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Practical GC/MS Analysis of Oxidation Dye Components in Hair Fiber as a Forensic Investigative Procedure*

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ABSTRACT: The purpose of this study was to improve the reliability of discrimination (or identification) of dyed hair by analyzing chemical substances present in the hair, as an addition to the conventional macroscopical and microscopical examinations and ABO blood group examination. Oxidation hair-dye components such as *p*-phenylenediamine (PPDA), toluylene-2,5-diamine (T-2,5-DA), *o*-aminophenol (OAP), *m*-aminophenol (MAP), *p*-aminophenol (PAP) and *p*-amino-*o*-cresol (PAOC) were selected as the object of study. After alkaline-digestion, hair samples were adjusted to a pH of 12.6 to 12.8, and applied onto an Extrelut column. After 15 min, the components were simultaneously extracted and derivatized with *n*-hexane including 1% heptafluoro-*n*-butyryl (HFB) chloride. Their HFB derivatives within a condensed sample were diluted in ethyl acetate, and analyzed by gas chromatography-mass spectrometry (GC-MS) with full mass scanning or selected ion monitoring. For estimating their levels, 2,4,6-trimethylaniline was used as the internal standard. Standard curves obtained by preparing a 5 cm segment of control hair spiked with authentic substances were linear, ranging from 0.1 to 4.0 μg for PPDA and T-2,5-DA, and from 0.01 to 0.4 μg for OAP, MAP, PAP and PAOC. The coefficient of variation of inter-day precisions (each $n = 4$) was below 16% for PPDA, below 20% for OAP and PAP and below 24% for T-2,5-DA, MAP and PAOC. These components were detectable even at 6 ng for PPDA, T-2,5-DA, MAP and PAP, 8 ng for OAP, and 4 ng for PAOC. Recovery percents using this procedure ranged from 54 to 86%. By using actual dyed hair samples this method was shown to provide high sensitivity for accurate detection.

KEYWORDS: forensic science, criminalistics, forensic chemistry, human hair, oxidation hair dye components, aromatic diamines, aminophenols, gas chromatography-mass spectrometry

Hair dyes are widely used for cosmetic reasons. Although various kinds of hair dyes are commercially available, oxidation species containing phenylenediamines, aminophenols, dihydroxybenzenes, nitro compounds and their salts as the main components are most commonly used since they facilitate simple and rapid hair coloring.

In forensic or criminal science, identification or discrimination of dyed hair is important when investigating a crime scene. Forensic analysis of hair involves morphological properties, ABO blood group examination, electrophoresis of hair protein, trace element analysis and DNA typing tests (1,2). However, these tests are sometimes insufficient for the identification or discrimination of dyed hair, and for this reason, further additional information is required. Hair-dye analysis may provide yet a further factor in hair investigation, as has been explained by Fujita et al. (3). Our previous reports described primitive studies where dyed hair was identified by gas chromatographic-mass spectrometric detection of *p*-phenylenediamine (PPDA), toluylene-2,5-diamine (T-2,5-DA), *o*-aminophenol (OAP), *m*-aminophenol (MAP) and *p*-aminophenol (PAP) (4,5). These attempts indicated that the peak ratios between analytes played an important role in such identification. However, the procedure of sample preparation was too laborious and time-consuming for routine use, and the prepared samples that contained various interfering substances were unsuitable for analysis using the capillary column-gas chromatographic system (CC-GC). Accordingly, a simple and rapid procedure preparing suitable samples for CC-GC analysis was required. Meanwhile, we devised a method in which extractive heptafluoro-*n*-butyrylation on an Extrelut column enabled the analysis of methamphetamine and its metabolites in body materials by gas chromatography-mass spectrometry (GC-MS) (6). This method is also applicable to the routine analysis of hair. As a result of our cumulative experience regarding hair analysis, we have been able to clearly identify certain components, including hair-dye components (7). This led us to examine whether this method is practically applicable to forensic investigation for identifying and discriminating dyed hair as an addition to conventional procedures. In this study, strands of hair which had been colored with hair dye products were obtained from three volunteers and examined.

