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New Spectrophotometric and Spectrofluorimetric Methods for Determination of Prazosin HCl in API and Drug Product

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Abstract: ***New simple and sensitive spectrophotometric and fluorimetric methods have been developed for the determination of prazosin hydrochloride (PRZ) in its active pharmaceutical ingredient (API) and drug product. The spectrophotometric method (Method I) is based on formation of a binary complex with rose Bengal (RB) at 572 nm. The absorbance-concentration plot is rectilinear over the range 2.5 - 25 µg/mL. The first fluorimetric method (method II a) depend on measurement of native fluorescence intensity of PRZ at λ emission 388 nm using λ excitation 340 nm. The fluorescence-concentration plot is rectilinear over the range 0.05– 1.4 µg /mL. The second method (method II b) was based on the quantitative quenching effect of PRZ on the native fluorescence of rose Bengal (RB) at the same pH. The quenching of the fluorescence of rose Bengal (RB) was measured at 575 nm after excitation at 483 nm. The fluorescence-concentration plot is rectilinear over the range 0.5–8 µg/mL. The proposed methods were successfully applied to the analysis of commercial tablets. Statistical comparison of the results with the reference method revealed good agreement and proved that there were no significant differences in the accuracy and precision between the two methods respectively***

Keywords: Prazosin hydrochloride (PRZ), Rose Bengal (RB), Clark and Lubs buffer, Quenching effect

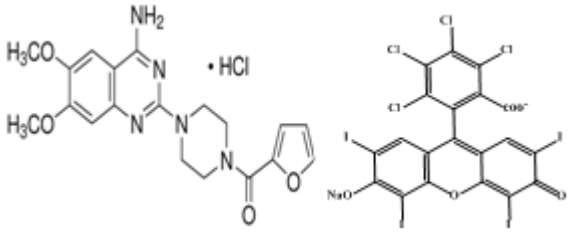
1. Introduction

Prazosin HCl (PRZ); $C_{19}H_{21}N_5O_4$.HCl; Piperazine, 1-(4-amino-6, 7-dimethoxy-2-quinazolinyl)-4-(2-furannyl-carbonyl)-monohydrochoride, is a sympatholytic alpha-adrenergic blocker used in the treatment of anxiety, hypertension, refractory pulmonary oedema and panic disorders. It reduces peripheral resistance and blood pressure by vasodilation of peripheral vessel (by blockade of α_1- adrenergic receptors) in arterioles and veins without increasing the heart rate or significantly impairing sympathetic function [1]. The vasodilator effect may be related not only to the direct relaxant action on vascular smooth muscles but to the blockade of postsynaptic α-adrenoceptors [2].

The drug and its formulations are official in the United States Pharmacopeia [3], and the British Pharmacopeia [4]. PRZ is an antihypertensive1 and a potent vasodilator agent [5] in the quinazoline family and also has been found to be value in the treatment of heart failure [6] Many analytical techniques have been previously reported for Prazosin analysis in its pharmaceutical formulations by reversed phase HPLC [7], HPLC-MS [8], HPLC in presence of degradation product [9-11], thin layer chromatography [12,13], different UV methods [14- 16],UV and Voltammetry method[17]. Beside spectrophotometric and fluorimetric method [18-21], polarographic and Capillary electrophoresis methods [22-24].

Rose Bengal (RB) dye (4, 5, 6, 7-tetrachloro-2', 4', 5', 7'-tetraiodofluorescein disodium salt, C.I. name: Acid Red 94). It has been used as ion-pair forming agent for determination of chlorphenoxamine hydrochloride, anhydrous caffeine [25], oxybuprocaine hydrochloride [26] and ranitidine hydrochloride [27]. It was also used for spectrophotometric and conductometric methods for macrolide antibiotics determination in pure and pharmaceutical dosage forms[28], also it has been used for determination of barium through formation of ternary complex with 18-crown-6[29]. Among the various methods available for the determination of drugs, spectrophotometric and spectrofluorimetric continue to be the most convenient analytical techniques, because of their inherent simplicity, low cost and wide availability in most quality control laboratories. Chromatographic methods require highly sophisticated and expensive instruments and solvents that may not be available in some quality control laboratories in the developing countries.

