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RP-HPLC method development and validation for estimation of "RIVAROXABAN" as API and Active dosage form.

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I. INTRODUCTION

1.1 Introduction of dissolution study

Introduction The dissolution test has evolved to become a definitive tool used to characterize the performance characteristics of solid oral dosage forms. As dosage forms have become more unique over the last fifty years, the dissolution apparatus has required continuous improvement and modification to provide suitable conditions for performance testing of a wide variety of products. However, probably 99% of dissolution testing is performed on traditional tablets and capsules.

1.2 Dissolution - Definition

Academic definition: Dissolution is the process by which a solid substance enters into a solvent to form a solution.

Pharmaceutical definition: Dissolution is a test used throughout the life cycle of a pharmaceutical product to evaluate the rate of release of a drug substance from the dosage form.

1.3 What do we test?

Dissolution is not just about orally ingested products such as tablets and capsules. We also test : suspensions and powders coated beads and granules ointments, creams, gels transdermal patches implants, stents medicated contact lenses wound care products bone cement powders for inhalation chewing gums, etc….

Basically, the dissolution test mimics the first few stages of this process under very controlled laboratory conditions (in vitro). • For immediate release products: – Wetting in the stomach – Disintegration in the stomach – Deaggregation in the stomach – Dissolution in the stomach and intestine – Permeation through the intestinal wall – Absorption into the blood stream – Transit to the therapeutic site (via liver) – Decomposition and elimination.

2. Introduction to chromatography

Chromatography was first invented by Michael Tswestt, Russian botanist in 1906 for the separation of colored substance into individual component. Chromatography is defined as a procedure by which solute are separated by a dynamic differential migration process system consisting of two or more phases, one of which moves continuously in a given direction and in which the individual substances exhibit motilities by reason of differences in adsorption, partition, solubility, vapour pressure, molecular size or ionic charge density. The individual substance thus obtained can be identified or determined by analytical methods.

2.1.1 Principle of chromatography

Chromatography is based on the principle of separation of compounds into different bands (color graphs) and then identification of those bands. The preferential separation is done due to differential affinities of compounds towards stationary and mobile phase. After separation of the compounds, they are identified by suitable detection methods. The differences in affinities arise due to relative adsorption or partition coefficient in between components towards the both phases.

2.1.2 Separation process

The chromatographic method of separation involves the following steps:-

- Adsorption or retention of substances on the stationary phase.
- Separation of the adsorbed substances by the mobile phase.
- Recovery of the separated substances by the continuous flow of the mobile phase.

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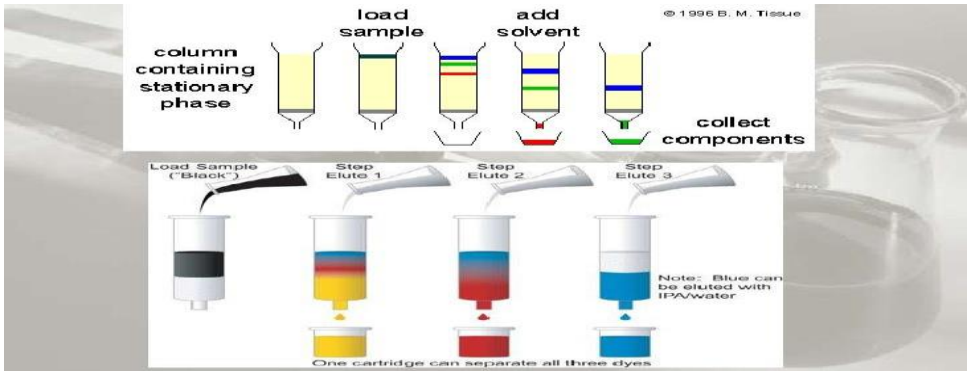

Figure 1 Principle of chromatography

2.1.3 UV- visible Spectroscopy

It may be defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength, approximating monochromatic radiation. Absorption spectrophotometry is the measurement of the absorption of electromagnetic radiation of definite and narrow wavelength range by molecules, ions and atoms of chemical substance. The technique most commonly employed in analytical field includes ultraviolet, visible, infrared and atomic absorption spectroscopy.

1.1.4 Beer's Lambert's Law

The fundamental law that governs spectrophotometric method is that "When a beam of monochromatic light is passed through transparent cell containing a solution of an absorbing substance, reduction of intensity of the light may occur, the rate of reduction in intensity with the thickness of the medium is proportional to the light and the concentration of the absorbing substance."

Mathematically, Beer's Lambert's Law is expressed as

A= log Io/It = abc

Equation 1 Beer'sLamberts law

Where,

A = Absorbance the solution at particular wavelength of light beam

Io =Intensity of incident light beam

It = Intensity of transmitted light beam

a = Absorptivity of molecule at the wavelength of beam

b = Path length of cell in cm

c = Concentration of solution in (gm/lit)

1.1.5 Chromatographic techniques

- Gas Chromatography (GC)
- High performance liquid chromatography (HPLC)
- Size exclusion Chromatography
- High performance thin layer Chromatography (HPTLC)
- Paper Chromatography
- Thin layer Chromatography (TLC)
- Ion exchange chromatography

1.2 High performance liquid chromatography (HPLC)

What does HPLC stand for?

HPLC is an analysis technique that yields high performance and high speed compared with traditional column chromatography because of the forcefully pumped mobile phase. Recently, ultrafast analysis using a high-pressure –resistant apparatus has been attracting attention. UHPLC (Ultra High Performance Liquid Chromatography) technique is based on the same method of separation as classical column Chromatography. i.e. adsorption, partition, ion exchange and gel permeation but it differs from column Chromatography, in that the mobile phase is pumped through the packed column under high pressure. It is the most popular technique today among the different chromatographic procedures. Due to significant evolution of (LC) instruments providing the superior quantitative result. The development of HPLC has enable LC to achieve great success in providing following features.

- Speed of separation
- High resolving power
- Repetitive and reproducible analysis

1.2.1 HPLC is the method of choice for the analysis

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- Non-volatile substances (for volatile substances GC is an alternative)
- Substances with high polarity or ionic samples
- Substances with high molecular weight
- Thermally unstable and decomposable substances

1.2.2 Types of HPLC

A) Normal –phase chromatography

Mechanism: Retention by interaction of the stationary phase's polar surface with parts of the sample molecules.

Stationary phase: SiO_2- $A_{12}O_3$,-NH_2,-CN,-Diol,-NO_2, etc.

Mobile phase Heptane, Hexane, Cyclohexane, CHC13, CHC13, CH2C12, Dioxane, Methanol, etc.

Application: Separation of non-ionic, non –polar to medium polar substances.

Disadvantage: Lack of reproducibility of retention times as water or protic organic solvents change the hydration state of the silica or alumina of stationary phase.

B) Reversed –phase chromatography

Mechanism: Retention by interaction of the station phase non-polar hydrocarbon chain with non-polar parts of the sample molecules.

Stationary phase: n-octadecyl (RP_{-18}) ,n-octyl (RP-8),ethyl (RP-2),phenyl,(CH_2)n-CH,

Mobile phase: Methanol or acetonitrile/water or buffer, etc.

Application : Separation of non-ionic and ion forming non-polar to medium polar substances(carboxylic acids , hydrocarbons).If ion forming substances (as carboxylic acids) are to be separated, a P^H control by buffers is necessary.

Table 1 Normal vs. Reversed Phase Chromatography

Content	Normal	Reverse
Packing polarity	High	Low
Solvent polarity	Low	High
Elution order	Non polar first	Polar first
Effect of increasing Solvent polarity	Decrease RT of polar Drug	Increases RT of non Polar drug

1.3 Instrumentation of HPLC»

- The various components of HPLC are:
- Mobile Phase Reservoir and solvent System treatment
- Pumps (Displacement Pump, Reciprocating Pump, Pneumatic Pump)
- Sample Injectors
- Liquid chromatographic column (Analytical Column)
- Detectors

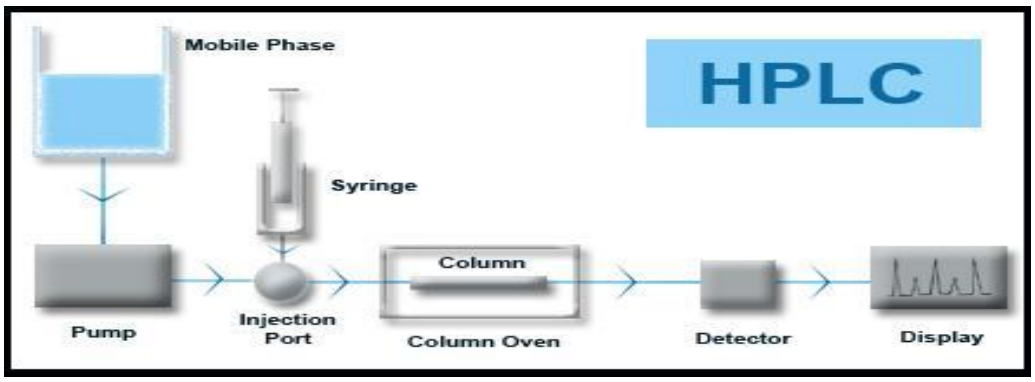

Figure 2 Instrumentation of HPLC

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1.3.1 Mobile phase reservoir and solvent system treatment

Modern HPLC apparatus is equipped with one or more glass or stainless steel reservoirs. It is often equipped with a means of removing dissolve gases usually O2 and N2 that interfere by forming voids in column and detector system.

Degasser may consist of:

- Vacuum pumping system
- A distillation system
- Devices for heating and stirring the solvent

Isocratic system: A separation that employs a single solvent of constant composition is called as isocratic system.

Gradient system: In this technique the proportion of the two or more solvent is varied in a programmed way.

1.3.2 Pump

Pumps are required to deliver a constant flow of mobile phase at pressure ranging from 1-550 bars. Pumps capable of pressure up to 8000 psi provide a wide range of flow rates of mobile phase, typically from 0.1 to 10ml/min. The various types of the pump used are:

- Constant displacement pumps or syringe pump
- Dual piston reciprocating pump
- Pneumatic pump or constant pressure pump.

1.3.3 Injection system

Injection pores are of basically of two types.

- Those in which the sample is injected directly into the column
- Those in which the sample is deposited into the column inlet and then swept by a valving action into the column by the mobile phase.

