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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF ALPRAZOLAM AND PROPRANOLOL HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, precise and highly selective spectrophotometric method was developed for simultaneous estimation of Alprazolam and Propranolol hydrochloride in pure as well as tablet dosage form. The simultaneous equation method is based on measurement of absorbance at 263 nm and 289 nm as two wavelengths selected for quantification of Alprazolam and Propranolol Hydrochloride using 0.1 N HCl as a solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness. A double-beam shimadzu UV-visible spectrophotometer, 1800 with a pair of 1 cm matched quartz cells was used to measure the absorbance of the solutions in developed method. The method was validated as per ICH guidelines. Linearity ranges from 5-25 µg/ml for Alprazolam and 10-50 µg/ml for Propranolol hydrochloride of the drugs. % RSD calculated was less than equal to 2 which indicates accuracy and reproducibility of the method. Recovery study indicates that these drugs could be quantified simultaneously without interference of excipient present in formulation. The developed UV spectroscopic method is suitable for the analysis of ALP and PRP in combined dosage form. The accuracy was found between 98-100% for ALP and 99-100% for PRP respectively. The precision (%RSD) was found to be 0.308 for ALP and 0.875 for PRP respectively. The LOD was found to be 0.041µg/ml for ALP and 0.094µg/ml for PRP respectively.

KEYWORDS

Alprazolam, Propranolol Hydrochloride, Simultaneous Equation, Method Validation and UV Spectrophotometer.

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INTRODUCTION

Alprazolam (ALP), is a short-acting drug of the benzodiazepine[1], used to treat moderate to severe anxiety disorders and panic attacks and is used as an adjunctive treatment for anxiety associated with moderate depression. Alprazolam possesses anxiolytic, sedative, hypnotic, anticonvulsant, and muscle relaxant properties[2-4]. Alprazolam may be habit-forming, and long-term use and abuse may

cause a physical dependence to develop along with withdrawal reactions during abrupt or rapid discontinuation[5-6]. The side-effect of alprazolam may occur in some patients and are more likely to higher the dosage taken. Some side-effects may disappear with continued treatment. If signs of an allergic reaction occur such as hives, difficulty breathing, swelling of face, lips, tongue, or throat[7-8]. Propranolol (PRP), chemically (RS)-1-(isopropyl amino)-3-(1-napthyloxy) propan-2-ol is a non selective beta blocker, completely absorbed from gastrointestinal tract. It is mainly used in the treatment of hypertension by blocking the action of epinephrine on both β_1-β_2- adrenergic receptors and also used in the management of hypertension, angina pectoris, myocardial infarction, migraine, glaucoma etc[9-11].

Literature survey revealed that ALP has been estimated with other drugs using UV, HPLC, LCMS, Flourimetry and HPTLC. Similarly PRP has been determined along with other drugs by UV, HPLC, and HPTLC. The present study is to estimate ALP and PRP using a simple, sensitive, accurate, precise and more economical UV spectroscopic method[1-11].

MATERIAL AND METHODS

Instruments

For weighing, a calibrated weighing balance (Shimadzu) of 1mg sensitivity was used. A Shimadzu UV-visible double beam spectrophotometer- 1800 was used. All the glass wares and were made of borosilicate and were calibrated.

Material

Pure standards of ALP and PRP were obtained as a gift sample and their marketed combination (Beta Anxit-20) was purchased from the market. Methanol and 0.1 N HCl of analytical grade was used as the solvent. A double-beam shimadzu UV-visible spectrophotometer, 1800 with a pair of 1 cm matched quartz cells were used to measure the absorbance of the solutions.

UV Spectroscopic Method

Solvent Selection

Alprazolam and Propranolol hydrochloride is soluble in 0.1 N HCl and methanol so, for a good result 0.1 N HCl is used as the solvent.

Preparation of Standard Stock Solution

The standard stock solution of Alprazolam(ALP) and Propranolol hydrochloride (PRP) was prepared by transferring accurately weighed 10 mg of Alprazolam and Propranolol hydrochloride Separately into 10 ml volumetric flask containing 0.1 N HCl. Then volume was made up to the mark by using 0.1 N HCl to give a concentration of 1000µg / ml. From this, 1ml of the solution was transferred to a 10 ml volumetric flask and make up the volume with 0.1 N HCl to give a concentration of each 100µg/ml, which is a standard stock solution and it is further diluted with 0.1 N HCl to get concentration range of 10µg/ml of each Alprazolam (ALP) and Propranolol hydrochloride (PRP).

