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Analytical Method Developed And Validated For The Estimation Of Propafenone Hydrochloride In Pharmaceutical Dosage Form

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Abstract

In this study, an simple, accurate, precise , economical and robust. RP-HPLC method was developed and validated for the estimation of Propafenone Hcl in pharmaceutical dosage form as per ICH guidelines. Chromatographic separation of peak achieved by isocratic elution on reverse phase HPLC system. (Agilant 1260 infinity II HPLC). An analysis carried out with Eclipse XDB –C18 150 X 4.6mM 5µ column was used at flow rate 1.0 ml/min and mobile phase in the ratio of methanol : 10mM ammonium acetate buffer (70:30% v/v) at 246 nm detection wavelength. In the column over temperature 40º and injection volume 20 µL. The results obtained from this study proved that the developed method was suitable for routine analysis.

Keywords : Agilant 1260 infinity II HPLC, column over temperature 40º, Eclipse XDB –C18 150 X 4.6mM 5µ column, Propafenone.

Introduction

Rates of degradation

A rigorous evaluation of the kinetics of degradation is generally not the focus of stress testing studies, but useful information can be gained by kinetic evaluation. Kinetics of degradation obtained from stress testing reveals the order of a reaction whether or not a solid-state degradation is catalyzed by humidity or if the reaction is autocatalytic. Such information can be very useful in designing stability studies and interpreting early time point results. In addition to following the kinetics of the disappearance of the parent compound, it can be very important in developing an understanding of the degradation pathways to follow the rates of formation of the different degradation products. Examination of the degradation profile from early time points (e.g., 0-5% degradation) can reveal which products are the primary and which are secondary. This can be especially important, as it is not uncommon for the degradation profiles observed during real-time stability studies to contain a mixture of both and secondary degradation products .[33]

Structures of the major degradation products

There are no clear regulatory requirements for structure elucidation of degradation products observed during stress testing. The structures of the major degradation products of a drug compound are a prerequisite to understand the degradation pathways and identification of degradation pathways involves both the conditions of degradation and the structures involved. Understanding the degradation pathways allows an assessment to be made of the sites in the compound that are susceptible to degradation under different conditions.

Degradation products arising in significant amounts during manufacture and storage should be identified, tested and monitored against appropriately established acceptance criteria. Examination of some degradation products generated under stress conditions may not be necessary if they are not formed under accelerated or long-term storage conditions. [34]

Structural information of stress-induced degradation products can be used to assess the potential for formation of toxic degradation products. Both ICH guidelines on impurities (drug substance and drug product) specifically address the issue of potential

toxic impurities and requires analytical procedures to be developed for those potential impurities that are expected to be unusually potent, producing toxic or pharmacological effects at a level not more than the identification threshold.

Determining the structures of the major degradation products can reveal whether or not a known carcinogen or toxic compound is or might possibly be formed. An understanding of the parts of the molecule that are labile or susceptible to degradation can help in the design of less reactive, more stable analogs. The development of a stable formulation is also aided by an understanding of the reactive parts of the drug molecule. Drug-excipient compatibility studies (which are a form of stress testing) often lead to new, unknown degradation products. The rational development of a stable formulation is greatly aided by a chemical understanding of the reactions leading to degradation.

Pathways of degradation

Establishing the pathways of degradation is critical; therefore, to develop an understanding of the intrinsic stability of the molecule, and degradation pathway information provides a scientific foundation for the validation of the stability indicating power of the analytical methodology. Determination of structures of degradation product provides the critical information needed to allow proposal of plausible degradation pathways. Stress-testing studies should also be conducted on the formulated drug product because drugs can and often degrade differently in the presence of excipients. Stressing the placebo in parallel with DP as a control for excipients' decomposition can be used to monitor thy decomposition's effect on degradation

pathways of active ingredients.

Introduction of Chromatography

Chromatography is probably the most powerful and versatile analytical technique available to the modern chemist, its power arises from its capacity to determine quantitatively many individual components present in mixture in single analytical procedure. Its versatility comes from its capacity to handle a very wide variety of samples that may be gaseous, liquid or solid in nature. The sample can range in complexity from a single substance to multicomponent mixture containing widely differing chemical. The beginning of chromatography started with the work of botanist Michael Tswett in the year 1896. Tswett define chromatography as "The method in which the components of a mixture are separated on an adsorbent column in a flowing system". Recently, the IUPAC has defined chromatography as: "*A method used primarily for the separation of thecomponents of a sample, in which the components are distributed between two phases, one of which is stationary while other moves. The stationary phase may be a solid or a liquid supported on a solid or a gel may be packed in a column, spread as a layer or distributed as a film. The mobile phase may be gaseous or liquid*".

High Performance Liquid Chromatography (HPLC)

HPLC is a popular method of analysis because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. Modern HPLC has many applications including separation, identification, purification and quantification of various compounds. Although HPLC is widely considered to be a technique mainly for biotechnological, biomedical and biochemical research as well as for the pharmaceutical industry. These fields currently comprise only about 50% of HPLC users. Currently HPLC is used by a variety of fields including cosmetics, energy, food and environmental industries.

Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification times of compounds being isolated by column chromatography.

High pressure liquid chromatography was developed in the mid-1970's and has quickly improved with the development of column packing materials and the additional convenience of on-line detectors. New methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as terms such as micro-column, affinity columns and fast HPLC

began to emerge.

Fig. 6: HPLC (Agilent 1200 series)

1.11.1. Advantages of HPLC

The major advantage of HPLC includes

1. It provides specific, sensitive and precise method for the analysis of different complicated samples.
2. There is ease of sample preparation and sample introduction.
3. There is increased speed of analysis.
4. It offers advantage over gas chromatography in analysis of many polar, ionic substances, high molecular weight substances, metabolic products and thermo-labile as well as non-volatile substances.

1.11.2 Most commonly used method in HPLC[45]

a) Normal phase chromatography

Normal phase HPLC was the first kind of HPLC chemistry used and separates based on polarity. This method uses a polar stationary phase and a non-polar mobile phase and is used when the analyte of interest is fairly polar in nature. The polar analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increase in analyte polarity, and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. The interaction strength not only depends on the functional groups in the analyte molecule, but also on steric factors and structural isomers. Use of more polar solvents in the mobile phase will decrease the retention time of the analyte while more hydrophobic solvents tend to increase retention time. Particularly polar solvents in a mixture tend to deactivate the column by occupying the stationary phase surface. This is somewhat particular to normal phase because it is most purely an adsorptive mechanism (the interactions are with a hard surface rather than a soft layer on a surface).

Mechanism: Retention by interaction of the stationary phase polar surface with polarparts of the sample molecules.

1. **Stationary phase**: It is a bonded siloxane with polar functional group like SiO_2,Al_2O_3, -NH_2, -CN, -NO_2, - Diol.
2. **Mobile phase**: Non-polar solvents like heptane, hexane, cyclohexane, chloroform,ethyl ether, dioxane.
3. **Application**: Separation of non-ionic, non-polar to medium polar substances.
4. **Sample elution Order**: Least polar components are eluted first.

B) Reverse phase chromatography

Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and a moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}.

The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Retention time is increased by the addition of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvent. Reversed phase chromatography is so commonly used that it is not uncommon for it to be incorrectly referred to as "HPLC" without further specification.

RP-HPLC operates on the principle of hydrophobic interactions which result from repulsive forces between a relatively polar solvent, the relatively non-polar analyte and the repulsive non-polar stationary phase. The driving force in the binding of the analyte to the stationary phase is the decrease in the area of the non-polar segment of the analyte molecule exposed to the solvent.

Mechanism: Retention by interaction of the (stationary phase) non-polar hydrocarbonchain with non-polar parts of sample molecules.

1. **Stationary phase**: It is bonded siloxane with non-polar functional groups like n-octadecyl (C-18) or n-octyl (C-8), ethyl, phenyl, $-(CH_2)_n$-diol, $-(CH_2)_n$-CN.

2. **Mobile Phase**: Polar solvents like methanol, acetonitrile, water or buffer (sometimeswith additives of THF or dioxane).

3. **Applications**: Separation of non-ionic and ion forming non-polar to medium polarsubstances (carboxylic acids hydrocarbons).

4. **Sample elution order**: Most polar components are eluted first.

1.11.3. Components of the HPLC system

High performance liquid chromatography consists of following major components

a) HPLC gradient mixers

b) HPLC pumps

c) HPLC columns

d) HPLC detectors

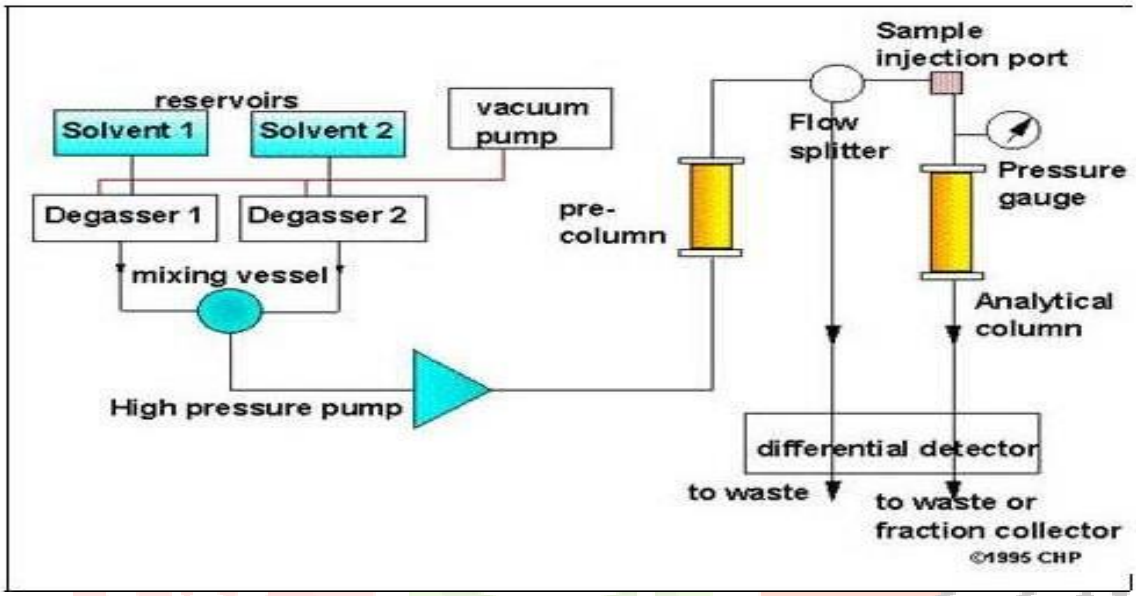


Fig. 7: Schematic diagram of HPLC instrument.

a) HPLC gradient mixers

HPLC gradient mixers provide a very precise control of solvent composition to maintain a reproducible gradient profile. This can be complicated in HPLC by the small elution volumes required by many systems. It is much more difficult to produce a constant gradient when mixing small volumes than when mixing large volumes. For low-pressure systems, it requires great precision in the operation of the miniature mixing valves used and low dispersion flows throughout the mixer. For multi-pump high-pressure systems, it requires a very precise control of the flow rate while making very small changes of the flow rate.

b) HPLC pumps

Because of the small particles used in modern HPLC column packing, modern LC pumps need to operate reliably and precisely at pressures of 10,000 psi or at least 6,000 psi. To operate at these pressures and

remain sensibly inert to the wide variety of solvents used HPLC pumps usually have sapphire pistons, stainless steel cylinders and return valves fitted with sapphire balls and stainless steel seats. For analytical purposes, HPLC pumps should have flow rates that range from 0 to 10 ml/min, but for preparative HPLC,

flow rates in excess of 100 ml/min may be required. It is extremely difficult to a very constant flow rate at very low flow rate.