Thus, the aim of the present work was to investigate economical, simple, precise, sensitive and environmental friendly two analytical methods (I-II a,b) for the determination of PRZ in drug product; Both methods (I-II a, b) involve the formation of intense red ion-association complex between drug and halo fluorescein derivative (RB) as reagent.



(a) (b)

Figure 1: Chemical structure of (a) Prazosin hydrochloride and (b) Rose Bengal.

2. Experimental

2.1. Apparatus

A Shimadzu UV-visible 2450 PC Series Spectrophotometer (Tokyo – Japan) with two quartz cells 1 cm path length, connecting to an IBM compatible computer and HP desk jet printer are used for all absorbance measurements of data using the following spectral parameters: Scan mode: absorbance, Speed: fast and Slit width: 2 nm. Fluorescence spectra and measurement were recorded using an Agilent Cary Eclipse Fluorescence Spectrophotometric, equipped with the xenon flash lamp, grating excitation and emission monochromators for all measurements and an Agilent Cary Eclipse recorder. Slit width for both monochromators were set at 10 nm. A 1 cm quartz cell was used. Digital pH meter PW 9409 Pye Unicum was used for checking the pH of the buffer solutions used.

3. Materials and Reagents

Prazosin HCl was kindly supplied by Pfizer Company Cairo, Egypt. The purity of the drug was found to be 99.23% according to the reference method [3]. Minipress tablets each contain 1.0 mg prazosine HCl/tablet. Batch 00556 P is product of Pfizer Company Cairo, Egypt. They were obtained from local pharmacy. Rose Bengal (C.I. 45440) (HOPKIN & WILLIAMS, England) 2×10^{-4} M aqueous solution for spectrophotometric method and 1×10^{-4} M, aqueous solution for spectrofluorimetric method. Both solutions were freshly prepared in distilled water and further diluted with the same solvent to the appropriate concentration. Clark and Lubs buffer solutions of pH 4.1 to 5.9 was prepared by mixing appropriate volumes of 0.1 M potassium hydrogen phthalate and 0.1 M sodium hydroxide and adjusting the pH to 5.5 using pH meter.
Freshly distilled water was used.

3.1. Preparation of stock and standard solutions

A stock solution of PRZ was prepared by dissolving 10.0 mg of PRZ in 100.0 mL of distilled water and was further diluted with the same solvent as appropriate for method I. For method II, dissolved 10.0 mg of PRZ in 100.0 mL of distilled water and was further diluted with Clark and Lubs buffer solution pH5.5 to prepared 10µg/ml. The standard solution was stable for 2 weeks when kept in the refrigerator.

3.2. Pharmaceutical Formulation Samples

For tablets, twenty tablets were weighed and pulverized well. A weighed quantity of the powdered tablets equivalent to 10.0 mg of PRZ was transferred into a small conical flask and extracted with 3 × 30 mL of distilled water. The extract was filtered into a 100 mL volumetric flask. The conical flask was washed with few mLs of water. The washings were passed into the same volumetric flask and completed to the volume with the same solvent. Aliquots covering the working concentration range were transferred into a series of 10 mL volumetric flasks. The procedure described under "Construction of calibration graph" was applied. The nominal content of the tablets was determined either from the previously plotted calibration graph or using the corresponding regression equation.

4. Construction of the Calibration Curves

4.1. Spectrophotometric method (Method I)

Aliquots of the standard drug solution in the concentration range listed in table 1 were transferred into 10mL calibrated flasks, 1 ml of 2×10^{-4} M dye solution the solution was diluted with water, and then diluted to the mark with Clark and Lubs buffer solution pH 5.5. The absorbance of the solution was measured at 572 nm against the corresponding reagent blank treated similarly.

4.2. Spectrofluorimetric method

4.2.1. Native fluorescence method (Method IIa)

The native fluorescence study of PRZ, Aliquots containing different amounts of the analyses, between 0.05-1.4 µg mL-1(50-1400 ng mL-1) were pipette into 10 mL calibrated flasks, and diluted to the mark with Clark and Lubs buffer solution pH 5.5. The fluorescence emission was measured at 388 nm using excitation wavelength of 340 nm, against a blank solution.

