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Analytical method development, validation and determination of phenazopyridine in its pharmaceutical dosage using diazonium salt of p-amino benzene sulphonic acid

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ABSTRACT

Phenazopyridine (PPD) an azo dye class of drug is known to provide symptomatic relief for muscles. A simple visible spectrophotometric method is developed and validated for PPD in its pharmaceutical dosage (pyridium), using diazonium salt of 4-Amino benzene sulphonic acid (PABSA) at low temperature. The maximum absorbance of PPD is found to be at λ_{max} *=422 nm. On reaction with diazonium salt of PABSA, PPD forms red colored azo derivative with maximum absorbance at* λ_{max} *=508nm. The solution obeys Beer's law in the concentration, range of 1-15* $\mu g / mL$ *.The coefficient correlation for the linear curve equation Y=0.032x-0.003is 1.00. Analytical, statistical and optical parameters were calculated from the average absorbance value of 0.2902 and found to be within limits. An average recovery of the solution was made to 95.5%.*

Key words: Phenazopyridine, para-amino benzene sulphonic acid, Azo dyes, validation, spectrophotometry.

INTRODUCTION

Phenazopyridine (PPD) (Fig-I) is chemically 3-(phenylazo)-2, 6-pyridine diamine monohydrogen chloride. It is widely used in combination with anti-biotics and anti- infective drugs. It belongs to azo dye class of drugs, which is in very bright orange color. When administered orally, it colorizes urine to orange color. It has analgesic effect on mucosa of urinary tract during excretion of urine [1-2]. It has been determined by HPLC [3-4], spectrophotometry [5-7] and GC-MS[8].

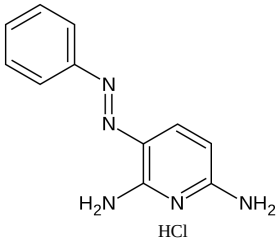


Figure-I Phenazopyridine

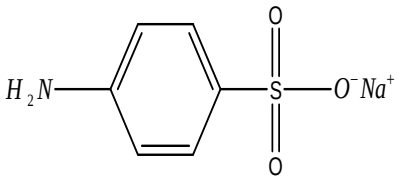


Figure-II Sodium salt of PABSA

The reagent used in this method to determine PPD is diazonium salt of para aminobenzene sulphonic acid (PABSA) commonly known as sulfanilic acid. PABSA is a white crystalline solid at room temperature and is used in quantitative analysis of nitrate and nitrite ions by calorimetry [9-10]. It readily forms diazonium salt, which is used to make dyes and indicators. In the present method PPD is accurately determined and validated in its pharmaceutical dosage by using diazonium salt of PABSA as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Para-Amino benzene sulphonic acid (Sd fine), Phenazopyridine, Pyridium, Chloroform, Potassium dihydrogen phosphate, Methanol, NaOH and HCl used were of analytical grade.

Instrumentation

The instruments used in this method are
UV-Visible spectrophotometer-3000+ (Labindia)
UV-Visible spectrophotometer-117(Systronics)
Analytical balance (Mattler toledo B2048)

Preparation of PABSA solution

0.175 gm of PABSA was accurately weighed and transferred into 100 ml standard flask. Few drops of Concentrated NaOH was added, to neutralize the sulphonic acid group present in PABSA. The p^H of the solution was adjusted to neutral. The concentration of the resultant solution was 0.001M. The solution was labeled as reagent solution. Further the beaker was kept in ice cold water.

Preparation of diluents buffer solution

3.4 gm of Potassium di hydrogen phosphate was taken into 500ml of standard flask, and was made up with distilled water. Further, the solution p^H was adjusted to 6.9 with 0.1M NaOH and 0.1M HCl solution and the solution were labeled as diluents solution.

Preparation of standard solution of PPD

0.214 gms of PPD (API) was accurately weighed and transferred into 100ml standard flask and made up with the diluents solution. The solution is labeled as 0.01M of standard solution of PPD.

Preparation of dosage solution of PPD

10 tablets of PPD (Pyridium), 200 mg each was finely powdered and thoroughly mixed, of this 0.214 gm was accurately weighed and transferred to 100 ml of standard flask and made up with the diluents solution. The solution was sonified and filtered for the removal of placebo and labeled as 0.01M dosage solution.