Pump Pressure

Pumps vary in pressure capacity, but their performance is measured on their ability to yield a consistent and reproducible flow rate. Pressure may reach as high as 6000 lbf/inch^2 (~40 MPa, or about 400 atmospheres). Modern HPLC systems have been improved to work at much higher pressures and therefore be able to use much smaller particle sizes in the columns (< 2 micrometers). These "Ultra High Performance Liquid Chromatography" systems or UHPLCs can work at upto 15,000 lbf/inch^2 (-100 MPa or about 1000 atmospheres).

c) HPLC columns

Column is often referred to as the heart of the HPLC separation process. HPLC columns are packed with very fine particles (usually a few microns in diameter) to attain the low dispersion that give the high plate counts expected of modem HPLC. LC columns, in general, achieve their separation by exploiting the different intermolecular forces between the solute and the stationary phase and those between the solute and the mobile phase. The column will retain those substances that interact more strongly with the stationary phase than those that interact more strongly with the mobile phase.

C18 and C8 HPLC columns

1. Classic reversed-phases for all general purpose applications.
2. Excellent peak shape and efficiency compared to competitive columns.
3. Classic reversed-phase retention and selectivity.
4. C18 is generally more retentive than the C_8
5. Various factors that govern the retention of component are as follows**:**

Types of detectors

1) Ultraviolet detector

a. Most widely used.

b. Principle-Absorption of UV visible light as the eluent from the column is passed through a small flow cell held in radiation beam.

c. Suitable for Gradient elution.

2) Fluorescence detector

a. Principle-Enable fluorescent compounds present in mobile phase to be detected by passing the column eluent through a cell irradiated with ultraviolet light and measuring any resultant fluorescent radiation.

b. Very sensitive and selective.

3) Refractive index detector

a. Principle-These are differential refractometer which respond to change in the bulk property of the refractive index of the solution of the component in the mobile solvent system.

b. It is less sensitive.

4) Electrochemical detector

a. Principle- These are based on standard electrochemical principles involving Zmperometry, voltametry and polarography.

b. These are very sensitive for substances that are electro active, i.e. those that undergo oxidation or reduction at a suitable potential.

5) Photodiode array detector (PDA)

A photodiode array (PDA) is a linear array of discrete photodiodes on an integrated circuit (IC) chip. For spectroscopy it is placed at the image plane of a spectrometer to allow a range of wavelengths to be detected simultaneously. In this regard it can be thought of as an electronic version of photographic film. Array detectors are especially useful for recording the full UV-visible absorption spectra of samples that are rapidly passing through a sample flow cell, such as in an HPLC detector.

1.11.4 Method development in HPLC

Methods for analyzing drugs by HPLC can be developed, provided one has knowledge about the nature of the sample namely, its molecular weight, polarity, ionic character, pKa values and the solubility parameter.

An exact recipe for HPLC method development cannot be provided because method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase.

The water soluble active phamaceutical ingredients is further differentiated as ionic or non ionic which can be separated by reverse-phase. Similarly, the organic soluble API can be classed as polar and non-polar and equally separated by reverse phase. In some cases the non-polar API may have to be separated using adsorption or normal phase HPLC, in which mobile phase would be non-polar organic solvent.

Validation of analytical techniques [49]

Introduction to validation

Validation is a concept that has been evolving continuously since its first formal appearance in United States in 1978. The concept of validation has expanded through the years to encompass a wide range of activities from analytical methods used for the, quality control of drug substances and products to computerized system for clinical triallabeling or process control. Validation is the overall expression for a sequence of activities in order to demonstrate and document that a specific product can be reliably manufactured by the designed processes, usually, depending on the complexity of today's pharmaceutical products, the manufacturer must ensure; “that products will be consistently of a quality appropriate to their intended use”.

Validation is a proof that a process works and this must be done using scientific ad statistical principles. This is done to establish process capability and to confirm end acceptability. Validation determines process variables and the acceptable limits for these variables and accordingly sets up appropriate in process controls, which specifies alert and action levels.

ICH guideline **Q2 (R1)** Method Validation Parameters are as follows:-

a) Accuracy

b) Precision

c) Specificity

d) Linearity

e) System suitability

f) Ruggedness

g) Robustness

a) Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which

is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

It is measured as the % of analyte recovered by assay or by spiking samples in a blind study. Accuracy should be established across the specified range (that is, line of working range) of the analytical procedure. For the assay of the drug substance, accuracy measurements are made by comparison of the results with the analysis of a standard reference material or to compare the results obtained from a second well-characterized independent procedure, the accuracy of which is stated and/or defined.

ICH Guidelines Q2(R1) recommend assessment of accuracy at three levels covering the specified range (i.e. three concentration levels and three replicates at each level of the total analytical procedure). The data should be reported as the percent recovery of the known amount added or as the difference between the mean and true values with confidence intervals.

The % recovery was then calculated by using formula.

$$\% \text{ Recovery} = \frac{(A - B)}{C} \times 100$$

Where,

A: % Total amount of drug estimated

B: % Amount of drug found on preanalyzed basis

C: % Amount of pure drug added.

b) Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous samples. It is expressed as standard deviation or coefficient of variation.

Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability: Repeatability expresses the precision under the same operating conditionsover a short interval of time. It is also termed as intra-assay precision.

Intermediate precision: Intermediate precision expresses within-laboratories variations:different days,

different analysts, different equipment, etc.

Reproducibility: Reproducibility expresses the precision between laboratories(collaborative estudies, usually applied to standardization of methodology).

c) Specificity

It is the ability to assess unequivocally the analyte in the presence of components which may he expected to be present in the sample under consideration. This might include degradants, impurities, matrices, excipients etc.

This definition has the following implications:

(i) Identification: to ensure the identity of an analyte.

(ii) Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

(iii) Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

e) Linearity

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to the concentration of analyte in the sample within a given range. A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighing of synthetic mixtures of the drug product components, using the proposed procedure.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods.

f) System suitability

System suitability is a Pharmacopoeial requirement and is used to verify whether the resolution and reproducibility of the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

g) Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety expressed as % RSD. The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under variety conditions such as different laboratories and different days etc.

h) Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Perform experiments by changing conditions such as temperature (±5 °C), change in wavelength (±2 nm), ionic strength of buffers and level of additives to mobile phase.

Table No. 8: Validation Parameter([50])

Type of analytical procedure characteristics	IDENTIFICATION	TESTING FOR IMPURITIES Quantition Limit	ASSAY -dissolution (measurement only) -content /potency

Accuracy	-	+ -	+
Precision			
Repeatability	-	+ -	+
Intermediate Precision	-	+ (1) -	+ (1)
Specificity (2)	+	+ +	+
Detection Limit	-	- (3) +	-
Quantition Limit	-	+ -	-
Linearity	-	+ -	+
Range	-	+ -	+

- Signifies that this characteristic is not normally evaluated

+ Signifies that this characteristic is normally evaluated

(1) In cases where reproducibility has been performed, intermediate precision is not needed.

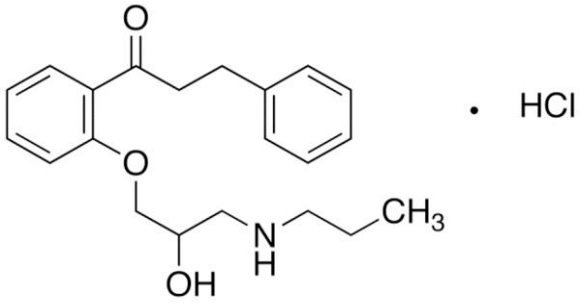
(2) Lack of specificity of one analysis procedure could be compensated by other supporting analytical procedure(s).

(3) May be needed in some cases.

2. DRUG PROFILE

Propafenone Hydrochloride

Structure:



IUPAC Name: 1-[2-[2-hydroxy-(propylamino)propoxy]phenyl]-3-phenylpropan-1-one; hydrochloride

Molecular formula: $C_{21}H_{28}ClNO_3$

Molecular weight: 377.909 g/mol,

Description: Colourless crystals or white crystalline powder

Solubility: Soluble in ethanol and water

Melting point: 171-174°C

Category: Anti-arrhythmic agent.

Use : Used in the management of supraventricular and ventricular arrhythmia.

Experimental work

Determination of wavelength for detection of Propafenone Hydrochloride

The working standard solution of Propafenone Hydrochloride (10 µg /ml in methanol) was scanned in the range of 200-400 nm against solvent blank and spectrum was recorded. UV spectrum shows λ max at 246.0 nm, was selected as wavelength of detection for HPLC study. The UV spectrum of Propafenone Hydrochloride is depicted in figure 8.

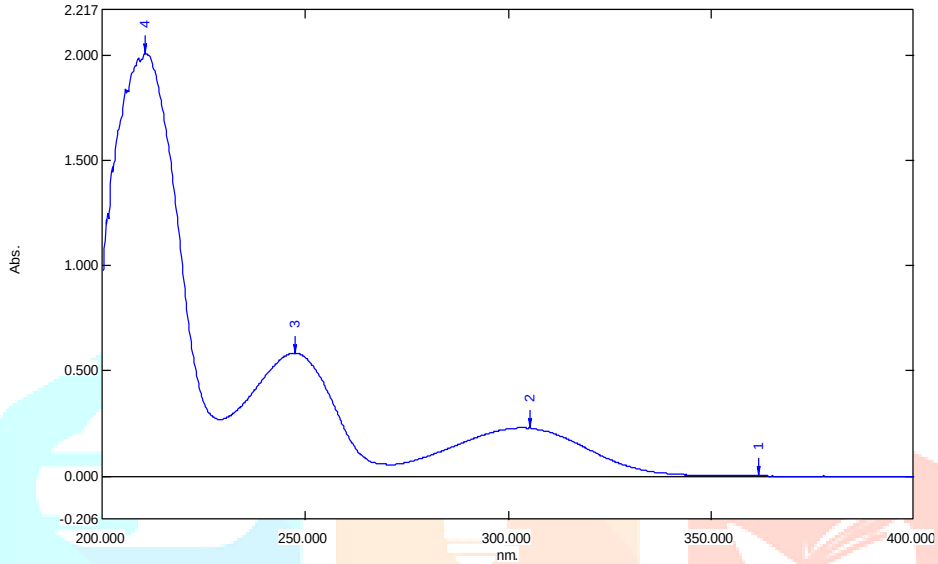


Fig. 4: UV-Spectrum of Propafenone Hydrochloride in methanol

7.4. Preparation of Standard Solutions

7.4.1. Stock standard solution of Propafenone Hydrochloride

Accurately weighed 10.0 mg of Propafenone Hydrochloride was dissolved and diluted to 10 ml methanol in a volumetric flask (conc. 1 mg/ml).

7.4.2. Working standard solution of Propafenone Hydrochloride

One ml of above solution was diluted with diluent in a 10.0 ml volumetric flask (conc. 100µg/ml). One ml of this solution was further diluted to 10.0 ml with diluent to give (10 µg/ml) concentrations.

