Research Article

Stability Indicating Method Development and Validation for Determination of Alprazolam and Propranolol by RP-HPLC

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ABSTRACT

A simple, economic new stability indicating high performance liquid chromatographic method for Alprazolam and Propranolol in tablet dosage form. The chromatographic separation was achieved using Phenomenex C18 (150mm x 4.6mm, 5µm) column as stationary phase with Phenomenex Security Guard Cartridges C18, 4x3mm and methanol: phosphate buffer pH 7 (70:30) as mobile phase at 1.0ml/min flow rate. Detection was carried out at 221nm on Younglin Acme 9000 HPLC system. ALP and PRO were eluted at 3.8 min and 5.06 min respectively. The method was validated in accordance with ICH guidelines. The described method was linear over concentration range of 0.8-1.2 µg/ml (r2-0.996) for ALP and 64-96 µg/ml (r2-0.991) for PRO. The amounts of ALP and PRO in marketed formulation were 108% and 100% respectively. The developed method has adequate sensitivity, reproducibility and specificity for determination of ALP and PRO in tablet dosage form. The stability of method was demonstrated by forced degradation studies under influence of acid, alkaline, oxidative Stress, photolytic and thermal stress as per ICH Q1A (R2) guidelines. Results indicated ALP susceptible for acid hydrolysis, oxidative stress and PRO susceptible for alkaline hydrolysis and oxidative stress. Developed method is simple and stability indicating can be used for routine estimation of ALP and PRO in tablet dosage form.

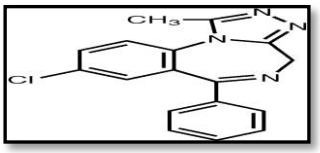
Keywords: Alprazolam, Propranolol hydrochloride, High performance liquid chromatography, Forced degradation study.

INTRODUCTION

Alprazolam (ALP) is a anxiolytic drug belonging to the class of benzodiazepine, chemically it is 8-Chloro-1-methyl-6-phenyl-4H-(1,2,4)triazolo(4,3-α)(1,4)-benzodiazepine (Fig.1a). It is used in the treatment of anxiety disorders, agoraphobia, panic disorders and depression [1-3].

Propranolol hydrochloride (PR0) is 1-[(1-methyl ethyl) amino] 3- (1-napthylenoylxy) 2 Propranolol hydrochloride (Fig.1b) is a nonselective β blocker, it blocks the action of epinephrine on both β1- and β2-adrenergic receptors. It is used for the treatment of angina pectoris, cardiac arrhythmia, hypertension, anxiety attacks and glaucoma [1-3].

The combination of ALP and PRH is approved in the treatment of anxiety. Literature survey revealed that few analytical methods reported for estimation of ALP alone and combination with other drugs UV-Spectrophotometric[4], RP-HPLC[5-6], HPTLC[6] similarly methods were reported for the determination of Propranolol by UV-Spectrophotometric[7-8], RP-HPLC[9-10], individual and combination with other drugs. Spectrophotometric[11], HPLC[12] method have been reported for simultaneous estimation of ALP and PRO, costly solvent are used, so need to develop economical and accurate method for estimation of ALP and PRO. The objective of present work was to develop stability indicating method for determination of alprazolam and propranolol by RP-HPLC



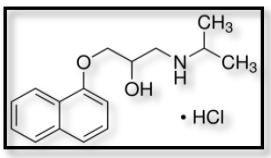


Figure 1: Structure of Alprazolam and propranolol

MATERIALS AND METHODS

Chemicals and Reagents

Alprazolam and Propranolol hydrochloride gift samples provided by Ipca Lab. Mumbai. Tablets of AZEN-P (Alprazolam 0.25mg, Propranolol hydrochloride 20 mg) were purchased from local market. Water LiChrosolv® (Merck Specialties Pvt. Ltd., Mumbai.), Methanol LiChrosolv® (Merck Specialties Pvt. Ltd., Mumbai.), Acetonitrile LiChrosolv® (Merck Specialties Pvt. Ltd., Mumbai.), Di potassium hydrogen phosphate anhydrous AR (Merck Specialties Pvt. Ltd., Mumbai.), Potassium dihydrogen orthophosphate AR (S.D. Fine-chem. Ltd., Mumbai).

