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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF PRAZOSIN HYDROCHLORIDE

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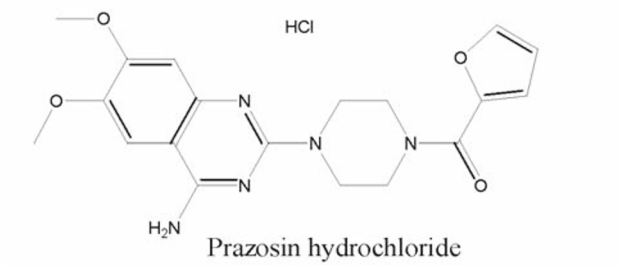
This work was aimed at creating a new, fast, and accurate UV spectrophotometric method for quantifying prazosin hydrochloride in pure and tablet dosage forms. A phosphate buffer solution of pH 6 was used as a diluent. The highest absorbance of prazosin hydrochloride was measured at 247 nm, and the linearity ranged from 2 to 8 μg/mL. The regression equation for prazosin hydrochloride was y= 0.087x + 0.236, with a correlation value 0.987. The percentage of recovery ranged from 99.6 to 101%. The relative standard deviation for intraday precision and interday precision was determined to be less than 2. The LOD and LOQ of prazosin hydrochloride were determined to be 0.0375 and 0.113 μg/mL, respectively. International Council for Harmonisation criteria validated the spectrometric technique and was suitable for routine quantitative measurement of prazosin hydrochloride in pure and tablet dosage forms.

Keywords: *prazosin hydrochloride, method development, phosphate buffer solution.*

Introduction. Chemically, prazosin is [4-(4-amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl]-(furan-2-yl) methanone. The molecular formula is $C_{19}H_{21}N_5O_4$, and the molecular weight is 383.401 g/mol. This quinazole derivative is the first of a new chemical class of antihypertensive compounds and is a very potent and selective α1 adrenergic antagonist. It reduces peripheral resistance and blood pressure by vasodilation of peripheral vessels (blocking α1-adrenergic receptors) in arteries and veins without increasing the heart rate. It can partially relieve obstructive and irritative symptoms by increasing urine flow and decreasing retention [1]. The drug and its formulations are officially in the British Pharmacopeia and United States Pharmacopoeia [2].

A review of the literature on prazosin hydrochloride found that UV spectroscopic [1–4], RP-HPLC [6–11], and LC-MS/MS methods [12] have been reported to date. Only four UV spectrophotometric methods have been reported for the pharmaceutical dosage form consisting of prazosin hydrochloride. In existing methods, one method was reported with a larger concentration range of 5–80 μg/mL, and organic solvent methanol was also used for dilutions [1]. The diluent of the existing method for a standard solution was somewhat complex, consisting of 0.1 N CHCOOH: Methanol (30:70) [2]. N-Bromo succinimide was used in another technique to quantify prazosin hydrochloride [3]. In a further method, a second derivative spectroscopic method was developed to estimate polythiazide and prazosin simultaneously, which was helpful in the case of binary mixtures [4]. Also, other methods, such as HPLC [6–11] and LC-MS/MS [12], were not cost-effective. These drawbacks were overcome to avoid uncertainty in the method and for economic and accurate quantification of the drug. The study was aimed at developing a simple, specific, and precise UV spectrophotometric method for quantifying prazosin hydrochloride in pure and tablet dosage forms.



Prazosin hydrochloride

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Department of Pharmaceutical Analysis, GITAM School of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh (State), India; email: sraja61@gmail.com. Abstract of article is published in Zhurnal Prikladnoi Spektroskopii, Vol. 91, No. 3, p. 468, May–June, 2024.

Materials and Methods. The absorbance of prazosin hydrochloride was measured using the Shimadzu UV-1780 spectrophotometer, which consists of matched quartz cells combined with UV probe software. For measuring purposes, an electronic balance, Shimadzu (Uni Bloc), was employed. Pipettes and volumetric flasks made of borosilicate glass were used in the experiment. The chemicals utilized were all of analytical grade. Methanol (Merck Life Science Private Limited), di-sodium hydrogen orthophosphate (Thermo Fisher Scientific India Pvt. Ltd.), citric acid (Merck Specialities Private Ltd.), and distilled water were the chemicals used. 63.2 mL of a 71.5 g/L solution of disodium hydrogen phosphate and 36.8 mL of a 21 g/L solution of citric acid were mixed to get a phosphate buffer solution of pH 6 [5]; 10 mg of prazosin hydrochloride drug was accurately weighed and transferred into 10 mL of the volumetric flask, and the volume was made up to the mark with methanol as a diluent (1000 μg/mL). Further, a working standard solution (100 μg/mL) was prepared by diluting the stock solution with phosphate buffer pH 6. Further, a working standard solution (100 μg/mL) was prepared by diluting the stock solution with phosphate buffer (pH 6.0).

