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New method development and validation for the determination of propafenone HCl in pure drug and its tablet dosage form by RP-HPLC

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ABSTRACT

A simple, rapid, accurate, precise, robust and reproducible reverse phase high performance liquid chromatographic method was developed for the determination of Propafenone HCl in pure drug and pharmaceutical dosage form. The quantification was carried out using enable Inertsil ODS-3 Vs column in a binary mode with mobile phase comprising of Acetonitrile: Methanol: Water (90:10) in the ratio of 75:25 %v/v at flow rate 0.7ml/min, detection was carried out at 245 nm using PDA detector with injection volume 20µl, the retention time was found to be 3.438min. The proposed method was validated as per ICH guidelines (Text and Methodology, Q2 (R1), 2005). The method produced a linear response in the concentration range of 1-5µg/ml (R2 0.999). The recovery studies were carried out and found to be within 101%. %RSD was found to be 2%. LOD and LOQ of propafenone HCl for the method were found to be 3.64µg/ml and 11.04µg/ml respectively. The proposed method was statistically evaluated and can be applied for the routine analysis, quality control of raw materials, formulation of different strengths, dissolution studies and bioequivalence studies for the same formulation of propafenone HCl.

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INTRODUCTION

Propafenone HCl is 1-[2-2[Hydroxy-3-propylamino]propoxy] phenylpropane-1-one hydrochloride (drug bank. Ca/drugs/DB01182), structure is shown in Figure 1, molecular Weight 377.909 g/mol, occurs as colourless crystals or white crystalline powder with a very bitter taste, solid in nature, Soluble in ethanol and water (with warming), Melting Point: 171-174°C. Propafenone HCl is used as an Anti-arrhythmic agent. Propafenone works by slowing the influx of sodium ions into cardiac muscle cells, causing a decrease in excitability of the cells. Propafenone is more selective for cells with a high rate but also blocks normal cells more than class1a or 1b. Propafenone differs from the prototypical class 1c antiarrhythmic in that it has additional activity as a beta-adrenergic blocker which can cause bradycardia and bronchospasm. Drugs that may interact with amiodarone, ketoconazole, ritonavir, saquinavir, erythromycin and grapefruit juice. Side effects of Propafenone HCl are dizziness, anxiety, loss of balance or coordination, nausea, vomiting and constipation. From the extensive literature survey, there are few methods, Implementation of QbD approach (Monika, L. *et al.,* 2013), Quantification of Propafenone HCl in presence of its degradation (Neha Sharma, S. *et al.,* 2013), Simultaneous determination of propafenone (Afshar M, R. *et al.,* 2004), RP-UFLC method (Sagar Suman Panda, B. *et al.,* 2014), it was revealed that there were a very few methods reported for pharmaceutical dosage forms. Therefore, here an attempt was made to develop a simple, cost-effective and accurate method.

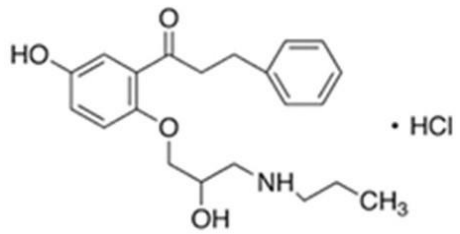


Figure 1: Structure of Propafenone HCl

METHODOLOGY

Determination of solubility of the drug

The drug was dissolved in different solvents and solubility was checked. Solubility was found good in methanol and slightly soluble in water.

Selection of Mode of Separation

The selection of a mode of separation depends on the nature of the sample, molecular weight, polarity and solubility. The drug selected in the present study was polar; hence the RP- HPLC method was preferred because of its suitability.

Preparation of Propafenone HCl drug solution

Accurately weighed 10mg of Propafenone HCl was transferred to the 10ml volumetric flask. The volume was made up to mark with the same solvent to 10ml (1000 µg/ml). Then 1ml of the above solution was diluted to 10ml with the solvent system (100µg/ml). The resultant standard solution (10µg/ml) was filtered through a 0.45 µm membrane filter and degassed under ultra-sonic bath before use. From the above standard solution, several working standard solutions are made by serial dilution technique.