Instruments

Chromatographic separation was performed on Younglin 9000 HPLC system. Chromatographic system equipped with Quaternary Gradient HPLC system (SP930D), fitted

with UV-VIS Detector. Phenomenex C18 (150 x 4.6mm, 5µm) column as stationary phase with Phenomenex Security Guard Cartridges C18, 4x3mm. The mobile phase was degassed by sonication using ultrasonic bath (Microclean-103). Shimadzu UV Spectrophotometer 1800 was used for wavelength selection. The standard substances were weighed on, electronic balance Shimadzu, Japan AY220. Vigo Melting point apparatus was used.

Preparation of Standard Stock Solution

Standard Stock Solution of ALP

10mg of standard ALP was weighed and transferred to a 10ml volumetric flask then dissolved in the methanol LiChrosolv®. The volume was made up to the mark with same solvent to obtain conc. of 1000µg/ml of ALP. From the resulting solution 1ml was diluted to 10ml with same solvent to obtain conc. of 100µg/ml of ALP,1 ml from above solution was diluted up to 10 ml with same solvent to obtain conc. 10µg/ml and labeled as 'Std Stock ALP'.

Standard Stock Solution of PRO

10mg of standard PRO was weighed and transferred to a 10ml volumetric flask then dissolved in the methanol LiChrosolv®. The volume was made up to the mark with same solvent to obtain conc. of 1000µg/ml of PRO. From the resulting solution 8 ml was diluted to 10ml with same solvent to obtain conc. of 800µg/ml of PRO and labeled as 'Std Stock PRO'.

Combined Standard Stock Solution of ALP and PRO

5ml of 'Std Stock ALP' (10µg/ml) and 5ml of 'Std Stock PRO' (800µg/ml) mixed to get conc. of 5µg/ml of ALP and 400µg/ml of PRO. The solution was labeled as 'Std Stock MIX'.

Selection of Analytical Wavelength

To investigate the appropriate wavelength for simultaneous determination of ALP and PRO, individual solutions in the mobile phase were scanned in the range of 200-300nm.

Chromatographic Condition

Analytical Column: Phenomenex C18 column (150 mm × 4.6 mm, 5 µm)

Mobile Phase: Methanol Phosphate Buffer pH7 (70:30)

Flow Rate: 1ml/min

Column temperature: Ambient

Injection Volume: 20 µl

Detection Wavelength: 221nm

Preparation of phosphate buffer pH7

Accurately weighed 0.136gm of potassium dihydrogen phosphate and 0.174gm of dipotasium hydrogen phosphate transferred in the 100ml volumetric flask and dissolved in HPLC water, then volume was made up to the mark with HPLC water.

Preparation of Mobile phase

Various combination of mobile phase including water, methanol, acetonitrile, phosphate buffer were tried finally Methanol and phosphate buffer of pH7 in the ratio (70:30) was selected for analysis of ALP and PRO.

Identification of Separated Peak of the Drugs

For identification of peak of the drugs; the standard solutions of ALP (10µg/ml) and PRO (10µg/ml) were injected separately into HPLC system and retention time were matched with retention time of mixture.

Method Validation

The method was validated according to ICH guidelines with respect to specificity, accuracy, precision, linearity, robustness, ruggedness, system suitability.

Specificity

The chromatogram of standard solution of mixture of ALP and PRO was compared with formulation to observe the interference of excipient.

Linearity

From the 'Std Stock MIX' (ALP 5µg/ml & PRO 400µg/ml) solution, 1.6, 1.8, 2, 2.2 and 2.4ml were transferred in a series of 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the conc. of 0.8, 0.9, 1, 1.1 and 1.2µg/ml of ALP and 64, 72, 80, 88 and 96µg/ml of PRO.

