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Determination by gas chromatography/mass spectrometry of *p*-phenylenediamine in hair dyes after conversion to an imine derivative

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Abstract

In this paper we describe an analytical method for the determination of *p*-phenylenediamine (PPDA) in hair dyes. In the adopted methodology the analyte is transformed into the corresponding imine derivative by treatment with benzaldehyde, and then analyzed by gas chromatography (GC) combined to mass spectrometry (MS), operating in SIM conditions. The direct and simultaneous chemical derivatization of the two amino functions of the analyte with benzaldehyde enhances the instrumental responses enabling the use of a sensitive and accurate method. Concentrations of PPDA in a set of commercial hair coloring creams are determined making use of *N*-benzylidene-4-methylbenzene-amine as a very stable internal standard which is easily prepared by condensation of 4-methylbenzene-amine with benzaldehyde. The regression calibration curves for PPDA in hair dyes are linear within 0.1 ± 25 mg/ml with 0.99 as a typical correlation coefficient.

Keywords: Oxidation hair dye components; Aromatic diamines; Gas chromatography/mass spectrometry; Imines

1. Introduction

Commercial hair coloring products contain a mixture of *p*-phenylenediamine, benzenediols, aminophenols and other activated aromatic substrates which are dye precursors involved in the process of hair dyeing. These dye intermediates have shown mutagenicity in vitro and carcinogenic properties in vivo [1–7]. Because of the toxicity of their components, the coloring paste composition is under control of the European Council Directive (76/768/EEC). Therefore, a number of analytical procedures have been developed to separate and determine dye intermediates in coloring products and/or hair, including GC/MS [8–10], HPLC [11–14], CE [15–16] and MEKC [17]. Some of these methods require laborious and time expensive extractions of hair dye components followed by their chemical derivatization [8–10].

Among the known protocols, the extractive heptafluoro-*n*-butyrylation on an extrelut column is one of the most attractive procedures [18]. Complete separation of eight dye components, including three isomers of aminophenol and three aromatic amines was achieved by this methodology which nevertheless suffers from rather low levels of reproducibility and recovery.

In the formation of the hair color, *p*-phenylenediamine or *p*-aminophenol react with hydrogen peroxide to produce imine derivatives which in turn react with a coupler (e.g., 3-aminophenol,1,3-benzenediol) to furnish the target dye. The stability of these aromatic imines [19] suggested us to convert the PPDA into an imine, taking in account that coloring product composition should not affect the reaction

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of PPDA with aldehydes. Application of the gas chromatography/mass spectrometry technique operating in SIM mode to determine the so derivatized PPDA obtained from commercially available hair dyes is the central aspect of this work.

2. Materials and methods

2.1. Chemicals

Benzaldehyde (+99%, chlorine free), *p*-phenylenediamine (+99%), and *p*-toluidine (+99%, powder), were purchased from Sigma–Aldrich (Milan, Italy).

Thin layer chromatography (TLC) was performed on neutral Al_2O_3 precoated glass plates (Merck, Darmstadt, Germany), using $CHCl_3$/MeOH (9:1, v/v) as eluent, and visualizing the spot of residual benzaldehyde with a 5% solution of 2,4-dinitrophenylhydrazine in ethanol.

2.2. Standard solution

To plot the calibration curves, two solutions of 40 mg/ml of *N*-benzylidene-4-methylbenzene-amine (solution A) and 140 mg/ml of N^1,N^4-dibenzylidenebenzene-1,4-diamine (solution B) in tetrahydrofuran (THF) were prepared.