RESULTS AND DISCUSSION

Selection of Detector Wavelength

A standard solution of propafenone HCl of concentration 10µg/ml was prepared and scanned in the UV region, i.e., 200 to 400 nm to detect the maximum wavelength.

Performance of trails

Different trials were performed with 10µg/ml solution by taking ACN: WATER in the ratio of (90:10), ACN:WATER (80:20)METHANOL:WATER (80:20) ACN:METHANOL: WATER (75:25). Hence after different trails of ACN: METHANOL: WATER at a ratio of 75:25 is selected for the further analysis and shown in figure 1.

Chromatographic conditions

The mobile phase consisted of ACN, Methanol and Water and in the ratio of 75:25. Contents of the mobile phase were filtered before use through a 0.22µm membrane filter and sonicated for 15min. The mobile phase was pumped with 0.7ml/min flow rate from the solvent reservoir to the column, and the injection volume was 20µl. The column oven temperature was maintained at 30°C. The eluents were detected at 245 nm and optimised conditions shown in Table 1.

Method Validation

Linearity

From the standard stock solutions of 0.1, 0.2 0.3, 0.4 and 0.5ml were pipette out and transferred into a 10ml volumetric flask, and the volume was made up with methanol. The solution was passed through 0.45µm membrane filter for filtration. The concentration of the solution was 1, 2, 3, 4 and 5µg/ml. The calibration curve was plotted between concentration and peak area and shown in figure 2.

Precision

From the standard stock solution 0.3ml was pipette out and transferred into six 10ml volumetric flasks, and the volumes were made with methanol. These solutions were filtered through a 0.45µm membrane filter. The solutions concentration was 3µg/ml of propafenone HCl. The same procedure is followed for inter-day precision, and intraday precision and chromatograms for inter and intraday was shown Figure 3 & 4 and results for Precision were shown in Table 2 & 3.

Acceptance criteria

The % RSD of responses of six replicate injections of intraday and interday should be not more than 2.

Accuracy

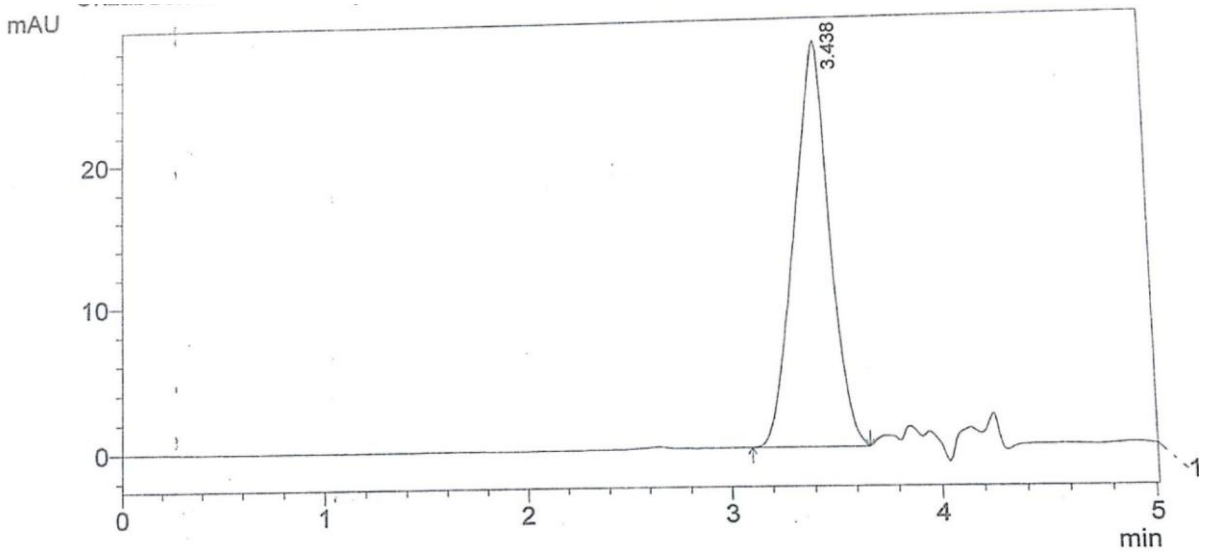
The average weight of tablets was determined by weighing 10 tablets and powdered. Tablet powder was equivalent to 50mg of Propafenone HCl was weighed and transferred into 10 ml volumetric flask about 5ml of methanol solution was added and degassed for 15 min for the complete dissolution of the drug, volume was made up to 10 ml with methanol and mixed. Above solution was filtered through Whatman filter paper number 41 i.e., primary sample stock solution (1000µg/ml) from this above solution 1 ml is pipetted out and made to 10ml (100µg/ml), i.e., secondary stock solution and further serial dilutions were made for accuracy and assay studies.

