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Determination of aromatic amines in hair dye and henna samples by ion-pair extraction and gas chromatography–mass spectrometry

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Abstract

A gas chromatography–mass spectrometry (GC–MS) method has been proposed for the determination of carcinogenic and toxic aromatic amines in hair dye, henna and dyed hair samples. The method includes ion-pair extraction of aromatic amines from aqueous samples with bis-2-ethylhexylphosphate (BEHPA) released after solving the samples in acidic solution followed by sonication, derivatisation of compounds with isobutyl chloroformate (IBCF) and their GC–MS analysis in both electron impact (EI) and positive and negative ion chemical ionisation (PNICI) mode as their isobutyloxycarbonyl (*iso*BOC) derivatives. The obtained recoveries of aromatic amines ranged from 92.2 to 98.4% and the precision of this method, as indicated by the relative standard deviations (RSDs) was within the range of 0.7–4.2%. The detection limits obtained from calculations by using GC–MS results based on signal-to-noise ratio (S/N)= 3 were within the range from 0.02 to 0.20 ng/g. In the present study, the commercially available 54 permanent hair dye, 35 modified or natural henna and 15 dyed hair samples were analysed for the aromatic amines by the proposed method and the method was shown to be suitable to determine the aromatic amine ingredients and metabolites of these commercial products.

Keywords: Ion-pair extraction; Aromatic amines; GC–MS; Hair dye and henna

1. Introduction

Aromatic amines like phenylenediamines, toluenediamines, aminophenols, toluidines, and chloroanilines are biologically active compounds widely used in the formulation of commercial oxidative (permanent) hair dyes as primary intermediates and/or couplers [1]. Aromatic amines in commercial hair dyes are absorbed percutaneously during normal use [2,3] and they can be toxic [4], mutagenic *in vitro* [5] and carcinogenic in experimental animals [5–7]. *In vitro* and animal studies support the carcinogenic potential of certain hair dye ingredients [5,8–11]. These include 4-methoxy-*m*-phenylenediamine, 2,4-diaminoanisole (2,4-DAAS), 4-chloro-*m*-phenylenediamine (4-C-*m*-PDA), 2,4-toluenediamine (2,4-TDA), 2-nitro-*p*-phenylenediamine (2NPPD) and 4-amino-2-nitrophenol (4A2NP) [9]. As reported in literature [5–11], different isomers of aromatic amines have different toxic and/or carcinogenic effects.

The contents of intermediates of oxidative hair dyes in hair colouring formulations are restricted according to Annex III of the EU Cosmetic Directive. In addition, the use of certain substances is banned in these formulations according to Annex II of the Directive because of their toxicity and/or carcinogenicity [4]. Therefore, the monitoring of levels of individual isomers of aromatic amines in commercial hair dye and henna samples is important to protect human health because of human exposure to these compounds.

The most widely used techniques to determine aromatic amines in hair dyes are high-performance liquid chromatography (HPLC) coupled with different detectors [12–19], gas chromatography (GC) [20,21] and micellar electro kinetic capillary chromatography (MEKC) [12]. The other analytical techniques have been described in the literature for the determination of aromatic amines in environmental samples [6,22–24], aqueous food simulants [25,26], biological fluids [27–29,] and aromatic amines released from azo colorants [6,30–34]. GC–MS has been recognised as the method of choice for the analysis of aromatic amines in hair dye and henna samples, due to the high efficiency of GC in separation and superior capacity of MS in structural identification. A pre-concentration step

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[22,26,28,35,36] is necessary to obtain good sensitivity, and derivatisation step [37] is generally required to improve the gas chromatographic properties because of the polarity of the amines.

The aim of this study was to develop analytical technique that could be applied to hair dye, henna and dyed hair samples to determine aromatic amines by adapting our previous method for the analysis of amines in environmental samples [22,23]. This paper presents an analytical procedure, proposed to enable the precise determination of individual isomers of aromatic amines in commercially available oxidative hair dye, henna and dyed hair samples. This procedure offers several significant advantages over the other techniques available, such as higher selectivity in isolation, resolution and sensitivity. In the proposed method, the compounds of interest were isolated from aqueous solution with chloroform containing bis-2-ethylhexylphosphate (BEHPA) as ion-pair reagent after dilution with acidic solution and derivatised with isobutyl chloroformate (IBCF). Aromatic amines were then analysed as their isobutyloxycarbonyl (*iso*BOC) derivatives by GC–MS.

2. Experimental section

2.1. Chemicals and reagents

All the reagents were of analytical grade. Bis(2-ethylhexyl)-phosphate, isobutyl chloroformate (IBCF), aniline (ANI), *N*-methylaniline (NMA), *o*-toluidine (OT), *m*-toluidine (MT), *p*-toluidine (PT), 4-chloroaniline (PCA), 4-ethylaniline (4-EA), 4-nitroaniline (4-NA), 2-aminophenol (2-AP), 3-aminophenol (3-AP), 4-aminophenol (4-AP), 2,4-toluenediamine (2,4-TDA), 2,6-toluenediamine (2,6-TDA), 3,4-toluenediamine (3,4-TDA), 2-nitro-*p*-phenylenediamine (2NPPD), diphenylamine (DPA), chloroform, toluene, di-sodium hydrogen phosphate, sodium hydrogen carbonate, sodium sulphate, hydrochloric acid and isobutylalcohol were purchased from Merck. 1,2-Phenylenediamine (1,2-PDA), 1,3-phenylenediamine (1,3-PDA) and 1,4-phenylenediamine (1,4-PDA) were supplied by Aldrich. 4-Aminobiphenyl (4-ABP) was supplied by Reidel-de Haen. Pyridine was purchased from J.T. Baker. 1-Naphthylamine (1-NPA) and 2-naphthylamine (2-NPA) were purchased from Sigma. 4-Chloro-*o*-phenylenediamine (4-C-*o*-PDA) and *o*-anisidine (2MOA) were purchased from Alfa Aesar. The hair dye and henna samples were supplied from various cosmetic shops, and hair samples were provided by hairdressers in Turkey as dyed in various colours

2.2. Samples extraction and derivatisation

The hair dye and henna samples (5–20 mg) were added to an Erlenmeyer and the compounds of interest were then extracted with 100–250 ml of 0.1 M aqueous HCl by sonication at 40 °C for 15 min. The pH of aqueous samples was adjusted to 1 by drop-wise addition of 0.1 M HCl and then, our previous method was applied to 10 ml of aqueous phases with minor modifications [22,23]. The aqueous samples were extracted with chloroform (3 × 3 ml) and separated using a separatory funnel. The pH of the aqueous phases was adjusted to 8, as it has been selected to be the optimised pH, by drop-wise addition of 1 M Na_2HPO_4 and then were extracted three times using 0.1 M BEHPA in chloroform (3 × 3 ml). The chloroform extracts containing the compounds to be analysed were back extracted with 0.1 M HCl solution for 3 min using ultrasonic bath. Then, the pH of the aqueous layer containing these amines was again adjusted to 8 and extracted three times with 0.1 M BEHPA in chloroform (3 × 3 ml). The chloroform layer (1 ml) was taken into a vial after drying over anhydrous sodium sulphate and then evaporated to dryness under nitrogen stream. Acetonitrile, pyridine and isobutyl alcohol (100, 200 and 20 μl) and then IBCF (300 μl) were added to residue and the vial was then closed and kept at room temperature for 10 min. Then, the mixture is evaporated under a gentle stream of nitrogen and the derivatised compounds are diluted in toluene. The toluene layer is separated and dried over anhydrous sodium sulphate after shaking with slightly alkaline methanol (1 ml) followed by 1.5 ml of 0.5 M NaOH for 1–2 min and centrifuging at 4000 × *g* for 2–3 min. The solution was then concentrated to a volume of 1 ml under a gentle stream of nitrogen and the final solutions (0.5–1 μl) were injected into GC–MS in splitless mode (5 min purge off). Although they were stable when stored at −15 °C, the *iso*BOC derivatives of the compounds of interest were analysed as soon as they were prepared. Aromatic amines are then analysed as their *iso*BOC derivatives by GC–MS in triplicate and quantitative analysis was performed in SIM mode.

Hair samples dyed in various colours (0.5–1 g) were denaturised with 100 ml of 2 M HCl by sonication at 65 °C for 20 min and then the pH of aqueous samples was adjusted to 1 by drop-wise addition of NaOH solution. The aqueous phases containing the compounds of interest were filtered and then analysed in the same way as the aqueous samples.

2.3. Instrumental analysis

A Thermo-Finnigan MAT 4500 GC–MS/MS instrument operating in the electron impact (EI) and positive and negative ion chemical ionisation (PNICI) mode was used. Column identification: ZB–5 ms, Column length: 30 m, ID: 0.25 mm, Film thickness: 0.25 μm. Temperature program: Initial Temp. 120 °C (2 min hold) 6 °C/min to 150 °C (3 min hold) at 3 °C/min to 290 °C (3 min hold) at 8 °C/min to 290 (2 min hold). Scan Range: 25–650 *m/z*, Electron energy: 70 eV for EI and 30 eV for PNICI.

