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ANALYTICAL METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS ESTIMATION OF PROPRANOLOL & PRAZOSIN IN SYNTHETIC MIXTURE

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Abstract

A Simple, rapid, accurate, precise and economical, reproducible & cost effective UV Spectroscopy method have been developed and validated for the simultaneous determination of Propranolol (PRO) and Prazosin hydrochloride (PZH) in a synthetic mixture using the simultaneous equation method has been developed.

The method is based on the simultaneous equations for analysis of both the drugs using 0.1%W/V HCl in methanol as a solvent, the absorption maxima for Propranolol & Prazosin were found to be 289 nm and 329 nm respectively.

A linear response was observed in the range of 12-72µg/mL and 12-72µg/mL with a correlation coefficient of 0.998 & 0.999 for PRO and PZH respectively. For this two developed and validated methods the %RSD for precision was found to be less than 2%. Accuracy was determined by recovery studies and the mean recovery was 102.31 ± 0.001 and 102.5 ± 0.00574 for PRO and PZH, respectively.

Developed method was then validated for different parameters like accuracy, precision, sensitivity and linearity as per ICH Q2 (R1) (International Conference on Harmonization) guidelines.

The LOD and LOQ were found to 0.162µg/ml and 0.491µg/ml for PRO at 289nm &0.204µg/ml and 0.618µg/ml for PZH at 329nm respectively.

Keywords: Propranolol, Prazosin, UV Spectroscopy, Simultaneous estimation.

Introduction

Propranolol and Prazosin are available in tablet dosage form. Chemically, Propranolol (PAR) hydrochloride [1-[(1-methyl ethyl amino)-3-(1-napthyloxy) propan-2-ol is non selective adrenergic β-adrenergic agonist which competes with

sympathomimetic neurotransmitters such as catecholamines results in reduction in releasing heart rate, cardiac output, systolic & diastolic blood pressure.[1] Hence, it is used to treat tremors, angina, hypertension, rhythm disorders & myocardial infarction. Prazosin hydrochloride [2-{4-[(furan-2-yl) carbonyl] piperazin-1-yl}-6, 7-dimethoxy quinazolin-4-amine is antihypertensive agent of the quinazoline family. Acts by inhibiting α- adrenergic receptors on smooth muscle inhibits vasoconstrictor effect of locally released catecholamines, resulting in peripheral vasodilatation.[2] Hence, used to prevent strocks, heart attacks & kidney problems. Prazosin reduced arterial pressure and Propranolol lowered heart rate.[3]The hypotensive effect of Prazosin 2.5 mg/day combined with Propranolol 10mg/day was significantly greater than that of Propranolol alone and resulted in good control of blood pressure.[3]Propranolol & Prazosin are listed in the Merck Index.[4] as well as also official in I.P[5] and USP.[6]

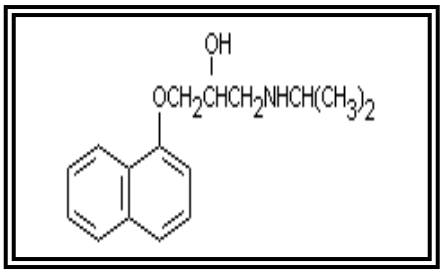

Fig: 1 Propranolol[1]

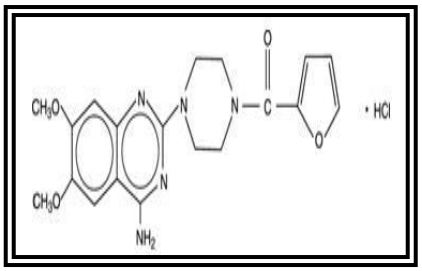

Fig:2 Prazosin Hydrochloride[2]

Literature survey reveals officials & various non officials analytical methods for determination of Propranolol such as UV Spectrophotometry[9],[10] HPLC[9] and HPTLC methods from pharmaceutical preparations. Few analytical methods for determination of Prazosin using UV Spectroscopy [12], HPLC[12],[14] and HPTLC in plasma[13] and pharmaceutical formulation have been reported.

However, there are no reported methods for simultaneous estimation of both drugs in combinations. This paper presents two simple, precise, reproducible and economical methods for the simultaneous analysis estimation of both the drugs in combination.

