

METHOD DEVELOPMENT AND VALIDATION FOR ESOMPRAZOLE SODIUM INJECTION BY RP-HPLC

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CHAPTER 1

INTRODUCTION

1) INTRODUCTION

Esomeprazole sodium belongs to the class of proton pump inhibitors (PPIs). It is omeprazole's enantiomer. Its chemical name is[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl sulfinyl]-1H benzimidazole-1-yl. It works by preventing enzyme activity in the gastric parietal cells, along with it reduces the concentration of gastric acid and delays the flow of hydrogen ions into the stomach. Esomeprazole alone or in combination with other drugs having better pharmacokinetic profile than omeprazole has been estimated using a variety of methods, such as UV and RP HPLC method. The study and skill of identifying materials according to the elements of composition they contain is known as analytical chemistry. The scientific field of pharmaceutical analysis examines the analytical tools for evaluating the purity, safety, and quality of chemicals and pharmaceuticals. It includes methods for figuring out the identity, potency, quality, and purity of novel chemicals. It also includes methods for detecting, sorting, and calculating the proportions of each component in a sample of matter.

Finding the safety and effectiveness of medications is mostly dependent on quality assurance. It contains very specific and sensitive analytical methods for design, as well as pharmacokinetics and drug metabolism investigations, all of which are critical for assessing bioavailability and clinical response. Both quantitative and qualitative analyses of pharmaceuticals and pharmaceutical substances, from bulk to final dose forms, are included in the phrase "pharmaceutical analysis." Therefore, it is employed as a diagnostic tool in contemporary medicine through the examination of chemical components in the human body that may change when a disease is present. A drug product's quality can be defined as the sum of all the factors that influence its dependability, safety, and effectiveness, either directly or indirectly.

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1.1 SIGNIFICANCE OF QUALITY CONTROL

The pharmaceutical sector remains an essential component of the health care system, conducting research and producing sustaining and revitalising goods.

Strict requirements pertaining to the quality, safety, and effectiveness of modern medications are necessary for their use in humans. The availability of appropriate product quality control

methodologies is required for the practical assessment of safety, efficacy, and the preservation of these features.

1.2 ANALYTICAL TECHNIQUES

Only analytical monitoring can guarantee a medication's safety and effectiveness. A medicine's overall purity must be evaluated throughout its quality due to analytical monitoring. A medicine's overall purity needs to be evaluated during usage, distribution, and storage.

The goal might be accomplished if the specifications to be used are founded on a verified process that can show the quality relationship between the chemical being examined and that which was first evaluated pharmacologically and toxicologically.

1.3 OPTICAL METHODS

Some of the optical methods are

- X-ray spectroscopy
- Uv-visible spectroscopy
- Infrared spectroscopy
- Atomic absorption spectroscopy
- Flame photometry
- Nuclear magnetic resonance spectroscopy
- Electron spin spectroscopy

1.4 ELECTRON ANALYTICAL METHODS

Some of the electro analytical methods are

- Amperometry
- Voltammetry
- Potentiometry
- Conductometry

1.5 SEPARATION METHODS/CHROMATOGRAPHY

Some of chromatographic methods are:

- Gas-liquid chromatography
- Gas-solid chromatography
- Liquid-liquid chromatography
- Liquid-solid chromatography

- Thin-layer chromatography
- Paper chromatography
- Gel permeation chromatography

One of the most crucial choices an analyst has to make is which approach is optimal for a certain analysis. He must comprehend the theoretical foundations, the specifics of the application, the situations in which each approach is reliable, and how to overcome these problems in order to accomplish this.

THE INSTRUMENTAL SEPARATIVE TECHNIQUES ARE DIVIDED INTO TWO CATEGORIES.

- 1. Chromatography**
- 2. Electrophoresis**
- 3. Mass spectroscopy**

The colour and graph indicate colour writing. Tweet coined the term when he used a chromatography column to separate colour bands of plant pigments that were transmitted along the tube's length. The stationary phase, also known as the immobile solid or liquid phase, consisted of an adsorbent powder that had been cleaned with a liquid solvent called the mobile phase.

CHROMATOGRAPHY

Chromatography is a method for breaking down a mixture into its component parts according to how well each one can elute through or along the stationary phase using a mobile phase. The sample is placed on the edge of the stationary phase, which has a strong affinity for the stationary phase, and a mobile phase is allowed to flow over it in order to sweep the sample down the length of the stationary phase component. The stationary phase can be either a liquid or a solid. are not as rapidly swept along the length of the stationary phase as those that are adsorbed to it less firmly.

The Greek letter chromos, which means colour, and the word graph, which means colour writing, are the roots of the word chromatography. The word chromatography comes from the Greek letter chromos, which means colour, and the word graph, which means colour writing. The mobile phase travels the entire length of the tube containing the stationary phase, which is a liquid or solid phase that is immovable.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High-performance liquid chromatography (HPLC) was created in the late 1960s and early 1970s based on an understanding of the theoretical underpinnings of previous chromatographic techniques, particularly chromatography in a column. The method employs adsorption partition (including reverse phase partition ion exchange and gel permeation) and the same separation methods as classical column chromatography. In HPLC, the mobile phase is pushed at high pressure through the packed column, which sets it apart from

column chromatography. The primary advantages of HPLC over conventional (gravity feed) column chromatography are improved separation resolution and material quantifiability.

