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METHOD DEVELOPMENT AND VALIDATION OF PARA-PHENYLENEDIAMINE IN PURE AND MARKETED DYES BY USING RP-HPLC

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Keywords:

HPLC, Para-Phenylenediamine, Validation, Method development

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ABSTRACT: To estimate the presence of para-phenylenediamine in various commercial hair colors, a straightforward, speedy, precise investigation used High-Performance Liquid Chromatography to find PPD in henna and determine its presence. In order to do the chromatography, stationary phase Phenomenox C18 100 mm x 4.6 mm, 5µm column system was used for UV detection with a242 nm wavelength. The mobile phase Methanol with water (HPLC grade) (70:30) was used as the mobile phase for the technique, which was created and validated for the detection of PPD in samples of commercial hair color. With a run time of 7 minutes, a retention time of 2.088 seconds, and a flow rate of 0.55 ml/min, the method was validated over a broad linear range of 5-28 g/ml. The PPD content of hair colors may not exceed 6%. The procedures were approved in line with ICH guidelines.

INTRODUCTION: Paraphenylenediamine (PPD) is a chemical substance that is widely used as a permanent hair dye. PPD is used in hair dye because it is a permanent dye that gives a natural look, and the dyed hair can also be shampooed or permed without losing its color [1-5]. PPD-containing hair dyes have been associated with cancer and mutagenicity. Apart from that, PPD has potential toxicity which includes acute toxicity such as allergic contact dermatitis and subacute toxicity. The limit of PPD in hair dyes is 6% [6-10]. The oxidation of PPD on the surface and within the skin is responsible for the initiation of allergic reactions [11-14].

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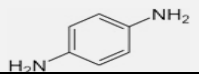
TABLE 1: DRUG PROFILE

Drug	Para phenylenediamine
Solubility	Highly soluble in NaOH, Poorly soluble in water Sparingly soluble in ACN Soluble in methanol
Molecular Formula	$C_6H_4(NH_2)_2$
IUPAC name	Benzene-1,4-diamine, paraphenylenediamine 1,4-Diaminobenzene 1,4-Phenylenediamine
Pka	2.97
Structure	

Instruments and Materials used: PPD pure drug was obtained as a gift sample from the pharmaceutical industry, in India. Marketed hair dyes from the local market. HPLC-grade Methanol and HPLC-grade water were used. Prior to the introduction of the mobile phase into the HPLC system, it was sonicated with a LABOTECH Sonicator (LMUC-2, L8486), filtered using vacuum filtration, and adjusted pH was confirmed

with an ELICO Ph meter. For the determination of tolvaptan by the chromatographic method, a SHIMADZU brand HPLC system with a binary pump (LC20AD) was used, the column was a SUNSILC18, the sample was injected with a Rheodyne injector, followed by a UV detector and dem Equipped with the system is installed with soft ware called LCSOLUTIONS for data interpretation.

METHOD DEVELOPMENT:

Preparation of Standard Solution: Weighing 10 mg of pure para phenylene diamine in a volumetric flask 10 ml, dissolving it in a small amount of HPLC grade methanol, and adding more methanol until the desired 1000 g/ml was obtained. Pipette 0.1 milliliters of 1000 g/ml into a 10 ml volumetric flask with the solution. Until the necessary concentration of 100 g/ml is attained, add solvent.

Determination of λ_{max}: The correct wavelength selection affects the sensitivity of methods that use V-Visible Spectrophotometry detectors. For drug detection, the ideal wavelength is one that offers the highest absorbance and the best response. The standard 10µg/ml solution of Paraphenylene diamine was scanned from 200-400nm to get the λ_{max}. It shows maximum absorbance at 243nm.

TABLE 2: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

System	ShimadzuLC-20AD
Column	Phenomeno x C18 100mm x 4.6mm, 5µ.
Mobile phase	Methanol: Water (HPLC grade) (70:30)
Injection volume	20.0µL
Retention time	3.4
Detector	242nm
Flow rate	0.5ml/min
Runtime	15min

Validation Parameters:

Specificity: Examining interference in the procedure that was optimized. At the retention durations of these medications using this method, we shouldn't observe interference peaks in the placebo or blank groups. This approach was therefore said to be precise. The blank solutions were injected to accomplish the specificity.