Determination of absorption maxima

The prepared standard solutions (10µg/ml) were scanned in the UV-VIS spectrophotometer in the wavelength range of 200-400 nm and an overlain spectrum was recorded. Using the overlain spectra, the wavelength maxima of both drugs, i.e. 263 nm (λ_1 for ALP) and 289 nm (λ_2 for PRP), were selected as two sampling wavelengths for simultaneous equation method. The prepared stock solutions were then diluted to get the solution of 5-25 µg/ml and 10-50 µg/ml for Alprazolam and Proprazolam hydrochloride respectively. The absorbance of these solutions were measured at the selected wavelengths and absorptivities were determined (Table No.1).

Vierodt's Method of Simultaneous Equations

This method is based on absorption of drugs at the wavelength maximum of the other. The concentrations of the drugs were calculated from the following equations:

$$C_x = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2} \quad \text{.....Eq. 1}$$

$$C_y = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2} \quad \text{.....Eq. 2}$$

Where, A_1 and A_2 are absorbance of mixture at 263 nm and 289 nm respectively, ax_1 and ax_2 are absorptivities of ALP at λ_1 and λ_2 respectively, ay_1 and ay_2 are absorptivities of PRP at λ_1 and λ_2 respectively. C_x and C_y are the concentrations of ALP and PRP respectively.

Quantitative Analysis of Tablet Dosage Form

20 Tablets of marketed formulation of Alprazolam 0.25 mg and Propranolol Hydrochloride 20 mg (Beta Anxit-20) respectively were weighed, their average weights determined. The correct amount of drug powder equivalent to label claim was weighed and transferred to 10 ml volumetric flask, dissolved in 0.1 N HCl and sonicated for 15 min. The volume was then made up to the mark using same solvent, from this 1 ml was taken and diluted to 10 ml with 0.1 N HCl which gives 0.25µg/ml of ALP and 20µg/ml of PRP. Absorbance of these sample solutions was recorded at 263 nm and 289 nm and then concentration of both the drugs was calculated using Equation 1 and 2 and the results are given in Table No.2.

Method Validation

The developed method was validated as per ICH guidelines for the following parameters:

Linearity

From the each 'Std Stock DMP' (100µg/ ml) and 'Std Stock PRP' (100µg/ml), 0.5, 1, 1.5, 2, 2.5 ml for ALP and 1, 2, 3, 4, 5 ml for PRP were transferred in a series of 10 ml volumetric flasks. The volume was made up to the mark with 0.1 N HCl to obtain the concentration of 5, 10, 15, 20, 25µg/ml and 10, 20, 30, 40, 50µg/ml for ALP and PRP respectively.

Calibration curves of ALP and PRP was constructed by plotting the Absorbance of ALP v/s Conc. of ALP and Absorbance of PRP v/s Conc. of PRP. The correlation coefficient (r^2) of least square linear regression for ALP and PRP was calculated.

Range

The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.

Accuracy

Recovery study was carried out by the standard addition method by adding a known amount of ALP and PRP to the pre-analyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated. 1 ml of tablet solution was transferred to 3 different 10 ml volumetric flask separately and 8, 10, 12µg/ml standard solution was added respectively and the volume was made up to the mark with 0.1 N HCl. Absorbance were noted for these samples. Standard deviation and % RSD was calculated. Accuracy is reported as % recovery, which was calculated from the expression as equation given below:

% Recovery = Observed value / True value ×100

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intra-day and inter-day precisions. Intraday precision was determined by analyzing the drugs at concentration (10µg/ml) for both the drugs and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis being carried out daily, for two consecutive days.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (10µg/ml) for both the drugs. Absorbance of each was measured.

Robustness

The robustness of the developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberate and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Limit of Detection

Detection limit was determined based on the standard deviation of absorbance of same

concentration that is a standard solution of ALP (10µg/ml) and PRP (10µg/ml) and LOD calculated by LOD = 3.3 (SD/S) Where, SD- standard deviation; S= slope of the curve.

Limit of Quantification

Quantification limit was determined based on the standard deviation of absorbance of same concentration that is standard solution ALP(10µg/ml) and PRP(10g/ml) prepared six times and LOQ calculated by LOD = 10(SD/S) Where, SD= standard deviation; S= slope of Curve.

RESULTS AND DISCUSSION

Linearity

The linearity of this method was determined at ranges from 5-25µg/ml and 10-50µg/ml for ALP and PRP respectively. The regression equation was found to be.