BASIC PRINCIPLE OF HPLC

Variations in the distribution of chemicals in the stationary phase and mobile phase phases are used in the separation process known as high performance liquid chromatography (HPLC). The thin layer that forms on the surface of tiny particles is referred to as the stationary phase, and the liquid that flows over them is referred to as the mobile phase. Every component in a sample has a distinct distribution equilibrium depending on phase solubility and molecule size under a particular dynamic solution.

Consequently, the components move at varying speeds during the stationary phase, causing them to become separated from one another. The column is a tub of stainless steel (or resin) filled with spherical solid particles. A liquid pump continuously feeds the mobile phase into the column inlet at a steady rate. Samples are injected from a sample injector, which is placed close to the column inlet. The injected samples move through it, switching between the stationary phase and the mobile phase.

SELECTIVITY OF HPLC

Most of the drugs can be analysed by HPLC method because of several advantages.

- ◆ Speed (analysis can be accomplished in 20 minutes or less)
- ◆ Greater sensitivity (Various detectors can be utilised.)
- ◆ Improved resolution (wide variety of stationary phase)
- ◆ Reliable columns (wide variety of stationary phase)
- ◆ Ideal for substances of low volatility.
- ◆ Easy sample recovery, handling and maintenance.
- ◆ Easy programming of the numerous functions in each module.
- ◆ Time programming operation sequence, such as initiating operation of detector lamp and pump to obtain stable baseline and equilibrated column.
- ◆ Before the work day begins.
- ◆ Excellent reproducibility of retention time

1.6 VALIDATION

A crucial step in efficient quality assurance is validation. A specific written proof that provides a high degree of assurance regarding the reliable production of a process employing equipment is referred to as validation.

Importance of validation

Due to the examination of a statistically insignificant number of samples, normal quality control cannot always guarantee the product's quality.

The validation should demonstrate that a system or product is adequate and reliable enough to meet predefined requirements, or that each finished product consistently maintains the same level of quality from batch to batch.

Comparing trends or results of cGMP complaints and taking appropriate action in the event of non-compliance are two uses for retrospective validation.

In the pharmaceutical sector, validation is essential to guaranteeing the efficacy, safety, and uniformity of goods and procedures.

In the analytical department, validation is particularly crucial to guaranteeing that the techniques used to assess a drug's quality are trustworthy and supported by science.

This is so because both product quality and patient safety are directly impacted by a drug's quality attributes.

OBJECTIVE OF VALIDATION

The fundamental goal of validation is to provide a standardised system for production and process control that is intended to ensure that the drug products have the identity, quality, and purity that they purport or are represented to possess.

1. Assurance of quality
2. Government regulation

TYPES OF VALIDATION

1. Equipment validation
2. Process validation
3. Cleaning validation
4. Analytical method validation
5. Facility validation including utilities

ANLYTICAL METHOD VALIDATION

Analytical Method Validation (AMV) is a critical process in the pharmaceutical, chemical, and other sectors that ensures a testing method is accurate, dependable, and appropriate for its intended use. This method is often required to comply with regulatory standards established by the International Council for Harmonisation (ICH), the United States Pharmacopoeia (USP), or other global guidelines.

Key Parameters for Validation

Analytical method validation focuses on several parameters, depending on the type of analysis (e.g., qualitative or quantitative)

Importance of Specificity

- ◆ **Product Quality:** Ensures that impurities or degradation products do not interfere with the measurement of the active ingredient.
- ◆ **Regulatory Compliance:** Required for pharmaceutical and chemical testing to meet regulatory guidelines (e.g., ICH, USP).
- ◆ **Accuracy and Precision:** Supports the reliability of quantitative results by ruling out interference from other substances.

Precision in Analytical Method Validation

Definition:

Precision refers to the degree of agreement or consistency among multiple measurements of the same sample under prescribed conditions.

It demonstrates the reproducibility of the analytical method and is typically expressed as the standard deviation (SD), relative standard deviation (RSD, also known as %RSD), or variance.

Types of Precision

1. Repeatability (Intra-day Precision):

- ◆ Assess precision under the same operating conditions over a short time frame.
- ◆ Typically involves analyzing multiple replicates of the same sample on the same day by the same analyst using the same equipment.

2. Intermediate Precision (Inter-day Precision):

- ◆ Evaluates variations within the same laboratory.
- ◆ Includes changes in analysts, equipment, or days of analysis.

3. Reproducibility:

- ◆ Assesses precision across different laboratories.
- ◆ Typically used for collaborative studies or method transfers.

1.7 ACCURACY

Definition

Accuracy is the measure of how close the results of an analytical method are to the true value or accepted reference value of the analyte being measured. It reflects the correctness of the method and its ability to provide an exact result for the intended purpose.