Experimental

Control Hair Specimens

Natural hair specimens (about 70 to 100 μg/cm and 80 to 100 μm in diameter) were provided by a Japanese volunteer (Mongoloid) for use as a control.

Reagents and Chemicals

Extrelut (E. Merck, Darmstadt, Germany) was washed twice with distilled dichloromethane and dried at 150°C for 3 h. PPDA, *m*-phenylenediamine (MPDA), T-2,5-DA, OAP, MAP, PAP, *p*-amino-*o*-cresol (PAOC), resorcinol (RES) and *p*-nitro-*o*-phenylenediamine (PNOPD) as dye components in addition to heptafluoro-*n*-butyryl (HFB) chloride were obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan). 2,4,6-trimethylaniline as the internal standard was purchased from Wako Pure Chemical Industries (Osaka, Japan). The solvents used were all distilled twice using

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TABLE 1—*Main components indicated in the product contents.*

Commercial Product	Components			
A	PPDA	OAP	PAP	
B	PPDA	MPDA	MAP	
C	T-2,5-DA	MAP	PAP	PAOC

A = 6G, Hoyu Co., Nagoya; B = 67G, Yamahatsusangyo Co., Osaka C = N4, Shiseido Co., Tokyo.

PPDA = *p*-phenylenediamine, MPDA = *m*-phenylenediamine, T-2,5-DA = toluylene-2,5-diamine, OAP = *o*-aminophenol, MAP = *m*-aminophenol, PAP = *p*-aminophenol, PAOC = *p*-amino-*o*-cresol.

an all-glass apparatus. The three brands of hair dye products shown in Table 1 were commercially obtained.

Standard Solutions

Authentic compounds as hair dye components were respectively dissolved in 1 M hydrochloric acid to give a concentration of 1 mg/mL. The solutions were diluted to the appropriate concentrations with distilled water for the experimental procedures.

Internal Standard Solution (IS Solution)

2,4,6-trimethylaniline was dissolved in 1 M acetic acid to give a concentration of 1 mg/mL. For use, the solution was diluted with distilled water (0.5 ng/μL).

Procedure of Extractive Heptafluoro-n-*butyrylation*

A 5 cm hair specimen was washed twice with water, twice with ether/ethanol (1:1 v/v), dried at an ambient temperature, cut into 5 mm pieces and then placed into a 10 mL centrifuge tube. The hair was digested with 1 mL of 1 M sodium hydroxide by heating at 70°C for 15 min. After cooling, 10 μL of IS solution were added to the sample which was acidified with 0.1 mL of concentrated hydrochloric acid and then centrifuged at 3000 rpm for 5 min. To the supernatant was added 0.1 mL of borate buffer (0.04 M, pH 9.2) adjusted to pH 12.6 to 12.8 with 1 M sodium hydroxide and this was then applied to a column (10 cm × 0.6 cm inside diameter) filled with 0.9 g of Extrelut. After 15 min, extractive derivatization was performed by passing through 7 mL of *n*-hexane containing 1% HFB chloride. After condensation under a nitrogen stream at room temperature, the eluate was diluted with 1 mL of ethyl acetate. A 1-μL aliquot of the solution was used for a gas chromatography-mass spectrometer.

Preparation of Standard Curves

For digestion, 1 mL of 1 M sodium hydroxide, containing either 0.1, 1.0, 2.0 or 4 μg of PPDA and T-2,5-DA, and either 0.01, 0.05, 0.2 or 0.4 μg of MPDA, OAP, MAP, PAP and PAOC, was added to the respective 5 cm control hair specimens. The sample was prepared for analysis in the manner as described above. For quantitative analysis, respective molecular ions of hair dye components and the IS were monitored. The standard curves for different solutions were obtained by plotting the peak area ratio of each component to the IS, against the respective concentration ($n = 4$, inter-day).

Recovery Rates of Hair Dye Components

To estimate the percentage recovery of hair dye components from hair using this method, standard samples were prepared at both 1.0 and 4.0 μg of PPDA and T-2,5-DA, and at both 0.05 and 0.4 μg of MPDA, OAP, MAP, PAP and PAOC. For the estimations of different solutions, respective 1 mL samples of 1 M sodium hydroxide (excluding hair) containing authentic hair dye components at the corresponding concentrations were treated as references in the manner described above ($n = 4$, inter-day).

