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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC AND HPLC METHODS FOR SIMULTANEOUS ESTIMATION OF PROPRANOLOL HYDROCHLORIDE AND CLONAZEPAM IN TABLET DOSAGE FORMS

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ABSTRACT

The aim of this study was to develop and validate analytical methods, specifically UV Spectrophotometry and High-Performance Liquid Chromatography (HPLC), for the simultaneous estimation of Propranolol Hydrochloride and Clonazepam in tablet dosage form. The focus was on using the UV Spectrophotometric method (simultaneous equation method) due to its simplicity, accuracy, precision, rapidity, and cost-effectiveness. Propranolol Hydrochloride and Clonazepam have distinct absorption maxima (λ max) at 291 nm and 222 nm, respectively, allowing their simultaneous determination in a mixture. This method proves ideal for routine analysis in quality control laboratories, leveraging its practical advantages such as inexpensive reagents, solvents, and readily available instrumentation. The accuracy and precision of the method were validated through recovery and repeatability studies, highlighting its reliability for routine analytical applications.

Keywords:-Propranolol hydrochloride , Clonazepam , UV Spectrophotometric , High performance liquid chromatography (HPLC).

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INTRODUCTION

Analytical chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials. It encompasses both qualitative analysis, which provides information on the identity and purity of the chemical species in a sample, and quantitative analysis, which determines the amount of one or more components within the sample. [1] Techniques in analytical chemistry range from basic methods such as weighing and titration to advanced techniques involving highly specialized instruments. Analytical method development and validation play crucial roles in the discovery, development, and manufacture of pharmaceuticals. [2] The objective of method development is to create and validate analytical methods such as UV Spectrophotometry, High-Performance Liquid Chromatography (HPLC), and High-Performance Thin Layer Chromatography (HPTLC) for drug products containing multiple active ingredients. Among these, ultraviolet-visible (UV-Vis) spectroscopy is one of the most frequently employed methods in pharmaceutical analysis due to its simplicity, accuracy, and cost-effectiveness.

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Official test methods derived from these processes are essential for quality control laboratories to ensure the identity, purity, potency, and efficacy of drug products. Spectrophotometric techniques for multicomponent samples leverage the property that absorbance at all wavelengths follows Beer-Lambert's law. [3] The absorbance ratio method, a modification of the simultaneous equation procedure, and difference spectroscopy, which measures the absorption difference (ΔA) between equimolar solutions of an analyte in different chemical forms, are fundamental techniques in this context. Additionally, derivative spectrophotometry involves converting a normal spectrum into its first, second, or higher derivative spectrum to enhance analytical accuracy. Propranolol, a beta-blocker, is used to treat tremors, angina, hypertension, heart rhythm disorders, and other cardiovascular conditions. Clonazepam is indicated as monotherapy or as an adjunct in the treatment of Lennox-Gastaut syndrome, akinetic, and myoclonic seizures. It is also valuable for patients with absence spells who have not responded to succinimides. [4] This study focuses on the simultaneous estimation of Propranolol Hydrochloride and Clonazepam in tablet dosage forms using UV Spectrophotometric methods, emphasizing the importance of developing reliable and efficient analytical techniques for pharmaceutical analysis

MATERIALS AND METHODS

All the solvents used in spectrophotometric analysis were of analytical reagent grade Potassium di-hydrogen phosphate, methanol, toluene, ortho-phosphoric acid, sodium hydroxide, formic acid, were of analytical grade. A Schimadzu spectrophotometer, Model no.1700 with 1 cm matched quartz cells were used for the experimental work. The absorption spectra of the reference and test solution were carried out in a 1cm quartz cell over the range of 200-400 mm. A Schimadzu electronic analytical balance (AUX-220) was used for weighing the samples. An ultrasonic cleaner was used for sonicating the tablet sample solution. UV method was developed and validated for the following combination of drug. Clonazepam and Propranolol hydrochloride

Development Of Uv Spectrophotometry For Simultaneous Estimation Of Clonazepam And Propranolol Hydrochloride

Preparation of Standard Stock Solution

A Stock solution 1000µg/ml each of Clonazepam and Propranolol hydrochloride were prepared by dissolving separately 100 mg of drug in methanol. For simultaneous estimation of Clonazepam and Propranolol hydrochloride, 2µg/ml of Clonazepam and 10µg/ml of Propranolol hydrochloride were prepared by diluting appropriate volumes of standard stock solutions in methanol. The scanning of the solution Clonazepam and Propranolol hydrochloride were carried out in the range of 400 – 200 nm to obtain overlain spectra. Absorbance were measured and absorptivities of standard solutions were calculated at selected wavelengths l1 (222nm) and l2 (291 nm) respectively. [5]

Preparation and analysis of Formulation

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed quantity of powder equivalent to 10 mg of Propranolol hydrochloride and 0.5 mg of Clonazepam were transferred to 100 ml volumetric flask and dissolved in about 25 ml of methanol.