3. Results and discussion

3.1. Interpretation of mass spectra of model compounds

Firstly, aromatic amine standards were derivatised with IBCF and their *iso*BOC derivatives were analysed by GC–MS. Total Ion Chromatogram (TIC) of derivatised amine standards are shown in Fig. 1.

Identification of the components was performed by interpreting their mass spectra obtained in the EI mode. All the

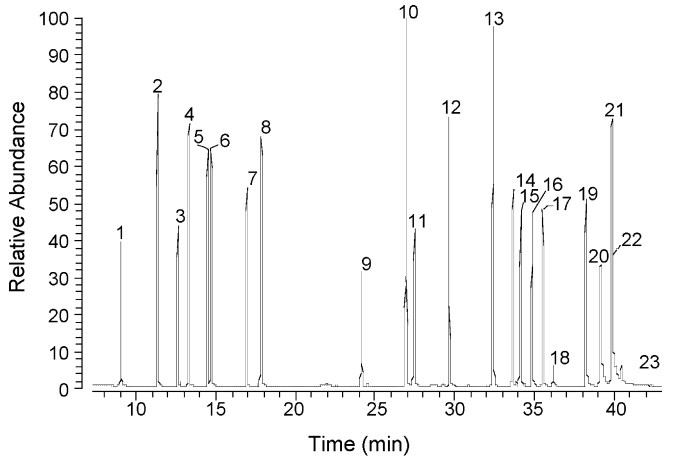

Fig. 1. TIC of isobutyloxycarbonyl derivatives of aromatic amine standards: (1) *N*-methylaniline; (2) aniline; (3) diphenylamine; (4) *o*-toluidine; (5) *m*-toluidine; (6) *p*-toluidine; (7) *o*-anisidine; (8) 4-chloroaniline; (9) 2-aminophenol; (10) α-naphthylamine; (11) 4-nitroaniline; (12) β-naphthylamine; (13) 1,3-phenylenediamine; (14) 3-aminophenol; (15) 4-aminophenol; (16) 3,4-toluenediamine; (17) 4-aminobiphenyl; (18) 4-chloro-*o*-phenylenediamine; (19) 2,4-toluenediamine; (20) 1,2-phenylenediamine; (21) 2,6-toluenediamine; (22) 1,4-phenylenediamine; (23) 2-nitro-*p*-phenylenediamine.

other *iso*BOC derivatives of aromatic amines were interpreted in the same way and expected fragmentation patterns were obtained. For example, interpretation of mass spectrum of *iso*BOC derivative of *o*-anisidine was carried out as follows; the ions observed at *m*/*z* 167 and 150 corresponds to the loss of isobutene ($CH_2C(CH_3)_2$) by a McLafferty-like rearrangement and the elimination of the free radical $(CH_3)_2CHCH_2O$ from the molecular ion of *iso*BOC derivative of *o*-anisidine observed at *m*/*z* 223, respectively. The molecular ion of *o*-anisidine at *m*/*z* 123 is formed by the elimination of isobutyloxycarbonyl group from the molecular ion of *iso*BOC derivative of *o*-anisidine observed at *m*/*z* 223. The loss of the butyl groups also gives the ion at *m*/*z* 57 when using IBCF. These peaks are characteristic for *iso*BOC derivatives. The ions observed at *m*/*z* 108 and 94 are formed by the loss of CH_3 and OCH_2 groups from the molecular ion observed at *m*/*z* 123, respectively. Identification of the compounds was also confirmed by GC–MS in PNICI mode using methane as reagent gas. The EI–mass spectra of *iso*BOC derivatives of (a) *o*-anisidine and (b) 4-aminobiphenyl are shown in Fig. 2.

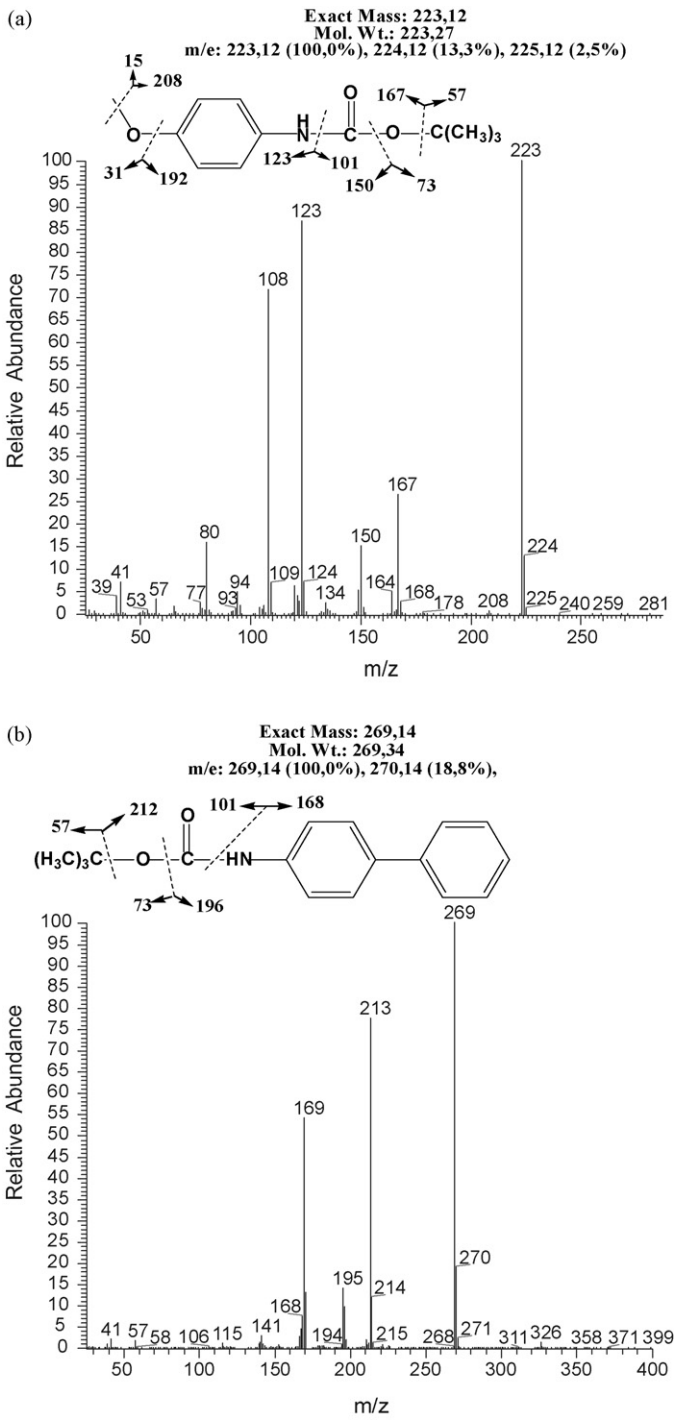

Fig. 2. Mass spectra of *iso*BOC derivatives of (a) *o*-anisidine and (b) 4-aminobiphenyl.

3.2. Recovery of the compounds of interest from hair dye and henna samples

Firstly, a standard solution containing 100 mg/l of each of the amines was prepared in acetone and 5 ml of this solution was derivatised with IBCF. The derivatised compounds were then diluted in toluene to give a final concentration of 100 μg/ml. Seven calibration standards with the concentrations in the range from 5 to 100 μg/ml were prepared by diluting the derivatised standard solution (100 μg/ml) and used for the calibration. Calibration standards of aromatic amines were analysed as their *iso*BOC derivatives by GC–MS in SIM mode in triplicate and calibrations were performed by calculating the peak areas of the compounds. The calibration curves of the compounds of interest were performed for five replicate runs in the investigated concentration range using standard solutions at different concentrations and the calibration graphs were linear for the concentration ranges stated. The linearity of the proposed method for the analysis of the compounds of interest in hair dye and henna samples was calculated in the investigated concentration range, as the determination coeffi-

Table 1
The optimum extraction pHs, absolute recoveries of aromatic amines for overall procedure with RSD at selected pH, calibration curves, R^2, LOD and QI