1.3.4 Precolumn

Some HPLC instruments are equipped with a pre column, which contains a packing chemically identical to that in the analytical column. Particle size is large hence the pressure drop across the pre column is negligible with respect to the analytical column. The precolumn is mainly used to remove the impurities from the solvent, and thus prevent contamination of the analytical column.

Column packing materials: Two basic types of packing have been used in HPLC.

A) Based on size

Pellicular: It consists of no porous, spherical glass or polymer beads with a diameter of 30 to 40 µm.

Porous particles: These particles have sizes ranging from 3-10 µm. and are composed of silica alumina or an ion exchange resin. These particles are often wetted with thin organic films which are physically or chemically bounded to the surface.

B) Based on chemical composition

- **Normal phase partition chromatography**: In type of chromatography the stationary phase is polar and mobile phase is comparatively non polar.
- **Reverse phase partition chromatography**: The stationary phase is less polar than the mobile phase. The stationary phase is silica, chemically bounded through a Siloxane (Si-O-Si –C) linkage to a low polar functional group.

Theses phases are prepared by treating the surface silanol group of silica with reagent.

1.3.5 Detectors

A detector is required to sense the presence and the amount of sample in the sample component in the column effluent. The output of the detector is an electrical signal that is proportional to some property of the mobile phase and/or the solute. A detector that measures property which is possessed by both mobile phase and solute is called bulk property detector e.g. Refractive Index detector. Alternative if the property is possessed essentially by the solute e.g. absorption of UV/visible light of electrochemical property, the detector is called a solute property detector.

The most commonly used detectors in the HPLC analysis of pharmaceutical substance are describe below.

- Photometric detectors. (Single wavelength detector, Multi- wavelength detector, Variable wavelength detector, programmable detectors, Diode array detectors)
- Fluorescence detectors
- UV-Visible detectors

1.4 Method development

Method development involves evaluation and optimization of the various stage of sample preparation, chromatographic separation, detection and quantification. Method development is a challenging and time-consuming process requiring much experience, creativity, logical thinking, and experimentation. With all the software and automated systems available today, method development is still very much a trial- and error approach, expedited by a logical sequence of generic scouting runs and fine- tuning steps to achieve the requisite resolution and method performance.

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1.4.1 Consideration before method development

Choice of the instrument which is suitable for the analyte such as:-

- Gas Chromatography (GC)
- High Pressure Liquid Chromatography (HPLC)
- Combined GC and LC Mass Spectrometry (GCMS)
- HPLC-MS
- LC-MS-MS

Choice of the chromatographic conditions such as:-

- Mobile Phase
- Column
- Auto sampler conditions
- Flow rate
- Injection volumes
- Choice of the internal standard.

1.4.2 Factors affecting the choice of Analytical Method

Analytical techniques have different degrees of sophistication, sensitivity and selectivity, as well as, different cost and time requirements. An important task for the analyst is to select best procedure for a given determination this will require careful consideration of the following criteria.

- The type of analytical required: elemental or molecular, routine or occasional.
- Problem arising from the nature of the material to be investigated, e.g. radio-active substance, corrosive substance, substances affected by water.Possible interference from components of the material other than those of interest.
- The concentration range to be investigated.
- The accuracy required.
- The facilities available, particularly the instrument.
- The time required to complete the analysis.
- The number of analysis of similar type which have to be performed.

1.4.3 Selection of Analytical Method[9]

First stage in the selection or development of method is to establish what is to be measured and how accurately it should measure. Unless one has series of methods at hand to assess quality of the product, validation program may have limited validity .The selected method must have the following parameters:

- As simple as possible,
- Most specific,
- Most productive, economical and convenient,
- As accurate and precise as required,
- Multiple source of key component (reagents, column ,TLC plates)should be avoid,

2.4.4 Strategy for HPLC method development

First Reverse-phase should be tried and if not successful. Normal-phase should be taken into consideration. It is also important that before making experiments with Ion-exchange or Ion-pair chromatography, first ion suppression by p^H control and Reversed-phase chromatography should be tried. Ion-pair chromatography should be preferred to Ion-exchange chromatography. The analysis in HPLC is either in qualitative or quantitative determination different components present in the sample. The qualitative analysis determines the sample quality and quantitative analysis involves comparison of standard and sample (their area or height).It is based on two requirements they are reproducible chromatogram and linear response of the detector for analytes of interest.

- Steps in a common strategy for HPLC method development are summarized below:
- Define method and separation goals
- Gather sample and analyte information
- Initial method development scoutingruns and getting the first
- Chromatogram
- Method fine-tuning and optimization
- Method validation

1.5 Method development in HPLC

Method development and optimization in liquid chromatography is still an attractive field of research for theoreticians (researchers) and attracts also a lot of interest from practical analysts. Complex mixtures or sample required systematic method development involving accurate modeling of the retention behavior of the analyte. Among all, the liquid chromatographic method, the reversed phase systems based on modified silica offers the highest probability of successful result. However, a large number of (system) variables (parameters) affect the selectivity and resolution.

"Best column, best mobile phase, best detection wavelength, efforts in separation can make a world of difference while developing HPLC method for routine analysis. Determining the ideal combination of these factors assures faster delivery of desired results- a validated method of separation.

Before proceeding with development of method for a particular sample it is absolutely essential to have detailed information about the sample and separation goal should be clearly defined.

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1.5.1 Information about sample

- Number of components present in the sample
- pka values of different components
- UV spectra of each analyte
- Concentration range of each component
- Solubility behavior
- Nature of sample (solid, liquid, semisolid)

1.6 System suitability parameters

A system suitability test is an integral part of gas and liquid chromatographic method. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The test is based on concept that the equipment, electronic, analytical operation and sample to be analyzed constitute an integral system that can be evaluated as such. It is the verification of the system to ensure system performance before or during the analysis. Parameter such as plate count, tailing factor, reproducibility and resolution are determined and compared against the specification set for the method. The area under curve (AUC) of five replicate injections should not be more than 2% of relative standard deviation (RSD).

Table 2 Separation goal and its remarks in Chromatography

Aim			Remarks
RESOLUTION (RS)			For precise and accurate quantitative method,
SEPARATION TIME			For routine procedure<5-10 min
%RSD			RSD<2.0%
QUANTITATION PRESSURE			<150bars
PEAK HEIGHT			Narrow peaks for large S/N ratio
SOLVENT CONSUMPTION			Minimum per sum is desirable

1.6.1 Retention time (RT)

RT is the time of elution Retention of peak of maximum after injection of compound.

1.6.2 Theoretical plates (N)

It is also called as column efficiency. A column can be considered as being made of large number of theoretical plates where distribution of sample between liquid-liquid or solid-solid phase occurs. The number of theoretical plates in column is given by the relationship

N = 16(tw) 2

Equation 2 Theoretical plates

Where,

t = the retention time

W= the width at the base of the peak.

HETP =LN

Equation 3 Theoretical plates

Where

L= length of column

Theoretical Plates should be more than 2000

1.6.3 Resolution(R)

It is a function of Column efficiency and is specified to ensure that closely eluting compounds are resolved from each other to establish the general resolving power of the system. For the separation of two components in mixture the resolution is determined by equation.

R=2(t2-t1)w1=w2

Equation 4 Resolution

Where t2 and t1 is the retention time of second and first compounds respectively, where as W2 and W1 are the corresponding widths at the bases of peak obtained by extrapolating straight sides of the peaks to baselines. R should be more than 2 between peak of interest and the closest eluted potential interferences (impurities, excipients, degradation products or internal standard).

1.6.4 Tailing factor (T)

It is the measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing become more pronounced.

T=W x /2 f

Equation 5 Tailing factor

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Where, W0.05 is the width of peak at 5% height and F is the distance from the peak maximum to the leading edge of the peak height from the baseline. Tailing factor should be less than 2.

1.6.5Capacityfactor (k)

It is calculated by the formula,

$$K = t / ta -1$$

Equation 6 Capacity factor

Where t is the retention time of the drug ta is the retention time of non-retarded component, air with thermal conductivity detection.

1.7 Validation of analytical method ,

Validation is defined as "documented evidence which gives a high degree of confidence that a process, system, facility will consistently produce a product meeting its predetermined specifications and quality attributes. Method validation process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Result of method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

1.7.1 Validation parameter

- Accuracy
- Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range
- Robustness

A) 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 sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

B) 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. The accuracy of an analytical method should be established across its range. In the case of the assay of a drug in the formulated product, accuracy may be determined by application of the analytical method to synthetic mixture of drug product component to which known amount of analyte have been added within the range of the method. Average recovery should be 99 to 101 % of drug at each level.

C) Specificity

Specificity is the ability to measure unequivocally the desired analyte in the presence of components such as excipient and impurities that may also expected to be present. In case of assay, demonstration of specificity requires that the procedure is unaffected by presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate level of impurities or excipients, and demonstrating that the assay result is unaffected by the presence of these extraneous materials. If impurities of degradation product standards are unavailable, specificity may be demonstrated by comparing the test result of the samples containing impurities of degradation product to second well characterized procedure. These comparisons should include samples stored under relevant stress conditions (e.g., light, heat, humidity, acid or base hydrolysis and oxidation).

D) Linearity

Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range, using the relationship between detector response (peak area or height) and sample concentration (or amount). For assay method, this study is generally performed by preparing standard solution at five concentration level, from 50 to 150% of the target analyte concentration. Five levels are required to allow detection of curvature in the plotted data. The standards are evaluated using the chromatographic conditions determined during the specificity studies.

Acceptability of linearity data is often judged by examining the correlation coefficient and y- intercept of the linear regression line for the response versus concentration plot. A correlation coefficient of > 0.999 is generally considered as evidence of acceptable fit to the data to the regression line.

E) Range

Range of an analytical method is the interval between the upper and lower analytical concentration of a sample that has been demonstrated to show acceptable levels of accuracy, precision, and linearity.

F) Ruggedness

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- The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of condition such as different laboratories, instrument, lots reagents, temperature, analyst etc. It is normally expressed as the lack of influence on test results of proportional and environmental variables of the analytical method. For ruggedness study, the conc. of analyte is measured using different parameters such as.
- Different operator in same laboratory
- Different equipment in same day
- Different source of segment and solution
- Different laboratory

G) Robustness

Robustness is a measure of the performance of a method when small, deliberate changes are made to the specified method parameters. The intent of robustness validation is to identify critical parameters for the successful implementation of the method.

