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Original Article

Determination of phenylenediamines in hair colors derivatizated with 5-(4, 6-dichlorotriazinyl) aminofluorescein via micellar electrokinetic chromatography

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ABSTRACT

Phenylenediamines (PDs), which are reported to cause allergic dermatitis and possess genotoxicity and carcinogenicity, are the ingredients used in permanent hair dyes. The fluorescent derivatization strategy coupled with micellar electrokinetic chromatography (MEKC) were established to analyze four PDs, including *o*-phenylenediamine (OPD), *m*-phenylenediamine (MPD), *p*-phenylenediamine (PPD) and toluene-2,5-diamine (PTD). Additionally, 5-(4, 6-dichlorotriazinyl) aminofluorescein (DTAF) was used as a fluorescent reagent derived at amino groups of PDs and underwent nucleophilic substitution reaction to improve the detection sensitivity. The derivatization condition reacted at 90 °C for 10 min in alkaline conditions. The optimized separation conditions were 20 mM borate (pH 8.0) containing 10 mM Brij 35 and 35% (v/v) methanol. The limits of detection (S/N = 3) for MPD, PTD, PPD and OPD were 25, 25, 50 and 100 nM, respectively. Compared to MEKC-UV, the sensitivity enhancements were 30- to 81-fold when PDs were derived with DTAF. The high-sensitivity MEKC-LIF method was successfully established and applied to determine PDs in commercial hair colors for quality control and in real hair samples for evaluating the location of PDs in dyed hair samples, as well as in percutaneous absorption samples for evaluating the ability of PDs to penetrate skin.

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1. Introduction

Phenylenediamines (PDs) are the main ingredients that are used in permanent hair dyes. PDs are polymerized in the cortex of the hair, and the color of the dyed hair appears. The most common ingredient of permanent hair dyes, *p*-Phenylenediamine (PPD), has been used for decades. However, the safety of PPD is questioned. PPD can cause immune response and induce allergic dermatitis [1,2]. In Germany, 18–20% of women are engaged in hair salons and exposed to PPD for a long time, which can easily lead to allergic dermatitis [3]. Schmidt et al. observed that the rate of allergicdermatitis morbidity was increasing when a higher toluene 2,5-diamine (PTD) content was observed in the hair-dyed mouse [4]. In the in vitro study, PPD and H_2O_2 were added to the rat liver metabolic activation system (S-9) that contained *Salmonella typhimurium* strain TA98 to perform the Ames test. The results showed that Bandrowski's base was produced via PPD oxidation and lead to gene mutation [5]. To reduce the harm to the human body, PDs should not exceed the concentration in hair dyes that is approved by the national law. o-Phenylenediamine (OPD) and m-phenylenediamine (MPD) have been classified as banned components. Due to a lack of clinical data that PPD and PTD lead to cancer, the concentration limits were set at 2% (w/v) and 4% (w/v), respectively, by Taiwan's law. Therefore, it is important to develop a simple and sensitive analytical method for PDs and apply it to quality control of permanent hair dyes in pharmaceutical cosmetics and to determine the hair dyes that were acquired via the in vitro percutaneous penetration method to evaluate the percutaneous penetration ability of permanent hair dyes with different formulations.

In previous studies, several analytical instruments were used to determine PDs including gas chromatography coupled mass spectrometry (GC-MS) [6–8], high-performance liquid chromatography (HPLC) [9,10] and capillary electrophoresis (CE) [9,11,12]. Using GC-MS to analyze PDs results in a low detection limit. The advantages of CE are low solvent consumption and good resolution. The determination of PDs using MEKC were investigated by Wang et al. In the micellar electrokinetic chromatography (MEKC) mode, cetyltrimethylammonium chloride was added to reverse the electroosmotic flow (EOF), and PDs can be separated within 10 min [9]. However, the disadvantages of CE were poor detection sensitivity due to the short optical pathlength and small amount of sample injection. To improve the detection sensitivity and increase the applicability of the method, fluorescent derivatization was used in this study. After derivatization, the PD derivatives were determined in permanent hair dyes, hair samples and percutaneous absorption samples via micellar electrokinetic chromatography combined with laser-induced fluorescence detector (MEKC-LIF).

2. Materials and methods

2.1. Chemicals and reagents

All reagents were analytical grade. o-Phenylenediamine (OPD) and *m*-phenylenediamine (MPD) were purchased from Chem Service (West Chester, PA, USA). *p*-Phenylenediamine (PPD), toluene 2,5-diamine (PTD) and 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF) were obtained from Sigma–Aldrich (St. Louis, MO, USA). 1-Aminoadamantane hydrochloride (1-AD) was from Alfa Aesar (Heysham, Lancashire, UK). Sodium tetraborate decahydrate was from Merk (Darmstadt, Germany). Polyoxyethylene lauryl ether (Brij 35) was from Acros organics (New Jersey, USA). Methanol was obtained from Avantor Performance Materials (Center Valley, PA, USA). Deionized distilled water was purified using a Milli-Q system (Millipore, Bedford MA, USA).

2.2. Apparatus

The CE method was performed using a Beckman P/ACE™ MDQ capillary electrophoresis system equipped with a laser-induced fluorescence detector (Beckman Instruments, Fullerton, CA, USA). The excitation wavelength was set at 488 nm, and the emission wavelength was collected at 520 nm. All operations, including electropherogram acquisitions, were computer-controlled using the Beckman P/ACE MDQ 32 Karat system (Fullerton, CA, USA). Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50-μm i.d. and 40 cm effective length (total length of 50.2 cm) were used for separation and maintained at 25 °C. The thermal cycler was used to carry out fluorescence derivative reaction that was purchased from VWR International, Ltd. (Lutterworth, Leicestershire, England).