Preparation of 50% solution

From the 100µg/ml of the sample stock solution, 0.3ml of the solution was transferred into 10ml of the volumetric flask (3µg/ml); to which 0.15ml of Propafenone HCl working standard (100µg/ml)

Table 1: Chromatographic condition for method optimisation

S.No	Chromatographic parameters	Chromatographic conditions
1	Mode of separation	Isocratic Mode
2	Mobile phase	Solvent-A: Acetonitrile (75) Solvent-B: Methanol: Water 90:10 (25)
3	Column	Inertsil ODS-3Vs
4	Flow rate	0.7ml/min
5	Detection Wavelength	245nm
6	Injection Volume	20µl
7	Column oven temperature	Ambient (30°C)
8	Runtime	5min



Retention time	Peak Area	Theoretical plates	Tailing factor
3.438	327289	2585.580	0.848

Figure 2: Typical optimized chromatogram [ACN: (Methanol: Water 90:10) 75:25]

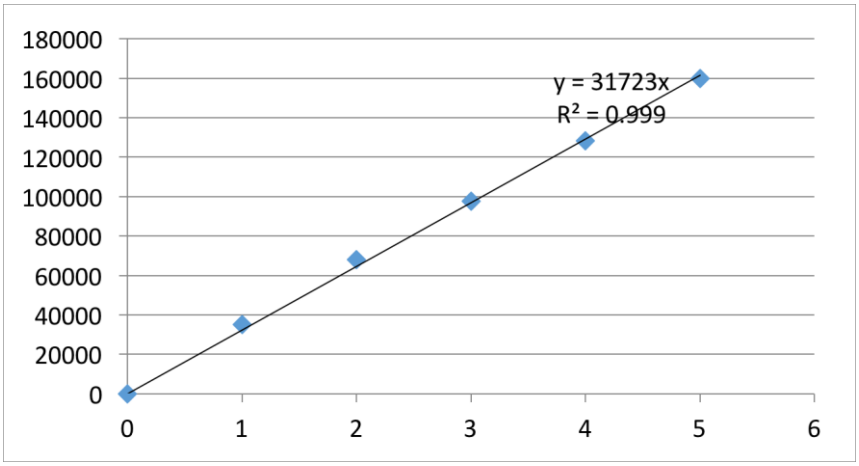
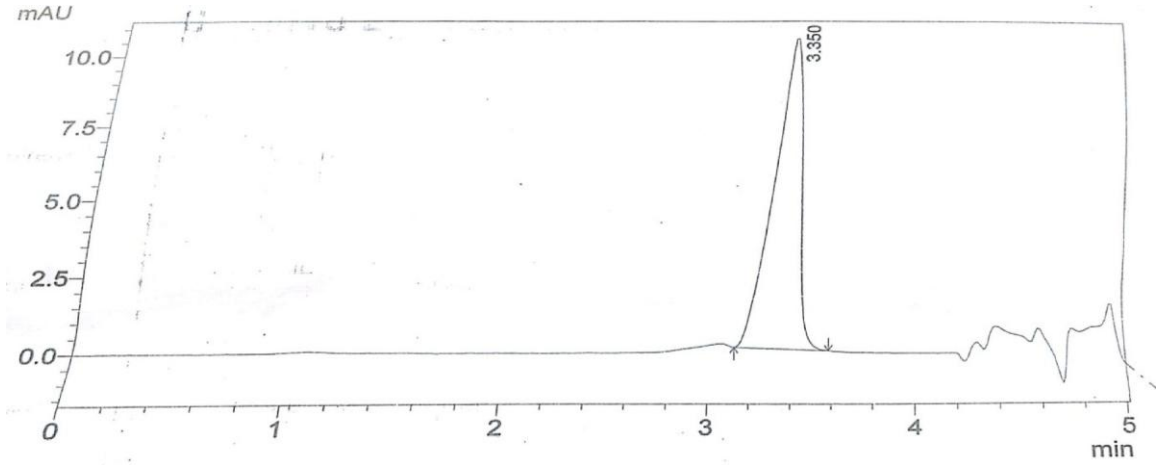


