

Article

GC-MS Analysis with In Situ Derivatization for Managing Toxic Oxidative Hair Dye Ingredients in Hair Products

Geon Park ¹, Won-Young Cho ¹ , Jisu Park ², Yujin Jeong ², Jihwan Kim ¹, Hyo Joon Park ¹, Kyung Hyun Min ^{2,*} and Wonwoong Lee ^{1,*}

¹ College of Pharmacy and Research Institute of Pharmaceutical Sciences, Woosuk University, Wanju 55338, Republic of Korea; ready1838@naver.com (W.-Y.C.)

² School of Pharmacy and Institute of New Drug Development, Jeonbuk National University, Jeonju 54896, Republic of Korea

* Correspondence: khmin1492@jnbu.ac.kr (K.H.M.); wwlee@woosuk.ac.kr (W.L.); Tel.: +82-63-219-5651 (K.H.M.); +82-63-290-1573 (W.L.); Fax: +82-63-219-5638 (K.H.M.); +82-63-290-1560 (W.L.)

Abstract: Hair care products that have oxidative hair dye ingredients have been widely used to permanently change hair color for the characteristic and younger appearance of people and/or their companion animals. In the European Union and the Republic of Korea, these ingredients have been carefully used or prohibited for cosmetic products according to their genotoxic potential. There is a growing demand for reliable quantification methods to monitor oxidative hair dye ingredients in hair care products. However, accurately quantifying oxidative dyes in cosmetic samples is challenging due to their high reactivity and chemical instability under both basic and ambient conditions. For this reason, for the quantification methods, elaborate sample preparation procedures should be accompanied by chemical derivatization to avoid chemical reactions between hair dye ingredients, before instrumental analysis. Therefore, this study utilized a gas chromatography–mass spectrometry (GC-MS) method combined with in situ chemical derivatization to quantify 26 oxidative hair dye ingredients in hair care products. In situ derivatization using acetic anhydride provided the characteristic $[M-CH_2CO]^+$ ions at m/z (M-42), produced by the loss of a ketene from the hair dye ingredient derivatives. These characteristic ions can be used to establish a selective ion monitoring (SIM) mode of GC-MS. The established method was successfully applied to hair dye products ($n = 13$) and hair coloring shampoos ($n = 12$). Most products contained unintended hair dye ingredients including catechol without labeling. It was cautiously speculated that these unintended hair dye ingredients might be caused by biodegradation due to various enzymes in natural product extracts. This study presents a reliable GC-MS method with in situ derivatization to quantify 26 oxidative hair dye ingredients in hair care products, addressing challenges related to their chemical instability. This method is crucial for public health and regulatory compliance.

Keywords: oxidative hair dye ingredients; hair dye products; hair coloring shampoo; in situ derivatization; gas chromatography–mass spectrometry



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1. Introduction

Since ancient times, people have sought to maintain a youthful and attractive appearance, while trendsetters have aimed to create distinctive looks. As a result, numerous cosmetic products have been continuously developed, manufactured, distributed, and used. Among these, hair care products containing oxidative hair dye ingredients (commonly known as permanent hair dyes) have been widely used to achieve long-lasting hair color

changes. These oxidative hair dyes function based on two key components, called first agents (primary intermediates and couplers) and second agents (oxidizing agents). First agents can be further categorized into primary intermediates and couplers, which can form colors and modify reactions, respectively. Second agents play as an alkalizing agent to promote the oxidation of primary intermediates. The interaction between different primary intermediates and couplers produces characteristic hair colors, and the highly reactive functional groups in their chemical structures contribute to their strong reactivity (Figure 1) [1–3].