Standard compounds	Opt.ext. pH	Absolute recoveries (%) with RSD at selected pH 8	Calibration curves ($y = ax + b$)	R^2	LOD (ng/g)	QI 1, 2, 3 *m/z*
NMA	7.78	96.8 (0.7)	$y = 9.8 \times 10^5 x + 1.5 \times 10^7$	0.9997	0.02	151,106,207
ANI	8.01	95.3 (1.3)	$y = 3.2 \times 10^6 x + 1.7 \times 10^7$	0.9995	0.09	93,137,193
DPA	8.93	97.4 (1.8)	$y = 1.4 \times 10^6 x + 7.3 \times 10^6$	0.9996	0.03	169,141,115
OT	8.48	97.8 (1.5)	$y = 2.5 \times 10^6 x + 1.5 \times 10^7$	0.9990	0.20	150,106,207
MT	8.50	95.8 (2.1)	$y = 2.6 \times 10^6 x + 1.3 \times 10^7$	0.9987	0.20	107,207,151
PT	8.50	98.3 (1.6)	$y = 3.9 \times 10^6 x + 1.1 \times 10^7$	0.9995	0.20	207,151,107
2MOA	9.00	97.3 (1.1)	$y = 3.2 \times 10^6 x + 7.0 \times 10^6$	0.9997	0.10	223,108,123
PCA	8.50	96.2 (2.3)	$y = 2.9 \times 10^6 x + 1.5 \times 10^7$	0.9983	0.10	127,171,227
2-AP	7.76	97.6 (1.4)	$y = 8.9 \times 10^6 x + 5.8 \times 10^6$	0.9986	0.02	153,209,309
1-NPA	7.90	98.4 (1.5)	$y = 3.1 \times 10^6 x + 2.2 \times 10^7$	0.9983	0.04	243,143,187
4-NA	8.18	95.2 (2.7)	$y = 1.8 \times 10^6 x + 1.0 \times 10^7$	0.9994	0.12	182,108,238
2-NPA	8.02	96.3 (1.5)	$y = 2.7 \times 10^6 x + 1.5 \times 10^7$	0.9995	0.04	243,187,143
1,3-PDA	8.12	94.8 (1.5)	$y = 2.1 \times 10^6 x + 4.5 \times 10^6$	0.9999	0.07	308,152,252
3-AP	8.00	94.2 (1.2)	$y = 2.8 \times 10^6 x + 7.4 \times 10^6$	0.9998	0.02	153,209,309
4-AP	8.06	96.1 (1.4)	$y = 1.4 \times 10^6 x + 9.9 \times 10^6$	0.9998	0.02	153,209,309
3,4-TDA	8.20	92.8 (2.4)	$y = 1.8 \times 10^6 x + 9.3 \times 10^6$	0.9995	0.03	248,322,122
4-ABP	8.21	96.2 (1.6)	$y = 1.6 \times 10^6 x + 9.2 \times 10^6$	0.9996	0.10	269,213,169
4-C-*o*-PDA	8.26	92.4 (3.4)	$y = 2.1 \times 10^6 x + 8.1 \times 10^6$	0.9984	0.20	342,268,142
2,4-TDA	8.50	94.1 (1.6)	$y = 1.4 \times 10^6 x + 9.1 \times 10^6$	0.9985	0.03	322,166,266
1,2-PDA	8.00	93.5 (1.8)	$y = 3.8 \times 10^6 x + 2.3 \times 10^7$	0.9994	0.07	108,308,234
2,6-TDA	8.06	98.2 (1.4)	$y = 4.5 \times 10^6 x + 1.3 \times 10^7$	0.9996	0.03	322,166,222
1,4-PDA	8.22	97.2 (2.6)	$y = 3.1 \times 10^6 x + 1.3 \times 10^7$	0.9997	0.07	308,196,252
2NPPD	8.24	92.2 (4.2)	$y = 3.7 \times 10^6 x + 3.8 \times 10^6$	0.9988	0.20	353,297,197

Number of experiments (n) = 7; Quantifiers ions: QI_1, QI_2, QI

cients (R^2) and calibration curves ($y = ax + b$), shown in Table 1. The limit of detection (LOD) of each of the amines was estimated for seven replicate runs by comparing the signal-to-noise ratio (S/N) of the lowest detectable concentration to S/N of 3.

In order to evaluate recoveries of amines from aqueous solution, a solution of a known concentration of amines mixture was prepared by diluting the standard solution (100 mg/l) and known volumes of this solution were spiked into the distilled water that had previously been shown to contain no amines, to give final concentrations in the range from 10 to 100 ng/l of each amine. Spiking the known volumes (10, 25, 50 and 100 μl) of amines solution (100 μg/l) with hair dye and henna samples (5, 10 and 25 mg) which were analysed for the compounds of interest was also performed to evaluate absolute recoveries from hair dye and henna samples (plus the blank value). Quantitative analyses of the amines in known amounts of these samples were performed in six replicate using GC–MS in SIM mode. The concentrations of each amine were calculated by peak areas and these areas were compared with the calibration graphs of the standards. To investigate optimum extraction pHs of each aromatic amines, standard solutions at different concentrations were analysed for the compounds of interest by the proposed method in triplicate at different pHs within the range from 6.0 to 11.0 and optimum extraction pHs of the most of amines were found to be around 8. The extraction pH of the method was therefore selected to be 8 and recoveries of amines at selected pH were evaluated. The absolute recoveries of aromatic amines for overall procedure described in this work were estimated considering recoveries of the compounds of interest from aqueous solution with RSDs at selected extraction pH and the recoveries of amines from hair dye and henna samples. The optimum extraction pHs, absolute recoveries of aromatic amines for overall procedure with RSDs at selected pH, calibration curves ($y = ax + b$), correlation coefficients (R^2), limits of detection (LOD) and quantifier ions (QI) are shown in Table 1.

3.3. Determination of aromatic amines as their isoBOC derivatives in hair dye and henna samples

In order to confirm the viability, the proposed method was applied to the oxidative hair dye and henna samples to determine aromatic amines. The commercially available 54 oxidative hair dye samples in different colours collected from Turkey were analysed for the contents of toxic and/or carcinogenic aromatic amines by the proposed method in triplicate and the most of the target compounds were determined. Structural identification of *iso*BOC derivatives of aromatic amines was performed by interpretation of the mass spectra and possible match with reagent grade standards' spectra and existing library data obtained in EI mode and also confirmed by PNICI. Quantitative analyses of the identified amines were performed in SIM mode using their peak areas and these areas were compared with the calibration graphs. TIC of *iso*BOC derivatives of aromatic amines isolated from one of the dark brown hair dye samples is shown in Fig. 3. The concentration levels of aromatic amines determined in hair dye creams, from light yellow to dark black shades, are shown in Table 2. The henna samples (35), available as natural or modified henna, were analysed in triplicate by GC–MS in both SIM and PNICI mode and the concentration levels of aromatic amines determined, from yellow to black shades, are shown in Table 3. All of hair dye and henna samples have different aromatic amine

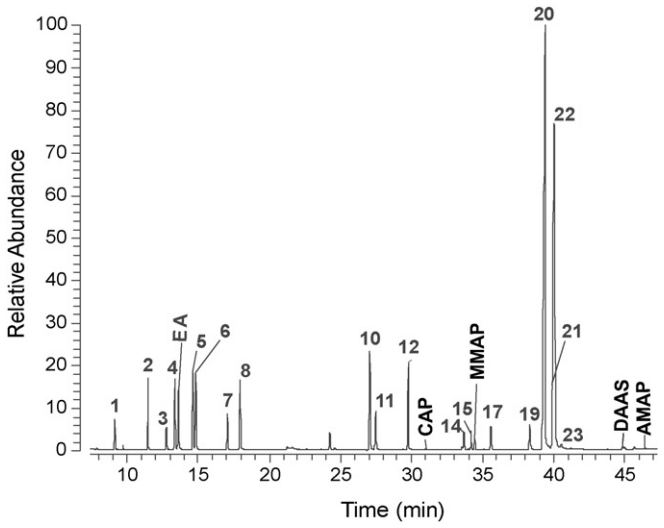

Fig. 3. TIC of isobutyloxycarbonyl derivatives of aromatic amines isolated from one of the dark brown hair dye samples: (1) *N*-methylaniline; (2) aniline; (3) diphenylamine; (4) *o*-toluidine; (EA) ethylaniline; (5) *m*-toluidine; (6) *p*-toluidine; (7) *o*-anisidine; (8) 4-chloroaniline; (10) α-naphthylamine; (11) 4-nitroaniline; (12) β-naphthylamine; (CAP) *Ø*-chloro-*Ø*-aminophenol; (13) 1,3-phenylenediamine; (14) 3-aminophenol; (15) 4-aminophenol; (MMAP) *Ø*-methoxymethyl-*Ø*-aminophenol; (17) 4-aminobiphenyl; (19) 2,4-toluenediamine; (20) 1,2-phenylenediamine; (21) 2,6-toluenediamine; (22) 1,4-phenylenediamine; (23) 2-nitro-*p*-phenylenediamine; (DAAS) *Ø*-diaminoanisol; (AMAP) *Ø*-aminomethyl-*Ø*-aminophenol.

ingredients and each of them contains primary intermediates at higher levels than that of their restricted values and/or the values of some aromatic amines which are banned in their use are shown in bold form in Tables 2 and 3.

1,4-Phenylenediamine (1,4-PDA) was found in all the analysed hair dye creams at highest concentrations to be up to 90301 μg/g, in 24 of the 25 henna up to 70413 μg/g and in 6 of the 10 natural henna samples up to 60670 μg/g. In addition, 1,3-phenylenediamine (1,3-PDA) was found in all the anaysed hair dyes and in 31 of the 35 henna samples at quantities up to be 40624 and 21562 μg/g, respectively. 1,2-Phenylenediamine (1,2-PDA) was also found at relatively higher concentrations in 31 of the 35 hennas which were up to 8320 μg/g and up to be 8010 μg/g in 49 of the 54 hair dye samples.

