METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PROPRANOLOL AND ALPRAZOLAM IN A COMBINED TABLET DOSAGE FORM

Shriya.N*[1], Dr. S. Shobha Rani[2], Dr. M. Ajitha[3], Sumit Agarwal[4], Dr. Y. Sridhar Reddy[5], Karthik.M[6], Mohammad Naseeruddin[7]

1. Research Scientist, Department of Pharmaceutical Analysis, CPS, IST, Jawaharlal Nehru Technological University, Hyderabad.
2. Professor, Pharmaceutical Sciences, CPS, IST, Jawaharlal Nehru Technological University, Hyderabad.
3. Professor, Pharmaceutical Sciences, CPS, IST, Jawaharlal Nehru Technological University, Hyderabad.
4. Director, Sain Medicaments Pvt Ltd, Telangana.
5. Senior Research Scientist, Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad.
6. Analytical Scientist, Analytical Research and Development, Dr. Reddy's Laboratories Ltd, Hyderabad.
7. Senior Executive, Analytical Research and Development, Aizant Drug Research Solutions Pvt. Ltd. Telangana.

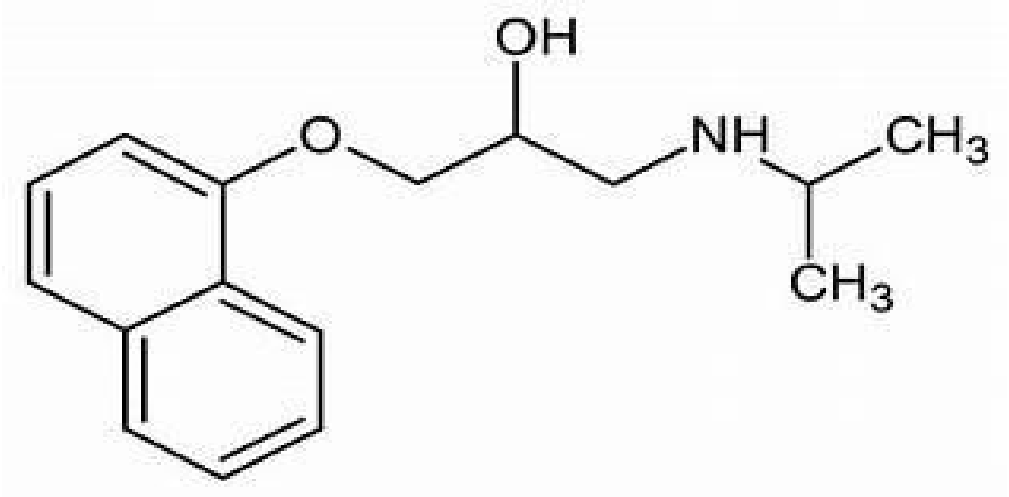
Corresponding Author:
Shriya.N*
Department of Pharmaceutical Analysis, CPS, IST,
Jawaharlal Nehru Technological University, Hyderabad.

Abstract
A RP-HPLC method was developed and validated for the simultaneous estimation of Propranolol and Alprazolam in a combined tablet dosage form by using Waters HPLC 2965 System with Auto-Injector and PDA detector with the Empower 3 software. Waters symmetry shield RP C18, 150 x 4.6 mm, 5 µm column was used as stationary phase and pH 4.5 Phosphate buffer and Methanol in ratio 70:30 % v/v was used as mobile phase with isocratic flow. Flow rate 1.0 mL/min was chosen, Injection volume was10 µL. Detection wavelength of 280 nm was chosen based on isosbestic point of Propranolol and Alprazolam. Runtime of chromatogram 7 minutes was chosen based on separation of drugs. The retention times of Propranolol and Alprazolam were found to be 5.5 minutes and 3.7 minutes respectively. Validation results have shown that the method met all the validation parameters acceptance criteria as per ICH Q2 guidelines and is suitable for estimation of Propranolol and Alprazolam in a combined tablet dosage form.
Key words: Propranolol, Alprazolam, Simultaneous Estimation, Combined Tablet Dosage Form, RP-HPLC.