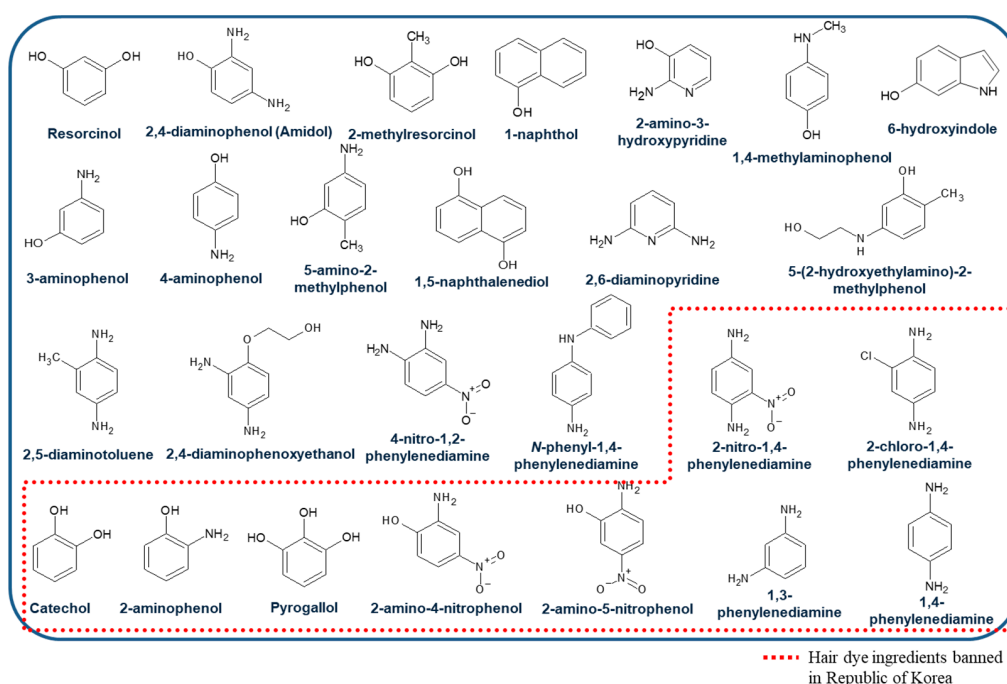


Figure 1. Chemical structures of 26 hair dye ingredients (primary intermediates and couplers).

According to the International Agency for Research on Cancer (IARC), it is well known that some oxidative hair dyes carry mutagenic toxicity [4–7]. The European Commission has banned several oxidative hair dye ingredients (such as 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, catechol, 1,3-phenylenediamine, and 2-aminophenol) for use in hair dye products [8]. Furthermore, in the Republic of Korea, the Ministry of Food and Drug Safety (MFDS) announced that the production of hair dye products (containing 2-aminophenol, 2-aminophenol hemisulfate, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 2-amino-5-nitrophenol sulfate, 1,3-phenylenediamine, 1,3-phenylenediamine hydrochloride, 1,3-phenylenediamine sulfate, 1,4-phenylenediamine, 2-nitro-1,4-phenylenediamine, 2-chloro-1,4-phenylenediamine sulfate, catechol, and pyrogallol) would be prohibited according to their genotoxic potentials [9]. Therefore, the development of analytical tools to accurately and precisely determine hair dyeing agents in various hair care products is essential, since many people have been daily and occupationally exposed to oxidative hair dye ingredients.

Although the selective and sensitive fluorescence determination method was developed to determine 1,3-phenylenediamine in hair dye and water samples [10], chromatographic separation methods have been widely employed to determine hair dye ingredients in hair care products [11,12]. Furthermore, analytical methods combined with mass spectrometry (MS) have been substantially utilized to sensitively and selectively detect hair dyeing agents in various cosmetic products [13–17]. In particular, since liquid chromatography–mass spectrometry (LC-MS) enables the determination of analytes

with minimum sample preparation, the dilute-and-shoot method has been employed as a suitable analytical approach for detecting vulnerable hair dye ingredients in hair care products [18]. Nevertheless, a reliable quantification of hair dye ingredients might be challenging, since most hair dye ingredients are highly unstable and chemically reactive, not only under basic conditions but also under ambient conditions, including storage in a refrigerator or a cold autosampler. To accurately and precisely quantify hair dye ingredients in hair care products, the chemical reaction between hair dye ingredients should be prevented using chemical derivatization combined with a delicate sample preparation process.

This study describes a reliable GC-MS method combined with in situ derivatization to quantify oxidative hair dye ingredients in hair care products, addressing challenges related to their instability and ensuring compliance with cosmetic safety regulations.

2. Experimental

2.1. Chemicals and Materials

Acetic anhydride, hydrochloric acid (HCl), sodium carbonate, 4-nitro-1,2-phenylenediamine, 2-nitro-1,4-phenylenediamine, 2-amino-5-nitrophenol, 5-amino-2-methylphenol 3-aminophenol, 2-aminophenol, 4-aminophenol, 1,3-phenylenediamine, 1,4-phenylenediamine, *N*-phenyl-1,4-phenylenediamine, 4-methylaminophenol, 2,6-diaminopyridine, amidol (2,4-diaminophenol dihydrochloride), 1,5-naphthalenediol, 1-naphthol, resorcinol, 2-methylresorcinol, 1,2,3-trihydroxybenzene (pyrogallol), 2-amino-3-hydroxypyridine, 6-hydroxyindole and isotopically labeled aniline- d_5 (internal standard; IS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 5-(2-Hydroxyethyl)amino-2-methylphenol, 2-amino-4-nitrophenol, 2,4-diaminophenxyethanol, 2,5-diaminotoluene, 2,4-diaminophenol hydrate (amidol), 2-chloro-1,4-phenylenediamine, and catechol were purchased from TCI (Tokyo, Japan). All authentic chemical standards and reagents used in this study were analytical grade or better. Ethyl acetate (EA), *n*-hexane, and methanol (MeOH) were purchased from J. T. Baker (Phillipsburg, NJ, USA). De-ionized water (DW) was obtained using an Evo-CB water purification system (Mirae ST, Anyang-si, Republic of Korea).