Preparation of reagent solution (Diazotation of PABSA)

Freshly prepared cold HNO_2 solution was added to PABSA solution. The flask was kept in ice cold water for about 5 minutes. The resulting solution is labeled as diazonium salt of PABSA.

Method development

5ml of dosage solution and 5ml of reagent solution was taken in 20 ml of standard flask and left to react for about 5 minutes, a red color develops in the flask, soluble in ethyl alcohol. The resultant solution obtained is labeled as sample solution of PPD, which is also known as azo dyes derivative of PPD. The maximum absorbance of the standard solution was recorded at λ_{max} =422nm, and for the sample solution at λ_{max} =508 nm. The sample solution obtained in the method developed is subjected to ICH guidelines to validate.

Validation of the method developed

Linearity and range

Eight different concentrations 1, 3, 5,7,9,11,13 and 15 ppm were made from the sample solution, these solution were subjected to absorbance and the values obtained were recorded (Table-I) and linearity plot was obtained at 508 nm.

The sample solution obeys Beer's law in the concentration range 1-15 ppm (Figure-II).The regression for the equation, Y=0.032x-0.003 was 1.00, established from the absorbance values of the sample solution.

Table-I Linearity and range

PPD Derivative (ppm)	Absorbance value
01	0.031
03	0.094
05	0.150
07	0.222
09	0.290
11	0.349
13	0.413
15	0.479

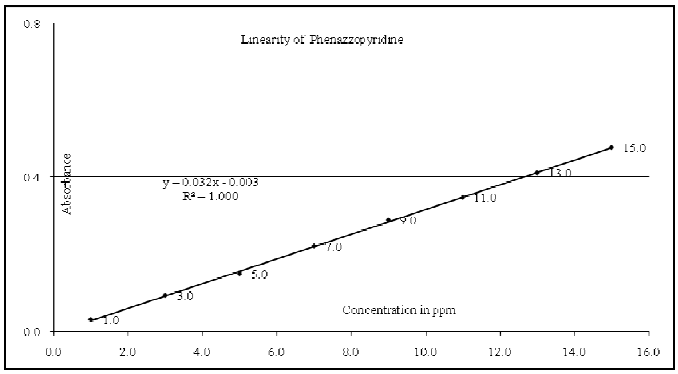


Figure-III Linearity & range of PPD

Specificity

In the present method, PPD reacts selectively with diazonium salt of PABSA in the pharmaceutical dosage, but not with placebo. This shows that the method is specific as per ICH guidelines.

Accuracy

A recovery study in the method developed, confirms that the method is accurate. From the freshly prepared sample solution 5ml was taken and concentration of 04. 06 and 08 ppm were made and their absorbance values were recorded and listed in table-2. An average recovery of 95.5% was made.

Table- II Accuracy of the method at λ_{max} =508 nm

Concentration of Derivative(ppm)	Label claim(mg)	Amount found(mg)	% of Recovery (Absorbance)
04	200	195.2	97.6 (0.125)
06	200	195.8	97.9 (0.188)
08	200	182	91.0 (0.233)

Repeatability

From the freshly prepared sample solutions of 5, 10 and 15 ppm and 5, 10 and 15 ppm of standard solution were made. Intraday precision and Inter day precision were recorded at 508 nm and 422 nm. The absorbance values recorded are given in table-III.

Table- III Repeatability

Formulation	Concentration Taken (ppm)	Intra-Day Precision (ppm found)	Inter-Day Precision (ppm found)
PPD(In dosage)	5	4.781	4.921
	10	9.295	9.820
	15	15.082	15.18
PPD (sample solution)	05	4.979	5.105
	10	10.100	9.862
	15	14.910	15.11

Ruggedness

Sample solution of 10 ppm, absorbance values were recorded on different days and were also recorded by two different analysts as given in table-IV.

