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SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF PARA PHENYLENEDIAMINE IN HAIR DYES

Nayaka Raghavendra Babu*[1], Syeda Sana Mahveen[1], Yenumula Padmavathi[1], Ananddas Ramesh Priyadharshini[1], Pola Ravi Kumar[1], Ancha Divya[1], Anil Kumar Tallam[2]

[1]*Department of Pharmaceutical analysis, G. Pulla Reddy College of Pharmacy, Hyderabad, India.
[2]KVK Tech, Newtown, Pennsylvania, United States of America

*Corresponding author e-mail: nayakaraghavendrababu@gmail.com

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ABSTRACT:
A new visible spectrophotometric method was developed for the determination of paraphenylenediamine (PPD) in pure and marketed forms using β-naphthol as chromogenic reagent. This is based on reaction of PPD with β-naphthol in acidic media to yield reddish-pink coloured chromogen exhibiting absorption maximum at 552.4 nm. Beer's law is obeyed in the concentration range of 200-1000 ng/mL with coefficient of determination as 0.9976. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 64.2 ng/mL and 194.8 ng/mL respectively. The developed method has been validated as per the ICH Q2 (R1) guidelines. The results demonstrate that the method is linear, precise and accurate. The proposed method was successfully applied for determination of PPD in different brands of hair dyes with good recovery and reproducibility.

Key words: β-naphthol, paraphenylenediamine, spectrophotometric determination.

1. INTRODUCTION:

Hair colouring [1-6] also known as hair dyeing is the most commonly employed process of changing the hair colour. The main purpose to include the habit of this cosmetic is to cover gray or white hair and to change to such colour which will be considered as more stylish or trendy and finally to give back the natural hair colour which has been bleached by hairdressing practices or sun bleaching. It classifies hair dyes into permanent, demi-permanent, semi-permanent, and temporary [7-15]. The three hair dye brands used in this research are as follows: Bigen, Black Rose and Indica.

The p-Phenylenediamine (PPD) is an organic compound chemically known as benzene-1,4-diamine. Its empirical formula is $C_6H_4(NH_2)_2$ representing molecular weight of 108.144 g/mol. It is a derivative of aniline and appears as a white solid, but samples can darken due to air oxidation. Soluble in water, slightly soluble in alcohol, ether, chloroform and benzene and it has melting point of 142 ˚C. PPD is mainly used as dye intermediate or chemical intermediate. Literature review reveals that there are various HPLC [16], GC-MS [17] and spectrophotometric methods [18-25] have been developed for the estimation of PPD in hair dyes. While spectrophotometric methods are the most widely used instrumental methods of predilection in industrial laboratories, a very few chemometric methods with 3-amino phenol, α-naphthol are reported but there are no chemometric methods using β-naphthol as reagent. Therefore, the need for a fast, low-cost and selective method is obvious especially for routine quality control analysis

of hair dyes containing PPD. Now a days no one are using special convectional reagents such as β-naphthol as chromogenic agent because chemometric methods have lack of sensitivity. The present study was planned to develop a sensitive method using β-naphthol as chromogenic reagent.

2. MATERIALS AND METHODS

Chemicals and Reagents: PPD(pure form) was supplied from Sd Fine Chemicals ltd (SDFCL, Mumbai). Different samples of hair dye (Black rose, Bigen, Indica) were purchased from local market. β-naphthol, sodium nitrite and ammonium sulphamate (analytical reagent grade) were obtained from Sd Fine Chemicals Ltd (SDFCL, Mumbai). Concentrated hydrochloric acid (HCl) was obtained from Finar chemicals (FC) Ltd.

Instruments: UV-visible spectrophotometer (Shimadzu UV 1800), Digital balance (Shimadzu BL220H). Ultrasonic bath sonicator (PCI Analytics 6.5li200H), Hot Air Oven (Tempo Equipment Private Limited).