Seven volumetric flasks of 10 mL were used in this experiment. From the working standard solution, 0.2- to 0.8-mL samples were put into volumetric flasks and combined with a phosphate buffer of pH 6 to get 2–8 μg/mL solutions. The solutions were scanned with a UV-Visible spectrophotometer within the 200–400 nm UV range.

Method Validation. Linearity is the ability of an analytical method to produce the observed concentration results of the tested samples proportionally to the theoretical concentration of analyte in the measured samples either directly or by a suitable mathematical transformation. Appropriate volumes of samples from prazosin hydrochloride, the usual working solution, were transferred to a volumetric flask (10 mL). The volume was adjusted using buffer, and solutions with concentrations (2–8 μg/mL) were achieved. The absorbance of each solution was measured, and a calibration curve was created by graphing absorbance vs concentration.

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The lowest concentration of analyte in a sample can be determined with acceptable precision and accuracy under the stated experimental conditions [1]:

$$\text{LOD} = 3.3 \times \text{Standard deviation/slope},$$

$$\text{Limit of quantitation (LOQ)} = 10 \times \text{Standard deviation/slope [1]}.$$

The accuracy of an analytical method was determined by applying the technique to analyze samples having known amounts of analyte. The accuracy was calculated from the test results as a percentage of analyte detected by the assay. The accuracy of the developed method was achieved by calculating the percentage recovery of prazosin by the standard addition method at 50, 100, and 150% [1].

Prazosin hydrochloride (brand name: Prazonol 5 mg) was used for assay. Ten tablets were weighed, and 10 mg were transferred to a volumetric flask (100 mL) and dissolved in diluent. The flask was sonicated for 10 min. The solution was filtered and diluted with water. Aliquots of sample solutions were placed in a volumetric flask of 10 mL. Diluent was used to make up the volume. The absorbance was measured at 247 nm.

Precision was calculated by interday and intraday variation. In the intraday study, solutions of the same concentration (6 μg/mL) were prepared and analyzed six times on a similar day. In the interday precision investigation, the identical concentration solutions were made and studied for 3 consecutive days, and absorbances were recorded. Percentage RSD was determined.

Ruggedness is the degree or measure of reproducibility under different situations, such as in different laboratories, different analysts, different machines, environmental conditions, operators, etc. The ruggedness studies were determined by changing the analyst as an extraneous influencing factor. The acceptance limit for the calculated %RSD of the peak area was less than 2.

Results and Discussion. The ability to obtain test findings that were proportional to the analyte concentration in samples within an appropriate range was termed linearity. The developed approach showed linearity in the 2–8 μg/mL range. The overlain spectra of prazosin hydrochloride were plotted in Fig. 1. For prazosin hydrochloride, the linearity equation was $y = 0.087x + 0.236$, with a correlation coefficient of 0.987. From the obtained linearity data, the coefficient of correlation was found to be less than 1. Hence, the results were within the acceptable limits. The linearity result was illustrated in Table 1, and the graph was plotted in Fig. 2. The method developed was found to be linear.

Accuracy was the degree to which the measured value agrees with the accurate value. The mean percentage recovery of prazosin hydrochloride was found to be between 99.87 and 100.71%, and it can be concluded that the results were within