Examples of typical variations are:

- Influence of variations of pH in a mobile phase
- Influence of variations in mobile phase composition
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

H) Limit of detection (LOD)

LOD is the smallest quantity of an analyte that can be detected, and not necessarily determined, in quantitative fashion. Approaches may include instrumental or non-instrumental procedures and could include those based on:

1. Visual evaluation
2. Standard deviation response and the slope
3. Calibration curve

The detection limit (LOD) may be expressed as**:**

$DL = 3.3\ \sigma / S$

Equation 7 (LOD)

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

I) Limit of quantitation (LOQ)

LOQ is the lowest concentration of analyte in a sample that may determine with acceptable accuracy and precision. Approaches may be include instrumental or

Non-instrumental procedures and could include those based on:

Visual evaluation

Standard deviation response and the slope

Calibration curve.

The quantitation limit (QL) may be expressed as:

$QL = 10\ \sigma / S$

Equation 8 (LOQ)

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Table 3 Characteristics to be validated in HPLC and their acceptance criteria

Characteristics	Acceptance criteria
Accuracy	Recovery 98-102%
Precision	RSD<2%
Specificity	No interference
Detection of limit	S/N> 2or 3
Quantitation of limit	S/N >10
Linearity	Correlation coefficient r>0.999
Range	80-120%

II. LITERATURE REVIEW

Literature survey was done on Capecitabine. There were some report for the analysis of single component and multi component in dosage form.

1) Samudrala Vijayasanthi, et, al.,(2012)

A rapid and sensitive reverse phase high performance liquid chromatographic method was developed for the estimation of capecitabine in pure and pharmaceutical formulation. Capecitabine chromatographed on reverse phase C18 column in a mobile phase consisting of Ammonium acetate buffer and acetonitrile in ratio 65:35 v/v. The mobile phase was pumped at flow rate of 1.2 mL/min with detection at 240 nm. The detector response was linear in the concentration of 20-120 µg/ml. The limit of detection and limit of quantitation was found to be0.0026µg/mL and 0.0088 µg/mL respectively. The mean recovery of the drug was 99.82%.

2) A. K. M. Pawar, et, al., (2016)

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To develop and validate a simple, selective, rapid, precise and accurate high performance liquid chromatographic method for determination of capecitabine in bulk and its pharmaceutical formulation. RP-HPLC method was performed by using a mobile phase consisting mixture of methanol and ammonium acetate buffer (p^H 4.5) in proportion 60:40 v/v. A ZORBAX eclipse plus C18 column was used.HPLC analysis was carried out at a wavelength of 241 nmwith a flow rate of 1ml/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a correlation coefficient 0.9984. The retention time of capecitabine was found to be 2.77min. Accuracy of the method was determined through recovery studies which were found to be within 97.57-102.22%.

3) Makula Ajitha, et, al., (2012)

To develop and validate a simple, specific, efficient and sensitive RP-HPLC method for determination of capecitabine from bulk using mobile phase consisting mixture of methanol and buffer 70:30 v/v at flow rate of 1.0ml/min.A phenomenex C18 column was used as stationary phase. The retention time forcapecitabine was 4.1 min. Linearity was found in concentration range of 10 to 50µg/ml, with good linearity response greater than 0.997. The mean % recovery obtained is 99.996%.

4) M. Prasad Rao et al., (2016)

An isocratic reverse phase high performance liquid chromatographic method has been developed and validated for the determination of capecitabine bulk and its pharmaceutical formulation. Separation was achieved with a develosil ODS-MG-5 column and buffer and methanol (450:550) v/v as eluent and purified water, methanol and acetonitrile (600:350:50)v/v as diluent at flow rate ml/min. The described method of capecitabine is linear over a range of 6µg/ml to 306µg/ml. The method precision for the determination of assay was below 2% RSD.

5) Pani Kumar AD et al., (2011)

A simple rapid accurate and stability indicating RP-HPLC method was developed for the determination of capecitabine in pure and tablet dosage form.The method showed a linear response for concentration in the range of 70-120µg/ml using 0.05M phosphate buffer and acetonitrile 50:50% w/v as the mobile phase with detection at 240 nm and flow rate of 1 ml/min and retention time 4.108 min. The method was statically validated for accuracy, precision, linearity, ruggedness, robustness, stability and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed, the %RSD from recovery studies was found to be less than one.

6) SreenivasaRao T. et al., (2012)

A validated method for the determination of capecitabine has been developed by using reverse phase high performance liquid chromatography and UV spectrophotometry in pharmaceutical dosage forms. Spectrophotometric determination was carried out at an absorption maximum of 307 nm using Ethanol. The linearity over the concentration range of 5-25 µg/ml with correlation coefficient 0.999 is obtained. Chromatographic separation was carried out using a mobile phase of acetonitrile and phosphate buffer(pH adjusted to 3.2 with Ortho phosphoric acid) in the ratio of 55:45 v/v on agilent C18 column.

7) K. Ravi Kumar et. al.,(2010)

A simple, rapid and selective RP-HPLC method has been developed for quantification Capecitabine from bulk drug and pharmaceutical formulations using a mobile phase consisting mixture of Buffer and acetonitrile in the ratio 80:40 v/v at the flow rate of 1.2 ml/min. The Hypersil BDS C8 column was used as stationary phase. The retention time for capecitabine was 15min. Linearity was observed in the concentration range of 40 to 60 µg/ml, with good linearity response greater than 0.997. The mean % recovery obtained is 100.1%. The proposed method is precise, accurate, selective and rapid for the determination of Capecitabine in capsules.

8) P. Ravisankar et, al., (2013)

A simple, specific, accurate, and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of capecitabine in tablet dosage forms. A welchrom C18 column was used as stationary phase, with mobile phase containing methanol: Acetonitrile: Water 50:30:20 v/v(pH adjusted to 4.6 using triethylamine) was used. The flow rate was 1.0 ml/min and effluents are monitored at 245 nm. The retention time of capecitabine is 4.123 min. The method was validated for specificity, linearity, accuracy, precision, limit of quantification, limit of detection, robustness in accordance with ICH guidelines. Recovery of Capecitabine in tablet formulation was found to be 99.88 %.

III.JUSTIFICATION AND OBJECTIVE

3.1Justification

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Analytical method is intended to establish the identity, purity, physical characteristics and potency of the drug that we use. Method is developed for drug testing during manufacturing and quality release operation as well as during long term stability studies.

It is an anticoagulant medication used to treat and prevent blood clots and to prevent stroke in people with nonvalvular atrial fibrillation through directly inhibiting factor Xa. It effectively blocks the amplification of coagulation cascade ,preventing the formation of thrombus .

Literature survey exposed very few analytical procedures for routine analysis of rivaroxaban.

Therefore, present research an attempt was made to develop and validate simple, precise and economic method for routine analysis of rivaroxaban in bulk and tablet dosage form.

3.2 Objective

1. To develop RP-HPLC method for RIVAROXABAN.
2. To validate RP-HPLC method for bulk drug as per ICH Q2R1 guideline.
3. To explore an applicability of method in pharmaceutical formulation.

IV. PLAN OF WORK

- Literature survey
- Selection of drug
- Procurement of drug
- Characterization of drug
- Selection of wavelength
- HPLC method development
- Selection of mobile phase
- Optimization of method
- Selection of chromatographic condition
- Development of RP-HPLC method
- Validation as per ICH Guideline Q2R1
- Dissertation writing and submission

V. DRUG PROFILE

Name: RIVAROXABAN

Structure:

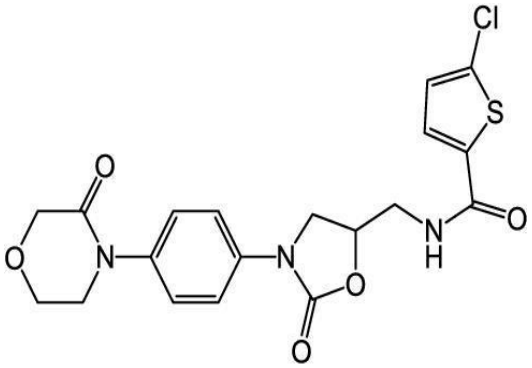

Figure 3 Structure of rivaroxaban

Description: rivaroxaban is used to treat or prevent deep venous thrombosis, a condition in which harmful blood clots form in the blood vessels of the legs. These blood clots can travel to the lungs and can become lodged in the blood vessels of the lungs, causing a condition called pulmonary embolism. This medicine is used for several days after hip or knee replacement surgery while you are unable to walk. It is during this time that blood clots are most likely to form.

IUPAC name: (S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]oxazolidin-5-yl]methyl} thiophene-2-carboxamide

Chemical formula: $C_{19}H_{18}ClN_3O_5S$

Molecular mass: 435.88g/mol^{-1}

Physical state: white to yellowish powder

Melting point: 230℃

Solubility: Slightly Soluble in organic solvent. It is soluble practically in water and aqueous media.

pka: Strongest acid 13.40

$t_{1/2}$: ~ 5-6 hours

Therapeutic uses: rivaroxaban is used help prevent strokes or blood clots in people who have atrial fibrillation.

Mechanism of action: rivaroxaban inhibits free and clot-bound FXa, and prothrombinase activity. rivaroxaban has no direct effect on platelet aggregation, but indirectly inhibits platelet aggregation induced by thrombin. By inhibiting FXa, rivaroxaban decreases thrombin generation and thrombus development.

Pharmacokinetic: The pharmacokinetic properties of rivaroxaban have been described in detail. In healthy male volunteers, rivaroxaban was rapidly absorbed after oral administration, with a time to maximum plasma rivaroxaban concentration of about 1 hour. The elimination half-life for rivaroxaban in healthy subjects is approximately 12 hours.

VI. MATERIAL AND METHODS

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6.1 Reagents and chemicals used

Table 4 List of chemicals

Ingredients	Grade	Suppliers
RIVOROXABAN	API	Dr. Reddy's Laboratory.
Acetonitrile	HPLC	Dodal Indusrty, Aurangabad
Water	HPLC	Rankem.

6.2 Marketed Preparation

Table 5 List of brand name and Formulation of RIVAROXABAN

Brand Name	Formulation	Available strength	Address of Manufacturing
XARELTO	Tablet	RIVAROXABAN 2.5mg	Pfizer.