Toluene-2,5-diamine (2,5-TDA) was found in concentrations up to 8209 μg/g in 26/54 of hair dye formulations and up to 123 μg/g in 10/25 of henna samples whereas it was not detected in natural henna samples. Although the use of toluene-2,4-diamine (2,4-TDA) and 2,6-TDA is banned in hair colouring formulations according to Annex II of the EU Cosmetic Directive, 2,4-TDA, anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity [9], was detected in 35 hair dye and 16 henna samples and 2,6-TDA was detected in 46 hair dye and 22 henna samples. 1,4-Phenylenediamine and toluene-2,5-diamine are the most frequently reported hair dye allergens and it has been reported that 2,5-TDA was elicited allergic reactions in concentrations that were 10-fold lower than the legal EU limit of 10% [38].

2-Nitro-*p*-phenylenediamine (2NPPD), which is thought to contribute to the increased incidence of bladder cancer, was found in 28 hair dyes and 13 hennas at quantities up to 83.77 and 24.15 μg/g, respectively. 4-Chloro-*o*-phenylenediamine (4-C-*o*-PDA), which is known to induce tumors of the urinary bladder and forestomach and hepatocellular carcinomas, was detected in 34 hair dyes in concentrations up to 321 μg/g in wine red dye and in 17 hennas up to 13.46 μg/g. Aminophenols (APs) were also found at relatively higher concentrations in most of the analysed hair dye and henna saples. 4-Aminophenol (4-AP), used as primary intermediates, was found in 53 hair dyes and 31 hennas at quantities up to be 56210 and 3689 μg/g, respectively.

It has been reported [5] that the content of primary starting materials including *p*-phenylenediamine, *p*-toluenediamine, substituted *p*-diamines, *o*- or *p*-aminophenols in hair dyes which contain couplers and primary starting materials at an approximate molar ratio of 1:1, ranges from 0.05% (light shades) to 1.5% (dark shades). Whereas, in this study, the content of total primary intermediates was calculated within the range of 0.10% (light yellow) and 9.60% (dark black) for hair dyes, 0.11% (yellow) and 8.26% (burgundy) for henna samples and they were found only in 6 of the 10 natural henna samples within the range of 0.09% (red) and 6.11% (chestnut).

4-Aminobiphenyl (4-ABP), a recognised human urinary bladder carcinogen [15], was found in concentrations up to 8.12 μg/g in 28 of the 54 hair dye and up to 2.23 μg/g in 11 of the 25 henna samples, whereas it was only found in 4 of the 10 commercial natural henna samples in concentrations up to 2.87 μg/g. The other isomers of aminobiphenyl (2 and 3-ABP) were also detected in 34 of the 54 hair dye, 16 of the 35 henna samples and the total concentrations of 2-and 3-ABP were found up to be 135.39 μg/g in hair dye and 46.73 μg/g in henna samples. 1,4-PDA is a key constituent of permanent hair dyes used for the development of most shades and may be a source of ABP contamination in hair dyes [15].

2-Naphthylamine (2-NPA), which may cause effects on the blood, resulting in the formation of methaemoglobin and effects on the bladder, resulting in the inflammation and blood in urine, was detected in 20 hair dyes and 15 hennas in concentrations up to be 5.47 and 4.15 μg/g, respectively. In addition, 1-naphthylamine (1-NPA) was detected in 33 hair dyes in concentrations up to 5421 μg/g and 17.64 μg/g in 14 henna samples. *o*-Toluidine (OT), which is suspected to have carcinogenic potential, evidenced or indicated by *in vitro* and/or animal studies [28], was also found in 34 of the 54 hair dye at quantities up to 1547 μg/g. Moreover, *o*-anisidine (2MOA) was detected in 32 of the 54 hair dye in concentrations up to be 88.05 μg/g in wine red colour and in 17 of the 35 henna up to be 1.87 μg/g.

Furthermore, only one of the isomer of *Ø*-diaminophenetol (DAP) was detected in 17 hair dye and henna samples. One of the isomers of *Ø*-amino-*Ø*-nitrophenol (ANP) was detected in 13 hair dye and 15 henna samples. Either one or two different isomers of *Ø*-diaminoanisols (DAAS) and *Ø*-aminomethyl-*Ø*-aminophenols (AMAP) and only one isomer of *Ø*-diaminodiphenylamines (DADA), *Ø*-methoxymethyl-*Ø*-aminophenols (MMAP) and *Ø*-chloro-*Ø*-aminophenol (CAP) were detected in hair dyes, whereas they were not detected in henna samples. In addition, either one or two different isomers of *Ø*-methoxy-*Ø*-toluidines (MHT) and one isomer of

Table 2
The concentrations of aromatic amines determined in commercial oxidative hair dyes

Compounds	Hair dyes																			
	Dark black (μg/g)					Black (μg/g)					Dark brown (μg/g)					Brown (μg/g)				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
NMA	1.15	0.27	–	–	–	0.25	58.14	725	–	251	4.16	508	–	203	105	122	457	62.14	–	513
ANI	1.85	–	214	354	1687	2.36	1540	4512	8963	1.52	185	5.47	8.15	870	22.7	14.12	2520	128	65.24	52.46
DPA	1.10	2.12	–	–	654	252	1.88	9.88	–	5.63	0.10	3.46	7.82	–	13.11	3.55	–	1.48	212	36.14
OT	9.12	–	0.88	–	–	687	3.14	1547	2.56	0.01	112	–	123	–	–	1.36	–	–	–	368
MT	0.23	3.75	0.48	1.24	3.88	241	–	36.40	3562	452	223	416	1.48	120	335	145	201	25.4	162	256
PT	–	4.12	–	0.03	212	1.35	–	1.66	4215	124	5.55	312	2.27	60.23	113	562	1033	3.78	1205	48.15
2MOA	**7.85**	**4.18**	**0.07**	**0.04**	–	–	**0.05**	**0.42**	–	–	0.08	**0.23**	**1.25**	–	–	**1.02**	–	–	–	**0.36**
PCA	–	1.24	–	–	154	145	1.15	–	8720	0.04	–	5.88	3.55	402	562	1320	762	362	105	168
2-AP	–	0.47	512	8380	2587	6250	34.2	0.53	1.66	3.56	3.55	0.66	1003	45.12	764	1254	642	398	304	5874
1-NPA	0.22	0.78	1.85	–	–	0.58	0.04	0.05	5.21	0.02	0.24	0.45	1.66	0.13	–	–	22.14	1.16	5421	–
4-NA	0.15	–	5.55	–	–	1.65	13.5	2331	1.36	2.12	225	53.20	8.04	414	32.45	56.44	1066	408	286	3.51
2-NPA	–	**0.15**	–	**5.47**	–	**0.52**	–	**0.04**	**0.17**	–	–	**0.08**	–	–	–	–	**0.61**	–	–	**0.08**
1,3-PDA	**10170**	**20010**	**16587**	**14634**	**40624**	**4512**	**1698**	**587**	**6921**	**7512**	**18170**	**19320**	**10610**	**16021**	**962**	**54.12**	**2608**	**1912**	**36501**	**1023**
3-AP	3115	–	1478	124	874	575	132	1467	182	6354	1205	892	524	1230	1276	320	287	11030	452	162.4
4-AP	2306	3458	1540	5874	2546	3854	548	886	521	552	5306	2088	456	2611	3062	56210	1540	2102	808	10450
3,4-TDA	–	0.45	5478	–	2.58	–	6854	–	–	8.15	3.77	3.25	2547	2031	1564	2341	504	13.77	–	1470
4-ABP	**8.12**	–	**3.24**	**0.01**	**0.21**	**0.08**	**0.02**	–	–	–	**0.09**	**0.01**	–	–	–	–	**0.07**	**1.88**	–	**0.03**
4-C-*o*-PDA	0.21	0.14	–	–	0.14	–	–	0.04	–	0.04	5.21	0.04	1.77	0.53	0.25	2.14	0.33	–	–	0.02
2,4-TDA	0.35	–	2.15	–	145	574	–	0.03	–	2.14	0.02	1.22	2.15	–	56.19	1.25	4.45	–	–	0.09
1,2-PDA	**330**	**87.41**	**254**	**567**	–	**2360**	**324**	**65.4**	**1.66**	**–**	**145**	**6.21**	**4870**	**508**	**6.17**	–	**5.41**	**812**	**1232**	**70.12**
2,6-TDA	–	–	124	4217	152	–	1.63	23452	212	–	5874	3621	89.1	16.22	354	242	365	38.14	12.03	1.12
1,4-PDA	**90301**	**51474**	**44471**	12457	**29682**	48751	54110	13470	15742	28440	26150	41004	23005	23001	19582	8245	34210	26470	10413	7342
2NPPD	**0.35**	–	**0.04**	**0.13**	3.58	–	–	**114**	–	**0.07**	**0.04**	**0.01**	**0.01**	**0.01**	–	**0.36**	**0.04**	**0.01**	–	–
DADA	**0.06**	–	–	**0.03**	**0.05**	**0.04**	–	–	**0.03**	**0.03**	**0.11**	–	–	–	**0.01**	–	**0.03**	–	**0.44**	**0.01**
MMAP	**0.44**	–	**0.03**	–	**0.07**	**0.06**	–	–	**0.01**	–	–	–	–	–	–	–	**1.12**	–	**0.23**	–
CAP	**0.02**	**0.07**	**0.17**	**0.02**	–	–	–	**1.42**	–	–	**0.01**	**0.01**	**0.02**	**0.56**	–	**0.05**	**0.02**	–	–	–
DAAS	**5.03**	**1.13**	**2.14**	–	–	–	**0.08**	**1.05**	–	–	**0.01**	–	–	**0.48**	–	–	–	**0.03**	–	–
DAAS	**0.07**	–	**0.02**	–	–	–	**0.02**	**0.07**	–	–	**0.02**	–	–	**0.33**	–	–	–	**0.12**	–	–
DAP	**0.12**	–	**1.35**	–	–	**0.01**	**0.01**	–	–	–	–	**0.03**	–	–	–	–	**0.03**	–	–	**0.51**
ANP	**0.03**	–	–	**1.12**	–	–	–	–	–	–	**0.01**	–	–	–	–	–	**2.54**	–	–	–
AMAP	**0.07**	–	**0.04**	–	**1.22**	**1.18**	–	**0.06**	**0.04**	–	–	–	**0.02**	–	**0.32**	**0.88**	–	–	–	**0.18**
AMAP	**0.05**	–	**0.03**	–	**0.05**	**0.07**	–	**0.08**	**0.01**	–	–	–	–	–	**0.05**	–	–	–	–	–
2,5-TDA	8.23	5.75	19.42	–	93.88	–	–	96.4	562	–	–	–	–	–	1812	119	–	413	1620	–
EA	2745	214	3618	–	357	–	5471	–	145	4.18	1762	1.55	54.12	2.11	–	8.45	17.56	870	1034	515
2-ABP	122	–	0.51	1.13	4.58	1.16	–	1.26	3.27	2.14	1.09	2.01	0.13	0.18	1.72	1.06	2.04	1.05	1.67	1.87
3–ABP	13.21	–	0.16	0.42	2.05	0.25	–	1.18	1.86	0.42	0.88	0.93	–	–	1.64	–	1.43	–	0.44	0.09
Total concentration (mg/g)	109.15	75.27	74.31	46.62	79.79	68.21	70.79	49.19	49.76	43.72	56.38	68.25	43.32	47.54	30.63	71.03	46.25	45.05	49.84	28.36

