Future Journal of Pharmaceutical Sciences

RESEARCH

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Combined and comparative analytical studies with stability studies and validation for estimation of prenoxdiazine HCl in pharmaceutical dosage form

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Abstract

Background A precise, accurate, economical and reproducible UV, RP-HPLC and UPLC method has been developed for the estimation of Prenoxdiazine hydrochloride in pharmaceutical and bulk dosage form. Each of the developed method has been validated for the analysis of Prenoxdiazine hydrochloride.

Result The validation of the analytical methods was carried out based on various parameters like precision, linearity, accuracy, specificity, LOD, and LOQ for all the three methods. The parameters were found statistically significant for the analysis of Prenoxdiazine hydrochloride. The method was found sensitive in the presence of analytical impurities. The retention time of the Prenoxdiazine HCl was found to be 2.5 and 2.9 min, using common solvents acetonitrile and methanol, in UPLC and HPLC, respectively.

Conclusion The method may be useful in the wide range of analysis for the estimation of Prenoxdiazine hydrochloride. It is found to be robust upon the change in solvent flow rate, column temperatures. Further, the study has investigated the stability of the sample under stressed condition like thermal, oxidative, and photolytic. Here, we tried to develop and compare cost-effective methods for Prenoxdiazine HCl using most common industrial methods which are never studied.

Keywords Prenoxdiazine HCl, Libexin, UPLC and HPLC, Validation, Stability studies

Introduction

Prenoxdiazine hydrochloride (HCl) is chemically known as 1-[2-[3-(2, 2-diphenylethyl)-1, 2, 4-oxadiazol-5 yl] ethyl] piperidine (Fig. 1) [1]. Prenoxdiazine HCl acts as a non-narcotic bronchodilator due to its antitussive nature which is used to treat chronic obstructive pulmonary diseases and chronic bronchial asthma in children [2]. The drug profile of Prenoxdiazine HCl is depicted in Table 1. From the commercial point of view, we have developed the widely used qualitative and quantitative analysis methods of patented drug Prenoxdiazine HCl, although more sophisticated methods like LC–MS, LC–MS–MS, and Gas chromatography can be tried further. The stability of a drug in formulation refers to the ability of a particular formulation to maintain its specifications related to its identity, strength, quality, and purity [3, 4]. Impurities in pharmaceuticals are unwanted chemicals that may remain with the drug or may develop during stability studies or upon aging of both drug and its formulation.

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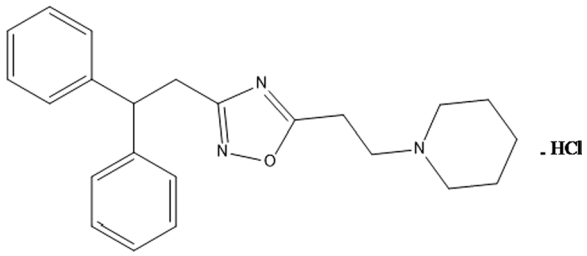


Fig. 1 Chemical structure of prenoxdiazine HCl

The presence of these impurities even in small amounts may influence the efficacy and safety of pharmaceutical products. For analysis of drug and its substances, the sensitive methods such as Liquid chromatography–mass spectrometry (LC–MS) and Gas chromatography–mass spectrometry (GC/MS) are preferred but are expensive.

The high-performance liquid chromatography (HPLC) and Ultra-performance liquid chromatography (UPLC) are found to be more reliable and cost effective [5]. UPLC is relatively a technique giving new possibilities in liquid chromatography and has been designed in a special way to withstand very high system back-pressures. UPLC photodiode array (PDA) a detector detects and quantifies the lower concentrations of analyte in the given sample, traces impurities with maximum sensitivity, and compares spectra across wavelengths. UPLC has been successfully exploited for the estimation of various drugs in its bulk form and formulations namely Bambuterol HCl and Montelukast Sodium [6], Duloxetine HCl [7], Erlotinib [8], Erythropoietin [9], etc. Spectrophotometry is used for both qualitative and quantitative investigations of samples. The wavelength at the maximum of the absorption band will give a piece of information about the structure of the molecule or ion and the extent of the absorption is proportional to the amount of the species absorbing the light [10]. In HPLC Separations is archived by partition, absorption, or ion exchange processes, depending on the type of stationary phase used [11]. Hence, the present study focused on the development and validation of a selective, fast, cost-effective stability-indicating Reverse-Phase-UPLC (RP-UPLC) method for the determination of Prenoxdiazine HCl.

Methods

Materials

Prenoxdiazine HCl was obtained as a gift sample from Khandelwal Laboratories Pvt. Ltd., Mumbai. The solvents acetonitrile (ACN), methanol (MeOH), and potassium dihydrogen orthophosphate employed in the present study are HPLC grade and were purchased from Sigma-Aldrich, Bengaluru, India, S. D. Fine-Chem limited, Mumbai, India, and Fluka, respectively. The other reagents and chemicals like HCl, sodium hydroxide, and hydrogen peroxide used in the present study are analytical grade and were purchased from Merck (Merck Eurolab, Fontenay-sous-Bois, France).