6.3 HPLC

6.3.1 Selection of Analytical Technique

HPLC was selected as analytical technique for estimation of RIVAROXABAN

Instruments

The analysis of the drug was carried out on Shimadzu HPLC system with UV Detector. Equiped with PUROSPHER STAR RP 18 column (4.0mm x 55mm; 3 µm), a 5µl injection volume and running Labsolution 6.86 software.

a) Chromatographic conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table 6 Chromatographic condition

1	HPLC	Shimdazu
2	Software	Labsolution 6.86
3	Column	Purospher star RP 18 (55mm ×4.0mm, 3um)
4	Stationary phase	PUROSPHER RP 18
5	Mobile phase	Solution A: Solution B(75:20v/v)
6	Detection Wavelength	250nm
7	Flow rate	1.0 ml/min
8	Column Temperature	45ºC
9	Sample size	50µl
10	Run Time	20 min
11	Filter paper	0.45µm

6.4.1 Method development of RP- HPLC

Mobile phase selection

The aim is to find the correct concentration of the mobile phase. The mobile phase and its strength is a measure of its ability to pull analytes from the column. In reverse phase HPLC, with aqueous mobile phases such as Solution A: Solution B (75:25 v/v). The retention

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time is also important criteria for selection of mobile phase.

Selection of column

RP 18 column was selected for analysis as it is short and recommended to reduce method development time. RP 18 columns afford shorter retention. Generally RP 18 column is used for better results of analysis in reverse phase chromatography.

6.5 Characterization of standard drug

- Melting Point
- Solubility
- UV spectrum and wavelength
- IR Spectroscopy
- HPLC chromatogram and retention time

6.6 Experimental Work

6.6.1 Determination of wavelength

Preparation of standard stock solution

20 mg of RIVAROXABAN was weighed accurately and transferred to 100ml volumetric flask containing a mixture of acetonitrile and water in the ratio of 50:50v/v. The volume was made up to the mark using same mixture of solvent to obtain resulting solution of 1000ug/ml. Then 1.0ml was pipette out and diluted up to the 10ml which will give resultant solution of 100ug/ml. The absorbance of the latter was recorded using UV visible spectrophotometer in the range 200-400nm.

6.6.2 Method optimization for RIVAROXABAN tablets 2. 5 mg dissolution study

Dissolution Parameters:

Dissolution Medium	0.05 M Sodium Phosphate Buffer with 0.05 % SLS,pH 6.8
Apparatus	Paddle
Speed	75 RPM
Temperature	37.0 ± 0.5 ° C
Medium	0.05 M Sodium Phosphate Buffer with 0.05 % SLS , pH 6.8
Volume	1000 ml
Time point	30 min

Optimization of chromatographic condition

The following chromatographic conditions were established by trial and error and were kept constant throughout the analysis.

Table 7 Chromatographic conditions

Column	PUROSPHER STAR RP 18	55mm x 4.0mm, 3µm or equivalent.
Detection of wavelength	250nm	
Flow rate	1ml/min	
Temperature	45ºC	
Sample size	10µl	
Mobile phase	Solution A: Solution B(75:25)	

Preparation of solution:

0.05 M Sodium Phosphate Buffer with 0.05 % SLS, pH 6.8 solution preparation:

Weigh accurately 5.0g Sodium lauryl sulphate in to Approximately 9 Liters water, add 60 g monobasic sodium phosphate and dissolve the contents. Adjust the pH to 6.8 with 10N sodium hydroxide solution and make up to 10 liters with purified water.

Preparation of Mobile phase:

Buffer:

Weigh accurately 1.36g of Sodium dihydrogen phosphate monohydrate into a beaker containing 1000 ml of Milli - Q- grade water and

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mix to dissolve. Filter through 0.45 micron or finer porosity membrane filter and degas.
Solution A: Mixture of buffer, Acetonitrile in the ratio of 90:10 v / v and degas.
Solution B: Acetonitrile
Mobile phase: Mixture of solution A and solution B in the ratio (75:25 v / v)

Standard stock solution:
Weigh accurately 22.0 mg of rivaroxaban a working standard, into a 200 ml clean,dry volumetric flask , and add 100 ml of Acetonitrile and sonicate to dissolve. Make up to volume with dissolution media and mix.

Standard solution:
Dilute 5.0 ml to 100 ml with Dissolution media.

Sample solution:
Set the parameters of instrument as mentioned dissolution conditions . Place one tablet each in six vessels containing the dissolution medium, which has been equilibrated at 37 ° C10.5 ° C and start the dissolution test . At the specified time interval withdraw the sample solution from each vessel and filter through 0.45µm nylon or PVDF syringe filter, by discarding first 5 ml of filtrate.

6.7 RP- HPLC method development and validation
6.7.1 System suitability testing
Inject the dissolution medium (Blank) into the liquid chromatograph, record the chromatogram.
A. Inject the standard solution, Six times into the liquid chromatography, record the chromatograms and evaluate the system suitability.
B. 5% RSD for peak areas of rivaroxaban from six injections from standard should be not more than 1.0% other parameters are shown in **Table 10.**

6.7.2 Method validation
A) Linearity
From stock solution 1ml to 100ml , 2.5ml to50ml, 5ml to 50ml ,4ml to 25ml, 5ml to 25ml, 6ml to 25ml,6.5ml to 25ml of solution, and volume was made up to the mark with dissolution media to get final concentration 0.225,1.375,2.75,4.4,5.5,6.6,8.25 µg/ml. Sample were injected and peaks were recorded at 280nm as the graph plotted as concentration of drug verses peak area. Results were recorded for equation of line; correlation coefficient and intercept are shown in **Table 12, 13 & Figure 6.**
Y= mX+c
Equation 9 equation of line
Where,
Y- Area
X- Unknown concentration
m- Slope of graph
c- Intercept

B) Precision:
1) Intra-day precision:
Set the parameters of instrument as mentioned dissolution conditions . Place one tablet each in six vessels containing the dissolution medium, which has been equilibrated at 37 ° C10.5 ° C and start the dissolution test . At the specified time interval withdraw the sample solution from each vessel and filter through 0.45µm nylon or PVDF syringe filter, by discarding first 5 ml of filtrate. Inject the sample solutions into the liquid chromatography and record the chromatogram .The results are shown in **Table 13**.
2) Inter-day Precision:
The same procedures were performed as intra day procedure with different analyst**,** system, column & chemical. The Results are shown in **Table 13.**
Acceptance criteria precision the relative standard deviation should not be more than 2% for test.

C) Robustness
The following parameters were changes for Robustness study. 1.Chnages in flow(±10), Changes in Temp(±10), Changes in RMP(±10), Changes in Dissolution volume(±10), The Results are shown in **Table 14.**

D) Detection Limit
Based on the standard error of the predicted y value for each x in a regression, the detection limit (DL) was calculated as
LOD=3.3×STEYXSLOPE
E) Quantitation limit
Based on the standard error of the predicted y value for each x in a regression, the quantitation limit (QL) was calculated as,
LOQ=10×STEYXSLOPE
F) Accuracy (%recovery)
Method of analysis : Set the conditions as mentioned in the Method of analysis .

Standard stock solution :
Weigh accurately 20 mg of rivaroxaban working standard , into a 100 ml clean . dry volumetric flask , and add 20 ml of Acetonitrile and sonicate for 5 min to dissolve .dilute upto the mark with the solvent mixture.filter through 0.45micronmeter nylon syringe filter .discard first 3ml of solution.

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Preparation of standard solution :

Transfer 5 ml of this solution into 100 ml of volumetric flask and make up the volume up to the mark with dissolution medium .

rivaroxaban stock solution for accuracy :

Weigh accurately 2.5 mg of rivaroxaban API , into a 100 ml clean , dry volumetric flask and add 50 ml of Acetonitrile and sonicate to dissolve . Make up to volume with dissolution media and mix.

Sample solution (50 %):

Set the parameters of instrument as mentioned dissolution conditions. Transfer 1.5mg of tablet and 110 mg placebo in each three vessels containing 1000 ml dissolution medium, which has been equilibrated at 37 ° C + 0.5 ° C and start the dissolution test.At the specified time interval withdraw the sample solution from each vessel and filter through 0.45um nylon or PVDF syringe filter, by discarding first 5 ml of filtrate.

Sample solution (100%):

Set the parameters of instrument as mentioned dissolution conditions. Transfer 2.5mg of tablet and 110.01 mg placebo in each three vessels containing 1000 ml dissolution medium , which has been equilibrated at 37 ° C10.5 ° C and start the dissolution test . At the specified time interval withdraw the sample solution from each vessel and filter through 0.45µm nylon or PVDF syringe filter , by discarding first 5 ml of filtrate .

Sample solution (150%):

Set the parameters of instrument as mentioned dissolution conditions . Transfer 3.75 mg tablet of accuracy stock solution and 110.02 mg placebo in each three vessels containing 1000 ml dissolution medium , which has been equilibrated at 37 ° C ± 0.5 ° C and start the dissolution test . At the specified time interval withdraw the sample solution from each vessel and filter through 0.45µm nylon or PVDF syringe filter , by discarding first 5 ml of filtrate .The result are shown in **Table 16**

VII. RESULT AND DISCUSSION

7.1 Preliminary studies on rivaroxaban

7.1.1 Melting point

Melting point was determined using melting point apparatus by capillary method and observed to be 230ºC (Reported M.P. 203ºC -230ºC).

7.1.2 Solubility

The bulk rivaroxaban drug soluble in water and aqueous media and insoluble in organic solvent(observed by visually).

7.1.3 Infra red spectroscopy

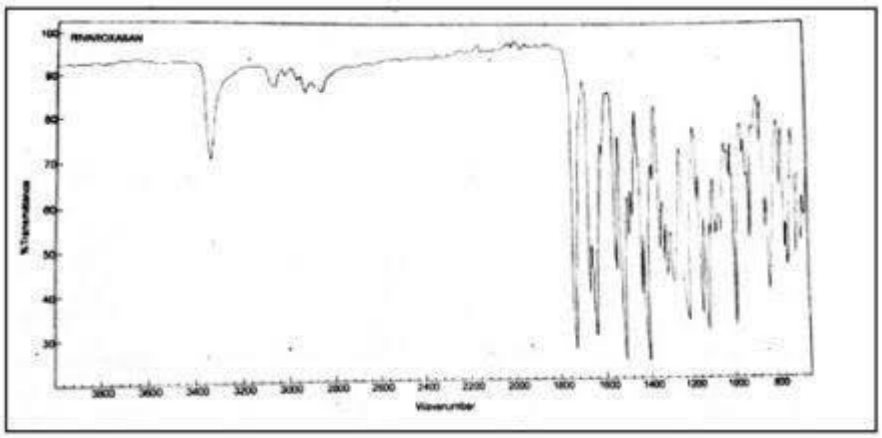

Table 9 IR spectroscopy of rivaroxaban

7.1.4 UV spectroscopy

UV absorption of 100ug/ml solution of rivaroxaban using mixture Methanol: water (50:50v/v) was generated and absorbance in the range of 200-400nm recorded. λmax of RIVAROXABAN was found to be 250nm.