4.2.2.The quenching fluorescence method (Method IIb)

For spectrofluorimetric method, 1.0 mL of 1× 10−4 M RB was used and dilution of the standard solution with Clark and Lubs buffer solution pH 5.5 to obtain the working concentration range of 0.5 –8 µg/mL. The fluorescence intensity of the resulting solution was measured at 577 nm after excitation at 483 nm. The difference in the fluorescence intensity (ΔF) was plotted vs. the final concentration of the drug (µg/mL) to get the calibration curve. Alternatively, the regression equation was derived.

4.2.3. Determination of Stoichiometric Ratio

The stoichiometric ratio of the formed products was investigated by Job's continuous variation method [33]. Equimolar stock solutions of PRZ and RB (1×10^{-4} M) were prepared in the previously specified solvent. Series of 2-mL portions of the stock solutions of the drug and the reagent were made up comprising different complementary proportions in 10mL volumetric flasks containing 1ml of the appropriate buffer. The solutions were further manipulated as described under spectrophotometric method (Method I).

5. Results and Discussion

Fluorescein and its derivatives have been used as ion-pairing agents for the determination of many pharmaceutical compounds using spectrophotometric or spectrofluorimetric methods, among these agents, Rose Bengal (RB) has been utilized for the determination of the cited drug prazosin through spectrophotometric and spectrofluorimetric measurements. The purposes of the present study were to develop simple and sensitive spectrophotometric and spectrofluorimetric methods for the determination of PRZ in

its API and drug products without prior extraction procedures or using organic solvents.

In the present study, PRZ was found to form an ion pair red complex with RB at pH 5.5 with maximum absorbance at 572 nm (Fig. 2). The formed complex is mainly due to the electrostatic interaction between the studied drug and anionic functional group of RB under acidic pH5.5.

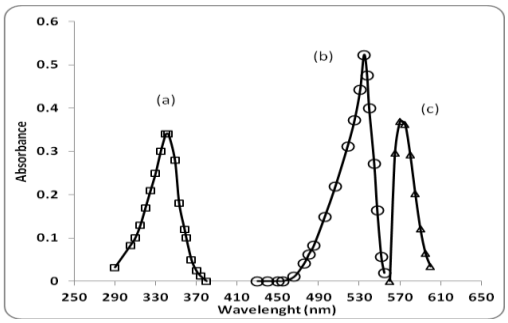


Figure 2: Absorption spectra of (a) PRZ.HCl (10.0 µg/mL) in Clark and Lubs buffer Solutions of pH 5.5, (b) reagent blank vs. solvent, (c) ion-pair

Complex vs. reagent blank

Absorption spectrum shows that the binary complexes formed between Rose Bengal and with the cited drug has higher value of molar absorptivity for the spectrophotometric method presented in Table 1. Investigation was carried out to establish the most favorable conditions for complex formation in the spectrophotometric method of Prazocin with rose bengal to achieve maximum color development, for ion-pair formation.

PRZ is freely soluble in water, the solution exhibited an intense native fluorescence in different media, such as water, using Clark and Lubs buffer solutions in the pH range 4.1 to 5.9. Highest fluorescence intensity was obtained in buffer solution pH 5.5; at λ emission 388 nm upon using 340 nm as λ excitation Figure (3).

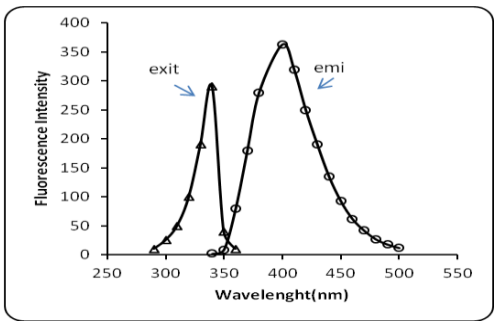


Figure 3: Excitation (340nm) and Emission Spectra (388nm) of PRZ. HCl (1.2µg/mL) in Clark and Lubs buffer solutions of pH 5.5

