

## Analytical Method Development and Validation for the Simultaneous estimation of Alprazolam and Propranolol in their combined dosage form

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### Abstract

To develop and validate simple, rapid, economic and accurate spectrophotometric methods for the estimation of the Alprazolam and Propranolol which can be applied successfully for the analysis of both the drugs in pure as well as in their combined dosage form.

A double-beam Shimadzu UV-Visible spectrophotometer, 1800, with a pair of 1cm matched quartz cells was used to measure the absorbance of the solutions in both developed methods viz. simultaneous equation method and absorption ratio method. 0.1N HCl was selected as solvent for the preparation of solutions. After scanning 10µg/ml solution of each drug separately in the range of 200-400 nm, wavelength of maximum absorption of ALP (262.5 nm) and PROP (288.5 nm) were selected for simultaneous equation method and isobestic point (283 nm) and absorption maxima of ALP (262.5 nm) were selected for absorption ratio method. The methods were validated statistically as per ICH guidelines.

Linearity ranges from 5-25 µg/ml for both drugs. %RSD calculated was less than equal to 2 which indicates accuracy and reproducibility of the method. Recovery studies indicate that these drugs could be quantified simultaneously without interference of the excipients present in formulation.

The developed UV spectroscopic methods are simple, precise, less time consuming, economical and accurate and thus are suitable for the analysis of ALP and PROP in combined dosage form.

**Keywords:** Alprazolam, Propranolol, Simultaneous Equation, Absorption Ratio, Validation

### Introduction

Alprazolam (ALP), chemically 8-chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine (Fig 1) is a potent short-acting drug of the benzodiazepine class which acts by enhancing the effects of a neurotransmitter GABA in the body and thus primarily used in the treatment of moderate to severe anxiety disorders and panic attacks and also possesses anxiolytic, sedative, hypnotic, anticonvulsant and amnesic properties[1-4] whereas Propranolol (PROP), chemically (*RS*)-1-(isopropylamino)-3-(1-naphthoxy) propan-2-ol (Fig 2) is a non-selective beta blocker and is mainly used in the treatment of hypertension by blocking the action of epinephrine and norepinephrine on both  $\beta_1$ - and  $\beta_2$ -adrenergic receptors and also used in the management of hypertension, angina pectoris, myocardial infarction, migraine, glaucoma etc. [5-7]

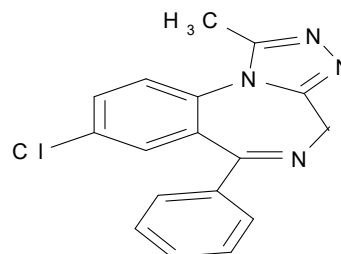


Fig 1: Chemical structure of Alprazolam

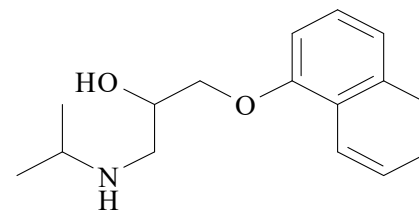


Fig 2: Chemical structure of Propranolol

Literature survey revealed that ALP has been estimated with other drugs using UV [8], HPLC [9-11], LCMS [12-13], fluorimetry [14] and HPTLC [9-10]. Similarly PROP has been determined along with other drugs by UV [15-16], HPLC [17-20] and HPTLC [21]. Since, no spectrophotometric method has been reported yet for simultaneous estimation of ALP and PROP, so the present study is focused on a successful attempt to estimate ALP and PROP using more economical UV spectrophotometric method..

## Materials and Methods

Pure standards of ALP and PROP were obtained as gift sample and their marketed combination (Ambulax-M) was purchased from the market. 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.

### Preparation of Standard Stock Solutions

Accurately weighed 10 mg of ALP and PROP were dissolved separately in small amount of 0.1N HCl in 10ml volumetric flask. The drug was dissolved with sonication and the final volume was adjusted with 0.1N HCl up to the mark to get a solution of 1000 µg/ml each and then further diluted to get 10µg/ml.