2.3. Standard preparation and sample pretreatment

A standard stock solution of OPD, MPD, PPD, 1-AD and DTAF (10 mM) was prepared in methanol, and PTD (1 mM) was prepared in deionized distilled water. The working solutions were diluted using methanol from stock solutions. Then, all solutions were stored in the dark at −20 °C. The sample pretreatment procedures were as follows. (1) Hair color products (Sample P1 and P2). The ingredients of Sample P1 were PTD, *m*-aminophenol (MAP), *p*-aminophenol (PAP), ammonia hydroxide and hydrogen peroxide. The ingredients of Sample P2 were PPD, resorcinol, MAP, PAP, ammonia hydroxide and hydrogen peroxide. Hair color samples (100 mg) were added to 1 mL of methanol, vortexed for 5 min, sonicated for 5 min and finally centrifuged for 10 min at 12,000 rpm. The supernatant was filtered using a 0.45-μm PVDF filter (Millipore, Bedford, MA, USA) followed by 400-fold dilution with methanol prior to the derivatization procedure. (2) Hair samples. This study was approved by the Institutional Review Board (KMUHIRB-E (II)-20160162). Uncontaminated hair samples were collected from healthy volunteers. The hair washing procedure was as follows: 1% SDS solution for 5 min, distilled water for 5 min and methanol for 5 min. After dying hair using sample P1 for 30 min, the hair was cleaned with distilled water. The hair was allowed to dry at room temperature. After washing and drying, hair (10 mg) was cut into small sections (1–2 mm) and extracted using the following procedure: sonication of hair in 1 mL of 0.1 M HCl for 2 h, then left standing at room temperature for 12 h. The supernatant was neutralized using 1 mL of HCl (1 M), filtered using a 0.45-μm PVDF filter followed by a 5-fold dilution with methanol prior to derivatization procedure.

(3) Percutaneous absorption samples. The vertical diffusion cell (i.d.: 9 mm; receptor volume: 4 mL) was purchased from Hanson (CA, USA), and the Strat-M™ membrane (i.d.: 25 mm) was obtained from Merck (Darmstadt, Germany). In the percutaneous absorption experiment, the membrane was placed between two compartments of vertical diffusion cells. The top compartment was covered with 100 mg of hair color product (Sample P1). The bottom compartment was filled with receptor fluid (phosphate buffered saline buffer). The membrane was kept at 32 °C by heating receptor fluid using a heating circulator (Firstek, Taipei, Taiwan) and stirred using a Helix™ stirrer. After 30 min, the excess dye was removed using wipes and collected in 1.5 mL of methanol. The excess dye solution was sonicated for 5 min and centrifuged at 12,000 rpm for 5 min. The supernatant was evaporated in a centrifugal vaporizer (EYELA CVE-2000, Japan) and reconstituted with 0.5 mL of methanol. The membrane was cut into small sections and collected in 1 mL of methanol. The solution was sonicated for 5 min and centrifuged at 12,000 rpm for 5 min. Then, the supernatant was evaporated in a centrifugal vaporizer and reconstituted with 0.5 mL of methanol. The receptor fluid was collected and evaporated in a centrifugal vaporizer and reconstituted with 0.8 mL of methanol.

2.4. *Derivatization procedure*

A total of 20 μL of DTAF (2.5 mM), 35 μL of 1-AD (100 μM) and 25 μL of derivatization buffer (20 mM borate, pH 8.5) were added to 20 μL of samples containing a mixture of analytes in a vail. Then, after being capped and homogenized, the derivatization solution was heated using the thermal cycler. The derivatization reaction was reacted at 90 °C for 10 min. After derivatization, the derivatization solution was kept at 4 °C until the CE analysis.

2.5. *Electrophoretic procedure*

The separation buffer was a borate buffer (20 mM, pH 8.0) containing 10 mM Brij 35 and 35% (v/v) methanol. Between each experimental run, the capillary was rinsed with 1 M NaOH for 5 min and deionized water for 5 min at 30 psi. At the beginning of each experiment, the capillary was rinsed with 1 M NaOH and deionized water for 3 min, followed by running buffer for 3 min. The samples were hydrodynamically injected at 0.5 psi for 15 s. The applied voltage for separation was 20 kV.

3. Results and discussion

3.1. *Selection of derivatization conditions*

Derivatization reagents, with the excitation wavelength of 488 nm and the emission wavelength of 520 nm, can react with amino groups including fluorescein isothiocyanate (FITC) [13–18], 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) [19–21], 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF) [22–25], 5-carboxyfluorescein succinimidyl ester (CFSE) [25,26]. Among them, the advantages of DTAF were short reaction time, less hydrolysate formation and good stability of the derivatives. DTAF has a chlorine-containing s-triazine structure and can reacted with amine via the nucleophilic substitution reaction (Fig. S1). The reaction needs to be carried out in an alkaline condition to obtain the higher fluorescence intensity. In this study, a borate buffer with pH 8.0, 8.5, 9.0 and 9.5 was selected to explore the derivatization effect. The results are shown in Fig. 1. When the pH value of the borate buffer increased from 8.0 to 8.5, the peak height of MPD and OPD decreased with the increase of pH value, while the peak height of PTD and PPD increased. The nucleophilicity of the amine group in the molecular state ($-NH_2$) is superior to that of the ionic state ($-NH_3^+$). PPD and PTD were partially dissociated at pH 8.0, such that the reactivity with DTAF was decreased. However, when the pH value is higher than 8.5, the height of the four derivatives clearly decreases. The reason is that when DTAF is at the higher pH value condition, more hydroxyl ions react with DTAF to form the hydrolysis products, which leads to reduced derivatization efficiency of PDs [27,28]. Finally, pH 8.5 was chosen as the optimum derivatization condition.