Introduction

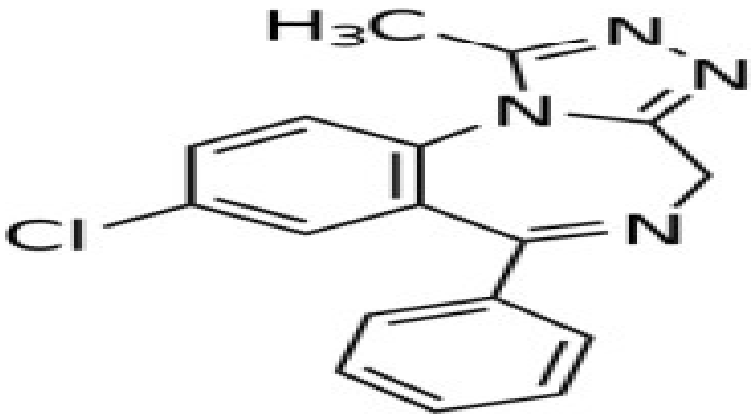
Propranolol is a beta-blocker used to treat a variety of conditions including angina (chest pain due to heart problems), arrhythmias, heart attacks, high blood pressure (hypertension), anxiety, tremors, migraines, and pheochromocytoma. It works by influencing how the body responds to nerve signals, particularly those affecting the heart. By slowing the heart rate and making it easier for the heart to pump blood, Propranolol helps prevent arrhythmias, stabilize blood pressure, and reduce the risk of heart attacks. Additionally, it dilates blood vessels to improve blood flow, which helps alleviate angina and prevent migraines. Although the exact mechanism for reducing tremors is not fully understood, Propranolol is thought to interfere with nerve signals to the muscles involved in tremors. In cases of anxiety, it counteracts the effects of adrenaline and noradrenaline neurotransmitters that increase heart rate and contribute to feelings of anxiety. For pheochromocytoma, a type of tumour that produces excess adrenaline, Propranolol may be used to manage high adrenaline levels and lower the risk of dangerously high blood pressure during tumour removal surgery.

Fig. 1: Chemical structure of Propranolol.



Alprazolam interacts with benzodiazepine receptors, specifically targeting both BNZ 1 receptors, which are involved in regulating sleep, and BNZ 2 receptors, which influence muscle relaxation, anticonvulsant effects, motor coordination, and memory. These benzodiazepine receptors are believed to be linked with gamma-aminobutyric acid-A (GABA-A) receptors. By binding to these receptors, Alprazolam enhances the effects of GABA, increasing its binding affinity for the GABA-A receptor. When GABA attaches to the GABA-A receptor, it opens chloride channels, allowing chloride ions to flow into the neuron. This influx of chloride ions results in the hyperpolarization of the neuronal membrane, which decreases the likelihood of neuronal firing or excitation.

Fig. 2: Chemical structure of Alprazolam.



Materials and Methods

Chemicals and Reagents:

Propranolol and Alprazolam reference standards of purity 99.6 % and 99.8 % respectively were supplied by USP. The Zolent Plus tablets used in the assay which contained 10 mg of Propranolol and 0.5 mg of Alprazolam were obtained from local market. Potassium di Hydrogen Phosphate is procured from Rankem, Methanol, Hydrochloric Acid, Potassium Hydroxide were sourced from Merck, Milli Q water.

Instruments:

HPLC: Waters HPLC 2965 System with Auto-Injector and PDA detector with the software Empower 3
Balance: Sartorius
pH Meter: Analab Scientific
Sonicator: Leela Sonic

Chromatographic Conditions:

Column: Waters symmetry shield RP C18, 150 x 4.6 mm, 5 µm column.
Mobile phase: pH 4.5 Phosphate buffer and Methanol in the ratio 70:30 % v/v.
Flow: Isocratic
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Detection wavelength: 280 nm
Colum Temperature: 35°C
Sample Temperature: 20°C
Run Time: 7 Minutes

Preparation of Solutions:

pH 4.5 Phosphate Buffer Preparation:
Dissolved 13.61 g of Potassium Dihydrogen Phosphate in 1000 mL of Milli Q water, pH was adjusted to 4.5 with diluted HCL or KOH solution.