Instrumentation

UPLC H class system (Waters) equipped with a PDA detector was used for the qualitative and quantitative analysis of Prenoxdiazine HCl. The signals and results were processed using Empower software version 3. The UPLC columns (i) Waters Acquity UPLC HSS (4.6 × 50 mm, 1.8 µm) (ii) Waters acquity UPLC column CSH, C_{18} (2.1 × 100 mm, 1.7 µm) were used. The composition of 10 mM phosphate buffers (pH 7) (A) and ACN (B) was used as mobile phase. The flow of mobile phase linear gradient elution as follows: 0–0.5 min, 50% A and 50% B; 0.5–3 min, 30% A and 70% B; 3.1–5 min, 50% A and 50% B. The flow rate of sample elution was fixed as 0.4 mL/min and the injection volume was set as 5 µl.

Table 1 Drug profile of prenoxdiazine HCl [12–14]

Sr. Nos.	Drug profile	
1	Generic name	Prenoxdiazine HCl
2	Molecular formula	$C_{23}H_{27}N_3O.HCl$
3	IUPAC name	1-[2-[3-(2,2-diphenylethyl)-1,2,4-oxadiazol-5-yl] ethyl] piperidine
4	Molecular weight	397.94 g/mol
5	Description	White, odorless, crystalline
6	Melting point	192°–193 °C
7	Solubility	Completely soluble: acetonitrile; soluble on sonication: methanol; insoluble: water
8	State	Solid
9	Category	Antitussive, cough suppressant
10	Log P	5.08
11	pH	4.8

The chromatographic analysis was performed at ambient temperature. The detection wavelength was evaluated at 220–400 nm by scanning the sample over various wavelengths using Shimadzu Ultra-Violet (UV)-1800 and UV-1700.

Shimadzu LC-20AT HPLC system equipped with UV–Visible detector SPD-20A and Syncronis C18 (250 mm × 4.6 mm, 5 µm) column with a mobile phase of MeOH: Water (80:20) ratio was used for the analysis. The isocratic mode was chosen due to its simplicity in application and robustness concerning longer column stability. This configuration provides a large number of theoretical plate values for most of the separation. The percentage of the assay was found to be 101%. The method was validated as per the International Conference on Harmonization (ICH) guidelines.

Optimization of the mobile phase, optimization of detection wavelength and preparation of stock solution

Selection of solvent

Prenoxdiazine HCl is soluble in MeOH and ACN on sonication. Hence, MeOH was selected as a solvent for the method development.

Selection of wavelength (λ_{max})

A working standard solution of Prenoxdiazine HCl (80 ppm) was prepared from the standard stock solution and scanned over the wavelength range of 200–400 nm. The wavelength maximum (λ_{max}) was selected by observing the spectra for the highest absorbance.

Preparation of standard stock solution (1000 ppm)

100 mg of Prenoxdiazine HCl was accurately weighed and transferred to a 100 mL volumetric flask (VF) and dissolved in MeOH. After the complete dissolution of the drug, the volume was made up to 100 mL with MeOH. The resulting solution was 1000 ppm.

Preparation of test sample solution (80 ppm)

0.8 mL of the standard stock solution was taken and transferred to a 10 mL VF and the solution was made up to the final mark. The resulting solution obtained was 80 ppm. Similarly, other solutions were prepared from 1000 ppm.

Selection of wavelength, mobile phase, and preparation of stock and test solutions for RP-HPLC

Selection of elution mode

Reverse-phase (RP-HPLC) is used for the analysis of ionic compounds and moderate to nonpolar compounds. Reverse-phase chromatography is simple, convenient, efficacious, stable, and reproducible. C_{18} column was selected because it is the least polarity in comparison to C_4 and C_8 columns. C_{18} column allows eluting polar compounds more quickly in comparison to nonpolar compounds. In addition to this, the UV detector was used, which allows easy detection of the compounds in UV transparent organic solvents.

Selection of wavelength (detection limit)

The selectivity of the HPLC method that uses a UV detector depends on the proper selection of wavelengths. The sample solution of Prenoxdiazine HCl (80 ppm) was scanned in the range of 200–400 nm. Data were obtained overlay spectra of the drug. The wavelength of 259 nm was selected as a detection wavelength.

Selection of mobile phase

The various solvents were tried as mobile phases for the separation of Prenoxdiazine HCl based on the solubility profile.

Preparation of stock solution of prenoxdiazine HCl: (1000 ppm)

100 mg of Prenoxdiazine HCl was accurately weighed and transferred to a 100 mL VF and dissolved in 10–15 mL of MeOH and volume was made up to 100 mL MeOH. The resulting solution was 1000 µg/mL.

Preparation of test sample solution

The accurately measured solution of Prenoxdiazine HCl (0.4, 0.6, 0.8, 1.0, and 1.2 mL) from the stock solution was transferred to a 10 mL VF and made up to the mark with MeOH. The solutions prepared were 40, 60, 80, 100, and 120 µg/mL.

Preparation of diluents, mobile phase and standard solution for RP-UPLC

Preparation of diluent

To 800 mL of ACN, 200 mL water was added and the resulting solution was sonicated for 5 min for degassing.

Preparation of the mobile phase

The mobile phase was prepared such that it consists of two phases, phosphate buffer pH 7 (A) and ACN (B). Accurately weighed 1.36 g of potassium dihydrogen orthophosphate was added to 1000 mL of water, and the pH of the solution was adjusted to pH 7 with diluted 10 mM NaOH and filtered through 0.22 µ nylon filter. 1000 mL of HPLC grade ACN was filtered through a 0.22 µ nylon filter. Both the phases were degassed by sonication for 5 min.