In case of assay of drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which the known amount of analyte have been added within the method. If it is not possible to obtain all product components, it may be acceptable either to add known quantities of the analysis to the

drug product or to compare results with those of a second, well characterized method, the accuracy of which has been stated or defined accuracy studies for drug substance and drug product are recommended to be performed at the 80,100 and 120% level of label claim as stated in the guideline for submitting sampling and analytical data for method validation.

ICH Requirements for Accuracy in Analytical Method Validation

The **International Council for Harmonisation (ICH)** specifies the requirements for accuracy in its guideline **ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology**. These requirements ensure that the analytical method reliably measures the true value of the analyte.

Purpose of Accuracy Testing

- ◆ To demonstrate the reliability of the method to provide results that are close to the true or reference value.
- ◆ To ensure the method is suitable for its intended application, such as assay or impurity quantification.

1.8 LINEARITY

Definition

Linearity refers to the ability of an analytical method to produce results that are directly proportional to the concentration of the analyte within a specified range. It demonstrates that the method provides consistent and predictable responses for varying concentrations of the analyte.

ICH Definition

According to ICH Q2(R1):

"The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample."

1.Proportional Relationship:

A linear method exhibits a straight-line relationship between analyte concentration and the instrument's response (e.g., absorbance, peak area).

1. Assessment Range:

Linearity is evaluated over the range where the method is intended to be used.

2. Statistical Analysis:

- Assessed using regression analysis, with the slope, intercept, and correlation coefficient (R^2) being key metrics.

Linearity Evaluation

1. Concentration Levels:

Prepare at least 5-6 concentration levels (e.g., 50%, 75%, 100%, 125%, and 150% of the target concentration).

2. Plotting Data:

- Plot a graph of the analyte concentration (x-axis) against the response (y-axis).

3. Regression Analysis:

- ◆ Perform linear regression to determine:

Slope

Intercept

Coefficient of determination (R^2)

Acceptance Criteria for Linearity

- ◆ The response should be directly proportional to concentration within the defined range.
- ◆ Residuals (differences between observed and predicted values) should be minimal and randomly distributed.
- ◆ No significant deviation from linearity should occur across the tested range.

Determination of Linearity in Analytical Method Validation

The determination of linearity involves experimentally evaluating the relationship between the concentration of an analyte and the response obtained from the analytical instrument. This is a critical step in method validation to ensure the method reliably measures the analyte across a specified range.

Steps to Determine Linearity

1. Define the Range

- ◆ Select the concentration range over which the method will be used.
- ◆ Typically, this range includes levels around the target concentration (e.g., 50%, 75%, 100%, 125%, and 150%).

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Perform the Analysis

- ◆ Analyze each standard solution using the analytical method.
- ◆ Record the instrument's response (e.g., peak area for chromatography, absorbance for spectroscopy).

1.9 LIMIT OF DETECTION (LOD)

Definition

The Limit of Detection (LOD) is the lowest amount of an analyte in a sample that can be detected but not necessarily quantified as an exact value. It represents the method's sensitivity to detect the presence of the analyte.

Determination of Limit of Detection (LOD)

Determining the Limit of Detection (LOD) involves establishing the lowest concentration of an analyte that can be detected by the analytical method. The LOD is crucial for applications requiring trace-level sensitivity, such as impurity testing or environmental analysis.

ICH Definition

According to ICH Q2(R1):

"The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value."

1.10 LIMIT OF QUANTITATION (LOQ) IN ANALYTICAL METHOD VALIDATION

Definition

The Limit of Quantitation (LOQ) is the lowest concentration of an analyte that can be reliably quantified with acceptable accuracy and precision under the stated experimental conditions. Unlike the Limit of Detection (LOD), the LOQ is the lowest level at which both the detection and accurate quantification of the analyte are possible.

ICH Definition

According to ICH Q2(R1):

"The limit of quantitation (LOQ) is the lowest concentration of an analyte that can be quantitatively determined with suitable precision and accuracy.

Purpose of LOQ

- ◆ To determine the smallest concentration of an analyte that can be measured with confidence and reported within predefined limits for accuracy and precision.
- ◆ It is crucial for methods used in quantitative analysis, where reliable and reproducible measurements are needed at low levels of the analyte.

1.11 RUGGEDNESS

Definition

The ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the analysis of the samples under a variety of condition, such as different, analyst, different instrument, different lots of reagents, different elapsed assay times, different assay temperature, different days etc.

Determination

The ruggedness of an analytical method is determined by the analysis of aliquots from homogenous lots in different laboratories by different analysis, using operational and environmental condition that may differ but are still within the specified parameters of the assay. The degree of reproducibility of the results is that determined as a function of assay variables. This reproducibility may be compared to the precision of assay under normal condition to obtain a measure of the ruggedness of the analytical method.

1.12 SYSTEM SUITABILITY SPECIFICATION AND TESTS

The accuracy and precision of HPLC data collected begin with a well-behaved chromatographic system. The system suitability specifications and tests are parameters that provide assistance in achieving this purpose.