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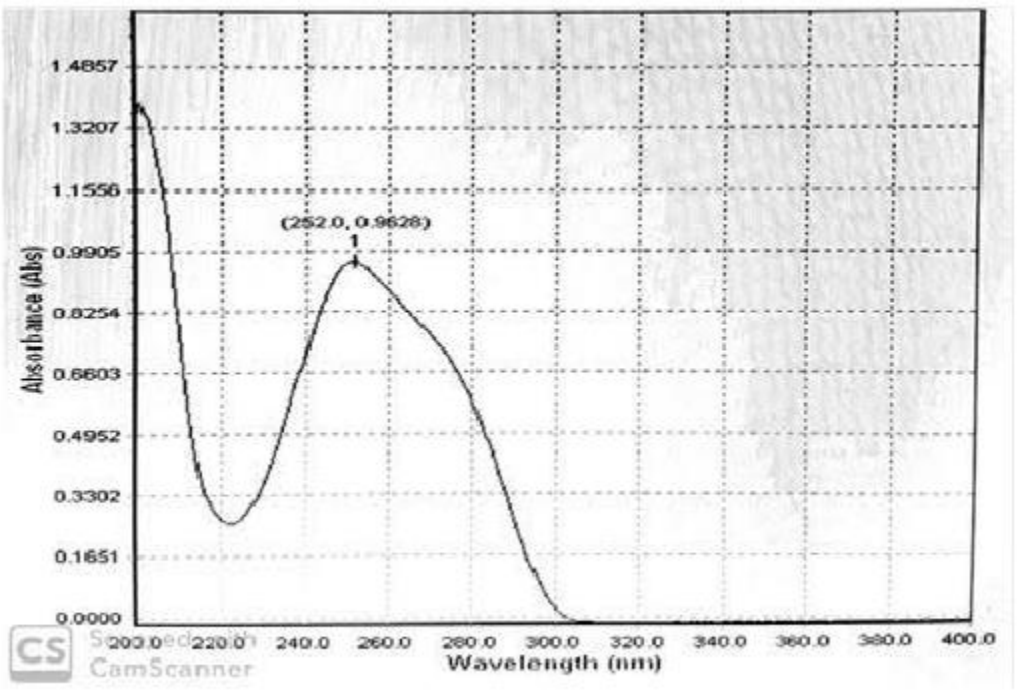

Figure 5 Detemination of absobance

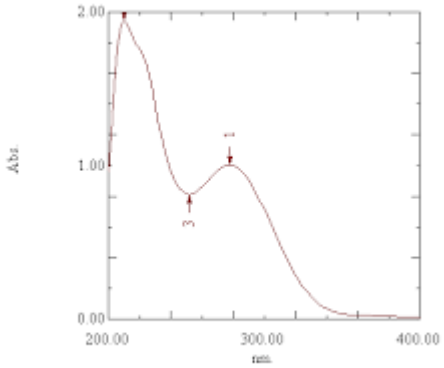

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Figure 6 UV spectra of capecitabine

7.2 HPLC method development

7.2.1 Trials and errors

Mobile phase- Solution A: Solution B (75:25v/v)

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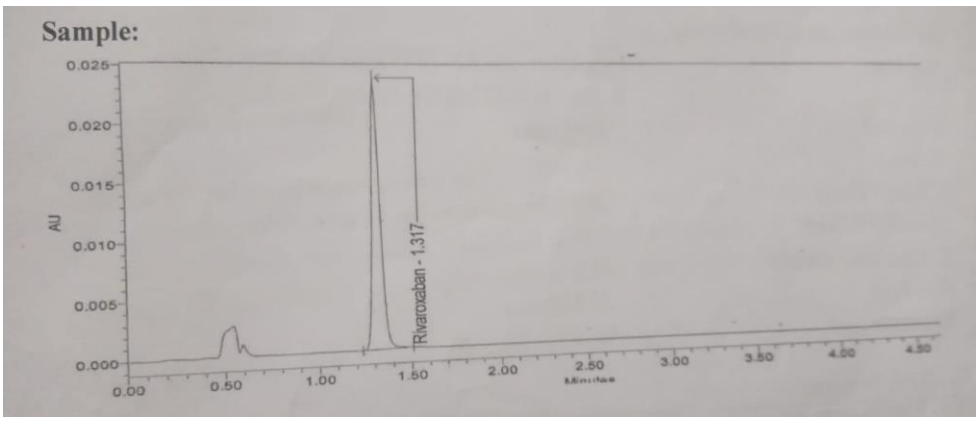

Figure 7 Chromatogram of trials and errors

Name	Retention Time(min)	Mean Area	No. of Theoretical Plates
RIVAROXABAN	1.317min	3128244	113225

Table 10 Results of trials and error of rivaroxaban

7.2.2 Chromatographic condition for trials and error

- Column: RP-18 (55mmx4.0mm,3µm)
- Flow rate: 1.0 ml /min
- Wavelength: 250 nm
- Injection volume: 5µl
- Column temperature: 45°C
- Run time: 20 min
- Mobile phase: Solution A:Solution B (75:25v/v)

7.3 RP-HPLC Method Validation

7.3.1 System suitability test

To optimize the chromatographic conditions, the effect of chromatographic variables such as composition of mobile phase, flow rate and the column were studied. The resulting chromatograms were recorded and the chromatographic parameters such as peak area, resolution and theoretical plates were integrated. The conditions obtained most excellent resolution; symmetry factor and theoretical plate were selected for further estimation.

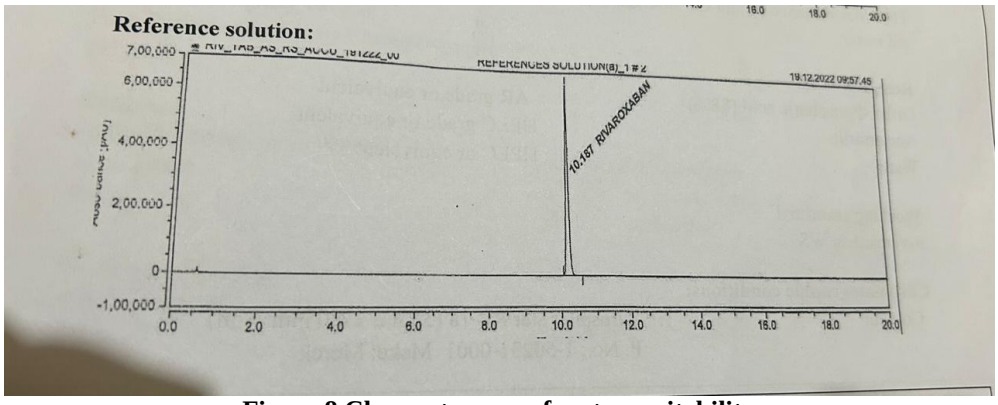

Figure 8 Chromatogram of system suitability

Table 10 System suitability parameter

Sr.no	System suitability parameter	Mean observation	Standard limits	Inference
1	Retention time	10.187 min	NLT 2.0min	Passed
2	Area	604376	NLT 2000	Passed
3	Theoretical plate	6573	NLT 2000	Passed

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4	Tailing factor	1.135	NMT 2.0	Passed
5	% RSD	0.59	NMT2.0%	Passed

7.3.2 Linearity

The linearity of an analytical procedure is its ability to elicit test results that are proportional to the concentration of analyte in sample. From the stock standard solution, aliquots of 1 to 100, 2.5to 50, 5to 50, 4to 25, 5 to 25, 6 to 25, 6.5 to 25ml were taken in volumetric flasks and diluted up to the mark with mobile phase such that to obtain concentration of rivaroxaban in the range 12.5-75 µg/ml. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area verses the drug concentration.

LINEARITY STUDY OF RIVAROXABAN

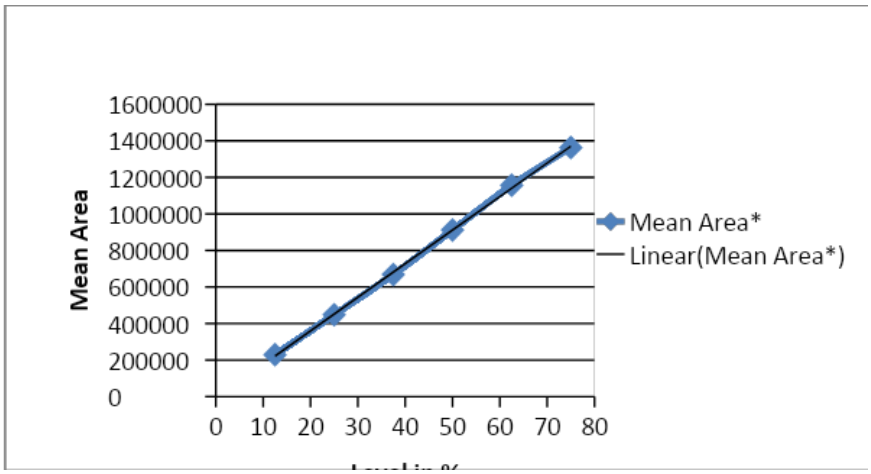

Figure 9 calibration curve of rivaroxaban

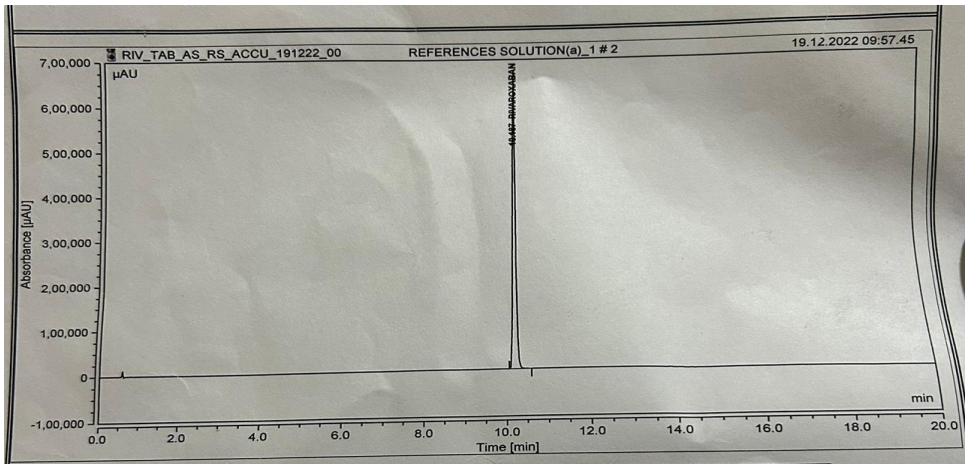

Figure 10 Chromatogram of linearity

Table 11Linearity data of rivaroxaban

Sr. no.	Conc. ug/ml	Mean Area*
1	0.225	30405
2	1.375	150676
3	2.75	303282
4	4.4	479842
5	5.5	586968

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6	6.6	721784
7	8.25	874126

Regression equation data Y=mx+c	
Slope (m)	18383
Intercept (c)	-8057.7
Correlation coefficient	0.9994

Table 12 Regression equation data of Capecitabine

7.3.3 Precision

The method was established by analyzing various replicate standard of Capecitabine. All the solution analyzed thrice in order to record any intra-day and inter-day variation in the result that concluded.