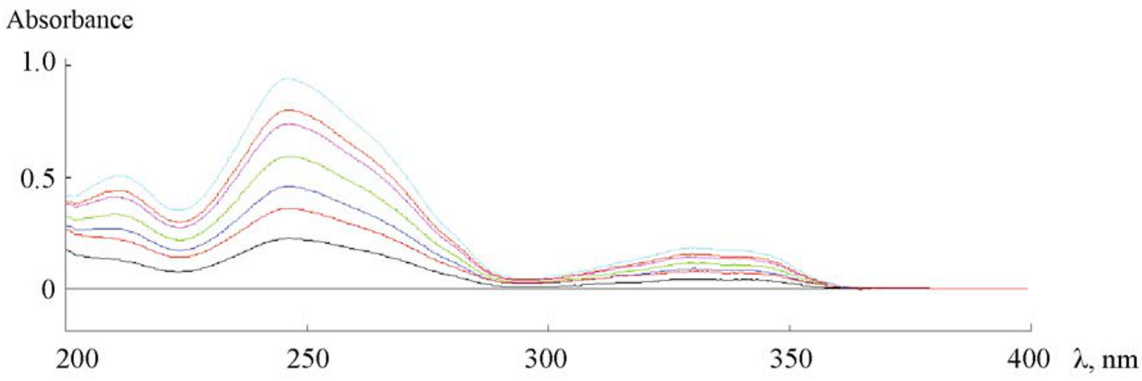

Fig. 1. Overlain spectra of prazosin hydrochloride.

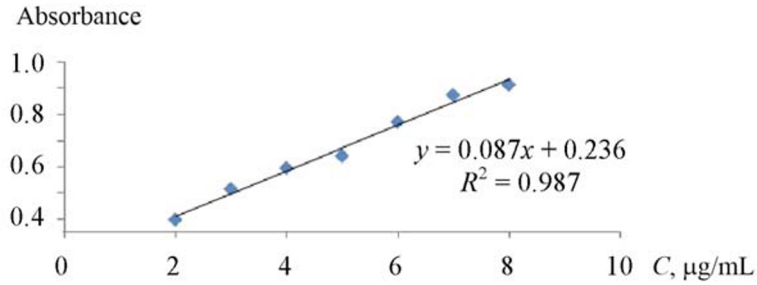

Fig. 2. Linearity graph of prazosin hydrochloride.

TABLE 1. Linearity Study of Prazosin Hydrochloride

Concentration, μg/mL	Absorbance
2	0.397
3	0.516
4	0.597
5	0.643
6	0.772
7	0.876
8	0.916

the limits. The observed data were within the range, indicating that the suggested analytical method might have had good recovery and accuracy. Accuracy results were shown in Table 2.

Precision was defined as the degree of agreement between individual test findings when the procedure was tested on several uniform samples. The precision of the analytical method was determined by assaying six determinations at a test concentration (6 μg/mL). The percentage relative standard deviation (%RSD) calculated statistically (Table 3) was found to be less than 2% (i.e., 0.314%), thus indicating the high precision of the proposed method.

The percentage relative standard deviation for interday precision was found to be 0.18–0.36%. The percentage relative standard deviation of precision studies was less than 2 and within the acceptable range. The intraday precision was summarized in Table 3.

TABLE 2. Accuracy Study of Prazosin Hydrochloride

Level, %	Total amount, µg/mL	Absorbance	Amount found, µg/mL	Recovery	Mean	SD	%RSD
50	3	0.500	3.03	101	100.4	0.7	0.6
50	3	0.499	3.02	100.7	100.4	0.7	0.6
50	3	0.496	2.98	99.6	100.4	0.7	0.6
100	4	0.590	4.06	101.5	101.2	0.4	0.39
100	4	0.589	4.05	101.4	101.2	0.4	0.39
100	4	0.587	4.03	100.7	101.2	0.4	0.39
150	5	0.678	5.08	101.6	101.2	0.35	0.34
150	5	0.677	5.06	101.3	101.2	0.35	0.34
150	5	0.675	5.04	100.9	101.2	0.35	0.34

Note. SD: standard deviation, RSD: relative standard deviation.

TABLE 3. Intraday and Interday Precision of Prazosin Hydrochloride for Concentration Taken 6 µg/mL

Absorbance	Concentration found, µg/mL	Assay, %	SD	%RSD
	Intraday			
0.762	6.04	100.7	0.0024	0.314
0.765	6.08	101.3	0.0024	0.314
0.763	6.05	100.9	0.0024	0.314
0.767	6.10	101.7	0.0024	0.314
0.760	6.02	100.3	0.0024	0.314
0.762	6.04	100.7	0.0024	0.314
Interday				
0.761	6.03	100.5	0.0014	0.18
0.763	6.05	100.9		
0.764	6.06	101	0.0014	0.18
0.760	6.02	100.3		
0.762	6.04	100.7	0.0028	0.36
0.766	6.09	101.5		

Note. SD: standard deviation, RSD: relative standard deviation.