An appropriate volume of the solution B was added to 1.25 ml of the solution A in a screw capped vial, and the resulting mixture was diluted to 5 ml with THF. Thus, 10 different concentration levels of N^1,N^4-dibenzylidenebenzene-1,4-diamine were prepared: solutions **1a**-**5a** contained 10 mg/ml of internal standard and 0.1, 0.5, 1.0, 3.0 and 5.0 mg/ml of N^1,N^4-dibenzylidenebenzene-1,4-diamine, respectively; solutions **1b**-**5b** contained 10 mg/ml of internal standard and 3.0, 5.0, 10, 20 and 25 mg/ml of N^1,N^4-dibenzylidenebenzene-1,4-diamine, respectively. One microliter of each solution was injected for the GC/MS analysis.

Calibration standards were analyzed by GC/MS and the peak area ratios (PPDA/internal standard) were plotted against the concentration of PPDA in the calibration standards. Calibration curves were obtained from weighted least-square linear regression analysis of data.

To test the linearity of analysis, the two solution series **1a**-**5a** and **1b**-**5b** were injected three times during the same day (**1a**-**5a**: $y = 0.040x - 0.004$, $R^2 = 0.997$; $y = 0.040x - 0.004$, $R^2 = 0.998$; $y = 0.040x - 0.003$, $R^2 = 0.997$. **1b**-**5b**: $y = 0.119x - 0.412$, $R^2 = 0.992$; $y = 0.121x - 0.420$, $R^2 = 0.991$; $y = 0.117x - 0.413$, $R^2 = 0.990$).

2.3. Derivatization of hair dye components with trifluoroacetic anhydride

Trifluoroacetic anhydride (5 ml) was added to 2 g of black hair coloring cream dissolved in THF (15 ml). The resulting mixture was stirred for 5 h at room temperature. After this time, the solvent was evaporated under vacuum and the resulting residue was treated with a saturated aqueous solution of $NaHCO_3$ (pH 8) and extracted with chloroform (3 × 5 ml). The combined organic extracts were dried over Na_2SO_4 and evaporated to dryness. The obtained product was dissolved in diethyl ether (10 ml) and stored at 4 °C. One microliter of this solution was injected for the GC/MS analysis.

2.4. Preparation of the N^1,N^4-dibenzylidenebenzene-1,4-diamine

Benzaldehyde (975.6 mg, 9.20 mmol) was added to a magnetically stirred solution of *p*-phenylenediamine (497.1 mg, 4.60 mmol) in THF (20 ml). The resulting mixture was treated with a catalytic amount of 4-methylbenzenesulfonic acid (23.74 mg, 0.138 mmol) and then Na_2SO_4 (236.13 mg, 18.4 mmol) was added as drying agent.

After 5 h at 70 °C, the reaction mixture was filtered and the solvent evaporated under vacuum, to give a yellow oil which was dissolved in $CHCl_3$ (2 ml). The product was recovered in 81% yield after precipitation from *n*-hexane (15 ml). The purity of the final compound was checked by GC/MS and ^{1}H NMR.

^{1}H NMR ($CDCl_3$), δ (ppm): 7.29–7.31 (m, 4 H; Ar–H), 7.46–7.52 (m, 6 H; Ar–H), 7.90–7.95 (m, 4 H; Ar–H), 8.52 (s, 2 H; HC=N).

m.p.: 136–138 °C.

GC/MS (EI): 284 [$M^{\bullet+}$, 100%]; 283 (40); 207 (9); 180 (5); 77 (7).

2.5. Preparation of *N*-benzylidene-4-methylbenzene-amine

Benzaldehyde (4.18 g, 39.40 mmol) was added to a magnetically stirred solution of *p*-toluidine (4.0 g, 37.40 mmol) in THF (50 ml). The resulting mixture was treated with a catalytic amount of 4-methylbenzenesulfonic acid (193.0 mg, 1.12 mmol) and then Na_2SO_4 (10.6 g, 74.8 mmol) was added as drying agent. After 5 h at 70 °C, the reaction mixture was filtered and the solvent evaporated under vacuum to give a yellow oil which was dissolved in $CHCl_3$ (6 ml). The product was recovered in 84% yield after precipitation from *n*-hexane (60 ml). The purity of the final compound was checked by GC/MS and ^{1}H NMR.