After the immediate dissolution, the volume was made up to the mark with methanol. The solution was sonicated for about 15 minutes and filtered through Whatmann filter paper No: 41 and was diluted to prepare the concentration of 100 µg/ml of propranolol hydrochloride & 10 µg/ml of Clonazepam. Absorbance of this solution were measured at 222 nm (lmax of Clonazepam) and 291 nm (l max of propranolol hydrochloride) and the amount of drugs present in the average weight of tablet were obtained by substituting the values in respective simultaneous equation.

$$C_{CLZ} = \frac{A_2ay_1 - A_1ay_2}{Ax_2ay_1 - ax_2y_2}$$

$$CPRO = \frac{A1ax2 - A2ax1}{ax_2ay_1 - ax_1ay_2}$$

Where

i. ax1 and ax2 are absorptivities of Clonazepam at l1 and l2 respectively ii. ay1 and ay2 are absorptivities of Propranolol hydrochloride at l1 and l2 respectively iii. A1 and A2 are absorbance of mixtures at l1 and l2 respectively iv. CCLZ and CPRO are concentrations of Clonazepam & Propranolol hydrochloride

Validation of the proposed method

The proposed method was validated for the parameters like linearity, accuracy and precision as per ICH guidelines. Appropriate dilutions were made from the standard stock solutions to get the concentration of 2–10 µg/ml of Clonazepam and 10–30 µg/ml of Propranolol hydrochloride. [6] The absorbance of the solutions was measured at 222nm and 291nm for Clonazepam and Propranolol hydrochloride respectively against methanol as blank. The calibration curve was constructed by

plotting absorbance versus concentration. Linearity graph was plotted over a concentration rage of 2 – 10 µg/ml of Clonazepam and 10 – 30 µg/ml of Propranolol hydrochloride. The regression analysis was carried out for the calibration graphs to find out correlation coefficient, Y-intercept and slope of the regression line which estimates the degree of linearity. The correlation coefficient was found to be 0.9928 and 0.9989 for Clonazepam and Propranolol hydrochloride respectively.

Limits of detection (LOD) and Limits of Quantitation (LOQ)

In accordance with the standards of the International Commission on Harmonization (ICH), the technique was used to define detection and quantification limits based on the Standard Deviation of response and calibration slope of the compounds. [7] LOD and LOQ values were multiplied by 3.3 and by 10 correspondingly, respectively, [(standard repeatability deviation) / (regression equation pitch)]; and then dividing by 3.3 and 10.

Accuracy

By conducting a recovery study, researchers were able to assess the accuracy of the procedure. The solutions were injected in triplicate to ensure accuracy at 80%, 100%, 120% was spiked with a known amount of standard drug, and the content was re-analysed using the established technique. The % recovery was computed for each of the three injections at the given concentration. That is recovery study for selected drugs was carried out at 3 above levels. [8] The average percentage recovery at each spike level must be 98.0 percent or above, and it must not be higher than 102 percent The acceptability criterion for percentage relative standard deviation (percent RSD) should not be higher than two standard deviations from the mean.

Precision

Using intra-day and inter-day variance, researchers were able to estimate the precision of their procedure. Standard and sample solutions were examined on the same day in the intra-day experiments, with percentage relative standard deviation (percent RSD) being computed. [9] Within-day tests were carried out in which standard and sample solutions were examined on successive days, and the percentage relative standard deviation (percent RSD) was determined and shown. In contrast, the percentage relative standard deviation (percent RSD) of the absorbance obtained for the medication in single and multicomponent formulations should not be more than 2.0 in the case of UV.

