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Sensitive Spectrophotometric Method for the Determination of Prazosin

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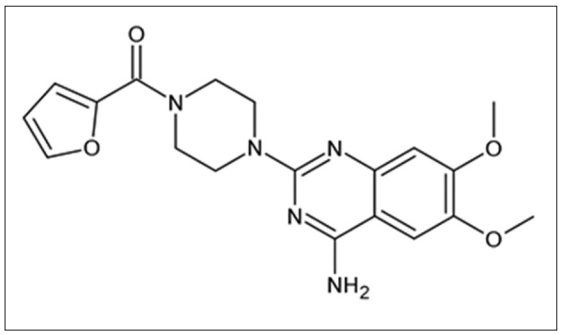
ABSTRACT

A simple, sensitive, precise, and economical spectrophotometric method has been developed for the determination of prazosin in pharmaceutical and biological fluid samples. Prazosin undergoes diazotization when treated with sodium nitrite and hydrochloric acid. The excess of nitrous acid during the diazotization is removed by the addition of urea solution. The diazonium cation reacts with the coupling reagent, β-naphthol by electrophilic substitution at the o-position of the coupling agent to produce an orange azo product. This orange product shows maximum absorbance at 480 nm. The method obeys Beer's law in the concentration range of 40-225 μg.ml^{-1} of prazosin. The optical characteristics of the proposed method such as molar absorptivity, Sandell's sensitivity, slope, and intercept were found to be 1.1286 L.mole^{-1} cm^{-1}, 0.0025 μg.cm^{-2}, 0.00243, and 0.0047, respectively. Validation studies are statistically significant as all the statistical parameters are within the acceptance range (% relative standard deviation [SD] < 2.0 and SD < 2.0) for both accuracy and precision study. The proposed method was found to be simple, sensitive, accurate, precise, rapid, and economical for the routine quality control application of prazosin in pharmaceutical formulations.

Key words: *Prazosin, Sodium nitrite, Hydrochloric acid, β-naphthol, Spectrophotometric method.*

1. INTRODUCTION

Prazosin is used to treat high blood pressure, anxiety, and panic disorder (Scheme 1). It is an alpha-adrenergic blocker that is specific for the alpha-1 receptors. Prazosin is chemically called 2-[4, 2-furoyl pipe-razin-1-41]-6, 7-dimetho-xyquinazolin-4-amine. Prazosin can be synthesized from 2-amino-4, 5-dimethoxybenzoic acid. The antihypertensive characteristics of prazosin make it a second-line choice for the treatment of high blood pressure.



Scheme 1: Structure of prazosin.

Several techniques have been reported for the determination of prazosin which include spectrophotometric [1,2], high-performance liquid chromatography (HPLC) [3], reversed phase-HPLC [4], liquid chromatographic [5], solid phase extraction [6], spectrofluorimetric [7], and ion selective electrode [8] methods for the estimation of prazosin. However, the above-mentioned methods are very complex and expensive equipment is involved. Spectrophotometric methods provide simple, rapid, reliable, sensitive and accurate procedure for determination of drugs [9,10].

The present investigation has made to develop simple, cost-effective, selective, accurate, and rapid for the determination of prazosin in bulk and dosage form.

2. EXPERIMENTAL

2.1. Instrumentation

A Shimadzu ultraviolet-visible double-beam spectrophotometer (model 2450) with 1 cm matched quartz cells was used for all the spectral measurements.

2.2. Chemicals and Reagents

0.1 N sodium nitrite, 1% β-naphthol, 1% urea solution, 0.5 N sodium carbonate, methanol, 0.1 N hydrochloric

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acid, and β-naphthol which were procured from Merck were used in the present study. All the chemicals used were of analytical grade. Double distilled water was used for all the experimental studies.

2.3. Preparation of the Sample Solution

Powdered tablet equivalent to 50 mg of the drug is weighed accurately and transferred to a 50 ml beaker and mixed well with 30 ml of methanol. The solution is filtered and transferred to a 50 ml volumetric flask, and the volume is made up to 50 ml with methanol. The concentration of the drug solutions is now 1 $mg.ml^{-1}$. This stock solution is further diluted to obtain the working concentration of 100 $\mu g.ml^{-1}$.

Known volumes of the drug formulation prepared as above and transferred into a series of 10 ml volumetric flasks and 2 ml of 0.1 N hydrochloric acid solution, 1.0 ml of 0.1 N sodium nitrite solution are added. The resultant solution in each flask is shaken well and allowed to stand for 5 min at 0-5°C temperature for diazotization. Then 1.0 ml of 1% urea solution, 1.0 ml of 0.5 N sodium carbonate, and 1.0 ml of 1% β-naphthol solution are added and volume is made up to 10 ml with methanol. The absorbance of the resultant solution is measured at 480 nm (Figure 1). The amount of prazosin in the pharmaceutical formulation is evaluated from the calibration plot.