Low Limits of Detection

To estimate the detection limits by this method, each 5 cm control hair specimen containing quantities (1, 2, 4, 6 and 8 ng) of dye components (MPDA, PPDA, T-2,5-DA, OAP, MAP, PAP and PAOC) was treated in a similar manner to that described above. The lower limits of detection were based on a signal-to-noise ratio of more than 5.

Conditions of GC-MS

GC-MS analyses were performed on a GC-MS QP5000 (Shimadzu, Kyoto, Japan) that was operated in the positive electron impact (EI) mode with an XTI®-5 capillary column (30 m × 0.25-mm inside diameter, 0.25-μm film thickness, Restek Corporation, Bellefonte, PA). The septum at the injection port used was Thermolite™ (Restek). The injection port temperature was 210°C, whereas the flow rate of the helium carrier gas was 1.7 mL/min at 100 kPa. The purge time for the splitless injection was 1.0 min. The oven was maintained at an initial temperature of 70°C for 1 min, then programmed at 20°C/min to a final temperature of 290°C, where it was maintained until termination. The interface temperature was set at 250°C. The ionization energy was set at 70 eV. Full mass spectra were collected from within the scan range of 50 to 650 amμ at the scan cycle of 0.5 s. For the selected ion monitoring (SIM), the ions at m/z 303 and 500 were chosen for PPDA and MPDA, those at m/z 317 and 514 for T-2,5-DA, those at m/z 304 and 501 for OAP, MAP and PAP, those at m/z 318 and 515 for PAOC, and that at m/z 331 for IS, with a dwell time of 100 ms in each instance.

Analysis of Dyed Hair Provided by Volunteers

Specimens of dyed hair were provided by three volunteers as shown in Table 4. Each 5 cm piece of hair was subjected to analysis of hair dye components in the manner described above. To assess the sensitivity of this method, hair cut into 1-, 2-, 5-, 10-, 15-, 20-, 25-, 30- and 35-mm pieces was also analyzed. The assessment was performed as described in the section entitled ''The low limits of detection.'' The same hair dye products as those used by the volunteers were also analyzed in order to assess the results of our hair analysis.

Results

In order to obtain qualitative information, the nine common components of hair dyes and 2,4,6-trimethylaniline as the IS were each analyzed by GC-MS at 1 μg/mL for this study. Among them, no data were obtained for RES or PNOPD. The other compounds, PPDA, MPDA, T-2,5-DA, OAP, MAP, PAP and PAOC, were detectable as HFB derivatives, and their retention times, principal ions and relative intensities are shown in Table 2.

For regular routine tests, SIM performance was examined by monitoring the respective molecular ions and principal ions. All peaks were simultaneously distinguishable at the baseline level on chromatograms (Fig. 1). Standard curves ($r^2 \geqq 0.995$) were linear in a range between 0.1 and 4 μg for PPDA and T-2,5-DA, and in

TABLE 2—*Retention times and principal ions for standard hair dye components, including the internal standard.*

Derivative	Mol. Wt.	Rt, min	Principal Ions (relative intensity, %)						
PPDA.2HFB	500	8.20	108 (100)	169 (17)	303 (93)	304 (10)	331 (11)	481 (15)	500 (68)
T-2,5-DA.2HFB	514	8.37	69 (100)	169 (10)	317 (89)	318 (13)	345 (59)	514 (62)	
MPDA.2HFB	500	7.82	69 (100)	169 (46)	303 (44)	304 (5)	331 (85)	481 (20)	500 (86)
OAP.2HFB	501	5.38	135 (100)	169 (26)	288 (21)	304 (9)	332 (5)	501 (18)	
MAP.2HFB	501	6.38	69 (100)	169 (37)	288 (13)	304 (42)	332 (64)	501 (41)	
PAP.2HFB	501	6.63	69 (100)	169 (42)	288 (7)	304 (56)	332 (7)	501 (36)	
PAOC.2HFB	515	6.84	69 (100)	169 (28)	302 (12)	318 (46)	346 (50)	496 (8)	515 (39)
2,4,6-TMA.HFB*	331	6.73	134 (36)	162 (100)	331 (47)				

Mol. wt. = molecular weight, Rt = retention time, HFB = heptafluoro-*n*-butyryl.
* 2,4,6-trimethylaniline, internal standard.