Extraction of hair dye: Transfer accurately weighed quantity about 5 g of hair dye to round bottom flask. To this 60 mL of chloroform was added, connect to the reflex condenser. After completion of 5 h of extraction, transfer the solution to 250 mL beaker; rinse the flask with 3-4 mL of chloroform. To this 1 mL of acetic anhydride was added with continuous stirring, wait for 40 min. Heat it on the water bath until the solution evaporates to 25 mL. Filter the solution with vacuum filter. Dry the extract by keeping it in oven at 120 ˚C for 15 min. Remove and weigh it, calculate the percentage yield.

Percentage yield = $M_1 \times 0.5626 / M_2 \times 100$

where, M_1= Practical yield in grams, M_2= Weight of sample taken

Qualitative test for amines

Diazotisation test: Dissolve a little amount of sample in concentrated HCl and water. To this 10% sodium nitrite was added upon shaking, separation of reddish-brown solution indicates the presence of 1˚, 2˚, 3˚amine. To a little amount of above solution, cold solution of 2-naphthol in excess of 10% sodium hydroxide was added. Red dye indicates the presence of 1˚ aromatic amines.

Carbylamines test: To the small amount of sample add chloroform and alcoholic potassium hydroxide. Offensive smell indicates the presence of 1˚amines.

Preparation of β-naphthol (1%w/v): Dissolve 100 mg of β-naphthol in 10 mL of distilled water.

Preparation of 0.1% sodium nitrite: Dissolve 100 mg of sodium nitrite in 100 mL of distilled water.

Preparation of stock solution: Standard PPD, 10 mg was weighed and transferred to 10 mL volumetric flask and dissolved in water. The flask was shaken and was made up to the mark with water to give a solution of 1000 µg/mL. From this stock solution, 1mL was pipette out into another 10 mL volumetric flask and the volume was made up to 10mL with water to give 100 µg/mL. From this stock solution, 1 mL was pipetted out into another 10 mL volumetric flask and the volume was made up to 10 mL with water to give 10 µg/mL.

Preparation of 0.1% ammonium *sulphamate:* Dissolve 100 mg of ammonium sulphamate in 100 mL of distilled *water*.

Preparation of 0.1N HCl: Dissolve 0.85 mL of concentrated HCl in 100 mL of distilled water.

Calibration curve for PPD with β-naphthol (200-1000 ng/mL): From 10 µg/mL stock, aliquots of 0.2, 0.4, 0.6, 0.8 and 1.0 mL were

taken in 10 mL test tubes to which 1 mL of sodium nitrite (0.1%w/v) was added. This reaction was carried out by keeping the flask in an ice tray to maintain (0-5 ˚C) temperature for 5 min.

To this, 1 mL of 0.1N HCl, 1 mL of ammonium sulphamate (0.1%w/v) and then 1 mL of β-naphthol (0.04%)was added and wait for 5 min for colour development i.e. reddish-pink (whole procedure was carried out in an ice bath). Volume was made up to 10 mL with distilled water to give a solution of 200, 400, 600, 800 and 1000 ng/mL respectively. The absorbance of each solution was measured against blank (water) taken in visible region i.e., 400-700nm which showed a maximum absorbance at 552.4 nm. The method developed was validated according to the ICH guidelines.

3. RESULTS AND DISCUSSION

Linearity and range: The linearity of analytical method is ability to elicit test results that are directly proportional to the concentration of analyte in the sample within the range. The range of analytical method is the interval between upper and lower levels of analyte that have been demonstrated within a suitable level of precision, linearity and accuracy.

Under the optimum conditions, the calibration curve for the determination of PPD by its reaction with β-naphthol was constructed by plotting the absorbance as a function of corresponding concentrations are200, 400, 600, 800, 1000 ng/mL. The results are reported in **Table 1 and Fig.1**.