Table –IV Intermediate precision (Ruggedness)

Concentration of sample solution	Factor Considered	Absorbance
10 ppm	Day-1	0.300
	Day-2	0.311
	Analyst-1	0.298
	Analyst-2	0.310

Robustness

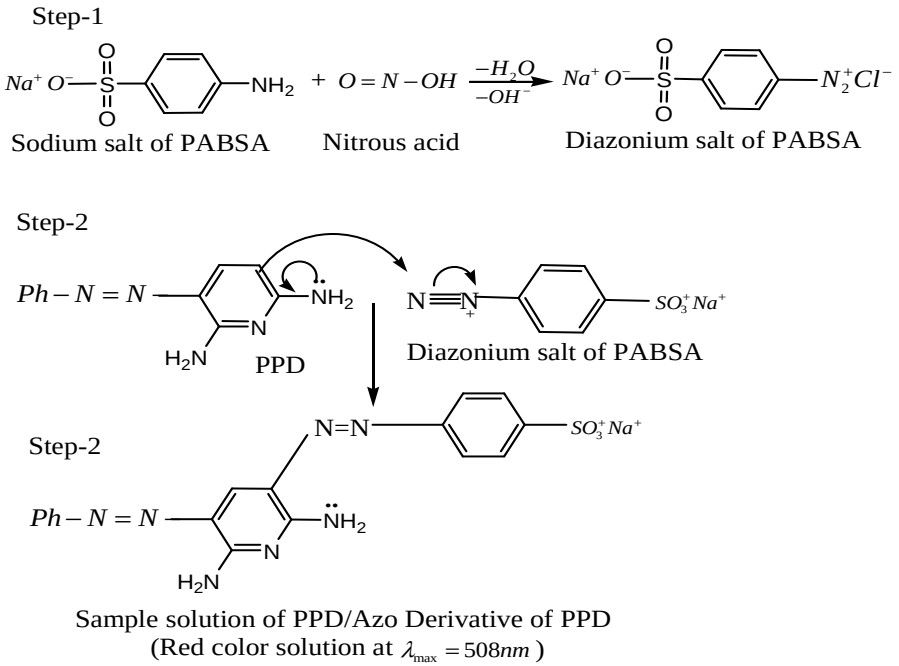
This step involves scanning of the sample solution at different wavelength, temperature and p^H .The values of absorbance recorded are given in table-V.

Table -V Robustness

Concentration of sample solution	Factor Considered	Absorbance
20 ppm	λ_{max} =505 nm λ_{max} =512 nm	0.60 0.61
	P^H =6.1 P^H =7.9	0.59 0.62
	$At\ 25^0C$ $At\ 30^0C$	0.58 0.61

RESULTS AND DISCUSSION

Phenazopyridine is validated selectively from its pharmaceutical dosage in the present method as per ICH guidelines [11-12]. PPD react with diazonium salt of PABSA, to form azo red dye which is soluble in ethanolic solution. The concentration of azo dye formed must be directly proportional to the amount of PPD in its dosage. The λ_{max} of PPD is 422nm, but on formation of azo red dye the solution shows λ_{max} =508nm. Which supports the formation of PPD azo dye derivative. To support the present method analytically ICH guidelines were followed [13-14] and the results are given as follows.The linearity equation and the range of the solution was Y=0.032x-0.003 and 1-15 $\mu g / mL$ respectively [Table-I and Figure III]. The coefficient correlation for the linear curve equation was 1.00.The method is specific which one of the primary requirements of validation procedure. An average recovery of was made to 95.5%[Table-II].Intra and Inter day precision was made, and from the absorbance values it suggest that the method is repeatable[Table-III].Intermediate precision on different day and by different analyst suggest that the method is rugged, since there is no much variation in the absorbance values of PPD and its azo derivative [Table-IV].The sample solution of PPD derivative on subjection at different temperature, p^H and λ_{max} proves that the method is robust[Table-V]. Finally the stability of the solution was found to be 12 hours, calculated as per ICH [15]. Optical data and statistical data was also calculated for the sample solution and given in table VI and VII respectively. Analytical data obtained in the present method was verified and were found under limit [16]. The absorbance of PPD solution in visible region was 422nm and the azo derivative solution absorbance value 508nm was obtained and given in figure IV and V respectively. A two step mechanism was proposed for the reaction between PPD and azo dye of PABSA, first step involves the reaction of PABSA with nitrous acid, to give diazonium salt of PABSA. In second step PPD react with diazonium salt of PABSA to give deep red coloration, due to the formation of azo red dye. The two step reaction is given in scheme-I. For step-II a possible mechanism was formulated, as step-I is reported mechanism, given in scheme-I.