Figure 3: Calibration Curve of Propafenone HCl

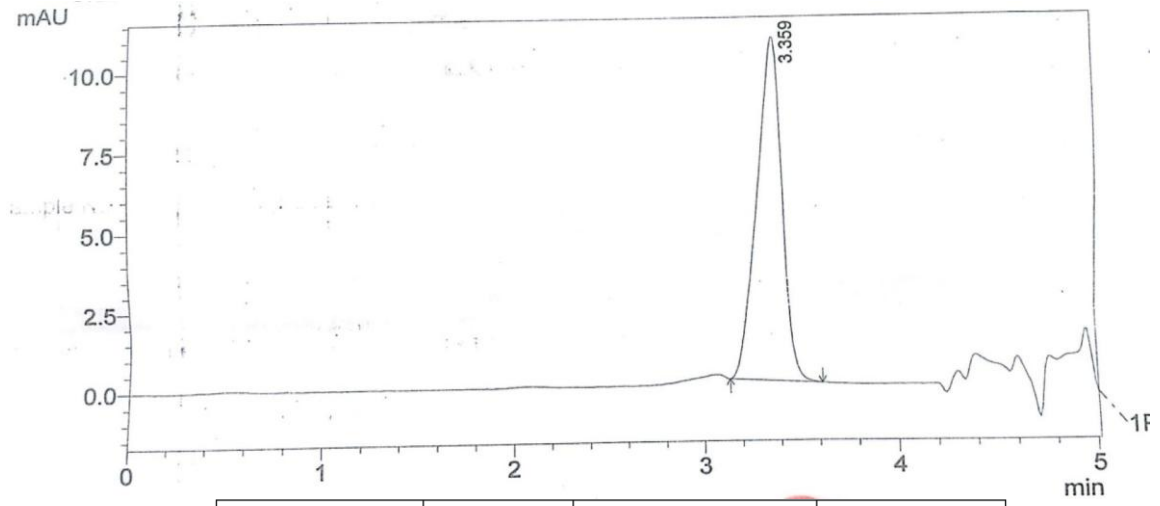
Table 2: Intraday precision studies of Propafenone HCl

S. No	Amount (µg/ml)	Amount found (µg/ml)	Percentage %	% Mean	SD **	% RSD
1	3	2.94	98.05	97.72	0.00089	0.000091
2		2.83	94.33			
3		2.95	98.33			
4		2.92	97.33			
5		3.00	100			
6		2.95	98.33			



Retention time	Peak Area	Theoretical plates	Tailing factor
3.350	90994	24367.927	0.853

Figure 4: Chromatogram for intraday precision



Retention time	Peak Area	Theoretical plates	Tailing factor
3.359	99117	2551.180	0.864