2.2. Sample Preparation Including In Situ Derivatization

A total of 26 authentic chemical standards for hair dyes were carefully weighed at 2 mg using an analytical balance (Mettler Toledo, Columbus, OH, USA). The weighed standard chemicals were transferred to 4 mL amber vials and mixed with 2 mL of DW or MeOH (stock solution). The stock solution was serially diluted using DW to appropriate concentrations. The diluted standard solutions were used to optimize sample preparation and instrumental analysis conditions, as well as to evaluate the quantification method.

The collected hair dye samples (500 mg) were weighed using an analytical balance and a micropipette. Each weighed sample was transferred into a 4 mL clear vial, spiked with 10 μ L of an IS solution at a concentration of 10 μ g/mL. To derivatize the hair dye ingredients, 100 μ L of 1 M sodium carbonate buffer and 200 μ L of acetic anhydride were added to the vial, and the mixture was thoroughly mixed. The mixed sample was vigorously vortexed for 1 min, followed by adding 3 mL of EA and another shaking for 3 min. The final mixed sample did not form emulsions and clearly separated into two distinct layers. The supernatant was transferred into a 4 mL vial and dried under a N_2 gas gentle stream. The hair dye derivatives were reconstituted with 100 μ L of EA and transferred into a vial for GC-MS analysis. When the concentration of hair dye ingredients in a sample exceeded the calibration range, the sample was diluted with DW prior to sample preparation to ensure accurate quantification.

2.3. Gas Chromatography–Mass Spectrometry Conditions

In this study, an Agilent 5975C mass spectrometer system connected to an Agilent 6890N gas chromatograph was used in electron ionization (EI) mode at 70 eV (Palo Alto, CA, USA). The pretreated samples were introduced into the injection port at 280 °C using split mode (ratio at 10:1). A chromatographic separation of hair dye derivatives was accomplished by a DB-17ms capillary column (30.0 m length × 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). Highly purified helium gas (99.999%) was used as a carrier gas at a flow rate of 1.0 mL/min. The oven temperature was initially held at 50 °C for 4.0 min, increased to 185 °C with a 30 °C/min rate, and then raised to 300 °C with a 5 °C/min rate, where it was held for 4 min. The temperatures for the MS transfer line and ion source were set at 280 °C and 230 °C, respectively. The hair dye derivatives were detected using scan mode (m/z 50–500) and quantified using selected ion monitoring (SIM) mode. A SIM mode was programmed based on characteristic retention times and fragment ions for hair dye derivatives.

2.4. Method Validation

This method was validated in terms of linearity, detection and quantification limits, precision and accuracy, and stability, and applied to commercially available hair care products. Since a hair dye product sample without oxidative hair dye ingredients was not feasible, all validation results were derived from an investigation using a hair shampoo, free from oxidative hair dye ingredients, to which known amounts of the analytes and the IS were added. The calibration curves for hair dye ingredients were constructed within the corresponding dynamic ranges in five replicates. The limits of detection (LODs) and quantification (LOQs) were investigated by calculating a signal-to-noise ratio at 3 and 10, respectively. The precision and accuracy of the method were evaluated based on intra- and inter-day assays. The intra- and inter-day assays were employed by analyzing samples spiked with standard solution at three concentration levels within the same day and five consecutive days, respectively. To understand the chemical instability of 26 hair dye ingredients, the stability of the method was investigated at 24 and 48 hr, after the standard solutions were made.

3. Results and Discussion

3.1. Optimization of In Situ Derivatization and Extraction Conditions

Since oxidative hair dye ingredients are chemically vulnerable due to their high reactivity, it is difficult to accurately quantify hair dye ingredients in hair care products. Therefore, in this study, an in situ chemical derivatization method was used to prevent the chemical reaction between oxidative hair dye ingredients with highly reactive functional groups in hair care products. Acetic anhydride has not only been widely used to protect thermally unstable hydroxy and amine groups in chemical structures [19–21] but could also be employed in aqueous phases [21,22]. Therefore, in situ derivatization using acetic anhydride was selected to directly apply to various types of hair care products including hair dye products and hair coloring shampoos.

The overall procedure for in situ derivatization was slightly modified based on the previous report [23]. The derivatization procedure was evaluated using representative hair dyes, such as 6-hydroxyindole, 3-aminophenol, 1,5-naphthalenediol, and *m*-phenylenediamine, which were selected on the basis of their chemical structures. Although these representative oxidative hair dye ingredients have multiple functional groups to protect, all targets were derivatized without significant by-products under the optimized derivatization conditions (Figures S1A–D and S2A–D).