Preparation of standard

A standard stock solution of Prenoxdiazine HCl was prepared by transferring an accurately weighed quantity of about 100 mg of Prenoxdiazine HCl in a 100 mL VF. Then, added about 75 mL of diluent, dissolved, and sonicated it for 5 min to achieve complete solubilization. After that diluted to the marked volume with the diluent and mixed well.

Parameters for the method validation

The optimized RP-UPLC, RP-HPLC, and UV methods were validated according to the methods described in ICH guidelines Q2 (R1) [15]. The validation characteristics addressed were specificity, linearity, and range, accuracy, precision, and robustness [16]

Forced degradation study

The forced degradation/stress study was conducted to evaluate the specificity and stability-indicating feature of the developed method. The standard solution of Prenoxdiazine HCl (100 µg/mL) was stressed deliberately in the solubilized form at acidic/alkaline pH conditions, and thermal, oxidative as well as photolytic conditions.

Acid degradation

To the 8 mL of 100 µg/mL standard Prenoxdiazine HCl solution, 1 mL 3 N HCl was added and the solution was kept at room temperature for 1 day. After 1 day, the sample was neutralized using 1 mL of 3 N NaOH and the volume was made up 10 mL with diluent. Similarly, the blank (without drug) sample was also studied.

Base degradation

To the 8 mL of 100 µg/mL standard Prenoxdiazine HCl solution, 1 mL of 1 N NaOH was added and the solution was kept at room temperature for 4 h. Then, the solution was neutralized with 1 mL of 1 N HCl and the final volume was made up 10 mL with diluents and RP-UPLC was performed. Similarly, the blank (without drug) sample was also studied.

Thermal degradation

1 mL of Prenoxdiazine HCl standard solution (100 µg/mL) was taken in a test tube and it was placed in an oven at 60 °C for a day. The sample was allowed to cool at room temperature and then quantitatively transferred to a 10 mL VF. Then, the final volume was adjusted with diluent and drug quantification was performed by RP-UPLC.

Oxidative degradation

To 9 mL of standard solution (100 µg/mL) in a test tube, 1 mL of 30% hydrogen peroxide (H_2O_2) was added. The mixture was kept at 25 °C for 3 days and later RP-UPLC was performed for the estimation of Prenoxdiazine HCl. Similarly, the blank (without drug) sample was also studied.

Photodegradation

10 mL of 100 µg/mL Prenoxdiazine HCl standard solution was taken in a 10 mL VF and it was kept in a UV cabinet and was exposed to the light of 1.2 million lux hrs under 200 watts. The degraded samples were injected into the RP-UPLC system and they were analyzed against an untreated control sample.

Results

Optimization of detection wavelength for UV method development

A working standard solution of 80 ppm was prepared from the standard solution of Prenoxdiazine HCl, and scanned over the wavelength range of 200–400 nm. The wavelength maximum was selected by observing the spectra for the highest absorbance. The λ_{max} was found to be 259 nm (Fig. 2) and the calibration curve has been plotted (Additional file 1: ST-9). Further, the method was validated based on repeatability (Additional file 1: ST-10), precision (Additional file 1: ST-11), accuracy, robustness, LOD and LOQ. Further, the validated method has been employed for the estimation of Prenoxdiazine HCl in an unknown sample (Additional file 1: ST-16).

Optimization of chromatographic conditions for RP-HPLC method development

RP-HPLC method was developed by running the various chromatographic trials (Additional file 1: SF-2). The chromatographic trials were by changing the mobile phase composition (Additional file 1: ST-16), flow rate (Table 3a), temperature (Table 3b), etc. The first trial was run by using ACN: Water as a mobile phase in the ratio of (50:50) (Additional file 1: SF-2a). The resulting HPLC chromatogram shows an improper peak shape. Other trails were taken on the same mobile phase but with different ratios. The results of the change in the ratio of the mobile phase tempted the peak splitting that was observed in ACN: Water in the ratio of 80:20 (Additional file 1: SF-2c), the solvents were changed and trials were rerun on the MeOH: Water in different ratios. Based on all the results of several trials, the mobile phase was optimized as MeOH: Water in the ratio of 80:20 (Fig. 3a). A sharp peak was obtained at 2.9 min in C18 column (250 mm × 4.6 mm, 5 µm) under the flow rate of 1.0 mL/min. Further, the developed method has been validated for system suitability (Additional file 1: ST-17), linearity (Fig. 3b), precision (Additional file 1: ST-19), and other parameters.