The solutions were filtered through syringe filter and 20µl injected into the HPLC system and their chromatogram were recorded for 10mins. Peak areas were recorded for all the peaks. Calibration curves of ALP and PRO were constructed by plotting the peak area *v/s* conc. The correlation coefficient (r^2) of least square linear regression for ALP and PRO was calculated.

Range

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

Accuracy

From the 'Sample Stock 1' solution 1 ml was transferred to four different 10ml volumetric flasks separately along with 0, 0.8, 1, 1.2 ml from the 'Std Stock MIX' (ALP-5µg/ml and PRO -400µg/ml) solution. The volume was made up to the mark with mobile phase. All the solutions were filtered through syringe filter and injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditions after getting a stable baseline. Peak areas were recorded for all the peaks and percent recoveries were calculated.

Precision

The precision of an analytical method was studied by performing Repeatability and intermediate precision.

Repeatability

Repeatability was determined by analyzing the standard solution of ALP (1µg/ml) and PRO (80µg/ml) six times and %RSD calculated

Intermediate Precision Intra-day Precision

Intra-day precision was determined by analyzing the standard solution of ALP (1µg/ml) and PRO (80µg/ml) at 9.00am and 6.00pm on same day following the procedure of repeatability.

Robustness

Combined standard solution of ALP (1µg/ml) and PRO (80µg/ml) was prepared and analyzed at different flow rates (0.9, 1.0, 1.1 ml/min) separately.

System Suitability

Sample solutions of ALP (1µg/ml) and PRO (80µg/ml) were prepared and analyzed six times. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with the recommended limit or not.

Assay of Tablet Formulation

Twenty Tablets (Alprazolam 0.25mg, Propranolol hydrochloride 20mg) were weighed and finely powdered. Tablet powder equivalent to 0.25mg of ALP & 20mg of PRO was transferred to 50ml volumetric flask. 5ml methanol was added, sonicated for 10min, diluted with methanol to volume, and filtered through whatman filter paper No. 41 ('Sample Stock1'). 2ml of resulting solution diluted upto10ml with mobile phase to obtain 1µg/ml ALP and 80µg/ml PRO ('Sample Stock2').

Sample Stock2 was filtered through syringe filter and injected into HPLC system. The amount of ALP and PRO present in the tablets were calculated using calibration curve equations, y= 142x+6.2 and y= 130.91x+1058.4 respectively.

Forced degradation study

Forced degradation study performed on ALP and PRO both standard sample and tablet formulation

Preparation of standard stock solution of ALP (API)

20mg of standard ALP was weighed and transferred to a 20ml volumetric flask then dissolved in the methanol LiChrosolv®. The volume was made up to the mark with methanol to obtain conc. of 1000µg/ml of ALP (Sample stock A).1 ml of above solution dilute up to 10 ml with methanol resulting concentration 100µ/ml. 4ml of this solution diluted with mobile phase up-to 10ml concentration of resulting solution 40µg/ml. solution filtered through syringe filter and injected in HPLC. This is chromatograph of fresh sample.

Preparation of standard stock solution of PRO (API)

20mg of standard PRO was weighed and transferred to a 20ml volumetric flask then dissolved in the methanol LiChrosolv®. The volume was made up to the mark with methanol to obtain conc. of 1000µg/ml of PRO (Sample stock A).1 ml of above solution dilute up to 10 ml with methanol resulting concentration 100µ/ml. 4ml of this solution diluted with mobile phase up-to 10ml concentration of resulting solution 40µg/ml. solution filtered through syringe filter and injected in HPLC. (Chromatograph of fresh sample as a reference)

To conduct forced degradation study on API, standard stock solution was prepared using above procedure. These solutions subjected to acid hydrolysis i.e. refluxing with 0.1 N HCL at 70^{0}C. The mixture was neutralized by using 0.1N NAOH. Alkaline hydrolysis i.e. refluxing with 0.1N NAOH 70^{0}C. The mixture was neutralized by using 0.1N HCL. Oxidation using 5% H_2O_2, heating at 70^{0}C for 24 hrs. Photolysis degradation using UV Cabinet for 24 hrs. Thermal degradation using hot air oven at 70^{0}C, 24hrs.