Figure 5: Chromatogram of interday precision

Table 3: Intraday precision studies of Propafenone HCl

S.No	Amount (µg/ml)	Amount found (µg/ml)	Percentage%	% Mean	SD **	% RSD
1	3	2.94	98.05	97.72	0.00089	0.000091
2		2.83	94.33			
3		2.95	98.33			
4		2.92	97.33			
5		3.00	100			
6		2.95	98.33			

** Average of six determinations

Table 4: Interday precision studies of Propafenone HCl

S.No	Amount (µg/ml)	Amount found (µg/ml)	Percentage %	Mean	SD*	% RSD
1	3	2.08	93.33	98.27	0.00008	0.0000814
2		2.96	98.66			
3		3.02	100.66			
4		3.00	100			
5		2.98	99.33			
6		2.93	97.66			

Table 5: Results for the accuracy of Propafenone HCl

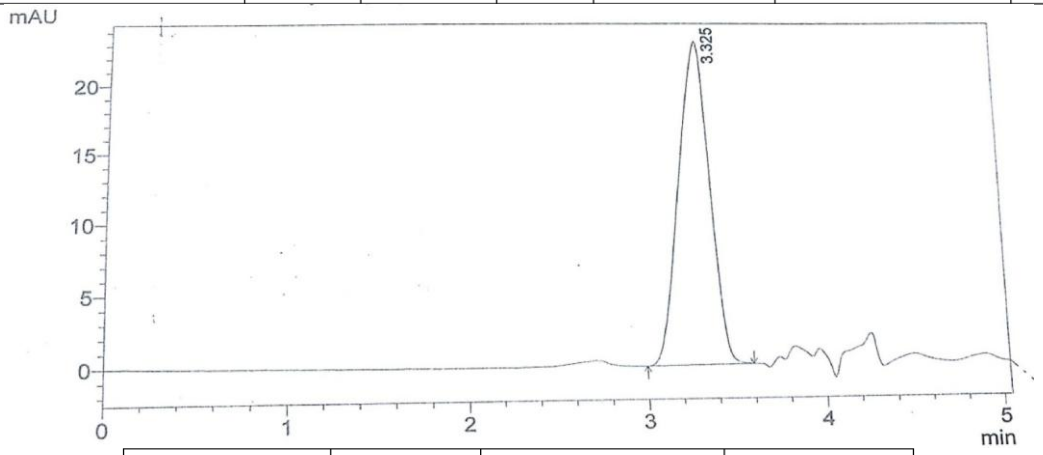
S. No	% Spike Level	Amount present (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery	SD	% RSD
1	50%	2.93	1.5	4.42	1.49	99.3	101.7	0.73	0.51
2				4.46	1.53	102			
3				4.49	1.56	104			
4	100%		3	5.98	3.05	101.6	100.8	0.1	0.07
5				5.95	3.02	100			
6				5.94	3.01	100.3			
7	150%		4.5	7.44	4.51	100.22	99.84	0.02	0.014
8				7.41	4.48	99.55			
9				7.42	4.49	4.49			

Table 6: LOD & LOQ studies of Propafenone HCl

S.No	Name of the drug	LOD (µg/ml)	LOQ (µg/ml)
1	Propafenone HCl	1.25	3.794

Table 7: Robustness studies for Propafenone HCl

Parameter	Normal	Variation	Rt	Tailing factor	Theoretical plates	%RSD
Wavelength Variation	245	240	3.315	0.862	2215.853	0.008
		250	3.360	0.865	2578.148	0.001
Flow Rate Variation	0.7	0.6	3.797	1.018	3281.126	0.003
		0.8	2.860	1.089	2925.864	0.004
Column oven Temperature Variation	30°C	25°C	3.233	1.004	3243.471	0.0032
		35°C	3.189	0.990	3143.059	0.0045



Retention time	Peak Area	Theoretical plates	Tailing factor
3.325	322664	2420.280	0.781

Figure 6: Chromatogram of the Sample solution

Table 8: Assay studies of Propafenone HCl

S.No	Label claim	Amount found	Assay
1	150mg	151.5mg	101%

was spiked (1.5µg/ml), about 4ml of methanol was added and degassed to dissolve for 20min with intermediate shaking. The volume was made up to 10ml with diluents. The solution was filtered through a 0.45microns membrane filter and injected into the HPLC system.