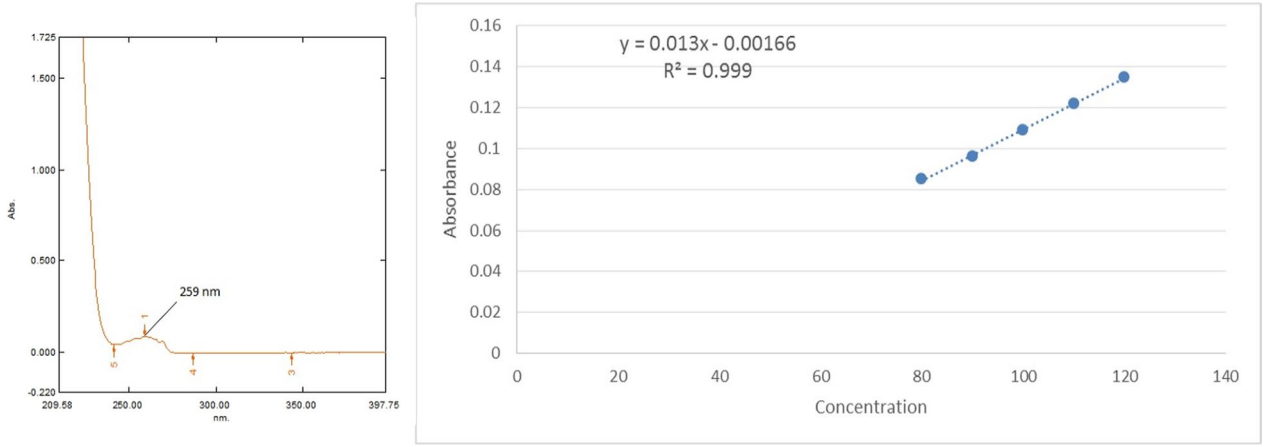
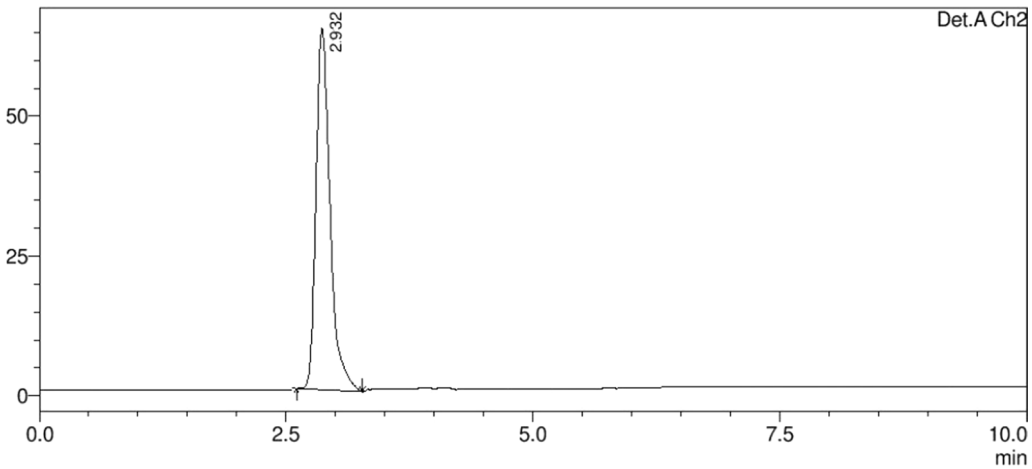
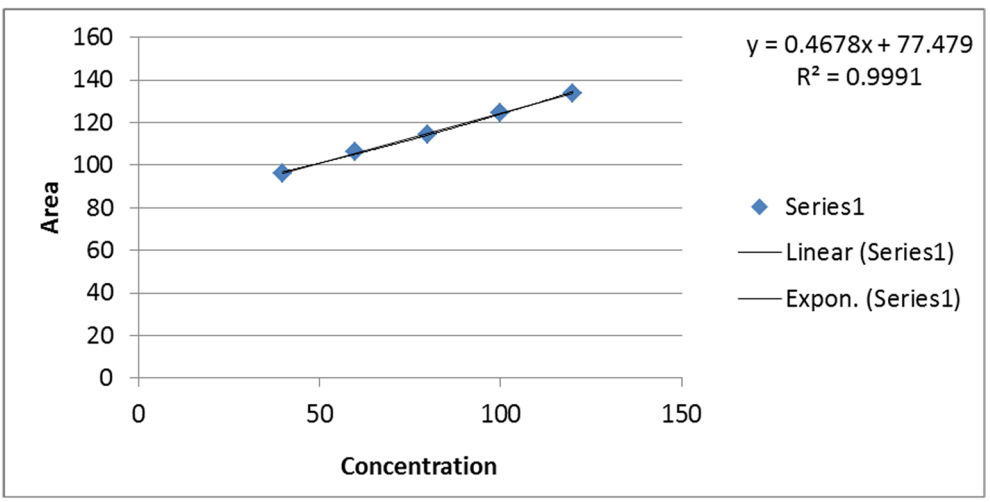


Fig. 2 Selection of wavelength at 259 nm and calibration curve for UV–visible spectroscopy



(a)



(b)

Fig. 3 **a** Optimized chromatogram of prenoxdiazine HCl at 259 nm, **b** calibration curve for HPLC

RP-UPLC method development

The objective of the present study was to develop a rapid and sensitive method of analysis for Prenoxdiazine HCl. The use of UPLC for analytical method development has received considerable attention because of its advantages over existing methods in the form of its speed, sensitivity, resolution, less solvent consumption, more productivity, and cost. The present study is an attempt to develop a precise, rapid, and sensitive method for the identification and quantification of Prenoxdiazine HCl. While developing an analytical method using RP-UPLC, the influence of mobile phase composition, the sampling rate of the detector, and diluents on the peak shape of the drug were evaluated. Also, various combinations of gradient time programs were tried and then finalized based on the sharpness of the Prenoxdiazine HCl peak (Fig. 4a).

Optimization of mobile phase composition

The first mobile phase tried was phosphate buffer and ACN (Additional file 1: SF-3). The resulting UPLC chromatogram indicates the tailing of the peak was decreased to a greater extent. Moreover, the interference was also observed between 6 and 8 min. In the next trials, mobile phase composition was varied and the results were analyzed. During optimization of the mobile phase, several chromatographic trials were run with the different mobile phase composition, i.e., phosphate buffer (pH 7.5): MeOH, acetate buffer (pH 7.5) and MeOH, phosphate buffer (pH 3) and acetonitrile (Additional file 1: SF-3a), phosphate buffer (pH 5): ACN (Additional file 1: SF-3b) and phosphate buffer pH (6.8): ACN (Additional file 1: SF-3c). Among these mobile phases, phosphate buffer (pH 7.5) and ACN have shown better performance concerning separation and sharpness of drug peak, hence the same composition was selected for the study.