^{1}H NMR ($CDCl_3$), δ (ppm): 2.43 (s, 3 H; CH_3), 7.15–7.32 (m, 4 H; Ar–H), 7.48–7.57 (m, 3 H; Ar–H), 7.90–7.99 (m, 2 H; Ar–H), 8.50 (s, 1 H; HC=N).

GC/MS (EI): 195 [$M^{+\bullet}$, 96%]; 194 (100); 180 (5); 118 (11); 91 (41); 77 (4); 65 (12).

2.6. Sample preparation

1.25 ml of the solution A (see standard solution paragraph) was added to a crimp top vial containing 0.50 g of hair colouring cream and benzaldehyde (0.11 g, 1.04 mmol) suspended in THF (3 ml). The resulting mixture was treated with a

catalytic amount of 4-methylbenzenesulfonic acid (5.93 mg, 0.031 mmol) and then Na_2SO_4 (443.0 mg, 3.12 mmol) was added as drying agent. After stirring for 5 h at 70 °C, the reaction mixture was filtered, the mother liquor was diluted to 5 ml with THF, and the resulting solution was stored at 4 °C. One microliter of this solution was injected for the GC/MS analysis.

2.7. GC/MS

The GC/MS analyses were performed using a GC system G1800 Series II (Agilent Technologies Inc., Palo Alto, CA, USA), equipped with an HP-5MS (30 m × 0.25 mm, PhMesiloxane 5%) capillary column. Complete characterization of the imines was carried out operating in scan mode. The confirmation of the identity of N^1,N^4-dibenzylidenebenzene-1,4-diamine was performed by EI (70eV, RT 24.03 min) mass spectrum, which shows the ions at *m*/*z* 284, 180, 104 and 77 corresponding to the species $[M]^{\bullet+}$, $[M - C_7H_6N]^{\bullet+}$, $[C_7H_6N]^{\bullet+}$, and $[C_6H_5]^{\bullet+}$, respectively. The quantitative analysis of the PPDA imine derivative was performed in selected ion monitoring (SIM) mode. For N^1,N^4-dibenzylidenebenzene-1,4-diamine were monitored the ions *m*/*z* 283 and 284, while for *N*-benzylidene-4-methylbenzeneamine, the internal standard, the ions *m*/*z* 194 and 195.

The GC/MS analyses were carried out in splitless mode (1 min purge off) using helium as carrier gas at a flow rate of 1 ml/min. The injection port temperature was 250 °C; the oven was maintained at an initial temperature of 60 °C for 2 min, and then programmed at 14 °C/min (11 °C/min for trimethylsilyl derivatives) to a final temperature of 280 °C, where it was maintained for 20 min.

2.8. Validation

1.25 ml of the solution A (10 mg/ml) were added to two crimp top vials containing 5 and 15 mg/ml of PPDA, respectively. The resulting solutions were then treated with benzaldehyde according the above described procedure. The obtained products were analyzed and measured against the calibration curve ($y = 0.040x - 0.004$, $R^2 = 0.998$; $y = 0.119x - 0.412$, $R^2 = 0.992$, respectively). To test repeatability and reproducibility of the analyses, 1 μl of each solution product was injected three times during the same day. The determined concentration levels were 4.89, 4.91 and 4.93 mg/ml for the sample containing 5 mg/ml of PPDA. Accuracy was 97.60% and the medium value (MV) determined was 4.88 mg/ml with a standard deviation (SD) of 0.02. For the sample containing 15 mg/ml of PPDA the accuracy was 98.33% and the determined concentration levels were 14.67, 14.78 and 14.80 mg/ml. In this case, MV was 14.75 mg/ml with SD of 0.07.

The limit of detection (0.05 mg/ml) was calculated after repeated injections of the appropriately diluted solution B.