The formed ion pair complex is not fluorescent; therefore, the decrease in the fluorescence of RB upon the addition of the drug was the basis fit the spectrofluorimetric measurement at 575 nm after excitation at 483 nm (Fig. 4)

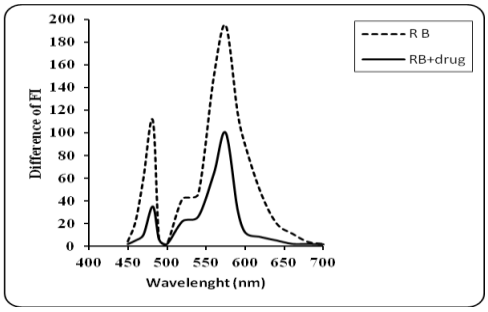


Figure 4: Excitation (483 nm) and Emission Spectra (575 nm) after reaction with RB ($1X10^{-4}$M) and PRZHCl8µgml^{-1}).

Optimization of Reaction Conditions

The spectrophotometric and spectrofluorimetric properties of the product as well as the different experimental parameters affecting its development and stability were carefully studied and optimized , to give the maximum sensitivity, adherence to Beer's law, and stability. Such factors were changed individually while the others were kept constant. These factors include; the effect of pH buffer, concentration of RB, time of reaction and diluting solvents.

Effect of pH

In spectrophotometric method, the influence of pH on the formation of ion-pair associate PRZ-HCl with halofluorescein dye was studied and optimized to obtain maximum color intensity (Fig. 5), by using a series of buffer solutions acetate, phosphate and Clark and Lubs buffer. The pH was varied over a pH range of 4-6 using Clark and Lubs buffer. Upon increasing the pH, the absorbance of the red ion associate was found to increase from pH 4 to 6 and attaining maximum absorbance at PH 5.5, this increase remains constant until pH 5.7, after which a slight decrease in absorbance was achieved. . Therefore, Clark and Lubs buffer of pH5.5 was chosen as the optimum pH throughout the study. The shape of the absorption spectra and the position of the absorption maxima of the ion pairs formed did not vary with pH; these results indicate that only one type of complex is formed.

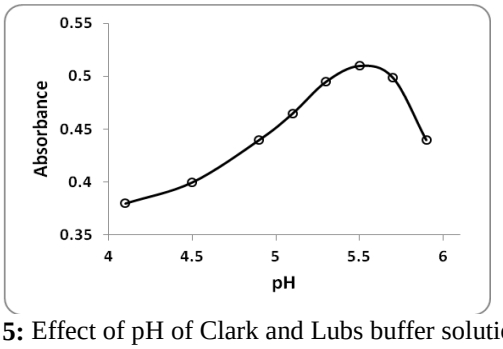


Figure 5: Effect of pH of Clark and Lubs buffer solutions for the ion associate of PRZ HCl -RB using (10.0µg/mL) of the cited drug.

The pH is a critical factor in the complex formation, since it affects the ionization of RB. The influence of pH using Clark and Lubs buffer solutions on the quenching of the fluorescence intensity of RB was studied over the pH range 4.1–5.9. It was found that increasing pH values resulted in a subsequent increase in ΔF up to 5.5. This increase remains

constant until pH 5.7. After which a slight decrease in ΔF was achieved. Therefore, The Clark and Lubs buffer of pH5.5 was chosen as the optimum pH throughout the study.

Effect of reagent concentration of RB:

The optimum concentration of the reagent was determined for the drug. In the spectrophotometric method, 1 mL of reagent concentration, (2×10^{-4} M) was suitable to develop the maximum complete color formation. Drug- reagent-buffer was the favorable sequence of addition for complete color development and highest absorbance at the recommended wavelength.

Effect of time of reaction and standing.

In spectrophotometric method; the intensity of the final color was stable for 48 h without precipitation of the complex. The effect of time on the quenching of the fluorescence intensity of Rose Bengal was also studied. It was found that the decrease in the fluorescence intensity of RB was immediate upon addition of PRZ and remained constant for more than 4 hours.