### 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 (Fig.3). Using the overlain spectra, the wavelength maxima of both drugs, i.e. 262.5 nm ( $\lambda_1$  for ALP) and 288.5 nm ( $\lambda_2$  for PROP), were selected as two sampling wavelengths for simultaneous equation method and 283.0 nm (isobestic point) was selected for absorbance ratio method. The prepared stock solutions were then diluted to get solutions of 5-25 µg/ml for both the drugs. The absorbance of these solutions were measured at the selected wavelengths and absorptivities were determined (Table 1).

**Table 1: Absorptivity of ALP and PROP at 262.5 nm, 288.5 nm and 283nm respectively.**

Components	Absorptivity at 262.5 nm	Absorptivity at 288.5 nm	Absorptivity at 283 nm
ALP	40	19.2	22.85
PROP	14.688	34.118	20.06

### Method A: Vierodt's Method of Simultaneous Equations

This method is based on absorption of drugs at the wavelength maximum of the other. It employs solving of simultaneous equations using Cramer's rule and matrices. The concentrations of the drugs were calculated from the following simultaneous equations:

$$C_x = \frac{A_1 a_{y2} - A_2 a_{y1}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \dots\dots\dots \text{Eq. 1}$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{y1} a_{x2} - a_{y2} a_{x1}} \dots\dots\dots \text{Eq. 2}$$

Where,  $A_1$  and  $A_2$  are absorbance of mixture at 262.5 nm and 288.5 nm respectively,  $a_{x1}$  and  $a_{x2}$  are absorptivities of ALP at  $\lambda_1$  and  $\lambda_2$  respectively and  $a_{y1}$  and  $a_{y2}$  are absorptivities of PROP at  $\lambda_1$  and  $\lambda_2$  respectively.  $C_x$  and  $C_y$  are the concentrations of PROP and ALP respectively.

### Method B: The Graphical Absorption Ratio Method (Q-Analysis)

This method is modification of simultaneous equation method which is based on measurement of absorbance at two wavelengths, one being the isoabsorptive point (298nm) and second be the  $\lambda_{max}$  of one of the component (288nm). The concentrations of drugs were determined by using the following equations,

$$C_x = (Q_m - Q_y) A_1 / (Q_x - Q_y) / a \dots\dots\dots \text{Eq. 3}$$

$$C_y = (Q_m - Q_x) A_1 / (Q_y - Q_x) / a_2 \dots\dots\dots \text{Eq. 4}$$

Where  $C_x$  and  $C_y$  are the concentration of ALP and PROP respectively,  $A_1$  is the absorbance of sample and  $a_1$  and  $a_2$  are absorptivities of ALP and PROP respectively at iso-absorptive wavelength,

$Q_m$  is absorbance of sample at  $\lambda_{max}$  / absorbance of sample at iso-absorptive wavelength

$Q_x$  is absorptivity of ALP at  $\lambda_{max}$  / absorptivity of ALP at iso-absorptive wavelength

$Q_y$  is absorptivity of PROP at  $\lambda_{max}$  / absorptivity of PROP at iso-absorptive wavelength

### Application Of The Developed Method On Tablet Dosage Form

Twenty tablets (Ambulax-M) were weighed, crushed and an accurately weighed sample equivalent to 10mg of PROP was taken in which 9.75 mg of pure ALP was added by standard addition method in order to bring both drugs in 1:1 ratio. The drug powder was dissolved in 0.1N HCl with sonication, filtered through Whatman filter paper and then volume was made up to 10 ml with 0.1N HCl to get stock solution of 1000 µg/ml of each drug and then further diluted to get 10µg/ml. All determinations were carried out three times at selected wavelengths and then the concentration of both the drugs was calculated using Equation 1 and 2 in Method I and Equations 3 and 4 in Method II and the results are given in Table 2.

### Analytical Method Validation

The developed method was validated according to the ICH guidelines [22-23] using following parameters.

#### Linearity

The linearity of this method was evaluated by linear regression analysis and both the drugs shows linearity in the concentration

range of 5-25  $\mu\text{g/ml}$ . Linearity graph is shown in Fig. 4 and regression coefficients are reported in Table III.

#### Limit of detection and quantification

Limit of detection (LOD) and Limit of quantification (LOQ) were calculated as  $3.3\sigma S$  and  $10\sigma S$ , respectively, where S is the slope of calibration curve and ' $\sigma$ ' is standard deviation of y-intercept of regression equation. (Table 3).