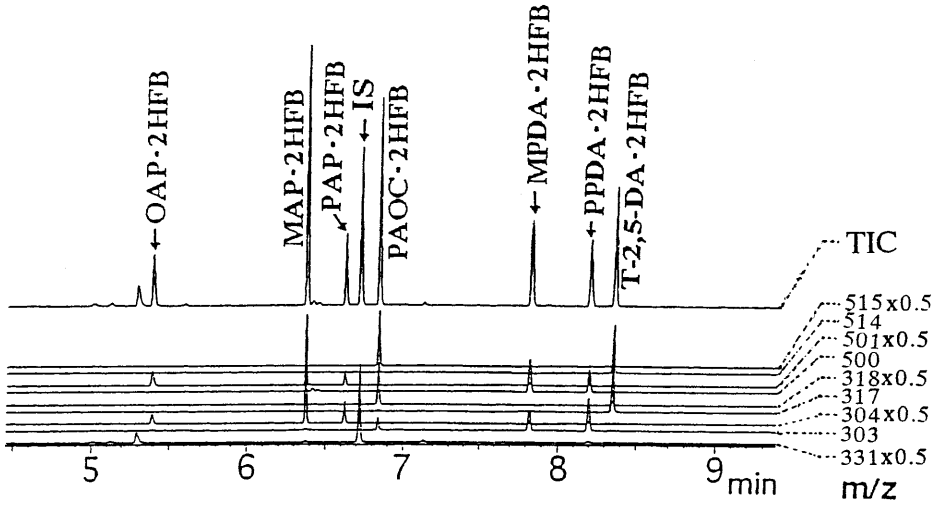

FIG. 1—*GC/SIM chromatogram of 7 heptafluoro*-n-*butyrylated hair dye components with the internal standard (IS) 2,4,6-trimethylaniline (7 hair dye components: 1 ng, IS: 0.5 ng). PPDA and MPDA at m/z 500 and 300, T-2,5-DA at m/z 514 and 317, OAP, MAP and PAP at m/z 501 and 304, PAOC at m/z 515 and 318, and the internal standard (IS) at m/z 331.*

TABLE 3—*Percentage recovery of hair dye components during procedure of extractive derivatization.*

Ingredients	Recovery (%, n = 4)	
	a	b
PPDA	76 (15)	54 (11)
T-2,5-DA	69 (12)	56 (13)
MPDA	72 (10)	58 (9)
OAP	66 (27)	64 (28)
MAP	61 (24)	59 (14)
PAP	86 (23)	86 (17)
PAOC	71 (19)	81 (20)

Added amounts of components, a = 1 μg for PPDA and T-2,5-DA; 50 ng for MPDA, OAP, MAP, PAP and PAOC, b = 4 μg for PPDA and T-2,5-DA; 400 ng for MPDA, OAP, MAP, PAP and PAOC.
The values in parentheses are coefficients of variation (%).

a range between 0.01 and 0.4 μg for MPDA, OAP, MAP, PAP and PAOC. The reliabilities expressed by coefficients of variation (c.v.) were below 11% for MPDA, below 16% for PPDA, below 20% for OAP and PAP, and below 24% for T-2,5-DA, MAP and PAOC (n = 4, inter-day). Variabilites in the values of inter-day and intra-day coefficients of variation for standard curves were minimum, lying within a few percent (1 to 4%), respectively. The detection limit by this method was 6 ng for PPDA, T-2,5-DA, MPDA, MAP and PAP, 8 ng for OAP and 4 ng for PAOC. Percentage recovery of hair dye components during the alkaline-digestion procedure ranged from 54 to 86%, as shown in Table 3 (n = 4, inter-day).

