

Development of an RP-HPLC Method for the Simultaneous Estimation of Propranolol Hydrochloride and Diazepam in Combined Dosage form

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ABSTRACT

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A simple, efficient, and reproducible RP-HPLC method for the simultaneous determination of propranolol hydrochloride and diazepam in bulk and in pharmaceutical formulations has been developed and validated. The separation was carried out on Waters C18 (250 × 4.6 mm i.d, 5 μ) column using acetonitrile: 0.4 % potassium dihydrogen ortho phosphate (adjusted to pH 3.52 with ortho phosphoric acid) in the ratio of 60:40 v/v as eluent. The flow rate was 1 ml/min and effluent was detected at 229 nm. The retention time of propranolol hydrochloride and diazepam were 2.330 and 6.663 min. respectively. The linear dynamic range was 2-24 μg/ml and 0.25- 3.0 μg/ml for propranolol hydrochloride and diazepam, respectively. Percentage recoveries for propranolol hydrochloride and diazepam were 100.03 and 99.72 %, respectively. All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The developed method is also found to be precise and robust for the simultaneous determination of propranolol hydrochloride and diazepam in tablet dosage forms.

Keywords: Propranolol hydrochloride, Diazepam, and Simultaneous determination

INTRODUCTION

Propranolol hydrochloride (PH): It is a non-selective beta-blocker mainly used in the treatment of hypertension. Propranolol hydrochloride is used in the treatment or prevention of many disorders including acute myocardial infarction, arrhythmias, angina pectoris, hypertension, hypertensive emergencies, hyperthyroidism, migraine, pheochromocytoma, menopause, and anxiety. Chemically propranolol hydrochloride is 1-naphthalen-1-yloxy-3-(propan-2-ylamino)propan-2-ol hydrochloride (Fig. 1a).

Diazepam is a member of the benzodiazepine family. Benzodiazepines are sedatives that cause dose-related depression of the central nervous system. They are useful in treating anxiety, insomnia, seizures, and muscle spasms. Chemically it is 7-chloro-1-methyl-5-phenyl-3H-1, 4-benzodiazepin-2-one (Fig. 1b).

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Fig.1a: Structure of Propranolol hydrochloride

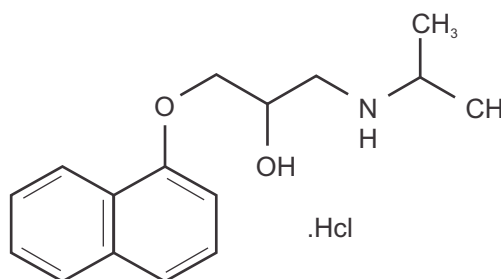
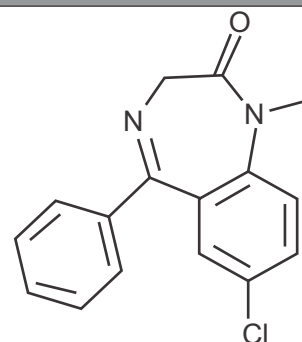


Fig.1 b: Structure of diazepam



Literature survey reveals a few spectrophotometric and bioanalytical methods for the estimation of both drugs as a single component and in combination with other drugs¹⁻⁶ however no method has been reported for analysis of these drugs in combined dosage form. The objective of present communication is to develop simple, rapid, and precise RP-HPLC method for the estimation of propranolol hydrochloride and diazepam in combined tablet dosage form.

EXPERIMENTAL

Equipment

The HPLC system consisted of a solvent delivery module Lc-10AT vp Shimadzu liquid chromatograph pump with 20 μ l loop and model SPD 10A vp UV-Visible detector.

Propranolol hydrochloride and diazepam reference substances were gifted by M/S Trident Pharmaceuticals, Hyderabad, India. Tablet dosage forms (BETAPRP- DZ, Concern Pharma) were procured from the local market, each tablet containing 20 mg of propranolol hydrochloride and 2.5 mg of diazepam. HPLC grade acetonitrile was procured from E.Merck (India) Ltd., Mumbai. Potassium dihydrogen ortho phosphate (AR grade) was obtained from S.D. Fine Chemicals, Mumbai.

Chromatographic conditions

Waters C₁₈ column (250 \times 4.6 mm, i.d, 5 μ) was used for separation. The mobile containing acetonitrile and 0.4 % of potassium dihydrogen ortho phosphate (adjusted to pH 3.52 with orthophosphoric acid) in the ratio of 60:40 v/v was delivered at a flow rate of 1.0 ml/min with detection at wavelength 229 nm. The Injection volume was 20 μ l and the analysis was performed at ambient temperature.