To extract hair dye ingredient derivatives from aqueous phase matrices after derivatization, a simple liquid extraction (SLE) was performed. In this study, several conditions for an SLE method were optimized in terms of solvent type, volume, and repetition number of extraction. Different extraction solvents, such as ethyl acetate (EA), *n*-hexane, and dichloromethane, were evaluated using 2 mL volume without repeated extractions. As shown in Figure 2A, when EA was used as an extraction solvent, overall oxidative hair dye ingredient derivatives could be thoroughly extracted from aqueous sample solutions compared to other extraction solvents. Therefore, EA was chosen to extract acetylated hair dye ingredient derivatives from sample solutions due to its moderate polarity and structural compatibility with the acetylated derivatives. Furthermore, solvent volume and the repetition number for extraction were comprehensively investigated to select the optimized extraction conditions. Interestingly, when 3 mL of EA was used without repeated extractions, most of the targeted hair dye derivatives showed higher extraction efficiencies compared to other conditions (Figure 2B). It was speculated that an increase in the extraction solvent and the repetition number might lead to an increase in the loss of volatile hair dye ingredient derivatives during N₂ gas drying. Therefore, in this study, 3 mL of EA was used as an extraction solvent to extract hair dye ingredient derivatives from samples without repetition.

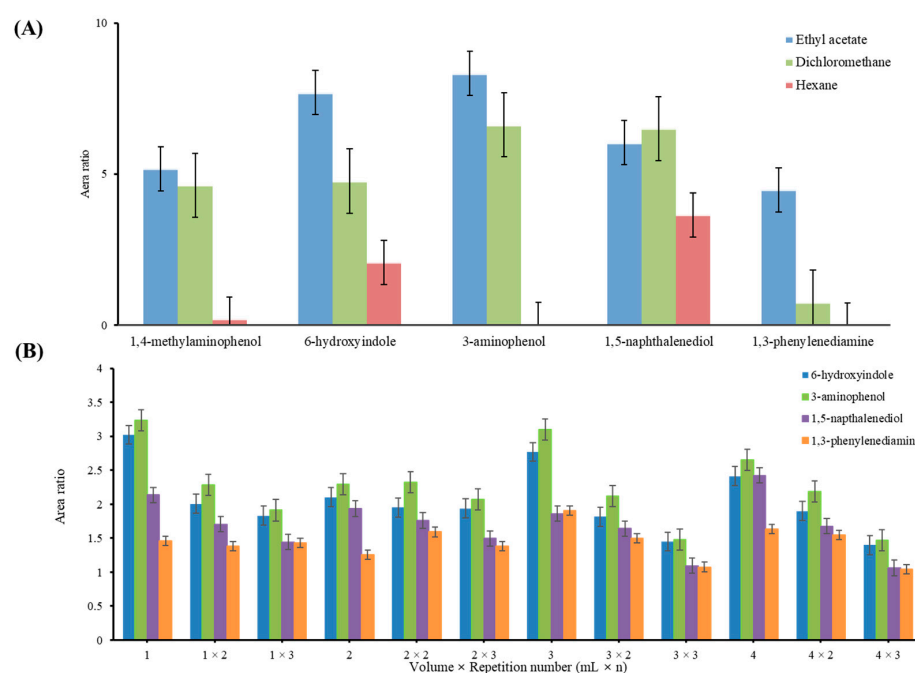


Figure 2. Influences of (A) types and (B) volumes of extraction solvents.

3.2. GC-MS-SIM Conditions

Under the optimized sample preparation conditions, 26 oxidative hair dye ingredients were detected using GC-MS (Figure 3). Overall, oxidative hair dye ingredients were successfully separated under the optimized GC conditions, except for resorcinol, 2,4-diaminophenol, and IS, and 5-(2-hydroxyethyl)amino-2-methylphenol and 2-amino-5-nitrophenol, respectively. Nonetheless, since these co-eluted analytes (resorcinol (m/z 110), 2,4-diaminophenol (m/z 67), and IS (m/z 98), and 5-(2-hydroxyethyl)amino-2-methylphenol (m/z 154) and 2-amino-5-nitrophenol (m/z 136)) provided different and characteristic fragment ions on EI-mass spectra (Figure S3A–E), they could be identified and quantified separately in complex sample matrices. In particular, the EI-mass spectra of hair dye derivatives exhibited the characteristic $[M-CH_2CO]^+$ ions at m/z ($M-42$), which could be

formed by the loss of a ketene from the derivatives. These ions, along with retention time and additional fragment patterns, can be used as reporter ions to identify and confirm the acetyl derivatives of hair dye ingredients. To accurately and precisely quantify hair dye ingredients, a SIM method for individual hair dye ingredients was constructed based on the corresponding characteristic fragment ions and appropriate retention time (RT) windows. Furthermore, the relative ratios between fragment ions of hair dye ingredient derivatives were examined to confirm the analytes during GC-MS analysis. Several low-abundance hair dye ingredient peaks, such as 2,4-diaminophenol, 1-amino-3-hydroxypyridine, pyrogallol, 2,4-diaminophenoxyethanol, and 4-nitro-1,2-phenylenediamine, exhibited high detection limits (Table 1), which might be due to poor derivatization and/or extraction efficiency. SIM conditions for 26 oxidative hair dye ingredients were summarized in Table S1.