7.4.3. Preparation of sample solution

Accurately weighed quantity of tablet powder equivalent to 10mg Propafenone HCl was transferred to 10 ml volumetric flask and dissolved in about 7 ml methanol using ultrasonication for 20 min. The volume was made up to the mark with methanol. The solution was filtered through Millipore Nylon filter (0.45µ) and 1.0 ml of filtrate was diluted to 10.0 ml with mobile phase. One ml of this solution was further diluted to 10.0 ml with mobile phase to obtain a concentration of 10µg /ml.

Chromatographic conditions

Different solvent and buffers of different pH were tried by permutation and combination to obtain adequate retention of the drug. Finally, mixture of methanol and ammonium acetate buffer, in the ratio of 66:34%v/v was found to yield satisfactory retention time of Propafenone HCl at 4.192 min, with sharp symmetrical peak and well resolved from all the degradation products. One of the chromatogram of standard solution of Propafenone HCl is depicted.

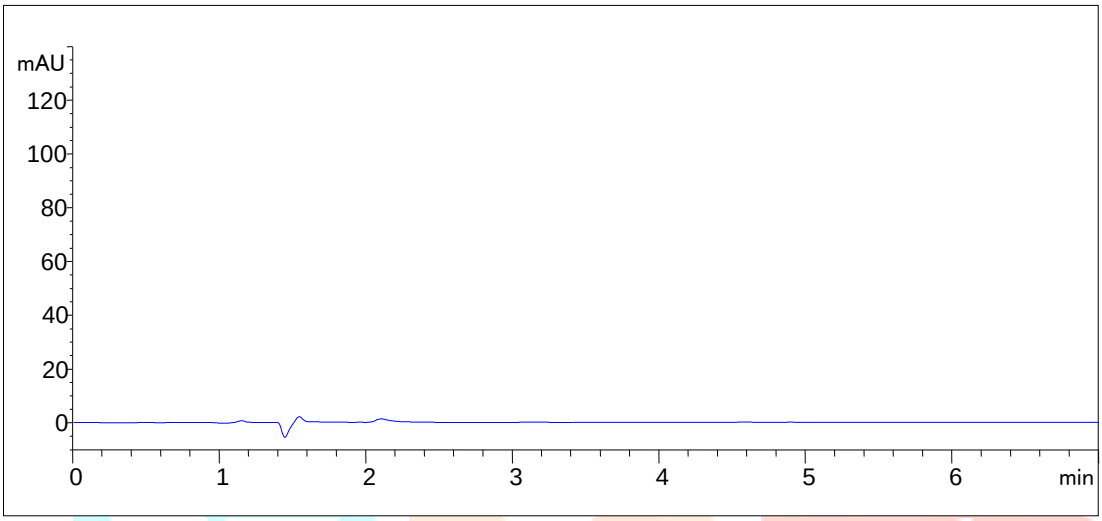


Fig : HPLC chromatogram of blank solution

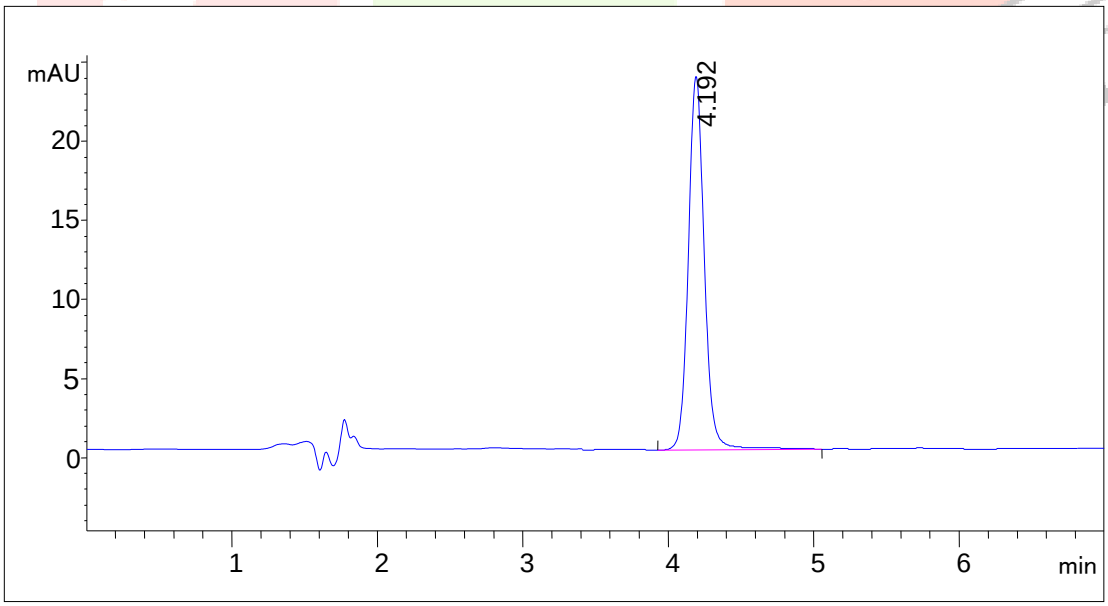


Fig. HPLC chromatogram of standard solution of Propafenone HCl

The following chromatographic conditions were maintained throughout the method development.

Instrument	:Agilent-1260 Infinity II HPLC system
Column	:Eclipse XDB-C18 150 x 4.6mm ,5µm,

Mobile phase	:Methanol : 10 mM Ammonium Acetate Buffer (70:30 %v/v)
Detection wavelength	:246 nm
Flow rate	:1.0 mL/min
Column oven temperature	:40ºC
Injection volume	:20 µL

Stability of Standard and Sample Solutions

Stability of working standard and sample solutions of Propafenone HCl was studied by injecting solution at different time intervals to maximum for 24 hrs. The results are shown in table no 8.

Study of Stability of standard and sample solutions

Time (hr)	Area	
	Standard solution	**Sample solution**
0 hr	179.1	180.1
1 hr	180.3	182.3
3 hr	180.1	182.2
5 hr	179.0	181.1
8 hr	181.0	180.8
24 hr	182.1	179.5
Mean	**180.3**	**181.0**
± SD	**1.171**	**1.112**
%RSD	**0.649**	**0.614**

Validation of proposed method

According to the guidelines of ICH Q2 (R1) all the parameters as discussed below were analyzed and validated accurately following the procedure of the proposed method.

System suitability test parameters

For system suitability test parameters, six replicate injections of working standard solution of Propafenone HCl (10µg/ml each) were injected and analyzed under optimized chromatographic conditions. The results of system suitability test parameters study is depicted in Table No. 9.

Table No. 9: System suitability test parameters of Propafenone HCl

Sr. No	Retention Time (min)	Capacity Factor (k')	Area	Symmetry	Plates

1	4.19	1.80	182.3	0.81	7279
2	4.19	1.80	183.9	0.83	7415
3	4.10	1.82	186.4	0.82	7429
4	4.21	1.82	184.0	0.84	7435
5	4.20	1.82	184.0	0.84	7435
6	4.19	1.80	183.5	0.80	7509
Mean	**4.17**	**1.81**	**184.0**	**0.821**	**7252**
±SD	**0.04**	**0.01**	**1.334**	**0.016**	**75.23**
%RSD	**0.95**	**0.61**	**0.725**	**1.989**	**1.014**

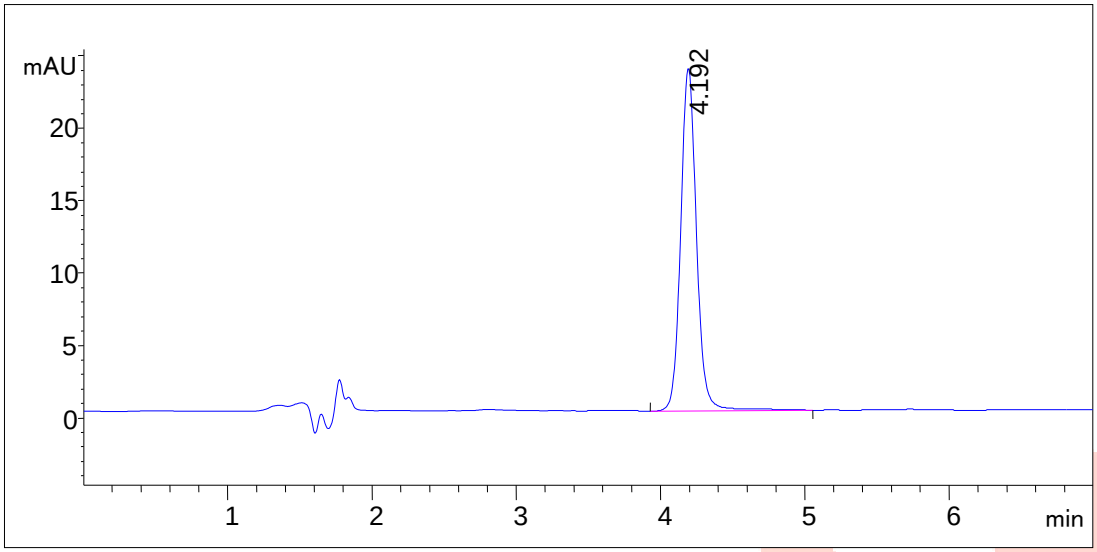


Fig.11: HPLC Chromatogram of System Suitability Study.

Linearity:

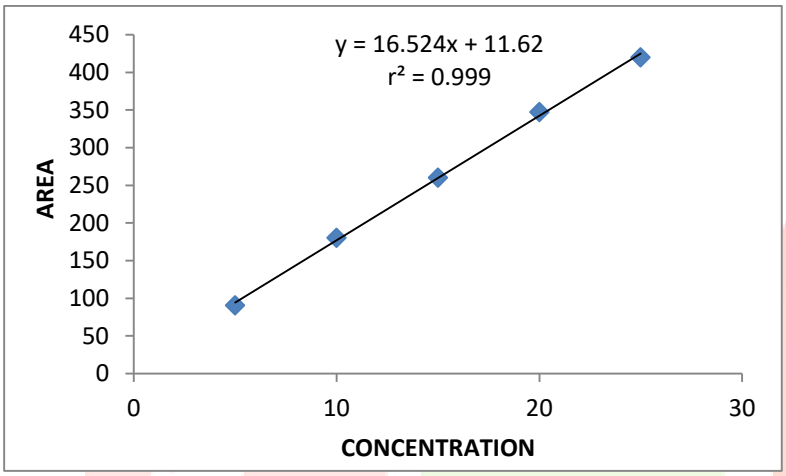
Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. Linearity should be evaluated by visual inspection of a plot of signal as a function of analyte concentration. The correlation coefficient, y-intercept, slope of the regression line and the residual sum of squares should be calculated.

Linearity of Propafenone HCl

One ml of standard solution of Propafenone HCl was transferred in a 10.0 ml of volumetric flask and the volume was made up to the mark with mobile phase to give 100µg/ml concentration of Propafenone HCl. Four aliquot portions of this solution (0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml) were further diluted separately to 10.0 ml with mobile phase to give concentration range of 5-25µg/ml. All the solutions were analyzed using the standard chromatographic conditions and the responses were measured as peak areas. The calibration curve was obtained by plotting peak area *vs* concentration.

Study of Linearity of Propafenone HCl

Sr. No.	Conc. (µg/ml)	Peak Area
1	**5**	90.4
2	**10**	180
3	**15**	260
4	**20**	347
5	**25**	420



Study of linearity of Propafenone HCl

Precision

The working sample solution of Propafenone HCl (10 µg/ml concentration) was used for the comparison with sample solutions by area normalization method. An accurately weighed six quantities of tablet powder equivalent to 10 mg of Propafenone HCl was transferred to different 10 ml volumetric flasks and dissolved in adequate quantity of methanol using ultra sonication for 20 minutes. The solutions were filtered through Millipore Nylon filter (0.45µ). The volume of each flask was adjusted to 10.0 ml with methanol.