Table 2 (*Continued*)

Compounds	Hair dyes																		
	Chestnut (μg/g)				Red (μg/g)				Wine red (μg/g)					Dark yellow (μg/g)					
	1	2	3	4	1	2	3	4	1	2	3	4	5	1	2	3	4	5	
NMA	74.12	1.07	1.54	2.22	24.12	218	96.52	20.84	0.23	7.87	8.14	–	–	2.16	11.08	1.13	223	18.26	
ANI	88.45	0.47	113	3.25	15.44	2.42	730	847	736	452	5.40	83.02	21.55	142	103	6.15	11.70	2.78	
DPA	22.32	–	1.04	–	2.10	3.58	1.82	2.12	2.20	19.08	1.58	–	5.10	0.40	13.41	2.12	14.23	22.10	
OT	0.18	–	0.83	1.12	1.02	13.50	128	–	120	523	3.34	1.56	–	172	203	2.23	–	1.16	
MT	3.89	1.72	1.28	1.23	40.19	24.56	448	312	103	1.4	–	1061	512	635	816	1.08	81.20	235	
PT	0.33	0.12	57.16	–	5.55	312	3.27	23.20	211	128	–	1005	–	1.05	412	0.27	68.23	313	
2MOA	**3.87**	**0.10**	**1.15**	**0.05**	**18.14**	**1.53**	**1.28**	–	**12.41**	**0.31**	**88.05**	**0.11**	–	**1.08**	**0.21**	**0.25**	–	**0.03**	
PCA	73.71	3.54	50.16	1.25	77.21	80.85	73.55	414	412	–	27.22	824	–	158	7.08	3.15	0.82	462	
2-AP	912	146	1028	8.25	83.45	16.66	713	84.12	184	1.53	14.2	–	–	155	1066	1123	65.62	1264	
1-NPA	2.84	0.12	4.27	–	814	157	1.61	–	0.18	–	0.04	–	–	7.24	1.45	12.12	3.45	2.87	
4-NA	3.27	2.51	8.91	3640	145	13.20	6.01	208	451	22.87	23.5	1.32	14.71	3150	432	18.04	6212	120	
2-NPA	**1.03**	–	**0.07**	–	–	**1.43**	–	–	–	–	–	**0.17**	–	**1.23**	–	–	–	**2.02**	
1,3-PDA	**10234**	**8030**	**1887**	**7170**	**171**	**220**	**20110**	**14041**	**5200**	**10587**	**10098**	**2921**	**3752**	**2314**	**1204**	**9514**	**12040**	**113**	
3-AP	2134	1312	3078	163	2845	381	1125	752	742	167	501	163	1074	3005	1692	5240	3030	4676	
4-AP	1374	9480	1403	2060	1306	142	11.58	19.12	3854	886	548	1521	1052	22.06	32.08	1488	1202	–	
3,4-TDA	3.87	115	–	6.12	–	78.14	–	178	231	–	154	12.15	–	0.71	4.25	1547	185	412	
4-ABP	–	**0.07**	**0.04**	**0.25**	**1.57**	–	–	–	**0.19**	–	**1.23**	**0.04**	–	**2.12**	–	–	–	**1.33**	
4-C-*o*-PDA	–	0.50	–	–	0.22	–	1.25	–	154	321	1.23	2.12	22.4	1.22	2.30	0.07	3.53	0.05	
2,4-TDA	–	14.24	0.15	125	2.23	–	1.16	–	1.74	3.03	–	2.22	–	1.62	0.93	1.15	–	–	
1,2-PDA	**148**	**57.41**	**802**	**114**	**8010**	**3200**	**124**	**34.55**	**156**	**6584**	**1124**	**17.66**	**3.07**	–	**314**	**45.12**	**113**	**4.25**	
2,6-TDA	117	0.87	204	47.12	174	21.15	29.1	6.21	1.25	22.34	–	52	–	0.74	0.21	9.1	1.22	0.54	
1,4-PDA	17456	11740	29471	32340	24050	28004	3305	10301	22751	12410	14010	20742	17440	20150	21004	10205	11501	12582	
2NPPD	**1.14**	–	**1.02**	–	**3.14**	**83.77**	–	–	**1.02**	**0.14**	**4.13**	–	**2.17**	**104**	–	–	–	**1.13**	
DADA	–	–	–	**0.11**	**1.21**	**2.87**	**30.11**	–	–	–	–	**0.04**	–	–	–	**0.06**	**1.87**	**0.01**	
MMAP	–	–	**0.04**	**1.83**	**1.01**	**2.23**	**0.07**	–	–	**1.05**	–	**0.01**	–	**0.08**	**0.12**	–	–	0.07	
CAP	**0.12**	–	**0.11**	–	**2.01**	**0.25**	**0.12**	–	–	–	–	–	**0.02**	–	**0.21**	**1.02**	**1.56**	–	
DAAS	–	–	**3.15**	**0.02**	**0.20**	–	**2.08**	–	–	**1.05**	**0.03**	**0.06**	–	**0.01**	–	–	**0.13**	–	
DAAS	–	–	–	**0.09**	–	–	–	–	–	**0.07**	**0.02**	–	–	–	–	–	**0.31**	–	
DAP	–	**0.01**	–	–	–	**0.18**	–	**0.07**	**1.18**	–	–	–	–	–	–	**0.06**	–	–	
ANP	**0.12**	–	**0.02**	–	–	–	–	–	–	–	–	–	–	**1.16**	–	–	**0.53**	–	
AMAP	**1.25**	**0.43**	**0.14**	**0.58**	–	–	**0.02**	–	**0.18**	**0.02**	–	**0.04**	–	–	–	**0.14**	–	**0.30**	
AMAP	–	–	–	**0.05**	–	–	–	–	**0.07**	**0.04**	–	**0.01**	–	–	–	–	–	**0.05**	
2,5-TDA	1789	674	–	–	3467	–	–	–	8.16	1234	–	189	–	2901	–	–	2.18	–	
EA	1470	2060	1618	3014	63.52	88.55	34.14	–	132	140	2071	145	82.11	–	–	4.12	2.55	–	
2-ABP	–	1.17	–	12.08	2.42	–	1.14	–	0.57	–	2.19	0.23	–	3.84	–	–	–	2.13	
3-ABP	–	0.18	–	1.26	1.53	–	0.18	–	0.45	–	1.45	–	–	1.78	–	–	–	1.62	
Total Concentration (mg/g)	35.92	33.64	39.94	48.71	41.33	33.07	26.98	27.25	35.47	33.51	28.72	28.74	23.93	32.83	27.32	29.22	34.77	20.24	