Accuracy

The accuracy of the analytical method for ALP and PRP was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 263 nm and 289 nm results were expressed in terms of % recoveries.

Precision

The precision (measurement of intra-day, inter-day, repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 2.0 %. This indicated that method was highly precise.

Preliminary Analysis of Alprazolam and Propranolol hydrochloride

Preliminary analysis of Alprazolam and Propranolol hydrochloride such as description, solubility was performed.

Assay of Tablet formulation

Amount of drug present in tablet formulation was calculated using simultaneous equation at 263 nm and 289 nm for ALP and PRP Respectively, and $y= 0.029x + 0.115$ and $y= 0.017x + 0.112$ for ALP and PRP respectively. Amount of Alprazolam and Propranolol hydrochloride were found to be 99% and 100% of label claim respectively. This method can be employed for routine analysis of both the drugs.

Summary and Conclusion

Summary of UV Spectrophotometric Method for Alprazolam and Propranolol hydrochloride.

Table No.1: Absorptivity of ALP and PRP at 263 nm, 289 nm respectively

S.No	Components (10µg/ml)	Absorptivity at 263 nm	Absorptivity at 289 nm
1	ALP	0.413	0.237
2	PRP	0.187	0.299

Table No.2: Result of analysis of the tablet Mixture

S.No	Drug	Label Claim (mg / tab.)	Amount found (mg)	% Drug found
1	ALP	0.25	0.248	99.2
2	PRP	20	19.79	98.95

Table No.2: Linearity of Alprazolam and Propranolol Hydrochloride

	Alprazolam (ALP)		Propranolol Hydrochloride (PRP)	
S.No	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	5	0.257	10	0.299
2	10	0.423	20	0.462
3	15	0.571	30	0.654
4	20	0.715	40	0.826
5	25	0.855	50	1.015
6	Regression equation: $y= 0.029x + 0.115$		Regression equation: $y= 0.017x + 0.112$	
7	$R^2= 0.999$		$R^2=0.999$	

Table No.3: Results for accuracy

S.No	Drug	Amount present (µg/ml)	Amount of standard drug added (µg/ml)	Amount Recovered (µg/ml)	%Recovery
1	ALP	10	80%(8µg/ml)	17.799	98.88
		10	100%(10µg/ml)	19.8	99.00
		10	120%(12µg/ml)	21.96	99.81
2	PRP	10	80%(8µg/ml)	18	100
		10	100%(10µg/ml)	19.7	98.5
		10	120%(12µg/ml)	21.8	99.09

Intra-day precision

Table No.4: Intra-day precision

S.No	ALP		PRP	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	10	0.420	10	0.292
2	10	0.421	10	0.290
3	10	0.422	10	0.292
4	10	0.423	10	0.299
5	10	0.423	10	0.299
6	10	0.423	10	0.299
7	% RSD	0.309	%RSD	1.453

Inter-day Precision

Table No.5: Inter-day precision

S.No	ALP		PRP	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	10	0.420	10	0.292
2	10	0.421	10	0.290
3	10	0.420	10	0.291
4	10	0.423	10	0.299
5	10	0.423	10	0.299
6	10	0.423	10	0.299
7	% RSD	0.308	%RSD	1.451

Repeatability

Table No.6: Repeatability study

S.No	ALP		PRP	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	10	0.420	10	0.299
2	10	0.421	10	0.298
3	10	0.420	10	0.293
4	10	0.423	10	0.299
5	10	0.423	10	0.299
6	10	0.423	10	0.298
7	% RSD	0.308	%RSD	0.875

Limit of Detection

Table No.7: For Limit of Detection

S.No	LOD (µg/ml)	Con.
1	ALP	0.041µg/ml
2	PRP	0.094 µg/ml

Limit of Quantification

Table No.8: For Limit of Quantification

S.No	LOQ (µg/ml)	Con.
1	ALP	0.125 µg/ml
2	PRP	0.285 µg/ml

Robustness

Table No.9: Result for robustness

S.No	Wavelength	Absorbance	
		ALP(10 µg/ml)	PRP(10 µg/ml)
1	Wavelength (1)	0.420	0.299
2	Wavelength 2	0.4222	0.297
3	Wavelength 3	0.421	0.295
4	%RSD	0.237%	0.23%