It consists of the following factors:

- * Capacity factor
- * Precision/Injection repeatability
- * Relative retention
- * Resolution
- * Tailing factor
- * Theoretical plate number

1. Capacity factor (K')

$$K' = (t_R - t_0 / t_F)$$

The capacity factor is a measure of where the peak of interest is located with respect to the void volume i.e., elution time of the non-retained components.

2. Precision/Injection repeatability (RSD)

Injection precision expressed as RSD (relative standard deviation) indicates the performance of the HPLC which includes the pumping, column and the environmental conditions, at the time the samples are analysed. It should be noted that sample preparation and manufacturing variations are not considered.

3. Relative retention (α)

$$\alpha = K'_1 / K'_2$$

Relative retention is a measure of the relative location of two peaks. This is not an essential parameter as long as the resolution (R_s) is stated.

4. Resolution (R_s)

$$R_s = (t_2 - t_1) / (1/2) (t_1/2 + t_2/2)$$

R_s is a measure of how well two peaks are separated. For reliable

R_s is a measure of how well two peaks are separated. For reliable quantitation well separated peaks are essential for quantitation. This is a very useful parameter if potential interference peaks (s) may be concern.

5. Tailing factor

$$T = W_x / 2f$$

The accuracy of quantitation decreases with increases in peak tailing because of the difficulties encountered by the integrator in determine where/when the peak ends and hence the calculation of the area under the peak. Integrator variables are present by the analyst for optimum calculation of the area for the peak of interest. If the integrator is unable to determine exactly when an upslope for down slope occurs, accuracy drops.

6. Theoretical plate number (N)

$$N = 16(t_R/t_w)^2 L/H$$

Theoretical plate number is a measure of column efficiency, that is, how many peaks can be located per unit run-time of the chromatograph. N- Constant for each peak on the chromatogram with a fixed set of operating conditions.

H- Height equivalent of a theoretical plate.

L- Length of column.

RESEARCH ENVISAGED

2) RESEARCH ENVISAGED

Because the RP-HPLC method is important for drug estimation, the current work concentrated on developing a new, rapid, and sensitive RPHPLC methodology for the simultaneous determination of drugs. A review of The developed method was reviewed for reproducibility and broad relevance in compliance with ICH regulations (Code Q2A, Code Q2B), which are also necessary. The literature revealed that several methods for measuring esomeprazole in pharmaceutical formulations have been described, each with lengthy run durations and solvent use. Developing and validating a successful RP-HPLC technique for esomeprazole injection level determination was the aim of the project. The validation process has been created in accordance with ICH principles.

Pharmaceutical quality is maintained by rigorously inspecting raw materials, in-process samples, and finished products, and one of the many pharmacy specialties that is essential to this process is pharmaceutical analysis.

Although all goods and services should be of the highest calibre, medicinal products must be of highest priority because medicine deals with human life" Maintaining the quality of pharmaceuticals is also mandated by law and is crucial for companies functioning in the competitive market of today.

Drug quality can only be preserved by employing an analytical approach that is very accurate and precise and meets all other validation requirements.

The relative and absolute mistakes identify the precision and accuracy. With a simple method, errors will be reduced. Thus, accuracy and precision may be indirectly correlated with the method's simplicity. When building an analytical method, one of the most important factors should be it's relative simplicity.

Analytical technique validation is a crucial and necessary part of their development or use. It is deeply needed to support industrial product development and regulation, subsequently methods created and used for product analytical monitoring must be validated.

These days, new medications with a combination of drugs rather than just one are being introduced to the market since multi-drug therapy is more effective than single-drug therapy at treating illnesses.

A previous drug extraction from the excipients or a complex separation process is necessary for the analysis of multicomponent formulations in order to estimate the drugs effectively with the necessary accuracy and precision.

- ♦ choosing the right mobile phase and solvent for HPLC analysis. The chosen solvent should not interact chemically with the component of interest and should be easily accessible, affordable, and of HPLC quality.

- ◆ choosing appropriate wavelengths for sampling and detection.
- ◆ Determination of appropriate working concentration range.
- ◆ Study of the nature of formulation and possible measures to avoid interference of the excipients
- ◆ Standardization of developed method and analysis of selected formulation.

Validation of developed method

All the method, which has been developed, shall be validated according to ICH norms. The six pairs of parameters, which are applied to validate the developed method, are as follows:

- ◆ Accuracy/Precision
- ◆ Repeatability/Reproducibility
- ◆ Linearity/Range
- ◆ Limit of detection/limit of Quantitation
- ◆ Selectivity / Specificity
- ◆ Robustness/Ruggedness

CHAPTER 3

REVIEW LITERATURE

Neha A Jain et al., (2011) outlined a precise, specific, and accurate UV spectrophotometric technique for determining naproxen and esomeprazole concomitantly in a laboratory mixture of the two substances. Using methanol as a solvent, the procedures included the area under the curve (AUC) method in the range of 227–237 nm and 296.5–306.5 nm, the simultaneous equation formation at 232 nm and 301.5 nm, respectively, the absorption correction method at 232 nm (max of naproxen), the isoabsorptive point of naproxen & esomeprazole at 239.2 nm, and the absorption ratio method at 301.5 nm.