Standard stock solution

Stock solutions of propranolol hydrochloride and diazepam (1 mg/ml) were prepared separately using mobile phase as solvent. From the standard stock solutions, mixed standard solutions of different concentrations ranging from 2 to 24 μ g/ml of propranolol hydrochloride and 0.25 to 3.0 μ g/ml of diazepam were prepared by diluting with mobile phase. With the optimized chromatographic conditions, a steady base line was recorded. Twenty micro liters of each mixed standard solution was injected six times and chromatograms were recorded. The retention time of propranolol hydrochloride and diazepam were found to be 2.330 min 6.663 min, respectively. Calibration curves were constructed by plotting the average peak areas against the respective concentrations and found to be linear in the above range with the correlation coefficients (r^2) 0.999 and 0.998 for propranolol hydrochloride and diazepam, respectively.

Analysis of propranolol hydrochloride and diazepam in combined dosage form

Twenty tablets were weighed and average weight was determined and finely powdered. Tablet powder equivalent to 20 mg of propranolol hydrochloride and 2.5 mg of diazepam was accurately weighed and transferred to 10 ml volumetric flask. The contents were sonicated after adding 5 ml of mobile phase and the volume was made upto the mark with mobile phase. The sample solution was filtered through whatmann filter paper and an appropriate volume of the aliquot was transferred to 10 ml volumetric flask and the volume was made up to the mark. Twenty micro liters of the solution was injected into the chromatographic system and the peak areas were measured and the quantitation was carried out by keeping these values to the regression equation of corresponding calibration curve.

VALIDATION⁷⁻⁸

The method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines.

Linearity

The linearity of the method was determined at six concentration levels ranging from 2 to 24 μ g/ml of propranolol hydrochloride and 0.25 to 3.0 μ g/ml of diazepam. The regression equation of calibration curves were $Y = 177.07x + 23.561$ for propranolol hydrochloride and $Y = 168.75x + 16.949$ for diazepam.

Accuracy

The accuracy of the method was determined by recovery experiment. To carry out recovery studies, fixed amount of sample was taken and standard drug was added at three different levels (80, 100, 120 %). Each level was repeated three times.

Precision

Precision was studied to find out intra and inter day variation in the proposed method at three different levels on the same day and on three different days, respectively. The %RSD was calculated for intra day and inter day precision and found to less than 1%.

Limit of detection (LOD) and limit of quantitation (LOQ)

Calibration curves were prepared using concentrations in the range of 0.2 – 2.0 μ g/ml for propranolol hydrochloride and 0.05- 0.25 μ g/ml for diazepam (expected detection limit range). The Standard deviation of Y intercepts of regression lines were determined and kept in the following equation for the determination of LOD and LOQ. Detection limit = $3.3\sigma/S$; Quantitation limit = $10\sigma/S$; where, σ is the Standard deviation of Y intercept of regression lines and S is the slope of

Fig. 2: Overlay spectra of propranolol hydrochloride and diazepam

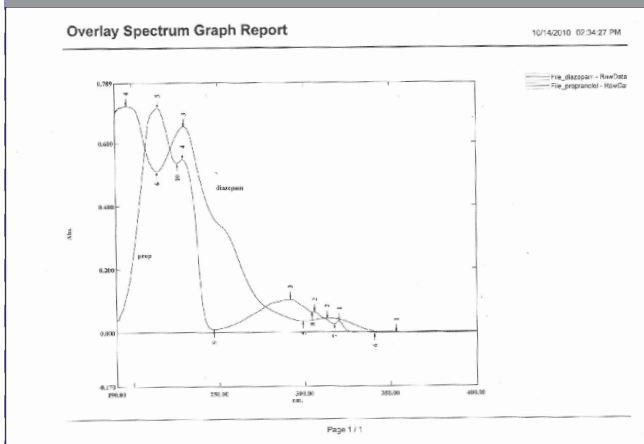
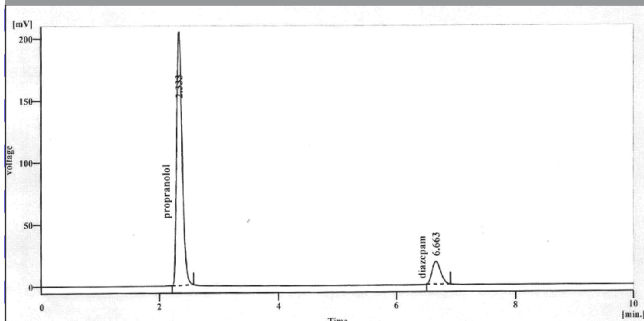


Fig.3: Typical chromatogram of propranolol hydrochloride and diazepam (Mixture containing 8µg/ml of propranolol hydrochloride and 1µg/ml of diazepam)



calibration curve. The LOD was found to be 0.30 µg/ml for propranolol hydrochloride and 0.08 µg/ml diazepam. Limit of quantitation was found to be 0.92 µg/ml for propranolol hydrochloride and 0.23 µg/ml for diazepam, respectively.