Materials and Methods

Instrument

- U.V. Visible Spectrophotometer: A Shimadzu model 1800 (Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell.
- Software: UV Probe version 2.31

Chemicals and Reagents

- The bulk drug Prazosin was obtained from West coast Pharmaceuticals, Ahmedabad and Propranolol was purchased from Swapnaroop drugs, Andheri, Mumbai. Analytical grade methanol was procured from Merck Fine chemicals (Mumbai).

Selection of a Solvent

- 0.1 %W/V HCl in methanol was selected as solvent for studying spectral characteristic of drugs.

Preparation of Standard Solution

(A) Preparation of standard stock solution for Propranolol (100µg/ml)

- Accurately weighed quantity of 10 mg of Propranolol was transferred into 100 ml volumetric flask and final volume was made with 0.1 %W/V HCl in methanol to get stock solution containing 100 µg/ml of Propranolol in 100ml volumetric flask.

Preparation of Working Standard Solution of Propranolol

- From the above stock solution pipette out 1.2 mL, 2.4mL, 3.6mL, 4.8mL, 6.0mL and 7.2mL of solution and transferred to 10 mL volumetric flask and make up the volume up to 10 mL with 0.1 %W/V HCl in methanol to Produce concentration 12, 24, 36, 48, 60, and 72 µg/mL respectively.

(B) Preparation of standard stock solution for Prazosin (100µg/ml)

- Accurately weighed quantity of 10 mg of Prazosin was transferred into 100 ml volumetric flask and final volume was made with diluent to get stock solution containing 100 µg/ml of Prazosin in 100ml volumetric flask.

Preparation of Working Standard Solution of Prazosin

- From the above stock solution pipette out 1.2 mL, 2.4mL, 3.6mL, 4.8mL, 6.0mL and 7.2mL of solution and transferred to 10 mL volumetric flask and make up the volume up to 10 mL with diluents 12, 24, 36, 48, 60, and 72 µg/mL respectively.

Selection of Analytical Wavelength

48µg/ml solution of Propranolol & 12µg/ml solution of Prazosin were prepared in 0.1% W/V HCl in methanol & spectrum was recorded between 200-400nm.Propranolol showed λ max at wavelength 289nm &Prazosin showed λ max at wavelength 329 nm.

Preparation of Calibration Curve

(A)Preparation of calibration curve for Propranolol:

This series consisted of different concentrations of standard Propranolol solution ranging from 12-72 µl/ml. Take 1.2, 2.4, 3.6, 4.8, 6, 7.2 ml of aliquots to the 10 ml volumetric flasks from 100 µg/ml of Propranolol stock solution and make up the volume to mark with mobile phase to get final concentration (12 - 72µg/ml) respectively. Plot the graph of Area V/S Concentration to get calibration curve.

(B) Preparation of calibration curve for Prazosin:

This series consisted of different concentrations of standard Prazosin solution ranging from 12-72 µg/ml. Take 1.2, 2.4, 3.6, 4.8, 6, 7.2 ml of aliquots to the 10 ml volumetric flasks from 100 µg/ml of Prazosin stock solution and make up the volume to mark with mobile phase to get final concentration (12-72µg/ml) respectively. Plot the graph of Area Vs Concentration to get calibration curve.

Preparation of Sample solution

About 10 mg of Synthetic mixture was weighed accurately and transferred into a 100 mL volumetric flask. The content was mixed with 0.1%W/V HCl in Methanol and sonicated for 20 min to dissolve the drug as completely as possible. The solution was then filtered through a Whatmann filter paper no. 41. The volume was adjusted up to mark with 0.1%W/V HCl in Methanol. The mixture contains 100µg/ml of Propranolol and 25µg/ml of Prazosin.

Validation [7]

Linearity and Range

The linearity response was determined by analyzing 6 independent levels of calibration curve in the range of 12-72 µg/ml & same for Prazosin. (n=3). Plot the calibration curve of Absorbance Vs Concentration & find out correlation co-efficient& regression line equations for Propranolol & Prazosin.