Preparation of 100% solution

From the 100µg/ml of the sample stock solution, 0.3ml of solution (3µg/ml) was transferred into 10ml of volumetric flask to which 0.3 ml of Propafenone HCl working standard was spiked (3µg/ml), about 4ml of diluent was added and degassed to dissolve for 20min with intermediate shaking. The volume was made up to 10ml with diluent. The solution was filtered through a 0.45microns membrane filter and injected into the HPLC system.

Preparation of 120% solution

From 100µg/ml sample stock solution 0.3ml of solution (3µg/ml) was transferred into 10ml of volumetric flask, to which 0.45ml of Propafenone HCl working standard was spiked (4.5µg/ml), about 4ml of diluent was added and degassed to dissolve for 20min with intermediate shaking. The volume was made up to 10ml with diluent. The solution was filtered through a 0.45microns membrane filter and injected into the HPLC system. The % mean recovery and its %RSD of all nine determinations were calculated and results shown in Table 4.

Acceptance criteria: The % recovery and mean % recovery should be 99.0%-102.0% and %RSD. For the three replicates at each %spiked level, % RSD should be not more than 2.

LOQ and LOD

The limit of detection and limit of quantification was calculated using an average value of slope and standard deviation.

Robustness

Deliberate variations were made to the optimised HPLC conditions, to evaluate robustness were:

1. Flow rate variations

2. Column oven temperature variations

3. Wavelength variations

Flow rate varied by ±0.ml /minute

The standard preparation of 10 µg/ml solution was injected into the HPLC system with a variation in flow rate, varied by ±0.1 ml/minute, i.e., 0.6 ml/min and 0.8ml/min. The chromatograms were shown in figure 2.

Wavelength varied by ±2nm

The standard preparation of 10µg/ml solution was injected into the HPLC system with variation in wavelength, varied by ±5nm, i.e., 240 nm and 250 nm. The chromatograms were shown in figure 3.

Column oven temperature varied by ±5°C

The standard preparation of 10 µg/ml solution injected into the HPLC system with variation in temperature, varied by ±5°C, i.e., 25°C and 35°C. Results for Robustness was shown in Table 6.

Assay

This prepared sample solution was injected in six replicates into the HPLC system, and the observations were recorded, chromatogram for the assay was shown in Figure 5 and Results in Table 7.

DISCUSSION

Propafenone HCl was determined by the RP-HPLC method, optimisation of chromatographic parameters was done. Parameters were optimised by altering the mobile phase ratio and flow rate at a wavelength of 245 nm. The trails for optimisation was conducted by using different mobile phases which include ACN:WATER (90:10), ACN:WATER (80:20), METHANOL:WATER (90:10), ACN:METHANOL:WATER (75:25). Out of all trails, 75:25 ratio of [ACN: (Methanol: Water 90:10)] at 0.7ml/min flow rate was selected for this proposed method.

The calibration was performed by using the external calibration method. The calibration curve using peak area Vs.concentration was plotted. The correlation coefficient was calculated as 0.999, the system precision was done on both intraday and interday, and the % RSD is below 2. The recovery studies were passed out to confirm the accuracy of the method by adding the standard drug to a previously analysed formulation. The average percentage recovery appeared as 101%. LOD and LOQ were calculated and were in within limits. Robustness was performed by deliberate changes in the optimised condition such as flow rate, temperature, wavelength, and chromatograms were noted. The percentage of propafenone HCl present in the formulation was occurred to be 101%.

CONCLUSION

Now concluded that the method showed tremendous sensitivity, reproducibility, accuracy and repeatability, which is proved the low percentage relative standard deviation. The results of recovery studies determine that there is no interference from the excipients used in the formulation. The RP-HPLC method can be effectively applied for the routine analysis of propafenone HCl pure and tablet formulation in quality control analysis.

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