Stress study on tablet formulation was performed by exposing tablets to oxidative, thermal, photolytic conditions. Stressed samples analyzed by validated HPLC method. Chromatograms of stressed samples were determined for peak purity.

RESULT and DISCUSSION

Wavelength selection

ALP (10µg/ml) and PRO (10µg/ml) in mobile phase were scanned separately in range of 200-400 nm.

λ_{max} of ALP 221nm and PRO 290nm.based on spectral characteristic and overlain spectra 221 selected as wavelength for analysis.

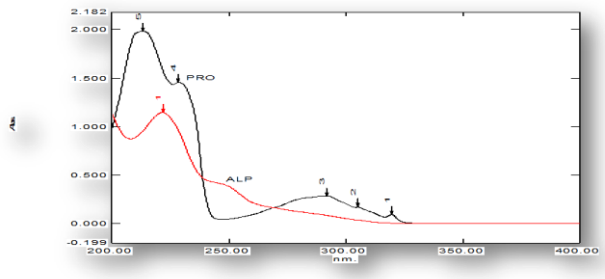


Figure 2: Overlain spectra of ALP and PRO between 200-400nm in mobile phase

Linearity and Range

Graphs were plotted concentration in µg/ml on X axis verses area on Y axis and the correlation coefficients were determined. The method was found to be linear in concentration range of 0.8-1.2µg/ml for ALP and 64-96 µg/ml for PRO.

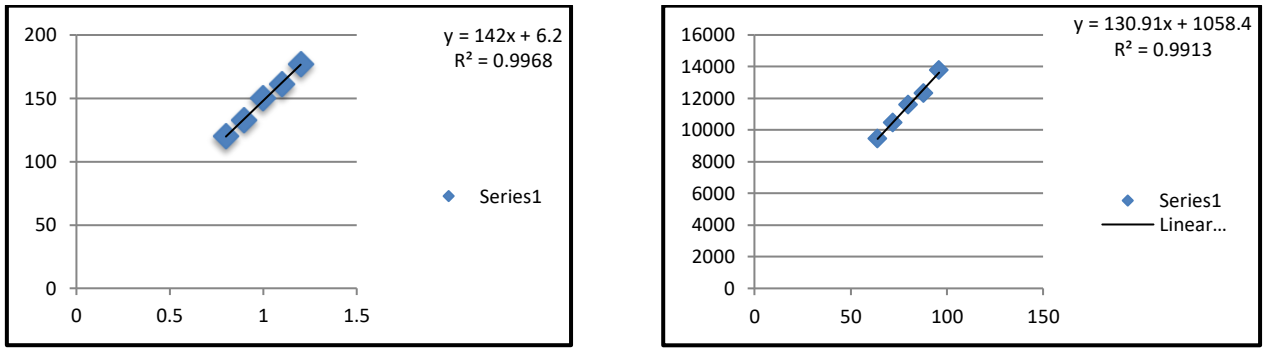


Figure 3: Calibration curve of ALP and PRO by RP-HPLC method

Accuracy

This technique involves the addition of standard drug solution to preanalyzed sample solution at 80%, 100%, and 120%. The % recoveries were 100-106 % (ALP), 105-107% (PRO).