Limit of detection and limit of quantification: The sensitivity of proposed method for measurement of PPD was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were determined according to the ICH guidelines for the validation of analytical procedure. The following formula were used

$$LOD=3.3\sigma/S$$
$$LOQ=10\sigma/S$$

where, σ = standard deviation (SD) of the response (intercept) S = slope of the calibration curve.

The calculated values of LOD and LOQ were found to be 64.2 and 194.8 ng/mL respectively

Table 1: Calibration curve data

S. No	Concentration (ng/mL)	Absorbance
1.	200	0.229
2.	400	0.408
3.	600	0.555
4.	800	0.686
5.	1000	0.838

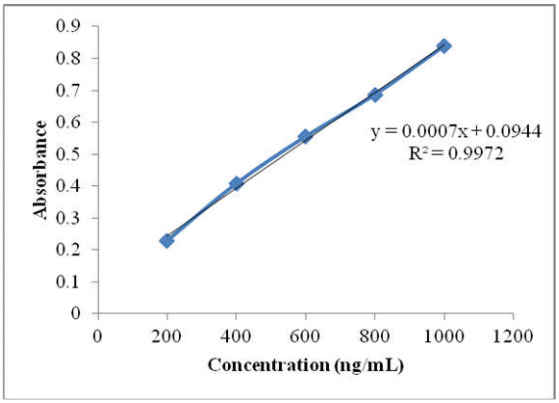

Fig.1 Calibration curve for PPD at 552.4 nm with β–naphthol

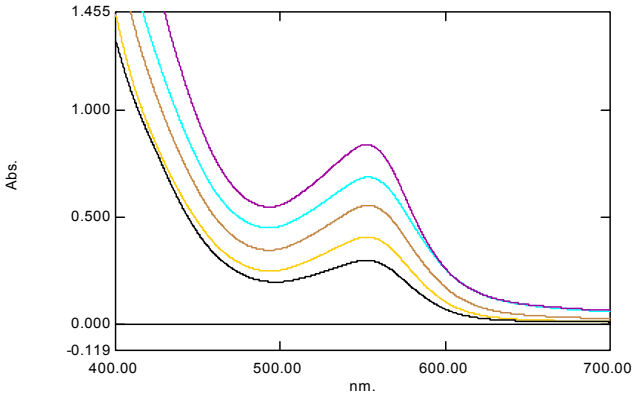

Fig.2 Overlay Spectra for PPD at 552.4 nm with β–naphthol

Precision: The precision of an analytical method is the degree of agreement among individual test results, when the method is

Table 2: Repeatability data of PPD at 552.4 nm using β –naphthol

Concentration (ng/mL)	Absorbance	Mean* ± SD	%RSD
600	0.556	0.554±0.001	0.32
600	0.555		
600	0.555		
600	0.553		
600	0.554		
600	0.551		

*Average of six determinations

applied repeatedly to multiple samplings of homogeneous sample. It provides an indication of random errors results and expressed as relative standard deviation (%RSD).

recorded at 552.4 nm. The %RSD was calculated. The obtained results were reported in **Table 2**.

Intraday and inter day precision: The intra-assay, inter-assay precision of the proposed method was determined on samples of drug solutions at varying concentration levels (480 ng/mL, 600 ng/mL, and 720 ng/mL) by analysing three replicates of each sample as a batch in a single assay run (single day)and in three consecutive days at 552.4 nm. The %RSD was calculated, and results were reported **in Table 3**.

