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HPTLC method development and validation for the estimation of Propafenone Hydrochloride in tablet dosage form

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ABSTRACT

A simple, selective, linear, precise and accurate HPTLC method was developed and validated for rapid assay of propafenone HCl in tablet dosage form. The separation was achieved on aluminum plate 60F$_{254}$, (10 × 10 & 20 × 10 cm) with 250 µm thickness as the stationary phase and the mobile phase consisted of chloroform: methanol: ammonia (8:2:0.2, v/v). The solvent system was found to give compact spot for propafenone HCl (R_f values of 0.49). Densitometric analysis was carried out in the absorbance mode at 254 nm. The linear regression analysis data for the calibration plots showed good linear relationship with respect to peak area in the concentration range 0.50-1.50 µg spot^{-1} of propafenone HCl (with r = 0.99774). The method was validated for limit of detection, limit of quantitation, accuracy, precision, robustness and recovery. The result and statistical analysis proves that the developed method is reproducible and selective for the estimation of said drug. The proposed method can be successfully applied for the estimation of propafenone HCl in tablet dosage forms.

Key-words: *Propafenone HCl; HPTLC; Densitometric analysis Validation.*

INTRODUCTION

Propafenone (**Fig. 1**) is chemically (R, S) 1-{2-[2-hydroxy-3 - (propylamino) propoxy] phenyl} - 3 - phenylpropan - 1 - one. Propafenone is a class of anti-arrhythmic medication, which treats illnesses associated with rapid heartbeats such as atrial and ventricular arrhythmias. It works by slowing the influx of sodium ions into the cardiac muscle cells, causing a decrease in excitability of the cells [1-2].

Literature survey showed some HPLC method [3] and HPTLC method [4-5] for the estimation of propafenone HCl in pharmaceutical dosages form. Most of these methods report the estimation of propafenone HCl from tablet formulation in the biological samples particularly from plasma [6-9].

This paper reports a simple, precise, rapid and cost effective HPTLC method for the estimation of propafenone HCl in its tablet dosage form [10].

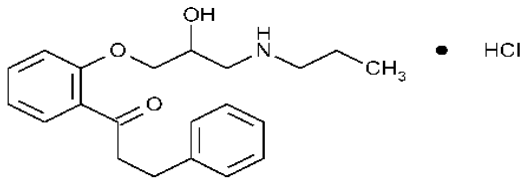

Fig. 1: Chemical structures of Propafenone HCl

MATERIAL AND METHODS

Instrumental and analytical conditions:

Standard experimental conditions were optimized in view to develop an assay method to quantify propafenone HCl as in its tablet dosage form. Samples was spotted in the form of band of 2 mm with Camag microlitre syringe on pre-coated silica gel aluminum plate 60F$_{254}$, (10 × 10 & 20 × 10 cm) with 250 µm thickness; using CAMAG LINOMAT 5 semiautomatic sample applicator and LINOMAT V automatic sample applicator with help of (Hamilton-100 µl Switzerland) syringe. The plates were prewashed with methanol so as to remove adhere impurity and activated at room 120^{0}C for 5 min prior to chromatography. Samples were applied as band at a distance of 8 mm from lower edge and the distance between two bands was 4 mm. The mobile phase consisted of chloroform: methanol: ammonia (8:2:0.2v/v/v) was optimized for good resolution with compact spots. The length of chromatogram run was 80 mm. Subsequent to the development; TLC plate was dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorption mode at 254 nm.

Reagents and chemicals:

Analytically pure propafenone HCl and tablet formulation was gifted by Emcure Pharmaceutical Limited, Pune. All chemicals and reagents used were of AR grade, from Merck Chemicals (Mumbai, India).

Preparation of Analytical solutions:

Preparation of mobile phase:

Mobile phase was prepared by mixing 8 ml chloroform, 2 ml of methanol and 0.2 ml of ammonia.

Preparation of standard stock solution:

The stock solutions (1000 µg/ml) of PFN was prepared by accurately dissolving 10 mg of the drugs with sufficient methanol in 10 ml volumetric flask and then the volume was made separately to 10 ml with methanol.

Preparation of standard solution:

5.0ml of PFN stock solution further diluted to 10 ml with methanol to get final concentration of 0.5 µg/µl of PFN. Then further take 5 ml and diluted to 10 ml to get concentration 0.25 µg/µl.

Preparation of sample stock solution:

Twenty tablets were weighed and average weight was calculated. The tablets were triturated to a fine powder. An

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accurately weighed quantity of powder equivalent to 100 mg of PFN was transferred to 10 ml volumetric flask. To it add 5 ml of methanol shake well and sonicated for 10 min. The resultant solution was filtered through 0.45µm membrane filter, diluted to volume with methanol to get stock sample solution containing 10 µg/µl of PFN.