RESULT AND DISCUSSION

PROPRANOLOL HYDROCHLORIDE AND CLONAZEPAM

UV Spectroscopic method was developed for simultaneous estimation of Propranolol hydrochloride and Clonazepam.

UV Spectrometric

The λ_{max} of Propranolol hydrochloride and Clonazepam were observed at 291 nm and 222 nm respectively. Fig. 1.1 and 1.2 depicts the λ_{max} of Propranolol hydrochloride and Clonazepam respectively. Fig. 1.3 represents the overlain spectrum of Propranolol hydrochloride and Clonazepam. [10]

Table:1 Linearity range (UV Spectrophotometry) of Clonazepam and Propranolol hydrochloride

S.No.	**Clonazepam**		**Propranolol hydrochloride**	
	Concentration in µg/ml	Absorbance at 222nm	Concentration in µg/ml	Absorbance at 291 nm
1	2	0.0305	10	0.358
2	4	0.617	15	0.523
3	6	0.874	20	0.725
4	8	1.105	25	0.884
5	10	1.491	30	1.072

Table: 2 Analytical performance (UV Spectrophotometry) of Clonazepam and Propranolol hydrochloride

Parameters	**Clonazepam**	**Propranolol hydrochloride**
Absorption maximum (λ max)	222 nm	291 nm
Beer's lamberts limit (µg/ml)	2 - 10	10 - 30
Coefficient correlation(r^2)	0.9928	0.9989

Regression Equation	y = 0.14295 x + 0.02071	y = 0.03575 x + 0.00257
Intercept (A)	0.02071	0.03575
Slope (B)	0.14295	0.00257
Limit of Detection (µg/ml)	1.5	0.93
Limit of Quantification (µg/ml)	4.5	2.83

Table: 3 Accuracy (UV Spectrophotometry) of Clonazepam and Propranolol hydrochloride

% Level of drug		**% Recovery Clonazepam**	**% Recovery Propranolol hydrochloride**
80		100.29	100.41
		100.34	100.32
		100.48	100.21
	Mean	100.37	100.31
	% RSD	0.1	0.1
100		100.29	100.47
		100.31	100.25
		100.49	100.38
	Mean	100.36	100.36
	% RSD	0.11	0.11
120		100.47	100.51
		100.34	100.09
		100.45	100.01
	Mean	100.42	100.26
	% RSD	0.07	0.22
Grand Mean		100.38	100.31
% RSD		0.03	0.05