Table 2 (*Continued*)

Compounds	Hair dyes															
	Golden blonde (μg/g)					Yellow (μg/g)					Light yellow (μg/g)					
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	6
NMA	12.22	4.57	42.14	–	5.03	34.5	1.34	36.25	4.12	2.81	28.74	–	1.23	16.52	10.86	9.15
ANI	7.12	15.20	11.4	5.20	2.48	28.25	597	113	8.45	15.90	51.54	1687	6.42	730	847	222
DPA	–	18.30	1.48	2.12	6.10	–	–	1.04	12.32	2.18	–	6.14	3.56	1.82	3.12	2.11
OT	13.23	–	–	–	168	11.72	–	0.03	76.54	1.02	3.65	–	3.50	3.28	–	–
MT	541	20.13	25.4	–	201	87.14	1.66	18.69	13.09	4.10	8.24	19.25	34.56	44.18	89.15	135
PT	564	2030	3.08	305	18.15	17.82	90.12	72.16	101	15.55	11.03	23.12	31.62	14.7	3.20	1.14
2MOA	**4.02**	–	–	–	–	**0.08**	**1.10**	–	1.87	–	–	–	**1.50**	**1.24**	–	–
PCA	23.20	17.62	31.62	10.12	4.13	4.20	1.54	20.16	3.72	84.21	156	3.54	10.81	2.55	14.14	15.62
2-AP	854	342	294	214	4774	8.05	106	928	512	60.45	6.80	287	16.04	213	842	664
1-NPA	–	–	1.12	–	–	–	0.12	–	2.84	–	–	1.32	–	1.62	–	–
4-NA	5614	8062	4508	2026	854	1640	2021	881	387	1145	685	22.36	43.20	16.01	2.08	0.45
2-NPA	–	**0.08**	–	–	**0.12**	–	–	**0.02**	**1.04**	–	–	**0.13**	**0.55**	–	–	–
1,3-PDA	**74.32**	**1008**	**2912**	**6501**	**1323**	**2270**	**4130**	**627**	**20234**	**231**	**9634**	**6204**	**130**	**624**	**1541**	**962**
3-AP	129	384	952	388	181	163	130	118	340	145	8544	8.14	1381	7025	78.52	124
4-AP	4571	932	2465	1036	2054	260	940	1403	137	136	5.14	146	11.42	13.58	109	306
3,4-TDA	1241	49.15	23.07	–	180	313	28.15	17.08	23.87	9801	1.27	2.55	48.14	–	–	20164
4-ABP	**1.12**	–	**2.08**	–	**0.08**	**0.20**	–	–	–	**1.52**	**1.25**	**0.32**	–	**0.14**	–	–
4-C-*o*-PDA	–	0.30	–	–	3.14	–	2.58	1.13	–	0.16	–	0.11	–	–	–	0.25
2,4-TDA	–	4.40	–	0.75	–	2.25	4.24	0.15	–	0.23	–	14.25	0.58	0.16	2.12	5.10
1,2-PDA	–	**5.40**	**18.12**	**132**	**60.12**	**1.58**	**87.41**	**1.54**	**96.25**	**74.23**	**14.78**	**0.71**	**12.74**	**12.04**	**34.55**	**86.17**
2,6-TDA	11.18	2.65	8.14	1.03	1.18	7.12	0.83	44.04	12.17	–	–	1.02	1.15	14.21	2.21	3.54
1,4-PDA	8815	10210	7670	19413	18342	9340	12740	17071	7456	6050	2457	21082	16304	9105	11301	582
2NPPD	–	–	–	**2.12**	–	–	–	**1.05**	–	**334**	**2.12**	–	–	0.08	–	–
DADA	–	–	–	**0.40**	–	–	**0.03**	–	–	**0.21**	–	–	–	–	–	**0.01**
MMAP	**0.13**	–	**1.16**	**0.20**	–	–	**0.14**	–	–	**0.11**	–	**0.07**	**0.23**	**0.07**	–	–
CAP	**0.01**	**0.04**	–	**0.12**	–	–	–	**0.12**	–	–	**0.02**	–	**0.05**	–	–	–
DAAS	**5.60**	–	**0.08**	–	**0.07**	**0.32**	–	–	**1.30**	**0.24**	–	**0.07**	–	**0.08**	–	–
DAAS	**0.03**	–	**0.02**	–	–	**0.02**	–	–	**2.16**	–	–	–	–	–	–	–
DAP	–	–	–	–	**0.53**	**0.14**	–	**0.33**	–	–	–	–	**0.12**	–	**0.02**	–
ANP	–	**2.50**	–	**0.42**	–	**0.04**	–	–	**0.15**	–	**1.12**	–	–	–	–	–
AMAP	**0.80**	–	**1.55**	–	**0.10**	**0.28**	**0.13**	–	**0.25**	–	–	–	**0.10**	**0.02**	–	**0.30**
AMAP	–	–	–	–	–	–	–	–	**0.02**	–	–	**0.05**	–	–	–	**0.02**
2,5-TDA	–	192	24.57	806	–	8209	–	243	–	1914	–	–	–	1.25	17.13	–
EA	–	112	1050	1034	504	113	1854	1963	–	89.52	–	–	18.05	–	–	–
2-ABP	4.61	–	3.12	–	0.89	0.52	–	–	–	2.43	3.55	–	–	0.87	–	–
3-ABP	1.24	–	2.18	–	0.55	0.38	–	–	–	1.64	2.93	–	–	0.41	–	–
Total concentration (mg/g)	22.49	23.41	20.05	31.88	28.68	22.51	22.74	23.56	29.43	19.78	21.62	29.51	18.06	17.84	14.90	23.28

Bold values signifies all of hair dye and henna samples have different aromatic amine ingredients and each of them contains primary intermediates at higher levels than that of their restricted values and/or the values of some aromatic amines which are banned in their use.