The solutions were further diluted appropriately with mobile phase to obtain a concentration of 10 µg/ml of Propafenone HCl. The solutions were analyzed using the optimized chromatographic conditions. The results of precision study Propafenone HCl are shown below.

Results of precision study of Propafenone HCl

Sr. No.	Wt. of tablet Powder (mg)	Peak Area	Amt. of drug Estimated (mg)	% label claim
1	17.1	184.68	10.18	101.8
2	17.2	187.65	10.34	103.4

3	17.3	185.54	10.22	102.2
4	17.2	182.43	10.06	100.6
5	17.1	183.34	10.11	101.1
6	17.2	184.73	10.18	101.8
Mean				**101.2**
Standard deviation				**0.010**
% Relative standard deviation				**1.022**

Accuracy

To ascertain the accuracy of the proposed methods, recovery study was carried out by standard addition method at 50%, 100%, 150% and 200% of the test concentration.

Accurately weighed quantity of pre-analyzed tablet powder equivalent to about 5 mg of Propafenone HCl (10% of label claim) was transferred individually to eight different 10.0 ml volumetric flasks (each level in duplicate). To each flask standard Propafenone HCl (in solution form) was added at 5.0 mg (50%), 10 mg (100%), and 15mg (150) except two flask (50%) so as obtain concentration range of 50-200% of label claim. The contents were dissolved in adequate amount of methanol using ultrasonication for 20 minutes. All these solutions were filtered through Millipore Nylon filter (0.45µ) and the volume was made up to the mark with methanol. One ml of each filtrate was diluted to 10.0 ml with mobile phase. One ml of this solution was further diluted to 10.0 ml with mobile phase. The solutions were filtered and analyzed using the optimized chromatographic conditions .The results of recovery study are shown below.

Results of recovery study of Propafenone HCl

Level of addition	**Wt. Of Tablet Powder (mg)**	**Wt. of pure drug added (mg)**	**Peak Area**	**Amt. of Drug Estimated (mg)**	**% drug Recovered**
50%	8.7	0.0	90.22	4.97	99.4
	8.6	0.0	91.11	5.02	100.4
100%	8.5	5.2	180.1	9.30	99.3
	8.4	5.1	182.6	10.0	100.7
150%	8.6	10.3	273.7	15.1	100.6
	8.7	10.2	274.0	15.1	100.7
200%	8.5	15.1	360.2	19.9	99.3
	8.4	15.2	362.5	19.9	99.9
Mean					**100.0**
Standard deviation					**0.636**
% Relative standard deviation					**0.637**

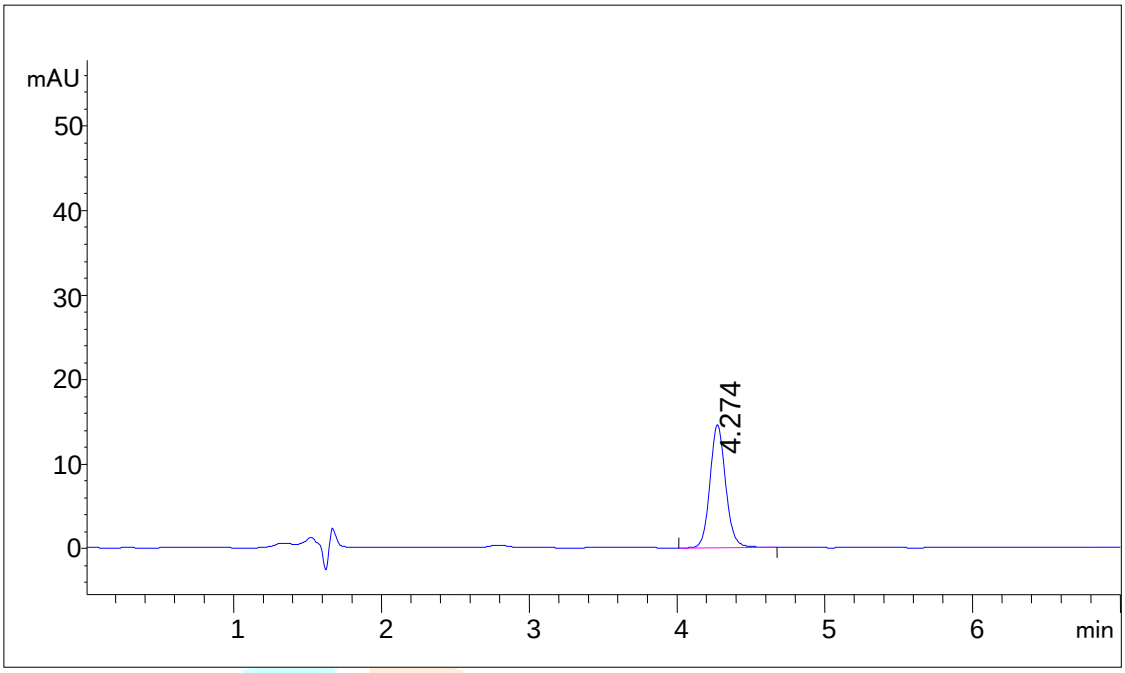


Fig.13: HPLC Chromatogram of Recovery Study of Propafenone HCl of at 50% level

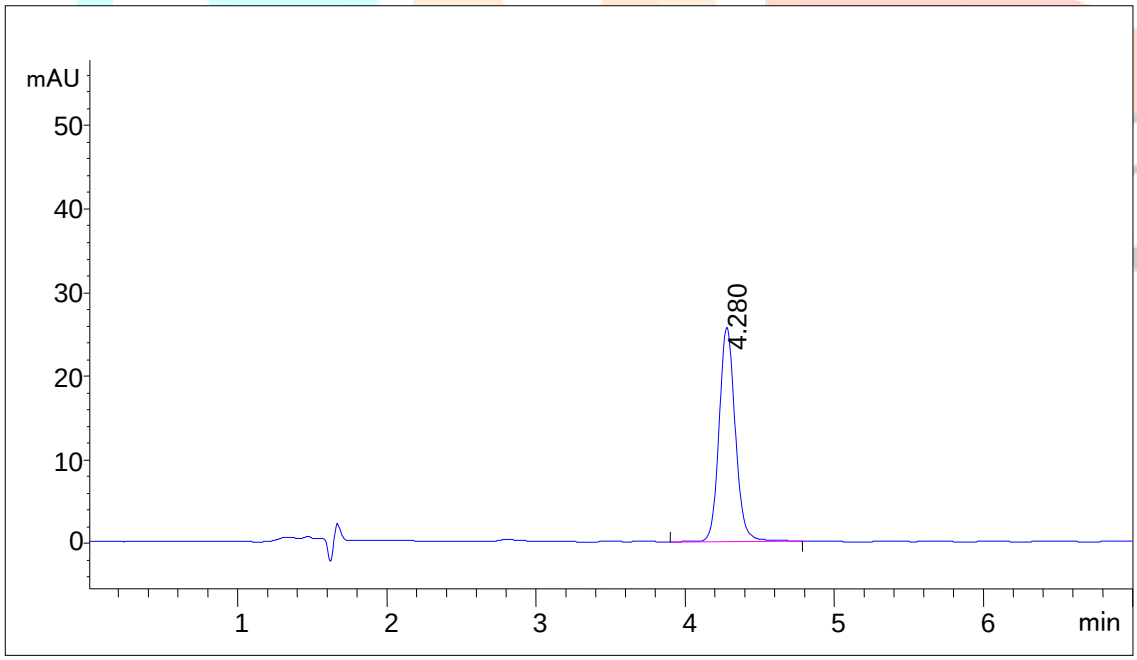


Fig.14: HPLC Chromatogram of Recovery Study of Propafenone HCl at 100%

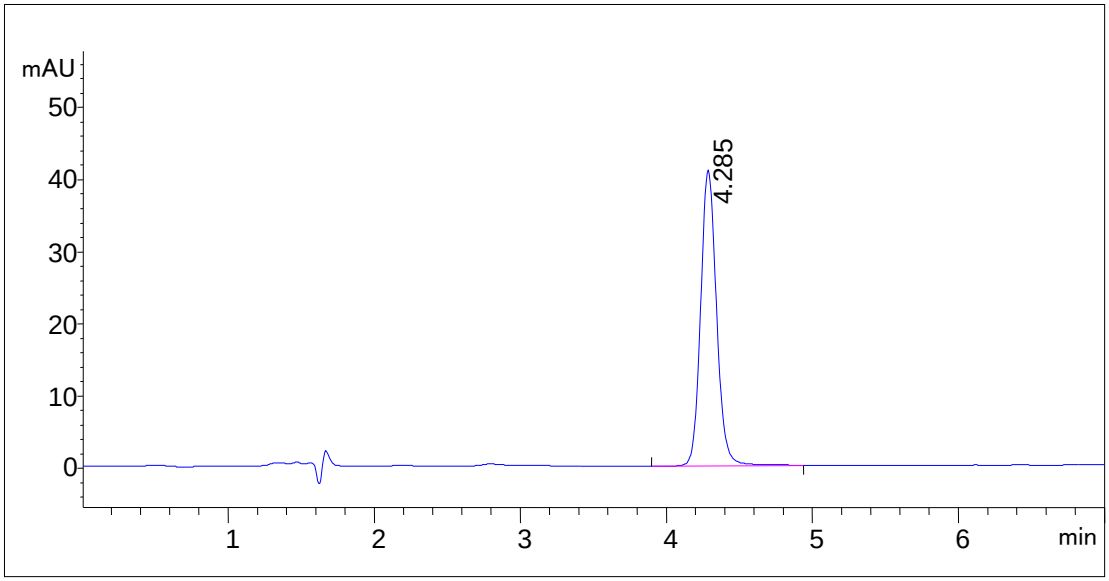
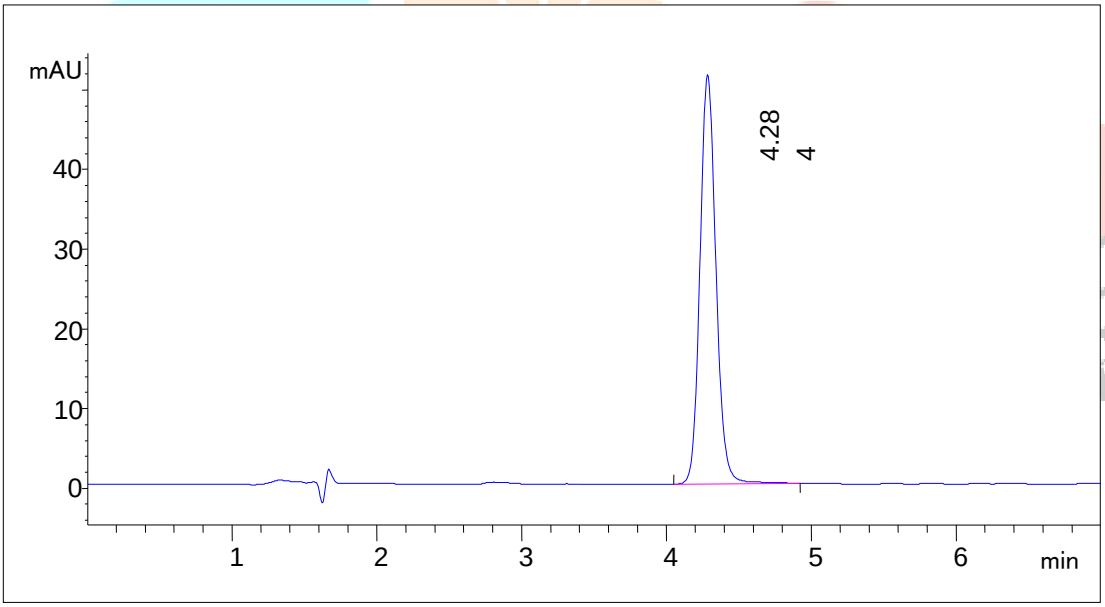


Fig.15: HPLC Chromatogram of Recovery Study of Propafenone HCl at 150% level



HPLC Chromatogram of Recovery Study of Propafenone HCl at 200% level

Robustness

The robustness method was studied by varying the chromatographic condition by making a small deliberate change in the detection wavelength by ±2 nm, change in flow rate by 0.2ml/min, change in mobile phase composition by ±2 % v/v and change in column temperature ±5 °Cand then chromatograms were recorded. The results of robustness study for Propafenone HCl are shown below.