Table 3: Intra-day precision data of concentration 480, 600 and 720 ng/ml (black rose, bigen, indica) brand using at β -naphthol 552.4 nm

Brands	Concn (ng/mL)	Absorbance			Mean* ± SD	%RSD	Absorbance			Mean* ± SD	%RSD
		Morning	AN	Evening			Day - 1	Day-2	Day-3		
Bigen	480	0.374	0.366	0.366	0.368±0.004	1.25	0.464	0.463	0.456	0.461±0.003	0.69
	600	0.487	0.486	0.483	0.485±0.002	0.42	0.524	0.520	0.519	0.521±0.002	0.50
	720	0.529	0.528	0.521	0.526±0.004	0.82	0.608	0.602	0.595	0.601±0.006	1.08
Black Rose	480	0.386	0.382	0.377	0..381±0.004	1.18	0.508	0.496	0.495	0.499±0.007	1.44
	600	0.555	0.546	0.545	0.587±0.005	1.00	0.543	0.552	0.546	0.547±0.045	0.83
	720	0.605	0.603	0.600	0.602±0.002	0.41	0.666	0.661	0.650	0.659±0.008	1.24
Indica	480	0.430	0.427	0.424	0.427±0.003	0.70	0.434	0.451	0.440	0.441±0.008	1.95
	600	0.586	0.581	0.579	0.582±0.004	0.61	0.529	0.520	0.515	0.521±0.007	1.36
	720	0.638	0.634	0.633	0.638±0.002	0.31	0.686	0.672	0.689	0.682±0.009	1.32

*Average of three determinations

Table 4: Accuracy & Assay data of PPD at 552.4 nm using β–naphthol

Brand	Accuracy					Assay		
	% Levels	Amount of sample added (mL)	Amount of standard added (ng/mL)	Mean absorbance	% Recovery ± SD	Label claim (mg)	Amount found (mg)	%Purity
Bigen	80	0.48	0.6	0.883	104.3±0.007	300	333	111
	100	0.6	0.6	1.000	108.7±0.004			
	120	0.72	0.6	1.035	101.7±0.004			
Black Rose	80	0.48	0.6	0.790	92.0 ±0.006	300	327	109
	100	0.6	0.6	0.851	90.7±0.002			
	120	0.72	0.6	0.963	93.9±0.004			
Indica	80	0.48	0.6	0.867	102.2±0.005	150	157	105
	100	0.6	0.6	0.907	96.61±0.004			
	120	0.72	0.6	1.032	101.2±0.017			

Repeatability: Repeatability assessment of an analytical method is performed by analysing six replicates of single concentration that is 600 ng/mL. Absorbance of samples were

Accuracy: Accuracy is the closeness of test results obtained by the method to the true value. The recovery was assessed by determining the agreement between the

measured standard concentration and added known concentration to the sample. The test was done by spiking the exacted dye powder with pure PPD at three different levels (80%, 100% and 120%) was calculated and reported in **Table 4**.

Assay: From 10 µg/mL stock, 0.6 mL was taken and transferred to 10 mL volumetric flask. It is spiked with required amount of standard pure dye solution. To this, 1 mL of 0.1N HCl and 0.1% of sodium nitrite , 0.1% 1 mL of ammonium sulphamate and then 1 mL of β-naphthol (0.04%)was added and wait for 5 min for colour development i.e. reddish-pink (whole procedure was carried out in an ice bath). Volume was made up to 10 mL with distilled water to give required concentration. The absorbance of a solution was measured against reagent blank (water) taken in visible region i.e., 400-700 nm which showed a maximum absorbance at 552.4 nm. The obtained results are given in **Table 5**. The results obtained for percentage purity are within the acceptable limit as per I.P.

haemolysis inhibition at 400mg concentration, which was significant at $p<0.005$.

Colour stability: The colour stability of PPD was checked in UV-Visible spectrophotometer. The solution was scanned at 552.4 nm in the duration of 0 to 30 min. The colour of 600 ng/mL solution of PPD was found to be stable for 30 min.

4. CONCLUSION

A new simple visible spectrophotometric method was developed using β-naphthol as chromogenic reagent for estimation of PPD in different brands of hair dyes. The developed method has been validated as per ICH guidelines and the validation parameters was found to be within the acceptance criteria. The proposed method was found to be economic, novel, simple, sensitive, accurate, precise and reproducible; it can be used for analysis of Paraphenylenediamine in pure and different brands of marketed hair dyes.

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6. CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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