Table 3
The concentrations of aromatic amines determined in commercial hennas

Compounds	Henna																												
	Black (μg/g)					Dark brown (μg/g)			Chestnut (μg/g)				Burgundy (μg/g)			Red (μg/g)			Golden Blonde (μg/g)					Yellow (μg/g)		
	1	2	3	4	5	1	2	3	1	2	3	4	1	2	3	1	2	3	1	2	3	4	5	1	2	
NMA	82.13	7.14	147	–	124	45.24	–	–	21.57	12.14	–	–	23.25	12.07	–	214	–	–	–	84.32	72.84	–	49.75	16.52	–	
ANI	76.12	245	84.52	53.02	11.25	88.15	11.70	–	19.20	–	5.20	2.48	–	114	83.12	2042	1730	8470	118	72.14	15.90	51.54	168	–	8.47	
DPA	1.15	–	3.08	–	2.10	–	–	4.10	4.30	0.48	–	–	2.45	–	11.04	–	–	2.12	–	–	2.18	–	–	0.82	–	
OT	1020	374	153	85.46	–	75.16	–	13.42	–	31.15	12.76	–	–	10.12	16.83	–	1.28	–	14.03	16.54	1.02	3.64	–	–	1.28	
MT	12.47	18.51	–	–	5.32	17.08	–	–	2.13	29.4	–	42.31	–	18.72	11.28	–	4.18	–	8.69	13.09	4.10	–	19.25	4.18	–	
PT	21.41	–	13.28	–	–	10.25	18.23	3.54	630	5.08	3.42	8.10	0.12	–	57.16	–	1.27	10.20	72.06	–	15.22	12.03	13.12	–	15.20	
2MOA	–	**0.85**	**0.31**	–	–	**0.26**	–	**1.03**	–	**0.08**	**0.13**	–	–	**0.07**	–	**0.53**	–	**0.04**	–	**1.87**	–	**0.03**	–	–	**0.12**	
PCA	–	77.28	–	541	–	80.15	10.82	–	43.62	1.62	8.12	–	40.25	3.54	10.16	158	73.42	1114	–	–	84.21	156	3.54	2.55	14.14	
2-AP	1034	958	723	–	–	123	15.62	16.64	212	194	82.14	14.74	80.25	134	12.28	56.66	28.43	34.12	128	512	60.42	16.80	187	–	84.52	
1-NPA	5.78	–	2.15	–	–	–	3.45	–	–	2.12	–	–	–	0.12	–	17.64	11.61	14.33	–	–	–	–	1.32	0.62	–	
4-NA	8.77	–	32.16	–	–	28.04	42.32	12.10	4048	508	1026	8.22	3640	2051	4191	–	6.01	–	8810	3870	1145	481	1752	1631	2808	
2-NPA	–	**0.30**	–	–	**4.15**	–	–	**1.02**	–	**0.04**	–	**0.06**	–	**0.07**	–	**1.43**	**0.08**	**0.15**	–	**1.04**	–	**0.04**	–	–	**0.57**	
1,3-PDA	**5240**	**1298**	–	–	**20052**	**8814**	**20040**	**2113**	**912**	**290**	**61.87**	**853**	**717**	**1030**	**2887**	**3420**	**5110**	**4641**	**622**	**1234**	**48.56**	**25.63**	**6201**	**62.40**	**84.42**	
3-AP	–	472	–	263	5134	215	1030	2136	350	1952	541	128	161	1012	3078	389	325	452	1180	1340	141	88.12	–	–	185	
4-AP	2754	1048	526	1321	2152	1132	916	–	452	102	1030	1054	206	980	453	149	18.57	140	1.58	–	1.26	5.30	14.3	13.58	15.62	
3,4–TDA	–	2.28	–	32.14	1.45	982	145	482	–	1.75	–	214	–	11.25	178	28.14	–	–	–	–	–	1.27	–	0.03	–	
4-ABP	**0.39**	**2.23**	–	–	**1.16**	–	–	**0.24**	–	**1.08**	–	**0.03**	**1.05**	–	–	**0.01**	–	–	–	**0.52**	–	–	**0.02**	**0.14**	–	
4-C-*o*-PDA	0.25	–	2.16	–	13.46	0.12	1.14	0.02	0.40	–	–	2.14	–	4.50	–	–	–	5.23	1.13	–	3.19	–	–	0.14	0.06	
2,4–TDA	–	–	13.03	–	24.32	12.15	–	–	–	1.40	–	0.15	–	–	0.15	–	1.13	–	1.15	0.06	0.23	–	14.25	–	2.12	
1,2-PDA	**254**	**2120**	**1584**	**89.66**	–	**512**	**198**	**394**	**6.40**	**28.12**	**138**	**80.12**	**1140**	**5741**	**8256**	**3212**	**1024**	–	**81.52**	**66.25**	**93.23**	–	–	**1020**	**1345**	
2,6-TDA	–	13.25	52.24	3.45	–	119	18.22	10.50	465	814	231	2.18	–	–	204	0.04	1.12	3.21	–	3.17	–	–	4.02	84.21	0.21	
1,4-PDA	12751	21180	23410	**61442**	–	20205	2501	30582	15210	**60670**	**70413**	28342	12340	21740	19471	18004	12105	11301	13210	17456	19050	21457	10082	19105	15801	
2NPPD	**4.02**	–	**5.19**	–	**12.18**	–	–	**24.15**	**1.14**	–	–	–	–	–	**1.02**	–	–	–	**1.05**	–	–	–	–	–	–	
DAP	**0.45**	**0.13**	–	**0.14**	–	–	**1.17**	**0.01**	**4.13**	**0.20**	–	**1.15**	**0.01**	–	–	**2.87**	**30.47**	–	**0.02**	–	**0.22**	–	–	–	**1.14**	
ANP	**1.06**	–	–	–	–	–	–	**0.02**	**0.03**	**0.06**	–	**0.10**	–	**0.04**	–	**2.23**	**0.07**	–	**0.03**	–	**0.11**	–	**0.07**	**0.07**	–	
DAMP	**4.03**	**0.14**	–	–	–	–	**2.23**	–	**0.04**	**1.17**	–	–	–	–	**0.02**	**0.13**	**0.04**	–	–	**1.23**	–	**0.04**	–	–	**2.23**	
MHT	**1.12**	**2.07**	**0.78**	–	–	–	–	**1.34**	**1.05**	–	**1.34**	**0.12**	**0.04**	–	–	–	**0.08**	**0.03**	–	–	**0.33**	**1.05**	**0.12**	**0.05**	–	
MHT	–	**1.13**	**1.44**	–	–	–	–	**0.22**	–	–	**0.25**	–	**0.01**	–	–	–	**1.13**	**0.04**	–	–	**0.22**	–	–	–	–	
2,5-TDA	15.39	89.46	12.34	–	–	–	–	–	23.14	69.87	1.12	–	–	–	1.56	–	–	–	2.56	–	123	–	–	5.78	26.19	
EA	154	845	120	56.47	86.13	–	–	–	12.14	47.53	0.10	–	132	18.62	198	19.36	60.13	–	85.12	–	–	125	–	15.12	–	
2-ABP	41.15	46.73	–	–	24.52	14.68	–	7.13	14.28	–	–	8.03	4.35	–	–	6.27	–	4.16	–	5.19	–	–	2.24	4.45	–	
3-ABP	15.73	27.35	–	–	7.12	8.93	–	4.16	13.17	–	–	7.11	0.27	–	–	2.78	–	0.89	–	1.64	–	—	0.16	1.12	–	
Total concentration (mg/g)	23.50	28.83	26.89	63.89	27.66	32.48	24.96	35.81	22.45	64.76	73.56	30.77	18.49	32.88	39.12	27.73	20.53	26.19	24.34	24.68	20.86	22.42	18.51	21.97	20.40	

Table 3 (*Continued*)

Compounds	Natural henna									
	Black (μg/g)		Dark brown (μg/g)			Chestnut (μg/g)			Red (μg/g)	
	1	2	1	2	3	1	2	3	1	2
NMA	14.56	72.63	–	14.56	2.45	8.64	1.45	–	35.87	23.46
ANI	113	186	65.13	41.32	1.13	–	14.32	52.11	32.42	28.14
DPA	–	–	–	–	–	–	–	1.23	–	–
OT	–	–	1.12	0.75	–	–	0.42	–	–	0.05
MT	2.56	0.76	17.08	–	2.11	1.83	–	17.84	–	4.15
PT	64.13	78.54	18.42	21.85	–	5.08	3.42	8.10	–	8.20
2MOA	**0.13**	**0.06**	**0.13**	–	–	**0.02**	**0.07**	—	–	–
PCA	1.59	11.48	–	0.23	1.45	0.65	–	0.83	11.42	22.14
2-AP	413	634	154	48.4	–	–	153	65.44	42.13	–
1-NPA	0.14	–	–	–	–	–	0.04	–	0.14	12.17
4-NA	0.14	0.66	46.79	145	–	4680	3.87	–	–	–
2-NPA	–	**0.08**	**0.04**	–	–	–	**0.01**	–	–	–
1,3-PDA	**9234**	**146**	–	–	**13056**	**10629**	**21562**	**17453**	**367**	**510**
3-AP	–	1.28	89.56	118	–	–	1162	2028	–	1081
4-AP	3689	2467	1834	–	718	412	965	763	915	1254
3,4-TDA	1230	41.13	–	10.16	–	8.63	–	12.67	–	0.04
4-ABP	**0.12**	–	–	**2.87**	–	**0.56**	–	**0.05**	–	–
4-C-*o*-PDA	0.03	–	–	–	–	1.63	–	1.05	–	–
2,4-TDA	–	0.11	0.05	–	–	1.40	–	0.15	–	–
1,2-PDA	**8320**	**5309**	**4320**	**4568**	–	**98.21**	**1432**	**714**	**11300**	**876**
2,6-TDA	0.33	–	0.52	1.42	–	–	–	1.14	–	–
1,4-PDA	–	9563	20760	19356	8762	**60670**	–	–	–	20301
2NPPD	–	**0.02**	–	**2.14**	**0.72**	**0.92**	**1.14**	–	**0.61**	–
DAP	–	**0.02**	–	–	**0.06**	–	–	**0.16**	–	–
ANP	–	–	–	**0.05**	–	–	**0.03**	–	–	**0.05**
DAMP	–	–	–	–	**0.01**	–	–	–	–	–
MHT	–	**0.02**	–	**0.02**	–	–	–	**0.31**	–	–
MHT	–	–	–	**0.05**	–	–	–	–	–	–
EA	9.16	63.12	28.93	86.14	12.14	8.13	10.10	–	27.48	–
2-ABP	7.43	–	–	6.62	–	4.58	–	–	–	–
3-ABP	5.14	–	–	3.26		2.45	–	–	–	–
Total concentration (mg/g)	23.13	26.58	27.33	24.43	22.56	76.53	25.31	21.12	12.73	14.12

Bold values signifies all of hair dye and henna samples have different aromatic amine ingredients and each of them contains primary intermediates at higher levels than that of their restricted values and/or the values of some aromatic amines which are banned in their use.