2.4. Assay Procedure

The prazosin formulations namely minipress and vasoflex containing 100 mg weight were taken. 10 tablets are weighed and powdered. The various aliquots of the standard prazosin solution ranging from 0.2 to 1.0 ml are transferred into a series of 10 ml volumetric flasks. To each flask, 2.0 ml of 0.1 N hydrochloric acid solution, and 1.0 ml of cold 0.1 N sodium nitrite solution are added. The resultant solution in each flask is well shaken and allowed to stand for 5 min at 0-5°C temperature for diazotization to complete. 1.0 ml of 1% urea solution is added to each flask, and the solution is shaken frequently to allow nitrogen gas to escape. Then, 1.0 ml of 0.5 N sodium carbonate solution and 1.0 ml of 1% β-naphthol solution are added, and the volume in each flask is made up to 10 ml with methanol. An orange color is formed. The maximum absorbance of the orange colored solution is measured at 480 nm against the reagent blank. Calibration graph (Figure 2) is obtained by plotting absorbance values against the concentration of prazosin solution. The calibration curve is found to be linear over a concentration range of 40-225 $\mu g.ml^{-1}$ of prazosin. The amount of prazosin in the sample is estimated from the calibration graph.

3. RESULTS AND DISCUSSION

3.1. Effect of Concentration of Hydrochloric Acid on the Diazotization and Coupling Reaction

The effect of hydrochloric acid concentration on the absorbance is studied by varying the volume of hydrochloric acid (0.1 N) and measuring the absorbance at 480 nm. The data are presented in Table 1. The data in Table 1 shows that 2.0 ml of hydrochloric produces maximum absorbance and hence the same concentration is maintained throughout the experimental work.

3.2. Effect of Concentration of Sodium Nitrite

To a series of 10 ml graduated tubes, different aliquots of prazosin solution were taken. To each tube, 2.0 ml of 0.1 N hydrochloric acid, 1.0 ml of 1% β-naphthol, 1.0 ml of 1% urea solution, and 1.0 ml of 0.5 N sodium carbonate solution are added, and varying amounts of sodium nitrite are added. The contents are made up to the mark and set aside for 5 min for completion of the reaction. The absorbance of the resultant solutions is measured at 480 nm and the data are presented in Table 2. The data in Table 2 indicate that 1.0 ml of

Table 1: Effect of concentration of hydrochloric acid solution on absorbance.

Volume of HCl (ml)	Absorbance at 480 nm
1.0	0.222
1.5	0.335
2.0	0.480
2.5	0.477

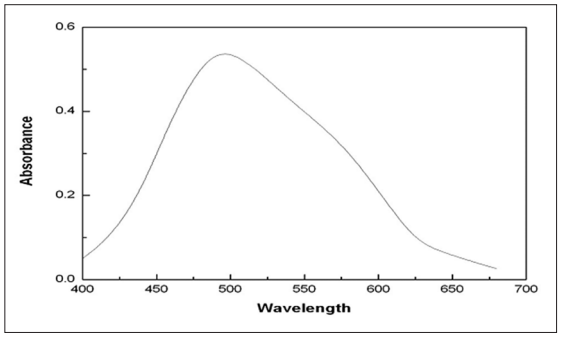

Figure 1: Absorption spectrum of diazotized prazosin treated with β-naphthol.

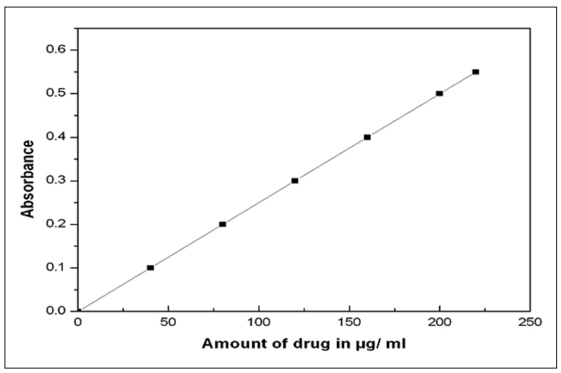

Figure 2: Calibration curve of prazosin.

sodium nitrite is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

3.3. Effect of Concentration of β-naphthol

Aliquots of prazosin ranging were taken into a series of 10 ml volumetric flasks. To each flask, 2.0 ml of 0.1 N HCl, 1.0 ml of 0.1 N sodium nitrite solution, 1.0 ml of 1% urea solution, and 1.0 ml of 0.5 N sodium carbonate solution were added and varying amounts of β-naphthol were added. The volume was made up to the mark. After completion of the reaction, the absorbance of the orange colored chromogen was measured at 480 nm against the corresponding reagent blank. The data are presented in Table 3. The data in Table 3 indicate that 1.0 ml of 1% β-naphthol is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

The data in Table 3 indicate that 1.0 ml of 1% β-naphthol is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies.

3. 4. Method Validation

3.4.1. Linearity

Under the above experimental conditions, linear calibration graphs were obtained by plotting the absorbance of the studied drug concentration versus absorbance within the specified range (Figure 2). The optical characteristics such as Beer's law limits, molar absorptivity, and Sandell's sensitivity are presented in Table 4. The regression analysis was made for the slope (b), intercept (a) and correlation coefficient (r) and the results are summarized in Table 4. The calibration was linear for prazosin at a concentration range of 40-225 $\mu g.ml^{-1}$ with correlation coefficient 0.998, intercept 0.0047, and slope 0.0024. The low values of % relative standard deviation (RSD) indicate the method is precise and accurate.