Pallavi Sripal Reddy et al., (2011) found that the RP-HPLC method for determining the presence of naproxen and esomeprazole in pharmaceutical formulations is straightforward, accurate, economical, and stable. On an X Terra RP-18 Column (150X4.6mm, 5 μ), naproxen and esomeprazole were separated by RP-HPLC using a mobile phase made up of buffer made with 0.005 mole of sodium perchlorate and 5 ml of n-butyl amine in milli-Q grade water with a pH of 8.7 that was combined with acetonitrile and methanol at a flow rate of 1.5 ml/min. The selected wavelength for detection is 305 nm. Esomeprazole and naproxen had retention durations of around three and six minutes, respectively.

Chandrakant Sojitra et al., (2012) released a high-performance liquid chromatography method that is quick, sensitive, specific, and easy to use for the simultaneous measurement of naproxen and esomeprazole in pharmaceutical formulations that contain both drugs. The SUPELCO 516 C18 column (250 x 4.6 μ m, particle size 5 μ m) was used to accomplish the separation. The mobile phase was composed of 0.01M phosphate buffer pH 4.5: acetonitrile: Methanol (40: 50: 10 v/v/v, with the addition of 0.1% triethyl amine) at a flow rate of 0.8 ml/min. The wavelength of detection was 303 nm. The SUPELCO 516 C18 column (250 x 4.6 μ m, particle size 5 μ m) was used to accomplish the separation. The mobile phase was composed of 0.01M phosphate buffer pH 4.5: acetonitrile: Methanol (40: 50: 10 v/v/v, with the addition of 0.1% triethyl amine) at a flow rate of 0.8 ml/min. 303 nm was the wavelength of detection.

A. Jaswanth et al., (2021) created and approved a straightforward, effective, and repeatable RP HPLC analytical technique for determining the amount of esomeprazole in a sterile dosage form. An acetonitrile and buffer combination in a 65:35 ratio was used as the mobile phase for the chromatographic separation on a C8, 250 mm $\times$ 4.6 mm $\times$ 5 μ m column. At 1.5 ml/min, the mobile phase was pumped, and effluent was measured at 280 nm. In the 50–150% μ g/ml range of Esomeprazole, where $r^2 = 0.999$, the technique was linear. The precision relative standard deviation as a percentage was 0.053%. The procedure was verified in accordance with ICH rules and the requirements for robustness, ruggedness, accuracy, precision, linearity, and specificity were all satisfied. Esomeprazole in a sterile dosage form can thus be routinely analysed using this approach without any problems.

Devi Thamizhanban et al., (2019) As a result, the method may be used successfully and interference-free to the regular analysis of esomeprazole in sterile dosage form 500/20 mg, 375/20 mg, using a single unit of tablet" Using Agilent's high performance liquid chromatography and an Xterra RP-18 column, a chromatographic separation was accomplished using the gradient elution technique for mobile phases 1 and 2. The mobile phases were methanol (700:200:100, v/v/v) and perchlorate buffer (pH 8.7): acetonitrile (700:300, v/v). The detection wavelength was set at 305 nm, and the flow rate was kept constant at 1.5 ml/min. Using the described approach, naproxen and esomeprazole were eluted at 3.3 and 6.1 minutes, respectively. The validation of analytical methods was carried out in compliance with the Q2 (R1) criteria of the International Conference on Harmonisation. Using r^2 values of 0.9996 and 0.9997, respectively, the technique was linear for naproxen and esomeprazole in the range of 60–1500 $\mu\text{g/ml}$ and 2–60 $\mu\text{g/ml}$.

For naproxen and esomeprazole magnesium, the sample recoveries were 100.38–101.39% and 99.67–99.94%, respectively, confirming that formulation additives did not interfere with the estimate. The stressed samples were examined using the devised approach, and the forced deterioration tests were conducted. Naproxen and esomeprazole in bulk and commercial combinations can be evaluated sans interference from degradants thanks to the purity angle of the peak being less than the threshold angle.

Farah Khan et al., (2016) esomeprazole (ESO) and diclofenac sodium (DIC) in pharmaceutical dosage forms simultaneously using a newly designed and verified RP-HPLC process. In this research, a BDS Hypersil C18 column (250 4.6 mm, 5 μm) and a mobile phase made of methanol and 50 mM phosphate buffer (35:65) were employed.. The flow rate was set at 1.0 mL/min, and both esomeprazole and diclofenac sodium were detected at a wavelength of 213 nm. A retention time of 4.13 minutes was obtained using etoposide (ETO) as the internal standard. In the range of 25–1000 ng/mL for ESO and 15.5–500 ng/mL for DIC, the procedure was linear. The intra-day and inter-day variability for both medications is less than 3%, and the approach exhibits strong linearity ($r^2 = 0.999$). For ESO and DIC, the limits of quantification were 25 and 15.5 ng/mL, respectively, whereas the limits of detection were 15 and 10.5 ng/mL. The suggested approach exhibits strong resilience, withstanding minor

intentionally altering the mobile phase's pH and flow rate. When used to determine drug concentrations in injection, tablet, capsule, and spiked human plasma dosage forms, the new approach demonstrated good performance and reproducibility.