Robustness study for Propafenone HCl

Sr. No	Parameter	Optimized condition	Used condition	Peak area	Retention time (t_R)	Plate count	Peak symmetry
1.	Flow rate (±0.2ml/min)	1.0ml	0.8	226.07	5.333	9193	0.83
			1.2	151.56	3.646	7058	0.83
2.	Detection wavelength (±2 nm)	246 nm	244	172.13	4.310	7989	0.84
			248	187.83	4.253	8008	0.83
3.	Mobile phase composition (±2 %v/v)	Mathanol : 10mM Amm. Acetate Buffer (70:30 %v/v)	68:32	174.84	4.808	8037	0.84
			72:28	188.50	3.879	7857	0.88
4	Column Temperature (±5 ℃)	40℃	35^0C	183.09	4.295	7793	0.84
			45^0C	180.29	4.245	7902	0.85

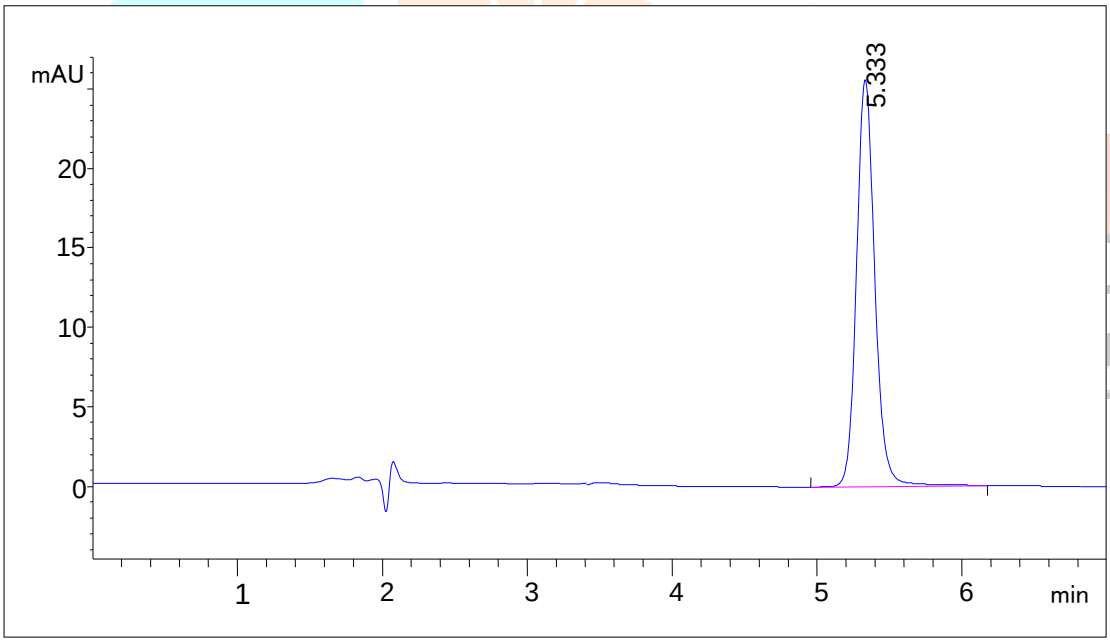


Fig.17: HPLC Chromatogram of Robustness Study of Propafenone HCl at flow rate of 0.8 ml/min

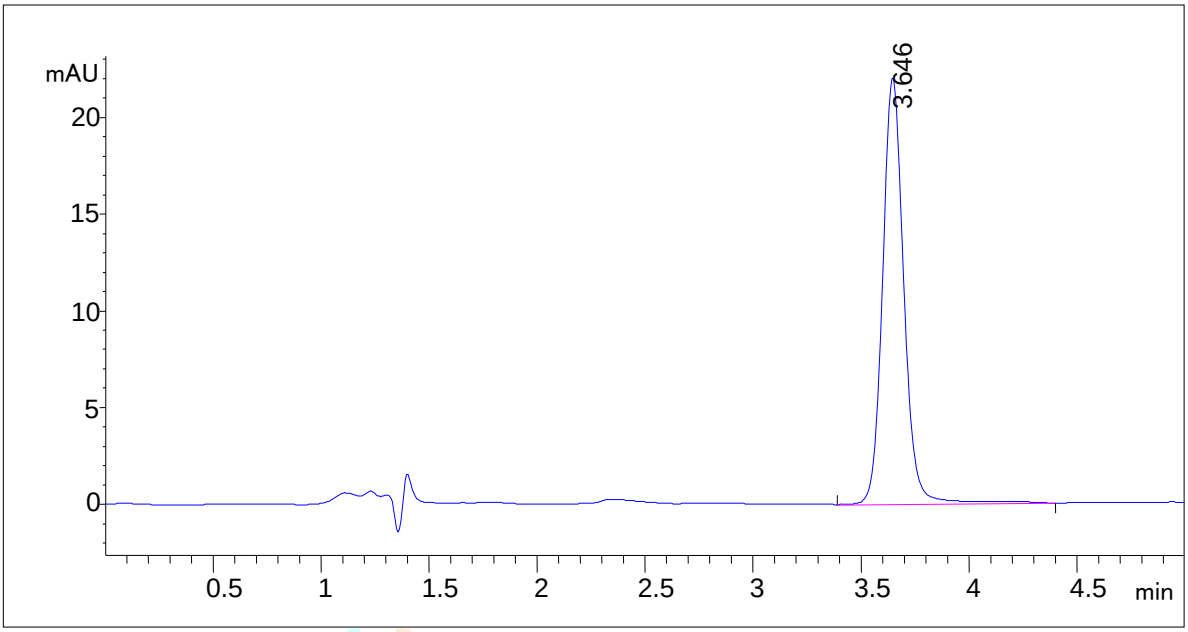
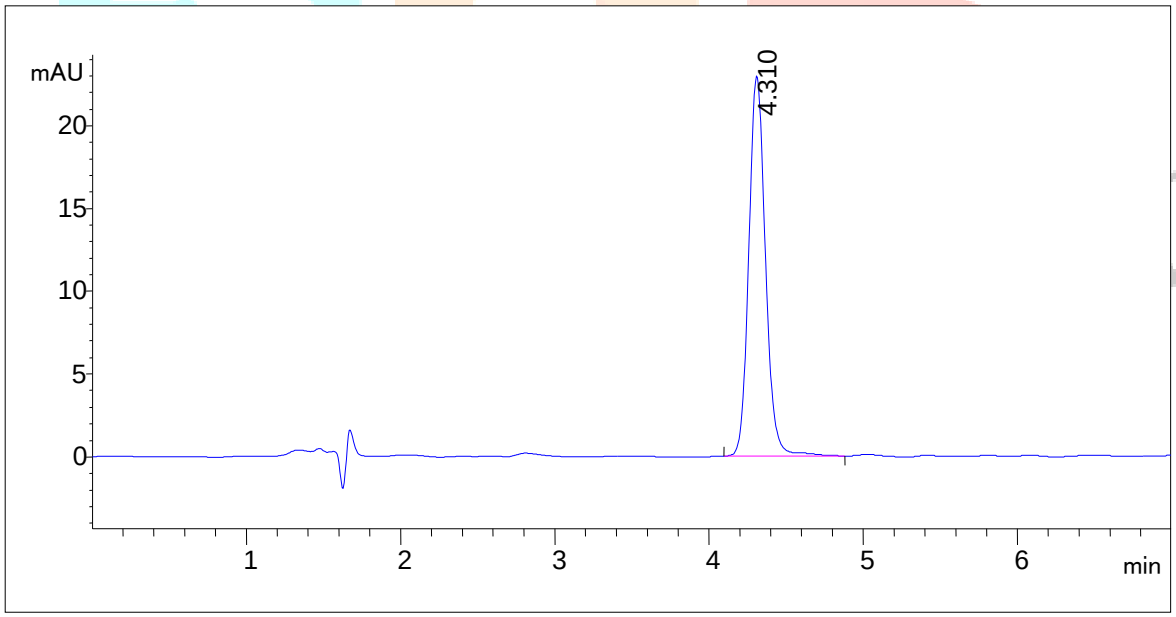
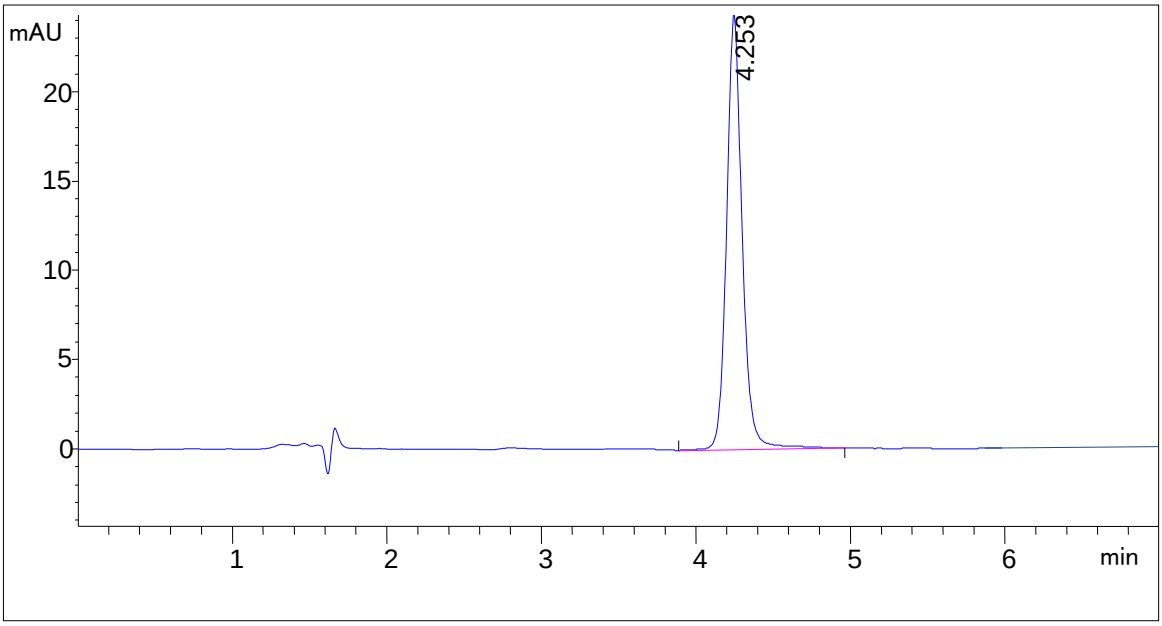