The higher the concentration of borate buffer solution in derivatization conditions, the lower peak height and wider peak width of the analytes were observed. While the borate buffer concentration was set at 10 mM, MPD and OPD peak height were 2–3 times lower compared with a 20-mM borate buffer. However, OPD derivative overlaps with other interferences. Ultimately, a 20-mM borate buffer was chosen for further derivatization conditions.

To prevent the aqueous solutions from forming DTAF hydrolyzate, methanol was selected as the solvent in the derivatization reaction by adjusting the proportions of methanol (20%, 50%, 75% and 100%, v/v) throughout the derivatized

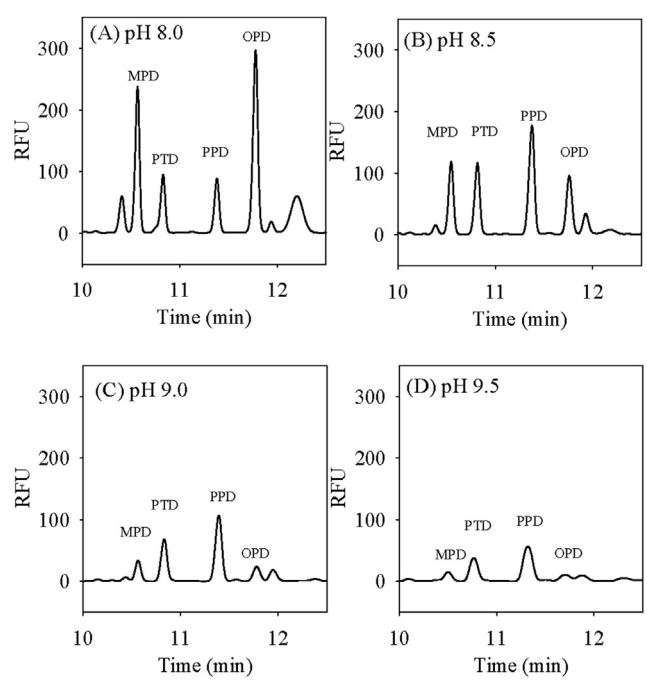

Fig. 1 – Electropherograms of different pH values in a derivatization buffer. pH value (A) 8.0, (B) 8.5, (C) 9.0, (D) 9.5 in 20 mM borate. Separation condition: rinse buffer: 20 mM borate (pH 8) containing 10 mM Brij 35 and 35% (v/v) MeOH; sample injection: 0.5 psi, 15 s; separation voltage: 20 kV; four analyte concentrations: 5 μM.

solution. The results showed that the peaks were broadened when 20% methanol was added. When increasing the proportion of methanol in the derivatized solution, the peak shape of PDs was improved, and the peak height also increased. When 75% methanol is added, four PDs can reach the baseline separation. However, when 100% methanol was added to the derivatized solution, the peaks of the analytes were not observed. The reason was that the pH value of derivatized solution was close to neutral and decreased the nucleophilic ability of PDs. Finally, 75% methanol was chosen as the optimum derivatization condition.

Four DTAF concentrations (0.25, 0.50, 1.00, 2.00 mM) were used for evaluating the derivatization efficiency. The electropherograms are shown in Fig. S2. DTAF reacted with themselves and produced byproducts that would interfere the derivatization efficiency of analytes. The peak heights of PTD and PPD decreased while DTAF concentration was above 0.5 mM. Therefore, 0.5 mM DTAF was chosen as the optimal condition.

The reaction temperature and time of derivatization affected reaction completion. In this work, reaction temperatures of 60, 70, 80, 90 and 100 °C were selected for evaluating the derivatization efficiency (Fig. S3). When reaction temperature was increased, fluorescent intensities of PTD and PPD increased. However, MPD and OPD decreased. Meanwhile, the relative standard deviation (RSD) of analyte intensities at different reaction temperatures were used to evaluate the stability of derivatives. When the reaction temperature was set at 90 °C, the lowest RSD were obtained (the RSDs of MPD, PTD, PPD and OPD were 3.14%, 1.29%, 1.40% and 2.47%, respectively). Considering the fluorescent intensities and stability of derivatization, the optimal reaction temperature was set at 90 °C. Four reaction times (5, 7, 10 and 12 min) were chosen to discuss the effect of derivatization. When the reaction time increased, the intensities of PTD and PPD derivatives increased. MPD and OPD had the highest peak heights when reaction time was set at 10 min. Therefore, the optimal reaction time was set at 10 min.

3.2. *Optimization of separation conditions*

According to the previous study, the maximum fluorescence of DTAF was under alkaline conditions [27]. Therefore, the pH value of separation buffer was chosen in alkaline conditions (pH 7.5, 8.0, 8.5 and 9.0). Higher fluorescence intensities were acquired when the pH value of borate buffer was increased. However, high pH value led to stronger EOF, and analytes were separated with poor resolution. Therefore, pH 8.0 was chose as the optimal condition.