Preparation of sample solution:

0.5 ml stock sample solution was further diluted to 10 ml with methanol to get concentration of 0.5 µg/µl of PFN. Then further take 5 ml and diluted to 10 ml to get concentration 0.25 µg/µl. Sample solution (2µl) was applied on TLC plate, developed and scanned under standard chromatographic condition.

Analysis of the marketed formulation:

To determine the content of commercial formulation the solution were prepared as described in preparation of sample solution. Mean peak area of the drug was calculated and the drug content in the tablets was quantified.

Method development and validation of HPTLC:

Linearity:

Standard solution of propafenone HCl (0.25 µg/µl) was prepared in methanol. 2, 3, 4, 5, and 6 µl of standard solution was applied to TLC plate so as to give concentration 0.5, 0.75, 1.0, 1.25, 1.50, and 1.75 µg spot^{-1} for propafenone HCl. The data of peak area plotted against corresponding concentration was treated by linear least-square regression analysis. **(Fig. 3)**

Precision:

Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions.

Interday precision and intraday precision were determined both in terms of repeatability (injection and analysis). The intermediate precision of method was checked by repeating the study on different days.

The repeatability of sample application and measurement of peak area was determined by performing six replicate measurements of the same band. The intermediate precision of method was checked by repeating the study on different days.

Accuracy:

The recovery studies were carried out by adding known amount of standard to samples at 80, 100 and 120% level and analyzed by the proposed method, in triplicate. This was done to check the recovery of the drug at different levels in the formulations by optimized method.

Limit of detection and limit of quantitation:

The limits of detection and quantitation of the developed method were calculated for propafenone HCl using the formula as given below.

Limit of Detection=3.3 x σ/S Limit of Quantitation=10 x σ/S Where, "σ" is the standard deviation of the response, "S" is the slope of the calibration curve.

Specificity:

The specificity of the method was ascertained by analyzing the standard drug and sample with respect to R_f value and spectra. The peak purity of propafenone HCl was assessed by comparing the spectra of diluents, mobile phase, standard and sample.

RESULTS AND DISCUSSIONS

The present investigation reported a new HPTLC method development and validation of estimation of propafenone HCl. The method developed was proceeding with wavelength selection. The optimized wavelength was 254nm. **(Fig . 2)**

In order to get the optimized HPTLC method various mobile phases were used. The mobile phase consisted of an aqueous solution of chloroform: methanol: ammonia (8: 2: 0.2 v/v) was used and the R_f value was about 0.49. The specificity of the method was determined for presence of components that may be unexpected to be present. The absence of additional peaks in the chromatogram indicates non interference of the excipients in the tablet dosage form. The linearity was determined in analyte concentration range of 0.5-1.50 µg spot^{-1}. The calibration curve obtained by plotting peak area versus concentration was linear and the correlation coefficient was found to be 0.99774 for propafenone HCl. **(Table 1, Fig. 4)**

The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The repeatability, interday and intraday were calculated for propafenone HCl. (**Table 3)**

The accuracy study was performed in 80%, 100% and 120%. The percentage recovery was determined for propafenone HCl and was found to be 99.12% (**Tables 4)**. Assay of propafenone HCl in its tablet dosages form was calculated. **(Table 2)**. A typical chromatogram showing the separation of propafenone HCl is shown in **Fig. 3**

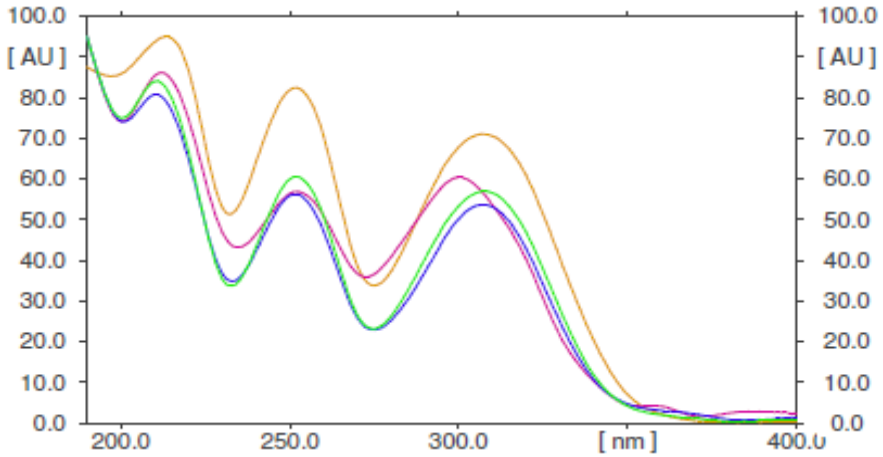

Fig. 2: Overlain spectra for selection of wavelength (254 nm) for Propafenone HCl

