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Quantitation of Propafenone HCl in presence of its Degradation product by RP-HPLC: Application to Pharmaceutical Dosage form

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Propafenone HCl in tablet dosage form. Isocratic elution at a flow rate of 1.0 mL min $^{-1}$ was employed on Inertsil ODS (250 × 4.6 mm, 5µm) column at ambient temperature. The mobile phase consisted of 0.01 M potassium dihydrogen phosphate (pH 3.0, ortho phosphoric acid):acetonitrile at 65:35 (v/v) and the detection wavelength were at 249 nm. Linearity was observed in concentration range of 50-150 µg/mL. The retention time for Propafenone HCl was 7.41 min. The method was validated as per the ICH guidelines and attempted for forced degradation study. The proposed method can be successfully applied for the estimation of Propafenone HCl in tablet dosage forms.

Key-words: *Propafenone HCl, RP-HPLC, Validation, Estimation, Dosages form.*

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 in the biological samples particularly from plasma [6-9].

The International Conference on Harmonization (ICH) guidelines entitled 'stability testing of new drug substances and product requires the stress testing of the drug substance should be carried out to elucidate the inherent stability characteristics of the active substance. An ideal stability indicating method is one that quantifies the drug seprated and also resolves its degradation product. Hence this paper reports a simple, precise, rapid and cost effective HPLC method for the estimation of Propafenone hydrochloride in its tablet dosage form [10].

The aim of the present work was to develop an accurate, specific, reproducible and stability indicating HPLC method for determination of Propafenone HCl as per the ICH guidelines [11].

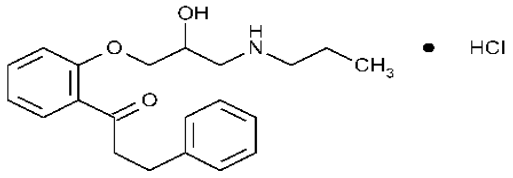

Fig. 1: Chemical structures of Propafenone HCl

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MATERIAL AND METHODS

Instrumental and analytical conditions:

A Thermo separation product HPLC system equipped with UV visible detector with auto sampler and running on chromquest software was used for the present study. The column used was, Inertsil C18 (250 × 4.6 mm, packed with 5 µm) and detection was performed at 249 nm. The injection volume of sample was 20 µL with run time of 15 minutes. An isocratic mobile phase containing of 0.01 M potassium dihydrogen phosphate (pH 3.0, ortho phosphoric acid : acetonitrile at 65:35 (v/v) was carried with the flow rate at 1 mL min^{-1}. The mobile phase was filtered through 0.45µm membrane filter and degassed before use.

Reagents and chemicals:

Analytically pure Propafenone HCl and tablet formulation was gifted by Emcure Pharmaceutical Limited, Pune. Milli-Q Water from Emcure house preparation. Ortho phosphoric acid was purchased from Research Lab., Fine Industries, Mumbai. Potassium dihydrogen phosphate, methanol and tri ethyl amine all were of analytical grade (Merck Ltd., Mumbai). Acetonitrile was purchased from RFCL Ltd, Mumbai.

Preparation of Analytical solutions:

Preparation of potassium dihydrogen phosphate buffer solution:

A weighed quantity of 1.36 gm potassium dihydrogen phosphate (KH_2PO_4) taken in a 1000ml volumetric flask. To this add 200ml of Milli-Q water and mixed in ultra sonicator and made up the volume to mark with Milli-Q water. Adjust the buffer to pH-3 with ortho phosphoric acid and filtered through 0.45µ membrane filter.

Preparation of mobile phase:

Mix a mixture of above buffer 650 ml (65%), 350 ml of acetonitrile (35%) and degassed in ultrasonic water bath for 5 minutes.

Preparation of standard stock solution:

The standard stock solution was prepared by taking 25mg of standard drug of Propafenone HCl in to 50ml volumetric flask, to which add 30ml of mobile phase and sonicated for about 10 min then the final volume was made up to 50 ml with the mobile phase. The filtered solution was further diluted in the diluents (mobile phase) to make the final concentration of 0.5mg/ml.

Preparation of standard solution:

Pipette out 5ml from the above stock solution into a 50ml volumetric flask and was diluted up to the mark with diluents (mobile phase).