Effect of diluting solvent

In spectrophotometric method; upon diluting the reaction solutions with water, transparent solution was obtained indicating the solubility of the PRZ - RB product in water. In+ order to select the most appropriate solvent for diluting the reaction solutions, different solvents were tested and compared with water; these solvents were methanol, isopropanol, acetonitrile, and 1,4-dioxane. The highest readings were obtained when water was used for dilution (Fig.7).

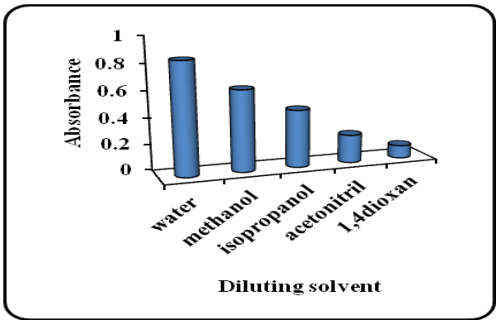


Figure 7: Effect of diluting solvent on the absorbance of PRZ HCl(20µg/ml)- RB

However, in spectrofluorimetric method different Clark and Lubs buffer solutions pH (4.1- 5.9) In order to select the most appropriate solvent for diluting the reaction solutions, and compared with water. The best reading for (ΔFI) were obtained when Clark and Lubs buffer solutions pH5.5 was used for dilution (Fig.8)

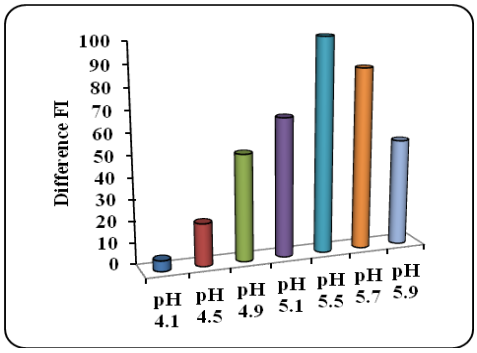


Figure 8: Effect of diluting pH of Clark andLubs buffer solutions on the decrease of the fluorescence intensity of RB using 8µg/mL of PRZHCl

Stability of the Chromophore and Fluorophore

After dilution of the reaction solutions, it was found that the absorbance of the chromogen (PRZ-RB) and the (ΔFI) of the fluorophore remained stable for at least 4 hours. This allowed the processing of large batches of samples, and their comfortable measurements with convenience. Thus, increased the convenience of the methods as well as made the method applicable for large number of samples.

Composition of the ion pair Complex

Job's continuous variation method was utilized for investigating the reaction between drug and reagent. A series of solutions was prepared by mixing equimolar solutions of the drug and reagent in varying proportions while keeping the total molar concentration constant. The absorbance spectrum of the resultant ion associate was measured at the respective λmax to determine the absorbance. . The results showed that the composition of the associate was equimolar (1:1). Then a plot of the absorbance against the mole fraction of the drug was constructed and presented graphically in Fig. 9.

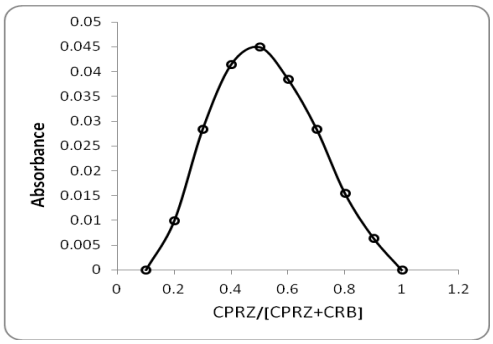


Figure 9: Job's method of continuous variation for PRZ-HCl ion associate

CPRZ+CRB=$1x10^{-5}$ M

The conditional stability constants (K_f) of the ion pair associates under the experimental conditions described above was calculated from the continuous variation data using the following equation:

$$K_f = \frac{A/A_m}{[1-A/A_m]^{n+1} C_M^n (n)^n}$$

Where A and Am are the observed maximum absorbance and the absorbance value when all the PRZ present is associated respectively, CM is the mole concentration of PRZ at the maximum absorbance and n is the stoichiometry with which

ion associate with PRZ. The log K_f value of PRZ-RB ion pair was 5.3cal mol^{-1}. The free energy change of the ion-pair associate was found to be - 7.274 Kcal mol^{-1}, according to the following equation

$$\Delta G = -2.203 \times RT \log K_f$$

Where R is the ideal gas constant
T is the temperature in Kelvin.