Table No. 1: Regression Statistics for analysis of Propafenone HCl

Range	r^2	Slop	LOD	LOQ
0.50-1.50 µg/spot	0.99774	366.5 + 3.911x	0.16 µg/spot	0.50 µg/spot

Table No. 2: For asssay of marketed formulation

Drug	Area of standard	Wt. of standard	Area of sample	Wt. of sample(mg)	% Purity	% lable claim

Propafenone HCl	8400	10 mg	7689	171.40	100 %	98.25 %

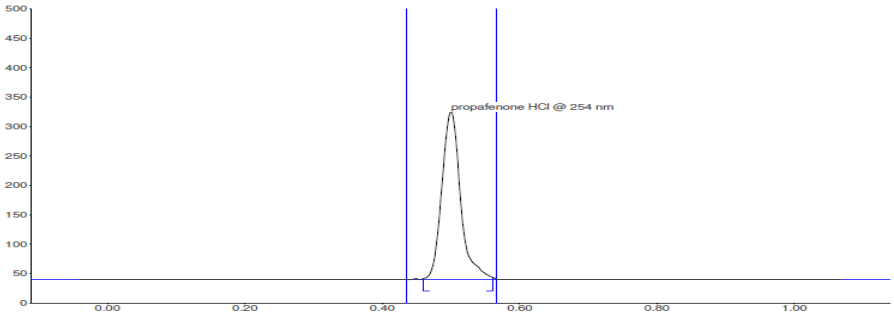

Fig. 3: Representative chromatogram of Propafenone HCl Standard

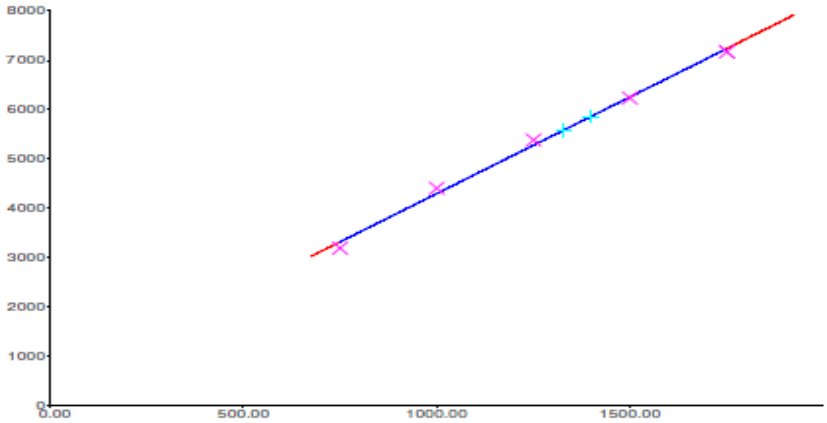

Fig. 4: Calibration curve of Propafenone HCl

Table No. 3: Repeatability of Propafenone HCl

Repeatability				
Rf value	**Mean Rf value**	**Area**	**Mean Area**	**% CV**
0.49		6113.33		
0.49		6152.00		
0.49		6281.45		
0.50	0.49	6409.92	6295.62	2.729
0.50		6521.42		
Intra-day repeatability				
0.50		6801.85		
0.50		6458.58		
0.51	0.51	6502.80	6541.40	2.518
0.51		6578.30		
0.51		6365.49		
Inter-day repeatability				
0.50		6537.33		
0.51		6138.80		
0.50		6123.69		
0.51	0.51	6166.70	6180.03	3.558
0.51		5933.63		

Table No. 4: Recovery analysis of Propafenone HCl

Drug	level of addition (%)	Amount of sample solution - I Applied (µl)	Amount of pure drug added (µl)	% Recovery ± SD	% RSD
	80	1	3.2	99.02 %	1.28
PFN	100	1	4	99.70 %	1.30
	120	1	4.8	98.65 %	0.82

* Each value corresponds to the mean of three determinations

CONCLUSION

The developed HPTLC method enables accurate, precise and specific for determination of propafenone HCl. Statistical analysis proves that the method is reproducible and selective for routine analysis of propafenone HCl in pharmaceutical dosage form without interference from excipients.

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