Linearity standard stock solution:

Accurately weighed and transferred about 31.52 mg of Propafenone HCl standard into a 50 ml volumetric flask. Added about 30 ml of mobile phase and sonicated to dissolve it. Made up the volume with mobile phase and mixed. Diluted 2,3,4,5 and 6 ml of this solution to 50 ml with mobile phase to get final concentration 25.12 µg/ml(50%), 37.68 µg/ml (75%), 50.24 µg/ml (100%), 62.80 µg/ml(125%), 75.36 µg/ml(150%) respectively .

Preparation of sample solution:

Twenty tablets were weighed and their average weight (257mg) was calculated. A quantity of the tablet powder (171mg) equivalent to 100 mg of Propafenone HCl was transferred into a 100ml volumetric flask. Add 30ml of mobile phase, sonicated for about 12 min and then the final volume was made up to 100ml with the mobile phase. Filter the resultant solution through 0.45µm membrane filter. Further 5ml of the above stock solution was pipette into a 100ml volumetric flask and the volume was adjusted up to the mark with mobile phase to give a concentration of 50µg/ml.

Preparation of placebo solution:

Accurately weighed and transferred about 71 mg of placebo into a 100 ml volumetric flask and dissolved in mobile phase by shaking the flask for 15 min. Filter the first 30ml of the filtrate through 0.45 µ filter paper. Pipette out 5 ml of the solution into a 100 ml volumetric flask and made up the volume with mobile phase.

Method development and validation of HPLC:

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

Linearity:

Express ability to obtain test results where directly proportional to the concentration of analyte in the sample. Construct the calibration curves for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations. A linear regression was applied and slope, intercept and correlation coefficient were determined.

From the linearity standard stock solution, the various dilutions of Propafenone HCl in the concentration of 50, 75, 100, 125 and 150µg/ml were prepared. Each of these solutions (20 µl) was injected six times in to the HPLC system under standard chromatographic condition and the peak areas and retention times were recorded.

Precision:

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

System precision and method precision were determined both in terms of repeatability (injection and analysis).

In order to determine precision, six independent sample solutions (20µl) from a single lot of formulation of Propafenone HCl (50µg/ml) were injected in to HPLC system, the retention time and peak area was determined and expressed as mean and %RSD.

Accuracy:

Accuracy was determined in terms of percentage recovery. The accuracy study was performed for 50%, 100% and 150% for Propafenone HCl. Standard with placebo solutions are injected in to HPLC system in triplicate and percentage recoveries of Propafenone HCl was calculated. The area of each level was used for calculation of % recovery.

Specificity:

Spectral purities of Propafenone HCl chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks.

Robustness:

Robustness of the developed method was investigated by evaluating the influence of small but deliberate variations. The robustness was performed for the flow rate variations (±0.1), the change in buffer pH (±2), and change in wavelength (±2) and even by change in the mobile phase (0±5%)

System Suitability:

System suitability tests were carried out on freshly prepared standard stock solutions of Propafenone HCl and it was calculated by injecting standards in five replicates and the values were recorded.

Solution staibility:

Solution staibility test was carried out initially on freshly prepared standard and sample solution then it was stored and inject in different a time interval (6, 12, 18 and 24hrs). The analytical solution was kept for a period of more than 24 hrs and observed solution stability for analytical work of the project.

Assay of 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.

Force degradation study:

Forced degradation study was carried out by attempting deliberate exposing the drugs to different stress conditions.

Acid and base induced degradation product:

The powder equivalent to 100 mg was accurately weighed and transferred to two 100 ml volumetric flask separately. To it add 10 ml of 0.1N HCl and 0.1N NaOH separately, content was mixed well and kept for constant shaking for 4hr at 80ºC. To the flask 10 ml of diluent was added, sonicated for 15min followed by filtration through 0.45 µm and then diluted to volume with diluent. 5.0 ml of resultant solutions was diluted to 100 ml with the diluent to give final concentration 50µg/ml of Propafenone HCl.

Hydrogen peroxide induced degradation product:

The powder equivalent to 100 mg was accurately weighed and transferred to a 100 ml volumetric flask. To it 10 ml of 50.0% H_2O_2 solution was added, content was mixed well and kept for constant shaking for 4hr at 80ºC temperature. To the flask 10 ml of diluent was added, sonicated for 15 minutes followed by filtration through 0.45 µm and then diluted to volume with diluents. 5.0 ml of resultant solutions was diluted to 100 ml with the diluent to give final concentration 50 µg/ml of Propafenone HCl.