Due to the similar structure and charge-to-mass ratio of the four PD derivatives, the difference in the electrophoretic mobility is small and cannot be separated via capillary zone electrophoresis. Therefore, the MEKC mode was used in this study. MEKC is a technology to change the mobility of analytes depending on different partition ability of analytes between the micelle and aqueous solution. When an anionic surfactant (such as SDS) is added to the separation buffer solution, the analysis time is longer than 25 min, and only one peak was observed. In addition, other surfactants, such as Triton X-100, Tween 20, Tween 60, Tween 80 and Brij 35, were tested to evaluate the separation efficiency of PDs. Only Brij 35 can produce better separation resolution. The CMC value of Brij 35 was 0.1 mM. When the concentration was greater than CMC, the micelles as a pseudostationary phase were formed. With the increase in the concentration of Brij 35, the resolution of four structurally similar analytes increased due to the alteration of the electrophoretic mobility of analytes. The electropherograms are shown in Fig. S4. All analytes were separated while adding 10 mM Brij 35.

The addition of an organic solvent can reduce the polarity of the separation buffer solution and alter the partition coefficient between the analyte and micelles to improve the resolution. Three organic solvents (acetonitrile, ethanol and methanol) were selected as buffer additives to evaluate the separation efficiency. When ACN was added, the four analytes had the same electrophoretic mobility and could not be separated. When ethanol was added, four analytes could be separated but the analysis time was longer than 20 min. Different percentages of methanol (0%, 20%, 30% and 40%) were taken to discuss the effect of methanol in separation solution. PDs cannot be separated without adding methanol. When the percentage of methanol increased, the resolution improved due to the change of partition coefficient of analytes and reduction of EOF. All analytes were separated completely after 35% methanol was used. However, when methanol with a concentration greater than 35% was used, the resolution of OPD derivative became worse. Therefore, 35% methanol was chosen as the optimal condition.

3.3. *Method validation*

To evaluate the quantitative applicability of the established CE method, five different concentrations of analytes were analyzed; 35 μM 1-AD was used as an IS to calculate the peak area ratios by dividing the corrected peak areas of each analyte. The corrected peak area, which was used with the standard, was calculated as follows: (peak area of the analyte/migration time of the analyte)/(peak area of the IS/migration time of the IS). Calibration curves were obtained from the linear peak area ratios by plotting the analyte concentration on the X-axis, and the peak area ratios on the Y-axis. The data are shown in Table 1. The calibration curve was linear in the range of 0.125 μM–5.000 μM with a correlation coefficient (r) of each curve was greater than 0.997 ($n = 5$). The precision and accuracy were calculated based on the analyses of PDs (at three different levels). The RSD values were lower than 4.56%, and RE values were lower than 4.97%, which indicates good precision and accuracy. Limit of detection (LOD) was determined by spiking the standard solutions with decreasing concentrations of each analyte until S/N was equal to 3. The limits of detection for OPD, MPD, PPD and PTD were 0.100, 0.025, 0.050 and 0.025 μM, respectively. In Table 2, the LOD of this study was clearly improved via fluorescent derivatization. Compared with MEKC-UV [9], the sensitivity improved by 30- to 81-fold.

3.4. *Selectivity experiments*

Except for PDs, several ingredients contained amino groups, which reacted with DTAF, such as o-aminophenol (OPA), m-

Table 1 – Regression analysis for determination of phenylenediamines in intra-day (n = 3) and inter-day analysis (n = 5).

Analytes	Regression equation	Correlation coefficient (r)
Intra-day analysis (n = 3)		
OPD	Y = (0.2940 ± 0.0061)X + (0.0056 ± 0.0118)	0.999
MPD	Y = (0.4037 ± 0.0095)X + (0.0317 ± 0.0192)	0.999
PPD	Y = (0.8354 ± 0.0611)X + (0.0126 ± 0.0335)	0.997
PTD	Y = (0.5294 ± 0.0348)X + (0.0092 ± 0.0249)	0.998
Inter-day analysis (n = 5)		
OPD	Y = (0.3003 ± 0.0020)X + (0.0085 ± 0.0136)	0.999
MPD	Y = (0.3943 ± 0.0043)X + (0.0488 ± 0.0094)	0.999
PPD	Y = (0.8855 ± 0.0098)X + (0.0443 ± 0.0335)	0.999
PTD	Y = (0.6016 ± 0.0121)X + (−0.0151 ± 0.0225)	0.999

Table 2 – Previous studies of PD analytical methods.

Compounds	Methods	LOD	Samples	References
OPD, MPD, PPD, PTD	MEKC-LIF	0.025–0.100 μM	hair colors; hair samples; percutaneous absorption samples	This study
OPD, MPD, PPD, PTD	MEKC-UV	1.73–2.96 μM	–	[9]
OPD, MPD, PPD, PTD	MEKC-UV	30.60–65.43 μM	–	[11]
OPD, MPD, PPD	CZE-ECD	0.11–1.08 μM	–	[12]
PPD	GC/MS	0.93 μM	Cosmetic products	[6]
PPD	GC/MS	0.93 nM (LOQ)	Biological fluids	[8]
OPD, MPD, PPD, PTD	HPLC-DAD	0.46–2.59 μM	–	[9]
PPD	HPLC-ECD	0.02 ng	Human urine; rabbits urine, feces, and blood	[10]

Abbreviations: LOD, Limitation of detection; LOQ, Limitation of quantitation; OPD, *o*-phenylenediamine; MPD, *m*-phenylenediamine; PPD, *p*-phenylenediamine; PTD, toluene-2,5-diamine; MEKC, micellar electrokinetic chromatography; CZE, capillary zone electrophoresis; ECD, electrochemical detection; DAD, diode-array detection.

aminophenol (MAP), p-aminophenol (PAP) and 4-(methylamino) phenol (4MAP), which typically appear in hair color products. To avoid interference from the other amino compounds with the quantitative results, OPA, MAP, PAP and 4MAP were chosen for use in selected experiments. The results are shown in Fig. 2. The migration order of these compounds was as follows: MAP, OAP, PAP and 4MAP. Four aminophenol related compounds exhibited different apparent electrophoretic mobility and migrated faster than PDs under the optimized condition. According to these results, the common amino related compounds did not interfere with the determination of PDs.