Rajendra Singh Rajput et al., (2022) The Oxbridge BEH Shield RP18 (4.6 x 250 mm), 5 μm , phosphate buffer pH 7.3, and acetonitrile (740:260 %v/v) as a mobile phase were used to develop a straightforward, specific, and well-defined stability indicating method for the quantitative estimation of esomeprazole in tablet dosage form. This method was successfully validated in accordance with the ICH guideline. The technique was determined to be robust, rugged, precise, linear, and specific. In accordance with ICH recommendations, stress degradation studies were conducted by subjecting the esomeprazole magnesium

delayed release capsules to acidic, alkaline, oxidative, thermal, humidity, and photolytic stress conditions.. In several in vitro tests, a clog-free dispersion-delivery technique emerged by passing esomeprazole pellets dispersion through feeding tubes with mild syringe pressure. Testing was done using a nasogastric tube (8-French [Fr]) and diluents (various water pHs, such as 5.5, 7.0, and 8.5). Using water as a tube delivery medium, the results demonstrated good esomeprazole pellet delivery. The recovery of the esomeprazole pellets dispersion in 8-Fr nasogastric tubes at varying water pH values (pH 5.5, 7.0, and 8.5) at "0 and 15" minutes of incubation was about 100%.

Raja Haranadha Babu Chunduri et al. (2016) A high throughput ultra pressure liquid chromatography–mass spectrometry (UPLC–MS/MS) method has been developed and validated for the simultaneous quantification of levosulpiride, rabeprazole, and esomeprazole in human plasma using lansoprazole as the internal standard (IS). This method has good sensitivity and selectivity. The methyltertbutylether ethylacetate (80:20 v/v) extraction procedure, which has a high recovery rate, was utilised to extract the analytes and IS from 50 mL of human plasma. On a Hypersilgold C18 column, chromatographic separation of the analyte and IS was accomplished using a gradient mobile phase made of 2 mM Mammmonium formate/acetonitrile.

Khandokar Farjana Urmi et al. (2022) outlined the creation and verification of a novel, precise, accurate, and specific stability-indicating RP-HPLC method for the simultaneous measurement of Naproxen (NAP) and Esomeprazole (ESP) in a modified-release bi-layer tablet the dosage strategy. The method development exercise established the approach's resilience by implementing the Analytical Quality by Design idea. A photodiode array (PDA) detector was used to track the detection at 280 nm in a C18, 250 × 4.6 mm ID, and 5 µm particle size column with a 10 µl injection volume. The column oven was kept at 30 °C, the flow rate was kept at 1.5 ml/min, and the mobile phase was made up of 50:50 (v/v) buffer to methanol. ESP and NAP were found to have retention durations of 8.9~0.1 and 5.9~0.1 minutes, respectively. The approach's accuracy, linearity, precision, specificity, system appropriateness, and solution stability were all evaluated. Over the concentration range of 8–12 µg/ml for ESP and 200–300 µg/ml for NAP, linearity was noted, and the correlation coefficient (R²) became apparent to be excellent > 0.999. Since the process was unique to ESP and NAP, the finest purity for ESP was 99.97%, while for NAP it was 100.00%. The technique was accurate, with a %RSD below 2. For ESP and 99, the accuracy recovery trial with placebo fell between 99.63 and 100.36%. When determining Esomeprazole and Naproxen simultaneously in a normal laboratory analysis, this that have been suggested swift, reliable, and economical procedure can be utilised as a quality control tool.

CHAPTER 4

DRUG PROFILE

4) Drug Profile - Esomeprazole sodium

♦ Molecular structure

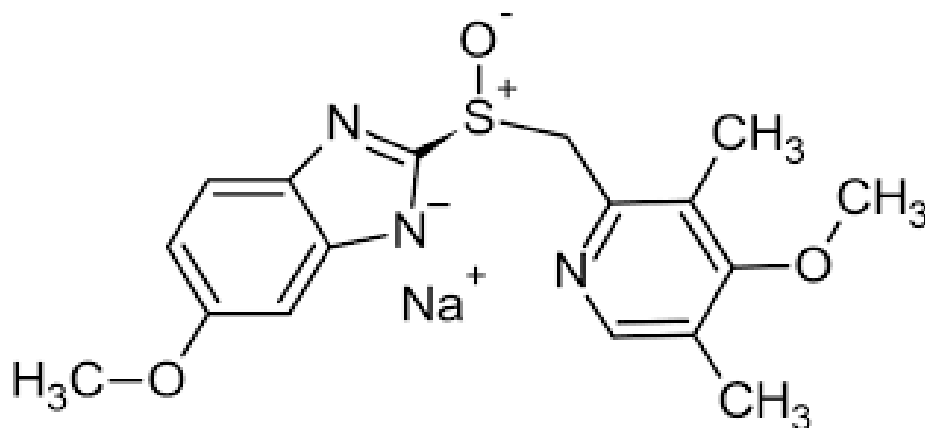


Figure 1: Molecular structure of esomeprazole.