Heat induced degradation product:

The powder equivalent to 100mg was taken into a Petridish and kept at 80ºC in vacuum oven for 48 hr and cooled to room temperature. The content was transferred to a 100 ml volumetric flask. To the flask 20 ml of solvent was added and sonicated for 10 minutes followed by filtration through 0.45 µm filter and then diluted to volume with diluents. 5.0 ml of resultant solutions was again diluted to 5 ml to give final concentration 50 µg/ml Propafenone HCl with solvent.

Photolytic induced degradation product:

The powder equivalent to 100mg was taken into a Petridis and kept in UV chamber at 254 nm for 48 hr. After exposing to UV radiations the Petridis was cool at room temperature. The content was transferred to a 100 ml volumetric flask. To the flask 20 ml of diluent was added and sonicated for 10 minutes followed by filtration through 0.45 µm filter and then diluted to volume with diluents. 5.0 ml of resultant solutions was again diluted to 100 ml to give final concentration 50 µg/ml Propafenone HCl with diluent.

RESULTS AND DISCUSSIONS

The present investigation reported a new RP-HPLC method development and validation of estimation of Propafenone HCl. The method developed was proceeding with wavelength selection. The optimized wavelength was 249nm.

In order to get the optimized RP-HPLC method various mobile phases were used. From several trials final method is optimized with the following conditions:

The mobile phase consisted of an aqueous solution of 0.01 M potassium dihydrogen phosphate; (pH 3.0 with OPA): ACN (65:35 v/v) was delivered at a flow rate of 1ml/min at ambient temperature and the retention time was about 7.4 minutes. The column used was Inertsil ODS 3V; (250 × 4.6 mm, 5µm). The flow rate was adjusted to 1 ml/min. The injection volume was 20µL. The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak. The absence of additional peaks in the chromatogram indicates non interference of the excipients in the tablet dosage form. The linearity was determined as linearity regression of the claimed analyte concentration of the range 50-150 µg/ml. The calibration curve obtained by plotting peak area versus concentration was linear and the correlation coefficient was found to be 0.9990 for Propafenone HCl. **(Table 1, Fig. 2)**

The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The % RSD for system precision **(Table 2)** was found to be 1.07 and the % RSD for method precision **(Table 3)** was found to be 0.70 for Propafenone HCl.

The accuracy study was performed in 50%, 100% and 150%. The percentage recovery was determined for Propafenone HCl and was found to be 100.22% **(Tables 4)**. The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, mobile phase composition, pH of buffer and flow rate **(Table 5)**. Theoretical plates and tailing factor were observed and were found to be 5716 (theoretical plates) and 1 (tailing factor) for Propafenone HCl **(Table 6)**.

Developed chromatographic method applied for the determination of Propafenone HCl in tablet formulation **(Table 7)**. A typical chromatogram showing the separation of Propafenone HCl is shown in **Fig. 3**.

The number of degradation products with their R_t values and percentage degradation of Propafenone HCl was calculated and listed in **Table 8.**

The chromatogram of the acid, base and hydrogen peroxide degraded sample showed additional peak. The percent drug degradation of Propafenone HCl at the level of 0.059% **(Fig. 4)**, 0.129% **(Fig. 5)** and 1.241% **(Fig. 6)** suggested that Propafenone HCl undergoes mild degradation under acidic, basic and oxidative condition respectively.

The sample degraded under dry heat (exposed to 80°C) and sample exposed to photochemical (UV-254nm) degradation showed additional peak other than the standard peaks of Propafenone HCl.. The percent drug degradation of Propafenone HCl at the level of 0.230% **(Fig. 7)** and 0.040% **(Fig. 8)** suggesting the stability of the drugs under heat and photochemical conditions.