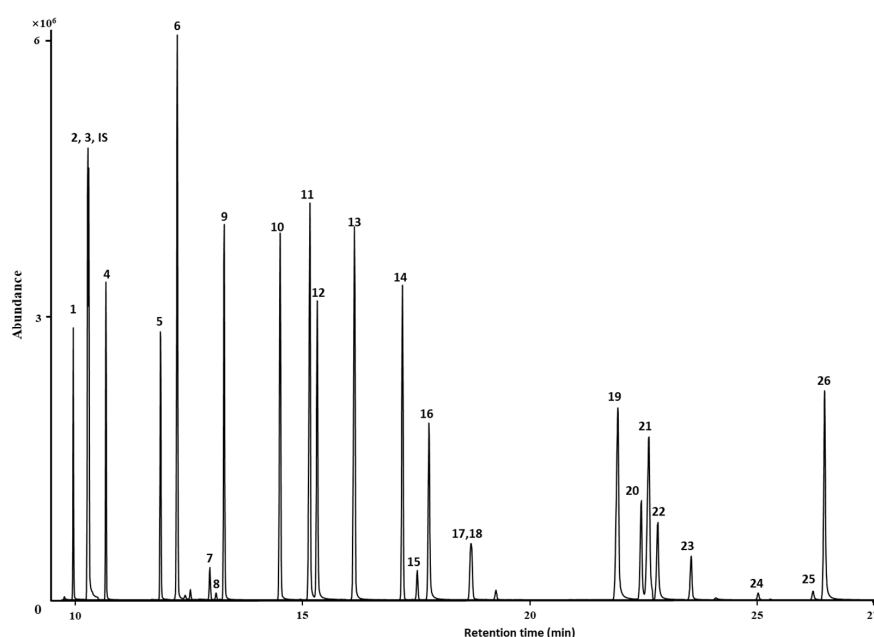


Figure 3. Overlaid selected ion monitoring (SIM) chromatograms for 26 hair dye ingredients and the internal standard using the quantification ions in a standard solution at 10 µg/mL. (Peaks are identified as follows: 1, catechol; 2, resorcinol; 3, 2,4-diaminophenol (amidol); 4, 2-methylresorcinol; 5, 1-naphthol; 6, 2-aminophenol; 7, 2-amino-3-hydroxypyridine; 8, pyrogallol; 9, 4-methylaminophenol; 10, 6-hydroxyindole; 11, 3-aminophenol; 12, 4-aminophenol; 13, 5-amino-2-methylphenol; 14, 1,5-naphthalenediol; 15, 2-amino-4-nitrophenol; 16, 2,6-diaminopyridine; 17, 2-amino-5-nitrophenol; 18, 5-(2-hydroxyethyl)amino-2-methylphenol; 19, 1,3-phenylenediamine; 20, 2-chloro-1,4-phenylenediamine; 21, 1,4-phenylenediamine; 22, 2,5-diaminotoluene; 23, 2-nitro-1,4-phenylenediamine; 24, 2,4-diaminophenoxyethanol; 25, 4-nitro-1,2-phenylenediamine; 26, *N*-phenyl-1,4-phenylenediamine; IS, aniline-*d*₅).

Table 1. Analytical characteristics of the established method for samples.

Analytes	Calibration Range (µg/g)	Linear Regression Equation	r ²	LODs (µg/g)	LOQs (µg/g)
Catechol *	0.5–100	y = 0.00065673x − 0.05655	0.999	0.34	1.01
Resorcinol	0.1–100	y = 0.00064491x + 0.00844	0.999	0.08	0.23
Amidol	5–100	y = 0.00000649x − 0.00247	0.999	2.58	7.75
2-Methylresorcinol	0.5–100	y = 0.00049549x − 0.14419	0.999	0.13	0.40
1-Naphthol	0.1–100	y = 0.00024251x − 0.00283	0.999	0.03	0.09
2-Aminophenol *	0.1–100	y = 0.00050966x − 0.02034	0.999	0.07	0.20
2-Amino-3-hydroxypyridine	0.5–100	y = 0.00035237x − 0.01697	0.999	0.27	0.81
Pyrogallol *	1–100	y = 0.00090479x − 0.55632	0.996	0.40	1.21
4-Methylaminophenol	0.5–100	y = 0.00051693x − 0.02538	0.999	0.13	0.38

Table 1. Cont.