System Suitability: The area for each of the five injections of the standard solutions was measured in the HPLC. It was discovered indicating that the area of five replicate injections % RSD fell within the predefined range.

Linearity: The ability of an analytical procedure is determined by its capacity to generate test results that are both within allowable bounds and directly proportional to the analyte concentration in the sample.

Preparation of 3-28 µg/ml Serial Dilution: From the 100 g/ml working standard solution, pipette 2.5, 5.0, 7.5, 1, 2, 25, 1, 50, 1.80, 2.40, 2.80 g/ml. These quantities should be transferred into a series of 10 ml volumetric flasks and diluted with HPLC grade methanol to achieve concentrations of 0.30, 4, 0.5, 0.7, 0.9, 1, 1.2, 1.5, 1.8, 2.4, and 2.8 g/ml. Plotting the calibration curve which was created by using concentrations on the X-axis and peak areas on the Y-axis allowed for the determination of the R2 value. By injecting PPD standard stock solution in the range of 3, 5, 7, 9, 10, 12, 15, 18, 24, 28 g/ml, linearity was tested, and the response was recorded.

Precision: The precision of an analytical method is defined as the amount of scatter between a series of measurements made with several samples of the same homogenous material under prescribed conditions. Precision was assessed using six replicates of a 10 g/ml concentration that were injected into an HPLC system. The percentage RSD was computed.

Accuracy: Recovery experiments evaluated the proposed method's accuracy. Three concentration levels of the standard solution of para phenylene diamine (50, 100, and 150% by volume) were spiked into the sample solution. Each concentration level had three replicates made, which were then injected into the HPLC machine and the result was recorded. Calculated was the recovery percentage.

Preparation of Standard Solution: Pipette portions of 0.5, 1.0, and 1.5 g/ml were transferred into a series of 10 ml volumetric flasks from the 100 g/ml working standard solution and diluted with HPLC grade methanol to produce concentrations of 5, 10, and 15 g/ml.

Preparation of 15µg/ml Sample Stock Solution: The sample PPD solution was created by combining 10 mg of PPD with 1000 g/ml of

HPLC-grade methanol in a volumetric flask having a capacity of 10 ml. Pipette 15 g/ml of HPLC grade methanol into a 10 ml volumetric flask after adding 1.5 ml of a 1000 g/ml solution.

Spiking Procedure: A 15 µg/ml sample solution of para phenylene diamine was added to 2 ml of the solution's mixture before being injected into an HPLC system. The system with 2 ml of a 10 g/ml standard solution spiked to 2 ml of a 15 g/ml sample solution of para phenylene diamine. After being blended, the solution was injected into an HPLC system with 2 ml of a 15 g/ml standard solution spiked to 2 ml of a 15 g/ml para phenylene diamine sample solution. To obtain concentrations at 80%, 100%, and 150% levels.

Robustness: An indication of an analytical procedure's reliability under ordinary usage is provided by its robustness, which assesses its ability to be unaffected by tiny but intentional changes in method parameters. In order to test the robustness of the approach, three replicates of a 15 g/ml paraphenylene diamine solution were injected under chromatographic conditions at a flow rate and mobile phase ratio of 0.5–0.1 ml/min and different mobile phase concentration ratios. We got chromatograms and determined %RSD.

LOD & LOQ: The lowest concentration of analyte in a sample that can be detected but not always precisely measured is the detection limit of a particular analytical procedure. On the other hand, the LOQ is the smallest amount of substance that can be precisely and accurately quantified.

Assay:

Preparation of Para phenylenediamine Sample Stock Solution (1000 µg/ml): Para-Phenylene diamine in order to achieve 1000 g/ml of PPD, 10 mg was dissolved in a 10 ml volumetric flask, and the remaining volume was filled with HPLC-grade methanol.

Preparation of Sample Working Solutions (15µg/ml): In order to obtain 15 g/ml of para-phenylenediamine, pipette 1.5 ml of the aforementioned sample stock solution into a 10 ml volumetric flask. Then, add diluent to the necessary volume.