Table 1: Result of Accuracy for RP-HPLC Method (n=3)

Sr. No.	Level of % Recovery	Amount of Sample (µg/ml)		Amount of Standard Drug Added (µg/ml)		Total Amount Found (µg/ml)		Amount Recovered (µg/ml)		% Recovery	
		ALP	PRO	ALP	PRO	ALP	PRO	ALP	PRO	ALP	PRO
1	0	0.5	40	-	-	0.47	36.14	-	-	-	-
2	80	0.5	40	0.4	32	0.87	70.14	0.401	34	100	106
3	100	0.5	40	0.5	40	0.998	78.14	0.528	42	105	105
4	120	0.5	40	0.6	48	1.11	87.64	0.640	51.5	106	107

Precision

Table 2: Result of Repeatability and intermediate precision Study for ALP and PRO

Inj.	Repeatability		Intermediate Precision			
	Peak Area(mV)		Peak Area at 9 am		Peak Area at 6 pm	
	ALP	PRO	ALP	PRO	ALP	PRO
1	155.96	11951.27	153	11542	155.96	11951.27
2	153.82	11845	151.1	11681.9	153.82	11845
3	154.84	11964.5	152.3	11677.8	154.84	11964.5
4.	151.5	10998	154	11700.2	153	11670.1
5.	152.75	11580	153.8	11650.3	152.8	11600.2
6.	154.05	11700	152.9	11600.5	154	11650.3
%RSD	1	1.4	0.69	0.51	0.76	1.3

Robustness

Table 3: Result of Robustness Study: Variation in Flow Rate (ml/min)

Inj.	Flow Rate (ml/min)	Analyte	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
1.	1	ALP	3.87	1.31	4250	4.6
		PRO	5.06	1.3	5517	
2.	0.9	ALP	3.88	1.31	4111	4.8
		PRO	5.06	1.3	6327	
3.	1.1	ALP	3.88	1.33	4295	4.5
		PRO	5.07	1.34	5696	

Assay

The amounts of ALP and PRO in marketed formulation were 108% and 100% respectively

System Suitability Testing

Table 4: Results of System Suitability Parameters

Analyte	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
ALP	3.8	1.31	4250	4.63
PRO	5.06	1.32	5517	

Table 5: Forced degradation study of ALP and PRO

Name	Condition	Time	% Recovery	% Degradation
ALP Standard	Acid hydrolysis / 0.1N HCL/heating at 70^{0}C	1 hrs	37.8	62.2
	Alkaline hydrolysis/0.1N HCL/heating at 70^{0}C	24hrs	90	10
	Oxidation/Room temp.	24 hrs	87	13
	Photolysis /UV Cabinet	24 hrs	98.42	1.58
	Thermal Hot air oven/70^{0}C	24hrs	98	2
ALP Sample	oxidation/5% H_2O_2	24 hrs	89	11
	Photolysis/ UV Cabinet	24 hrs	97	3
	Thermal/Hot-air oven/70^{0}C	24 hrs	99	1
PRO Standard	Acid hydrolysis/ 0.1N HCL/heating at 70^{0}C	24 hrs	98.3	1.7
	Alkaline hydrolysis/ 0.1N NAOH/heating at 70^{0}C	24 hrs	71.84	28.16
	oxidation/ 5% H_2O_2 /heating at 70^{0}C	24 hrs	73.9	26.1
	Photolysis/ UV Cabinet	24 hrs	85.8	14.2
	Thermal /Hot air oven/70^{0}C	24 hrs	95.5	4.5
PRO Sample	oxidation/5% H_2O_2	24 hrs	92.3	7.7
	Photolysis/ UV Cabinet	24 hrs	81	19
	Thermal/Hot-air oven/70^{0}C	24 hrs	94.5	5.5

CONCLUSION

Developed RP-HPLC method was simple, accurate, precise, linear, robust, economical can be applied for routine analysis of Alprazolam and Propranolol hydrochloride in tablet dosage form

Results of stress study shows there was no other co-eluting peak with main peak and method is specific for estimation of Alprazolam and Propranolol hydrochloride in presence of their degradation products. Developed method is stability indicating method can be used for routine estimation of ALP and PRO in tablet dosage form.

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