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A RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF PARA-PHENYLENEDIAMINE IN PURE FORM AND IN MARKETED PRODUCTS

Research Article

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ABSTRACT:

A rapid, precise, accurate, specific and sensitive reverse phase liquid chromatographic me
been developed for the estimation of Para-phenylene diamine in pure form and in marketed
The chromatographic method was standardized using a Octadecyl Saline C18 column
mm,5μm particle size) with UV detection at 245 nm and flow rate of 1 ml/min. The mobi
consisting of a mixture of Acetate buffer and Acetonitrile in the ratio of (50: 50 v/v) was sele
proposed method was validated for its sensitivity, linearity, accuracy and precision. The reter
for valsartan was 2.6 min. The % recovery was within the range between 98% to 1
percentage RSD for precision and accuracy of the method was found to be less than 2
method can be employed for routine quality control analysis of Para-Phenylenediamine in
and in marketed products.

KEYWORDS: Para-phenylene diamine, Estimation, Validation and RP-HPLC.

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INTRODUCTION:

P-Phenylenediamine (PPD) is an organic compound with the formula C_6H_4 $(NH_2)_2$. This derivative of aniline is a white solid, but samples can darken due to air oxidation. It is mainly used as a component of engineering polymers and composites. It is also an ingredient in hair dyes. P-Phenylenediamine is chemically 1, 4-Diaminobenzene (Fig. 1). PPD is soluble in water i.e., 10% at 400C, 87% at 1070C, 100% at 1400C. Molecular weight of PPD is 108.40g/mol, which is white crystalline solid, darkens upon exposure to air. PPD is used as precursor to aramid plastics and fibers such as Kevlar. These applications exploit PPD's difunctionality, i.e. the presence of two amines which allow the molecules to be strong together. This polymer arises from the reaction of PPD and terephthaloyl chloride. The reaction of PPD with phosgene gives the di-isocyanate, a precursor to urethane polymers. Also used as common hair dye and rubber antioxidant.

EXPERIMENTAL:

Chromatographic conditions:

The analysis of the drug was carried out on a Shimadzu SPD 20 A HPLC system equipped with a reverse phase Octadecyl Silane C18 column (250x4.6 mm, 5 µm), a 515 binary pump, a 20 µl injection loop, PDA detector 2996 and running on LC solutions software.

Buffer preparation:

Accurately weigh and transfer about 0.192g of ammonium acetate into 100ml purified water. Adjust pH to 5.6± 0.05 with glacial acetic acid solution.

Mobile phase preparation:

Mobile phase –A (50%): Prepare a filtered (through 0.45µm membrane filter) and degassed mixture of 10ml acetate buffer and 40ml of water as Mobile phase –A.

Mobile phase-B (50%): Prepare a filtered (through 0.45µm membrane filter) and degassed mixture of Acetonitrile as mobile phase B.

Diluent:

Prepare a filtered (through 0.45µm membrane filter) and degassed solvent of Acetonitrile as a diluent.

Sample preparations:

Standard stock solution preparation:

An accurately weighed quantity of 100 mg of Para-Phenylenediamine (PPD) was transferred into a 100 ml volumetric flask. 60 ml of Acetonitrile is added and sonicated for 20 minutes. Then made up to required volume with diluents to get the accurate volume of standard stock solution.

Working standard solution preparation:

From this, a working standard solution of the drug (10µg/ml) was prepared by diluting 1 ml of the above solution to 100 ml in a volumetric flask with the diluent.

Preparation of Sample Solution:

Accurately weighed quantity of sample powder equivalent to100mg of Para-Phenylenediamine (PPD) and transferred into a 100 ml volumetric flask containing 60 ml of diluent. The solution was sonicated for 20 min and shake for 10 min. The above solution was diluted to 100 ml with diluents. Take 10ml of the solution and centrifuge at 7500rpm for 15mins.The supernant liquid were filtered through 0.45µ m membrane filter.0.1 ml of above filtrate was taken into 10 ml volumetric flask and made up to the volume with diluent to get concentration of 10µg/ml. From these, the required concentration was prepared according to the method.

Procedure:

Take 1ml of working standard solution in 10ml volumetric flask and make up the solution with the diluent. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 ml/min. The detection of the drug was monitored at 245 nm. The run time was set at 5 min. Under these optimized chromatographic conditions the retention time obtained for the PPD was 2.64 min. A typical chromatogram of PPD is given in Fig 2.

Validation of the proposed method:

Validation of analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the intended analytical application. The method was validated for specificity, linearity, precision, accuracy, LOD, LOQ, ruggedness and robustness.

1. Specificity:

The specificity of the method can be defined as the ability to measure accurately the concentration of an analyte in the presence of all other sample materials. It is evaluated by analyzing the sample solution spiked with the heating to the appropriate levels. Results are furnished in Table-1.