For assessing practical utility, the specimens of dyed hair provided by the three volunteers were analyzed using this method. The results of qualitative and quantitative analysis of 5 cm strands of hair are shown in Table 4, with six components, PPDA, T-2,5-DA, OAP, MAP, PAP and PAOC being detected. No interfering peaks were found when analyzing natural hair. In Table 5, the analytical results of the commercial hair dye products used by the volunteers are also shown. Although MPDA was contained in the hair dye product used by volunteer 2, it could not be confirmed in her hair (Sample 2). The necessary lengths of qualitative analysis of hair from the three volunteers, in which hair dye components could be detected, were examined for the sensitivity of this method. As a result, PPDA, OAP and PAP were simultaneously detected in a 1.5 cm piece of hair from volunteer 1 (Fig. 2). T-2,5-DA, MAP, PAP and PAOC were simultaneously detected in a 1.5 cm piece of hair from volunteer 3 (Fig. 3). Furthermore, the length of hair from the three volunteers that was necessary for the detection of the six components acting as indicators was 1 mm for PPDA and T-2,5-DA, 15 mm for OAP, 10 mm for MAP, 5 mm for PAP and 15 mm for PAOC, respectively.

Discussion

Hair dye component analysis is very useful for the identification and discrimination of dyed hair. Examination of a piece of hair is often required in forensic hair analysis, especially where forensic hair comparison is essential. ABO blood group examination (absorption-elution technique) requires a piece of hair approxi-

TABLE 4—*Content of hair dye components in hair from volunteers measured by this method.*

Volunteer	Sex	Age	Days	Dm (μm)	Content of hair-dye components (μg)					
					PPDA	T-2,5-DA	OAP	MAP	PAP	PAOC
1	M	44	12	90–100	2.25		0.10		0.32	
2	F	48	20	80–100	0.75			0.04		
3	F	52	15	80–90		2.00		0.07	0.02	0.04

Samples 1-3: each comprising a 5-cm piece of hair; M = male; F = female.
Days = duration from time until sample collection.
Dm = diameter of hair shaft.

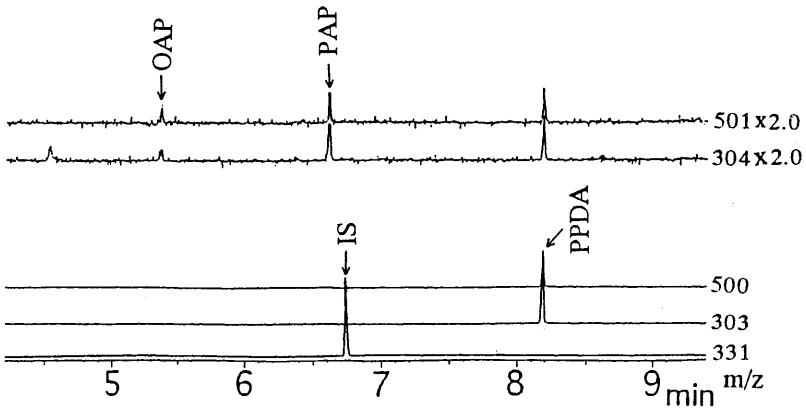

FIG. 2—*GC/SIM chromatogram of the extracts (HFB-derivatives) obtained from Sample 1. PPDA at m/z 500 and 300, OAP and PAP at m/z 501 and 304, and the internal standard (IS) at m/z 331. PPDA, OAP and PAP were simultaneously detected in a 1.5 cm piece of hair.*