Percentage purity was found to be 99%. According to the label claim, the drug content obtained from the values of sample solutions was found to be within the permissible range of 90–110%. The assay data of prazosin hydrochloride in form tablet Prazonol (label claim 5 mg) are amount found 4.95 mg, assay 99% w/w.

Ruggedness is the degree or measure of reproducibility under different situations, such as in other laboratories, different analysts, machines, environmental conditions, operators, etc. The %RSD was found to be 0.18 and 0.09, showing

TABLE 4. Ruggedness Data of Prazosin Hydrochloride for Concentration 6 µg/mL

Analyst	Mean absorbance	%RSD
1	0.763	0.18
2	0.762	0.09

Note. RSD: relative standard deviation.

TABLE 5. Summary of All the Results after Performing Different Validation Parameters

Maximum absorbance, nm	247
Linearity, µg/mL	2–8
Intercept, c	0.236
Slope, m	0.087*x*
Correlation coefficient R^2	0.987
Intraday precision (%RSD)	0.314
Interday precision (%RSD)	0.18–0.36
LOD, µg/mL	0.0375
LOQ, µg/mL	0.113
Assay (% Purity)	99
Ruggedness, %	0.09–0.18

Note. Regression equation: $y = mx + c$, where m is the slope; c is the intercept, and x is the concentration found in µg/mL. LOD: limit of detection, LOQ: limit of quantitation.

that it was within the limit. The ruggedness data also demonstrated that the values were within the limits. The results of ruggedness were illustrated in Table 4. The developed technique was validated according to International Council for Harmonisation (ICH) guidelines. The summary of the results was tabulated in Table 5.

Conclusions. A simple, low-cost, quick, and non-toxic UV spectrophotometric approach was created to identify and measure prazosin. Additionally, the present method was validated for linearity, precision, accuracy, LOD, and LOQ according to ICH recommendations. The LOD and LOQ of prazosin hydrochloride were determined to be 0.0375 and 0.113 µg/mL, respectively. The developed UV spectrophotometric method was simple, accurate, precise, and economical for estimating prazosin hydrochloride in pure and pharmaceutical dosage forms.

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REFERENCES

1. S. N. Shah, *Innov. J. Qual. Assur. Pharma Anal.*, **1**, No. 1, 110–114 (2015).
2. M. Ü. Özgür and S. Sungur, *Turk. J. Chem*., **26**, No. 5, 691–696 (2002).
3. K. Sreedhar, C. S. P. Sastry, M. N. Reddy, and D. G. Sankar, *Talanta*, **43**, No. 11, 1847–1855 (1996).
4. B. Panzova, M. Ilievska, G. Trendovska, and B. Bogdanov, *Int. J. Pharm*., **70**, Nos. 1–2, 187–190 (1991).
5. Indian Pharmacopeia 4.1 Buffer Solutions, **1**, 757–758 (2014).
6. M. Bakshi, T. Ojha, and S. Singh, *J. Pharm. Biomed. Anal*., **34**, No. 1, 19–26 (2004).
7. N. Sultana, M. S. Arayne, S. N. Shah, N. Shafi, and S. Naveed, *J. Chin. Chem. Soc*., **57**, No. 6, 1286–1292 (2010).
8. A. Shrivastava and V. B. Gupta, *Sci. Pharm*., **80**, No. 3, 619–632 (2012).
9. N. Sultana, M. Saeed Arayne, and S. N. Shah, *Med. Chem.*, **4**, No. 12, 770–777 (2014).

10. S. Naz, N. Sultana, M. S. Arayne, and N. Shafi, *Int. J. Pharm. Res. Dev*., **2**, No. 9, 6–12 (2010).
11. U. P. Panigrahy, K. Kumari, T. Reddy, and K. Abbulu, *Res. J. Pharm. Technol*., **13**, No. 4, 1779–1789 (2020).
12. J. C. L. Erve, S. C. Vashishtha, O. Ojewoye, A. Adedoyin, R. Espina, W. DeMaio, and R. E. Talaat, *Xenobiotica*, **38**, No. 5, 540–558 (2008).