2. Linearity:

Standard plots were constructed with five concentrations in the range of 1-5 µg/ to test linearity. The peak area of PPD was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. Results are furnished in Table-2.

3. Precision:

The precision of the assay was studied with respect both system and method precision.

A) System precision:

It was calculated from five replicate injections of freshly prepared PPD test solution in the same equipment at a same concentration. Peak area of PPD was determined and precision was reported as % RSD and the results are furnished in Table 3.

B) Method precision:

It was calculated from six replicate injections of freshly prepared PPD test solution in the same equipment at a same concentration. Peak area of PPD was determined and precision was reported as % RSD and the results are furnished in Table 4.

4. Accuracy:

The accuracy of the HPLC method was assessed by taking accurate sample stock solution at various concentrations ranging from 50% to 150% in triplicate form for each concentration. The results are furnished in table 5

5. Limit of detection (LOD):

Limit detection of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value. Limit of detection corresponds to the concentration that will give a signal-to-noise ratio of 3:1

$LOD = 3.3\sigma / S$

The **LOD** value was found to be **2.09µg/ml.**

6. Limit of quantification (LOQ):

Limit quantification of an individual analytical procedure is the lowest amount of analyte in a sample that can be determined quantitatively. Limit of quantification corresponds to the concentration that will give a signal-to-noise ratio of 10:1

$LOQ = 10\ \sigma/S$

The **LOQ** value was found to be **6.35µg/ml.**

7. Robustness:

Measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides indication of its reliability during its normal usage. This is to verify that the method performance is not effected by typical changes in normal experiments. The results are furnished in table 6 - 9.

A) Changing of mobile phase ratio
B) Changing of flow rate.

8. Ruggedness:

Ruggedness is the degree of reproducibility of test results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Working sample solution was prepared and injected on another instrument. Solutions were injected as per the test method. The results are furnished in table 10.

ASSAY PROCEDURE:

Separately Standard and Test preparation was injected into liquid chromatogram and the areas for major peaks were recorded by using the following formula. The results are furnished in table 11.

RESULTS AND DISCUSSION:

An accurate, simple, and reproducible RP-HPLC method was developed and Validated as per ICH guidelines for the estimation of Para-Phenylenediamine. Quantitative estimation of Para-Phenylenediamine was estimated by RP-HPLC method using A- 10ml acetate buffer (pH 5.6) & 40ml of water and B- Acetonitrile in the ratio of 50:50v/v as a mobile phase and column ODS C18 as a stationary phase and the peak was observed at 245nm which was selected as a wavelength for quantitative estimation.

CONCLUSION:

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Para-Phenylenediamine (PPD) and can be reliably adopted for routine quality control analysis of Para-Phenylenediamine in pure form and in marketed products.