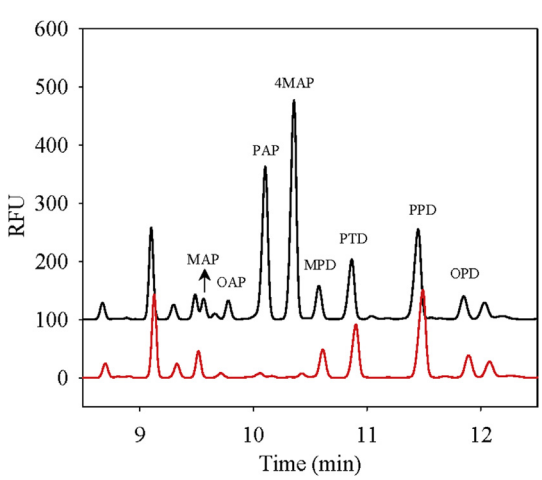

Fig. 2 – Electropherogram of a mixture of eight standard analytes derivatized with DTAF. Red line is a mixed solution, and black line is a mixed solution spiked with OAP, MAP, PAP and 4MAP. The separation condition was the same as in Fig. 1.

3.5. *Applications*

3.5.1. *Hair color samples*

Hair color products (Sample P1 and Sample P2) containing PDs were analyzed using the MEKC method. The PTD peak was still observed in Sample P1, and the PPD peak was observed in Sample P2. The electropherograms obtained from the analysis of Sample P1 and P2 are shown in Fig. 3. To further confirm the accuracy of the analysis, three amounts of standards were added to the sample to determine their recoveries. All of the recoveries were 99%–104% in Sample P1 and 99%–108% in Sample P2, which indicated that the cream matrix did not

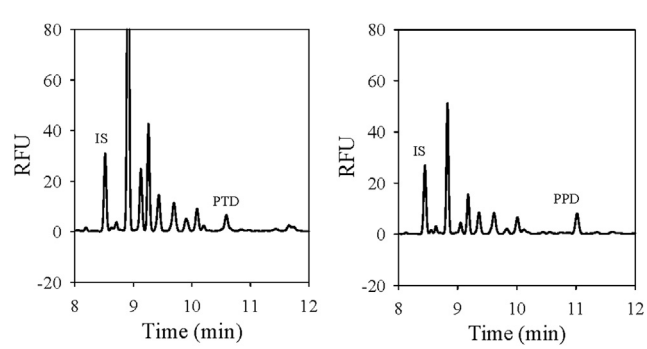

Fig. 3 – Electropherograms of (A) Sample P1 and (B) Sample P2. The separation condition was the same as in Fig. 1.

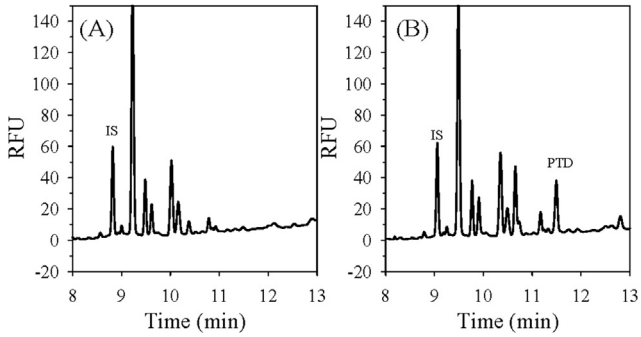

Fig. 4 – Electropherograms of hair sample (A) none dyed and (B) dyed hair. Separation condition was the same in Fig. 1.

affect the quantitative results obtained using the CE method. Quantitation of the PDs in Sample P1 and Sample P2 produced the following results: PTD (2.47%) in Sample P1; PPD (1.04%) in Sample P2. This method is suitable for the determination of PDs in hair color products.

3.5.2. *Hair samples*

To evaluate the residue of PDs in dyed hair samples, real undyed hair samples were collected and then dyed using Sample P1 before analyzing via the CE method. PTD was observed at 11.5 min, and many unknown peaks appear in the electropherogram (Fig. 4). All recoveries in hair samples were 99%–104%, and the reside amount of PTD was 507 ng/mg of hair. MS spectra of PTD in positive ionization mode showed the formation of $[M+H]^+$ (m/z of 581.13) (Fig.S5). These samples were further analyzed using nano LC-MS, and the results showed good agreement with our data.

