

UV –Visible Spectroscopy Method Development and Its Validation for the Analysis of Marketed Hair Dyes for Amine Content

K. Bhavyasri¹, K. AmukthaMalyada², M. Sumakanth³

¹Department of Pharmaceutical Analysis RBVRR Women's College of Pharmacy
Hyderabad, INDIA
Email: bhavya.khagga [AT] gmail.com

²Department of Pharmaceutical Analysis , RBVRR Women's College of Pharmacy
Hyderabad, INDIA
Email: amukthamalyada2910 [AT] gmail.com

³Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy
Hyderabad, INDIA
Email: Suma.mogili [AT] gmail.com

ABSTRACT---- To develop a simple rapid, accurate and spectrophotometric reproducible method were developed for estimation of PARA - PHENYLENE DIAMINE in different Marketed Hair dyes . The analysis of PPD was performed using NaOH solution as diluent and using folins reagent at 432nm respectively .The methods were linear in the concentration range from 0-50µg/ml. The methods were validated with respect to system suitability, linearity, precision, limit of detection, limit of quantification, accuracy, ruggedness and robustness . The developed method can be used for routine analysis of PARA PHENYLENE DIAMINE in marketed hair products. The methods were validated in accordance to the ICH guidelines.

Keywords---- ParaPhenylenediamine , folins reagent Spectrophotometric method

1. INTRODUCTION

Paraphenylenediamine (PPD) is a permanent hair dye it is chemical substance that is widely used . PPD is used in hair dye because that gives a natural look, and even after shampooing the hair dyed will not loose its colour .PPD hair dyes leads to cancer and mutagenicity. Apart from that, PPD also causes skin irritation and many such related allergies . Limit of PPD in hair dyes is 6%. The initiation of allergic reactions are by oxidation of PPD on the surface and within the skin .

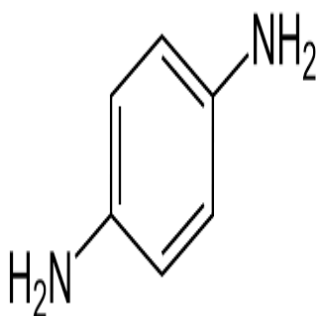


Figure 1 : Chemical structure of Para phenylenediamine

Natural black hair colour is due to melanin clusters dispersed within the colourless keratin-based cortex of the hair. Melanin is responsible for the colour determination of hair. White hair is due to age as we get older pigment cells in the hair die. Even though PPD has several disadvantages people are crazy about putting dyes on their hair as beauty has more significance in day to day life. this is because of the attraction in a high definition way and also it is the practice of changing hair colours. but before that skin sensitivity tests are important. Hair dyes and colours are substances that contain hundreds of chemicals that are combined to bring out the desired properties in the product. Many products brands do not contain PPD or PPD free, ammonia free that doesn't mean the hair is completely free from chemicals and also still cause allergic reactions. Ammonia and PPD free hair dyes contain Emollient oils, walnut oils, argan oil so help the texture to remain fine and the cuticles do not loose their moisture while dyeing .

1.1 MECHANISM OF ACTION OF HAIR DYES:

Hair shaft is the outer layer which is covered by Cuticle and Keratin cells , protein for the inner layers of hairs. Cortex contain more Keratin cells which helps to provide proteins to Melanin. Melanin gives Natural black colour . In a hair dye it generally consist of developer , primary intermediates , couplers .

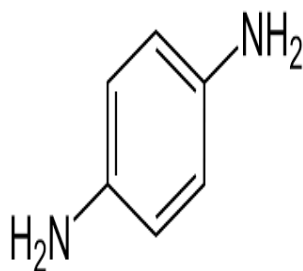
Developer : hydrogen peroxide

Primary intermediates : PPD

Couplers : Ammonia

Solution hair dye consist of Ph 11 which will help to expand or swell the hair shaft and thereby it leads to separation of keratin cells and thereby the color sieves into the cortex . Here the ammonia opens the cuticle for color to enter the cortex the swollen molecules join together and remain in the cortex making it impossible to escape and thereby oxidation of colour occurs on the hair and colour is produced

1.2 Structure of PPD :



Molecular formula : C₆H₄(NH₂)₂

Molecular weight : 108.4

2. MATERIALS AND METHOD

2.1 Chemicals: Para-Phenylenediamine , NaOH , Distilled water , Folins reagent

2.2 Instruments: ELICO SL 210 double beam UV-Visible Spectrophotometer, glass cuvettes, analytical weighing balance , Sonicator were used .