#### Inter-day and Intra-day Precision

Precision was studied by measuring absorbance of three replicate samples of 10  $\mu\text{g/ml}$  concentration in triplicate on same day (Intra-day precision) and on three consecutive days (Inter-day precision). The results are shown in Table 4

#### Accuracy (% Recovery)

The recovery studies were done by spiking the sample of known concentration (10  $\mu\text{g/ml}$ ) with known concentrations of the pure samples (80%, 100% and 120%) and then reanalyzed using the proposed methods. Percentage recovery was calculated using the equations for both the methods (Table 5).

## Results and Discussion

The methods discussed in the present work provide a convenient way to estimate ALP and PROP in pharmaceutical dosage form. Linearity was analyzed on selected wavelengths (262.5 nm ( $\lambda_{1\text{ALP}}$ ) and 288.5 nm ( $\lambda_{2\text{PROP}}$ ) and 283nm (isobestic point) and was found to be in concentration range 5-25 $\mu\text{g/ml}$  for both drugs (Table 3). Regression analysis was done and higher values of correlation coefficient ( $r^2$ ) indicate good linearity for both the drugs (Fig. 4).

The results of limit of detection (LOD) and limit of quantification (LOQ) shows the sensitivity of both the methods.

The precision of the proposed methods was determined by inter- and intra-day precision methods and the results are found to be 97-99.43% and 97.07-99.10% in case of inter-day precision and intra-day precision respectively (Table 4).

The amount of ALP and PROP in marketed formulation was determined by the proposed methods and ranges between 97.2-98.73% for simultaneous equation method and 98.7-99.56% for absorption ratio method (Table 2). % RSD calculated was less than 2 which indicates the accuracy and reproducibility of the method

The proposed method was validated according to the ICH guidelines. Low values of standard deviation show accuracy, repeatability and reproducibility of both the methods. The accuracy of the method was proved by performing recovery studies in which the results ranges from 98.56-100.36% in simultaneous equation method and from 99.7-100.16% in case of absorption ratio method (Table 5) which indicates that these drugs could be quantified simultaneously without the interference of the excipients present in the formulation.

Statistical analysis and drug recovery data showed that both methods are simple, rapid, economical, sensitive, precise and accurate and since no spectroscopic method has been reported therefore the proposed methods are suitable for the simultaneous determination of these drugs in pharmaceutical formulations therefore, the above method can be successfully applied in simultaneous estimation of ALP and PROP in marketed formulations with virtually no interference of the additives.

**Table 2: Result analysis of the tablet mixture**

Drug	Label claim (mg/ tab.)	Method 1		Method 2	
		Amount found (mg)	%Drug found $\pm$ SD	Amount found (mg)	%Drug found $\pm$ SD
ALP	0.25	0.243	97.2 $\pm$ 0.489	0.246	98.7 $\pm$ 0.637
PROP	10	9.873	98.73 $\pm$ 0.543	9.956	99.56 $\pm$ 0.946

Values expressed in mean  $\pm$  SD (n=3)

**Table 3: Optical characteristics**

Parameters	262.5 nm		288.5 nm		283 nm	
	ALP	PROP	ALP	PROP	ALP	PROP
Beer's law limit ( $\mu\text{g/ml}$ )	5-25	5-25	5-25	5-25	5-25	5-25
Regression equation	$Y = m X + c$					
Slope (m)	0.040	0.014	0.018	0.030	0.023	0.02
Intercept (c)	-0.007	0.004	0.005	0.017	-0.012	-0.001
Correlation coefficient ( $R^2$ )	0.999	0.998	0.999	0.998	0.999	0.999
Standard deviation SD	0.0005	0.0004	0.0003	0.0003	0.001	0.0005
Limit of detection ( $\mu\text{g/ml}$ )	0.041	0.094	0.055	0.033	0.143	0.0825
Limit of quantification ( $\mu\text{g/ml}$ )	0.125	0.285	0.166	0.1	0.434	0.25