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Fig 1: Structure of PPD

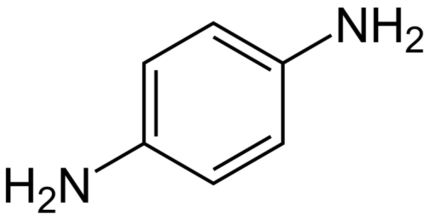

Fig 2: Chromatogram of Para-Phenylenediamine

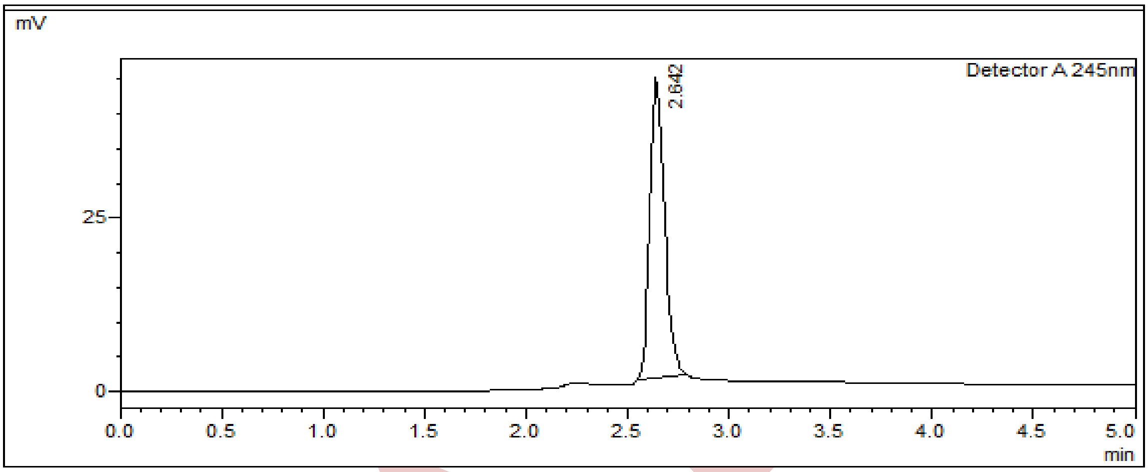

Fig no 3 chromatogram for method A

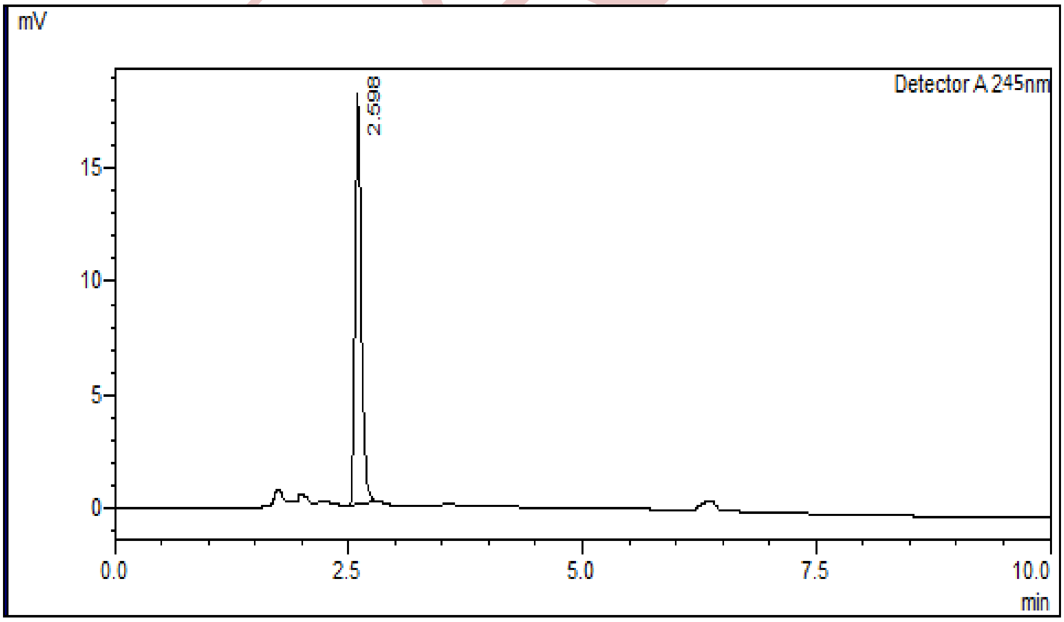

Fig 3: Assay sample –B

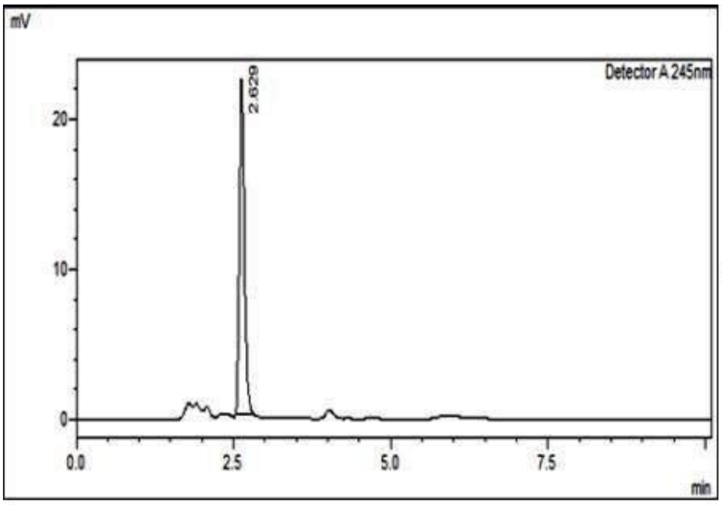

Fig 3: Assay sample –C

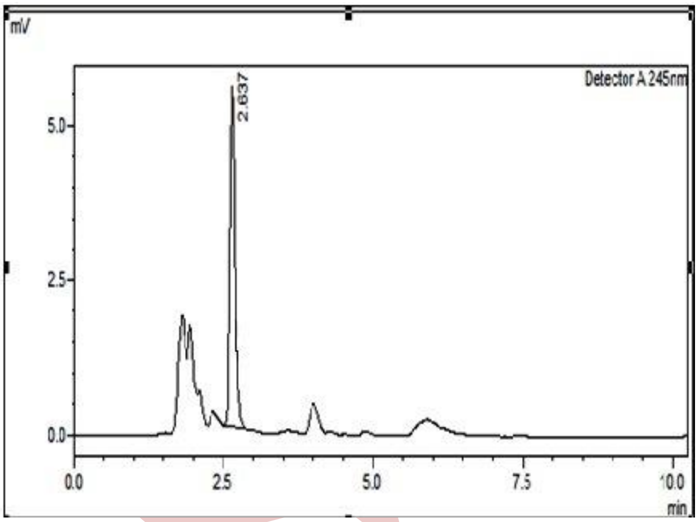

Fig No;4 Linearity curve

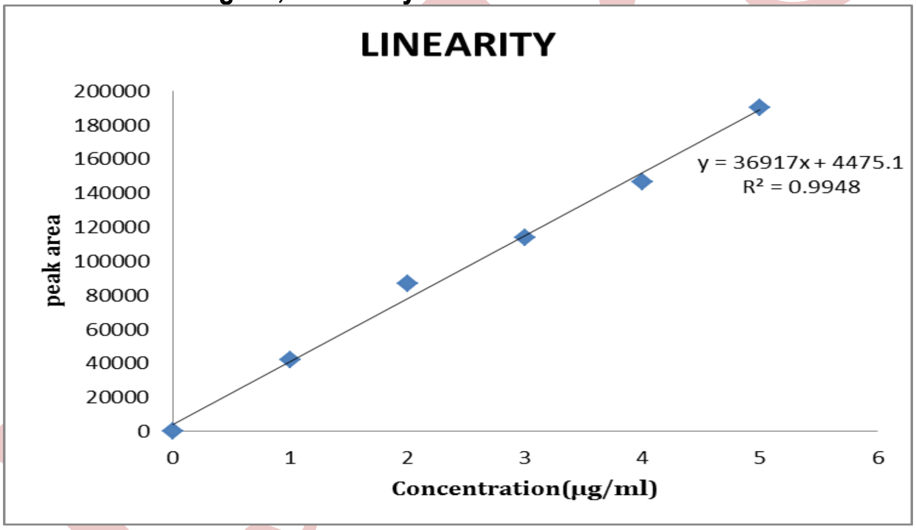

Table 1: Specificity of Para-Phenylenediamine

Sample	PPD	
	Peak area	Retention time
Standard	5419	2.642
Standard + v(heat)	5722	2.617

Table-2: Linearity data

S.no	Concentration (µg/mL)	Peak Area
1	1	42396
2	2	86884
3	3	114138
4	4	146951
5	5	190234
Slope		36917
R^2		0.9948

Table 3: System Precision

Sample no	Concentration (µg/ml)	Area
1	5	190234
2	5	188316
3	5	191011
4	5	190441
5	5	186769
Mean	189354.2	
SD	1765	
%RSD	0.93	

Table 4: Method Precision

Sample no	Concentration (µg/ml)	Area
1	2	84792
2	2	86884
3	2	79516
4	2	87609
5	2	84278
6	2	86165
Mean	84874	
SD	2907.13	
%RSD	0.034	

Table 5: Accuracy

S.N0	Concentration (%)	Peak Area	Rt	%Recovery	Mean