3. Results and discussion

The analytical technique GC/MS in SIM mode, applied to determine the concentration levels of *p*-phenylenediamine in hair dyes, requires complete derivatization of this intermediate to enhance the instrumental response [20]. The development of a rapid and sensitive derivatization procedure of hair dye intermediates, without pre-purification steps, is therefore essential for the accuracy in a quantitative determination and for obtaining the best recovery of the analytes. Amines are successfully analyzed by GC/MS after preparation of their trifluoroacetyl-, trimethylsilyl-, ethyloxycarbonyl- or butyloxycarbonyl-derivatives [21,22]. Pentafluoropropionylation and heptafluorobutyrylation are two protocols preferred when complex mixtures have to be analyzed. Consequently, we first investigated a trifluoroacetylation procedure to get complete chemical derivatization of dye components. The total ion chromatogram (TIC) obtained by injecting 1 μl of the organic solution arising from the treatment of an hair coloring cream with trifluoroacetic anhydride showed several peaks within 4–18 min which were recognized as myristyl myristate (R_t = 17.72 min), 2-amino-4-hydroxyethylaminoanisole sulfate (R_t = 15.72 min), cetearyl alcohol components (R_t = 12.10, 11.84, 11.37, 10.89, and 10.90 min), 1-naphthol (R_t = 8.07 min), and butylated hydroxytoluene (R_t = 8.04 min). Nevertheless, the trifluoroacetyl derivative of *p*-phenylendiamine (R_t = 8.60 min) gave a low intensity peak responsible for only a very small percentage of the recorded total ion current.

By considering the complexity of the TIC obtained from this preliminary experiment, and the very low response obtained in the case of trifluoroacetylated PPDA, we retained this procedure to be totally useless to the aim.

In another set of analyses, we tried to perform the simultaneous derivatization of all amino and phenol functions of hair dye intermediates making use of *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide and trimethylsilyl chloride [23], after dehydration of the hair dye creams. GC/MS analysis of the trimethylisilyl derivative mixtures provided a complete determination of phenolic components, but no derivatization of amino functions was observed. Even though this procedure allowed the contemporaneous characterization of four dye components, it did not account for a quantitative analysis of PPDA.

In analogy to the hair dyeing process, a complete derivatization of PPDA could be performed by a stable imine; because the content and the composition of the colouring products should not affect the reaction with an aldehyde. Therefore, benzaldehyde was chosen as the best reactant to prepare imine derivatives from PPDA, and the very good stability generally observed for aromatic imines [15] enabled us to propose a novel and very efficient protocol for the determination of PPDA in commercial hair dyes.

Time and reaction conditions useful to prepare the target imine were determined using different solutions of a pure sample of PPDA. In a typical procedure, 1 equivalent of