Table 4
The Concentration levels of aromatic amines determined in hair samples dyed in various colours

Metabolites	Dyed hair samples														
	Dark black (μg/g)			Black (μg/g)			Dark brown (μg/g)		Brown (μg/g)	Chestnut (μg/g)	Red (μg/g)		Wine red (μg/g)		Dark yellow (μg/g)
	1	2	3	1	2	3	1	2	1	1	1	2	1	2	1
NMA	14.14	70.17	102	11.42	71.10	54.21	32.10	80.16	58.25	63.24	77.24	93.50	108	202	110
ANI	9.40	31.47	531	173	212	648	68.15	42.33	38.14	205	65.25	41.62	185	107	24.69
DPA	0.20	–	2.83	83.46	–	3.18	–	0.11	0.08	0.23	1.42	1.04	2.14	12.75	10.16
OT	0.18	–	1.19	73.14	17.56	104	40.67	–	0.15	1.23	–	0.66	30.05	71.35	98.66
MT	–	0.07	1.21	0.05	–	7.16	90.55	105	21.16	1.98	10.57	2.20	18.10	9.42	103
PT	–	–	9.43	0.19	0.88	8.64	12.45	87.57	134	71.40	98.18	7.10	2.55	1.27	13.29
2MOA	**0.45**	**1.13**	**0.01**	–	–	**0.13**	**0.07**	**1.56**	**0.27**	**1.10**	**18.05**	**9.15**	**27.14**	**0.24**	**0.03**
PCA	–	–	1.78	0.87	1.75	–	–	1.02	15.97	4.16	89.79	11.10	75.21	3.55	42.13
2-AP	85.13	438	97.24	146	77.98	18.94	92.54	123.69	359	189	68.20	62.98	73.46	–	72.18
1-NPA	–	0.04	0.06	–	–	0.12	0.06	–	–	1.12	18.14	12.77	0.04	–	4.27
4-NA	0.55	–	0.46	1.24	1.49	–	71.04	2.48	70.51	5.98	59.40	16.98	18.11	5.01	1023
2-NPA	–	**0.09**	–	–	–	–	–	**0.03**	**0.03**	**0.27**	**0.47**	**1.05**	–	–	**0.13**
1,3-PDA	**418**	**575**	**1360**	**1430**	**508**	**198**	**615**	**1412**	**190**	**11012**	**70.10**	**184**	**361**	**810**	**9041**
3-AP	114	14.34	814	779	182	1205	824	1160	270	1340	740	378	515	289	845
4-AP	104	123	442	806	406	184	192	676	1949	5100	618	286	1006	311.58	119
3,4-TDA	–	2.14	98.75	–	754	1.16	0.87	0.68	454	0.32	1.18	36.18	29.10	–	–
4-ABP	**1.12**	–	**0.88**	**0.15**	–	–	**0.03**	–	**0.11**	–	**12.43**	**0.04**	**0.03**	–	**13.86**
4-C-*o*-PDA	–	–	0.02	–	–	0.10	–	0.02	0.07	–	0.83	–	13.57	2.20	–
2,4-TDA	–	–	0.45	85.88	7.43	–	–	0.12	12.45	1.34	1.16	–	0.07	0.16	0.08
1,2-PDA	**306**	**916**	**6.07**	**714**	**172**	**20.75**	**11.70**	**10.15**	**8.41**	**40.12**	**817**	**765**	**610**	**824**	**4.50**
2,6-TDA	58.99	–	84.71	–	–	180	98.10	154	21.8	46.03	84.12	28.13	4.82	19.13	6.21
1,4-PDA	20171	6034	7382	7862	10871	879	4082	7582	6210	18413	7340	10400	8550	6205	8371
2NPPD	**1.42**	–	–	**0.78**	–	**312**	**1.05**	–	–	**0.10**	**1.15**	**7.10**	**0.18**	**0.09**	**5.03**
DADA	**0.02**	–	**0.52**	**0.05**	–	–	–	–	–	**0.05**	**0.14**	**0.21**	–	**0.04**	**0.03**
MMAP	**0.13**	**0.18**	–	–	–	**0.06**	–	–	**0.18**	–	**0.04**	**0.06**	–	**0.12**	–
CAP	–	**2.07**	–	**0.04**	–	**0.08**	–	–	**0.09**	–	**0.07**	–	–	–	–
DAAS	**5.08**	–	**0.09**	**0.02**	**0.16**	–	–	–	–	–	–	–	**0.10**	**0.09**	–
DAAS	**0.02**	–	–	–	–	–	–	–	–	–	**0.04**	–	–	**0.03**	–
DAP	–	**0.12**	**0.17**	–	–	–	–	–	–	**0.05**	–	**0.07**	**0.19**	–	**0.04**
ANP	–	–	–	–	–	**0.05**	–	**0.08**	–	–	–	–	–	–	**1.52**
AMAP	–	**0.13**	**0.05**	–	–	–	–	–	**0.03**	–	–	–	–	**0.10**	**0.05**
2,5-TDA	98.45	0.68	311	0.41	2.82	11.74	4.67	10.13	84.26	86.58	1040	3.56	8.43	197	212
EA	76.43	54.46	1780	112	93.14	2.17	82.14	26.23	26.50	61.43	50.88	56.18	78.58	89.78	19.12
2-ABP	13.68	–	–	0.73	–	–	0.06	0.03	0.10	0.07	1.08	–	0.09	–	1.26
3-ABP	1.28	–	0.04	–	–	–	–	–	–	–	0.11	–	0.03	–	0.47

Ø-diamino-Ø-methylphenetols (DAMP) were detected in henna samples, whereas they were not present in hair dye samples. The concentration levels of these compounds including Annex II list of the EU Cosmetic Directive 76/768/EEC, are shown in Tables 2 and 3.

Some of the samples were also analysed by the proposed method after reduction of the dyes with tin (II) chloride and the obtained results revealed that the observation of the increases in concentrations of the compounds and determination of various aromatic amines different from those used in dye formulations such as DAAS, DAP, ANP, 4-ABP, 2-NPA, 3,4-TDA and 4-C-*o*-PDA may be due to the conversion of the primary intermediates to the related azo dyes to some extent and then, may result in the release of related and different aromatic amines by reduction of the azo bonds of the dye. The highest increases were observed especially in concentrations of 1,4-PDA, 4-ABP, DAS, DAP, AMAP and 1,4-PDA, 4-ABP, 4-AP, DAS when analysed after the reduction of hair dyes and henna samples, respectively. These findings agree with the reported results [30,32].

A number of samples including standard aromatic amine solutions were also analysed with gradient (C_{18}) RP-HPLC coupled with UV detector and the results indicated that the separation of all isomers of aromatic amines was not satisfactory by HPLC even though the separation of *iso*BOC derivatives of aromatic amines was better than underivatised compounds. Hence, the isolation procedure described in this work was combined with GC–MS to provide the required resolution for precise determination of individual isomers of aromatic amines in hair dye and henna samples.

3.4. Determination of aromatic amines as their isoBOC derivatives in hair samples dyed in various colours

The method was also applied to hair samples dyed in various colours in triplicate after denaturation to determine metabolites of hair dyes and most of the target compounds were determined although almost none of the compounds of interest were detected in washing water of dyed hair samples. The concentration levels of metabolites determined in dyed hair samples are shown in Table 4.

1,4-Phenylenediamine (1,4-PDA) was also found in all dyed hair samples at the highest concentrations to be up to 20171 μg/g. Furthermore, 1,3-PDA, ANI, 4-AP, 3-AP, EA, 2,5-TDA, 2-AP and 4-NA were found at relatively higher concentrations in almost all of the dyed hair samples, especially in dark shades to be up to 11012, 9681, 5100, 4340, 1780, 1130, 868 and 714 μg/g, respectively. In addition, 2,6-TDA, 3,4-TDA, 2-ABP were detected in dyed hair samples, whereas they were not detected in the related hair colouring formulations.

Determination of aromatic amines such as DAAS, DADA, AMAP, MMAP, DAP, ANP, 4-ABP, 2-NPA, 3,4-TDA and 4-C-*o*-PDA in hair samples dyed in various colours indicate that different colours of hair dyes, especially dark shades, were metabolised to various toxic and/or carcinogenic aromatic amines although they are not present in related dye formulations. Toxic and/or carcinogenic hair dye metabolites are shown in bold form in Table 4.

As shown in Tables 2–4, the determination of a great variety of aromatic amines in hair colouring formulations and hair samples dyed in various colours revealed the fact that studies on dermal absorption and bioavailability of aromatic amines stemming from hair dyes and modified hennas are required to be studied more closely to determine if these aromatic amines contribute to the increased risk of cancer reported in frequent users of hair dyes and hennas.

The proposed method, as described in our previous studies [22,23], has been successfully applied to hair dye, henna and dyed hair samples with some suitable modifications to determine the compounds of interest and the results revealed that the proposed method is enable to determine the levels of individual isomers of aromatic amines in commercial oxidative hair dye and henna samples. The obtained recoveries ranged from 92.2 to 98.4% and the precision of this method, as indicated by the relative standard deviations (RSDs) was within the range of 0.7–4.2%. The detection limits obtained from calculations by using GC–MS results based on S/N = 3 were within the range from 0.02 to 0.20 ng/g. The detection limits differ substantially for the various amines determined, but all were below our stated limits of detection. Excellent linearity was obtained in the concentration range from 1 to 100 ng/mg with R^2 in the range of 0.9983–0.9999.

4. Conclusion

Consequently, the method described in this study has been shown to be suitable with satisfactory accuracy and good reproducibility for the qualitative and quantitative determination of aromatic amines at the levels of ng/g in hair dye, henna and dyed hair samples.

Through the obtained results, it may be concluded that, the method developed can be proposed as a procedure for the precise determination of aromatic amine ingredients of commercial oxidative hair dye and henna samples to check their compliance with the Cosmetic Directives due to its excellent selectivity in isolation, higher resolution and sensitivity compared to the most of the other methods available.

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