Ruggedness

Table No.10: Result for Ruggedness

S.No	Analyst	Absorbance	
		ALP(10 µg/ml)	PRP(10 µg/ml)
1	Analyst 1	0.420	0.299
2	Analyst2	0.421	0.298
3	Analyst3	0.420	0.299
4	%RSD	0.1%	0.12%

Table No.11: For Summary

S.No	Parameters	Values	
		ALP	PRP
1	Beer's Law limit (µg/ml)	5-25	10-50
2	Absorption maxima (nm)	263	289
3	Standard regression equation	*y= 0.029x + 0.115*	*y= 0.017x + 0.112*
4	Correlation coefficient (R^2)	0.999	0.999
5	Accuracy	98.75-102%	99-100
6	Precision (% RSD) Repeatability	0.308	0.875
7	LOD (µg/ml)	0.041	0.094
8	LOQ (µg/ml)	0.125	0.285
9	Robustness (%RSD)	0.237	0.23
10	Ruggedness (%RSD)	0.1	0.12
11	Assay (%)	99.20	98.95

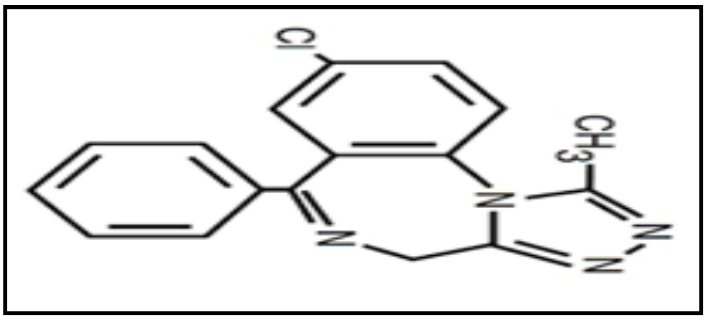

Figure No.1: Structure of Alprazolam

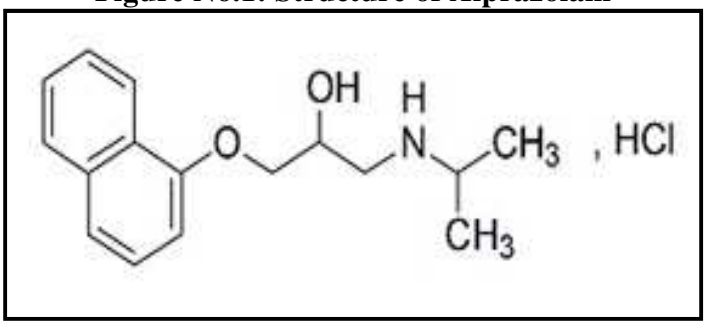

Figure No.2: Structure of Propranolol hydrochloride

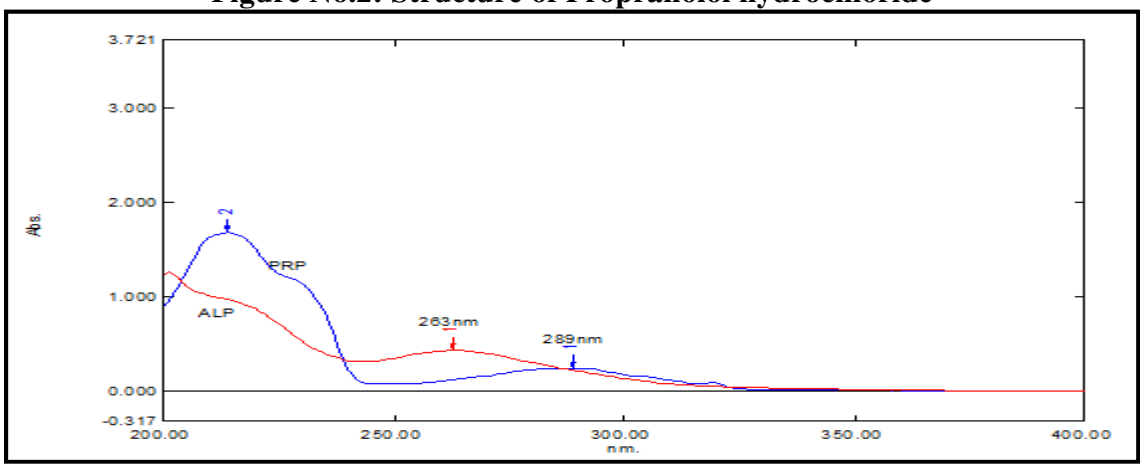

Figure No.3: Overlain spectra showing absorption maxima of ALP at 263 nm and PRP at 289 nm