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

Range	r^2	Slop	LOD	LOQ
50-150 µg/ml	0.9990	2311.6481x+11698.00	0.0280 µg/ml	0.0850 µg/ml

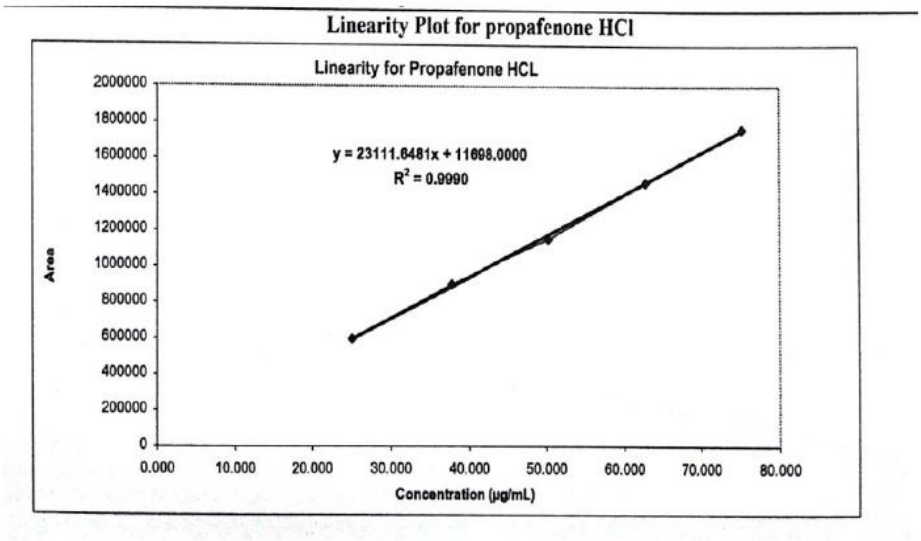

Fig. 2: linearity plot for Propafenone Hydrochloride

Table No. 2: System Precision values for Propafenone Hydrochloride

Injection No.	*Area of Standard*
1	1117108
2	1134248
3	1141526
4	1149731
5	1132139
Mean	1134950
%RSD	1.07

Table No. 3: Method Precision values for Propafenone Hydrochloride

Sr. No.	Area	Mean Area	% Assay	Mean assay	%RSD
1	1096916 1098481	1097699	99.17	100.11	0.70
2	1128628 1107850	1118239	99.98		
3	1143756 1141635	1142696	100.73		
4	1136105 1140886	1138496	100.44		
5	1103699 1097888	1100794	99.43		
6	1146376 1132645	1139511	100.89		

Table No. 4: Recovery Studies for Propafenone Hydrochloride

%	Sample	Area	Mean Area	Amount Recovery	% Recovery	Mean % Recovery	%RSD
50%	Sample-1	603488 605836	604662	52.8	**99.89**	**100.22**	**0.32**
	Sample-2	599869 601900	600885	52.47	**100.29**		**0.59**
	Sample-3	609386 605265	607326	53.04	**100.15**		**0.26**
100%	Sample-1	1153819 1143128	1148474	100.29	**100.29**		**0.04**
	Sample-2	1164091 1153806	1158949	101.21	**100.21**		**0.73**
	Sample-3	1153472 1168671	1161072	101.4	**101.4**		**0.05**
150%	Sample-1	1768448 1749259	1758854	153.6	**99.57**		**0.22**
	Sample-2	1741556 1748325	1744941	152.38	**100.74**		**0.19**
	Sample-3	1772937 1756957	1764947	154.13	**99.53**		**0.05**

Table No. 5: List of Robustness values for Propafenone Hydrochloride

Parameters	Area	Mean Area	% Assay	% Assay from method precision	% Variance from method precision
Flow rate (1.1ml/min)	1031364 1027862	1029613	101.08	100.11	0.97
Flow rate (0.9ml/min)	1249216 1249216	1249216	100.47	100.11	0.36
pH (3.2)	1156084 1147086	1151585	101.58	100.11	0.78
pH (2.8)	1145151 1146486	1145819	101.93	100.11	0.95
wavelength (251nm)	1080764 1102091	1091428	101.36	100.11	1.25
wavelength (247nm)	1160897 1164412	1162655	100.68	100.11	0.57
Organic solvent (+5%)	1064356 1067001	1065679	98.06	100.11	0.26
Organic solvent (-5%)	1078973 1076242	1077608	98.45	100.11	0.47

Table No. 6: System suitability Study

Parameters	Experimental value	Limit as per USP
Tailing Factor	1	Less than 2
Theoretical Plates	5716	More then 2000