3.5.3. *Percutaneous absorption samples*

Hueber-Becker et al. found that 0.54% of [^{14}C]-PPD was absorbed into the body after dyeing hair by radioactivity [29]. In in vitro studies, the amount of hair dye, aminopropyl methyl morpholinium methosulphate, in human skin via percutaneous absorption study was determined to be 0.9 μg/cm^2 [30]. Percutaneous absorption study can be used to evaluate the risk assessment of PDs. The results of the percutaneous absorption study of PTD are summarized in Table 3. A total of 0.63% of the applied dose was considered as bioavailable (the amount of membrane and receptor fluid). Among them, most dyes were in excess to form the polymer, 0.61% of the applied dose (7.29 ± 0.02 μg) was retained in the membrane, and 0.02% of the applied dose (0.24 ± 0.06 μg) was in receptor fluid. According to the results, a small amount of PTD penetrated the membrane in the in vitro study.

Table 3 – In vitro percutaneous absorption of PTD hair dye formulation.

Compartment	% of applied amount	Amounts of PTD (mean ± SD)
Surface excess	10.34%	127.75 ± 0.01 μg
Membrane	0.61%	7.29 ± 0.02 μg
Receptor fluid	0.02%	0.24 ± 0.06 μg

4. Conclusion

This study has developed derivatization reactions of PDs using the DTAF and MEKC-LIF method for the determination of PDs derivative. Compared with MEKC without derivatization reactions, the sensitivity enhancements were between 30-fold and 81-fold. The applicability was demonstrated through the determination of PD derivatives in commercial hair colors, hair samples and percutaneous absorption experiments without a further extraction procedure.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflicts of interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfda.2019.02.005.

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Determination of phenylenediamines in hair colors derivatizated with 5-(4, 6-dichlorotriazinyl)aminofluorescein via micellar electrokinetic chromatography

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[1]Hung-Yu Ko and Yi-Hui Lin are equal contribution in this work.

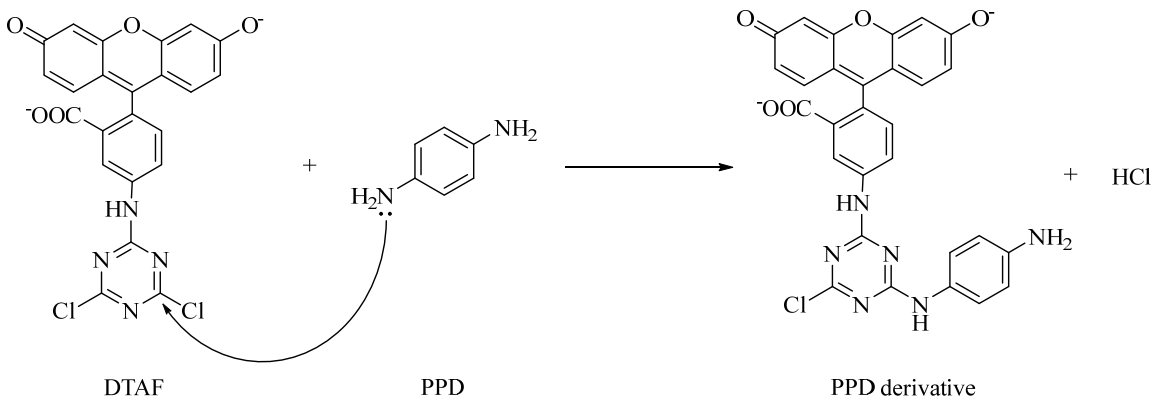

Fig. S1. Scheme of the derivatization reaction between DTAF and PPD.