Table 2: Effect of concentration of sodium nitrite.

Volume of sodium nitrite (ml)	Absorbance at 475 nm
0.5	0.330
1.0	0.492
1.5	0.489
2.0	0.467

Table 3: Effect of concentration of β-naphthol.

Volume of beta-naphthol (ml)	Absorbance at 475 nm
0.5	0.330
1.0	0.492
1.5	0.489
2.0	0.467

3.4.2. Robustness and ruggedness

The standard deviations of absorbance were calculated, the % RSD was found to be <2%. The low values of % RSD indicated the robustness of the method. The method of ruggedness was expressed as the percentage of relative standard deviation for the proposed method developed by two analysts in two different instruments in two different days. The results proved that there is no statistical difference between the above said two analysts and instruments which conclude the developed analytical method were robust and rugged.

3.4.3. Accuracy and precision

Precision and accuracy were evaluated using three samples of different concentrations. Day to day variability was assessed using three concentrations on three different days, over a period of 1-week. These results show the accuracy and reproducibility of the assay. Thus, it was tested on an intraday and interday basis. The % RSD values reported in Table 5 shows that proposed methods provides acceptable intraday and interday variation.

3.4.4. Limit of detection (LOD) and limit of quantification (LOQ)

Detection and quantification limits were taken as the lowest concentration used in the construction of the calibration curves in Table 4. LOD and LOQ were determined by the formula based on the standard deviation of the response and slope.

$LOD = 3 \times s/S$ and $LOQ = 10 \times s/S$.

Where "s" is the standard deviation of the intercept and S is the slope.

3.4.5. Effect of interferences

The importance of the proposed analytical method was proved by the study of the effect of the excipients,

Table 4: Optical characteristics of proposed methods.

Parameters	Value
λ_{max} (nm)	480
Beer's law limit ($\mu g.ml^{-1}$)	40-225
Molar absorptivity ($L.mole^{-1}\ cm^{-1}$)	1.1286
Sandell's sensitivity ($\mu g.cm^{-2}$/0.001 A.U)	0.0025
Slope (b)	0.00243
Intercept (a)	0.0047
Correlation coefficient (r)	0.998
%RSD	0.25
LOD	1.2316
LOQ	4.1014

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation

Table 5: Evaluation of interday and intraday accuracy.

Taken (µg ml^{-1})	Found	Recover (%)	±SD	%RSD
Interday				
5	4.98	99.66	0.0115	0.231
10	9.98	99.83	0.0152	0.153
15	14.96	99.75	0.0153	0.102
20	19.97	99.89	0.0084	0.042
Intraday				
4.98	99.80	0.0115	0.2323	4.98
9.98	99.84	0.0178	0.0782	9.98
14.95	99.72	0.152	0.1021	14.95
19.99	99.95	0.0085	0.0427	19.99

RSD: Relative standard deviation, SD: Standard deviation

Table 6: Determination of tamsulosin in presence of excipients.

Excipients	Amount taken (mg.ml^{-1})	*Found (mg.ml^{-1})	Recovery %	±SD
Glucose	15	14.97	99.86	0.0100
Sucrose	25	24.97	99.86	0.0152
Lactose	35	34.98	99.93	0.0057
Dextrose	45	44.97	99.94	0.0100
Talc	55	54.99	99.96	0.0152
Starch	65	64.97	99.96	0.0100

SD: Standard deviation

Table 7: Method accuracy from recovery assay.

Sample	Added (mg.ml^{-1})	*Found (mg.ml^{-1})	Recovery (%)	±SD
Serum samples	0.5	0.49	99.66	0.001
	0.7	0.69	99.71	0.001
	0.9	0.89	99.06	0.002
	1.1	1.09	99.75	0.002
Urine samples	2	1.97	99.26	0.006
	2.2	2.19	99.81	0.001
	2.4	2.39	99.88	0.002
	2.6	2.59	99.88	0.002

SD: Standard deviation

viz. glucose, sucrose, lactose, dextrose, talc, and starch which frequently come with the drug prazosin in its dosage forms. The results showed that there is no interference from the excipients which indicates a high selectivity in determining prazosin in its dosage form. These results are recorded in Table 6.

3.4.6. Assay in serum and urine samples

Sample was collected and stored in a freezer at −20°C. Urine samples were centrifuged for 5 min, and the clear supernatant was used as a stock sample solution. The stock solution was spiked with a suitable quantity of the drug and analyzed by the same procedure. The results are presented in Table 7.

4. CONCLUSION

The proposed method is found to be simple, precise, accurate, time-saving, reproducible, and can be conveniently adopted for routine estimation of prazosin in bulk drug samples and pharmaceutical formulations.

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***Bibliographical Sketch**

Prof. P. Venkateswarlu has more than 30 years of teaching and research experience in the Dept. of Chemistry, S. V. University, Tirupati. He guided 15 PhD students. He has more than 115 publications in internationally reputed journals which are indexed in Science Citation Index. He is the reviewer for many Elsevier journals.