Table No. 7: Developed chromatographic conditions of Propafenone HCl

Parameters	Method
Stationary phase (column)	Inertsil C18 (250 × 4.6 mm, packed with 5 µm)
Mobile Phase	0.01 M Potassium dihydrogen phosphate; pH adjusted to 3.0 with OPA: ACN (65:35 v/v)
Flow rate (ml/min)	1 mL/min
Run time (minutes)	12.0
Column temperature (°C)	Ambient
Volume of injection	20 µL

Detection wavelength (nm)	249

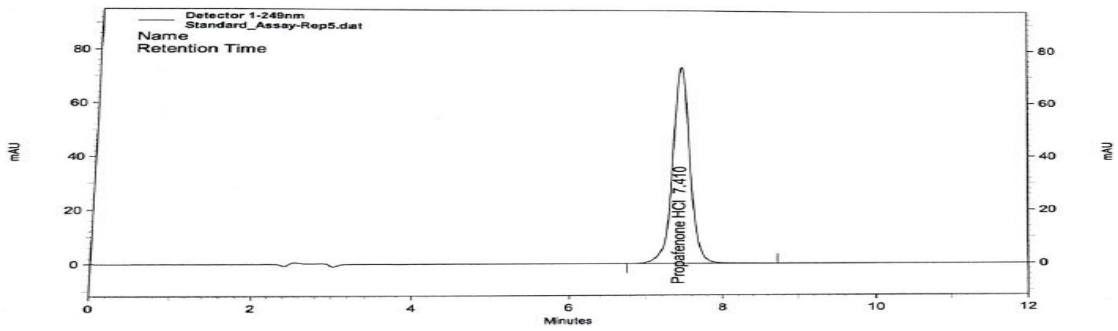

Fig. 3: Chromatogram of Propafenone HCl at optimized condition

Table No. 8: Forced degradation study results for Propafenone HCl

Stress exposure conditions	*Peak purity index*	*Threshold*	*Rt (min)*	*% Degradation*
Acidic (1M HCl) for 4.0 hrs at 80°C	1.0000	0.999947	6.88	0.059
Alkaline (1 M NaOH) for 4.0 hrs at 80°C	1.0000	0.999954	6.89	0.129
Oxidative (50% H_2O_2) for 4 hrs at 80°C	1.0000	0.999947	6.85	1.241
Thermal (80^{0}C) for 72 hrs in vacuum oven	1.0000	0.999950	6.82	0.230
Photolytic (UV) at 254 nm for 48 hrs	1.0000	0.999945	6.85	0.040