Effect of the sampling rate of the detector

Using phosphate buffer (pH 7.5) and ACN mobile phase, the effect of the sampling rate of the detector on the drug peak was assessed. Although the mobile phase composition maintained constant, the gradient time varied. An increase in the sampling rate of the detector doubled the drug peak response. The peak was very sharp without tailing and a blunt shape at the apex was also absent (Additional file 1: SF-4).

Influence of diluent composition

Effect of two different diluents comprising ACN: water (80:20) and MeOH: water (80:20) was evaluated on peak performance. Also, the solution stability was evaluated in these two diluents. The peak responses from these two at zero time were nearly similar but a prominent peak in front of the main drug peak was evident at 24 and 48 h when MeOH: water diluents were used. While no additional peaks were observed with ACN: water at 24 and 48 h suggesting the suitability of ACN: water (80: 20) (Additional file 1: SF-5). The impact of mobile phase and resolution of the chromatographic peak is shown in Additional file 1: ST-25.

Despite better chromatograms of the drugs obtained in phosphate buffer (pH 7.5) and ACN mobile phase, column (UPLC HSS columns) the performance was lost on usage due to the higher pH of the mobile phase. Because of the incompatibility of HSS columns at high pH, UPLC CSH, C_{18} (2.1 × 100 mm, 1.7 µm) column was used in the study. The pH of the mobile phase was lowered down to 7.0. Further, the flow rate of the mobile phase at 0.5 mL/min resulted in 12,000 psi system pressure on the use of the UPLC CSH column, hence the flow rate reduced to 0.4 mL/min.

Force degradation study

The degradation was observed at stress conditions, purity of drug peak in all condition passes the required standard [17]. The summarized results of force degradation study (Additional file 1: SF-6) are listed in Table 2.

Validation of UV method

Linearity and range

To observe the linearity five levels of concentrations ranging from 80 to 120 ppm (80, 90, 100, 110, and 120 ppm) were prepared and the absorbances for each concentration were recorded. Then, the graph of absorbance V/s concentration was plotted and the equation for a straight line with the correlation coefficient was determined. The linearity curve was repeated five times.

Precision

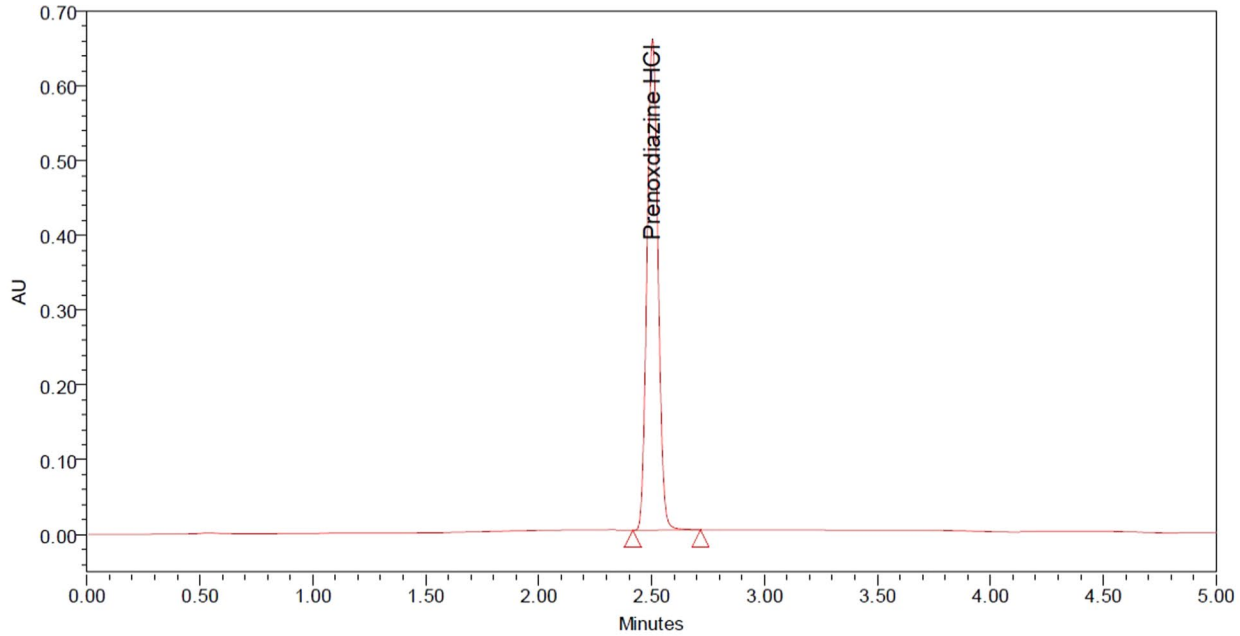
Repeatability Six replicates of standard solution (100 ppm) of Prenoxdiazine HCl were analyzed and % RSD was calculated. Acceptance criteria for % RSD should be less than 2%.

Intraday precision Three replicates of three concentrations (90, 100, and 110 ppm) of Prenoxdiazine HCl, a total of nine concentrations were analyzed in a single day and % RSD was calculated. Acceptance criteria for % RSD should be less than 2%.