Scheme-1 Formation of Azo derivative of PPD

Table-VI Optical parameter s involved in the method

Optical parameter	Coresponding value
Color of PABSA	White powder
Color of PPD in pyridium	Brown powder
Color of PPD in Buffer	Colorless
Color of PABSA diazonium salt.	colorless
Color of PPD azo derivative	Red (In methanol)
Color after 12 Hours	Pale red to Colorless
Absorption wave length of PPD	λ_{max} =422nm
Absorption wavelength of PPD azo derivative.	λ_{max} =508nm
Molar absorptivity	$0.02535 M^{-1} Cm^{-1}$
Optical density	0.02902
Transmittance	17.9%
Sandells sensitivity	$2.2\times10^{5}\ \mu g / ml / Cm^{2}$

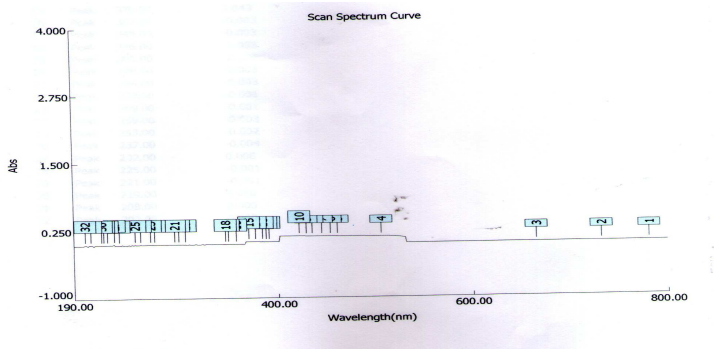


Figure-IV Absorbance spectrum of PPD at 422nm(standard)

Table-VII Statistical parameter of the proposed method

Statistical parameter	Corresponding value
Concentration of PPD	0.001M
Concentration of PABSA	0.001M
Recovery (Average)	95.5%
Limit of Quantization (LOQ=3SD/m)	0.00048 μ g/mL
Limit of Detection (10=SD/m)	0.32 μ g / mL
Length of the cell	1Cm
Signal-Noise ratio	1.610(Acceptable range)
Lower critical limit(LCL)	$3.0\times10^{-5}\ mg/L$
Upper critical limit(UCL)	$1.0\times10^{-4}\ mg/L$
Method detection limit(MDL)	$4.8\times10^{-5}\ mg/L$
Standard deviation (SD)	0.157, 0.024(variance)
Mean value	0.2535
Standard error value of mean	0.079
t-value	0.0032
p-value	0.9975
Average of absorbance	0.253
Concentration range	1-15 $\mu g/ml$
Linear equation	Y = 0.032x - 0.003
Intercept	0.003
Slope	0.032
Regression Co-efficient	1.00
Stability time	12 hours

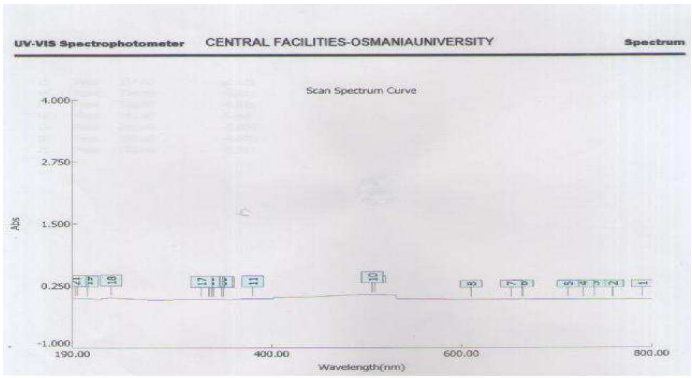


Figure-V Absorbance spectrum of PPD azo derivative at 508nm

CONCLUSION

The method is simple, accurate and economical and can be used for validation of PPD in its pharmaceutical dosage using diazonium salt of PABSA, which imparts red color to the solution, which is directly proportional to the concentration of the PPD in its dosage.

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