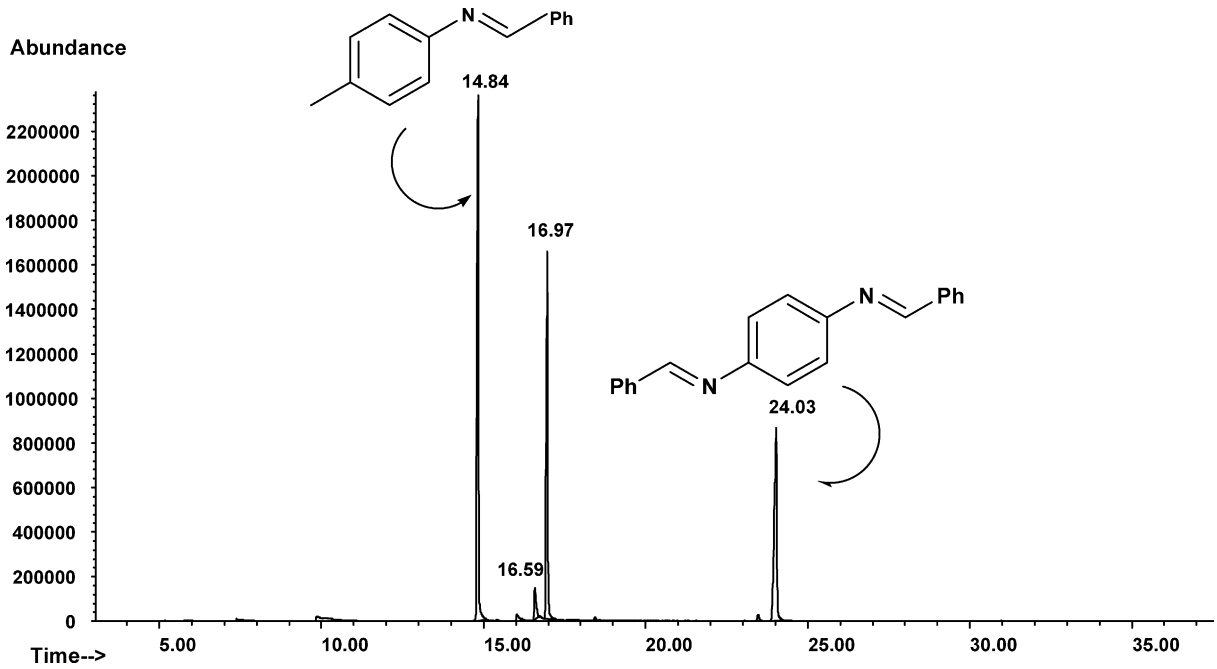

Fig. 1. Total ion chromatogram of imine derivatives: benzylidene-4-methylbenzeneamine (R_t = 14.84 min), cetearyl alcohol (R_t = 16.97 min) and N^1,N^4-dibenzylidenebenzene-1,4-diamine (R_t = 24.03 min).

PPDA was treated with a two-fold stoichiometric amount of benzaldehyde in dry THF. The mixture was maintained under magnetic stirring at 70 °C, and after 5 h TLC analysis of the crude mixture showed the complete conversion of PPDA. The obtained imine (N^1,N^4-dibenzylidenebenzene-1,4-diamine) was characterized by ^{1}H NMR and mass spectrometry (see experimental section). GC/MS analysis of the pure diimine derivative did not show peak splitting, indicating that condensation of the aldehyde with PPDA under the adopted experimental conditions affords only one product. From ^{1}H NMR data we determined the exclusive formation of only one isomer, the structure of which is reported in Fig. 1. In particular the 300 MHz spectrum recorded on a solution of the pure PPDA diimine dissolved in $CDCl_3$ was explicative of an (*E*)-geometry for both the C=N bonds. In fact, the couple of azomethine protons appeared in the spectrum as a narrow singlet downfield shifted and resonating at 8.52 ppm. This value exactly matches that already reported in literature for the same geometric isomer of the N^1,N^4-dibenzylidenebenzene-1,4-diamine [24]. *N*-Benzylidene-4-methylbenzeneamine was also recovered in form of the only one (*E*)-isomer, as demonstrated by the singlets attributable to the methyl and azomethine proton resonances, emerging in the ^{1}H NMR spectrum at ppm values typically referred for this type of geometry. The stability of N^1,N^4-dibenzylidenebenzene-1,4-diamine in THF was confirmed by subjecting this compound to GC/MS and ^{1}H NMR analyses, daily. No degradation products were observed after 15 days and more prolonged times of storage did not change the purity of the PPDA imine derivative.

Moreover, the investigated hair dyes products were preliminary subjected to GC/MS analyses performed on their extracts collected making use of different organic solvents and under different experimental conditions. The results demonstrated the total absence of *p*-toluidine, prompting us to select the corresponding imine derivative, the *N*-benzylidene-4-methylbenzene-amine, as the optimal internal standard.

The procedure of derivatization was extended to several commercially available hair dyes spiked with an appropriate amount of *N*-benzylidene-4-methylbenzeneamine. GC/MS analysis of the resulting solutions showed a marked enhancement of the instrumental response for N^1,N^4-dibenzylidenebenzene-1,4-diamine (Fig. 1).