Fig.18: HPLC Chromatogram of Robustness Study of Propafenone HCl at flow rate of 1.2ml/min

2. Detection wavelength

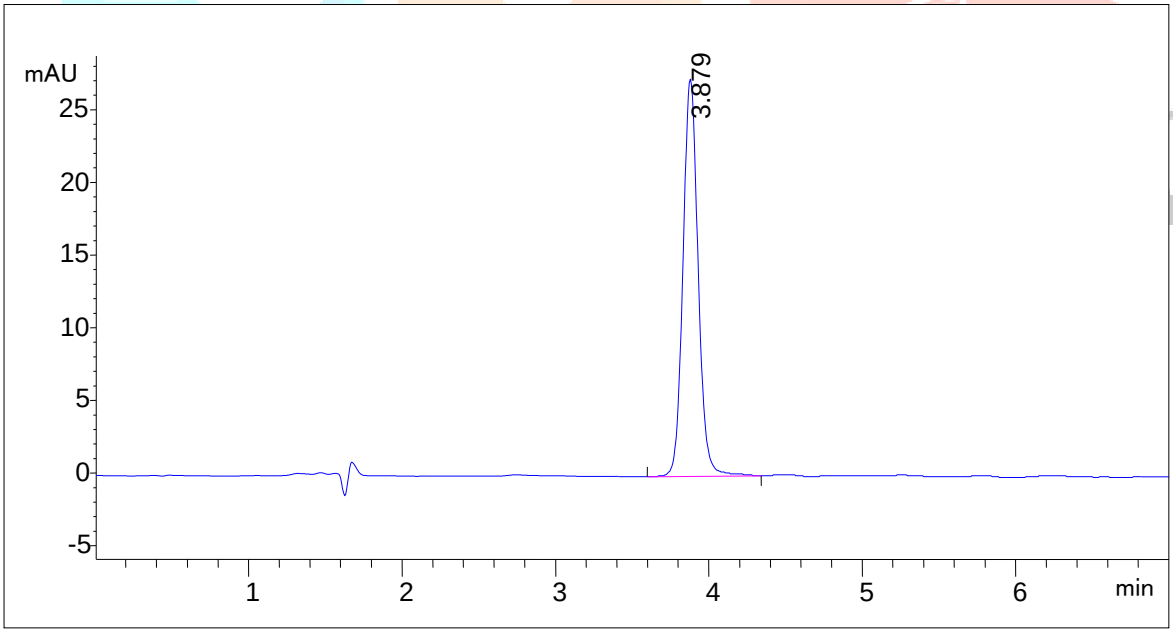


HPLC Chromatogram of Robustness Study of Propafenone HCl at detection wavelength of 244nm.

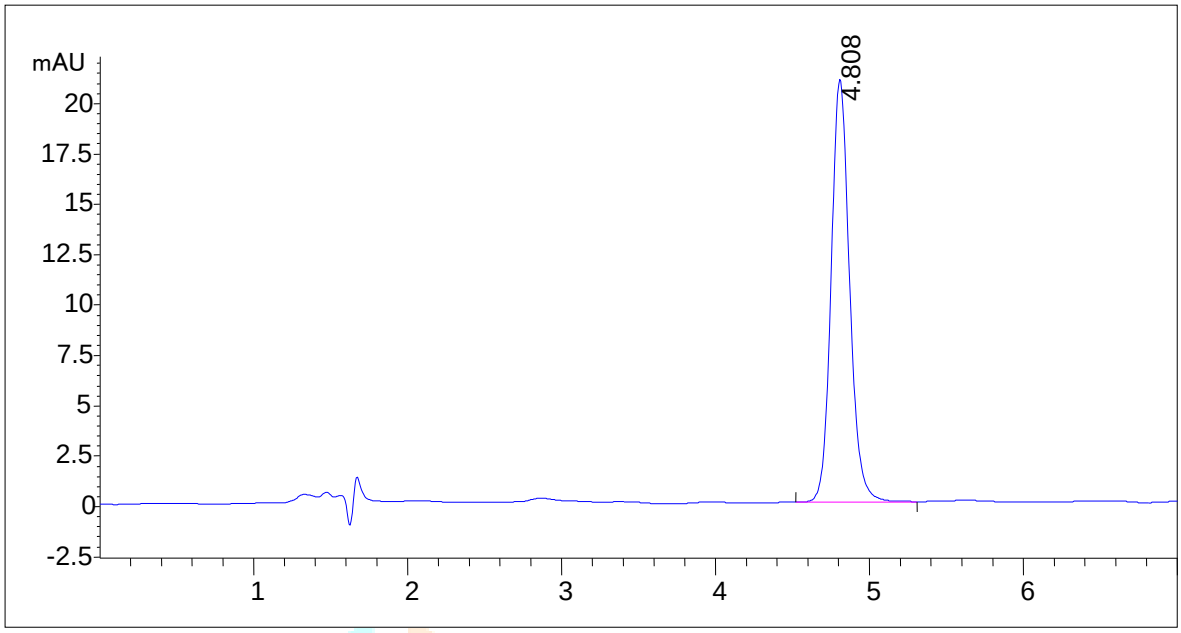


HPLC Chromatogram of Robustness Study of Propafenone HCl at detection wavelength of 248nm.

3. Mobile phase composition

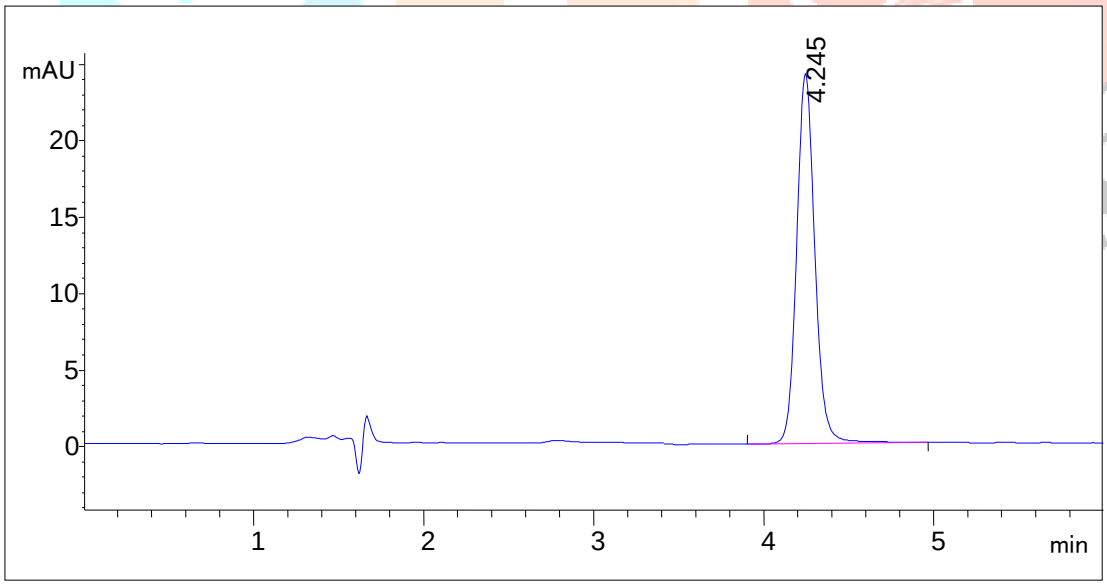


HPLC Chromatogram of Robustness Study of Propafenone HCl in mobile phase Methanol: 10mM Ammonium acetate buffer (72:28 %v/v).

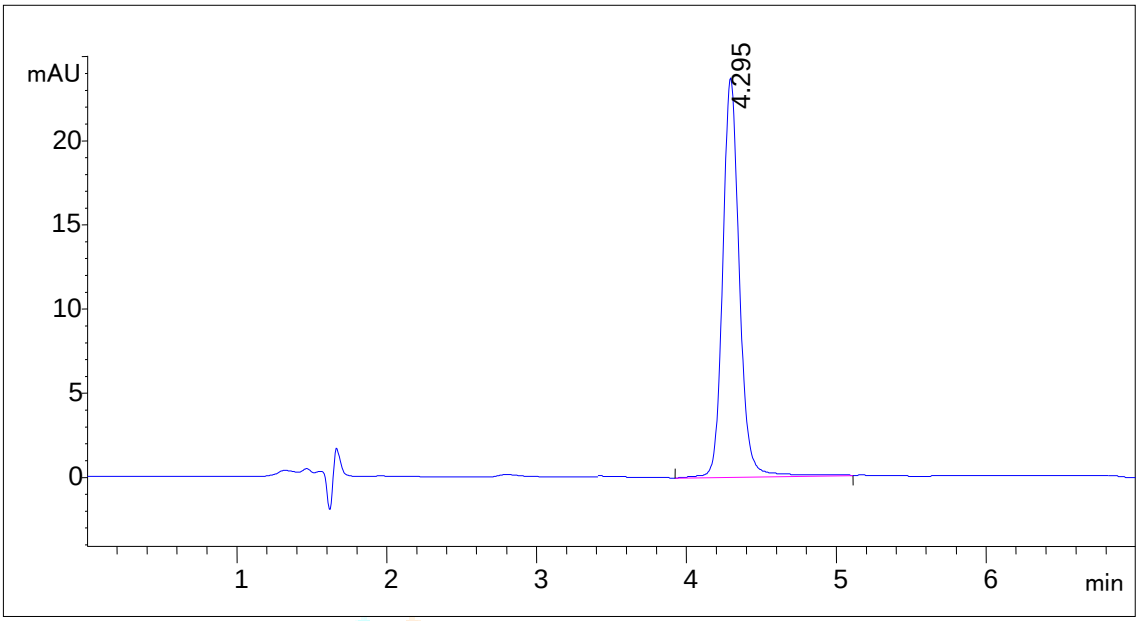


HPLC Chromatogram of Robustness Study of Propafenone HCl in mobile phase Methanol: 10mM Ammonium acetate buffer (68:32 %v/v).

3. Column temperature



HPLC Chromatogram of Robustness Study of Propafenone HCl at column temperature 35℃



HPLC Chromatogram of Robustness Study of Propafenone HCl at column temperature 45 ℃

Ruggedness

The study of ruggedness conditions was ascertained on the basis of three different conditions.

Inter-day study

The study was performed by replicate estimation of same sample of tablet formulation on three different days by proposed method.

7.8.6.2 Intra-day study

The study was performed by replicate estimation of same sample of tablet formulation on same day at three different intervals by proposed method.

7.8.6.3 Different Analysts

The study was performed by replicate estimation of same sample of tablet formulation by three different analysts by proposed method.

Ruggedness study for Propafenone HCl

Sr. No.	% Drug estimation		
	Intra day	**Inter day**	**Different analyst**
1	100.11	100.91	99.82
2	99.99	100.30	100.80
3	100.5	99.97	99.7
Mean	**100.2**	**100.39**	**100.1**
±SD	**0.267**	**0.4715**	**0.608**
% R.S.D.	**0.266**	**0.4697**	**0.608**

LOD and LOQ

LOD and LOQ for Propafenone HCl were evaluated by injecting a series of solutions duly diluted with known concentrations. Based on the response and slope of regression equation of the parameters of LOD and LOQ were calculated.