1	50%	41673	2.646	98	100%
2	50%	42396	2.661	100	
3	50%	44773	2.646	102	
4	100%	127011	2.638	100	101%
5	100%	129486	2.814	102	
6	100%	114138	2.617	100	
7	150%	183136	3.187	100	98%
8	150%	190234	2.615	96	
9	150%	186093	2.619	98	

Table 6: Mobile phase ratio: (40:60)

S.no	Concentration	Area	Rt	%RSD
1	1µg/ml	22487	2.64	**1.0**
2	1 µg/ml	22029	2.66	
3	1 µg/ml	22347	2.61	

Table 7: Mobile phase ratio: (60:40)

S.no	Concentration	Area	Rt	%RSD
1	1 µg/ml	24819	2.67	0.6
2	1 µg/ml	24864	2.60	
3	1 µg/ml	24553	2.66	

Table 8: Flow rate 0.8ml/min

S.no	Concentration	Area	Rt	%RSD
1	5 µg/ml	180741	3.069	1.11
2	5 µg/ml	183136	3.124	
3	5 µg/ml	179124	3.185	

Table 9: Flow rate 1.2ml/min

S.no	Concentration	Area	Rt	%RSD
1	5 µg/ml	80452	2.17	0.24
2	5 µg/ml	80113	2.13	
3	5 µg/ml	83679	2.26	

Table 10: Instrument change

	PPD	
	R_t (min)	Area
Trail 1	5.870	325062
Trail 2	5.870	328488
Trail 3	5.903	310749
Mean	321433	
S.D	9409.8	
%RSD	0.029	

Table 11: Assay Results

Sample Name	PPD		
	Area	% of PPD on pack	Amount obtained
Sample -A (Garnier)	33035	0.95% (i.e,0.38gm)	0.31gm
Sample –B (Godrej)	104707	10% (i.e,0.3gm)	4.7gm
Sample –B (Vasmol)	85738	4% (i.e,2gm)	1.85gm