Mechanism of the reaction

The stoichiometry of the reaction between the studied drug (PRZ), and RB was investigated by Job's method (33).The symmetrical bell shape of Job's plot indicated that the drug: reagent ratio was 1:1 for drug and reagent. Based on this ratio, the reactions pathways were postulated to be proceeded as shown in Fig.9 and scheme1which agrees with the fluorimetric method. The limiting logarithmic method [36] confirms the stoichiometry of the reaction between the studied drug and RB. The decrease in the fluorescence intensity of the reaction product was alternatively measured in the presence of either RB or PRZ. Plots of log [PRZ] vs log ΔF and log [RB] vs log ΔF gave two straight lines, the values of the slopes were 1.06:1.05 for PRZ: RB respectively (Fig.10). Hence, it is concluded that, the molar reactivity of the reaction is 1:1 drug: RB, based on the obtained molar ratio.

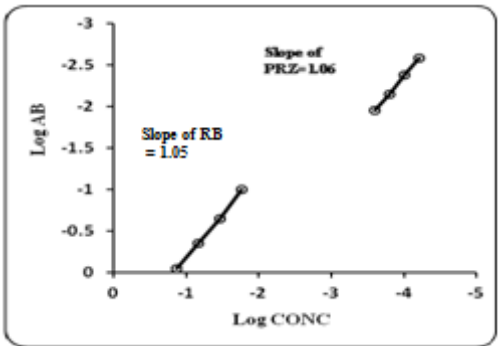
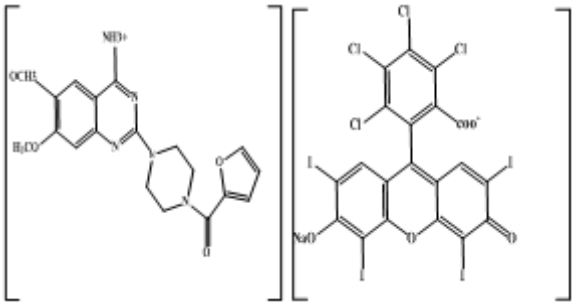


Figure 10: Limiting logarithmic plot for molar reactivity of PRZ with RB. C and ΔF are the concentration and difference of fluorescence intensity, respectively. For generating the first line (A),[RB]: 0.05 × 10^{-3}- 0.2X10^{-3} M; [PRZ]: 1 × 10^{-4} M. For generating the second Line (B), [PRZ]: 7.5 × 10^{-6} – 2.5 × 10^{-4}M; [RB]: 0.1×10^{3}M.



Scheme 2: Possible structure of ion-pair associate between the studied drug and RB

Analytical Performance

The absorbance-concentration plot was found to be linear over the range of 2.5−25 µg/mL. While, the native fluorescence-concentration plot is rectilinear over the range 0.05– 1.4 µg/ mL and difference in the fluorescence (ΔF) concentration plot was linear over the range of 0.5−8.0 µg mL^{-1} (Table 1).

Linear regression analysis of the data gave the following equations:
A = 0.0464C + 0.0712 (r = 0.9998) for method I
F = 286.58 C + 17.906 (r = 0.9998) for method IIa
ΔF= 7.839 C + 23.257 (r = 0.9998) for method IIb

Where A is the absorbance in 1 cm cell, C is the concentration of the drug (µg/mL), F is the native fluorescence of drug and ΔF is the native fluorescence of RB solution (F°) - fluorescence of the reaction product (F) and r is the correlation coefficient. The limit of quantitation (LOQ) was calculated according to ICH Q2 Recommendation [34] by establishing the lowest concentration that can be measured, below which the calibration graph is nonlinear and was found to be 2.7,0.06 and 0.55 µg/ mL for methods I , IIa, and IIb respectively.