Assay = (Sample Area / Standard Area) × 100% of the assessment

RESULTS AND DISCUSSION:

Specificity:

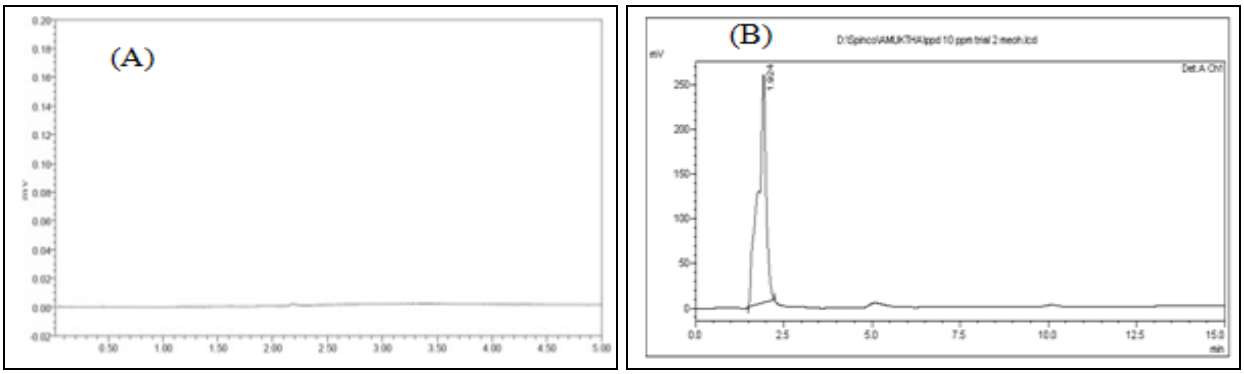


FIG. 1: (A) CHROMATOGRAM OF BLANK AND (B) CHROMATOGRAM OF STANDARD

Linearity:

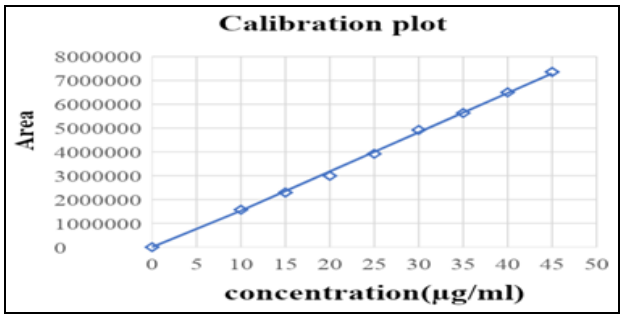


FIG. 2: CALIBRATION CURVE OF PPD

Precision:

TABLE 3: INTER-DAY PRECISION

Injections	Area	Ret. time	T. plate count	Tailing factor
1	1569030	2.456	2039.468	1.026
2	1569035	2.435	2042.298	1.043
3	1569033	2.428	2057.968	0.998
4	1569038	2.446	2056.985	0.976
5	1569040	2.459	2089.836	1.032
6	1569043	2.484	2075.547	1.063

TABLE 4: INTRA-DAY PRECISION

Injections	Area	Ret.time	T.platecount	Tailingfactor
1	1569142	2.479	2056.882	1.099
2	1569146	2.457	2097.556	0.988
3	1569148	2.489	2047.325	0.967
4	1569153	2.482	2082.425	0.943
5	1569152	2.496	2093.389	0.987
6	1569147	2.473	2073.786	0.956
	1569148			
SD	4.049691346			
%RSD	0.00025808%			

Accuracy:

TABLE 5: ACCURACY DATA

Concentration level (%)	Standard Conc. (µg/ml)	Spiked sample amount (µg/ml)	% Recovery	Mean% Recovery
	5µg/ml	15µg/ml	98.4%	
50%	5µg/ml	15µg/ml	99.6%	94.1%
	5µg/ml	15µg/ml	99.9%	
	10µg/ml	15µg/ml	99.3%	
100%	10µg/ml	15µg/ml	99.6%	96.6%
	10µg/ml	15µg/ml	98.5%	
	15µg/ml	15µg/ml	98.5%	
150%	15µg/ml	15µg/ml	99.7%	99.8%
	15µg/ml	15µg/ml	99.0%	