To test the repeatability of the analysis, the concentration level of PPDA in the samples **3**, **4** and **8**, obtained by weighting 0.5 g of black, brown and blond dye cream respectively, was measured three times by the proposed methodology after spiking each sample with a definite amount (10 mg/ml) of the internal standard and the concentration was estimated by using a calibration curve (Table 1).

Subsequently, in order to evaluate the analyte recovery, the concentration level of PPDA in the same samples **3**, **4** and **8** was determined after addition in each sample of a definite amount of a mixture of N^1,N^4-dibenzylidenebenzene-1,4-diamine (10 mg/ml) and *N*-benzylidene-4-methylbenzeneamine (10 mg/ml). In this case also, every sample was subjected three times to measurement.

From all samples, the analyte was recovered with percentage values ranging from 97.50 to 99.06% for the black

Table 1
p-Phenylenediamine (PPDA) concentration of samples **3**, **4** and **8** (0.5 g of cream) replicated three times each

Sample	Colour	Colour number	PPDA (mg/ml)[a]	MV	CV (%)
3	Black	V. 2.2	8.05 8.00 7.98	8.01	0.46
4	Brown	M. 5	3.49 3.47 3.45	3.47	0.57
8	Blond	T.M. 6	0.87 0.90 0.85	0.87	2.29

MV: medium value, CV: coefficient of variation.
[a] PPDA concentration measured in the final solution.

Table 2
p-Phenylenediamine (PPDA) concentration determined using 0.5 g of hair cream

Sample	Colour	Colour number	PPDA (mg/ml)[a]	PPDA/cream (g/kg)
1	Black	C. 2.2	4.40	44.0
2	Black	R.B. 2.2	7.48	74.8
3	Black	V. 2.2	8.01	80.1
4	Brown	M. 5	3.47	34.7
5	Brown	C. 5	2.31	23.1
6	Red	C. 7.46	0.16	1.6
7	Dark blond	C. 7.25	0.96	9.6
8	Blond	T.M. 6	0.87	8.7
9	Blond	C. 6.5	0.41	4.1
10	Blond	C. 9.03	0.31	3.1

[a] PPDA concentration measured in the final solution.

samples, from 97.80 to 98.67% for the brown samples, and from 98.10 to 98.80% for blond samples.

The method developed was applied to determine the content of PPDA in some commercially available black, red, brown, dark-blond, and blond hair dyes. All samples were purchased from the same producing company. Ten different hair dye samples were analyzed and the determined PPDA concentration levels are reported in Table 2.

N^1,N^4-Dibenzylidenebenzene-1,4-diamine and *N*-benzylidene-4-methylbenzeneamine are detected by GC/MS in SIM mode monitoring the ions at *m*/*z* 284 and 283 for N^1,N^4-dibenzylidenebenzene-1,4-diamine and *m*/*z* 195 and 194 for *N*-benzylidene-4-methylbenzeneamine.

The concentration levels of PPDA determined in all the examined hair dye creams, from blond to dark colour, range from 1.6 to 80.1 g/kg of cream. All these values are consistent with those established by the European Council Directive (76/768/EEC).

4. Conclusions

A sensitive and accurate analytical method for the determination of PPDA in commercial hair dyes was developed. The protocol combines an efficient sample derivatization, using the commercially available benzaldehyde, and a post-derivatization clean-up by filtration. In particular, the GC/MS technique operating in SIM mode, which involves the use of *N*-benzylidene-4-methylbenzeneamine as the internal standard, allows the estimation of concentration levels of PPDA up to 0.05 mg/ml. The good linearity, the excellent results for the recovery of PPDA and the high levels of sensitivity and precision recommend the use of the proposed methodology for the quantification of PPDA in all commercially available hair dyeing products.

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