A) Intra-day Precision

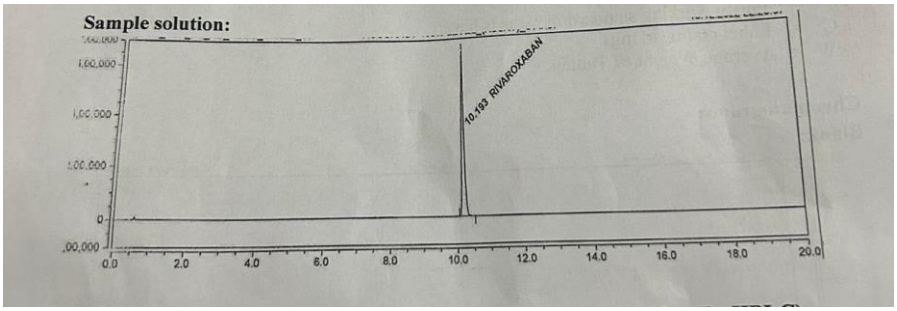

Figure 11 Chromatogram of intra- day precision

Result

Name	Retention Time(min)	Area	No. of Theoretical Plates	% Dissoluuution
rivaroxaban	10.193	3128252	112998	96

B) Inter-day precision

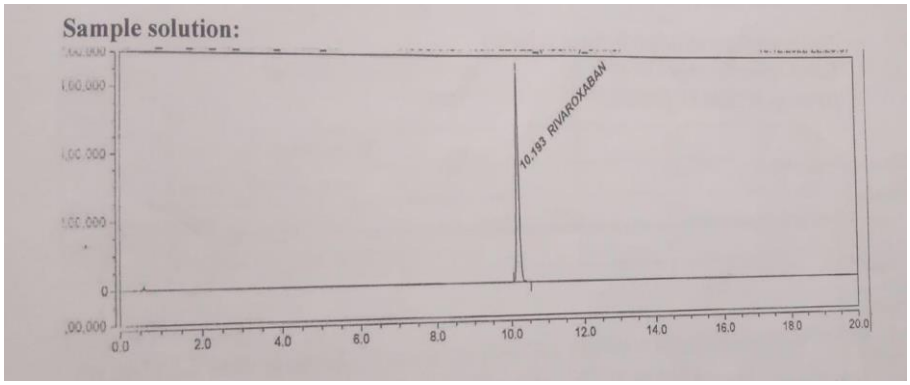

Figure 12 Chromatogram of inter-day precision

Result-

Name	Retention Time(min)	Area	No. of Theoretical Plates	% Dissoluuution
rivaroxaban	10.193	2801400	113235	97

Table 13 Result of Intra-day and Inter-day precision study of Capecitabine

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7.3.4 Robustness

The robustness of a method is its ability to remain unaffected by small but deliberate changes in method parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized parameter were done. The effect of changes in the flow rate, column temperature, medium volume, rate of rpm on retention time and tailing factor of drug peak was studied. Robustness parameters were also satisfactory; hence the analytical method would be concluded.

A)Flow variation (-10%,i.e.0.9 ml/min)

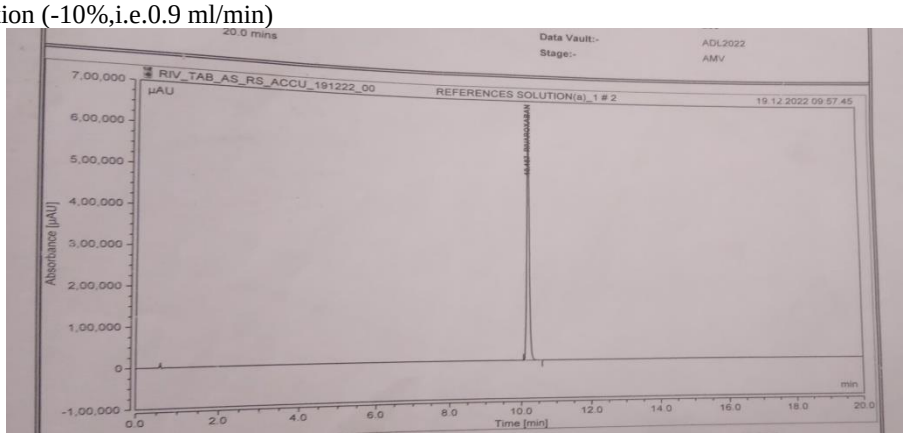

Figure 13 Chromatogram of flow variation -10%

Result-

Name	Retention Time(min)	Area	No. of Theoretical Plates	%Drug Release
rivaroxaban	10.187	3128244	113229	93

B) Flow variation (+10%,i.e.1.1 ml/min)

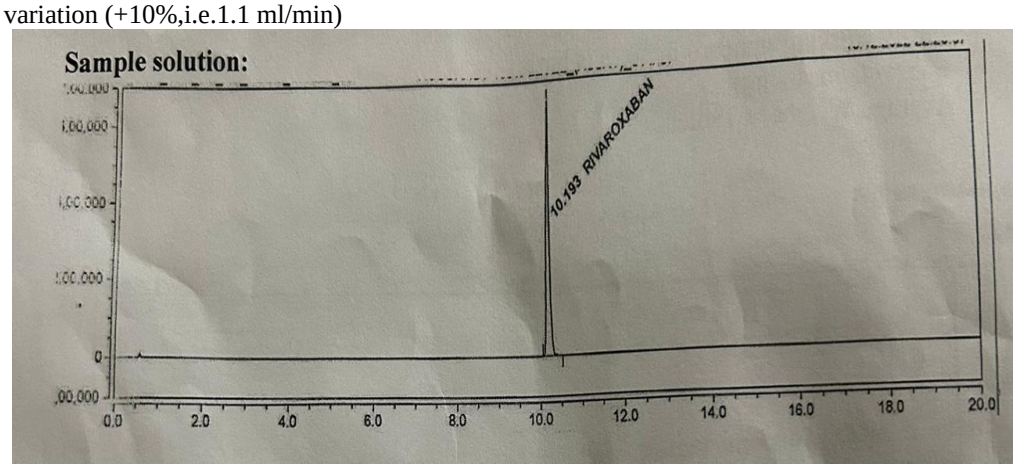

FIG: 14 Chromatogram of +10% flow

Figure **Name**	Retention Time(min)	Area	No. of Theoretical Plates	%Drug Release
rivaroxaban	10.193	3128256	113256	93%

C) Variation in Temperature (-2.0,i.e.28ºC)

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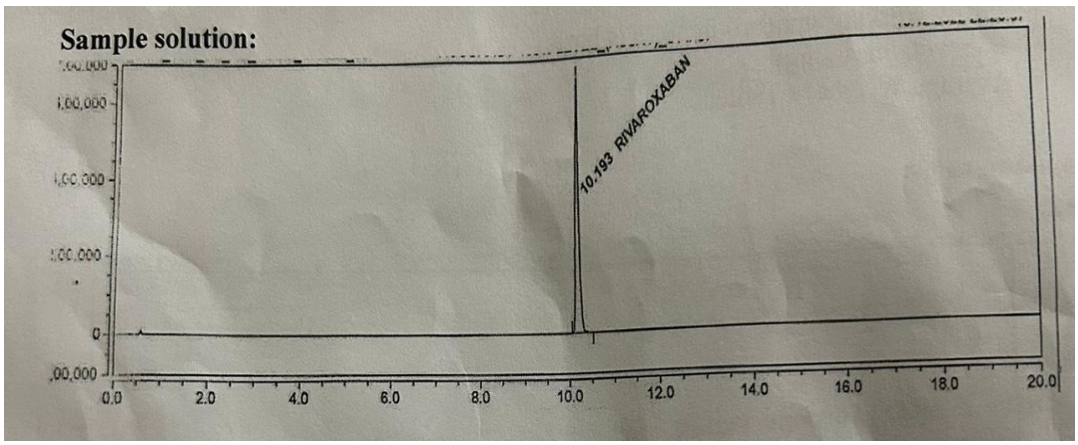

Figure 15 Chromatogram of temperature -2.0

Result-

Figure **Name**	**Retention Time(min)**	**Area**	**No. of Theoretical Plates**	**%Drug Release**
Rivaroxaban	10.193	3128265	113278	93%

D) Variation in Temperature (+2.0,i.e.32ºC)

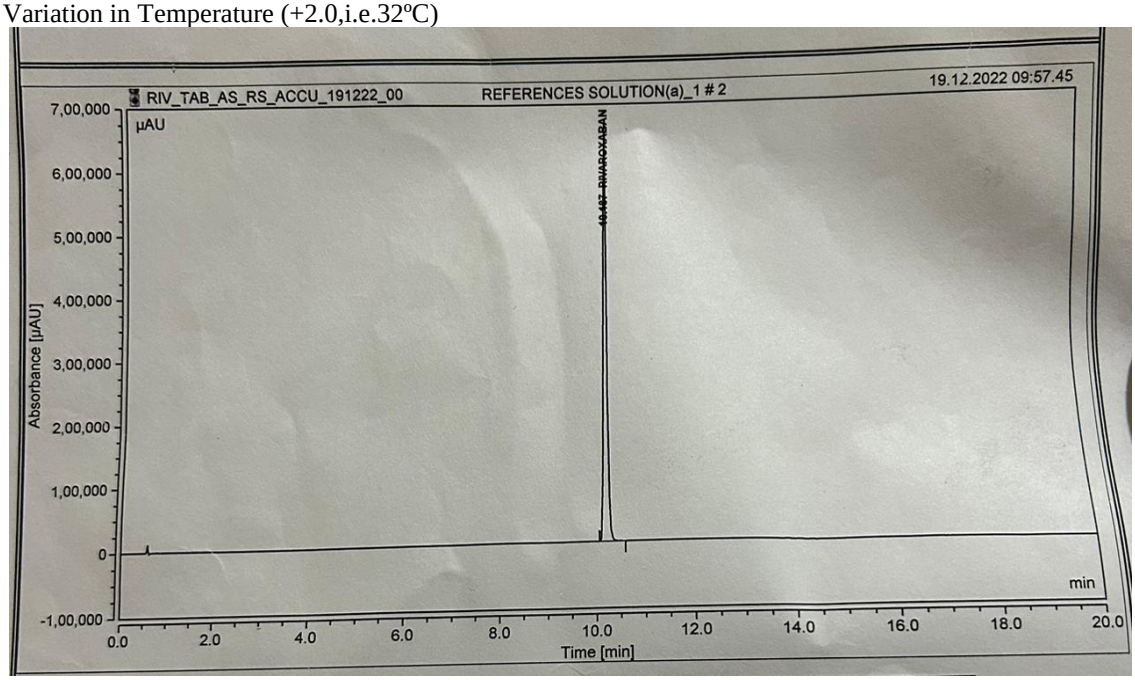

Figure 16Chromatogram of Temperature +2.0

Result

Figure **Name**	**Retention Time(min)**	**Area**	**No. of Theoretical Plates**	**%Drug Release**
Rivaroxaban	10.00	2831244	112998	93%

E) Variation in rpm (-2.0,i.e.73 rpm)

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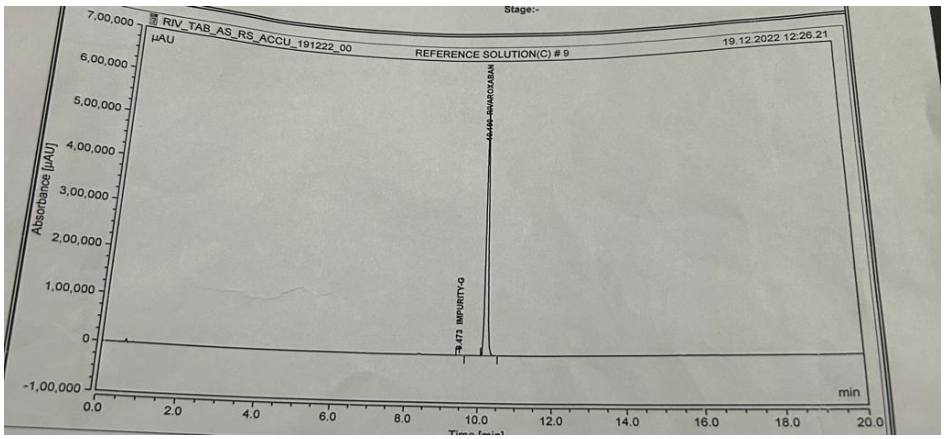

Figure 16Chromatogram of change 73 rpm

Figure **Name**	**Retention Time(min)**	**Area**	**No. of Theoretical Plates**	**%Drug Release**
Rivaroxaban	10.190	2801401	113225	91%

F) Variation in rpm (+2.0,i.e.77 rpm)

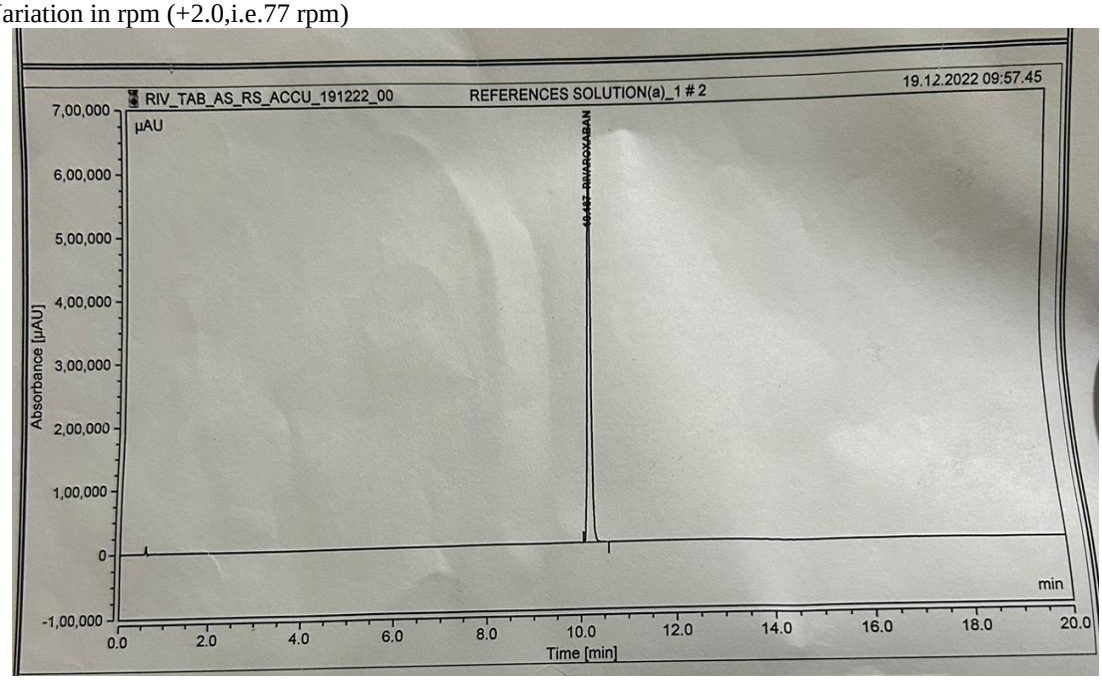

Figure 16Chromatogram of change rpm 77 rpm

Figure **Name**	**Retention Time(min)**	**Area**	**No. of Theoretical Plates**	**%Drug Release**
Rivaroxaban	9.493	2934244	113445	89%

G) Variation in Dissolution volume (+10%,i.e.990 ml)

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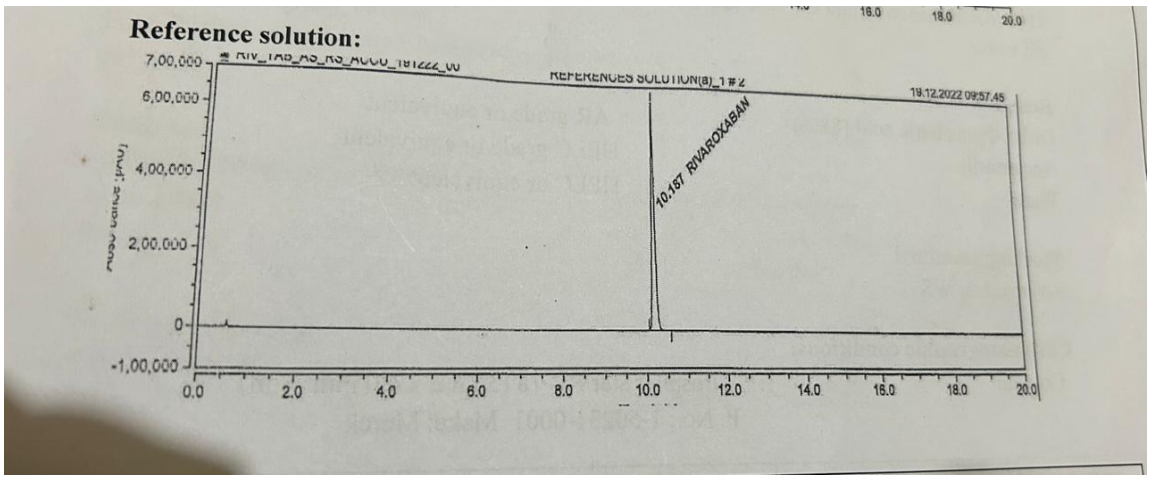

Figure 16Chromatogram of change in dissolution volume 990 ml

Result

Figure **Name**	**Retention Time(min)**	**Area**	**No. of Theoretical Plates**	**%Drug Release**
Rivaroxaban	10.187	3314596	116525	88%

H) Variation in Dissolution volume (-10%,i.e.810 ml)

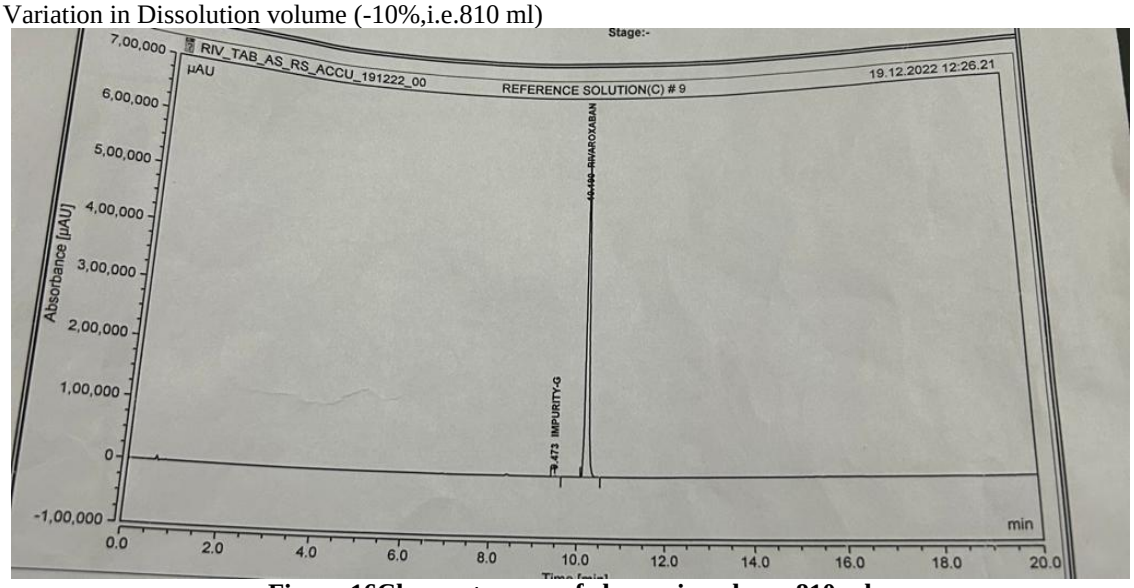

Figure 16Chromatogram of change in volume 810 ml

Result

Figure **Name**	**Retention Time(min)**	**Area**	**No. of Theoretical Plates**	**%Drug Release**
Rivaroxaban	10.190	3314597	113226	87%

Table 14 Result of robustness study of rivaroxaban

Parameter	**Conc.(µg/ml)**	**Amount of detected mean area**	**R.T**	**% assay Limit (90-102%)**

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Chromatogram of composition change Flow	-10%	3128244	10.187	93%
Chromatogram of composition change Flow	+10%	3128256	10.193	93%
Column Temperature change	-2	3128265	10.193	93%
Column Temperature change	+2	2831244	10.00	93%
Change in RPM	-2	2801401	10.187	91%
Change in RPM	+2	2934244	9.493	89%
Change in media	+10%	3314596	10.187	88%
Change in media	-10%	3314597	10.190	87%

7.3.4 Detection of limit

The LOD is the lowest limit that can be detected. Based on the standard error of the predicted y value for each x in a regression. The limit of detected (LOD) may be expressed as:

LOD=3.3×STEYXSLOPE

This parameter is not applicable for dissolution study.

7.3.5 Quantitation of limit

The LOQ is the lowest concentration that can quantitatively measured. Based on S.D. deviation of the response and the slope. The quantitation limit (LOQ) may beexpressed as:

LOD=10×STEYXSLOPE

This parameter is not applicable for Dissolution study.

7.3.6 % Recovery

Preparation of Solution:

Standard Stock Solution:

Weigh accurately and transfer 22.08 mg of rivaroxaban working standard into a 200 ml clean and dry volumetric flask and add 100 ml of acetonitrile and sonicate to dissolve make up to the volume of dissolution media and mix.

Standard Solution:

Transfer 5 ml of this solution into 100 ml of volumetric flask and make up the volume

Rivaroxaban stock solution for recovery study:

Weigh accurately 100.4 mg of rivaroxaban WS into a 100 ml clean and dry volumetric flask,and add 50 ml of acetonitrile and sonicate to dissolve.make up the volume with dissolution media and mix.

Sample solution (50%):

Set the parameter of the instrument as mention dissolution condition.transfer 1.5 mg of rivaroxaban and 110 mg placebo in each three vessel containing 1000 ml dissolution medium, which has been equilibrate at 37ºC±0.5ºC and start the dissolution test. At the specified time interval withdraw the sample solution from each vessel and filter through 0.45µm nylon or PVDF syringe filter, by discarding the first 5 ml of filtrate.

Sample solution (100%):

Set the parameter of the instrument as mention dissolution condition. Transfer 2.5 mg of rivaroxaban and 110 mg placebo in each three vessel containing 1000 ml dissolution medium, which has been equilibrate at 37ºC±0.5ºC and start the dissolution test. At the specified time interval withdraw the sample solution from each vessel and filter through 0.45µm nylon or PVDF syringe filter, by discarding the first 5 ml of filtrate.

Sample solution (150%):

Set the parameter of the instrument as mention dissolution condition. Transfer 3.75 mg of rivaroxaban and 110 mg placebo in each three vessel containing 1000 ml dissolution medium, which has been equilibrate at 37ºC±0.5ºC and start the dissolution test. At the specified time interval withdraw the sample solution from each vessel and filter through 0.45µm nylon or PVDF syringe filter, by discarding the first 5 ml of filtrate.

50% Accuracy

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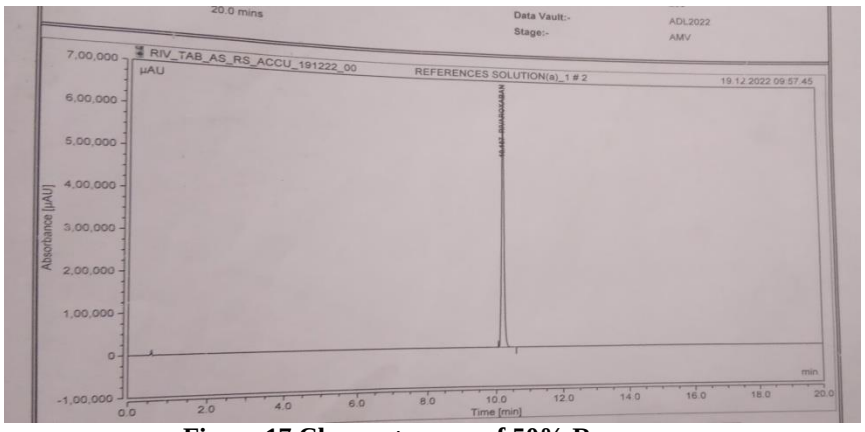

Figure 17 Chromatogram of 50% Recovery

Result-

Name	Retention Time(min)	Area	No. of Theoretical Plates
Rivaroxaban	10.187	1564122	117387

100% Recovery

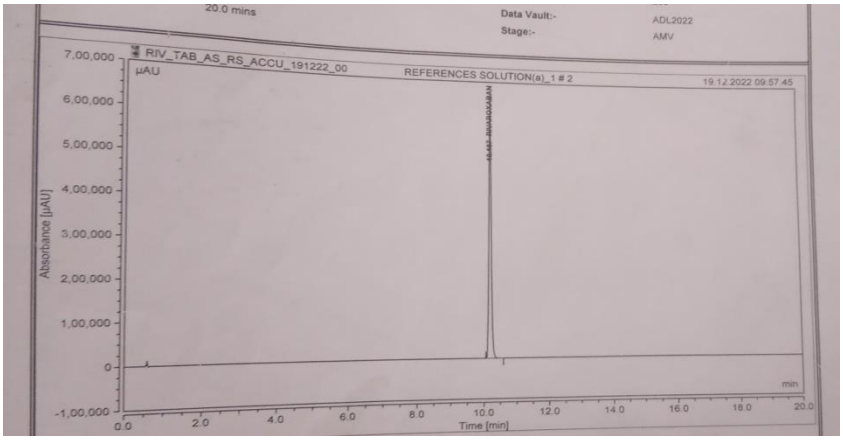

Figure 16 Chromatogram of 100% Recovery

Result-

Name	Retention Time(min)	Area	No. ofTheoretical Plates
Rivaroxaban	10.187	3128244	113226

150% Recovery

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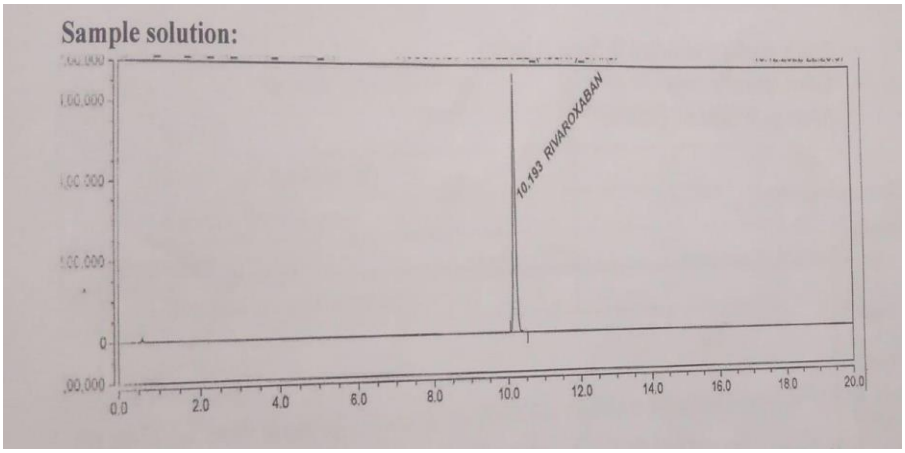

Figure 17 Chromatogram of 150% Recovery

Result-

Name	Retention Time(min)	Area	No. ofTheoretical Plates
RIVAROXABAN	10.193	4692366	112468

Accuracy Results of RIVAROXABAN Tablets2. 5 mg

Sr No	% Recovery Level	Wt.of Placebo (mg)	Amount of Sample Spiked (µg/ml)	Mean Area*	Amount Recovered (µg/ml)	% recovery
1	50	110	1.26	1564122	1.27	95
2	100	110.02	2.51	3128244	2.52	101
3	150	110.03	3.73	4692366	3.75	104

VIII. SUMMARY AND CONCLUSION

The present study was aimed to develop a sensitive, precise, and accurate HPLC method for analysis of RIVAROXABAN bulk drug Dissolution and in pharmaceutical dosage forms. In order to affect analysis of the component peaks, mixtures of methanol and water in different combinations were tested as mobile phase on a RP18 stationary phase. A mixture of Solution A and Solution B in a proportion of 75:25 v/v was proved to be the most suitable of all combinations since the chromatographic peaks were defined and resolved and almost free from tailing. The retention time obtained for rivaroxaban was 10.00 min each of the sample injected three times and reproducible retention times were observed in all cases.

System suitability parameter was studied with six replicate standard solution of the drug and calculated parameters are within the acceptance criteria. The tailing factor and the number of theoretical plates are in acceptable limit. The peak area of apixaban was reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 0.999) was observed between the concentration of rivaroxaban and respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was Y = 18383x- 8057.7 (where Y gives peak area and X is the concentration of drug).

When rivaroxaban solution containing 0.2mg/ml was analyzed the proposed method for finding out intra and inter day variations, % RSD was observed. The high recovery values obtained from the dosage form by the proposed method indicated accuracy. The absence of additional peaks indicated non-interference of common excipients used in tablets. The drug content in tablets was quantified using the proposed analytical method. The tablets were found to contain an average of 95-104% of the labeled amount of the drug. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and % assay. This indicates that the present method is robust. The lowest value of LOD and LOQ was obtained by the proposed method indicate the method is sensitive.

Hence the author proposed that the present HPLC method was sensitive and reproducible for the analysis of rivaroxaban in pharmaceutical dosage forms with short analysis time.

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