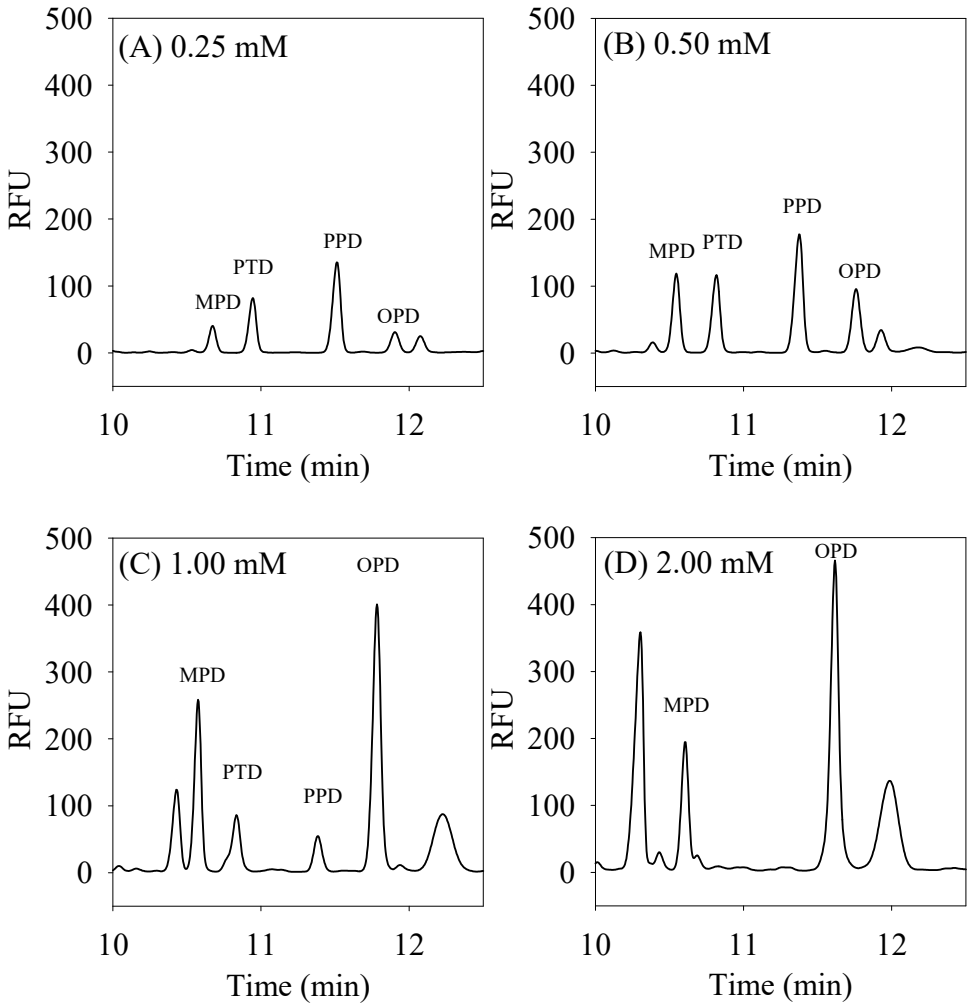

Fig. S2. Electropherograms of different DTAF concentrations in derivatization solution. (A) 0.25 mM, (B) 0.50 mM, (C) 1.00 mM and (D) 2.00 mM DTAF. Separation condition: rinse buffer: 20 mM borate (pH 8) containing Brij 35 and 35% (v/v) MeOH; sample injection: 0.5 psi, 15 s; rinse buffer pressure injection: 0.5 psi, 5 s; separation voltage: 20 kV; four analytes concentrations: 5 μM.

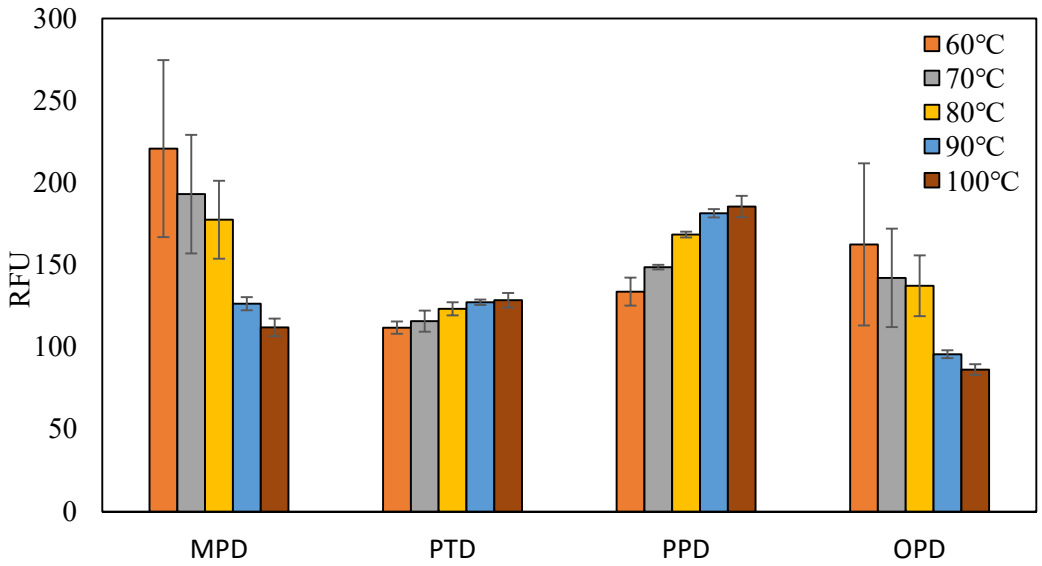

Fig. S3. Fluorescent intensities of four derivatives at different reaction temperatures.