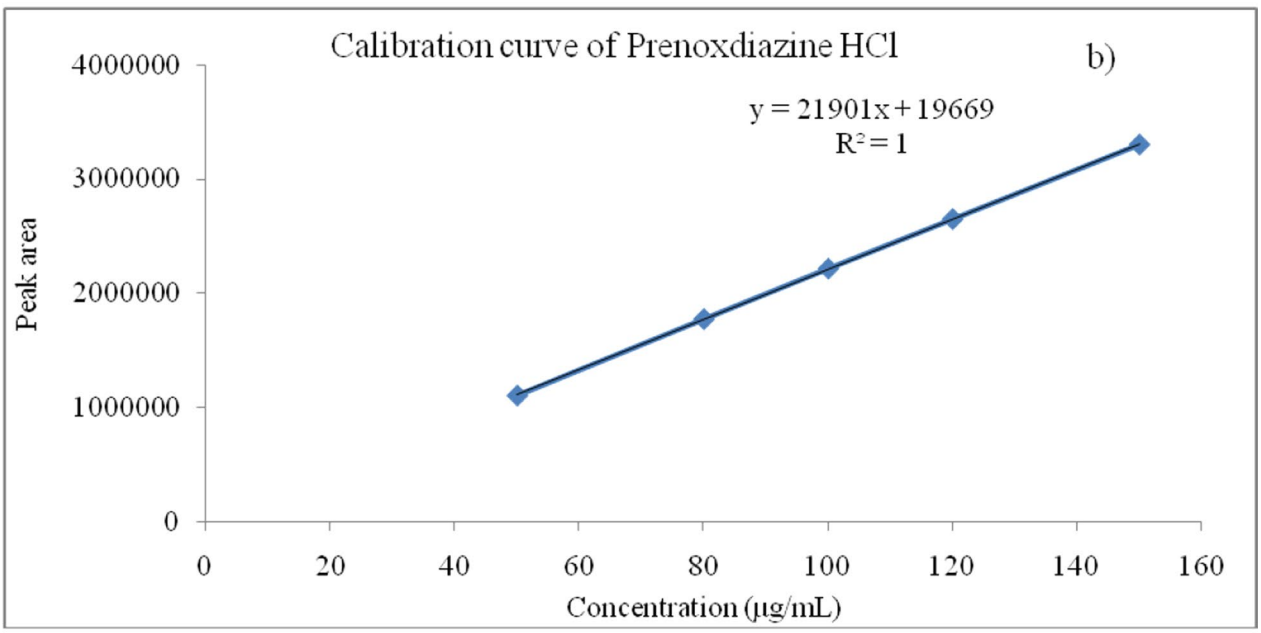
Intermediate precision

a. Interday precision

Three replicates of three concentrations (80, 100, and 120 ppm) of Prenoxdiazine HCl, a total of nine



(a)



(b)

Fig. 4 **a** Optimized chromatogram of prenoxdiazine HCl at 259 nm, **b** CALIBRATION curve for UPLC

determinations were analyzed at three consecutive days and % RSD was calculated. Acceptance criteria for % RSD should be less than 2%.

b. Different analyst

Three replicates of three concentrations (80, 100, and 120 ppm) of Prenoxdiazine HCl prepared by different analysts were analyzed in a single day and % RSD was calculated. Acceptance criteria for % RSD should be less than 2%.

Table 2 Forced degradation study using liquid chromatography technique

Stress condition	Optimized time period	% Reduction in area	Peak purity angle	Peak purity threshold
Untreated	–	–	0.12	0.587
Acid	1 day	16.34	0.586	1.143
Base	4 h	6.96	0.475	0.643
H_2O_2	3 days	8.15	0.586	1.110
Thermal	1 day	4.23	0.417	0.794
Photo	4.5 day	6.77	0.090	0.575

Table 3 Linearity and range of prenoxdiazine HCl in the HPLC studies

Sr. Nos.	Concentration (µg/mL)	Mean area ± SD ($n = 3$)
1	40	95.589 ± 0.430
2	60	106.402 ± 0.201
3	80	114.559 ± 0.150
4	100	124.388 ± 0.059
5	120	133.569 ± 0.295

c. Different instrument

Three replicates of three concentrations (80, 100, and 120 ppm) of Prenoxdiazine HCl were analyzed in two different instruments, UV-1800 and UV-1700 on a single day and % RSD was calculated. Acceptance criteria for % RSD should be less than 2%.

Accuracy

Accuracy was determined by spiking the drug product (which is previously analyzed) with three levels of known concentration (80, 100, and 120) of standard drug substance (Additional file 1: ST-12). From a 100 µg/mL test solution, a 4 mL solution was pipetted out and transferred to a 10 mL VF and diluted up to the mark with MeOH. It gives a concentration of 40 µg/mL. A total of nine test concentrations was prepared for a fixed amount of pre-analyzed samples (4 mL), increasing aliquots of Prenoxdiazine HCl (3.2, 4, 4.8 mL of 32, 40, 48 µg/mL) were added, respectively, and diluted up to the mark with MeOH.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in the method and indicates its reliability during normal usage. To confirm the robustness, change was induced in the detection wavelength (±0.5 nm) (Additional file 1: ST-13). % RSD for absorbance was calculated as 0.53, which is less than 2%.

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

The LOD and LOQ was estimated as 0.12 and 0.39, respectively, (Additional file 1: ST-14) from the set of five calibration curves used to determine method linearity.