- ♦ **Chemical name** - [(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl sulfinyl]-1H benzimidazole-1-yl.
- ♦ **Molecular formula** - C₁₇H₁₉N₃O₃S.
- ♦ **Molecular weight** - 367 Daltons
- ♦ **Description-** A solid that is off-white to light yellow in colour
- ♦ **Solubility** - Soluble in water, ethanol, DMSO,
- ♦ **Storage** - Store in well-closed containers, protect from moisture and humidity.
- ♦ **Melting point** - 229 °C
- ♦ **Category** - Proton Pump Inhibitor (PPI)
- ♦ **Pharmacology**

Mechanism of action-Esomeprazole exerts its stomach acid-suppressing effects by preventing the final step in gastric acid production by covalently binding to sulfhydryl

groups of cysteines found on the (H⁺, K⁺)-ATPase enzyme at the secretory surface of gastric parietal cells. This effect leads to inhibition of both basal and stimulated gastric acid secretion, irrespective of the stimulus. As the binding of esomeprazole to the (H⁺, K⁺)-ATPase enzyme is irreversible and new enzyme needs to be expressed in order to resume acid secretion, esomeprazole's duration of antisecretory effect that persists longer than 24 hours.

Indication and use

Esomeprazole injection is used to treat conditions where there is too much acid in the stomach. It is used for the short-term treatment (up to 10 days) of gastroesophageal reflux disease (GERD) with erosive esophagitis in adults and children 1 month of age and older. GERD is a condition where the acid in the stomach washes back up into the esophagus. This medicine is also used to lower the risk of rebleeding in patients with acute gastric or duodenal ulcer after endoscopy.

Esomeprazole is a proton pump inhibitor (PPI). It works by decreasing the amount of acid that is produced by the stomach.

This medicine is to be given only by or under the direct supervision of your doctor.

This product is available in the following dosage forms:

- ◆ **Dose- 20 mg or 40 mg.**
- ◆ **Pharmacokinetic-**Esomeprazole works by covalently attaching itself to the sulfhydryl groups of cysteines on the (H⁺, K⁺)-ATPase enzyme at the secretory surface of gastric parietal cells, which inhibits the last stage of gastric acid generation.. Esomeprazole works by covalently attaching itself to the sulfhydryl groups of cysteines on the (H⁺, K⁺)-ATPase enzyme at the secretory surface of gastric parietal cells, which inhibits the last stage of gastric acid generation.
- ◆ **Absorption** -
Peak plasma levels (C_{max}) happen about 1.5 hours after oral treatment (T_{max}). The area under the plasma concentration-time curve (AUC) increases threefold from 20 to 40 mg, and the C_{max} rises proportionately with increasing dose. Repeated once-daily dosage with 40 mg results in approximately 90% systemic bioavailability, compared to 64% following a single dose of 40 mg. The mean exposure (The AUC for esomeprazole increases from 4.32 µmolhr/L on Day 1 to 11.2 µmolhr/L on Day 5 after 40 mg once day dose. as a single 40 mg dose of Esomeprazole is administered with food, the AUC decreases by 43% to 53% as compared to fasting. Esomeprazole should be taken at least one hour before meal
- ◆ **Distribution** -
Plasma protein binding-Esomeprazole has a 97% binding rate to plasma proteins and remains stable at concentrations ranging from 2 to 20 µmol/l.
- ◆ **Apparent volume of distribution-**In healthy participants, the apparent volume of distribution at steady state is approximately 16 L.
- ◆ **Renal function-**Esomeprazole's pharmacokinetics are unaffected by renal function changes.

- ◆ **Liver cirrhosis**-Patients with hepatic cirrhosis may notice a small delay in the time to peak plasma concentration.
- ◆ **Hepatic dysfunction**-Esomeprazole's pharmacokinetics are unaffected by mild to moderate hepatic impairment. Severe hepatic impairment may increase the AUC and half-life of esomeprazole.

Dosage form

Table 1: Characteristics of drug

Brand name	Formulation	Contents	Manufacturer
Espentral	Injection	40mg/100ml	Skymap healthcare



Figure 2: Esomeprazole Sodium Injection

CHAPTER 5

PLAN OF WORK

1. Pre-formulation studies of drug

- ◆ Solubility
- ◆ Melting point
- ◆ Ph

2. Identification of pure drug

- ◆ Fourier transform infrared spectroscopy (FTIR)