Mobile phase preparation:
Mixed pH 4.5 Phosphate Buffer (70 %) and methanol (30 %) and degassed in ultrasonic water bath for 15 minutes.

Diluent preparation:
Mobile phase was used as diluent.

Standard Solution Preparation:
Weighed and transferred about 40 mg of Propranolol and 2 mg of Alprazolam reference standards into 100 mL volumetric flask, to this 20 mL of diluent was added, sonicated for 5 to 6 minutes then volume was made up with the diluent and mixed properly. 10 mL of the above solution was pipetted into 25 mL volumetric flask and volume was made up with the diluent and mixed properly. (Concentration of Propranolol and Alprazolam were 160 ppm and 8 ppm respectively).

Sample Solution Preparation:
4 tablets were transferred into 100 mL volumetric flask, around 20 mL diluent was added, then sonicated for 15 to 20 minutes, volume was made up with the diluent and mixed properly. 10 mL of the above solution was pipetted into 25 mL volumetric flask and volume was made up with the diluent and mixed properly. (Concentration of Propranolol and Alprazolam were 160 ppm and 8 ppm respectively).

Validation Parameters:

System Suitability:
Suitability of the system for the study was assessed by analysing replicate injections of standard solution of Propranolol and Alprazolam.

Linearity:
Linearity was determined by analysing standard solution of Propranolol and Alprazolam at various concentrations and then constructing a calibration curve based on those measurements.

Precision:
It involved preparing and analysing six independent samples.

Accuracy:
Was determined by recovery studies of Propranolol and Alprazolam.

Specificity:
Assessed by analysing the interference of other peaks (which includes blank, placebo, impurities) with Propranolol and Alprazolam peaks.

Robustness:
Robustness is tested by altering chromatographic conditions and assessing how these modifications affect the assay results of Propranolol and Alprazolam.

Results and Discussion

System Suitability:
The tailing factor for Propranolol and Alprazolam was 1.10 and 1.09 respectively, % RSD for peak areas was 0.4 for Propranolol & 0.3 for Alprazolam. Plate count was 7950 & 10149 for Propranolol and Alprazolam respectively. From these results it was found that system was suitable.

Table 1: Results of System Suitability

Parameter	Observed value for Propranolol	Observed value for Alprazolam
Tailing factor	1.10	1.09
%RSD (peak areas)	0.4	0.3
Plate count	7950	10149

Linearity:
The method demonstrated excellent linearity over the concentration range of 50 to 250 % with a Correlation Coefficient (R^2) of 1.000 for both Propranolol and Alprazolam.

Table 2: Results of Linearity for Propranolol

Concentration (µg/mL)	Peak Area
80.121	211236
160.232	423563
240.095	635802
320.124	843258
400.345	1055122
Square of correlation coefficient	**1.000**

Concentration (µg/mL)	Peak Area
% Y-intercept at 100% response	0.1

Table 3: Results of Linearity for Alprazolam

Concentration (µg/mL)	Peak Area
4.011	159609
8.039	320021
12.180	477258
16.123	639503
20.206	797025
Square of correlation coefficient	**1.000**
% Y-intercept at 100% response	**0.1**

Fig. 3: Linearity Graph of Propranolol.

