RESEARCH ARTICLE

Determination of Paraphenylenediamine in Hair Dyes Formulation Using Visible Spectroscopy

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ABSTRACT

Paraphenylenediamine (PPD), which stands for para-phenylenediamine, is a substance commonly present in hair dyes and is a potent sensitizer and reported to have adverse effects, including skin rashes, allergies, etc. This research study introduces a newly developed and rigorously tested visible spectrophotometric technique for accurately measuring the quantity of PPD in hair dye products. The proposed method relies on a colorimetric reaction between PPD and Folin–Ciocalteu phenol (FC) reagent in the presence of sodium hydroxide (NaOH), which results in a PPD-Folin's reagent complex that is blue in color. To create a standard calibration curve, various concentrations of PPD solutions within the range of 2 to 10 µg/mL, dissolved in 0.1N NaOH, were combined with 1.5 mL of FC reagent solution and scanned at 645 nm. The method was then used to estimate the amount of PPD in three marketed hair dye formulations. The technique demonstrated accuracy and precision, with a detection limit of 0.2445 µg/mL and a quantification limit of 0.7411 µg/mL. The level of PPD observed in three marketed formulations was between 10 to 12.5 µg/mL. During the study, it was observed that the black henna pack contained high concentrations of PPD.

Keywords: Para-phenylenediamine, Hair dye, Colorimetry, Folin–Ciocalteu phenol reagent, Visible spectroscopy, Black Henna.

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INTRODUCTION

Paraphenylenediamine (PPD) is a commonly used ingredient in hair dye formulations available on the market. It is a chemical compound that is used to impart a long-lasting color to the hair by penetrating the hair shaft and reacting with the melanin pigment present in the hair. PPD (Figure 1) is known for its ability to provide dark and intense color shades, which is why it is frequently used in permanent hair dyes.[1-4]

Nevertheless, PPD has been linked to a range of unfavorable responses, including skin irritation, allergic reactions, and in rare instances, anaphylactic shock. Immediate exposure to elevated levels of p-phenylenediamine can lead to serious dermatitis, eye discomfort, excessive tearing, asthma, gastritis, kidney failure, dizziness, trembling, seizures, and even a coma in humans. On the other hand, prolonged exposure in humans may lead to eczematoid contact dermatitis.[5,6]

Hence, it's crucial for individuals using hair dye to conduct a patch test before applying any hair dye product containing PPD in order to detect potential allergic reactions. Additionally, hair dye manufacturers are required to follow strict safety guidelines and label their products clearly to inform consumers about the potential risks associated with PPD.[7]

The literature also reports the determination of PPD in hair dye using spectrophotometric methods.[8-10] Herein, we report a reliable and precise method for the estimation of PPD in marketed hair dye formulations using visible spectroscopy.

The present method development is based on the colorimetric reaction of PPD (reducing agent) with Folin–Ciocalteu phenol (FC) reagent in the presence of sodium hydroxide, which results in a PPD-FC reagent complex that is blue in color. The blue color deepens as PPD concentration is raised while FC reagent concentration is held constant.

The FC reagent consists of phosphotungstic acid and phosphomolybdic acid. Under basic pH conditions, this reagent can undergo reduction by reducing agents such as phenolic and amino groups, resulting in the formation of a blue color that can be quantified using spectrophotometry. According to the literature, the Folin–Ciocalteu (F-C) reagent is employed in antioxidant assays based on electron transfer, which assesses the reducing capacity of an antioxidant. Additionally, it finds utility in determining the overall phenol/polyphenol content in plant-derived foods and biological samples. Moreover, the FC reagent is well-suited for quantifying total proteins, albumin, and globulin in human blood serum, measuring fibrinogen levels in blood plasma, or detecting gastric mucoprotein.[11-13]

The present approach involved mixing different concentrations of PPD solution with a set quantity and concentration of FC reagent solution. This mixture was then

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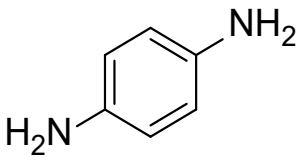

Figure 1: Structure of paraphenylenediamine

scanned at 645 nm and the quantitative analysis was carried out using visible spectroscopy.