6-Hydroxyindole	0.1–100	$y = 0.00065969x + 0.00183$	0.999	0.03	0.09
3-Aminophenol	0.5–100	$y = 0.00094849x - 0.34823$	0.999	0.06	0.17
4-Aminophenol	0.5–100	$y = 0.00106028x - 0.36194$	0.999	0.21	0.64
5-Amino-2-methylphenol	0.1–100	$y = 0.00114624x - 0.10384$	0.999	0.06	0.18
1,5-Naphthalenediol	0.1–100	$y = 0.00026184x - 0.00438$	0.999	0.02	0.05
2-Amino-4-nitrophenol *	0.5–100	$y = 0.00001830x - 0.00143$	0.999	0.14	0.41
2,6-Diaminopyridine	0.1–100	$y = 0.00016340x - 0.00196$	0.999	0.14	0.42
2-Amino-5-nitrophenol *	0.1–100	$y = 0.00002763x + 0.00227$	0.999	0.16	0.48
5-(2-Hydroxyethyl)amino-2-methylphenol	0.1–100	$y = 0.00049635x - 0.03$	0.996	0.10	0.30
1,3-Phenylenediamine *	0.1–100	$y = 0.00051658x - 0.02990$	0.998	0.12	0.37
2-Chloro-1,4-phenylenediamine *	0.1–100	$y = 0.00023605x - 0.01863$	0.999	0.05	0.15
1,4-Phenylenediamine *	0.1–100	$y = 0.00071032x - 0.02409$	0.999	0.15	0.45
2,5-diaminotoluene	0.1–100	$y = 0.00019091x + 0.00116$	0.999	0.15	0.45
2-Nitro-1,4-phenylenediamine *	0.1–100	$y = 0.00018237x + 0.00036$	0.999	0.24	0.73
2,4-Diaminophenoxyethanol	0.5–100	$y = 0.00006866x - 0.00313$	0.999	0.38	1.14
4-Nitro-1,2-phenylenediamine	1–100	$y = 0.00001374x + 0.00148$	0.999	1.34	4.00
N-Phenyl-1,4-phenylenediamine	0.1–100	$y = 0.00052814x - 0.02535$	0.999	0.05	0.14

* Hair dye ingredients banned in the Republic of Korea.

3.3. Method Validation

In this study, the target oxidative hair dye ingredients were selected based on the list in the Regulations on the Safety Standards, etc., of Cosmetics [9], which necessarily included 12 hair dye ingredients (such as 2-aminophenol, 2-aminophenol hemisulfate, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 2-amino-5-nitrophenol sulfate, 1,3-phenylenediamine, 1,3-phenylenediamine hydrochloride, 1,3-phenylenediamine sulfate, 1,4-phenylenediamine, 2-nitro-1,4-phenylenediamine, 2-chloro-1,4-phenylenediamine sulfate, catechol, and pyrogallol) that are banned by the Korean government. Since overall oxidative hair dye ingredients include various salt forms, a total of 26 oxidative hair dye ingredients were selected based on the desalted forms. The quantification results of the corresponding salt forms for individual oxidative hair dye ingredients were calculated based on the weighted values and calibration curves for desalted hair dye ingredients. To improve accuracy at low concentrations and minimize distortion in quantification results, calibration curves were constructed using a $1/x$ weighting factor. The calibration curves for the hair dye ingredients were established within their linear ranges, demonstrating determination coefficients (r^2) greater than 0.996. The LODs and LOQs were determined within 0.02–2.58 $\mu\text{g/g}$ and 0.05–7.75 $\mu\text{g/g}$, respectively. The intra-day assay results showed that precision and accuracy were in the ranges of 1.16–17.32% and 0.64–13.84%, respectively. The inter-day assay results were in the ranges of 0.64–14.59% for precision and 0.44–14.70% for accuracy. To evaluate chemical stability, underivatized hair dye ingredients were placed at different temperatures under conditions relevant to working environments. Samples were pretreated and analyzed by GC-MS at 24 and 48 h, and the results were compared to those of samples at 0 h. To assess sample losses due to glass vial adsorption and the hydrolysis of acetylated derivatives, the chemical stability of acetanilide was tested using glass vials. As shown in Table S2, no significant sample loss was observed due to the chemical instability of the acetylated derivatives or adsorption to the glass vials. Since most oxidative hair dyes were unstable at all investigated temperature conditions after 1 day, hair care products were not opened until analysis, and all working solutions for hair dye ingredients were made freshly every day. Most of the validation results showed that the analytical method used in this study was reliable for the quantification of 26 oxidative hair dye ingredients in hair care products. The overall validation results are summarized in Table 1 and Tables S3 and S4.