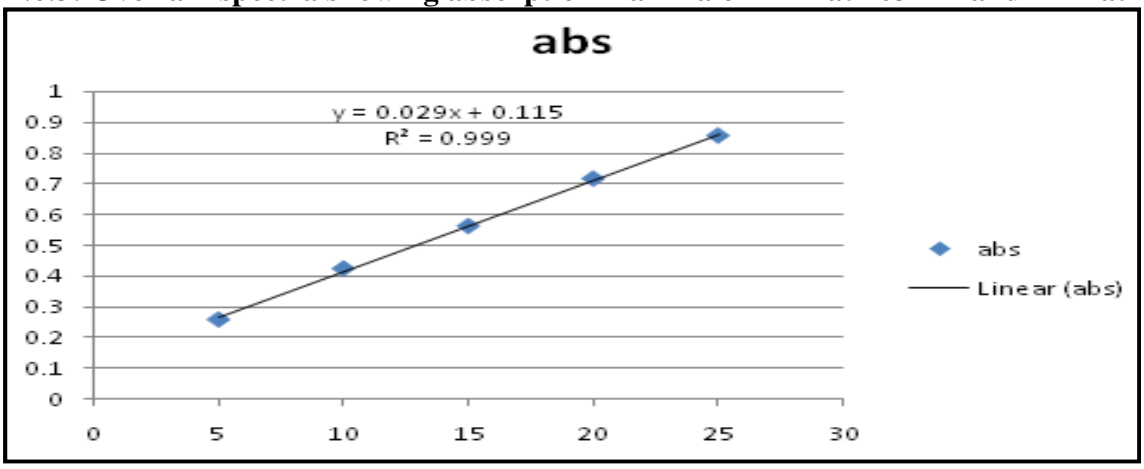

Figure No.4: Linearity of ALP

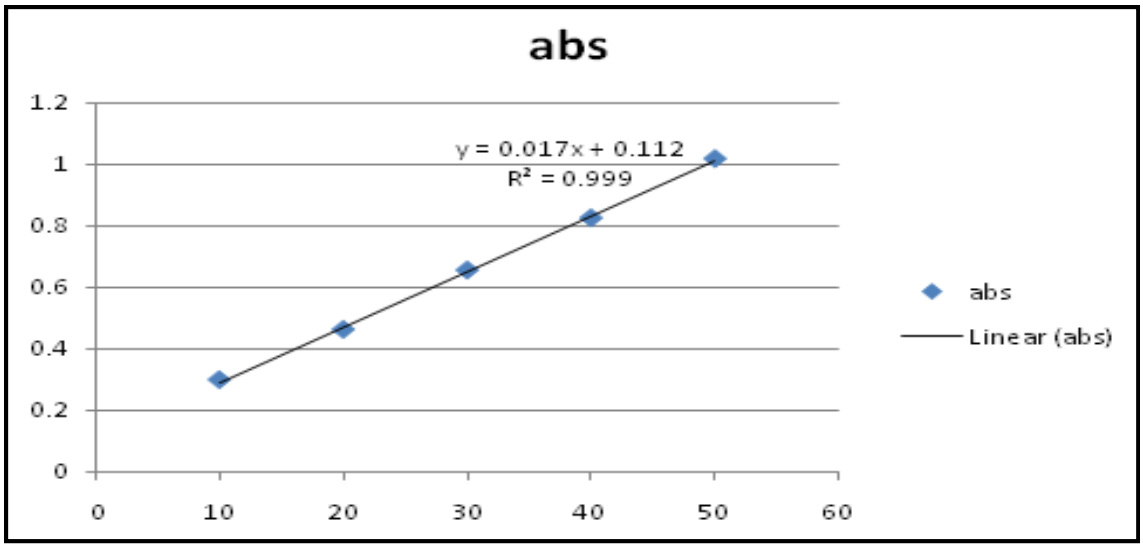

Figure No.5: Linearity of PRP

CONCLUSION

The UV-Spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Alprazolam and Propranolol hydrochloride in API and its dosage form without any interference from the excipients. This method can be effectively applied for the routine analysis of Alprazolam and Propranolol hydrochloride in API. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

ABBREVIATIONS

UV-Ultra Violet
API- Active Pharmaceutical Ingredient
ALP- Alprazolam
PRP- Propranolol hydrochloride
LOD- Limit of Detection
LOQ- Limit of Quantification

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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