MATERIALS AND METHODS

All the chemicals and reagents viz p-PPD, Folin–Ciocalteu phenol reagent (FC Reagent), distilled water (DW), sodium hydroxide (NaOH) of analytical grade were used for the experiment. Marketed Hair dye formulations were purchased from the local market. Single beam UV spectrophotometer (Aligent carry 60uv-vis, model GS2281), Ultrasonic Bath (Singen, Germany), and Digital weighing machine (Shimadzu model TX323L) were used for spectrophotometric analysis.

Method Development for Estimation of Paraphenylenediamine as API

Preparation of standard stock solution

To create the standard stock solution, precisely measured 10 mg of PPD was placed into a 100 mL volumetric flask. It was then dissolved in 20 mL of 0.1 N NaOH and subjected to sonication for 10 minutes. The volume was subsequently adjusted with the same solvent up to the mark, resulting in a final concentration of 100 µg/mL.

Preparation of FC reagent

For the FC Reagent preparation, 3 mL of FC reagent was combined with 3 mL of 0.1 N NaOH and 24 mL of distilled water.

Selection of wavelength

To determine the appropriate wavelength, 1-mL of the standard stock solution of PPD was transferred into a 10 mL volumetric flask. To this, 1.5 mL of FC reagent that had been diluted and made up to the mark with 0.1N NaOH was added, resulting in a concentration of 10 µg/mL. The resulting solution was subjected to UV scanning within the range of 800 to 400 nm, revealing that PPD exhibited its maximum absorbance (Figure 2) at 645 nm.

Method Validation for Estimation of Paraphenylenediamine As API

The validation process of this method encompassed assessments for linearity, accuracy, precision, and repeatability.

Linearity study

Different aliquots of PPD in the range 0.2–1.0 mL were transferred into a series of 10 mL volumetric flasks. To each solution, 1.5 mL of FC reagent that had been diluted and made up to the mark with 0.1N NaOH to get concentrations 2, 4, 6, 8, and 10 µg/mL, respectively. The solutions were scanned

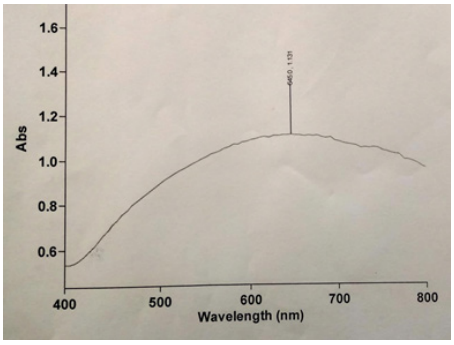

Figure 2: Visible spectrum of PPD[14]

Table 1: Linearity study of PPD[15]

Conc. (µg/mL)	*Absorbance*	*Standard deviation (SD)*	*%RSD*
2	0.2376	0.00020	0.0876
4	0.4521	0.00030	0.0675
6	0.6663	0.00026	0.0397
8	0.8944	0.00020	0.0232
10	1.1008	0.00026	0.0240
			Avg.%RSD– 0.0484

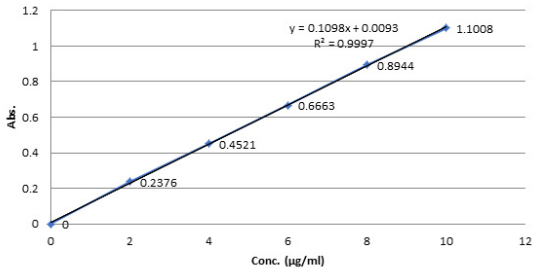

Figure 3: Calibration curve of PPD[16]

Table 2: Accuracy study of PPD[17]

S. No.	*level*	*Standard solution (mL)*	*Added Solution (mL)*	*Abs.*	*Mean*	*%Recovery*
1	80%	1.0	0.8	1.9858	1.9862	100.05
		1.0	0.8	1.9861		
		1.0	0.8	1.9867		
2	100%	1.0	1.0	2.1929	2.1934	98.91
		1.0	1.0	2.1935		
		1.0	1.0	2.1938		
3	120%	1.0	1.2	2.4218	2.4224	99.81
		1.0	1.2	2.4223		
		1.0	1.2	2.4231		