Table 4: Intra-day precision and accuracy of ALP and PROP

	METHOD-I				METHOD-II			
	Intra day		Inter day		Intra day		Inter day	
	ALP	PROP	ALP	PROP	ALP	PROP	ALP	PROP
Mean% ± S.D.	97.07% ± 0.266	98.52 ± 0.302	97% ± 0.251	97% ± 0.0481	98.66% ± 1.268	99.1% ± 1.267	99.43% ± 0.594	98.73% ± 0.558
Precision, %RSD	0.274	0.307	0.259	0.496	1.285	1.279	0.597	0.565

Values expressed mean ± SD (n=3)

Table 5: Recovery studies

Drug	Amount present (µg/ml)	Amount of standard drug added (µg/ml)	Method I			Method II		
			Amount recovered (µg/ml)	%Recovery ± SD	% RSD	Amount recovered (µg/ml)	% Recovery ± SD	% RSD
ALP	10	80%(8µg/ml)	18.01	100.06 ± 0.735	0.735	18.06	100.33 ± 0.564	0.562
	10	100%(10µg/ml)	19.85	99.25 ± 1.268	1.278	20.02	100.10 ± 0.579	0.578
	10	120%(12µg/ml)	22.04	100.18 ± 0.510	0.509	21.96	99.81 ± 0.588	0.589
PROP	10	80%(8µg/ml)	17.96	99.77 ± 1.033	1.035	18.01	100.06 ± 0.589	0.589
	10	100%(10µg/ml)	19.98	99.90 ± 1.027	1.028	19.97	99.85 ± 1.247	1.249
	10	120%(12µg/ml)	21.98	99.91 ± 0.969	0.97	22.01	100.04 ± 0.999	0.999

Values expressed mean ± SD (n=3)

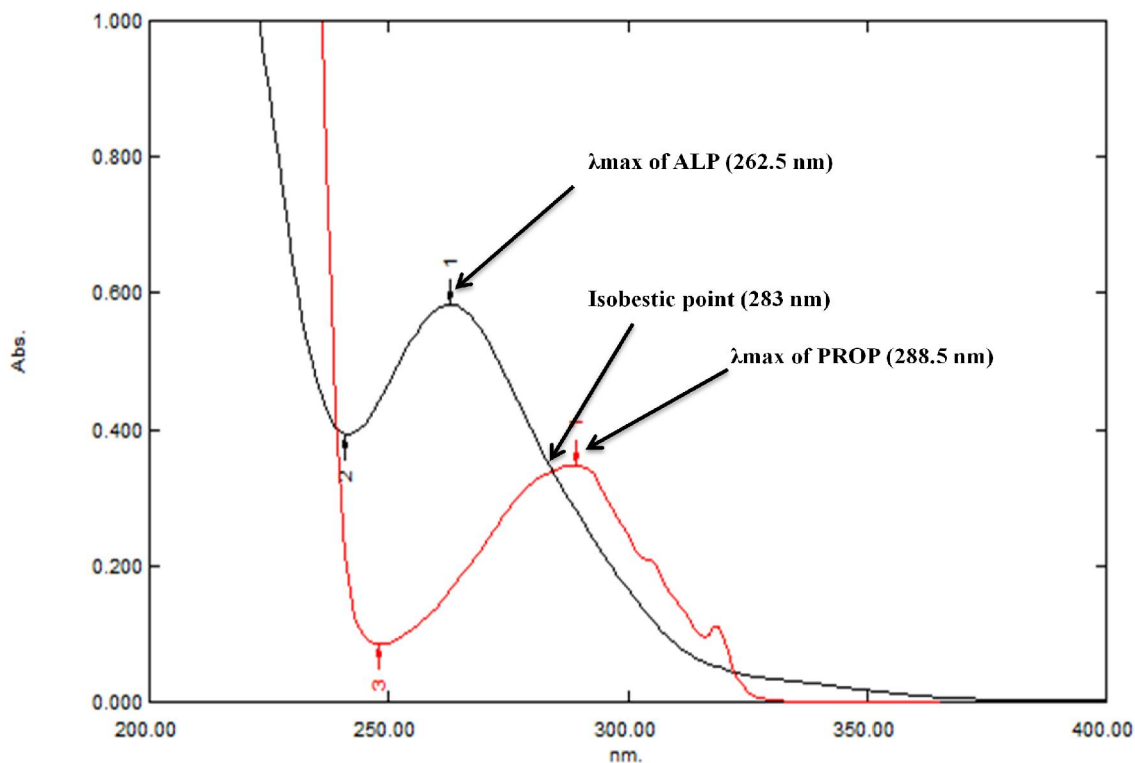


Figure 3: Overlain spectra showing absorption maxima of ALP at 262.5 nm and PROP at 288.5 nm &amp; Isobestic point at 283 nm.