Robustness

Robustness of the method was determined by making slight changes in the composition of organic phase ± 2% and the pH by ± 0.3, flow rate by ± 0.1ml and detection wavelength by ± 2 nm. It was observed that there were no marked changes in the retention time and area of the chromatograms and the %RSD was less than 1%, which demonstrated that the RP-HPLC method developed was robust.

Specificity

Commonly use excipients (starch, lactose, magnesium stearate) were spiked into a preweighed quantity of drug mixture. The chromatogram was taken by appropriate dilutions and the amount of each drug present in the sample mixture was determined.

Stability

In order to demonstrate the stability of both the standard and sample solutions during

Fig. 4: Calibration curve of propranolol hydrochloride

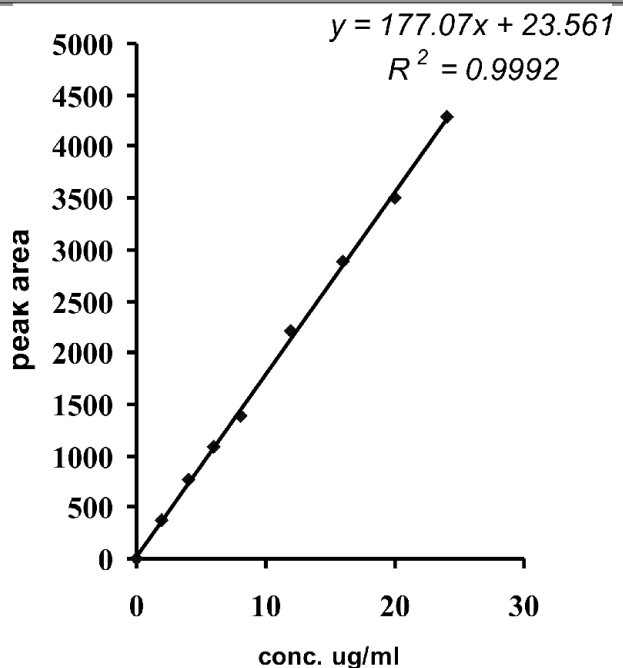
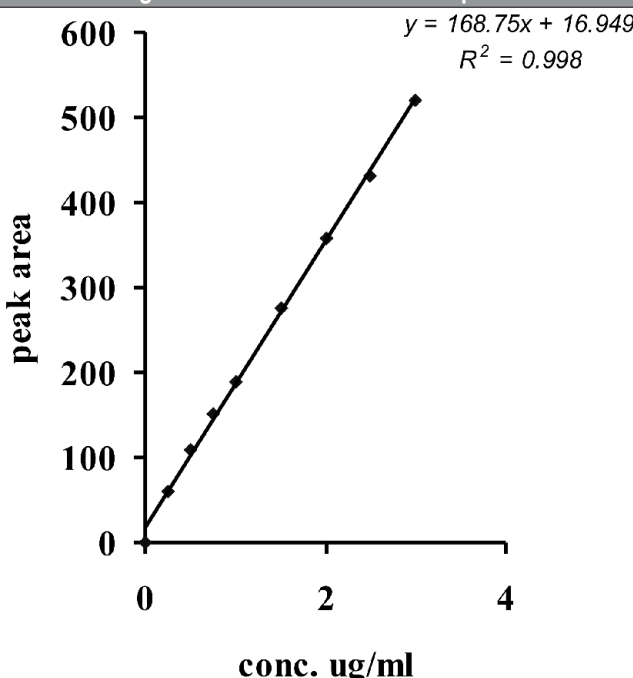


Fig. 5. Calibration curve of diazepam



analysis, both the solutions were analyzed over a period of 5 hours at room temperature. The peak areas and retention time of both the drugs remained almost unchanged and no significant degradation with in the indicated period.