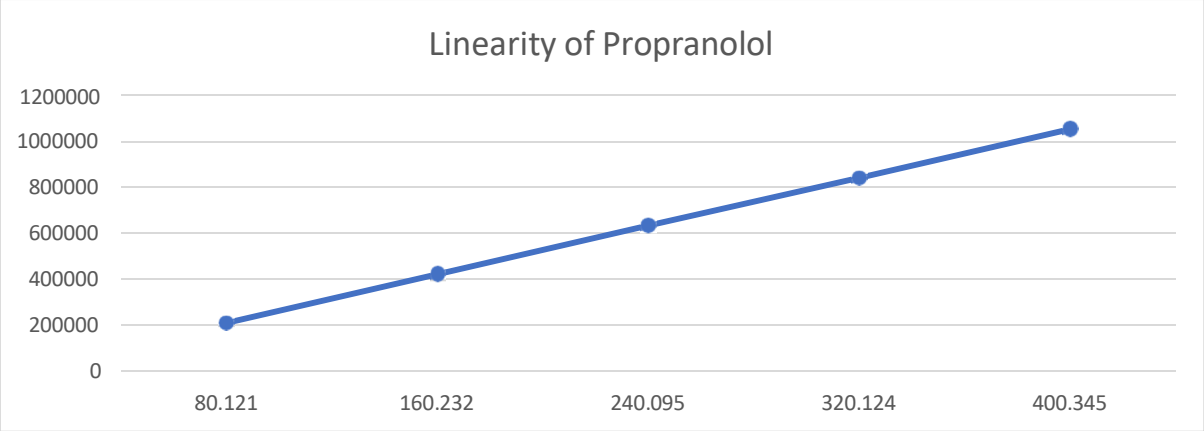
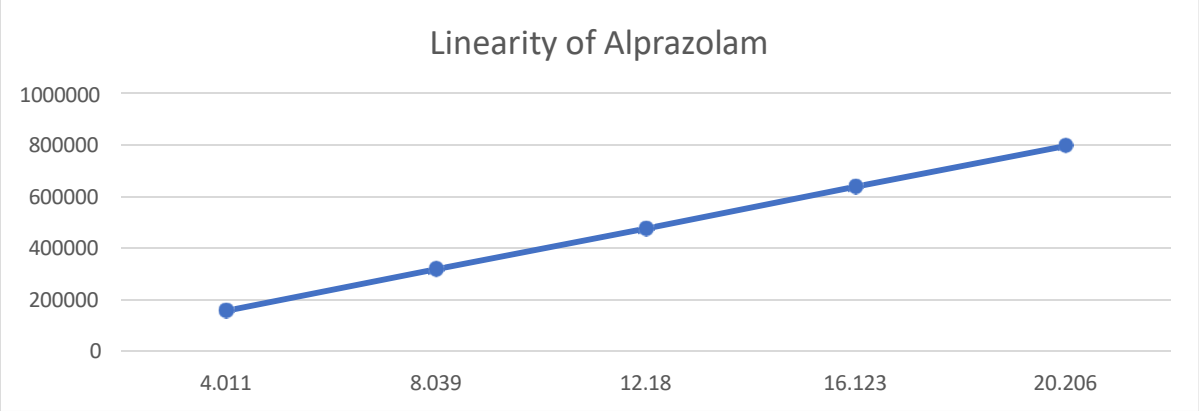


Fig. 4: Linearity Graph of Alprazolam.



Precision:
The % RSD of Propranolol and Alprazolam for six independent samples was found to be 0.5 and 0.7 respectively.

Table 4: Results of Method Precision

Sample Number	% Assay of Propranolol	% Assay of Alprazolam
1	100.5	101.2
2	100.3	100.6
3	100.1	100.7
4	99.9	99.6
5	99.8	100.5
6	101.1	101.6
Mean	**100.0**	**101.0**
% RSD	**0.5**	**0.7**

Accuracy:
Recovery studies indicated that individual and average recoveries ranged from 98.0 % to 102.0 %, with % RSD values not exceeding 2.0 across three concentration levels.

Table 5: Results of Accuracy of Propranolol

% Level	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean % Recovery	% RSD
50	20.5	20.3	99.0	99.8	1.4
	20.6	20.9	101.5		
	20.9	20.7	99.0		
100	40.2	40.0	99.5	99.2	0.6
	40.3	40.1	99.5		
	40.9	40.3	98.5		
150	60.8	60.2	99.0	99.0	0.2
	61.2	60.5	98.9		
	61.8	61.3	99.2		

Table 6: Results of Accuracy of Alprazolam

% Level	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean % Recovery	% RSD
50	1.15	1.14	99.1	99.4	1.3
	1.23	1.24	100.8		
	1.20	1.18	98.3		
100	2.12	2.13	100.5	100.0	0.8
	2.08	2.09	100.5		
	2.25	2.23	99.1		
150	3.13	3.11	99.4	100.3	0.8
	3.23	3.25	100.6		
	3.22	3.25	100.9		

Specificity:

There was no interference detected from impurity, blank and placebo peaks at retention times of Propranolol and Alprazolam peaks thus confirming the specificity of developed method. (Refer Figures 3, 4)

Fig. 5: Chromatogram of Standard

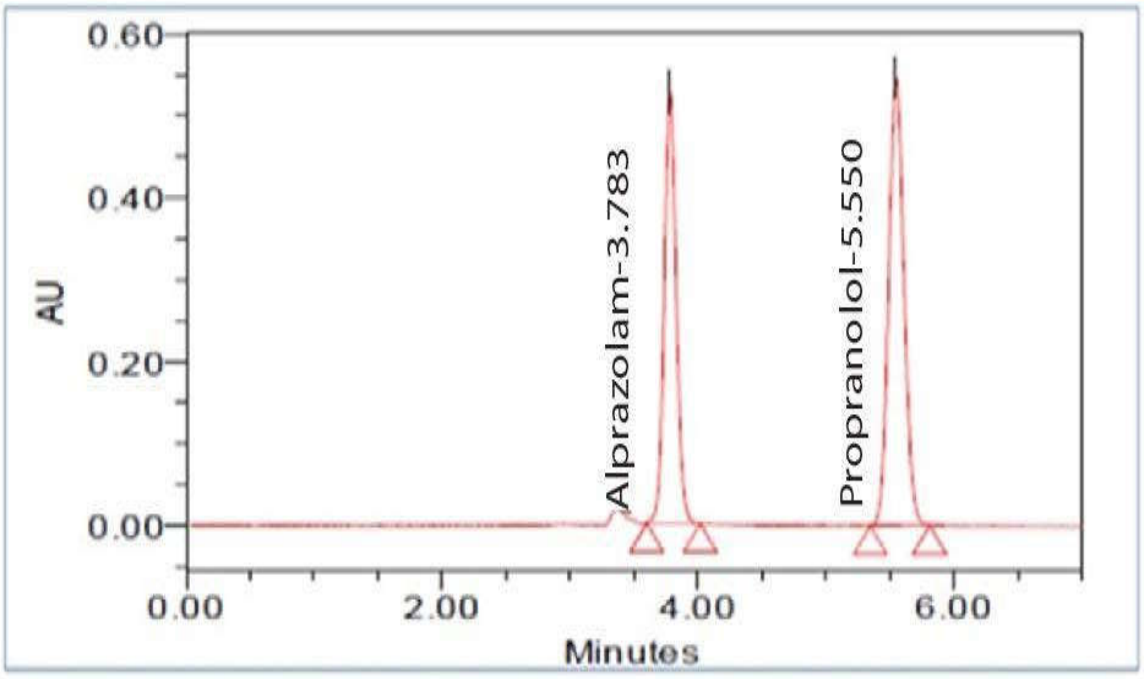
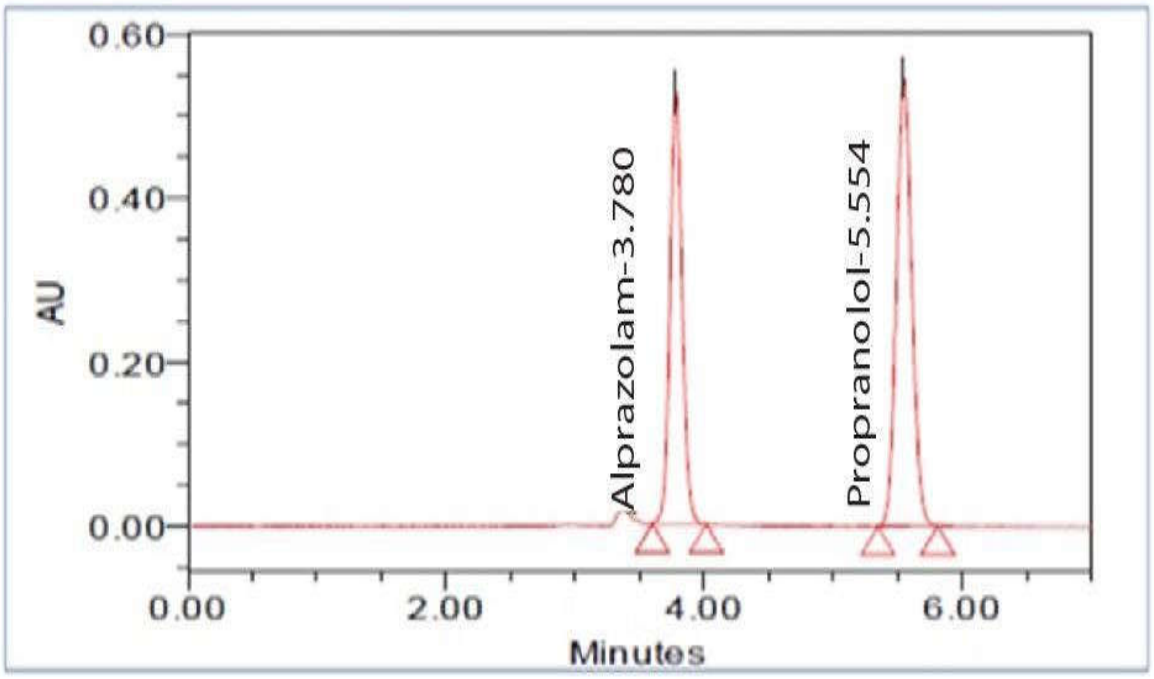