LOD and LOQ results of Propafenone HCl

Limit of Detection (LOD)	0.972µg/ml
Limit of Quantitation (LOQ)	2.94µg/ml

Specificity Study

The degradation study was performed not only on Propafenone HCl (API), but also in tablet formulation to determine whether any observed degradation occurred because of drug properties or was due to drug-excipient interactions.

Working standard solution of Propafenone HCl was freshly prepared (10µg/ml concentration) and used for comparison of results by peak area normalization method.

Accurately weighed quantity of tablet powdered equivalent to about of 10 mg Propafenone HCl were transferred to six different 10.0 ml volumetric flasks. The samples were then exposed to stress conditions like neutral, 0.1 N NaOH, 0.1N HCl, Photolysis, thermolysis, 3%H_2O_2. The solutions were then analyzed in similar manner as described under estimation of Propafenone HCl in tablets. The results of specificity study are shown in Table No. 15and the HPLC chromatograms of specificity sample of Propafenone HCl are shown in figure. 25-30.

Results of specificity study of Propafenone HCl

Standard and condition of exposure	Area	% labelled claim
Neutral (At room temp for 24 h)	180.54	99.9
0.1 N HCl (At room temp for 24 h)	185.11	102
0.1 N NaOH (At room temp for 24 h)	182.43	100
Thermal (At 70˚C for 24 h)	182.23	100
3% H_2O_2 (At room temp for 24 h)	183.34	101
Sunlight (24 h)	181.07	99.9

The HPLC chromatograms of specificity study of Propafenone HCl are as follows:-

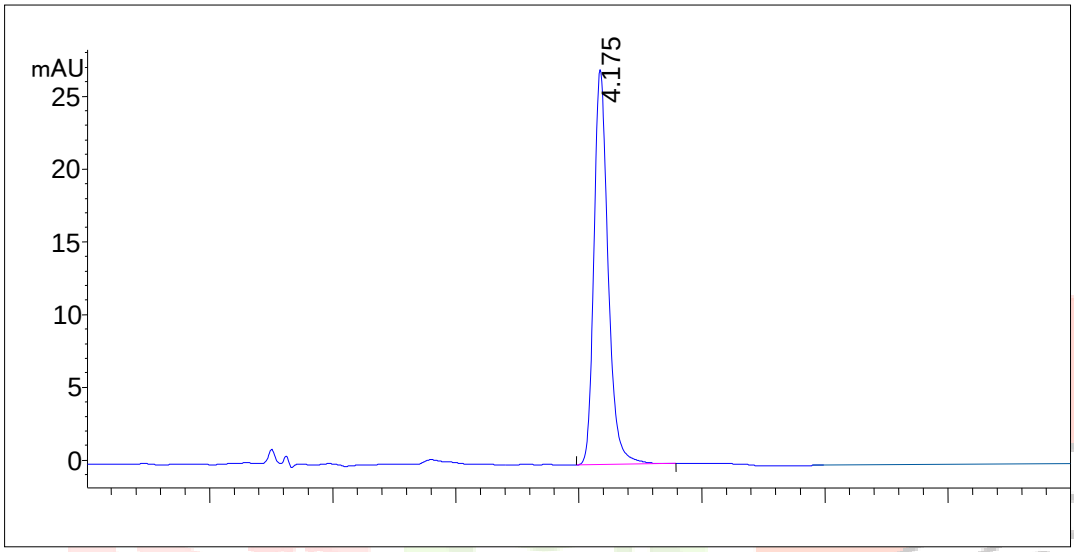
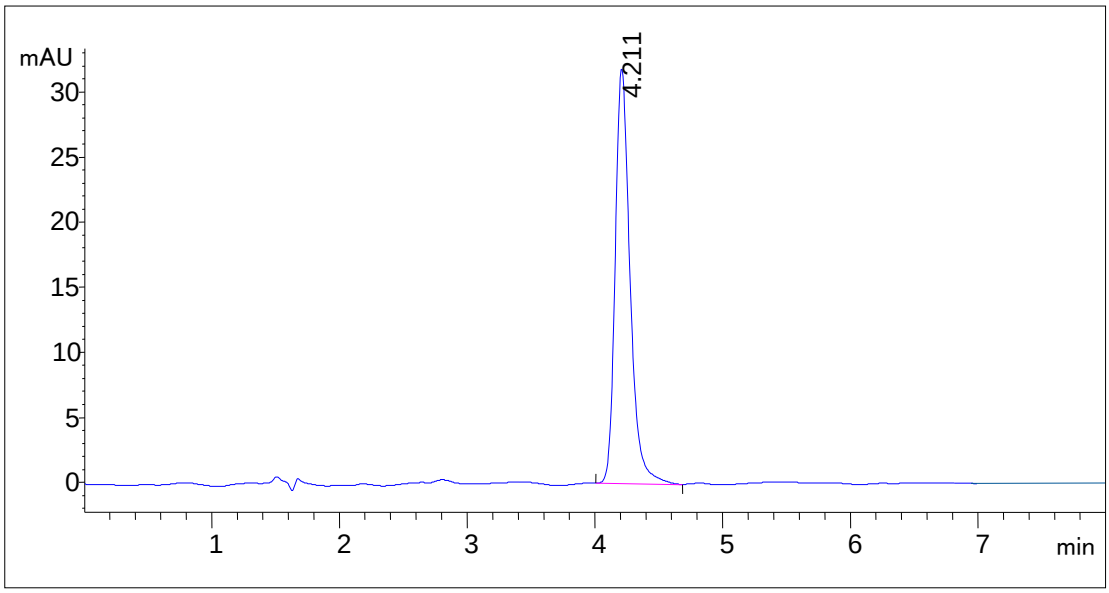


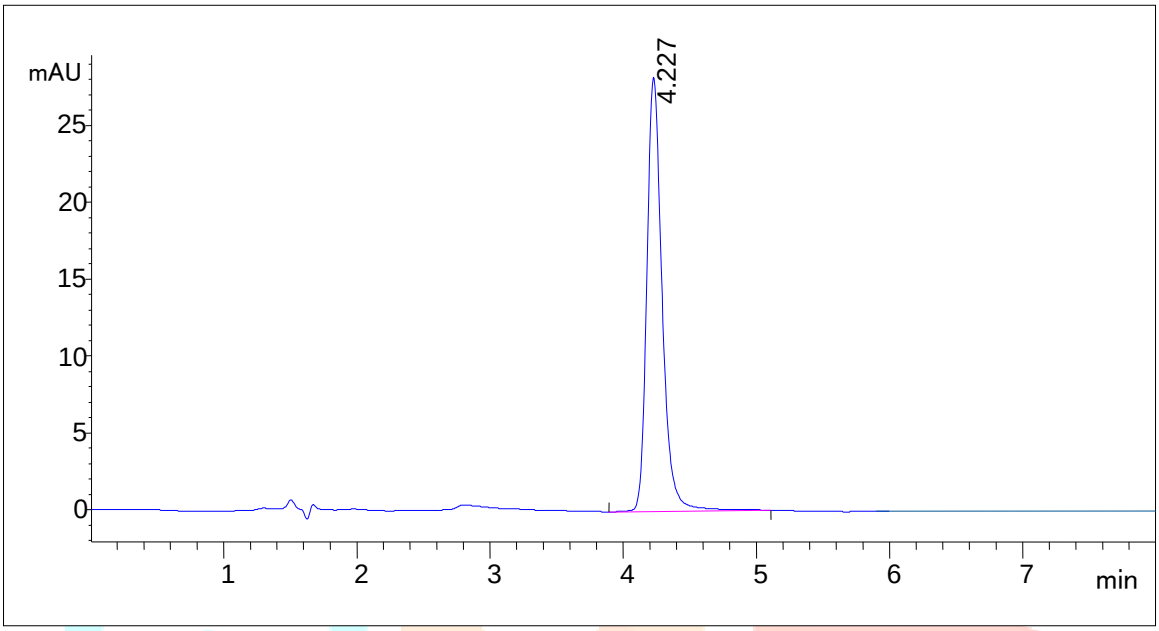
Fig 25: HPLC Chromatogram of specificity study of Propafenone HCl tablet in neutral (At room temp for 24 h)

2) 0.1N HCl



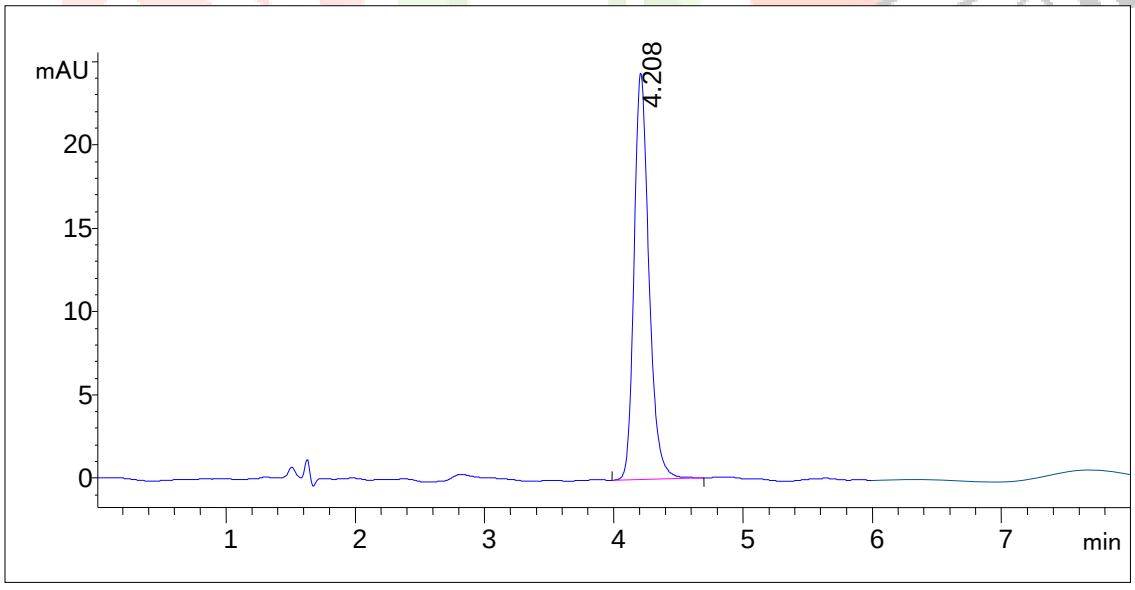
HPLC Chromatogram of specificity study of Propafenone HCl tablet in 0.1 N HCl (At room temp for 24h)

3) 0.1N NaOH



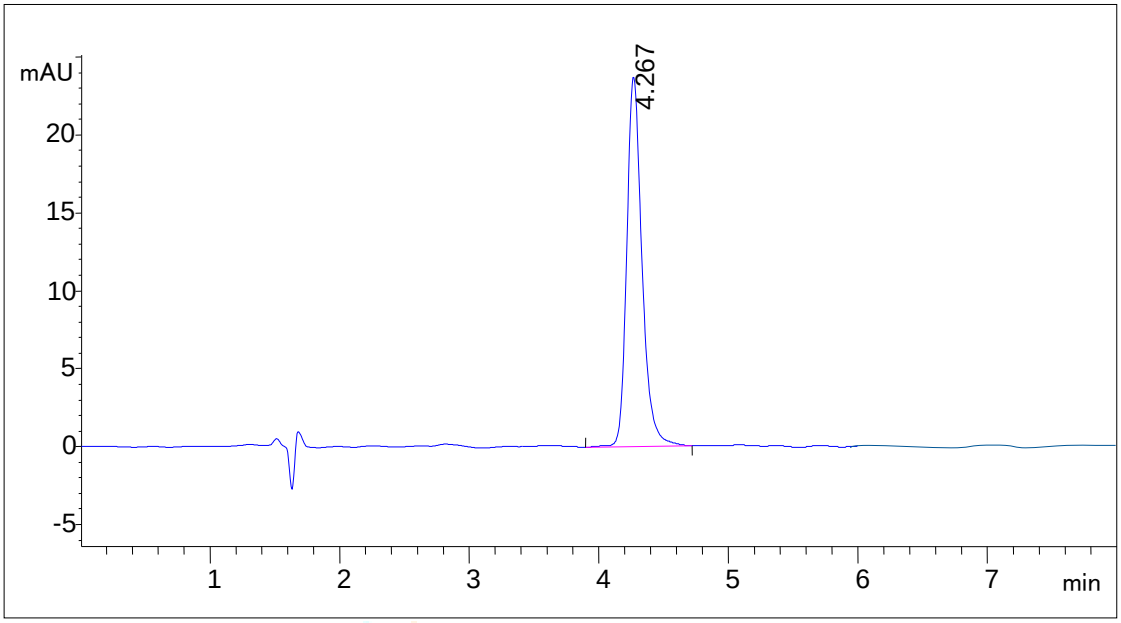
HPLC Chromatogram of specificity study of Propafenone HCl tablet in 0.1N NaOH (At room temp for 24h)

4) Thermolysis



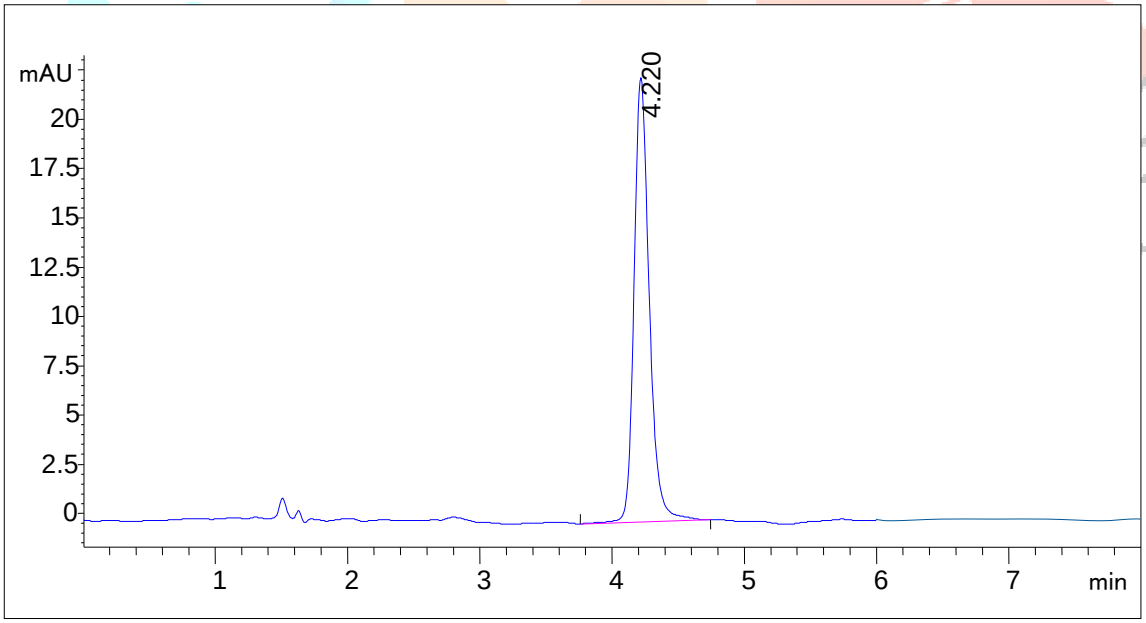
HPLC Chromatogram of specificity study of Propafenone HCl tablet in Thermal (At 70°C for 24 h)

1) Oxidation



HPLC Chromatogram of specificity study of Propafenone HCl tablet in 3% H_2O_2 (At room temp for 24h)

2) Photolysis



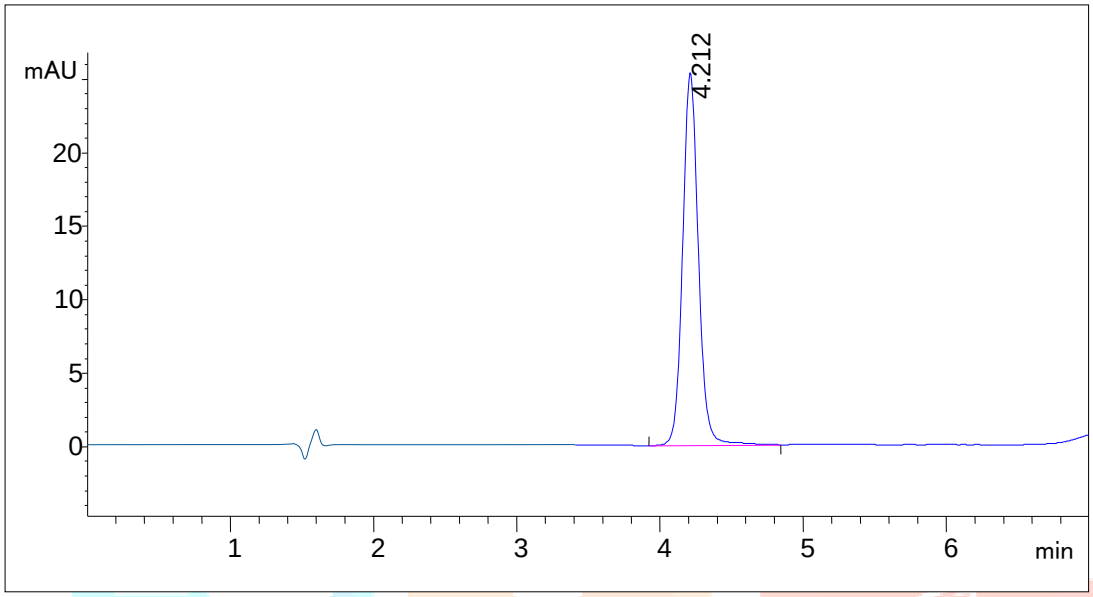
HPLC Chromatogram of specificity study of Propafenone HCl tablet in sunlight (24h)

Assay of tablet sample

The optimized HPLC method was then adopted for assay of Propafenone HCl in tablet formulation and it is summarized below.

A stock standard solution of Propafenone HCl (1 mg/ml) was prepared in methanol. The solution was further diluted appropriately with mobile phase to obtain a concentration of 10µg/ml of Propafenone HCl. A sample solution was prepared by dissolving quantity of tablet powder equivalent to 10 mg of Propafenone HCl in

adequate quantity of methanol using ultrasonication for 10 minutes. The volume of flask was adjusted to 10.0 ml with methanol. The solutions were further diluted appropriately with mobile phase to obtain a concentration of 10ug/ml of Propafenone HCl (on the basis of labeled claim). Chromatograms of standard and sample solutions were recorded under optimized conditions and the drug content was calculated. The results are summarized in Table No. 17.



HPLC chromatogram of assay of sample solution of Propafenone HCl

HPLC chromatogram of assay of sample solution of Propafenone HCl

Sr. No.	Wt. of tablet Powder (mg)	Peak Area	Amt. of drug Estimated (mg)	% label claim
1	17.2	183.34	10.11	101.1
2	17.2	185.11	10.20	102.0
3	17.3	182.23	10.04	100.0
4	17.2	182.43	10.06	100.1
5	17.1	182.89	10.08	100.1
6	17.2	184.11	10.15	101.5
Mean				**101.1**
Standard deviation				**0.008**
% Relative standard deviation				**0.808**

9.2 .SUMMARY

9.2.1 Development of Validated Stability Indicating RP-HPLC Method for Estimation of Propafenone Hydrochloride

9.2.2. HPLC method development

The chromatographic separation of Propafenone Hydrochloride and its degradation products was done on Eclipse XDB-C18 150 x 4.6mm, 5µm.

Several mobile phase compositions were tried to resolve the peaks of Propafenone Hydrochloride and their degradation products. Out of these combinations, the mobile phase containing Methanol:10 mM Ammonium Acetate Buffer (7030 %v/v) was found to be most satisfactory as it gave good resolution of drug and degradation products with reasonably symmetrical sharp peaks. Therefore, this mobile phase was selected throughout the analytical studies. A detection wavelength 246 nm was optimized as Propafenone Hydrochloride has substantially high absorbance at this wavelength. A flow rate of 1.0 ml/min at ambient (40 ˚C) temp was found to be optimum. The retention time of Propafenone Hydrochloride under optimized chromatographic conditions was found to be 4.192 min with sharp symmetrical peak (symmetry of 0.80). The capacity factor was 1.80 with theoretical plates was 7415. A typical HPLC chromatogram was obtained during determination of Propafenone Hydrochloride depicted in Fig 10.

- The standard and sample solutions of the drug show reasonably good stability over a period of about 24 hrs. with maximum % RSD of 0.649 %.
- The developed method was validated as per ICH guideline Q2R1 for system suitability, linearity, range, accuracy precision, robustness and ruggedness.
- The system suitability tests were performed by collecting data from six replicate injections of standard solutions. The drug was found to adequately retain at 4.192min with sharp symmetrical peak and high theoretical plate value of 7279 no. of plates indicating high column efficiency.
- The linearity of method was found to in range 5-25ug/ml in mobile phase with correlation coefficient r^2 = 0.999**.** A good accuracy of the method was verified through recovery test and the % of drug recovered was found to be 100 % indicating accuracy of the method.
- Precision of the methods was estimated by repeatability and intermediate precision study. The %RSD value was found to be 1.022%.
- The experimental values obtained in the determination of Propafenone HCl in tablet sample, indicated a good intra-day, inter day and by different analyst were very much reproducible with maximum % R.S.D 0.608 % which shows the ruggedness of the method in the hands expert analyst and at different time intervals different.
- The LOD and LOQ were found to be 0.972µg/ml and 2.94µg/ml

- In the specificity study the result of estimation of sample under different condition show that Propafenone HCl in quite stable under all conditions of exposure.such as acids, alkali, oxidation, heat and sunlight and then analyzing them by proposed HPLC method. The drug is found to be well resolve and free of interference from all the impurities and excipients present. .
- The results of assay of tablet obtained by proposed HPLC method with standard deviation 0.008 and % RSD 0.808 % which indicate method are quite concurrent and reproducible. Hence, it may be adopted for routine assay of Propafenone HCl free of interferences from its degradation products in tablet formulation.

CONCLUSION

In the project RP-HPLC method is developed and validated as per ICH Q2R1 guideline. The method is found to be accurate and precise over the range of their estimation in standard laboratory solution and sample solution from pharmaceutical dosage form.

In RP-HPLC Method the drug is found to be well resolved from other component (impurities and excipients) under the optimized chromatographic condition. Moreover, the method is in true sense can be said to be specific for estimation of Propafenone HCl due to its capacity to estimate the drug content unequivocally free of interference from its degradation products. The validation of method indicates that the method is simple, precise, accurate, rugged and reasonably specific for the estimation of Propafenone HCl in pharmaceutical dosage form.