The LOD can be calculated as:

$$\text{LOD} = 3.3 \times (\text{S.D.}/\text{Slope})$$

where SD = standard deviation of the y-intercepts of five calibration curves, Slope = mean slope of five calibration curves.

The LOD can be calculated as:

$$\text{LOQ} = 10 \times (\text{S.D.}/\text{Slope})$$

where SD = standard deviation of the y-intercepts of five calibration curves, Slope = mean slope of five calibration curves.

Analysis of drug in dosage form

The response of the sample solution was measured at 259 nm for the quantification of Prenoxdiazine HCl. The amount of Prenoxdiazine HCl present in the sample solution was calculated by fitting the responses into the regression equation for Prenoxdiazine HCl in the proposed method. 40 mg of Prenoxdiazine tablets was powdered and weighed and transferred to 100 ml of VF. For making up the volume MeOH was used as diluent. The absorbance value was put into the regression equation of the calibration curve and estimated the amount recovered (Additional file 1: ST-15).

Validation of RP-HPLC method

Linearity and range

The linearity response was determined by five independent levels (Table 3) of the calibration curve in the range of 40–120 µg/mL (Additional file 1: SF-1) for Prenoxdiazine HCl.

Precision

Repeatability The repeatability for Prenoxdiazine HCl (80 µg/mL) carried out based on five measurements of absorbance of the same solution (Additional file 1: ST-18) and % RSD was calculated as 0.436.

Intraday precision Three replicates of three mixtures of concentration of the standard solution (60, 80, and 100 ppm) of Prenoxdiazine HCl were prepared; a total of nine determinations was analyzed within a short period interval and % RSD was calculated.

Interday precision Three replicates of three mixtures of concentration of the standard solution (60, 80, and 100 ppm) of Prenoxdiazine HCl were prepared; a total of nine determinations was analyzed at three consecutive days and % RSD was calculated.

Intermediate precision

i. Different analyst

Three replicates of three mixtures concentration of the standard solution of Prenxdiazine HCl (60, 80, and 100 ppm) were prepared; a total of nine determinations were analyzed and % RSD was calculated.

Accuracy

The accuracy was determined by calculating the recovery of Prenoxdiazine HCl by the standard addition method (Additional file 1: ST-20). Powdered 100 mg of Prenoxdiazine HCl was weighed and transferred to a 100 mL of the VF, dissolved and diluted up to the mark with MeOH. The resulting solution was 1000 µg/mL. From 1000 µg/mL solution, 10 mL solution was pipetted out and diluted up to 100 mL with MeOH. The resulting solution was 100 µg/mL. From 100 µg/mL solution, 4 mL of solution was pipetted out and transferred to a 10 mL VF and diluted up to the mark with MeOH. It gives a concentration of 40 µg/mL. A total of nine test concentrations was prepared for a fixed amount of pre-analyzed samples (4 mL), increasing aliquots of Prenoxdiazine HCl (3.2, 4, 4.8 mL of 32, 40, 48 µg/mL) were added, respectively, and diluted up to the mark with MeOH.

Robustness

To evaluate the robustness of the method, a few parameters were deliberately varied. The parameters included are a variety of flow rates and changes in wavelength. The change was made at three levels and replicates 3 times. The flow rate change was in the range of (±0.1 mL) (Additional file 1: ST-21), and change in the wavelength was in range (±1 nm) (Additional file 1: ST-22). Then, the system suitability parameters were calculated for Prenoxdiazine HCl.

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

The LOD and LOQ was estimated as 0.041 and 0.123, respectively, (Additional file 1: ST-23) from the set of five calibration curves to determine the linearity of the method. The LOD can be calculated as:

$$\mathrm{LOD} = 3.3 \times (\mathrm{S.D./Slope})$$

where SD = standard deviation of the y-intercepts of five calibration curves, Slope = mean slope of five calibration curves.

The LOD can be calculated as:

$$\mathrm{LOQ} = 10 \times (\mathrm{S.D./Slope})$$

where SD = standard deviation of the y-intercepts of five calibration curves, Slope = mean slope of five calibration curves.

Analysis of drug in dosage form

The response of the sample solution was measured at 259 nm under the chromatographic condition mentioned earlier for the quantitation of Prenoxdiazine HCl in the dosage form. The amounts were determined by applying the values of the peak area to the regression equations of the calibration graph. The % assay was obtained as 101% (Additional file 1: ST-24).

Validation of RP-UPLC method

The developed RP-UPLC method was validated as per ICH Q2 (R1) guidelines. The following parameters were employed to validate the RP-UPLC method.

System suitability test

Before each validation run, the system suitability test of the chromatographic system was performed to verify whether the system is adequate for the analysis. To assess the system suitability, the parameters like retention time, theoretical plates, asymmetry, and the percentage of the relative standard deviation (% RSD) were investigated (Table 4). Five replicates injections of standard preparations were injected, then asymmetry, theoretical plate and % RSD were determined. For all the system suitability injections, the retention time was found to be 2.5 min, asymmetry, and % RSD were less than 2.0 and theoretical plates were greater than 10,000, suggesting the system suitability for RP-UPLC analysis Prenoxdiazine HCl analysis.

Table 4 Mean value of system suitability test UPLC

Sr. Nos.	Parameters	Result
1	Peak area	2,230,459
2	No. of theoretical plates	17,456
3	Retention time (min)	2.5
4	Asymmetry/USP tailing	1.1
5	% RSD	0.8

Specificity

The ability of the method to separate the drug from its degradation products and non-interference of excipients indicates the specificity of the method. The specificity of the method was evaluated by checking the interference of diluent with drugs. The overlaid chromatograms of diluent and Prenoxdiazine HCl revealed no interference of excipients with the drug (Additional file 1: SF-8).