Fig. 6: Chromatogram of Sample



Robustness:

Robustness of the method was proven as the minor modifications in chromatographic conditions has not affected the assay results of Propranolol and Alprazolam.

Conclusion

Pharmaceutical analysis plays a pivotal role in certifying quality of drugs and their formulations by the industry and by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out safe and effective drugs. The current good manufacturing practices (cGMP) and ICH guidelines insist for adoption of sound methods that are properly validated to ensure quality of drugs. The current method was properly developed and validated. The method validation results met all the acceptance criteria's as per ICH Q2 guidelines ensuring that the method was suitable for simultaneous estimation of Propranolol and Alprazolam in a combined tablet dosage form.

References

1. Rani G.T, Shankar D.G., Kadgapathi P, Satyanarayana B. A Validated RP HPLC Method for Simultaneous Determination of Propranolol hydrochloride and Alprazolam in Bulk and in Pharmaceutical formulations. *Journal of Pharmacy Research*. 2011, 2(4), 358-360.

2. Kapadia Samant, Tiwari Neha, Patani Pragnesh. Development and Validation of Stability Indicating HPLC Method for Simultaneous Estimation of Clonazepam and Propranolol HCL in their Combined Marketed Dosage form. *J Pharm Sci Bioscientific Res.* 2018, 8(1), 123-129.
3. K. Pravalika, Sashmitha Samuel.B, M. Bindu, Praharsha.Y, V.V.Sridevi. Method development and Validation for the Simultaneous estimation of Propranolol HCl and Clonazepam in combined dosage form using RPHPLC and UV spectrophotometric methods. *High Technology Letters.* 2022, 28(10), 1-13.
4. Rakesh R, Prajapati, Dr. J.B.Dave, Dr. C.N.Patel. Development and Validation of Stability Indicating High-Performance Liquid Chromatographic Method For Simultaneous Determination Of Alprazolam and Propranolol in Combined Dosage Forms. *International Journal Of Pharmacy & Technology*. 2011 (3)2, 2510-2523.
5. Patel Satish A, Patel Paresh U, Patel Natavarlal U. Development and Validation of Spectrophotometric method for Simultaneous Estimation of Diazepam and Propranolol hydrochloride in Combined Dosage Form. *International Research Journal of Pharmacy*. 2011, 2(8), 119-123.
6. D Meghana*, K Lahari, K Shantha Kumari, K Prakash. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Clonazepam and Propranolol Hydrochloride in Bulk and Pharmaceutical Dosage Forms. *Inventi Rapid: Pharm Analysis & Quality Assurance*. 2012, 4. 1-4.
7. Vinay B Patel, Jayant B Dave, et al. UV Spectrophotometric Method for Identification and Estimation of Clonazepam in Tablet Dosage Form. *International Journal of Pharmaceutical Research and Bio-Science*. 2012, 1(2), 62-70.
8. ICH Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonisation.