# DEVELOPMENT AND VALIDATION OF UV-MULTIVARIANT CALIBRATION TECHNIQUE AND HPTLC METHOD FOR THE ESTIMATION OF PROPAFENONE HYDROCHLORIDE IN ITS TABLET DOSAGE FORM

A Dissertation submitted to

**THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY**

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In partial fulfilment of the requirements for the award of the degree of

**MASTER OF PHARMACY**

**PHARMACEUTICAL ANALYSIS**

Submitted by

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## CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT AND VALIDATION OF UV-MULTIVARIANT CALIBRATION TECHNIQUE AND HPTLC METHOD FOR THE ESTIMATION OF PROPAFENONE HYDROCHLORIDE IN ITS DOSAGE FORM"** submitted to The Tamil Nadu Dr. MGR. Medical University, Chennai was carried out by **Register No. 261930402** in the Department of Pharmaceutical Analysis, Nandha College of Pharmacy, Erode-52. In the partial fulfilment for the award of degree of **Master of Pharmacy** in Pharmaceutical Analysis and my direct supervision and guidance.

This work is original and has not been submitted in part or full for any other degree or diploma in any university.

Place: Erode **Dr.M. JAGADEESWARAN**

Date:

# EVALUATION CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"DEVELOPMENT AND VALIDATION OF UV-MULTIVARIANT CALIBRATION TECHNIQUE AND HPTLC METHOD FOR THE ESTIMATION OF PROPAFENONE HYDROCHLORIDE IN ITS DOSAGE FORM"** submitted to The Tamil Nadu Dr. M.G.R. Medical University Chennai, was carried out by **Register No: 261930402** in Department of Pharmaceutical Analysis, Nandha College of Pharmacy, Erode-52 in the partial fulfillment of the degree of "Master of Pharmacy" in Pharmaceutical Analysis under the supervision of **Dr. M. JAGADEESWARAN, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutical Analysis, Nandha College of Pharmacy, Erode-52.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other University.

Internal Examiner External Examiner

# DECLARATION

I hereby declare that the work incorporated in thesis entitled "**DEVELOPMENT AND VALIDATION OF UV-MULTIVARIANT CALIBRATION TECHNIQUE AND HPTLC METHOD FOR THE ESTIMATION OF PROPAFENONE HYDROCHLORIDE IN ITS TABLET DOSAGE FORM"** was carried out under the guidance of **Dr.M. JAGADEESHWARAN, M. Pharm, Ph.D.,** Professor, Nandha College of Pharmacy, Erode. The project report is being submitted to Nandha College of Pharmacy, Erode, in partial fulfilment of the degree of Master of Pharmacy in Pharmaceutical Analysis.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other University.

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**Reg.Number: 261930402**

Department of Pharmaceutical Analysis

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# INTRODUCTION

# 1. INTRODUCTION[1-9]

Pharmaceutical analysis plays a vital role in the quality assurance and quality control of bulk drugs and their formulations. Pharmaceutical analysis is a specialized branch of analytical chemistry which involves separating, identification and determining the relative amounts of components in a sample of matter. It is concerned with the chemical characterization of matter both quantitative and qualitative.

The need and importance of drug analysis and assays involved in the development, manufacturing and therapeutic usage of drugs. This includes identification, characterization and determination of drugs in mixture such as dosage forms. The drug analysis procedures play essential roles in pharmacokinetics of drugs in human and animals. Bulk drugs are obtained by chemical synthesis, biosynthesis, isolation from plants or animals or biotechnological sources. Dosage form is the bulk drugs manufactured into dosage form with help of additives prior to their use in patients.

Drugs in different matrix are assayed by different methods such as chromatographic and spectroscopic techniques, separation techniques and radioimmunoassay etc.,

The modern trends in drug discovery and development with the aid of computer aided drug-designing promises the introduction of many newer drugs and multi components for various therapeutic activity.

**METHOD OF ANALYSIS**

**Generally analytical methods are classified into**

1. Chemical methods:
    a) Volumetric methods
    b) Gravimetric methods

2. Instrumental methods:

The analytical chemist to save time, to avoid chemical separation or to obtain increased accuracy, may make use of instrumental methods. This can be classified out as:

a) Spectrophotometer methods- UV, IR, NMR, Mass.
b) Fluorimetric methods
c) Polarimetric methods
d) Flame photometric method
e) Turbidimetric method
f) Chromatographic methods
g) Refractometric methods
h) Thermal methods
i) Electrochemical methods

**SPECTROPHOTOMETRY**

In chemistry, spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet and near-infrared, but does not cover time resolved spectroscopic techniques.

**COLORIMETRY**

This method of analysis is based on measuring the absorption of monochromatic light by coloured compounds in the visible path of the spectrum (380-800 nm). If the analytes are colourless, they are converted into coloured compounds by reaction with a suitable chromogenic reagent. They must be stable and have a constant composition and high colour intensity.

## ABSORPTION SPECTRUM

The absorption spectrum is a graphical representation of the amount of light absorbed by a substance at definite wavelengths. To plot an absorption curve, the values of the wavelength (λ) are laid off along the axis of abscissas and the values of the absorbance along the axis of ordinates. A characteristic of an absorption spectrum is a position of the peak (maxima) of light absorption, which is determined by the absorptivity at definite wavelength.

## BEER'S LAW PLOT:

A standard specimen of the analyte is taken and the solutions of this specimen with known concentration are prepared. The absorbances of all the solutions are measured at a definite wavelength (λmax) and the calibration curve is plotted by laying off the known concentration along the axis of abscissas and the absorbances corresponding to them along the axis of ordinates. The calibration curve is used to determine the unknown concentration of the analyte in solution. The functional groups of these drug determine the way of analysing it because they are responsible for the properties of the substances. The functional groups also determine the identification reactions and the methods of quantitative determination of drugs.

## METHOD DEVELOPMENT

Analytical method development and validation play an important role in the drug discovery, development and manufacture of pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency and performance of drug products.

Recent guidelines for the methods developments and validation for new no compendial test methods are provided by the FDA draft document, "Analytical procedures and methods validation: Chemistry, Manufacturing and Controls documentation". This recent document applies to the method development and validation process for process for products included in investigational new drugs (IND), new drug application (NDA) and abbreviated new drug

application (ANDA) submissions. Therefore, expectations from regulatory agencies for method development and validation are clear, in recent years, a great deal of effort has been devoted to the harmonization of pharmaceutical regulatory requirements in the United States, Europe and Japan.

As part of this initiative, the International Conference on Harmonization (ICH) has issued guidelines for analytical method validation. The recent FDA methods validation draft guidance document as well as USP both refer to ICH guidelines. Analytical guidance documents recently published by the ICH are the following:

- Stability testing (Q1)
- Validation of analytical procedures (Q2)
- Impurities in drug substances and products (Q3)
- Specifications for new drug substances and products (Q6)

The methods validation documentation requirements for IND and NDA submissions are outlined in the chemistry, manufacturing and controls (CMC) guidance document. The current trend continues to be in the direction of phase-dependent methods development and validation. Non validated screening methods are used to monitor the synthesis of active ingredients or to confirm their identity during discovery and preclinical research. Analytical methods are progressively optimized and a preliminary validation package is furnished as part of the IND application before phase I safety trials are initiated. All analytical methods should be fully optimized and validation completed before the NDA is submitted at the end of phase III studies. Method validation is a continuous process.

The steps of methods development and method validation depends upon the type of method being developed. However, the following steps are common to most types of projects

- Method development plan definition
- Background information gathering

- Laboratory method development
- Generation of test procedure
- Methods validation protocol definition
- Laboratory methods validation
- Validation test method generation
- Validation report

A well-developed method should be easy to validate. A method should be developed with the goal to rapidly test preclinical samples, formulation prototypes, and commercial samples. As the methods development and validation processes advance, the information gathered is captured in the design and subsequent improvement of the method. Ideally, the validation protocol should be written only following a thorough understanding of the methods capabilities and intended use. The validation protocol will list the acceptance criteria that the method can meet. Any failure to meet the criteria will require that a formal investigation be conducted. The required validation parameters, also termed analytical performance characteristics, depends upon the type of analytical method. Pharmaceutical analytical methods are categorized into five general types,

- Identification tests
- Potency assays
- Impurity tests: quantitative
- Impurity tests: limit
- Specific tests

The first four tests are universal tests, but the specific tests such as particle-size analysis and x-ray diffraction are used to control specific properties of the active pharmaceutical ingredient (API) or the drug product.

Only specificity is needed for an identification test. However, the full range of specificity, accuracy, linearity, range, limit of detection (LOD), limit of quantification (LOQ), precision and robustness testing are needed for more complex methods such as quantitative impurity methods. The validated test method is included in the validation report that summarizes the results of the validation studies. Both the validation report and test method are submitted as part of the NDA or ANDA.

The efficient development and validation of analytical methods are critical elements in the development of pharmaceuticals. Success in these areas can be attribute to several important factors, which in turn will contribute to regulatory compliance. Experience is one of these factors-both the experience level of the individual scientists and the collective experience level of the development and validation department. A strong mentoring and training program is another important factor for ensuring successful methods development and validation.

Companies must maintain an appropriate level of expertise in this important dimension of developing safe and effective drugs.

The drug development is mainly performed by the basis of High-performance thin layer chromatography, High performance liquid chromatography, UV/Visible spectroscopy methods.

Modern instrument HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. We are convinced that HPTLC is playing an important role in today's analytical world, not in competition to HPLC but as a complementary method.

One of the most obvious orthogonal features of the two techniques is the primary use reversed phase in HPLC versus unmodified silica gel in HPTLC, resulting in partition chromatography and adsorption chromatography respectively.

Unlike other methods, HPTLC produces visible chromatograms: complex information about the entire sample is available at a glance. Multiple samples are seen simultaneously, so reference and test sample can be compared for identification.

The wide variety of equipment, columns, eluent and operational parameters involved makes High performance liquid chromatography (HPLC) method development seem complex.

**MULTIVARIANT ANALYSIS**

Multivariant analysis is used to describe the analyses of data where there are multiple variable or observation for each unit or individual.

Often times these data are interrelated and statistical methods are needed to fully answer the objectives of our research.

Most of multivariant analysis deals with estimation, confidence sets, and hypothesis testing for means, variances, covariances, correlation coefficient and related, more complex population characteristics.

**TYPES OF MULTIVARIATE CALIBRATION:**

Many methods for multivariate calibration have been proposed. It turns out that many of the methods perform similarly. To avoid confusion due to use of many different methods, it is suggested that only the following should be considered.

- Multiple linear regressions (MLR)
- Principle component regression (PCR)
- Partial least square (PLS)
- Neutral networks (NN)
- Locally weighed regression (LWR)
- Radical basis function combined with PLS(RBF-PLS)

**CALIBRATION METHODS:**

1. **Classical calibration**

Classical calibration is to determine the concentration of a single compound using the response of a single detector. For e.g., a single spectroscopic peak area.

2. **Inverse calibration**

Although classical calibration is widely used, it is not always the most appropriate approach in analytical chemistry. The ultimate aim is usually to predict the concentration from the spectrum or chromatogram rather than vice versa. For e.g., can a response be predicted from the values of the independent factors.

**DISADVANTAGE:**

- In each instrument has different characteristics and, on each day, and each hour the response can vary.
- In multivariant calibration gives only a rough prediction, but if the quality of a product and it appears to exceed a certain limit.

**APPLICATION:**

- Multivariate chromatographic calibration technique is a powerful mathematical tool for optimizing chromatographic multivariate calibration and elimination of fluctuations coming from instrumental and experimental condition.
- Another multivariate HPLC-UV technique anticipated that under optimized condition considerable resolving power, sensitivity, rapidity, quality control and routine analysis of subject compounds is achieved.
- Analytical parameters such as selectivity, accuracy and precision have been established and evaluated statistically to assess the application of the method.

## HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

At the end of 50's TLC was introduced to the laboratories as a rapid non demanding techniques for simple quantitative investigation. Recent developments in the practice of thin layer chromatography have resulted in a breakthrough in performance which has led to the expression 'HPTLC'. These developments have not been the result of any specific advanced in instrumentation, but rather the culmination of improvement in the various operations involved in TLC.

It enjoys a practical application status as it combines the all of chromatography with quickness at a moderate cost- HPTLC is a major advancement of TLC principle requiring shorter & better resolution. The basic difference between conventional TLC & HPTLC is only in particle & pore size. Modern thin layer chromatography is powerful reliable & cost-efficient method for qualitative & quantitative analysis synonyms for modern thin layer chromatography are,

1. Planar chromatography
2. Instrumental thin layer chromatography
3. High performance thin layer chromatography
4. Planar liquid chromatography (PLC)

## PRINCIPLE

The principle of separation is adsorption. The mobile phase solvent flows through because of capillary action (against gravitational force). The components move according to their affinity towards the absorbent. The component with lesser affinity travels faster. Thus, the components are separated based on the affinity of the component towards the stationary phase.

In HPTLC high performance grade of silica in which the particle is small (about 5 μm) and very uniforms in size are used. These high-performance silica gel more efficient and

reproducible separation than conventional grades of silica. Consequently, the plates are smaller (10 cm) or (20 cm) & the development time is shorter typically only few min. If small volume (15 µm) of the samples all applied to the plates the spots in the development chromatography are very compact & all quantified by means of photo densitometer.

**VARIOUS STEPS INVOLVES IN HPTLC**

**PREPARATION OF HPTLC PLATES:**

**Cellulose:** fibrous cellulose slurry is prepared spread on the plate and it's dried at 110°C for 15 mins.

**Cellulose with starch binder:** 0.4 g of starch is mixed with 10 ml of water & which is mixed with 90 ml of boiling water & 2 % acid to prepare slurry thickness of the layer is 200 µm.

**Cellulose (microcrystalline):** Suspend 20gm cellulose in 60 ml of water, slurry is coated on plate with spreader & dried. Thickness of the layer is 400 µm.

**Cellulose (microcrystalline) with fluorescent:** Suspend 25 gm of cellulose in 40 ml of methanol & 20ml of water to prepare the plates. Layer thickness about 100µm.

**Silica gel or silica gel G:** Suspend 30 gm of silica gel Gin 60-65 ml water. Layer thickness is 250 µm.

**PREWASHING OF PRE-COATED PLATES:**

Sorbents with large surface area absorb not only water vapour & other impurities. From atmosphere but other volatile substance often condenses on it. This result in dirty zones & fail to give reproducible results. Hence it is always recommended to clear the plates before actual chromatography. It is called prewashing. It can be done by:

A) **Ascending:** Cleaning effect is superior however active dirt gets. Accumulated at solvent front 10-20 % of the upper portion.

B) **Dipping:** Quicker dipping process fields rather uniform clean layer but cleaning effect is often not as good as with ascending techniques.

C) **Continuous mode:** The plate is placed in a chamber closed by a lid having a slit.

**APPLICATION OF SAMPLE:**

The sample should be completely transferred in to the layer usually the volume of sample applied is 0.5 to 5 µL & size of thickness 0.5 to 1 mm thickness & concentration range is 0.1-1 µg/ml.

Advantage of application of sample as bands

1. Response of densitometer is higher.
2. Spot broadening in the direction of development is smaller.
3. In direction limits is about ten times better than in conventional TLC.

**MOBILE PHASE:**

Combination of solvents are used various components of mobile phase are measured separately and placed in a mixing vessel. Then introduced into the developing chamber. Mobile phase once used not commended for second use.

Choice of solvents depends on

1. Nature of the substance to be separated.
2. Materials; on which the separation is to be carried out.

**DEVELOPING AND DRYING:**

Ascending, Descending, Two dimensional, Radial, Antiradical, Multi model development chamber.

After developing the plate, mobile phase is removed from the chamber as quick as possible. The step should be performed in fumed cupboards to avoid contamination of lab atmosphere. Drying cupboards should have non perforated shelf. It is preferable to keep the plate horizontal.

## ADVANTAGE OF TLC/HPTLC

1. The substance that remains at the start during chromatography under chosen condition can easily be recognised.
2. Decomposition of analyte during chromatographic development can be detected by two-dimensional TLC.
3. Use of corrosive and UV absorbing mobile phase is feasible as detection is preceded by complete removal of mobile phase by evaporation.
4. Substance can be detected / identified by using universal detection/ visualization reagents (iodine vapours, methanolic sulphuric acid) or selective reagents.
5. Sample clean-up is often either unnecessary or necessary to a very limited degree.
6. No carry over, hence no contamination.

## FACTORS AFFECTING TLC SEPARATION AND RESOLUTION SPOT

1. Types of stationary phase (sorbent), its particle size and activity.
2. Types of plates (precoated or hand-made)
3. Mobile phase (solvent system)
4. Solvent purity.
5. Type and size of chamber.
6. Degree of chamber saturation.
7. Sample volume spotted.
8. Size (diameter) of the initial spot.
9. Solvent and mobile phase level in the chamber.
10. Separation distance.
11. Mode of development.

## METHOD VALIDATION

The word ‘Validation’ means ‘Assessment’ of validity or action of proving effectiveness.

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

Methods need to be validated or revalidated.

- Before their introduction into regular use.
- Whenever the condition change for which the method has been validated. e.g., instrument with different characteristics.
- Whenever the method is changed, and the change is outside the original scope of the method.

## REASONS FOR VALIDATION

- Setting standards of evaluation procedures for checking complaints.
- Retrospective validation is useful for trend comparison of results compliance to cGMP/GLP.
- Closer interaction with pharmacopoeia harmonization particularly in respect of impurities determination and their limits.
- To provide high degree of confidence that the same level of quality is constantly built into each unit of finished product from batch to batch.

## METHOD VALIDATION PARAMETERS:

### 1.SPECIFICITY

It is the capacity of an analytical assay to measure the analyte in a sample in the presence of the other components like degradants, excipients, impurities, matrix etc., expected to be present in the product. The parameter is calculated for to ensure that the assay provides an accurate statement of the identity, potency or purity of a product.

## 2. LINEARITY

Linearity is calculated by statistical method of linear regression analysis with standard preparation of minimum five concentration in the specified range.

It is the capability of an assay to obtain test results, which are directly proportional to the concentration of an analyte in the sample. The purpose of this parameter will identify the range of the analyte in analytical assay. It can be me assured as slope (m) and intercept of the regression line and correlation coefficient (R).

## 3. RANGE

It is a measure of the highest concentration of an analyte that can be measured with acceptance accuracy and precision. It is the upper limit of the linearity determination. If the relationship between response and concentration is not linear, the range may be estimated by means of a calibration curve.

## 4. ACCURACY

It is the closeness of agreement between the actual value of the drug and the measured value. Spike and recovery studies are performed to measure accuracy: a known sample is added to the excipients and the actual drug value is compared to the value found by the assay. Accuracy is expressed as the bias or the % error between the observed value and the true value (assay value/actual value x 100 %)

$$S\%\ Bias = \frac{\text{Measured value} - \text{true value} \times 100}{\text{True value}}$$

## 5. PRECISION

It is the closeness of agreement between the values obtained in an assay. It is expressed as the coefficient of variation (% CV). CV is the standard deviation of the assay values divided by the concentration of the analyte. Several types of precision can be measured: intra-assay precision (repeatability) is the % CV of multiple determinations of a single sample in a single

test run; inter-assay precision (also called intermediate precision) measures the % CV for multiple determinations of a single sample, controls and reagents analysed in several assay runs in the same laboratory.

Reproducibility is the precision between laboratories usually in collaborative studies and not directly relevant to assay validation in a manufacturing facility.

**6. DETECTION LIMIT**

The lowest quantity of an analyte in the sample is detected by the instrument but not necessary to determine the extract value of analyte quantity.

Detection limit was calculated by using the following formula:

$$\text{Detection limit} = \frac{3.3 \times \text{standard deviation of the response of the blank}}{\text{Slope}}$$

For the determination LOD initially standard deviation and slope of calibration curve was calculated. Then by using these values as per formula maintained in ICH guidelines these parameters were calculated.

**7.QUANTIFICATION LIMIT**

The lowest quantity of analyte in a sample is determined quantitatively by the instrument with appropriate precision and accuracy.

Quantification limit was calculated by using the following formula:

$$\text{Quantification limit} = \frac{10 \times \text{standard deviation of the response of the blank}}{\text{Slope}}$$

For the determining LOQ initially standard deviation and slope of calibration curve was calculated. Then by using these values as per formula maintained in ICH guidelines these parameters were calculated.

## 8. ROBUSTNESS

It is the capacity of an assay to remain unaffected by deliberate changes to various parameters of the method and gives an indication of its reliability during normal assay conditions. The variations could be in room or incubator temperature or humidity, variations in incubation times, minor variations in pH of a reagent etc.,

Under each of these conditions, the accuracy and precision or other assay parameter can be measured to see what variations can be tolerated in the assay conditions.

## 9. SYSTEM SUITABILITY TESTING

According to the USP, system suitability tests are fundamental part of chromatographic methods. For various analytical methods system suitability is an essential part. The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagent, and columns analysts) is suitable for the intended application.

**Table.1. Criteria for validation methods**

| Characteristics | Acceptable range |
|---|---|
| Accuracy | Recovery (98-102 %) |
| Precision, Repeatability | % RSD < 2 |
| Intermediate precision | % RSD < 2 |
| Specificity | No Interference |
| LOD | >2 or 3 |
| LOQ | >10 |
| Linearity | Correlation Coefficient (r) > 0.99 |
| Range | 80-120 % |
| Stability | >24 h or > 12 h |

# REVIEW OF LITERATURE

# 2. REVIEW OF LITERATURE[10-26]

**Nageswara Rao *et al.*,[10]** developed and validated a simple RP-HPLC method for the estimation of propafenone HCL in pure drug and pharmaceutical dosage form. The method is developed on Inertsil ODS-3Vs column with acetonitrile: methanol: water (90:10 v/v) as mobile phase in the ratio of 75:25 at a flow rate 0.7 ml/min, detection was carried out 245 nm. The recovery studies were found to be 101 %. LOD and LOQ was found to be 3.64 µg/ml and 11.04 µg/ml respectively.

**Amir Farag *et al.*,[11]** determined a new sensing platform based on NH2fMWCNTs (NH2-functionalized multi-walled carbon nano-tubes) for antiarrhythmic drug propafenone in pharmaceutical dosage form. Electrochemical studied were determined by cyclic, differential pulse, and square wave voltammetry, such as amount of modifier, pH, scan rate, accumulation time and potential were optimized. The linearity ranges from 0.1 to 10 µM, LOQ and LOD was found to be 0.03 µM and 0.01 µM respectively.

**Saleh Abd El Rasoul *et al.*,[12]** studied the propafenone HCl dissolving tablets containing subliming agent prepared by direct compression method. The prepared tablets were undergone physical characterization, in vitro dissolution and stability studies. It was successfully applied for physical and chemical properties with rapid disintegration in the oral cavity, rapid onset of action. The stability and storage condition were 25 and 40 °C for 3 months.

**Ji-Chun Qu *et al.*,[13]** performed a colorimetric method of propafenone by silver nanoprobe. In this method based on unique optical properties of silver nanoparticles (AgNPs). The propafenone induced the citrate to be desorbed from the surface of AgNPs, leading to aggregates the AgNPs, and by surface plasma absorption band shift and their results are yellow to red even purple color change. The concentration of propafenone can be determined by UV-Visible spectrophotometer. The limit of detection was carried out at 24 µM.

**Rafa Pietra *et al.*,[14]** studied the stability indicating of HPTLC method of propafenone HCl in tablets and GC-MS identification of its degradation products. In HPTLC separation method was carried out on silica $G_{60}F_{254}$. The mobile used as a mixture of chloroform: methanol: acetic acid (7:9.2:0.1 v/v/v). Linearity range was $r^2$ = 0.9987 and its degradation product was absorbed at 316 nm. LOD and LOQ were 0.02 and 0.08l ng/spot. The GC-MS technique was applied for identification of the degradants. The developed method has been validated as per ICH guidelines like linearity, accuracy (99.24 %), precision and specificity.

**Minoo Afshar *et al.*,[15]** developed and validated the capillary electrophoresis assay for the simultaneous enantioselective the propafenone and its major metabolites. It is based on liquid-liquid extraction at alkaline pH and CE in presence of pH 2.0 running buffer was 100 mM sodium phosphate, 19 % methanol, and 0.6 % highly sulfated β-CD. The run time of S-enantiomers are 30 min. The linearity range between 25 and 1000 ng/mL and LOD levels are 10 and 12 ng/mL. The enantiomers and its metabolites in invitro incubations comprising human liver microsomes. CE data can be evaluated for the enzymatic N-dealkylation and hydroxylation rates.

**Minoo Afshar *et al.*,[16]** developed and validated a simple and rapid high-performance chromatography method for the determination of propafenone HCl and its metabolites in human serum. Mobile phase was photoresist-phosphate buffer gradient at a flow rate of 1.7 ml $min^{-1}$ and UV-spectroscopy detected at 210 nm. The calibration curves were $r^2$ >0.999. The % RSD concentration range were 1.0-8.1 % and 4.2-11.5 % respectively.

**Zhiyan chi *et al.*,[17]** developed and validated the LC-MS-MS method of propafenone and its active metabolite 5- hydroxy propafenone (5-OHP) in human plasma. chromatographic separation of mobile phase was methanol, 5 mM ammonium acetate solution containing 0.2 % formic acid (68:32 v/v). The analytes were achieved by multiple reaction under positive

ionization mode. This method was applied to pharmacokinetic studied under propafenone and 5-OHP in healthy Chinese volunteers.

**Heitor Oliveira de Almeida Leite *et al.,*[18]** determined the stability indicating studies applied in the separation and characterization of the main degradation product of propafenone. The developed of HPLC method with diode array detection and liquid chromatography with mass spectrometry. This method was access to susceptibility of propafenone hydrochloride in stress condition. The oxidative degradation product was characterized by using liquid chromatography with mass spectrometry. Propafenone HCl was found to be highly oxidative and thermal degradation.

**Dhandapani *et al.,*[19]** performed the UV-spectroscopy method of propafenone hydrochloride in tablets. In extractive spectrophotometric, propafenone estimated at 301 nm using ethanol as a solvent. The correlation coefficient was 0.9819. In extractive spectrophotometric estimate by using methylene blue and phosphate buffer as an ion pair complexing agent and chloroform as a solvent, detected at 485 nm. The correlation coefficient range was 0.9769. In diphenhydramine citrate solution used to dissolve propafenone and detected at 301 nm, the correlation coefficient range was 0.9985.

**Adinarayana Andy *et al.,*[20]** developed and validated method of UHPLC-/MS/MS has been quantification of propafenone, 5-hydroxypropafenone and N-depropylpropafenone on human dried blood spot (DBS). The mobile phase was acetonitrile and formic acid. Total run time of analysis was 1.1 min. the linearity range of correlation coefficient of >0.99 for all drugs. The pharmacokinetic parameters AUC-∞ and $C_{max}$ were 6057 ± 1526, 2002 ± 515 and 525 ± 202 ng/mL and 653 ± 183, 295 ± 37.5 and 68.4 ± 13.6 ng/mL for PF, 5-OHPF and N-DPF.

**Madina Alikhodjaeva *et al.,*[21]** analyzed the propafenone hydrochloride in blood samples via HPLC method. TLC, HPLC, UV and Spectrophotometric methodology for

identification and quantitative determination of propafenone was performed. These methods were tested by studying the blood of volunteers who received therapeutic concentration (150 mg) of tablets. In TLC the chromatographic system was chloroform, ethyl acetate and ethanol (10:3:1 v/v/v) and its $R_f$ value = 0.42. In UV the maximum absorption at 248 and 304 nm. In HPLC the therapeutic concentration of blood (0.46 - 0.88 µg/L in a single 150 mg).

**Xiaoli Hu *et al.*,**[22] found the propafenone HCl by flow injection analysis with resonance light scattering detection (RLS). This method was based on the ion-association reaction of 12-tungstophosphoric acid (TP) with propafenone. The maximum scattering peak was located at 340 nm and the detection limit was 1.0 ng/mL. RSD of 2.1 % successive determination of 2.0 µg/ml. This method was successfully applied for serum samples and pharmaceutical samples.

**Sagar Suman Panda *et al.*,**[23] developed and validated a simple method of RP-UFLC method for estimation of propafenone hydrochloride. Separation mixture was carried on an enable $C_{18}G$ column using methanol: 10 mM TBAHS (95:05 V/V) as mobile phase and its absorbed wavelength was 247 nm. The retention time of propafenone was 2.692 min. The LOD and LOQ were 4.5 µg/ml and 9.75 µg/ml. %RSD values were found to be <2 %. Specificity was established after forced degradation was performed by using HCl, NaOH, $H_2O_2$, thermal and UV radiation.

**Monika Jadhav *et al.*,** [24] developed and validated the analytical method estimation of propafenone hydrochloride in tablet dosage form by using QbD approach. In HPTLC method include solvent methanol, precoated Aluminium backed TLC plate (10 cm x 10 cm), wavelength: 250 nm, saturation time: 20 min, mobile phase is methanol: ethyl acetate: triethylamine (1.5:3.5:0.4 v/v/v). In zero order spectrophotometric method were solvent - methanol, sample preparation tablet, wavelength: 247.4 nm, and for first order derivative spectrophotometric method it was scaling factor: 5 and delta lambda 4.

**Leticia Teixeira *et al.*,**[25] determined the developed and validated the analytical methods for pharmaceutical equivalence evaluation of propafenone HCl in coated tablets. In the tests parameters were average weight, hardness and disintegration test and no official method for assay and dissolution test using UV-spectrophotometry and to develop the specificity, linearity, precise and accuracy. The drug content was within a range of 90 to 110%.

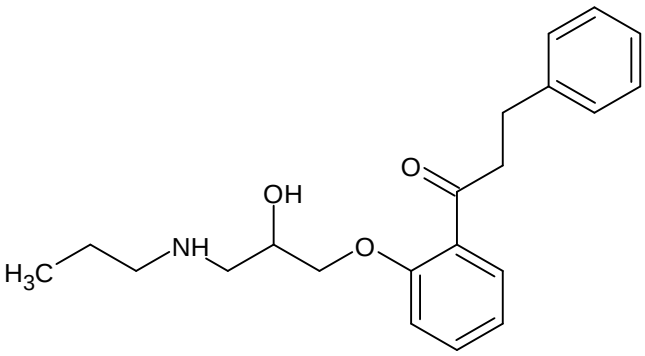
**Muggu Murali Krishna *et al.*,**[26] developed and validated a simple, rapid reverse phase high performance liquid chromatography method was determination of propafenone HCl in pure drug and pharmaceutical dosage form. The separation mixture was carried out Inertsil ODS-3Vs column and acetonitrile: methanol: water (80:20 v/v) as a mobile phase at a flow rate of 12 ml/min. The UV detection at 235 nm. The retention time was 3.33 min. the linearity ranges were $r^2 = 0.9997$. The %RSD was found to be 2 %. LOD and LOQ was 4.74 µg/ml and 12.04 µg/ml, respectively.

# DRUG PROFILE

# 3. DRUG PROFILE[27-29]

**PROPAFENONE HYDROCHLORIDE**

**Structure**




| | |
|---|---|
| **IUPAC Name** | **:**1- [2- [2- hydroxy-3-(propylamine) propoxy] phenyl]-3 phenylpropane -1- one hydrochloride |
| **Molecular formula** | **:** $C_{21}H_{27}NO_3HCl$ |
| **CAS Registry No** | **:** 34183-22-7 |
| **Molecular weight** | **:** 377.909 g/mol |
| **Category** | **:** Anti-arrhythmias |
| **Physical description** | **:** White crystalline power |
| **Solubility** | **:** Soluble in Dimethyl sulphoxide (DMSO), Methanol, Ethanol, Chloroform, slightly soluble in water |
| **Melting point** | **:** 171-174 ℃ |
| **Boiling point** | **:** 516.9°C |
| **Adverse effects** | **:** Difficulty breathing, constipation, blood dyscrasias, wheezing, chest pain, skin rash, unexplained fever, chills, weakness or sore throat |
| **Half-life** | **:** 2-10 hrs. |

| | |
|---|---|
| **Indication** | **:** Congestive heart failure, cardiogenic shock, sinoatrial, atrioventricular and intraventricular disorders of impulse conduction |
| **Mechanism of action** | **:** Propafenone works by slowing the influx of sodium ions into the 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 class 1a or 1b anti-arrhythmic medications |
| **Pharmaceutical formulation** | **:** Tablet, Capsules |
| **Label claim** | **:** 150 mg, 225 mg, 300 mg |
| **Brand Name** | **:** Rythmol, Pradil |
| **Storage condition** | **:** 2-8°C |

# AIM AND OBJECTIVE OF THE WORK

# 4. AIM AND OBJECTIVE OF WORK

The aim of this study is to develop and validate the "UV-Multivariant calibration technique and HPTLC method" for the estimation of propafenone hydrochloride in its tablet dosage form. The developed method is to be validate as per ICH guidelines.

The method development is to obtain a good separation and quantification with minimum time and effort. Based on the goal of separation and absorption, the method development is proceeded.

So, the major objectives of the present study are:

- ❖ To altering the chromatographic condition as compared with previously reported method in literature.
- ❖ To carry out the multivariant technique by using UV-spectrophotometric methods.
- ❖ To develop and validate the multivariant technique and HPTLC method for the estimation of propafenone hydrochloride in its tablet dosage form.

The following research work steps are given below:

**SPECTROPHOTOMETRIC METHOD**

The research work was performed on the following steps,

1. To check the solubility of the compounds.
2. To check the absorption maxima ($\lambda_{max}$) for the propafenone hydrochloride.
3. To check the multivariant technique of different absorbed wavelengths at 243, 245, 247, 249 and 251 nm.
4. To validate the method according to the standard parameters according to the ICH guidelines like linearity, precision, accuracy and specificity etc.,
5. To quantify the amount of drug present in the formulation.

## HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

The research work was performed based on the following steps,

1. To check the solubility of compounds.
2. To optimize the chromatographic conditions like mobile phase composition, saturation time, migration distance, wavelength.
3. To validate the developed method according to the standard parameters like linearity, precision, accuracy, specificity etc.
4. To identify the amount of drug present in the formulation.

# MATERIALS AND INSTRUMENTS USED

# 5. MATERIALS AND INSTRUMENTS USED

**Table.2. Materials**

| S. NO | CHEMICALS / REAGENT NAME | MANUFACTURER | GRADE |
|---|---|---|---|
| 1. | Methanol | S.D Fine-chem Ltd, (Mumbai) | AR |
| 2. | Chloroform | E. Merck (India) Ltd, (Mumbai) | AR |
| 3. | Ethanol | S.D Fine-chem Ltd, (Mumbai) | AR |
| 4. | Acetic acid | Loba chemie Pvt Ltd, (Mumbai) | AR |

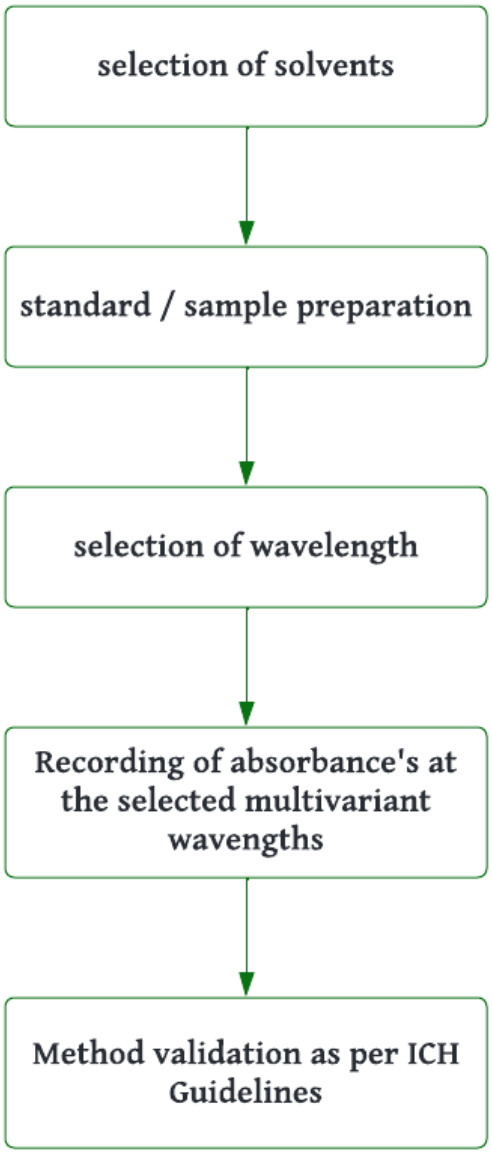
**Table.3. Instruments**

| S.NO | INSTRUMENTS NAME | MODEL | MANUFACTURER | SOFTWARE |
|---|---|---|---|---|
| 1. | UV-spectrophotometer | UV 1800 | Shimadzu (Japan) | UV probe |
| 2. | Digital balance | BL 220 H | Shimadzu (Japan) | - |
| 3. | Ultra Sonicator | 3.5 L 100 | Pci (Mumbai, India) | - |
| 4. | HPTLC | ➢ Semi applicator<br>➢ TLC scanner-3<br>➢ Twin through chamber | Camag | Wincat |

# EXPERIMENTAL WORK

# 6. EXPERIMENTAL WORK

## METHOD A: DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PROPAFENONE HYDROCHLORIDE



**Selection of solvents:**

The solvents were used for propafenone hydrochloride are Chloroform, 0.1N NaOH, Ethanol, Acetonitrile, Methanol, 1% Acetic acid, Dimethyl sulphoxide (DMSO), and Dimethyl formamide (DMF). The ethanol was selected as the solvent for propafenone hydrochloride because the spectrum was clearly observed and it is coming under the category of green solvent.

**Determination of $\lambda_{max}$:**

Dilute the stock solution with ethanol and measure the absorbance at the UV region from 200 – 400 nm. The $\lambda_{max}$ was obtained as 247 nm.

**Preparation of standard stock solution:**

The stock solution of propafenone hydrochloride was prepared by diluting 100 mg of reference drug into 100 mL of ethanol. Different concentrations of 4 to 20 µg/mL solutions of propafenone were prepared from the standard stock solution.

**Preparation of sample solution:**

Weighed and powdered 20 tablets of propafenone hydrochloride and the weight is equivalent to 10 mg was measured and diluted in 10 mL of ethanol to get 1 mg/mL solution. This solution was diluted and used for further analysis.

**Method validation:**

The validation parameters were performed in compliance with the ICH Q2B recommended conditions. The parameters are carried out by linearity, accuracy, precision and LOD & LOQ.

**Linearity**

The different concentrations of 4 to 20 µg/mL were prepared from the standard stock solution of propafenone hydrochloride. These solutions were scanned over a range of wavelength surrounding its absorbance maxima 243, 245, 247, 249, 251 nm. The absorbance

was recorded, and the standard graphs were obtained by plotting the concentration vs absorbance. The slope and intercept values were recorded with respect to their wavelength.

**Precision**

To assess the intraday and interday precision, 12 µg/mL solution was analyzed six times in a short interval of time in one day for intraday precision and on six separate days for inter day precision.

**Accuracy**

The suggested technique's recovery study was resolved by the standard addition method at 80 %, 100 % and 120 %. The stock solution of standard and the sample was prepared. Pipette out 0.5 mL of the standard into a three standard flask and add the sample 0.3, 0.5, 0.7 mL into above volumetric flasks and makeup to the volume of 10 mL with ethanol. The solution was measured under a UV spectrometer, and the % recovery was interpretated.

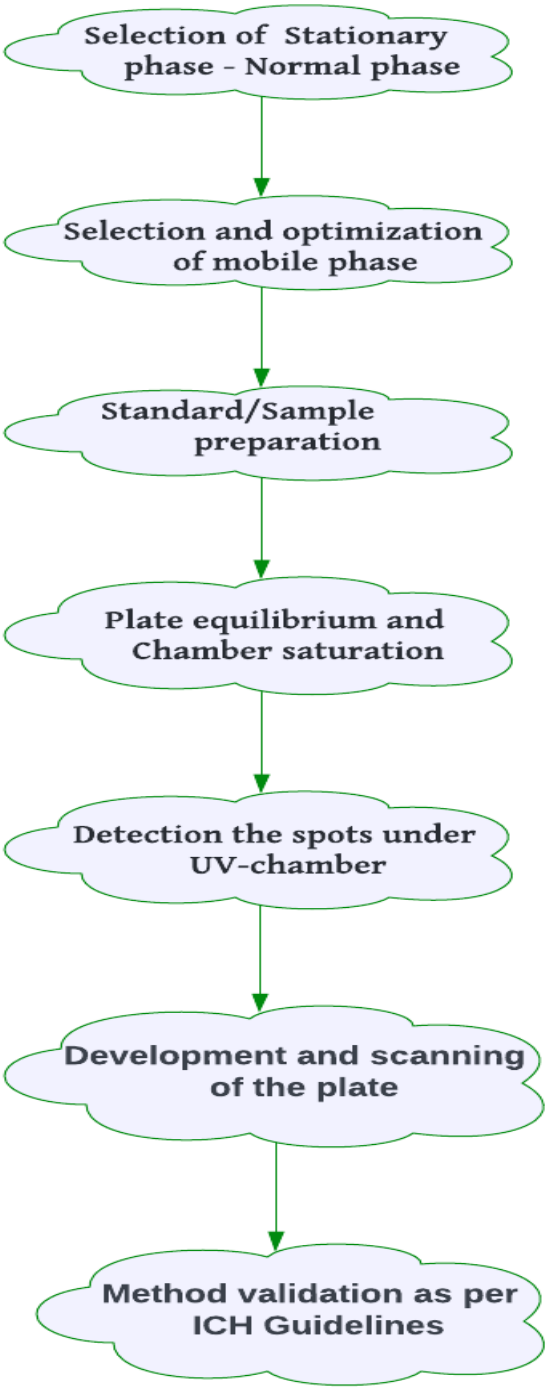
**LOD & LOQ**

Detection limit & quantification limit values were interpreted using the linear regression line's slope and intercept values. The sensitivity of the developed method was determined.

**Assay**

The drug content present in the formulation was analyzed and determined by statistical method.

**METHOD B: DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE DETERMINATION OF PROPAFENONE HYDROCHLORIDE**



**Selection of mobile phase by TLC method:**

The mobile phase selection was tried in different solvents and ratio by using Thin Layer Chromatography. The observed details are shown in Table 4. The trial 1,2,4 produce a tailing effect and finally, the trial 3 was chosen as a mobile phase solvent.

**Table.4. Selection of solvent system by TLC method:**

| | **Trial: 1** | **Trial: 2** | **Trial:3** | **Trial: 4** |
|---|---|---|---|---|
| **Solvent system** | Toluene: methanol (8.5:1.5 v/v) | Chloroform: methanol (9:1 v/v) | Chloroform: methanol: acetic acid (8:1.5:0.5 v/v/v) | Methanol: ethyl acetate: ammonia (1.8:5:0.5 v/v/v) |
| **Visualizing agent** | Iodine vapor | Iodine vapor | Iodine vapor | Iodine vapor |
| **$R_f$ value** | 0.113 | 0.176 | 0.68 | 0.744 |
| **TLC plate** |  |  |  |  |

**Preparation of stationary and mobile phase:**

Pre-coated silica gel $G_{60}F_{254}$ plate was selected as stationary phase and the mobile phase system consisting of chloroform: methanol: acetic acid (8:1.5:0.5 v/v/v) was used for the separation of propafenone hydrochloride.

**Preparation of standard stock solution:**

Standard stock solution containing of propafenone was prepared by dissolving 10 mg in 10 mL volumetric flask with methanol.

**Preparation of working standard solution:**

Working standard solution of propafenone from stock solution was applied on pre-coated TLC plate to get concentration ranging from 200 - 1000 ng/spot. The plate was developed in a developing chamber previously it was saturated with the mobile phase for 30 min. After development, the plate was air dried and standard zones were quantified by scanning at 251 nm. The calibration curves were constructed by plotting peak areas versus concentration for each drug.

**Preparation of sample solution:**

Twenty tablets containing 150 mg of propafenone was weighed and their average weight was calculated. An amount of powder equivalent to 100 mg of propafenone was extracted with 100 ml of methanol. It was then filtered and 1 ml of filtrate solution was diluted to 10 mL with methanol. A volume of 1.5 µL was spotted and a peak area was evaluated to calculate the propafenone contents in tablets.

**Chromatographic conditions:**

**HPTLC chromatographic conditions are summarized below:**

| | |
|---|---|
| Stationary phase | : Silica gel $G_{60}F_{254}$ |
| Mobile phase | : Chloroform: Methanol: Acetic acid (8:1.5:0.5 v/v/v) |
| Chamber saturation time | : 30 mins |
| Plate saturation | : 10 mins |
| Developing distance of the plate | : 80 mm |
| Bandwidth | : 5 mm |
| Slit dimension | : 5.00 x 0.45 mm |
| Scanning speed | : 20 mm/s |
| Detection of wavelength | : 251 nm |
| Number of tracks | : 10 |
| $R_f$ value | : 0.72 |

**Method validation:**

The developed methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures in terms of parameters like linearity, precision, accuracy, detection and quantification limits, specificity and robustness.

# RESULTS AND DISCUSSION

# 7. RESULTS AND DISCUSSION

## METHOD A: DEVELOPMENT AND VALIDATION OF PROPAFENONE HYDROCHLORIDE BY UV-MULTIVARIANT CALIBRATION TECHNIQUE

### Determination of $\lambda_{max}$ of propafenone hydrochloride

The absorption maxima ($\lambda_{max}$) of propafenone hydrochloride were observed at 247 nm by using ethanol as a solvent and is shown in Figure.1

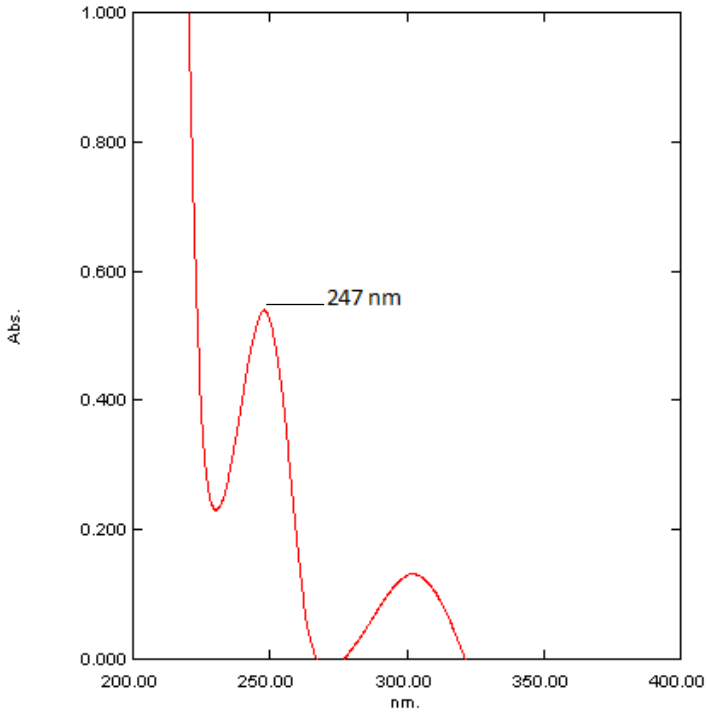



**Figure.1. $\lambda_{max}$ of propafenone hydrochloride**

### Linearity

The linearity was recorded at 243, 245, 247, 249 and 251 nm in the concentration range 4 to 20 µg/mL. The slope and intercept values of standard deviation showed the technique was precise. The $R^2$ values were found to be 0.9997 as presented in Table.5.

The linearity spectrum of propafenone was recorded at 243 nm. The calibration curve is showed in Figure.2 and their residual plot are showed in Figure.3. The overlay spectrum of different concentration at 243 nm was presented at Figure.4.

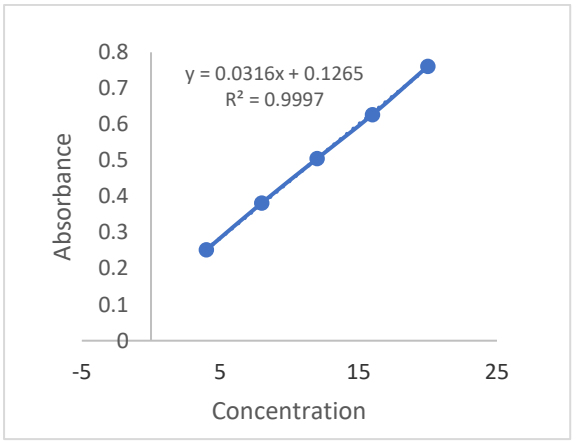



**Figure.2. Calibration curve at 243 nm**

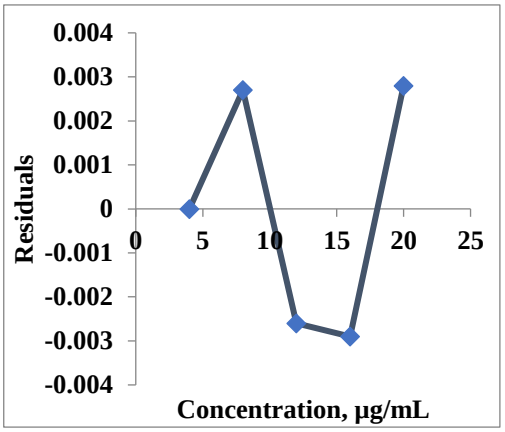



**Figure.3. Residual plot at 243 nm**

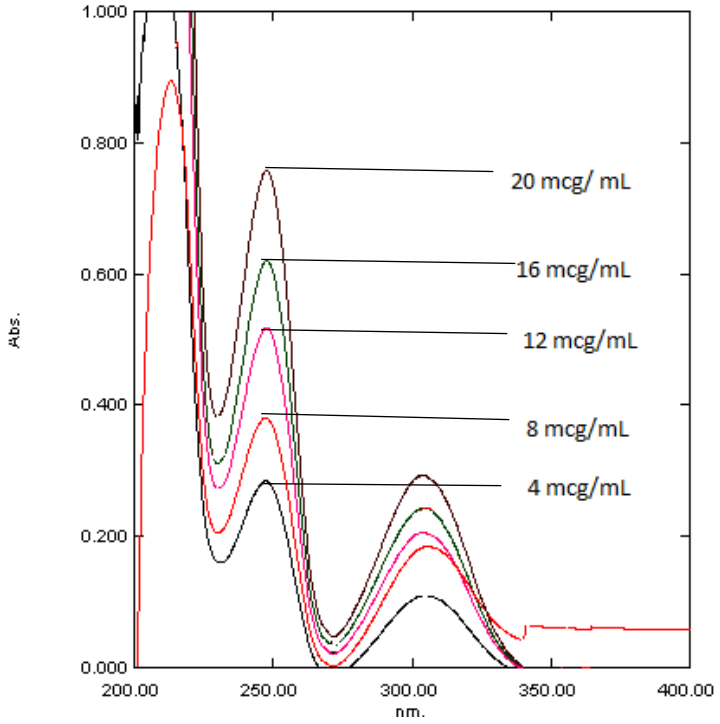



**Figure.4 Overlay spectrum at 243 nm**

The linearity observation of the propafenone was scanned at 245 nm. The calibration graphs were showed at Figure.5 and their residual plot are given in Figure.6. The overlay spectrum of different concentration at 245 nm was presented at Figure.7.

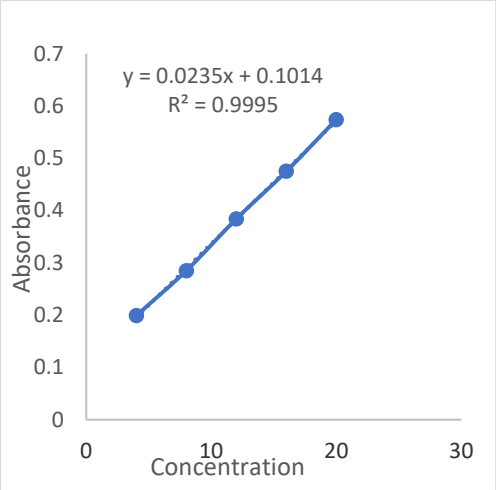



**Figure.5. Calibration curve at 245 nm**

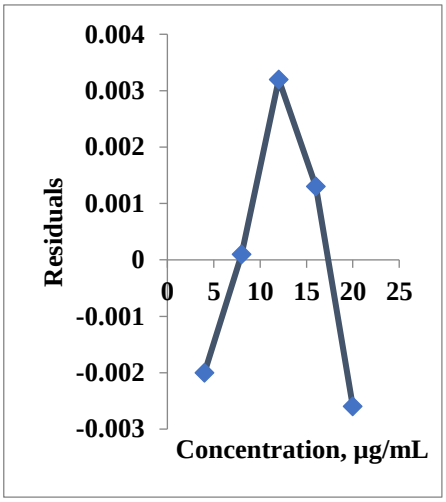



**Figure.6. Residual plot at 245 nm**

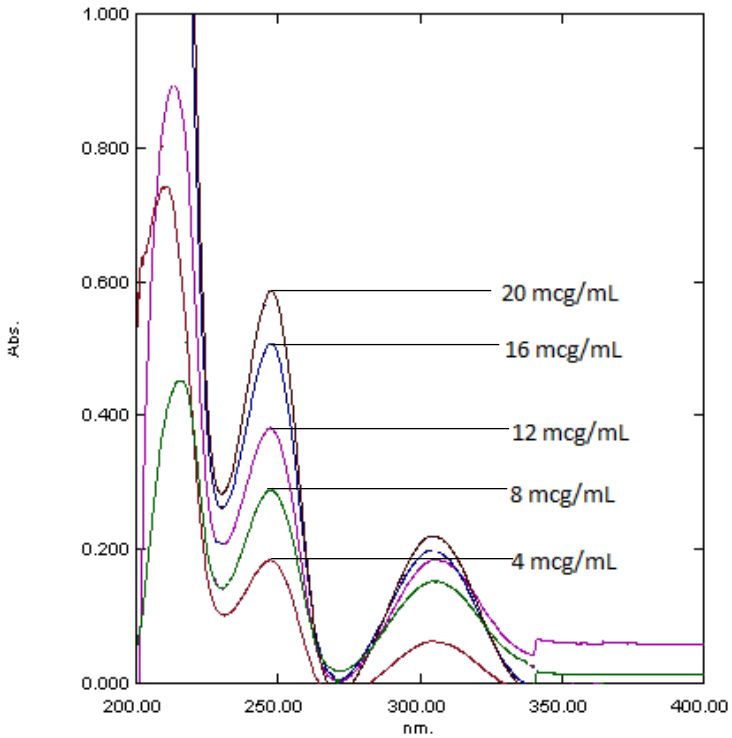
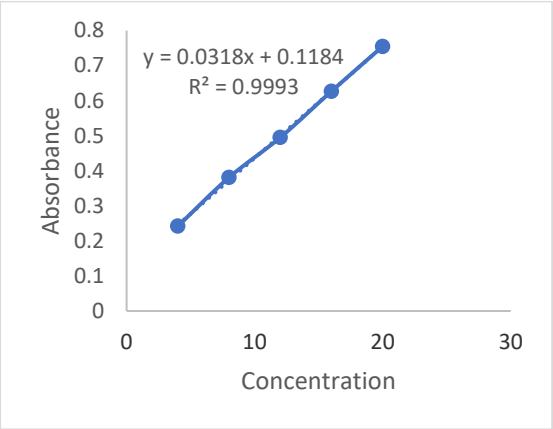



**Figure.7. Overlay spectrum at 245 nm**

The linearity observation of different concentration was measured at 247 nm. The calibration curve was showed in Figure.8 and their residual plot are showed in Figure.9. Finally overlay spectrum was presented at Figure.10.



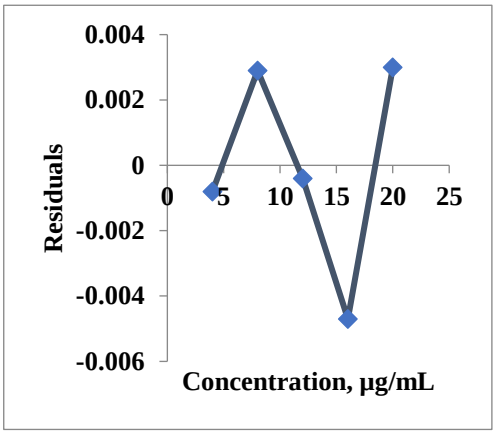




**Figure.8. Calibration curve at 247 nm**

**Figure.9. Residual plot at 247 nm**

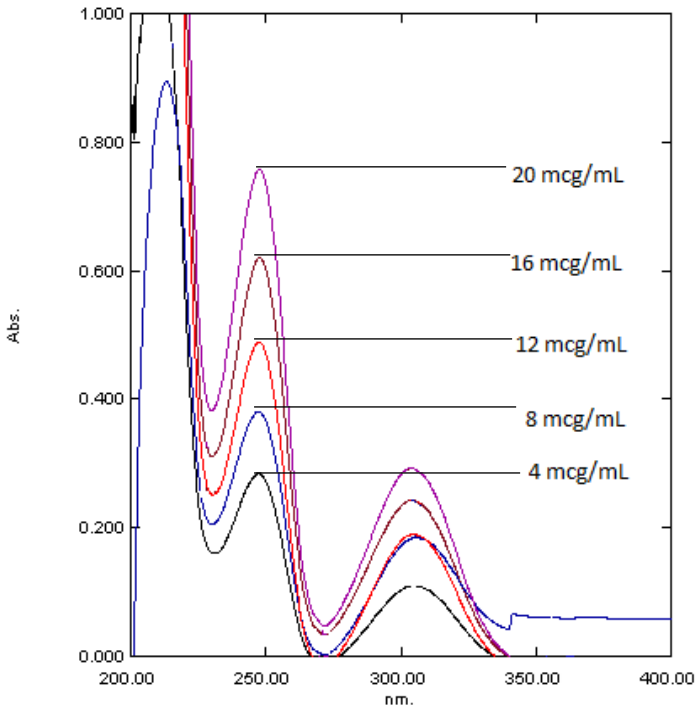



**Figure.10. Overlay spectrum at 247 nm**

The linearity calibration curves were observed at different concentration range over 249 nm. The correlation coefficient was plotted in Figure.11 and their residual plot was showed in Figure.12. The overlay spectrum was presented at Figure.13.

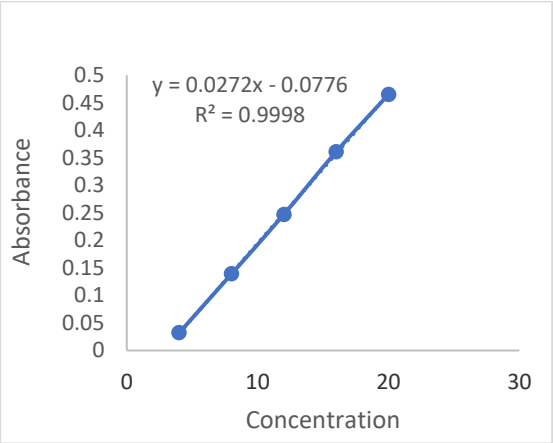



**Figure.11. Calibration curve at 249 nm**

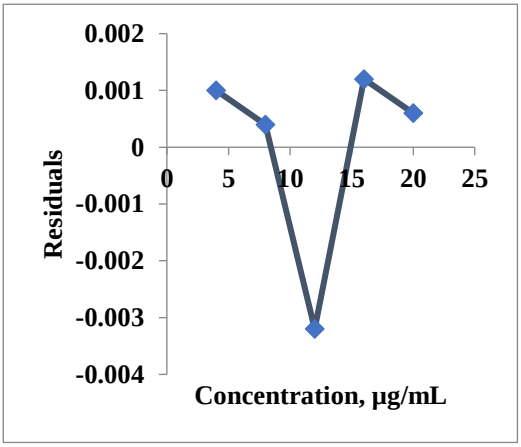



**Figure.12. Residual plot at 249 nm**

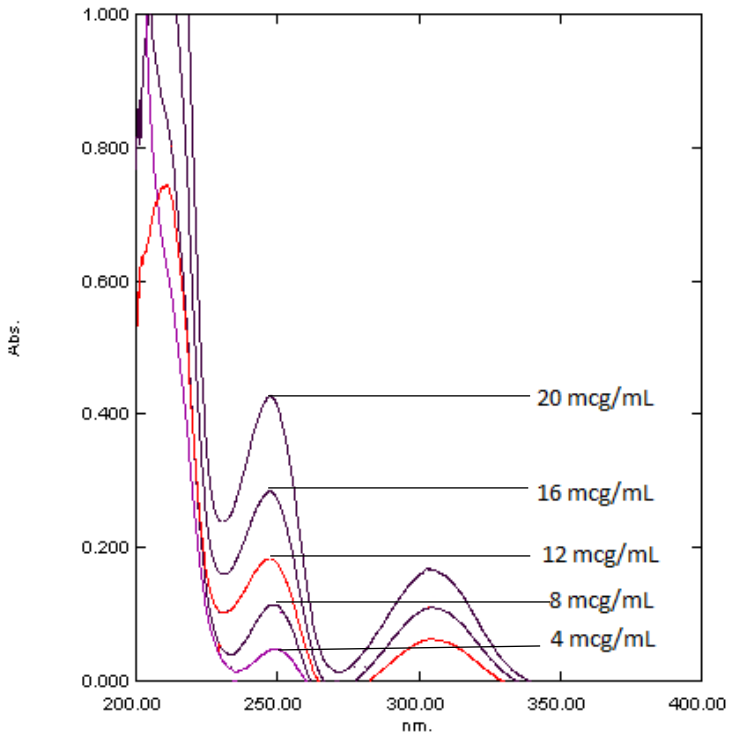



**Figure.13. Overlay spectrum at 249 nm**

The linearity of the propafenone was recorded at 251 nm. The calibration curves were showed in Figure.14 and their plot are showed in Figure.15. Finally overlay spectrum of different concentration were observed and presented in Figure.16.

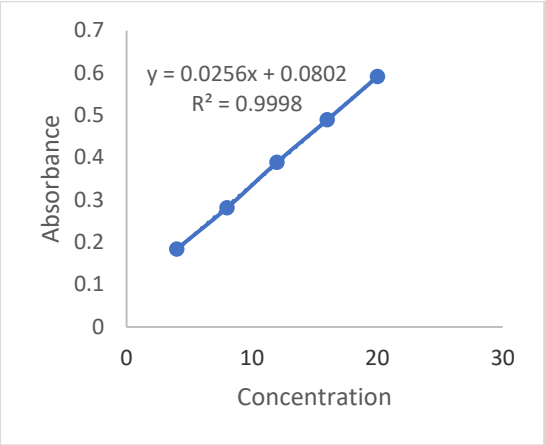



**Figure.14. Calibration curve at 251 nm**

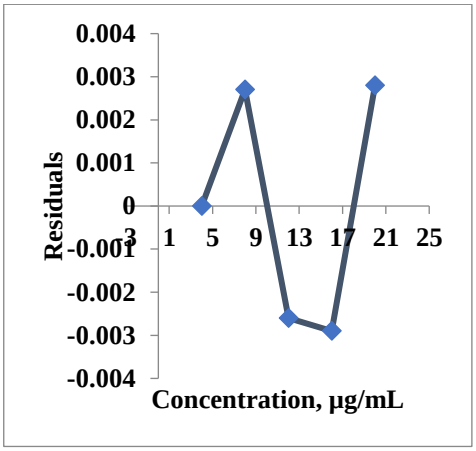



**Figure.15. Residual plot at 251 nm**

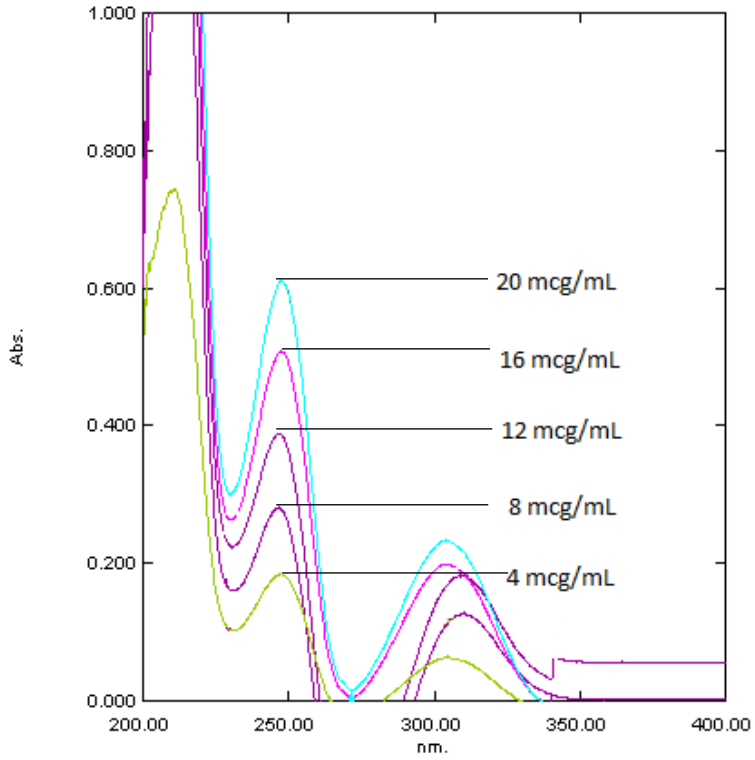



**Figure.16. Overlay spectrum at 251 nm**

**Table.5. Multivariant UV-calibration at five selected wavelengths**

| Concentration (µg/mL) | Absorbance (nm) | | | | |
|---|---|---|---|---|---|
| | 243 nm | 245 nm | 247 nm | 249 nm | 251 nm |
| **4** | 0.225 | 0.199 | 0.242 | 0.032 | 0.184 |
| **8** | 0.382 | 0.285 | 0.381 | 0.139 | 0.281 |
| **12** | 0.505 | 0.384 | 0.495 | 0.247 | 0.389 |
| **16** | 0.627 | 0.475 | 0.627 | 0.361 | 0.489 |
| **20** | 0.761 | 0.574 | 0.755 | 0.465 | 0.591 |

**Table.6. Linearity data showing statistical data at the selected wavelength**

| Wavelength (nm) | Regression equation | $R^2$ | LOD (µg/mL) | LOQ (µg/mL) | % RSD |
|---|---|---|---|---|---|
| **243** | Y=0.0316x+0.1265 | 0.9997 | 0.383 | 1.159 | 0.752 |
| **245** | Y=0.0235x+0.1014 | 0.9995 | 0.545 | 1.652 | 1.012 |
| **247** | Y=0.0318x+0.1184 | 0.9993 | 0.615 | 1.866 | 1.186 |
| **249** | Y=0.0272x+0.0776 | 0.9998 | 0.300 | 0.910 | 0.995 |
| **251** | Y=0.0256x+0.0802 | 0.9998 | 0.336 | 1.018 | 0.674 |

**Precision:**

The lower value of standard deviation shows that the technique was specific and percentage relative standard deviation for the intraday and interday precision obtained were in the range of 0.3811 & 0.3864 and showed in Table.7 & 8. It lies within the prescribed limit of less than 2 % at each wavelength. The low value of percentage relative standard deviation reveals the suggested techniques was accurate and precise.

**Table.7. Data for intraday precision**

| Concentration | Absorbance (nm) | | | | | |
|---|---|---|---|---|---|---|
| **(µg/mL)** | **243** | **245** | **247** | **249** | **251** | |
| **4** | 0.367 | 0.382 | 0.384 | 0.385 | 0.371 | 1.889 |
| **8** | 0.374 | 0.385 | 0.381 | 0.389 | 0.379 | 1.908 |
| **12** | 0.378 | 0.387 | 0.387 | 0.381 | 0.373 | 1.906 |
| **16** | 0.371 | 0.388 | 0.380 | 0.387 | 0.381 | 1.907 |
| **20** | 0.381 | 0.381 | 0.386 | 0.391 | 0.381 | 1.918 |
| | | | | | **Average** | 1.905 |
| | | | | | **SD** | 0.011 |
| | | | | | **% RSD** | 0.538 |

**Table.8. Data for interday precision**

| Concentration | Absorbance (nm) | | | | | |
|---|---|---|---|---|---|---|
| **(µg/mL)** | **243** | **245** | **247** | **249** | **251** | |
| **4** | 0.367 | 0.394 | 0.397 | 0.398 | 0.371 | 1.927 |
| **8** | 0.378 | 0.397 | 0.390 | 0.399 | 0.361 | 1.925 |
| **12** | 0.371 | 0.399 | 0.401 | 0.385 | 0.373 | 1.929 |
| **16** | 0.379 | 0.385 | 0.394 | 0.391 | 0.391 | 1.940 |
| **20** | 0.374 | 0.397 | 0.397 | 0.395 | 0.398 | 1.961 |
| | | | | | **Average** | 1.936 |
| | | | | | **SD** | 0.015 |
| | | | | | **% RSD** | 0.771 |

**Recovery studies:**

As per the ICH guidelines the mean % Recovery of the propafenone was found to be in the range of 99.83 % w/w, and it lies within limit of 97-103 % w/w. % Recovery data were

obtained in Table 9. The standard addition method of three different levels were tried 80 %, 100 % and 120 %.

**Table.9. Recovery studies of propafenone hydrochloride**

| Wavelength (nm) | Amount present (µg/mL) | Amount added (µg/mL) | Absorbance (nm) | Amount recovered (µg/mL) | % Recovery |
|---|---|---|---|---|---|
| 243 | 6 | 4.8 | 0.277 | 10.78 | 99.80 |
| | | 6 | 0.395 | 11.99 | 99.91 |
| | | 7.2 | 0.459 | 13.2 | 100.00 |
| 245 | 6 | 4.8 | 0.281 | 10.79 | 99.90 |
| | | 6 | 0.399 | 11.95 | 99.58 |
| | | 7.2 | 0.461 | 13.21 | 100.75 |
| 247 | 6 | 4.8 | 0.275 | 10.80 | 100.00 |
| | | 6 | 0.389 | 11.91 | 99.25 |
| | | 7.2 | 0.471 | 13.18 | 99.84 |
| 249 | 6 | 4.8 | 0.267 | 10.78 | 99.81 |
| | | 6 | 0.385 | 11.92 | 99.33 |
| | | 7.2 | 0.474 | 13.21 | 100.07 |
| 251 | 6 | 4.8 | 0.282 | 10.79 | 99.90 |
| | | 6 | 0.396 | 11.94 | 99.50 |
| | | 7.2 | 0.463 | 13.19 | 99.92 |

**LOD & LOQ:**

Detection and quantification limit values were calculated in Table.6. LOD & LOQ was found to be 0.435 µg/mL and 1.321 µg/mL respectively.

**Assay:**

The absorbance of the tablet formulation was recorded at 247 nm and it was showed in Figure.17. The percentage purity of propafenone were found to be 99.56 % and it is presented in Table 10. The % RSD value was found to be 0.3020.

**TABLE.10. Assay of propafenone hydrochloride**

| Brand Name | Label claim (%) | Amount estimated (mg) | % Assay |
|---|---|---|---|
| Pradil | 150 | 149.8 | 99.87 |
| | 150 | 149.8 | 99.53 |
| | 150 | 148.9 | 99.27 |
| **Average** | | 149.3 | 99.56 |
| | | **SD** | 0.300 |
| | | **% RSD** | 0.302 |

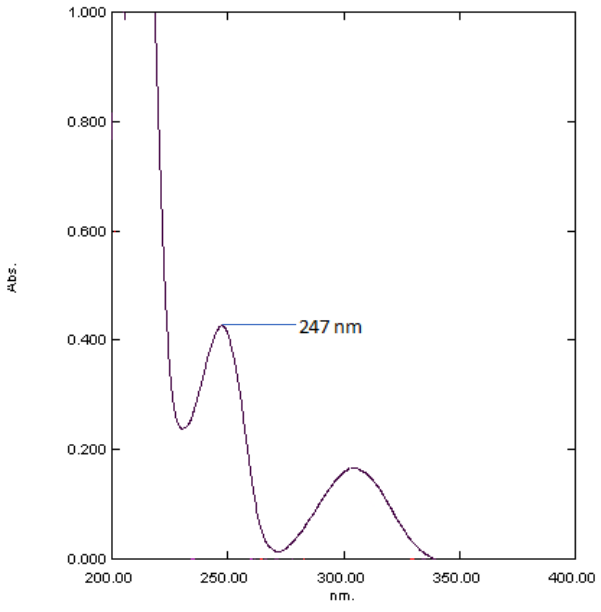



**Figure.17. Assay of propafenone in formulation**

## METHOD B: DEVELOPMENT AND VALIDATION OF PROPAFENONE HYDROCHLORIDE BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Experimental condition such as mobile phase composition and wavelength of detection were optimized to prove accurate, precise and reproducible results of propafenone hydrochloride determination. Good separation of the drugs ($R_f$ value of propafenone was 0.72) with good symmetrical peaks were obtained by using the mobile phase was Chloroform: Methanol: Acetic acid (8:1.5:0.5 v/v/v) and silica gel $G_{60}F_{254}$ plate, as a stationary phase. The spot of the drug was scanned at 251 nm.

### Chamber saturation time

The chamber saturation time was determined at different time intervals from 5 to 25 mins and the effect of chamber saturation time on the development pattern, peak shape and $R_f$ value was evaluated. It was found that saturation time of 10-15 min resulted in good resolution of components with good peak shapes. Hence, saturation time of 10 to 15 min was selected for the study.

### Plate equilibrium (pre-conditioning) time

The pre-conditioning time for the plate equilibrium was determined with different time intervals from 5-30 mins. In a twin trough chamber and peak area, $R_f$ value and peak shapes were calculated. The precision and reproducibility of peak area were good, after 10 min, time of plate saturation. The effect of plate equilibrium on the precision of peak area and $R_f$ are shown in Table.11.

**Table.11. Data showing a plate equilibrium time**

| Time (min) | % RSD | |
|---|---|---|
| | Peak area | $R_f$ |
| 5 | >2.10 | >2.20 |
| 10 | <1.0 | >1.20 |
| 15 | <1.60 | <1.9 |
| 20 | <1.9 | >2.0 |

**Composition of solvents in mobile phase**

The composition of solvents in mobile phase was determined on the basis of resolution of the drug, peak shape and $R_f$ value. It was found that increases in composition of chloroform or decrease in methanol concentration makes propafenone hydrochloride to move to the solvent front (no retention). The concentration of acetic acid determines the peak shape of the drug and in its absence, the peaks were highly asymmetric. Hence, several proportions of mobile phase were evaluated in order to achieve optimum separation of analyte. With the developed mobile phase, chloroform, methanol, acetic acid (8:1.5: 0.5 v/v/v) system, symmetrical peaks with good separation were obtained.

**Distance of solvent front**

The distance travelled by the solvent affects the Rf value of drugs. To optimized the solvent front, the mobile phase was allowed to run on the plates to various distances from 6.5 to 9.0. Table.12, shows the effect of distance travelled on the drug. The distance of solvent front was fixed as 8.5 cm.

**Table.12. Effect of solvent front**

| Solvent front (cm) | $R_f$ |
|---|---|
| 6.5 | 0.72 |
| 7.0 | 0.73 |
| 7.5 | 0.72 |
| 8.0 | 0.73 |
| 8.5 | 0.71 |
| 9.0 | 0.74 |

**Band width**

To determine the band width for the better $R_f$ and the peak area, the drug was spotted in various sizes (1 to 5 mm) of bandwidth. The chromatogram was developed and it was found that the best precision of $R_f$ and peak area was obtained at bandwidth 5 mm. The effect of bandwidth on precision is shown in Table 13.

**Table.13. Effect of bandwidth on precision**

| Drug | Peak area | %RSD | $R_f$ | %RSD |
|---|---|---|---|---|
| Propafenone hydrochloride | 200 | 0.87 | 0.72 | 0.92 |
| | 3396 | 0.11 | 0.73 | 0.96 |

**METHOD VALIDATION**

**Linearity**

To assess linearity, standard calibration curve for propafenone were constructed versus peak areas. The standard densitograms of propafenone are shown in Figures 19-23. The curve showed good linearity over the concentration range 200, 400, 600, 800, and 1000 ng/spot for

propafenone and its data were given in Table 14. The slope and intercept values, correlation coefficient was found to be 0.998 and showed in Figure 18.

**Table.14. Data for linearity**

| Concentration (ng/spot) | Peak area |
|---|---|
| 200 | 1676.6 |
| 400 | 2546.1 |
| 600 | 3491.8 |
| 800 | 4354.8 |
| 1000 | 5462.8 |

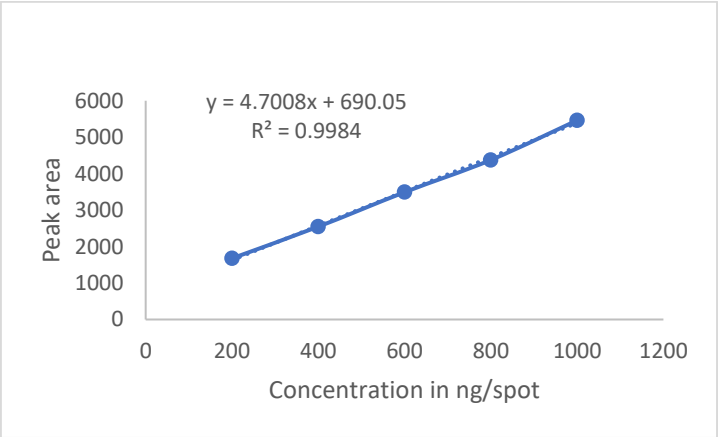



**Figure.18. Calibration curve of propafenone (200 – 1000 ng/spot)**

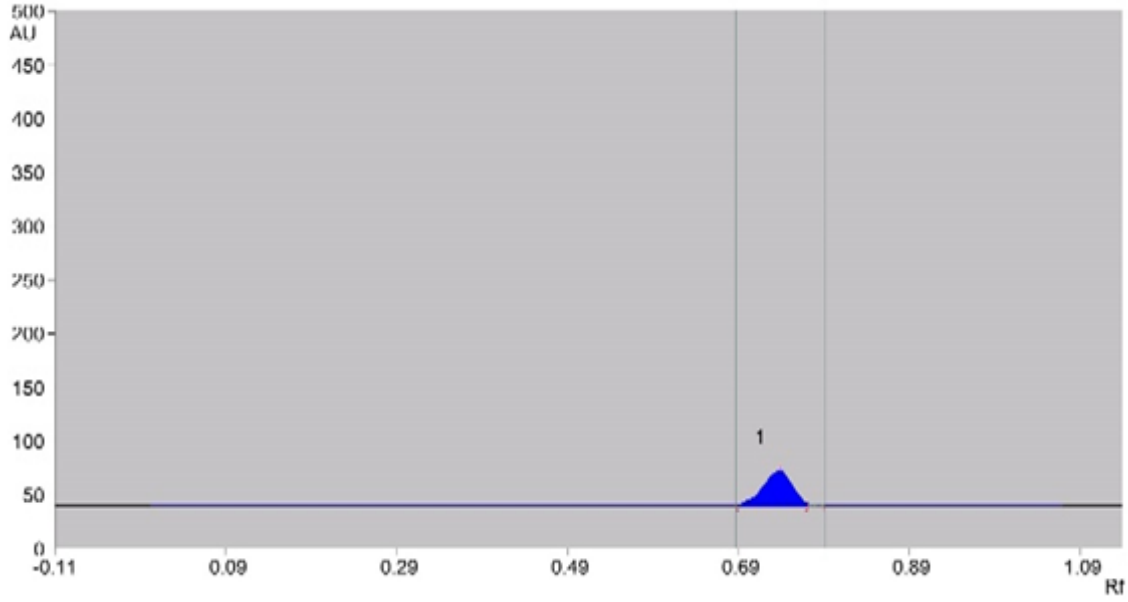



**Figure.19. Standard densitograms of propafenone (200 ng/spot)**

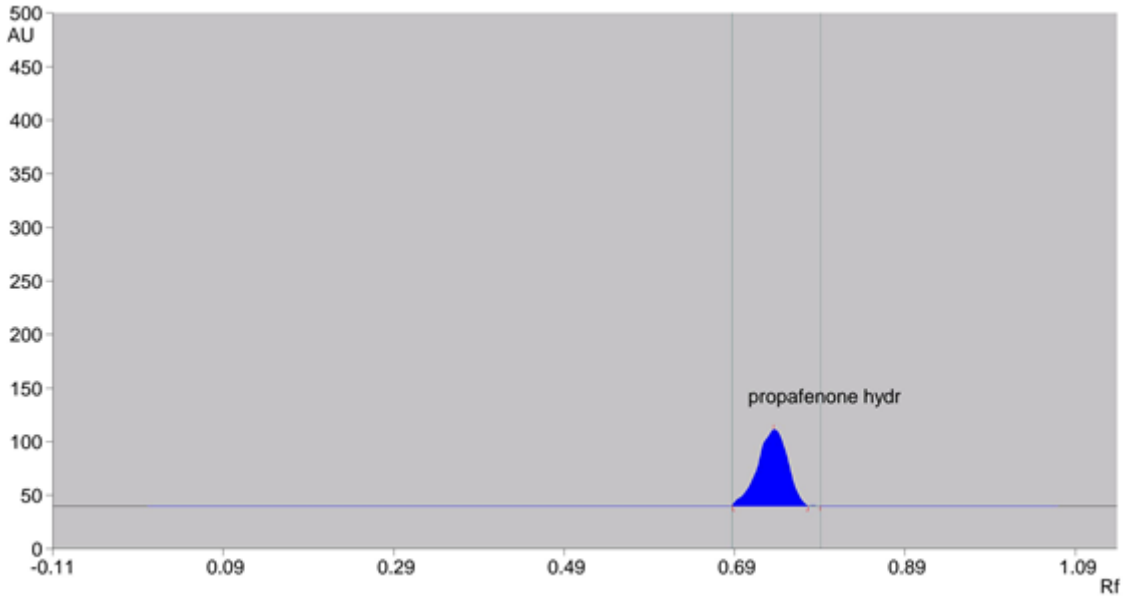



**Figure.20. Standard densitogram of propafenone (400 ng/spot)**

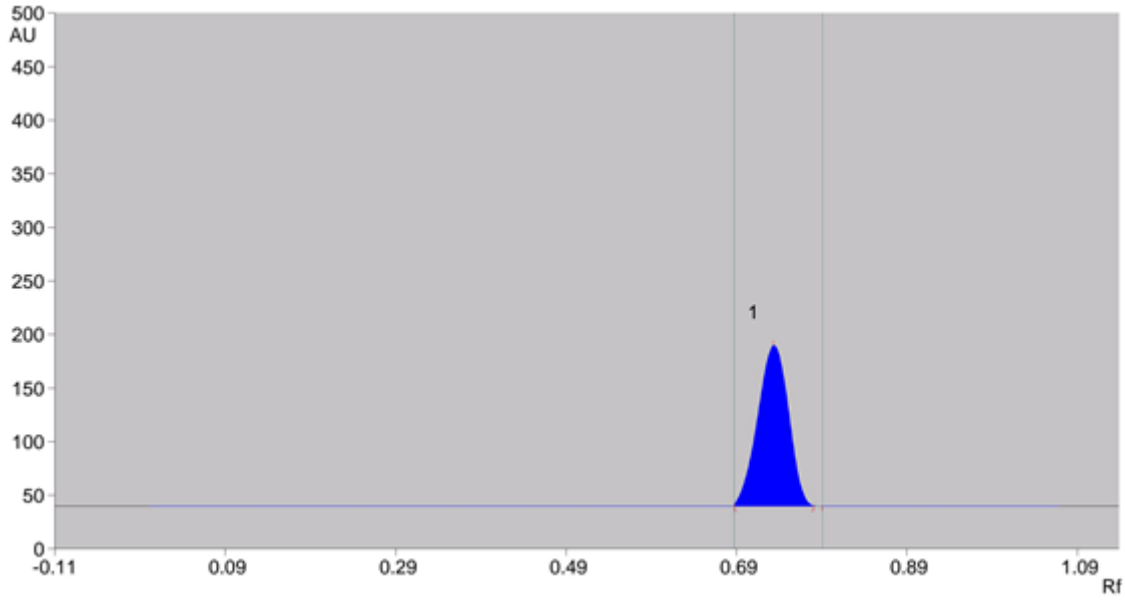



**Figure.21. Standard densitogram of propafenone (600 ng/spot)**

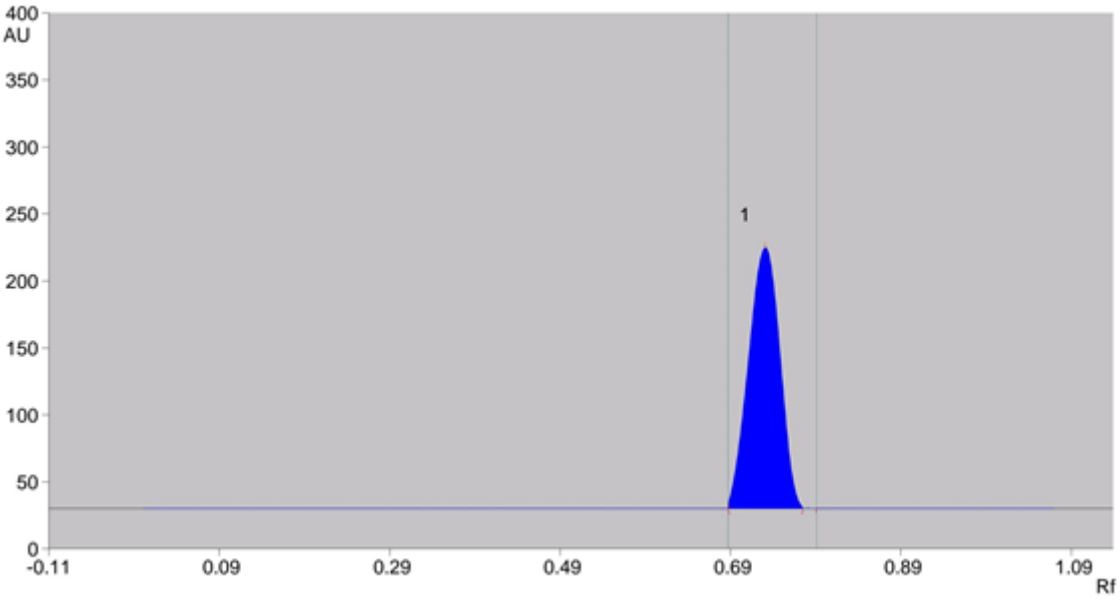



**Figure.22. Standard densitogram of propafenone (800 ng/spot)**

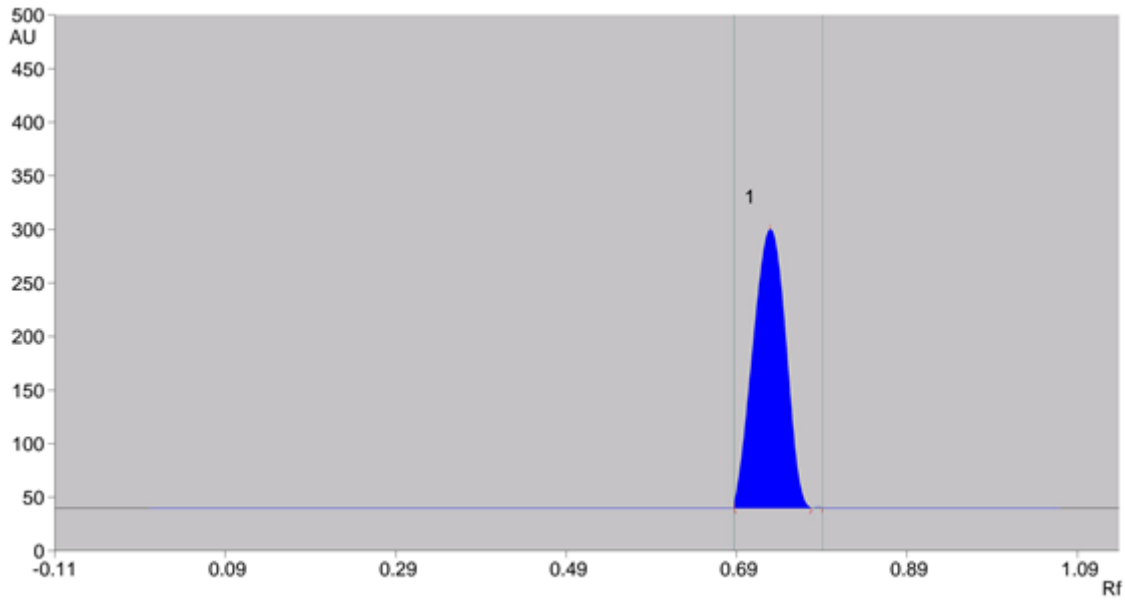



**Figure.23. Standard densitogram of propafenone (1000 ng/spot)**

**Precision**

The accuracy of the method was demonstrated by intermediate (inter-day) and repeatability (intra-day) precision and is expressed as the RSD of the results. The intra-day and inter-day precision studies were carried out at 3 concentration levels and determinations were made thrice at each concentration. Instrumental precision was studied, by repeatable application of same sample on TLC and the plate was measured six times without changing the position. The values obtained for precision studies indicate good repeatability and low inter-day variability. The precision of HPTLC method data is Table in 15.

**Table.15. Precision of HPTLC method for propafenone hydrochloride**

| Parameters | Intra day | Inter day |
|---|---|---|
| Mean peak | 3170 | 3381 |
| %RSD | 0.845 | 1.831 |

**Accuracy**

Accuracy of the HPTLC method was studied by standard addition technique. To the pre-analysed formulation, standard drugs were added at different levels at 80 %, 100 % and 120 %. The % recovery study results ranged from 99.83 % and showed in Table in 16, for propafenone showing the accuracy of the method.

**Table.16. Accuracy of HPTLC methods for propafenone**

| Level (%) | Amount added (mg) | Amount recovered (mg) | % Recovery |
|---|---|---|---|
| 80 | 8 | 7.97 | 99.62 |
| 100 | 10 | 9.98 | 99.8 |
| 120 | 12 | 12.01 | 100.08 |

**Limit of detection and limit of quantification**

The LOD and LOQ of drugs in HPTLC were taken from signal to noise ratio as 3:1 and 10:1, respectively. The LOD and LOQ values for propafenone were found to be 11 ng and 57 ng respectively and showed in Table in 17.

**Table.17. LOD and LOQ of drugs by HPTLC method**

| Drug | LOD (ng) | %RSD | LOQ (ng) | %RSD |
|---|---|---|---|---|
| Propafenone hydrochloride | 11 | 0.520 | 57 | 0.882 |

**Robustness**

Robustness studies of the developed method were studied by modifying the experimental condition at ± 2 min, equilibrium time (chamber saturation), ± 2 min. plate equilibrium time (plate saturation), ± 0.2 ml in mobile phase proportion, ± 0.2 cm solvent front and stability of solution. It was found that there was not much change in the Rf values, aera or symmetry of peaks. The % RSD was calculated and the values are shown in Table 18.

**Table.18. Robustness of HPTLC method for propafenone**

| Drug | Chamber saturation | | Plate saturation | | Mobile phase proportion | | Solvent front | |
|---|---|---|---|---|---|---|---|---|
| | Peak area | Rf | Peak area | Rf | Peak area | Rf | Peak area | Rf |
| Propafenone hydrochloride | 1355 | 0.74 | 2186 | 0.72 | 1479 | 0.71 | 2580 | 0.73 |

**Stability of drug**

The stability of the drug in solution and on the chromatoplate was studied by storing them on table top and inner refrigerator and checking the peak area at regular intervals. A 10% reduction in peak area from initial was taken into account to study the stability of drugs. The stability of drugs at room temperature is shown in Table 19. The drug stored under refrigeration were found to be stable for not less than 24 hr.

**Table.19. Stability data for propafenone**

| Drug | Drug | Peak area | |
|---|---|---|---|
| | | Initial | Final |
| In solution | Propafenone | 1576 | 1411 |
| On chromatoplate | Propafenone | 1245 | 1117 |

**System suitability parameters**

The HPTLC method was studied for its suitability for the analysis of formulation containing propafenone. The efficiency and asymmetric factor were calculated and are shown in Table 20.

**Table.20. System suitability of HPTLC method for propafenone**

| Drug | Efficiency (N) | %RSD | Asymmetric factor ($A_O$) | %RSD |
|---|---|---|---|---|
| Propafenone | 1856 | 0.856 | 0.72 | 0.522 |

**Application of the method**

The proposed methods were used for the assay of commercially available propafenone (150 mg) tablets. Six triplicate's determinations were performed on accurately weighed tablets. Experimental values obtained for determination of propafenone in samples is presented in Table 21. The densitograms of formulation is shown in Figure 24.

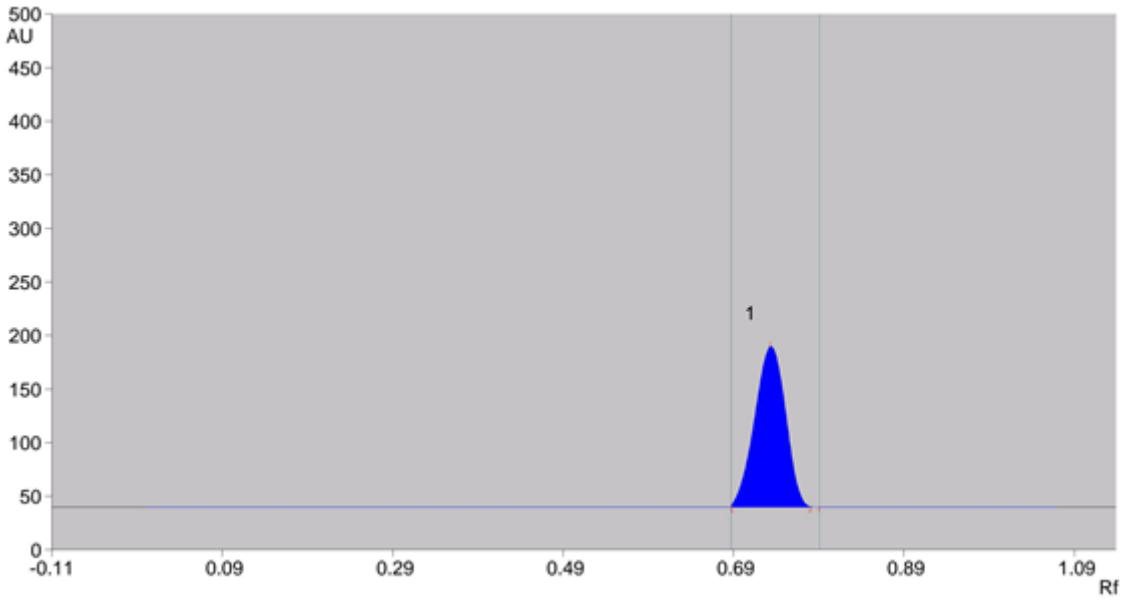



**Figure.24. Densitogram of propafenone in formulation**

**Table.21. Result of analysis of propafenone in formulation by HPTLC**

| Brand Name | Labelled amount(mg/tablet) | Estimated amount (mg/tablet) | %Label claim | %RSD |
|---|---|---|---|---|
| Pradil | 150 | 149.91 | 99.94 % | 0.76 |

# CONCLUSION

# 8. CONCLUSION

The UV-Multivariant calibration techniques and sensitive HPTLC method have been developed for the determination of propafenone hydrochloride in its tablet dosage form.

In UV-Multivariant calibration technique, ethanol was used as a solvent. The standard and sample solution were scanned at 247 nm and the concentration ranges are 4 – 20 µg/ml. The correlation coefficient values are $R^2$ = 0.9998, LOD = 0.451, LOQ = 1.36.55 and the percentage purity of propafenone hydrochloride formulation were found to be 99.56 %. The developed UV method was validated as per the ICH guidelines. The observed values are within the acceptable limits.

An optimized HPTLC method employing UV detection has also been developed and validated for the determination of propafenone in its tablet dosage form. In HPTLC – UV detection was observed at 251 nm. The pre-coated silica gel $G_{60}F_{254}$ used as a stationary phase and the mobile phase consisting Chloroform: Methanol: Acetic acid (8:1.5:0.5 v/v/v). The $R_f$ value was calculated at 0.72. The linearity ranges are 200-1000 ng/spot for propafenone. The linearity data revealed that the developed method is linear. The correlation coefficient $R^2$ was 0.9995. The precision and accuracy data lies within the reference range and the percentage label claim of propafenone hydrochloride was found to be 99.94 %.

Both UV-multivariant calibration and HPTLC method can be used for routine analysis of propafenone hydrochloride in its tablet dosage form without any interference of excipients. The newly developed densitometric method having high efficiency and quantification with less concentration levels when compared to previous method.

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