Table 1: Performance data of the proposed methods

Parameter	Spectrophoto-metric Method	Spectrofluorimetric method		Official USP Method (1)
		Native method	Quenching method	
Molar absorptivity ($lmol^{-1}$ cm)	1.6x10^4			
Sandell sensitivity (µg cm^{-2})	0.026			
Linearity range (µgml-1)	2.5-25	0.05-1.4	0.5-8	
LOD (µg/mL)	0.89	0.019	0.182	
LOQ (µg/ mL)	2.7	0.006	0.55	
Slope(b)	0.0464	286.58	7.839	
Standard deviation of slope(Sb)	0.114	3.573	19.197	
Intercept(a)	-0.0712	17.906	23.257	
Standard deviation of intercept(Sa)	1.744	2.821	56.961	
coefficient(r2)	0.9998	0.9998	0.9999	
Standard deviation of residuals(Sy/x)	200	0.492	3.33	
% Error	0.013	0.20	0.014	
Mean found(%) ± SD	100.04±0.031	100.02±0.05	100.06±0035	100.0±0.042
Student's t-value (2.228)	1.23	1.64	1.88	
Variance ratio F-test (5.1)	3.57	2.98	3.75	

Sb = standard deviation of the slope of regression line
Sa = standard deviation of the intercept of regression line.
Sy/x = standard deviation of the residuals.
% Error = RSD% / √ n.
Values between parentheses are the tabulated t and F values respectively, at p=0.05

LOQ was calculated from the following equation (27):

$$LOQ = 10\ Sa/slope$$

The limit of detection (LOD) was also calculated according to ICH Q2 Recommendation (34) and was found to be 0.89, 0.019 and 0.182 µg mL−1 for methods I, IIa, and IIb respectively. LOD was calculated from the following equation (27):

LOD =3.3 Sa/slope

The proposed methods were evaluated by calculating the accuracy as percent relative error and precision as percent standard deviation (RSD %) (Table 1).

Validation of the method

The proposed methods were tested for linearity, specificity, accuracy and precision.

Linearity

Under the described experimental conditions, the calibration graphs for the three methods were constructed by plotting the absorbance value in method I, the native of fluorescence intensity (FI) in method IIa and difference in the fluorescence intensity (ΔF) in method IIb vs. concentration in µg mL^{-1}. The regression plots showed a linear dependence of FI, ΔF and absorbance values on the drug concentrations over the range cited in Table 1. Regression equations, intercepts, slopes and correlation coefficients for the calibration data are presented in Table 1. The validity of the method was evaluated by statistical evaluation of the regression lines regarding standard deviation of the residual (Sy/x), standard deviation of the intercept (Sa) and standard deviation of the slope (Sb). The small values of the figures point out to the low scattering of the points around the calibration graphs and high precision (Table 1).

Accuracy

Statistical analysis [28] of the results, obtained by the proposed and the reference method for PRZ using Student's t-test and variance ratio F-test, shows no significant difference between the performance of the three methods regarding the accuracy and precision, respectively (Table 1). The reference USP method [3] is based on HPLC method. It is tedious, cost, and time consuming method.

Precision

Repeatability

The repeatability (Intra-day) was performed through replicate analysis of three concentrations of the drug (5, 10, 20 µg/mL) for Method I, (0.2, 0.6, 1.0 µg/mL) for Method IIa and (2, 4, 6) for method IIb in pure form on three successive times, and the results are shown in Table 2. The low values of standard deviations indicate high accuracy of the proposed methods, while low values of % RSD indicate high precision of the proposed methods (Table 2).

Intermediate precision (Inter-day) It was performed through repeated analysis of the drug in pure form, using the concentrations (5, 10, 20 µg/mL) for Method I, (0.2, 0.6, 1.0 µg/mL) for Method IIa and (2, 4, 6) for method IIb for a period of three successive days. The low values of standard deviations indicate high accuracy of the proposed methods, while low values of % RSD indicate high precision of the proposed methods (Table 2).