RESULTS AND DISCUSSION

The goal of this study was to develop a rapid HPLC method for analysis of propranolol hydrochloride and diazepam in its

Table 1: System Suitability and Validation Parameters of the proposed method

Parameters	Propranolol hydrochloride	Diazepam
Theoretical plates	3033	10525
Resolution	-----	20.231
Asymmetric factor	1.491	1.361
Retention Time (min)	2.330	6.663
Linearity range ($\mu\text{g/ml}$)	2 - 24	0.25 - 3.0
Regression Equation $Y=mx+c$	$Y=177.07x+23.561$	$Y=168.75x+16.949$
Slope (m)	177.07	168.75
Intercept (c)	23.561	16.949
Correlation coefficient	0.9992	0.998
Percent RSD		
Intra day (n=3)	0.23	0.34
Inter day (n=3)	0.38	0.46
LOD ($\mu\text{g/ml}$)	0.3	0.08
LOQ ($\mu\text{g/ml}$)	0.92	0.23

*Average of six determination, Y- Area under the curve and x is the concentration, RSD -Relative standard deviation, LOD -Limit of detection, LOQ - limit of quantitation

Table 2: Robustness data of the proposed method

Parameter	Proposed	Variation	Propranolol hydrochloride	Diazepam
			% RSD*	
pH of buffer	3.52	3.55	0.834	0.864
		3.49	0.783	0.739
Wavelength (nm)	229	231	0.675	0.784
		227	0.713	0.694
Mobile Phase	60:40v/v	62:38	0.783	0.569
		58:42	0.765	0.982
Flow rate (ml/min)	1ml/min	1.1	0.839	0.682
		0.9	0.782	0.876

*%RSD -Percent Relative Standard Deviation (Six determination)

Table 3: Results of analysis of formulation and recovery study of the proposed method

Drug	Amount mg/tablet		% Label claim	% Recovery
	Label claim	Amount found \pm SD		
Propranolol hydrochloride	20	19.99 \pm 0.08	99.99	100.03
Diazepam	2.5	2.501 \pm 0.1	100.04	99.72

*Average of six determinations, SD- Standard deviation

bulk and pharmaceutical formulations using a commonly used reverse phase C18 column. To develop an effective method for the analysis of the drugs, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, ideal mobile phase and its combination, optimum pH and

concentration of the standard solution were studied. UV overlain spectra of both propranolol hydrochloride and diazepam showed that both the drugs absorb appreciably at 229 nm, so 229 nm was selected as the detection wavelength (Figure 2). The mobile phase containing acetonitrile and 0.4% of potassium dihydrogen ortho phosphate (adjusted to

pH 3.52 with orthophosphoric acid) in the ratio of 60:40 v/v with a flow rate of 1.0 ml/min was selected for analysis after preliminary tests. The retention time of propranolol hydrochloride and diazepam were found to be 2.330 and 6.663 min, respectively. Resolution between propranolol hydrochloride and diazepam was found to be 20.231, which indicate good separation of both the compounds.

System suitability tests were carried out on freshly prepared standard solutions and the parameters are summarized in Table 1. The values obtained demonstrated the suitability of the system for analysis of these drugs in combined dosage form. Typical chromatogram of propranolol hydrochloride and diazepam is shown in Figure 3. The calibration curves of propranolol hydrochloride and diazepam were constructed by plotting the peak area of the drug (Y-axis) to the concentration (x-axis). It was found to be linear with a correlation coefficient of 0.999 and 0.998 which shows that good correlation exists between area of the peak and the concentration (Figure 4 and 5). This method was validated for its intra-day and inter-day precision. The results obtained were within the acceptable limit (Table 1). Robustness of the method was studied by changing the chromatographic conditions slightly and the results were presented in the Table 2. Detection limit for propranolol hydrochloride and diazepam was 0.3 and 0.08 µg/ml and quantitation limit was 0.92 µg/ml and 0.23 µg/ml, which suggest that a nanogram quantity of both the compounds can be estimated accurately. The RP-HPLC method developed in the present study was used to quantify propranolol hydrochloride and diazepam in combined dosage form and the results of assay were comparable with the corresponding labeled amounts (Table 3). High recovery values and no additional peaks, in the chromatogram indicate that the proposed procedure is free from interference of the commonly used excipients in the formulation. So the proposed method is accurate and specific and can be used for routine analysis of propranolol hydrochloride and diazepam in their combined dosage form.

CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of propranolol hydrochloride and diazepam in combination using simple mobile phase. The method gives good resolution between the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate, and precise.

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