Precision

(A) Repeatability: (n=6)

2.4 ml of working standard solution (100µg/ml) of Propranolol & 2.4 ml of working standard solution (100µg/ml) of Prazosin was transferred in to the volumetric flask & dilute up to the mark with 0.1%W/V HCl in methanol to get

24µg/ml of Propranolol &Prazosin. Each of the concentration was prepared 6 times. The absorbance of solution was measured six times using simultaneous equation spectrophotometry and % RSD was calculated.

(B) Intraday Precision: (n=3)

Different concentration of Propranolol (12, 48, 72 µg/ml) &Prazosin (12, 48, 72µg/ml) solutions was analyzed 3 times on the same day using simultaneous equation spectrophotometry & % RSD was calculated.

(C) Interday Precision: (n=3)

Different concentration of Propranolol (12, 48, 72µg/ml) & Prazosin (12, 48, 72 µg/ml) solutions was analyzed on 3 different days using simultaneous equation spectrophotometry & % RSD was calculated.

Limit of detection (LOD)

The LOD was estimated from the set of calibration curves use to determine method linearity. The LOD may be calculated as

LOD= 3.3σ/s

Where,

σ= Standard deviation of the Y-intercepts of the 5 calibration curves.

S= Mean of the slope of the calibration curves.

Limit of quantification (LOQ)

The LOQ was estimated from the set of calibration curves use to determine method linearity. The LOQ may be calculated as

LOQ= 10σ/s

Where,

σ= Standard deviation of the Y-intercepts of the 5 calibration curves.

S= Mean of the slope of the calibration curves.

Accuracy (Recovery study) (n=3)

Preparation of sample solution:10mg solution of synthetic mixture was taken in 100 ml volumetric flask & was diluted up to the mark with 0.1% W/V HCl in methanol. The solution was sonicated for 15 minutes. The solution obtained was 100µg/ml of Propranolol & 25µg/ml of Prazosin.

To a fixed amount of above test sample (1.2 mL i.e.12µg/ml), increasing aliquots of Propranolol working standard solution (were added at 80,100,120% level i.e. 0.96 ml, 1.2 ml, 1.44 ml of 100µg/ml) were added in 10 ml volumetric flask & dilute up to the mark with 0.1% W/V HCl in methanol. Absorbance of solution was measured at 289nm & 329nm. The amount of Propranolol was calculated at each level & % recoveries were computed.

To a fixed amount of above test sample (1.2 mL i.e.3µg/ml increasing aliquots of Prazosin working standard solution (were added at 80,100,120% level i.e. 0.96 ml, 1.2 ml, 1.44 ml of synthetic mixture containing 25µg/ml Prazosin) were added in 10 ml volumetric flask now add 0.9 ml (9µg/ml) to all the three flask as a standard addition & dilute up to the mark with 0.1% W/V HCl in methanol.

Absorbance of solution was measured at 289nm & 329nm. The amount of Prazosin was calculated at each level & % recoveries were computed.

The amounts of Propranolol &Prazosin were estimated by applying obtained values to the simultaneous equations. The experiment was repeated for three times. (n=3).

Simultaneous Equation Method

Procedure:

The absorbances of both the drugs were recorded at 289 and 329 nm and molar absorptivity (Є) for both the drugs was calculated from the formula:

€ = A/C

Where, A = absorbance,

C = concentration of analyte in µg/100mL

Table-1: Absorptivity data for Propranolol and Prazosin.

Sr. No	Conc. (µg/ml)	Propranolol				Conc. (µg/ml)	Prazosin			
		Ax1	A x2	€x2	€x2		A y1	A y2	€y1	€y2
1	12	0.220	0	183.3	0	12	0.088	0.329	733	274.1
2	24	0.481	0	200.4	0	24	0.194	0.678	808.3	282.5
3	36	0.802	0	222.7	0	36	0.313	1.068	869.4	296.6
4	48	1.074	0	223.7	0	48	0.412	1.387	858	288.9

5	60	1.405	0	234.1	0	60	0.518	1.697	863.3	282.8
6	72	1.627	0	225.9	0	72	0.630	2.056	863.5	285.5
	Average			215.01		**Average**			832.58	285.06

2.1.4 Development of Simultaneous Equation:

If sample contains two absorbing substance (X and Y) and each of which absorbs at the Wavelength maxima of the other. Then it is possible to determine both the drugs by the technique of simultaneous Equation.