3. METHOD DEVELOPMENT

3.1 Preparation of standard stock solution:

10ml volumetric flask was taken in that 10mg of Pure PPD was dissolve with diluent NaOH and make up to the mark which gives the concentration of 1000µg/ml.

3.1.1 Working standard solution preparation:

For preparation of 100µg/ml standard solution, pipette out 1ml from standard stock solution and transfer it to 10ml volumetric flask and make up to the mark with 0.1N NaOH. From this 100ug/ml pipette out 1ml and transfer into 10ml volumetric flask .And then pipette out 1ml of FC and 1ml of 0.1N NaOH and makeup the volume upto the mark with distilled water

3.2 Preparation of 0.1N NaOH:

0.4gms of NaOH is dissolved 100ml of distilled water and and transferred to 100ml volumetric flask

3.3 Preparation of FC Solution:

Take 1ml of FC reagent and transfer it into 10ml volumetric flask and to this add 1ml of 0.1N NaOH and add distilled water upto the mark .

3.4 Determination of wavelength of maximum absorption:

10ppm standard solution was prepared by taking 10ml volumetric flask and pipette 1ml of 100µg/ml of standard solution to this add 1ml NaOH, 1ml FC reagent and make up the volume upto the mark by distilled water and check the absorbance under UV Spectroscopy within the range 400-800nm using the diluent as blank. The maximum absorption were found at 432nm.

4. VALIDATION

ICHQ2(R1) guidelines was followed for analytical method validation. The following are the validation parameters performed for PPD.

4.1 Linearity:

Linearity of analytical procedure is defined as concentration of analyte in sample is directly proportional to obtained test result. A linear relationship should be developed across the range of analytical procedure. Linear standard solutions were prepared from the working standard solutions. From the working standard solution, serial dilutions were made to get 0-50ppm were prepared and absorbance was measured at 432nm using NaOH as diluent and as blank and the calibration curve is plotted.

4.2 Precision:

It is determined by keeping the same homogeneous sample for at least six times and noting the absorbance at lambda max. The consistency of homogenous sample. Then calculating the %RSD.

For performing precision, 50ppm standard solution of PPD was selected. The absorbance of 50ppm solution was checked at 432nm and this is repeated for 6 times and all 6 absorbance's were noted. The formula for calculating %RSD was given below.

$$\%RSD = (\text{standard deviation of the measurement} / \text{mean value of measurement}) * 100$$

4.3 Accuracy:

It is also Known as trueness. Accuracy is done by comparing the obtained test results with that of true value.

The accuracy of the proposed method was tested by recovery studies at 100%, 200%, and 300% by adding a known amount of pure drug to the pre-analyzed formulation of concentration 10µg/ml. The accuracy was determined by spiking standard solution to sample solution at three concentrations i.e., 100µg/ml, 200µg/ml, 300µg/ml. Standard concentrations equal to 100, 200, 300 percent is added to sample. 2ml of 200ppm sample was spiked to 2ml of 100ppm standard solution, 2ml of 200ppm of sample was spiked to 200ppm of standard solution, 2ml of 200ppm sample solution was spiked to 2ml of 300ppm of standard solution. At 432nm, absorbance was checked for three times. The below formula is used to calculate % Recovery.

$$\%Recovery = (\text{Amount found} / \text{Amount added}) \times 100$$

4.4 Robustness

Robustness of analytical procedure is minute changes in method are done to see the stability of the method. Robustness is performed by measuring the absorbance at 431, 432, 433nm i.e., ±1nm from the lambda max.

4.5 Ruggedness:

The results obtained by analysis of sample under different conditions must be reproducible. Different conditions may be different analyst, different instrument, different days etc.

In our research we did robustness studies were done by two different analysts.

4.6 Limit of Detection:

The analyte in sample that can be detected that is too less to quantify but can be detected. The formula for calculating LOD is given below:

$$\text{Limit of Detection} = 3.3 \cdot \sigma / S$$

where

σ = standard deviation

S = slope

4.7 Limit of Quantification:

The amount of analyte in the sample that can be just quantified. The formula for calculating LOQ is given below:

$$\text{Limit of Quantification} = 10 \cdot \sigma / S$$

where,

σ = standard deviation

S = slope

5. RESULTS AND DISCUSSION

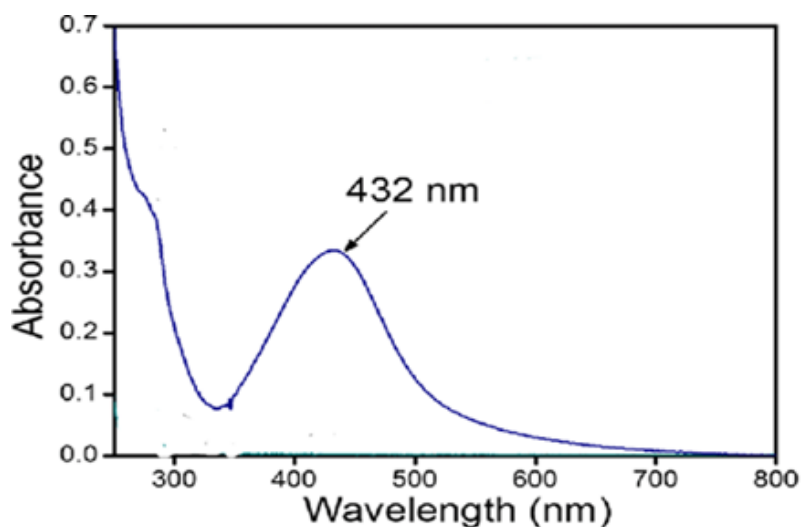


FIGURE:1 λ_{max} of NaOH

Table 1: Absorbance values for Calibration curve of PPD

Concentration	Absorbance
0ppm	0
2ppm	0.038
4ppm	0.128
6ppm	0.242
8ppm	0.346
10ppm	0.465
20ppm	0.586
25ppm	0.724
30ppm	0.823
35ppm	0.943
40ppm	1.032
50ppm	1.162

5.1 Linearity :

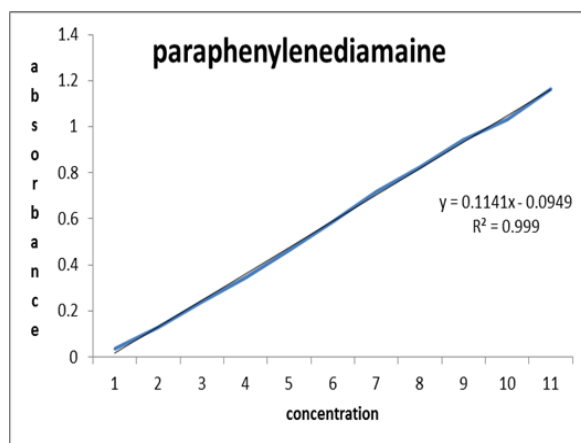


Figure 2: Calibration curve of PPD

5.2 Precision:

Table 2: Results of Precision

Concentration	Absorbance(x)
50	1.243
50	1.244
50	1.236
50	1.246
50	1.254
50	1.224
Average	1.241167
Standard deviation	0.010206
RSD%	0.822308

5.3 Accuracy:

Table 3: Results of Accuracy

% LEVEL	ABSORBANCE	% RECOVERY	MEAN % RECOVERY
100% (100ppm+200ppm)	1.004	97%	97%
	1.004	97%	
	1.024	98.9%	
200% (200ppm+200ppm)	1.1508	99.5%	99.5%
	1.1499	99.5%	
	1.1548	99.9%	
300% (300ppm+200ppm)	1.382	99.2%	98.5%
	1.3724	98.5%	
	1.3899	99.7%	

5.4 Robustness:

Table 4: Results of Robustness

Concentration	431nm	432nm	453nm
50ppm	1.2553	1.2422	1.2305
50ppm	1.2675	1.248	1.2415
50ppm	1.275	1.2542	1.2463
50ppm	1.2798	1.2461	1.2364
50ppm	1.2668	1.2542	1.2358
50ppm	1.2818	1.2242	1.2564
Mean	1.271033	1.244817	1.241483
SD	0.009864	0.011133	0.009881
%RSD	0.77603	0.89385	0.795884

5.5 Ruggedness:

Table 5: Results of Ruggedness

Concentration	Analyst-1	Analyst-2
50	1.2675	1.2422
50	1.2553	1.248
50	1.275	1.2542
50	1.2818	1.2461
50	1.2668	1.2242
50	1.2798	1.2542
Average	1.271033	1.244817
Standard deviation	0.009864	0.011133
RSD%	0.77603	0.89385

Table 6: Summary of Results

Parameters	PPD
Linearity range	0-50ppm
Slope	0.1141
Standard Deviation	0.010206
%RSD	0.822308
LOD	0.2951µg/ml
LOQ	0.8944µg/ml

6. CONCLUSION

A simple method has been developed for estimation of PPD in hair dyes. A method has been developed and validated according to ICHQ2(R1) guidelines. All the validation parameters have been performed and all the parameters were found to be within the limits.

7. ACKNOWLEDGEMENT

I want to acknowledge our beloved principal Prof. M. Sumakanth and Faculty of Department of Pharmaceutical Analysis for giving me the opportunity to perform the research work.

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