Figure: 1 UV spectrum for Clonazepam max

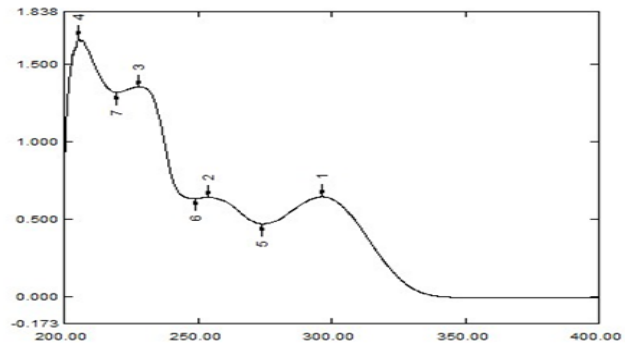


Figure: 2 UV spectrum for Propranolol hydrochloride max

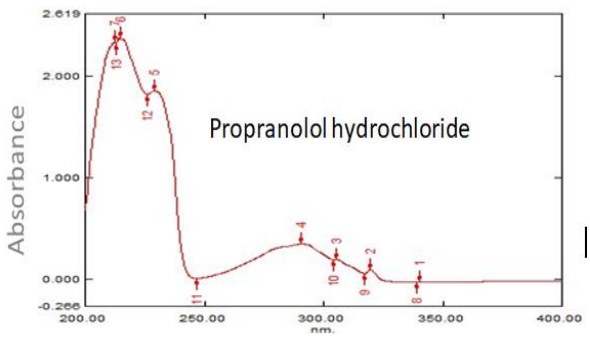
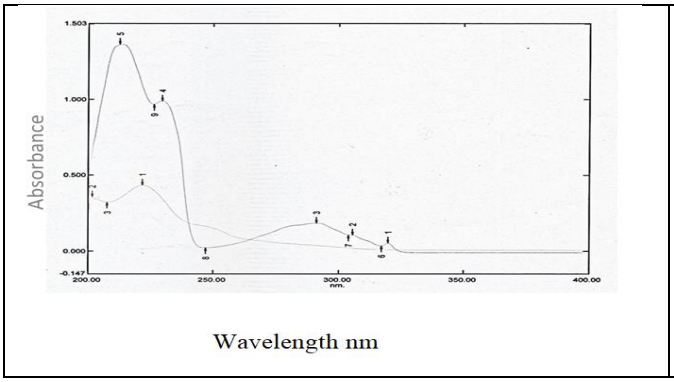
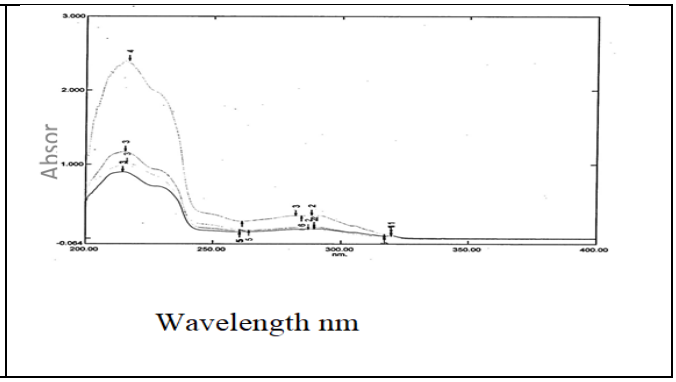


Figure: 3 Overlay spectrum of Clonazepam and Propranolol Hydrochloride

Figure: 4 Linearity graph of Clonazepam and Propranolol Hydrochloride





Five sets of standard solutions were prepared and scanned over the range of 400-200 nm. Propranolol hydrochloride and Clonazepam showed linearity in the range of 10-30 µg/ml and 2-10 µg/ml respectively. The linearity ranges are tabulated in Table 1. [11] Absorptivity values of Propranolol hydrochloride and Clonazepam were calculated. Analytical performance data are shown in Table 2

The correlation coefficient values were found to be 0.9989 for Propranolol hydrochloride and 0.9928 for Clonazepam. [12] The correlation coefficients were within the limit as all points lie on the same line and the relationship between concentration and absorbance of Propranolol hydrochloride and Clonazepam were linear in the range specified. [13] Linearity curves of Propranolol hydrochloride and Clonazepam are shown in Fig. 1.4 respectively

Accuracy

Accuracy of the proposed method was confirmed by recovery values from 100.29% - 100.49% for Clonazepam and 100.01% - 100.47% for Propranolol hydrochlorides with the grand mean values of 100.26% for Propranolol hydrochloride and 100.42 % for Clonazepam. [14] These reproducible results justified the accuracy of the method. These results are tabulated in Table 3.

Precision

Repeatability studies ensure the closeness of the result in all six determinations. The % relative standard deviation calculated from repeatability data are below 2% indicates the precision of the performed method. [15] The results of the market sample analysis are in good agreement with label claim. Repeatability data are furnished in Table 4

The proposed UV spectrophotometric method is simple, accurate, precise, rapid and economical for the simultaneous estimation of Propranolol hydrochloride and Clonazepam in tablet dosage form.[16] The proposed method used inexpensive reagent, solvent and instrument that are available in laboratory. Hence these methods can be conveniently adopted for routine analysis.

CONCLUSION

In conclusion, the UV spectrophotometric method using the simultaneous equation approach has been successfully developed and validated for the simultaneous estimation of Propranolol hydrochloride and Clonazepam in tablet dosage form. The method demonstrated excellent linearity, accuracy and precision, making it a reliable technique for the analysis of these two drugs in combination. The simplicity, rapidness, and cost-effectiveness of this method make it an ideal choice for routine analysis in quality control laboratories. Its practicality, which stems from the use of inexpensive reagents, solvents, and readily available instrumentation, further supports its adoption in routine analysis. Moreover, this method has the potential to be extended to other pharmaceutical formulations containing Propranolol hydrochloride, Clonazepam, or similar drug combinations, ensuring the quality and efficacy of various pharmaceutical products. Overall, the UV spectrophotometric method (simultaneous equation method) offers a valuable analytical tool for the simultaneous estimation of Propranolol hydrochloride and Clonazepam in tablet dosage form, contributing to the continued assurance of pharmaceutical product quality and patient safety.

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