The information required is:

λ1: Wavelength maxima for Propranolol

λ2: Wavelength maxima for Prazosin

ax1 and ax2: Absorptivity of Propranolol at 289 nm and329 nm

ay1 and ay2: Absorptivity of Prazosin at 289 nm and 329 nm

A1: Absorbance of at Propranolol 289 nm

A2: Absorbance of at Prazosin 329 nm

Let CX and CY be the concentration of Propranolol and Prazosin respectively in the diluted sample

Cx = (A2ay1 – A1 ay2) / (ax2ay1 – ax1 ay2)

Cy = (A1ax2– A2 ax1) / (ax2ay1 - ax1 ay2)

Table-2: Linearity data of Propranolol.

AT 289 nm			**At 329 nm**		
Con (µg/ml)	**Mean absorbance ±SD(n=6)**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD (n=6)**	**%RSD**
12	0.2202 +/- 0.00042721	0.20394215	**12**	0	-
24	0.4806+/- 0.00054772	0.113966408	**24**	0	-
36	0.8022+/- 0.00044721	0.055748391	**36**	0	-
48	1.0742+/- 0.00044721	0.041632247	**48**	0	-

60	1.4052+/- 0.00044721	0.031825619	**60**	0	-
72	1.6247+/- 0.00054772	0.033656296	**72**	0	-

Table-3: Linearity data of Prazosin.

AT 289 nm			**At 329 nm**		
Con (µg/ml)	**Mean absorbance ±SD(n=6)**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD (n=6)**	**%RSD**
12	0.0882+/- 0.00044721	0.50704489	**12**	0.3292+/- 0.00045	0.13585
24	0.1938 +/- 0.00044721	0.2307603	**24**	0.6782+/- 0.00045	0.06594
36	0.3132+/- 0.00044721	0.1427885	**36**	1.0684+/- 0.00058	0.05404
48	0.4122+/- 0.00044721	0.1084943	**48**	1.3872+/- 0.00058	0.04162
60	0.5182+/- 0.00044721	0.08630135	**60**	1.6956+/- 0.00058	0.03405
72	0.6332+/- 0.00044721	0.07062754	**72**	2.0552+/- 0.00045	0.02176

Table-4: Repeatability data of Propranolol.

AT 289 nm			**At 329 nm**		
Con (µg/ml)	**Mean absorbance ±SD(n=6)**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD (n=6)**	**%RSD**
24	0.481+/- 0.00063246	0.1314876	**24**	0	-

Table-5: Repeatability data of Prazosin.

AT 289 nm			**At 329 nm**		
Con (µg/ml)	**Mean absorbance ±SD (n=6)**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD (n=6)**	**%RSD**
24	0.1946+/- 0.000816497	0.419433175	**24**	0.6785+/- 0.00083666	0.1233105

Table-6: Intraday Precision data of Propranolol.

AT 289 nm			**At 329 nm**		
Con (µg/ml)	**Mean absorbance ±SD (n=3)**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD (n=3)**	**%RSD**

12	0.22133+/- 0.004527	0.68910	**12**	0	-
48	1.07466+/- 0.0005774	0.053727	**48**	0	-
72	1.62833+/- 0.011547	0.070913	**72**	0	-

Table-7: Intraday Precision data of Prazosin.

AT 289 nm			**At 329 nm**		
Con (µg/ml)	**Mean absorbance ±SD (n=3)**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD (n=3)**	**%RSD**
12	0.089+/- 0.001	1.1235955	**12**	0.3293+/- 0.0005774	0.1753
48	0.414+/- 0.002081	0.5024134	**48**	1.0683+/- 0.0005774	0.054040
72	0.635+/-0.002	0.3149606	**72**	1.69733+/- 0.0005774	0.03401

Table-8: Inter day Precision data of Propranolol.

AT 289 nm			**At 329 nm**		
Con (µg/ml)	**Mean absorbance ±SD**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD**	**%RSD**
12	0.30266+/- 0.002516	0.83147	**12**	0	-
48	1.091666+/- 0.0018248	0.38137	**48**	0	-
72	1.709+/- 00182483	1.0677	**72**	0	-

Table-9: Interday Precision data of Prazosin.

AT 289 nm			**At 329 nm**		
Con (µg/ml)	**Mean absorbance ±SD**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD**	**%RSD**
12	0.0926667 +/- 0.0015275	1.6484085	**12**	0.3620+/- 0.003	0.82872
48	0.4916667 +/	0.62133662	**48**	1.411+/-0.008717	0.6178

	0.0030551-				
72	0.7043333 +/- 0.0125033	1.7752011	**72**	2.10233+/- 0.02657	1.2641

Table-10: Accuracy data for Propranolol &Prazosin at 289nm & 329nm.

Drug	Amount of sample (µg/ml)	Amount spiked (µg/ml)	Amount of standard added (µg/ml)	Total amount (µg)	Total amount found (µg)	% Recovery+/- S.D (n=3)	%RSD (n=3)
Propranolol	12	9.6	0	21.6	22.10	102.31 +/- 0.001	0.1644737
	12	12	0	24	24.01	100.041+/- 0	0
	12	14.4	0	26.4	26.38	99.93 +/- 0.00057	0.0807107
Prazosin	3	9	6.6	12	12.33	102.5 +/- 0.0005774	0.1615719
	3	9.6	6.6	12.6	12.95	102.77 +/- 0.0005774	0.1536869
	3	10.2	6.6	13.2	13.47	102.043+/- 0.00057	0.1477859

Table-11: Assay Study Parameter.

Propranolol			Prazosin		
Concentration (µg/mL)	**Amount found (µg/mL)**	**% Assay**	**Concentration (µg/mL)**	**Amount found (µg/mL)**	**% Assay**
48	48.33	100.687	12	12.33	102.75

Table-12: Optical regression characteristics and Validation parameters.

Parameters	Propranolol		Prazosin	
Wavelength (nm)	289	329	289	329
Beer's Law Limit (µg/ml)	12-72(µg/mL)	-	12-72(µg/mL)	12-72(µg/mL)
Regression equation (y = mx+c)	Y=0.00232x-0.071	-	Y=0.009x-0.019	Y=0.0028x-0.000
Standard deviation of the	0.00114	-	0.00548	0.001732

Y- intercepts of the 5 calibration curves				
Mean slope of the 5 calibration curves	0.0232	-	0.009	0.028
Correlation coefficient (r2)	0.998	-	0.999	0.998
Method Precision (Repeatability) (%RSD, n=6)	0.480-0.482	-	0.194-0.196	0.678-0.679
Intraday Precision (%RSD, n=3)	0.221-1.628	-	0.088-0.637	0.329-1.698
Interday Precision (%RSD, n=3)	0.302-1.079	-	0.091-0.713	0.359-2.13

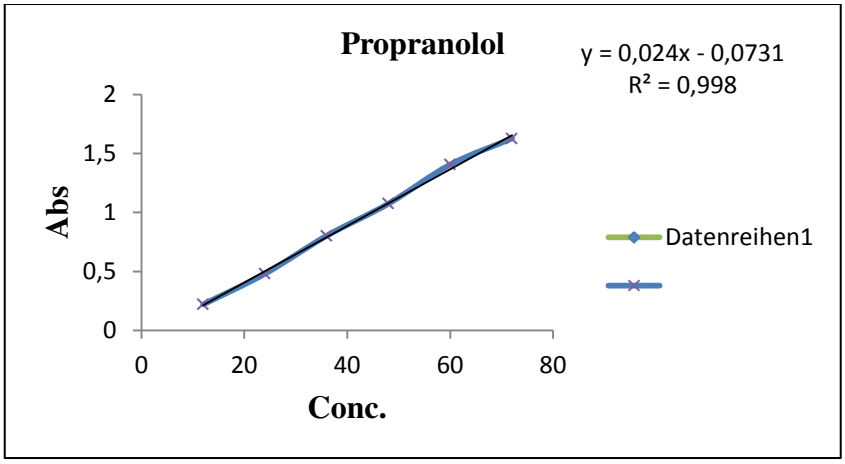

Fig.3. Calibration curve of Propranolol at 289nm.

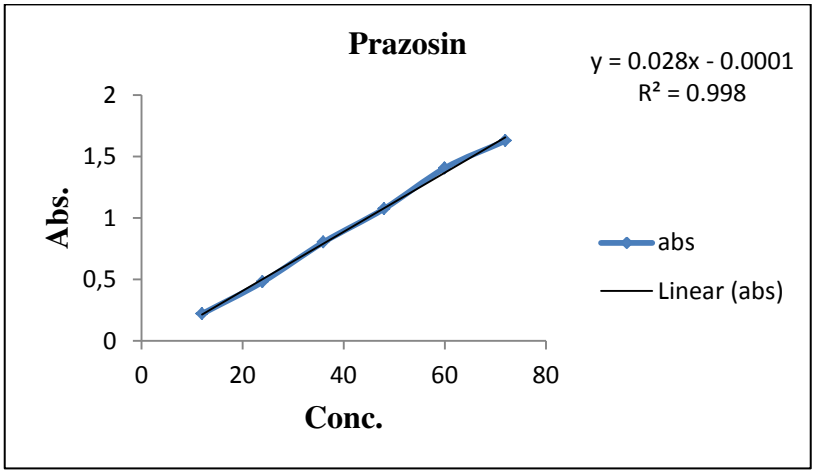

Fig.4. Calibration curve of Prazosin at 329nm.

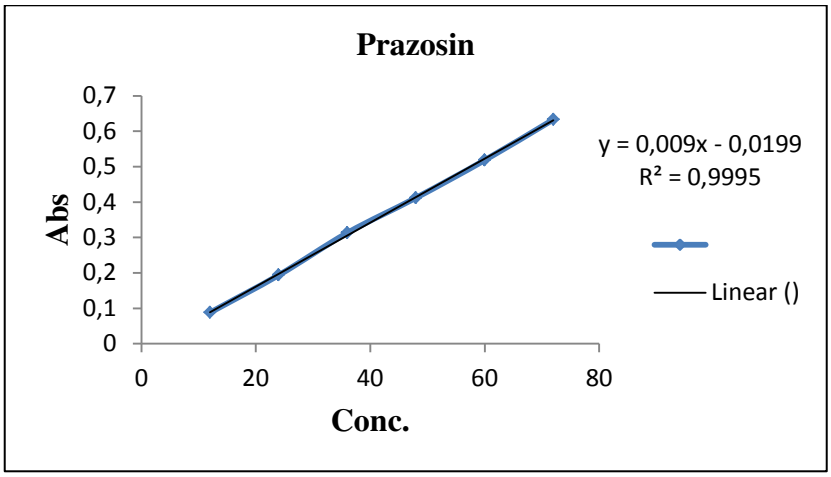

Fig.5. Calibration curve of Prazosin at 289nm.

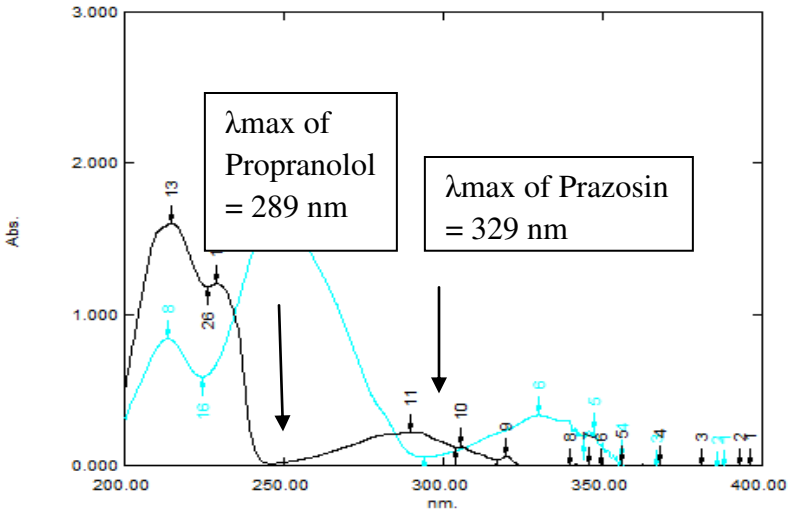

Fig.6. Overlay spectra of Propranolol (48µg/mL)&Prazosin (12µg/mL).