3.4. Application to Hair Care Products

A total of 25 hair care products, including oxidative hair dye products ($n = 13$) and hair coloring shampoos ($n = 12$), which have been widely used to color hair easily and simply by shampooing, were randomly purchased from the Korean domestic market, regardless of the country of manufacture. The collected hair care products were stored at room temperature and were not opened before analysis. Using this method, the quantification

results of 26 hair dye ingredients in hair care products were obtained. Since aniline- d_5 has a phenylamine structure similar to that of most hair dye ingredients and is not present in the sample matrices, it was used as the IS to mitigate sample losses during pretreatment and signal distortions during instrumental detection. Although aniline lacks a hydroxyl group in its structure, aniline- d_5 was found to be relatively suitable as an IS for hair dye ingredients. As shown in Figure 4A–C, the quantification method was successfully applied to hair care products to determine hair dye ingredients without severely distorted or dented peaks or significant interference effects from the complex matrix. A total of 26 hair dye ingredients in hair dye products ($n = 13$) and hair coloring shampoos ($n = 12$) were quantified in % (w/w) levels, considering their corresponding salt forms. However, quantification results obtained with this analytical method could not be compared with exact concentrations of the hair care products, since hair dye ingredient content was not indicated on the hair care product labels. All quantification results for the 26 oxidative hair dye ingredients in hair care products are summarized in Tables S5 and S6 and expressed as $\mu\text{g/g}$.

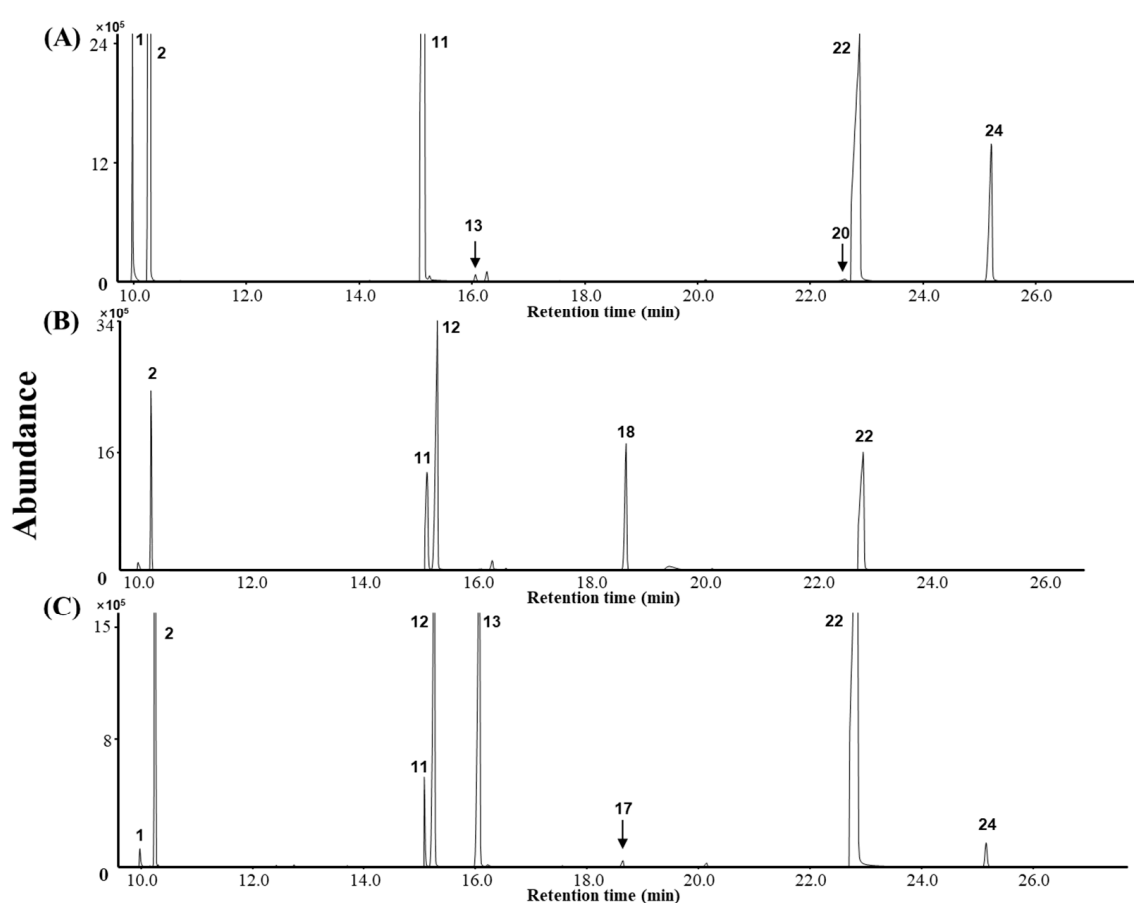


Figure 4. SIM chromatograms of representative hair care products: (A) hair dye product D, (B) hair dye product I, and (C) hair coloring shampoo D.