Robustness:

TABLE 6: ROBUSTNESS DATA OF CHROMATOGRAPHIC METHOD (0.55ML)

Injections	Area	Ret. time	T. plate count	Tailing factor
1	1559842	2.775	2356.676	1.042
2	1559739	2.773	2378.432	1.064
3	1559652	2.778	2401.332	1.053
	1559744.3			
SD	95.11221443			
%RSD	0.0060979%			

TABLE 7: ROBUSTNESS DATA OF CHROMATOGRAPHIC METHOD (0.6ML)

Injections	Area	Ret. time	T. plate count	Tailing factor
1	1548693	2.292	2041.289	0.983
2	1549548	2.289	2049.436	0.992
3	1546975	2.296	2039.928	0.989
	1548405.3			
SD	1310.3993			
%RSD	0.0846289%			

LOD & LOQ:

TABLE 8: LOD & LOQ DATA

Drug name	LOD	LOQ
PPD	0.000095646µg/ml	0.00028983µg/Ml

Assay: 15 µg/ml of standard and 15µg/ml of sample solutions were injected separately and the amount of para-phenylenediamine present in the marketed hair dye sample was determined.

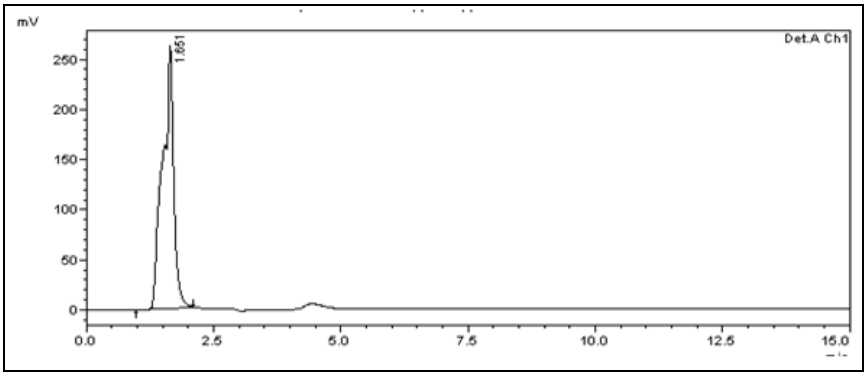


FIG. 3: CHROMATOGRAM OF SAMPLE

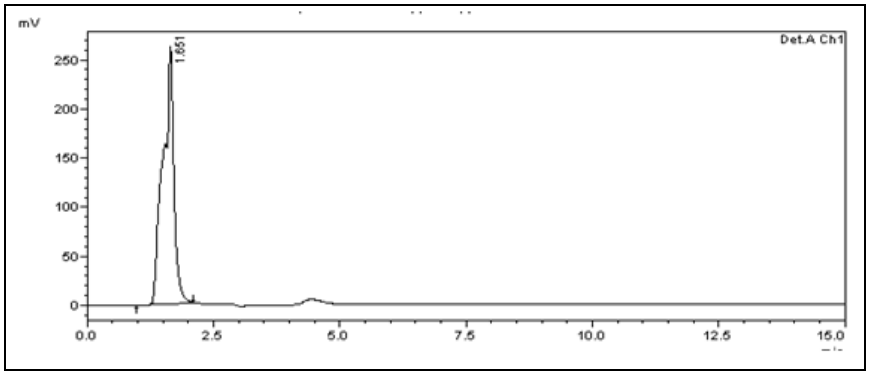


FIG. 4: CHROMATOGRAM OF STANDARD

CONCLUSION: The proposed RP-HPLC method was found to be simple, specific, precise, accurate, rapid, and economical for the estimation of PPD in Marketed products. The developed method was validated statistically according to ICH guidelines. The sample recovery in the formulation was in good agreement with their respective label claims. This method was found to be better than previously reported methods. Hence the above method can be used in quality control for routine analysis of tablets of PPD.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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