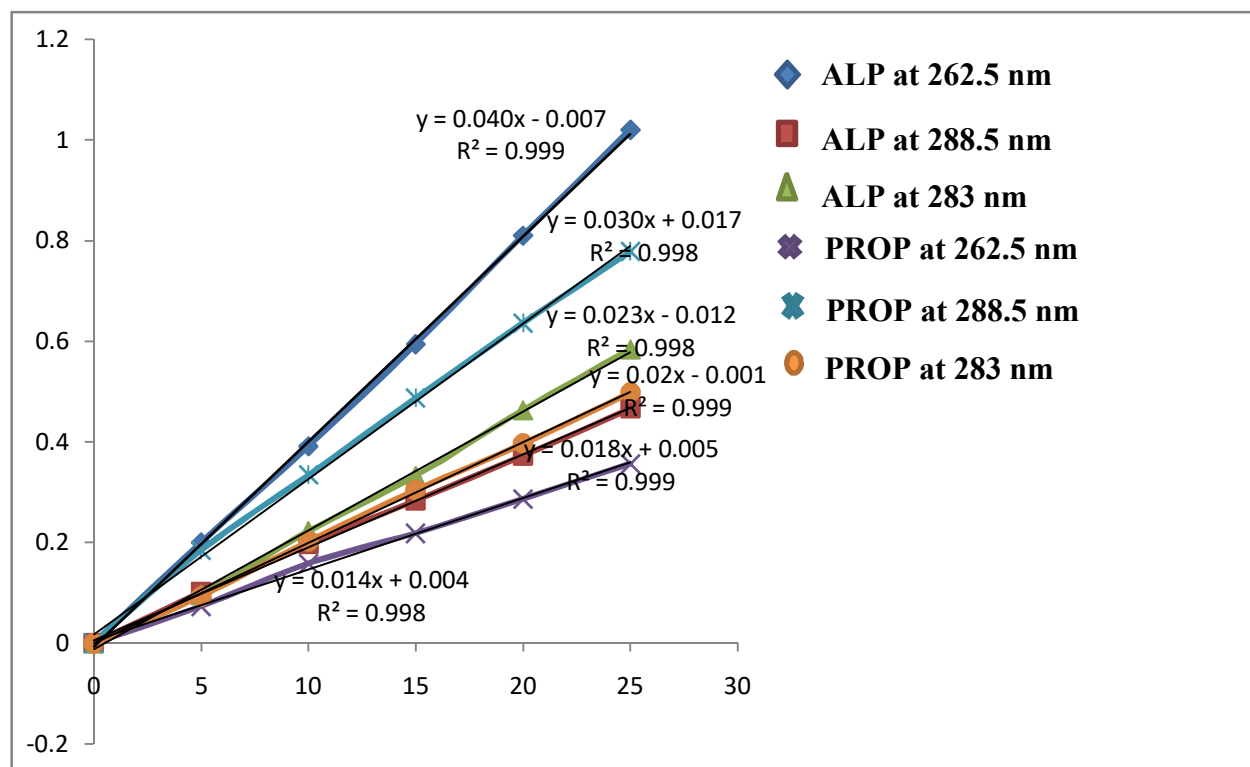


Figure 4: Linearity graphs of ALP and PROP at 262.5 ( $\lambda_{\text{max}}$  of ALP), 288.5 ( $\lambda_{\text{max}}$  of PROP) and 283 nm (Isobestic point).

## Conclusion

These results show that the proposed UV spectroscopic methods are simple, precise, economic, rapid and accurate therefore they are suitable for the analysis of ALP and PROP in the bulk and tablet dosage form without the interference of excipients.

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