Table 2: Validation of the proposed methods for the determination of prazosine HCl in drug substance

Conc. (µg/ml) Taken	Intra-days assay			Inter-days assay		
	Meth. I	Method II		Method I	Method II	
		Method IIa	Method IIb		Method IIa	Method IIb
	Found± %RSD[a]	Found± %RSD[a]	Found± %RSD[a]	Found± %RSD[a]	Found± %RSD[a]	Found± %RSD[a]
Meth. I						
5	4.9±0.32	4.95±0.3	5.10±0.1	4.9±0.2	2.0±0.3	5.2±0.6
10	10.1±0.2	9.93±0.6	9.91±0.3	9.91±0.3	4.01±0.2	10.1±0.3
20	19.9±0.2	20.1±0.5	19.9±0.2	19.93±0.1	5.98±0.3	20.1±0.3
Meth.IIa						
0.2	0.19±0.6	0.20±0.1	0.19±0.6	0.2±0.5	0.21±0.3	0.19±0.5
0.6	0.60±0.5	0.59±0.2	0.58±0.3	0.62±0.3	0.598±0.1	0.59±0.1
1.0	1.10±0.4	0.98±0.3	0.98±0.9	0.98±0.1	0.99±0.3	1.1±0.1
Meth. IIb						
2	1.91±0.1	2.0±0.3	2.0±0.3	2.0±0.3	0.21±0.3	1.98±0.6
4	3.97±0.2	3.98±0.5	3.9±0.5	4.01±0.2	0.598±0.1	3.91±0.3
6	6.01±0.3	5.9±0.6	6.1±0.5	5.98±0.3	0.99±0.3	5.97±0.3

[a] All the results are the average of three determination
method I : spectrophotometric method
method IIa: native of spectrofluorimetric method
method IIb: quenching of spectrofluorimetric method

Robustness and Ruggedness

The robustness of the proposed methods was examined against small, deliberate variations in the experimental parameters such as the change in the volume of ($0.2x10^{-3}$M) and ($0.1X10^{-3}$M) RB, (1.0±0.2 mL), the change in Clark and Lubs buffer solutions pH5.5±0.2. These minor change that may take place during the experimental operation did not affect the absorbance or fluorescence of the reaction product indicates that the method is robust as shown in (Table 3).

Table 3 Influence of small variations in the assay conditions on the analytical performance of the proposed methods (method I and method II) for determination of Prazosin HCl using Rose Bengal reagent.

Parameter	Recovery (mean% ± SD)[a]	
	Method I	Method IIb
RB concentration (%, w/v) 0.22%, 0.24% w/v	100.23 ± 0.123	
RB concentration (%, w/v) 0.12%, 0.14% w/v		100.45 ± 0.358
Volume of RB (1 ± 0.2ml)	100.54 ± 0.276	100.86 ± 0.164
The Clark and Lubs buffer solutions pH5.5±0.2.	100.02±0.452	100.06±0.312
[a] Values are mean of 3 determinations		

Pharmaceutical Applications

The proposed methods were applied to the determination of the studied drug in its drug product. Also the proposed method was tested for specificity and accuracy for tablets analysis (Table 4).

Table 4: Results of analysis of tablets by the proposed methods.

Pharmaceuticals	Spectrophotometric method	Spectrofluorimetric method		Official USP Method(3)
		Native method	Quenching method	
Minipress tablets 1.0mg PRZ.HCl/tab				
$\bar{X}$ ± SD	100.23±0.325	100.06±0.621	100.09±0.214	100.01±0.125
Student's *t* test (2.228)	1.954	1.320	1.251	
Variance ratio *F* test (5.1)	2.9	1.9	2.5	
Minipress tablets, batch #1415700, labeled to contain 1.0 mg				

Specificity

The specificity of the methods were investigated by observing any interference encountered from the common tablet excipients, such as talc, lactose, starch, avisil, gelatin, and magnesium stearate. These excipients didn't interfere with the proposed method.

Accuracy

The results of the proposed methods were compared with those obtained using the reference method (3). Statistical analysis (35) of the results obtained using Student's t-testand variance ratio F-test revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 4).

6. Conclusion

The present study describes two sensitive methods for the determination of prazosin HCl without interference from common tablet excipients. From economic point of view, the proposed methods are simple, rapid and inexpensive. They also have wider linear range with good accuracy and precision beside the use of water as diluting solvent. The most important advantage of the methods is that the ion-pair formed is measured directly without need for pretreatment of the drug and extraction with organic solvent. Hence, it can be applied for the routine quality control of the studied drug in its dosage forms.

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