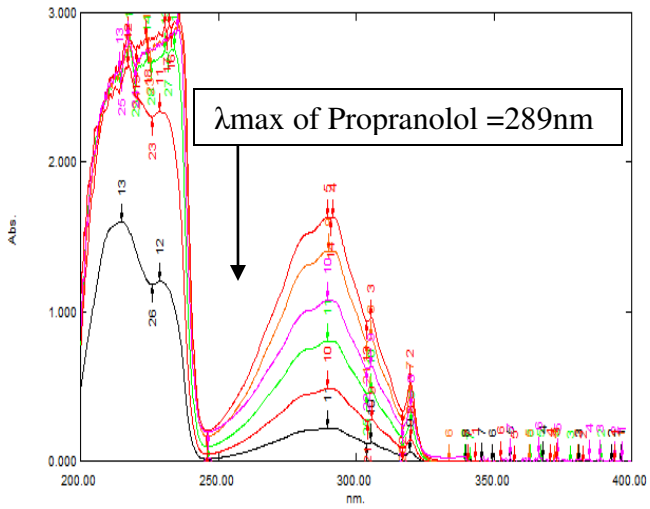

Fig.7. Overlay spectra of Propranolol (12-72µg/mL)

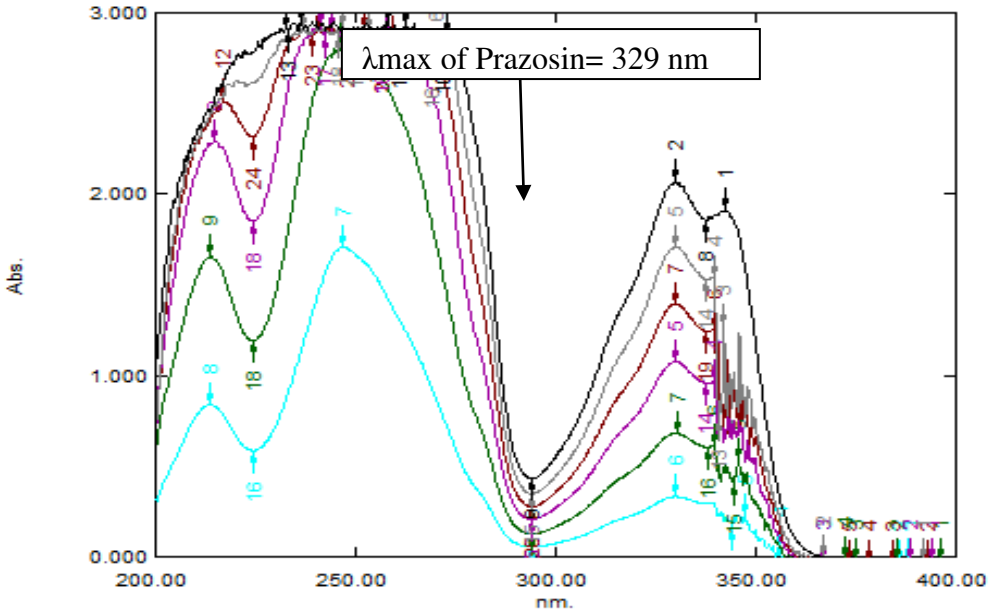

Fig.8. Overlay spectra of Prazosin (12-72µg/mL)

Results and Discussion

The calibration plot for the method was linear over the concentration range of 12-72µg/mL for PRO and 12-72µg/mL for PZH respectively. The determination of coefficients (R^2) was 0.998 and 0.999 for PRO and PZH respectively. The method was found to be precise and as the %RSD values for intraday and inter day were found to be less than 2% for PRO and PZH respectively. % recovery (99.93-102.77%) was found to be good at each added concentration, indicating that method was accurate. The LOD and LOQ were found to 0.162µg/ml and 0.491µg/ml for PRO at 289nm and 2.009 µg/mL and 6.088µg/ml for PZH at 289nm & 0.204µg/ml and 0.618µg/ml for PZH at 329nm respectively. Hence, the proposed method was specific for the estimation of Propranolol & Prazosin.

Conclusion

The proposed Spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of PRO and PZH in combined dosage form. The method utilizes easily available and cheap solvent for analysis of PRO and PZH hence, the method is economic for estimation of PRO and PZH in combinations. The common excipients and additives are usually added in the synthetic mixture do not interfere in the analysis of PRO and PZH in method, Hence it can be conveniently adopted for routine quality control analysis of the drugs in mixture or combined pharmaceutical formulation.

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