Precision

The precision of the method was determined by performing repetitive analysis of six independent test sample preparation and % RSD was calculated. The method is considered to be validated for precision when % RSD is less than 1%. The precision results of the developed RP-UPLC method were analyzed and % RSD is found to be less than 1%, hence complied with ICH standard (Additional file 1: ST-26).

Linearity

The linearity of the developed RP-UPLC method was investigated by analyzing the standard solutions of Prenoxdiazine HCl at five different concentration levels like 50, 80, 100, 120, and 150 µg/mL (Additional file 1: SF-7). The overlaid chromatographs of these concentrations and calibration curves were plotted. The plot of peak area vs. concentration was found to be linear over a concentration range of 50–150 µg/mL with a slope of 21,910, intercept of 19,669, and regression coefficient of 1. The high value of the regression coefficient suggested a good quality of linearity (Fig. 4b).

Robustness

The robustness of an analytical method is a measure of the ability to remain unchanged by performing small deliberate variations in method parameters and indicates reliability during its normal usage. The robustness of the developed RP-UPLC method was assessed by altering the flow rate and column oven temperature. The results of the above-mentioned deliberate changes are summarized in Additional file 1: ST-28. All the other chromatographic parameters were held constant. Then, the asymmetry (tailing factor) of Prenoxdiazine HCl was less than 1.30 illustrating the robustness of the method.

Solution stability

The stability of Prenoxdiazine HCl in solution was established by storing the drug solution at 25 °C and 2–8 °C for 72 h. These solutions were reanalyzed following 24 and 72 h, and results are analyzed and compared against the fresh sample. % recovery of drug stored at 25 °C and 2–8 °C was found to be > 99% thereby confirming the solution stability of Prenoxdiazine HCl (Additional file 1: ST-27).

Discussion

A precise, accurate, economical, and reproducible method has been developed for the estimation of Prenoxdiazine HCl in pharmaceutical and bulk dosage form. The drug and its bulk dosage form is been evaluated through comparative analytical methods like UV, RP-HPLC, and UPLC.

Various diluents, buffers (at different pH levels), and mobile phases were tested in order to ensure that the established method could be used commercially and that exact results could be obtained. Initially, the cost-effective approach (RP-HPLC) was developed and validated using MeOH and water. The applicability of the disclosed method was subsequently tested on a more sophisticated instrument (UPLC) with empower-3 software, but an improper peak and a seeking shorter run time forced to shift toward ACN and buffer as mobile phase. Though the UV and RP-HPLC methods are simple, specific, and reproducible, the analysis time was found to be minimum in UPLC, as expected. Forced degradation studies were performed to evaluate the ability of drugs to withstand in the pure form under stressed conditions. Conditions used should reflect the situations possibly to be encountered during actual formulation and handling. Prenoxdiazine HCl was subjected to acid, alkaline, oxidative, thermal, and photolytic stress conditions (Additional file 1: SF-6). Although the nature of degradant product is not reported in the current study. The results of the study suggested that degradation products were well separated from the standard peak of Prenoxdiazine HCl and the purity data (purity angle should be less than purity threshold) of Prenoxdiazine HCl confirmed homogeneity of the peak (Table 2). Although degradation was observed at stress conditions, purity of drug peak in all condition passes the required standard.

Conclusion

The present study reports the unique and novel analytical method for the analysis of patented drug Prenoxdiazine HCl. When compared to other analytical procedures, a more sophisticated, powerful, and simple methodology (UPLC) was selected due to the utilization of high pressure and small particle size. Additionally, the use of PDA detector and upgraded software revealed the stability of Prenoxdiazine HCl even at stress conditions. The method was validated to establish compliance with ICH guidelines. The statistical parameters and asymmetry were found to be under the acceptance criteria. The developed method proved to be superior in separating the main

drug peak from its degradants and diluent. Therefore, this method can be explored for routine analysis as well as stability analysis via RP-UPLC of Prenoxdiazine HCl in its bulk form and its formulation.

Abbreviations

RP-HPLC	Reverse-phase high-performance liquid chromatography
UPLC	Ultra-high performance liquid chromatography
ACN	Acetonitrile
MeOH	Methanol
LOD	Limit of detection
LOQ	Limit of quantitation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-023-00504-1.

Additional file 1: Supplementary data of UV–Vis spectra, Calibration curve, System Suitability, Overlay Spectra, Chromatographic trials as figures indicating SF-1–08. Supplementary data of Absorbance V/s Concentration of standard, Validation parameters, Selection of Mobile Phase and Optimization, Effect of mobile phase composition on the resolution as table indicating ST-09–28.

Acknowledgements
We are thankful to Khandelwal Laboratories, Mumbai, for providing the Sample, Piramal Pharmaceutical development services, Ahmedabad, for providing facilities. We are also thankful to the Principal, R C Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, for encouragement.

Author contributions
RS, RB, DP, and AB contributed equally for proceeding this research. Concept, guidance, and final manuscript was prepared by AN and checked by AS. We declare that all authors have read and approved the manuscript before submission.

Funding
Not applicable.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The authors declare no conflict of interest.

Competing interests
The authors declare that they have no competing interests.

Received: 12 November 2022 Accepted: 17 June 2023
Published online: 28 June 2023

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