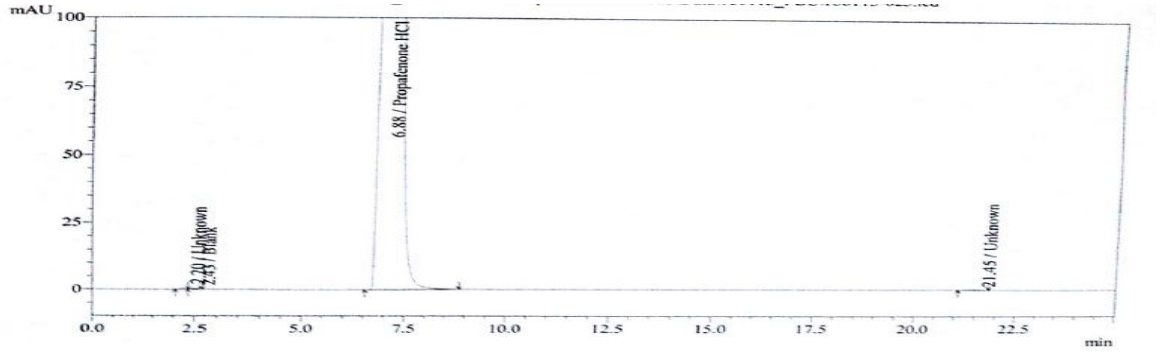

Fig. 4: Chromatogram of acid [1N HCl (Reflux for 4 h at 80°C)] treated sample

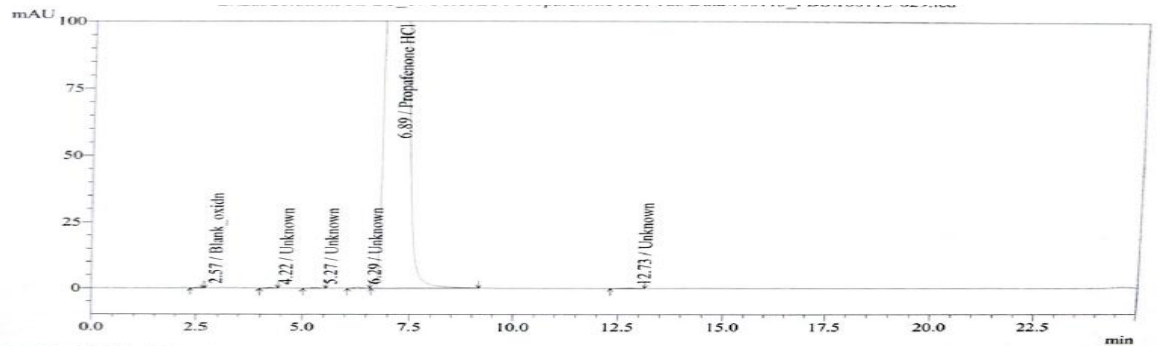

Fig. 6: Chromatogram of hydrogen peroxide (50%) [(Reflux for 4 h at 80°C)] treated sample

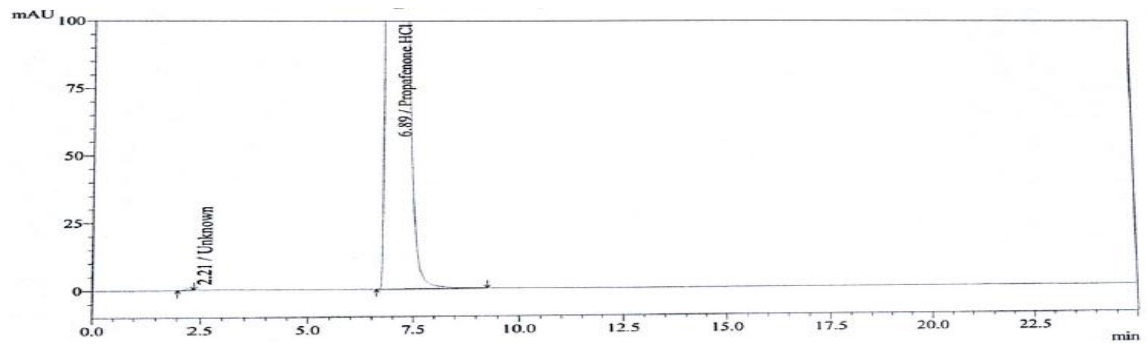

Fig. 7: Chromatogram of heat (80°C for 72 h) treated sample

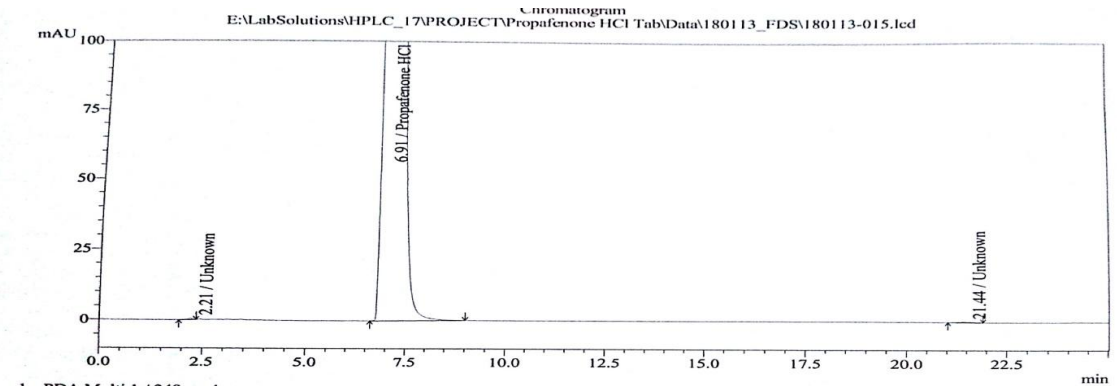

Fig. 8: Chromatogram of UV (254 nm) light [48 h] treated sample

CONCLUSION

The developed HPLC method enables accurate, precise, specific and stability indicating 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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