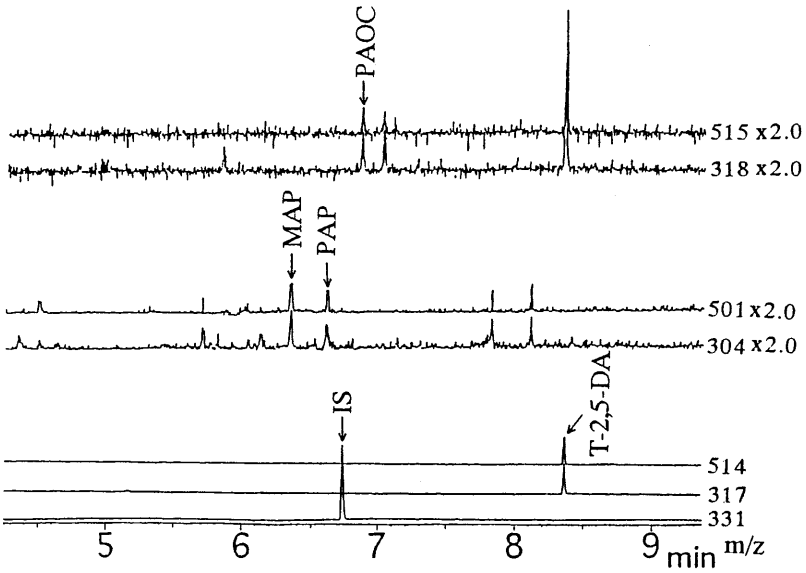

FIG. 3—*GC/SIM chromatogram of the extracts (HFB-derivatives) obtained from Sample 3. T-2,5-DA at m/z 514 and 317, MAP and PAP at m/z 501 and 304, PAOC at m/z 515 and 318, and the internal standard (IS) at m/z 331. T-2,5-DA, MAP, PAP and PAOC were simultaneously detected in a 1.5 cm piece of hair.*

mately 5 cm in length (80 to 100 μm in diameter). This method can be applied to hair specimens that have previously been subjected to the conventional macroscopical and microscopical examinations and to ABO blood group examination (5).

Accordingly, in this study, qualitative and quantitative analysis of dyes in a 5 cm piece of hair, simultaneous extraction from a human hair dyed with oxidation dyes and HFB derivatization of dye components for GC-MS measurements were all achieved. Furthermore, the sensitivity of this method for qualitative analysis was also examined using hair specimens from volunteers.

The major dye components in the hair specimens from the three volunteers comprised two diamines (PPDA and T-2,5-DA), while the content of aminophenols and aminocresol was very low (Table 4). It is known that PPDA and T-2,5-DA are the major dye components in most commercial hair dyes. This result corresponded with findings from analysis of commercial hair dyes, as shown in

TABLE 5—*Concentrations of components in commercial hair dye products* measured by this method.*

Commercial Product	Concentrations, mg/mL						
	PPDA	T-2,5DA	MPDA	OAP	MAP	PAP	PAOC
A	9.0	. . .	. . .	1.4	. . .	3.8	. . .
B	5.4	. . .	0.4	. . .	3.9	. . .	. . .
C	. . .	8.0	. . .	. . .	2.0	0.4	0.4

A = 6G, Hoyu Co., Nagoya; B = 67G, Yamahatsusangyo Co., Osaka, C = N4, Shiseido Co., Tokyo.

* These products were used when the volunteers had their hair dyed.

Table 5. The components detected in Samples 1 and 3 corresponded with those in each of the commercial hair dyes (Tables 4 and 5).

In a previous study, we demonstrated the presence of five components in oxidation hair dyes, namely PPDA, T-2,5-DA, OAP, MAP and PAP from dyed hair using GC/MS (4,5).

By this extractive heptafluoro-*n*-butyrylation on an Extrelut column, the lengthy laborious process of separate extraction procedures and separate analysis procedures for diamines and aminophenols was eliminated; MAP could be fully resolved from PAP, and PAOC could be detected. The whole procedure using this method took only about 3 h, even for multiple samples (usually six samples) because of its sensitivity, simplicity and speed. From these results, it was suggested experimentally that hair dye component analysis of a dyed hair specimen may be an index for use in forensic hair analysis, especially forensic hair comparison. However, analytical results obtained by this method alone are not acceptable as conclusive evidence in cases depending on forensic hair comparison. It is considered that useful evidence can be obtained by combining the analytical results obtained by this method with those obtained by morphological examination and ABO blood group examination.

We are now in the process of confirming the usefulness of this method in practical forensic cases.

Conclusion

By extractive heptafluoro-*n*-butyrylation on an Extrelut column, six hair dye components can be easily, rapidly and simultaneously detected from a single strand of dyed hair. The efficacy of this method was demonstrated using dyed hair from volunteers.

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