diamines (using approximately 100 ng/μl) the recovery was 80% or better for dyes A, B and E.

TABLE III

RESULTS OF THE DETERMINATIONS OF 1,4-DIAMINOBENZENE (*p*-PDA) 2,5-DIAMINOTOLUENE (2,5-TDA) AND 2,4-DIAMINOANISOLE (2,4-DAA) IN PERMANENT HAIR DYES

Numbers in parentheses indicate percent relative standard deviations (% R.S.D.)

Hair dyes	*Diamine (mg/ml)*		
	p-PDA	*2,5-TDA*	*2,4-DAA*
A	<0.005*	<0.005*	<0.02*
B	12.2 (9.6)	<0.005*	0.50 (20)
C	29.2 (9.5)	<0.005*	4.30 (2.5)
D	12.0 (11.8)	<0.005*	0.1 (30)
E	<0.005*	4.20 (2.4)	<0.02*
F	<0.005*	<0.005*	<0.02*
G	32.5 (20.4)	<0.005*	12.4 (10.5)

* Detection limits.

The recovery of dye F at this lower level was about 50% for *p*-PDA and 2,5-TDA. No 2,4-DAA was detected. These poor recoveries may be attributed to some unknown diamine–dye component interactions.

pH Change

The usual pH of all seven dyes was in the range of 10.2–10.6. One round of analytical runs was made by changing the pH of each dye to 13 before extraction with ethyl acetate. This change of pH did not affect the result of the analysis for any dye; thus implying that the diamines found in these analyses were already present as free base.

CONCLUSION

A simple and sensitive gas–liquid chromatographic method has been developed for direct determination of diamines in oxidation-type hair dyes. The amount of sample handling time and potential loss of sample during the determinations are reduced considerably. In spite of the complicated nature of the hair dye formulations, the chromatographic separations of the three diamines under considerations are good and are conveniently capable of integration by the computer. The reactive nature of the diaminic components in these dyes, apparently contributes to the poor precision for the determinations. The method is considered suitable for routine analysis of the three diamines in hair dyes.

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