Table 3: Intraday study of PPD[18]

Conc. (µg/mL)	*Absorbance*	*Intra day*
4	0.4521	0.4489
8	0.8944	0.8444
10	1.1008	1.0056

Table 4: Repeatability study.[19]

S. No.	*Absorbance*	*Conc. (µg/mL)*	*Mean (µg/mL)*
1	0.6660	5.98	6.065
2	0.6756	6.06	
3	0.6841	6.14	
4	0.6822	6.12	
5	0.6770	6.08	
6	0.6697	6.01	

Table 5: Study on the marketed hair dye formulation.

S. No.	*Marketed Hair Dye Formulation (F)*	*Abs*	*Mean*	*SD*	*%RSD*	*PPD found (µg/mL)*
1	F 1	1.3690	1.3633	0.005118	0.3753	12.39
		1.3618				
		1.3591				
2	F 2	1.1324	1.1321	0.0003	0.0269	10.26
		1.1322				
		1.1318				
3	F 3	1.2799	1.2810	0.0012	0.0937	11.58
		1.2823				
		1.2810				

Table 6: Summary of the results

Parameters	*Result*
Wavelength	645 nm
Linearity Range	2–10 µg/mL
Linear Regression Equation	y = 0.1098x + 0.0093
Slope	0.1098
Standard Error	0.0081
% Relative Standard Deviation (RSD)	0.0484
Limit of Detection (LoD)	0.2445 µg/mL
Limit of Quantification (LoQ)	0.7411 µg/mL

on a spectrophotometer in the visible range 400 to 800 nm (Table 1). The spectrum was recorded at 645 nm. The calibration plot was constructed as concentration *vs*. absorbance (Figure 3).

Accuracy

In pre-analyzed sample solutions, known quantities of the standard stock solution were added at different levels, specifically 80, 100, and 120% (Table 2). The solutions were subsequently reanalyzed using the proposed method.

Precision

The precision of the method was evaluated in terms of intra-day and inter-day variations. Intraday precision involved analyzing 4, 8, and 10 µg/mL PPD solutions three times within the same day (Table 3).

Repeatability

Repeatability was assessed by analyzing six different samples with a concentration of 6 µg/mL of PPD solution (Table 4).

Sensitivity

Following the ICH guidelines, the limits of detection (LoD) and quantification (LoQ) were calculated as LoD = 3.3σ/S and LoQ = 10σ / S, where σ represents the standard deviation of the response and S is the slope of the calibration curve. The calculated values were LoD = 0.2445 µg/mL and LoQ = 0.7411 µg/mL when a standard error was taken 0.0081 and slope was 0.1098.[20-22]

Application of the Proposed Method for Pharmaceutical Formulation

For the analysis of PPD in a commercial formulation, 100 mg of hair dye was placed in a 100 mL volumetric flask and filled to the mark with 0.1 N NaOH. After filtration, 1-mL of the solution was transferred to a 10 mL volumetric flask, and 1.5 mL of FC reagent was added. The volume was then adjusted to the mark with 0.1 N NaOH, and the solution was analyzed using a spectrophotometer in the UV range from 800 to 400 nm, with measurements recorded at 645 nm. The drug concentrations were calculated using a linear regression equation (Table 5).

RESULTS AND DISCUSSION

The result of the developed method is shown in Table 6. Thus a visible spectroscopy-based method for estimating PPD in hair dye formulations was developed and validated according to ICHQ2 (R1) guidelines.

CONCLUSION

In summary, a visible spectroscopy-based technique has been created and validated for assessing para-PPD levels in hair dye products. This method involves a colorimetric reaction between PPD and FC reagent, resulting in the formation of a blue PPD-FC reagent complex. The method exhibited both accuracy and precision, with a detection threshold of 0.2 µg/mL. It was successfully employed to quantify PPD in various commercially available hair dye formulations, including those containing henna. Notably, it unveiled significantly elevated PPD concentrations in black henna products, thereby heightening the potential for sensitization among consumers. Consequently, this approach represents a dependable and cost-effective means of ascertaining PPD content within hair dye formulations.

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