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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PROPRANOLOL HYDROCHLORIDE AND ETIZOLAM IN THEIR COMBINED DOSAGE FORM BY VIERODT'S METHOD

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ABSTRACT:

The present study deals with UV spectrophotometric method development and validation for the simultaneous estimation of Propranolol hydrochloride and Etizolam in bulk and combined dosage form by simultaneous equation method (Vierodt's method).The wavelengths selected are 255 and 288nm lambda max of Etizolam and Propranolol hydrochloride respectively. The linearity of propranolol hydrochloride and etizolam was found to be in the range of 5-50µg/ml. The % Recovery was found to 98.75% to 99.88% for Propranolol hydrochloride and 99.2% to 102.5% for Etizolam. The proposed method was validated as per ICH guidelines.

KEYWORDS:

Propranolol hydrochloride,Etizolam, simultaneous equation method .

INTRODUCTION

Propranolol (PROP) (**Fig 1**), chemically (RS)-1-(isopropylamino) 3-(1-naphthyloxy) propan-2-ol [1] is a nonselective beta blocker and is mainly used in the treatment of hypertension by blocking the action of epinephrine and norepinephrine on both β1 and β2 adrenergic receptors and also used in the management of hypertension, angina pectoris**,** myocardial infarction, migraine, glaucoma etc[2]. Etizolam (ETI) (**Fig 2**) belongs to an original chemical class of diazepines, namely thienotriazolodiazepines with antianxiety activity and chemically it is 4-(2-Chlorophenyl)- 2–ethyl–9–methyl–6H- thieno [3,2-f] [1,2,4] triazolo-[4,3-a][1,4] diazepine)[3]. Etizolam has anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant properties [4]. It is 6-10 times more potent than diazepam [5].

The combination of these drugs are used to treat anxiety. Literature review reveals that a few analytical methods that have been reported for the determination of etizolam is by using solid phase extraction with GC–MS[6], high performance liquid chromatography (HPLC)[7] and capillary gas chromatography–mass spectrometry[8]. UV [9], HPLC [10], HPTLC [11] and GC [12] are reported for the estimation of propranolol in single and other combined dosage forms. PROP is official in IP[13] and ETI is official in JP XV. However there is no analytical method reported for simultaneous estimation of both drugs in their combined tablet dosage form. Present work describes rapid, simple, sensitive, accurate and reproducible UV spectroscopic method. The method was validated in compliance with ICH guidelines [14-15]

MATERIALS AND METHODS

Pure standards of Propranolol hydrochloride and Etizolam were obtained from Yarro chem Products and their marketed combination (ETIZOLA BETA) was purchased from the market. 0.1 N HCl of analytical grade was used as the solvent. A double-beam JASCO 630 UV-visible spectrophotometer, with a pair of 1 cm matched quartz cells were used to measure the absorbance of the solutions.

Preparation of Standard Stock Solutions: Standard stock solutions of Propranolol and Etizolam were prepared separately by dissolving 10 mg of each drug in 10ml of methanol to get standard stock solution of 1000 µg/ml respectivelyand 1 ml was pipetted out and further volume was made up to 10 ml with 0.1N HCl to obtain concentration of 100µg/ml. Further dilutions were made in 0.1N HCl from stock solution to get concentrations of 5-30 µg/ml of propranolol hydrochloride and etizolam.

EXPERIMENTAL METHOD

Simultaneous Equation Method:

Determination of Absorption maxima.

Propranolol hydrochloride dilutions were prepared from 100ug/ml stock solution to get concentrations of 5-30µg/ml. The solutions were scanned at each wavelength i.e.255nm and 288nm λmax of FLU and λmax of PRO. The calibration curve was plotted. The concentration of PRO and ETI was calculated using following equations:

Vierodt's Method of Simultaneous Equations: This method is based on absorption of drugs at the wavelength maximum of the other. It employs solving of simultaneous equations using Cramer's rule and matrices. The concentrations of the drugs were calculated from the following simultaneous equations:

Where, A1and A2 are absorbance of mixture at 255nm and 288 nm respectively, ax1and ax2 are absorptivities of PRO at λ_1 and λ_2 respectively and a_{y1} and a_{y2} are absorptivities of ETI at λ_1 and λ_2 respectively. C_x and C_Y are the concentrations of PROP and ETI respectively.

$$C_x = \frac{A_2 a y_1 - A_1 a y_2}{a x_1 a y_2 - a x_2 a y_1}$$

$$C_y = \frac{A_1 a x_1 - A_2 a x_1}{a x_1 a y_2 - a x_2 a y_1}$$

Standard Mixture Solution of Etizolam and Propranolol hydrochloride:

Accurately weighed 20mg of Propranolol hydrochloride and 5mg of Etizolam were transferred into a clean and dry 100 ml volumetric flask and dissolved with sufficient volume of 0.1 N HCl. The volume was made up to 100 ml with 0.1 N HCl to get concentration of 200 µg/ml of Propranolol hydrochloride and 50µg/ml mixture of Etizolam.Aliquots from standard solution were withdrawn in the volumes of 0.5, 1.0, 1.5, 2.0 and 2.5 ml and transferred into different 10 ml volumetric flask. The volumes were made up with the 0.1 N HCl to get 10-50µg/ml of PROP and 2.5-20µg/ml of ETI in the mixture. Absorbance of working standard solutions of mixture of the drugs were taken at the wavelength of 255 and 288nm using 0.1 N HCl as blank. The concentration of both the drugs were calculated from the simultaneous equation

Application of the Developed Method on Tablet Dosage Form

Twenty tablets containing 0.5mg of Etizolam and 20 mg of Propranolol hydrochloride were weighed and finely powered. Accurately weighed 125.1mg of powder which is equivalent to 20mg of Propranolol hydrochloride and 0.5mg of Etizolam was transferred into a clean, dry 100 ml volumetric flask. To this 4.5 mg of standard Etizolam powder was added to achieve ratio. To this 20 ml of methanol was added and sonicated for 15 min.. The resulting suspension was then filtered through whatmann filter. The volume of filtrate was made up to 100 ml with 0.1 N HCl to get the concentration 200µg/ml of Propranolol hydrochloride and 50µg/ml of Etizolam. Further dilutions were made to get 20µg/ml of Propranolol hydrochloride and 5µg/ml of Etizolam. All determinations were carried out three times at selected wavelengths and then the concentration of both the drugs was calculated using the equation.

Analytical Method Validation

Validation of the UV method was done with respect to following parameters

1) Linearity and Range

The standard solutions of both PRO and ETI were scanned in the range of 400-200 nm against solvent 0.1N HCl and absorbance was measured at λmax of 288nm and 255nm respectively. The stock solution was diluted with distilled water to reach a concentration range 1-23 µg /ml for FLU and 4-48 µg/ml for PRO. The absorbance was plotted against the corresponding concentrations to obtain the calibration graphs.

2) Accuracy

Recovery studies was carried out by applying the method to drug sample to which known amount of PRO and ETI corresponding to 80, 100, 120% of label claim has been added (standard addition method).

3) Precision

The standard solutions of drug sample were prepared and analyzed. The tablet assay was performed to determine reproducibility and repeatability. The percentage relative standard deviation (RSD %) was found to be within limits.

Limit of Detection (LOD)

For determination of LOD, visualization method was followed. In visualization method lower dilutions of the standard drugs were made and absorbance at 255 and 288 nm were recorded.

RESULTS AND DISCUSSION

An attempt was made to develop a simple, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of Propranolol and Etizolam in their combined dosage forms. The molar absorptivity for both the drugs were calculated at the wavelengths 288 nm (λmax of Propranolol), 255 nm (λmax of Etizolam) . Both the drugs obeys Beer Lambert's law in the range of 5-50µg/ml.

The simultaneous equation used for analysis of Propranolol hydrochloride and Etizolam in sample formulation gives satisfactory results of 19.94 mg and 0.49 mg of Propranolol hydrochloride and Etizolam, which complies with the label claim of 20 mg of Propranolol hydrochloride and 0.5 mg of Etizolam respectively. The method has been further validated for limit of detection, linearity, range, precision and accuracy. The sensitivity of the method was found to be satisfactory. The lowest limit was detected by visualization method at the concentration of 0.6 µg/ml and 0.1 µg/ml for Propranolol hydrochloride and Etizolam respectively.The precision of method and system was determined by replicate injections of standard solution. In method precision the % RSD of the assay was found to be 0.326 % for Propranolol hydrochloride and 0.377 % for Etizolam. In system precision the %RSD of the absorbance was found to be 0.177% for Propranolol hydrochloride and0.377% for Etizolam. The precision on different days was verified and found to be satisfactory. The values were precise as found from the % RSD of the assay, which was 0.03186% for Propranolol hydrochloride and 0.240 % for Etizolam.As the values of % RSD for precision study obtained was within the acceptance criteria of less than 2%, the proposed method was found to be providing

good degree of precision and reproducibility. The accuracy was determined through recovery study of the drug by spiking the standard drug of Propranolol hydrochloride and Etizolam at three different levels of 80%, 100 % and 120 % with previously analyzed samples of known fixed concentration. The results showed percentage recovery of 98.75% to 99.88% for Propranolol hydrochloride and 99.2% to 102.5% for Etizolam which was in good agreement to acceptance criteria of 90- 110%. Based on the results obtained, it is found that the proposed methods are accurate, precise and reproducible. It can be employed for routine quality control analysis of Propranolol and Etizolam in combined tablet dosage forms.

CONCLUSION

The new, simple, sensitive and economical UV spectrophotometric methods were developed for the simultaneous analysis of Propranolol and Etizolam in bulk and in pharmaceutical formulations. The developed methods were validated and from the statistical data, it was found that the methods were linear, accurate and precise and can be successfully applied for the analysis of pharmaceutical formulations without interference of excipients.

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