As shown in Tables S5–S8, out of 14 hair dye products collected, 5 products matched the ingredients listed on the labels, while 9 other products contained the unlabeled ingredients. For example, although a total of 13 hair dye products collected should not contain catechol as an ingredient according to their labels, catechol was observed in 6 products. However, catechol could possibly be coming from other natural ingredients in hair dye products, since catechol can occur naturally in various natural substances [24] and might be degraded from salicylic acid by enzymes in natural product extracts [25]. Furthermore, seven other hair dye ingredients (including 4-aminophenol, 5-amino-2-

methylphenol, 5-(2-hydroxyethyl)amino-2-methylphenol, 1,3-phenylenediamine, 2-chloro-1,4-phenylenediamine, 2-nitro-1,4-phenylenediamine, and 2,4-diaminophenoxyethanol) were detected in seven different hair dye products without ingredient labeling. Nonetheless, most ingredients were observed at very low concentration levels, except for 4-aminophenol and 1,3-phenylenediamine. It was speculated that several ingredients might be mislabeled on hair product labels and/or trace amounts of ingredients could be adulterated due to the mixed production lines. In addition, it seemed that several hair dye products appeared to contain hair dye ingredients in high concentrations above the regulatory limits (Table S9). According to the regulatory limits in the Republic of Korea, most hair dye ingredients are permitted within the range of 0.02–4.5%. Since hair dye products are mixed with the oxidizing agents to be used, all hair dye ingredients in hair dye products were investigated within the prescribed concentration ranges.

Compared to hair dye products, although all hair coloring shampoos contained relatively low concentrations of hair dye ingredients, some hair dye ingredients, such as resorcinol, 4-aminophenol, 2-amino-5-nitrophenol, 1,3-phenylenediamine, 2,5-diaminotoluene, and 4-nitro-1,2-phenylenediamine, were found in high concentrations in some products. It was also cautiously speculated that, similar to the hair dye products, these unintended ingredients might be possibly caused by reasons such as mislabeling and/or mixed production lines. Interestingly, most of the hair care products commonly contained several hair dye ingredients, such as phenylenediamine isomers, aminophenol isomers, and resorcinol. Although some of these ingredients are banned for use in hair care products in the Republic of Korea, most of the hair care products used in this study were collected before the strengthened regulatory limits were released. Since the previously manufactured products can be sold in the Korean domestic market until the specified date (usually 2 years after notification), the banned dyes could be detected.

4. Conclusions

This study utilized a GC-MS-SIM method with in situ derivatization to determine 26 hair dye ingredients in hair care products. The in situ derivatization using acetic anhydride, commonly used for protecting hydroxy and amine groups, was performed to prevent chemical reactions between highly reactive hair dye ingredients, allowing accurate quantification. The derivatization resulted in the characteristic $[M-CH_2CO]^+$ ions at m/z ($M-42$), produced by the loss of ketene. A GC-MS-SIM method was optimized based on specific RT windows and fragment ions for the hair dye derivatives. The established method was successfully applied to commercially available hair dye products ($n = 13$) and hair coloring shampoos ($n = 12$). Although many hair care products contained unintended, unlabeled hair dye ingredients, these were generally detected at very low levels. Although most of the hair care products contained hair dye ingredients banned in the Korean market, it was found that all the products were manufactured before the MFDS notification date. This method provided reliable quantification results of hair dye ingredients in hair care products even at very low concentrations and could be a useful tool to supervise various hair care products containing the toxic hair dye ingredients banned in the Korean and European markets. This study will be helpful in improving cosmetic safety and ensuring public health.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/chemosensors13030094/s1>, Figure S1: Total ion chromatograms for 4 representative hair dye ingredients; (A) 6-hydroxyindole, (B) 3-aminophenol, (C) 1,5-naphthalenediol, (D) 1,3-phenylenediamine. Figure S2: EI-mass spectra for 4 representative hair dye ingredients: (A) 6-hydroxyindole, (B) 3-aminophenol, (C) 1,5-naphthalenediol, (D) 1,3-phenylenediamine. Figure S3: EI-mass spectra for co-eluted hair dye ingredients. Table S1: SIM conditions for 26 hair dye ingredients and IS. Table S2: Chemical stability of ac-

etanilide (*N*-phenylacetamide). Table S3: Intra- and Inter-day assay results of 26 hair dye ingredients. Table S4: Stability test results of 26 hair dye ingredients. Table S5: Quantification results ($\mu\text{g/g}$) of 26 hair dye ingredients in hair dye products ($n = 13$). Table S6: Quantification results ($\mu\text{g/g}$) of 26 hair dye ingredients in hair coloring shampoos ($n = 12$). Table S7: Hair dye ingredients on the labels of hair dye products and detected ingredients. Table S8: Hair dye ingredients on the labels of hair coloring shampoos and detected ingredients. Table S9: The regulatory limits for hair dye ingredients in the Republic of Korea (1 June 2024).

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