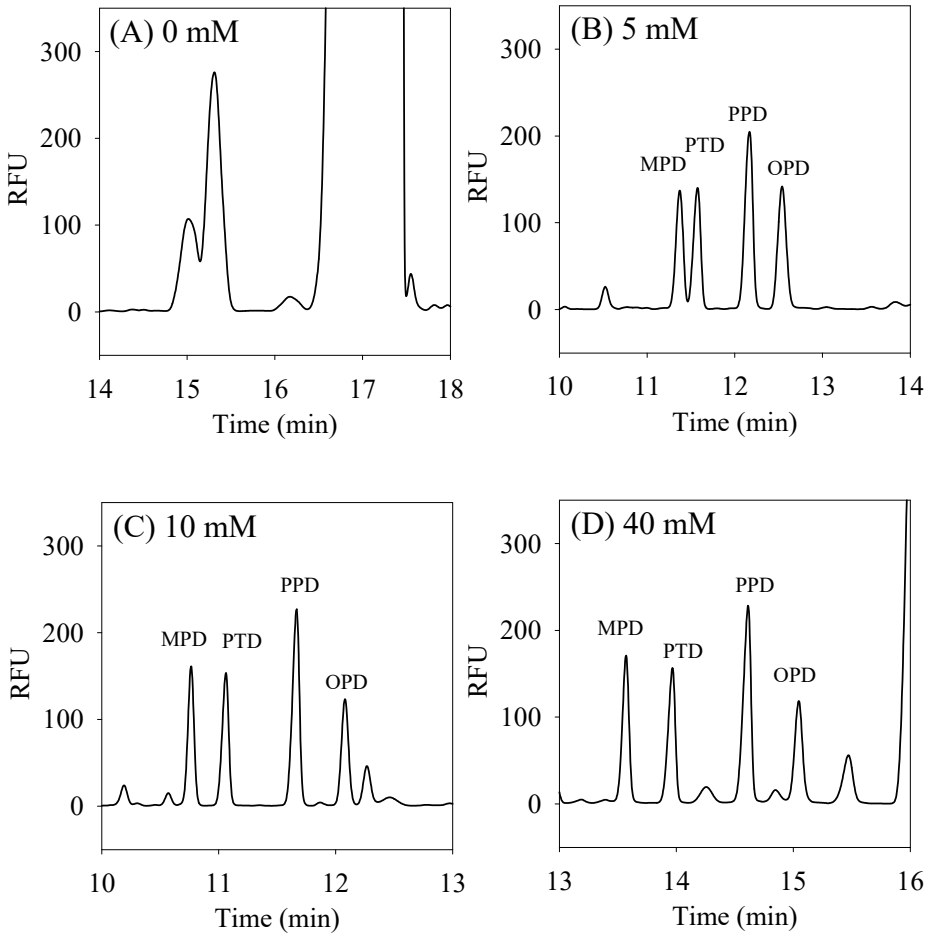

Fig. S4. Electropherograms of different Brij 35 concentrations. (A) 0, (B) 5, (C) 10, (D) 40 mM Brij 35. Separation condition: rinse buffer: 20 mM borate (pH 8) containing Brij 35 and 35% (v/v) methanol; sample injection: 0.5 psi, 15 s; rinse buffer pressure injection: 0.5 psi, 5 s; separation voltage: +20 kV; four analytes concentrations: 5 μM.

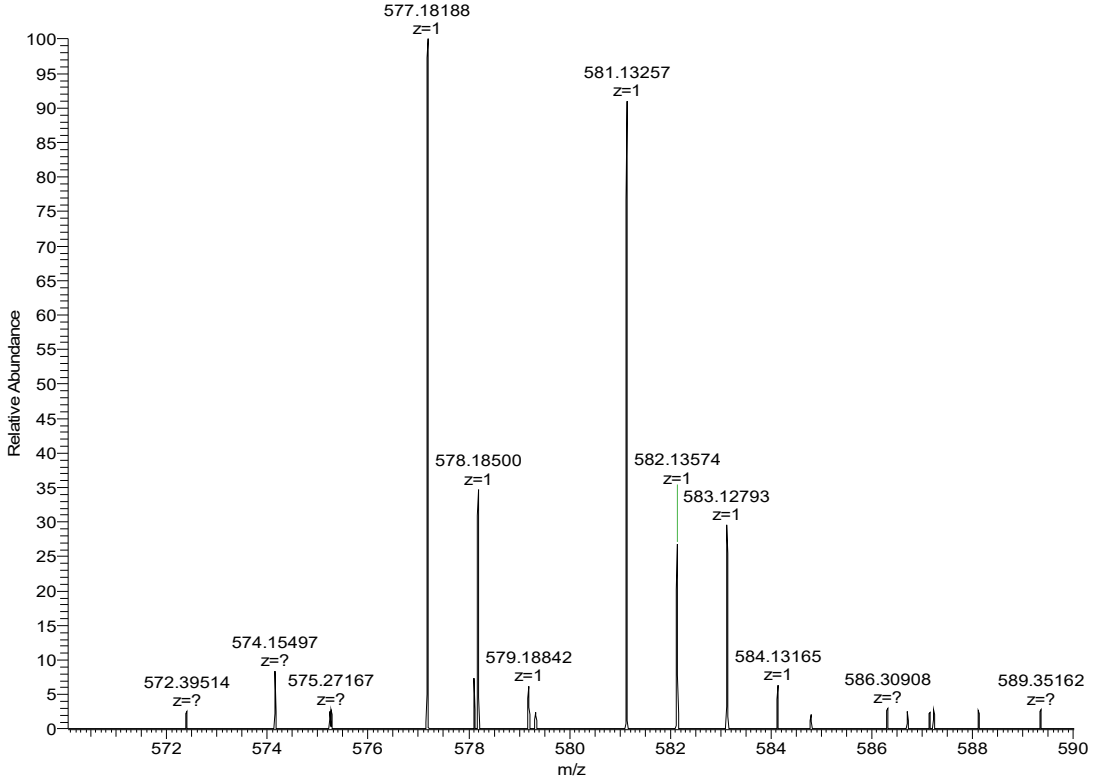

Fig. S5. The MS spectrum of the PTD derivative in dyed hair sample 1. Peak: [M+H$^+$]: PTD derivative (*m/z* 581.133).

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人體研究持續審查報告同意證明書

計畫中文名稱：檢測化粧品成分於人體含量之研究
計畫主持人：陳彥伶
機構名稱：高雄醫學大學/ 香粧品學系
經費來源：科技部
本會編號：KMUHIRB-E(II)-20160162
核准日期(審查通過日)：2018 年 10 月 15 日
計畫執行期間：自 2016/11/23 至 2019/10/31 止

未預期事件或藥品嚴重不良反應通報、後續定期追蹤之程序及應注意事項，請參閱背面。

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第二人體試驗審查委員會
主任委員：陳立宗

西元2018年10月15日

Approval of Clinical Trial/Research

Protocol Title: Determination of cosmetic ingredients in human body
Principal Investigator(s): Chen Yen-Ling
Institution: Kaohsiung Medical University
Source of Funding: Ministry Of Science and Technology
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Approval dated: 2018/10/15
Duration of Approval: from 2016/11/23 to 2019/10/31

See the back of this page for the procedures for reporting unanticipated problems, or drug serious adverse reactions, or interim, and other important notes.

Li-Tzong Chen

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