3. Selection of solvent for method development

- ◆ Determination of wavelength

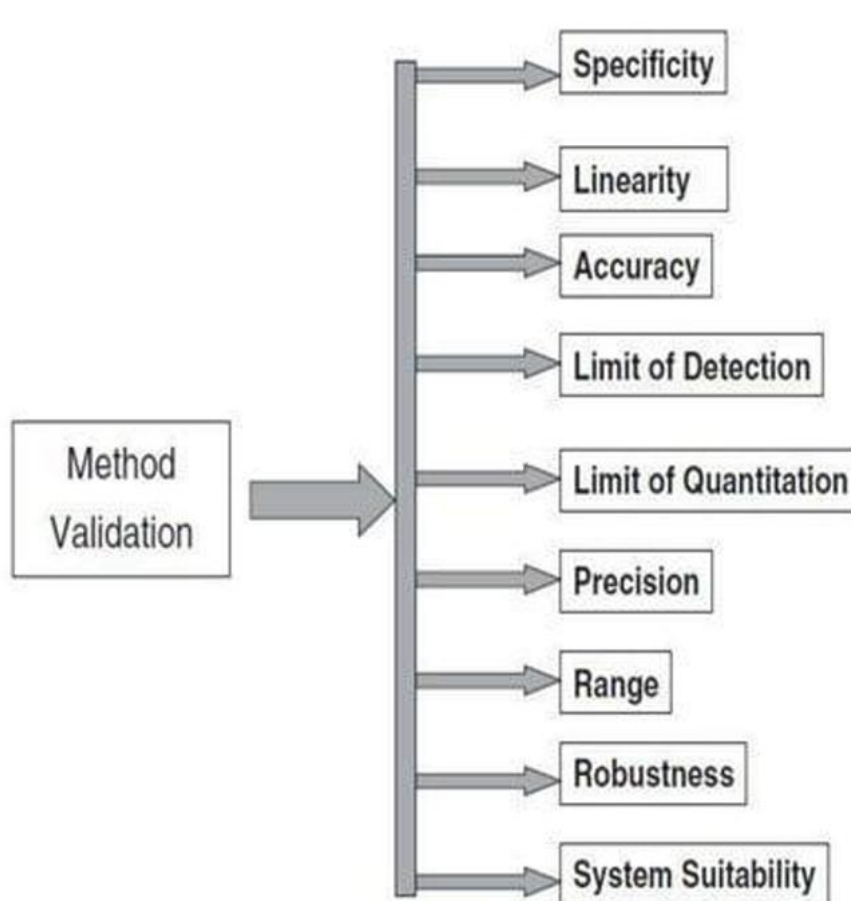


Figure 3: Aspects of Method Validation

Drug analysis is crucial to the discovery, production, and therapeutic use of drugs. Several appropriate methods are used, such as UV spectrophotometers, HPLC, and HPTLC, to simultaneously estimate the amount of drug contained in dose forms. etc. These are jagged and potent approaches. They also have excellent linearity, speed, and specific precision.

A pharmaceutical company relies on qualitative chemical analysis to ensure that the raw materials utilised and the final product produced fulfil the required specifications. The medications will come in single or multi-component dose forms. They proved to be successful because of their combined mechanism of action on the body. The number of medicine or drop formulations introduced into the market is expanding at a rapid pace. . These may be either new entire in the market or structure modification of the exciting drug or Nobel

doses form or multiple component dosage form the complex in the doses form including that of the multi component do this phone create a significant challenge to the electrical chemistry during the development of essay produce for its accurate estimations. Estimating individuals in many component dosas might be challenging due of the extraction or isolation technique.

Selection of detect detector wavelength.

Selection of extraction procedure.

Optimization of chromatographic condition.

Estimation of esomeprazole sodium

5.1 Materials and methods

Instrumentation:

S.No.	Name of Instrument	Model	Make
1	Semi micro balance	CPA225D	Sartorius
2	pH meter	Metlet Toledo	Thermos orion
3	HPLC	LC-20AT	Shimadzu
4	C 18 Column	Phenomenex	Spectro lab
5	Sonicator	USB	Shimadzu

Table 2: List of Instruments used in method validation.

Chemicals and reagents

S.No.	Chemicals/reagents	Make/grade
1	Glacial acetic acid	Merck (hplc grade)
2	Dipotassium hydrogen phosphate	Merck (GR grade)
3	Methanol	Merck (GR grade)
4	Water	Merck (GR grade)

Table 3: List of Chemicals and reagents used in method validation.

Working/ reference standards

S.No.	Name of working/ reference standards	% purity
1	Esomprazole sodium	99.56

Table 4: Working/ reference standards of esomeprazole sodium

5.2 POLARITY OF COMMON ORGANIC FUNCTIONAL GROUPS AND SOLVENTS

Selection of wavelength for detection of components

Solution of esomeprazole sodium were scanned in the UV region and spectrum was recorded. The solvent used was 0.02M dipotassium hydrogen phosphate, and acetonitrile in the ratio 55:45 It was seen that at 260nm all compounds have good absorbance, which can be used for the estimation of compounds by HPLC.

5.3 OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

1. Selection of wavelength for detection of components.

A spectrum was recorded after an Esomprazole sodium solution was scanned in the ultraviolet spectrum. Acetonitrile in a 55:45 ratio and 0.02M dipotassium hydrogen phosphate were the solvents utilised. It was observed that all compounds had high absorbance at 280 nm, which can be utilised for HPLC compound determination.

Selection of chromatographic method

The sample's molecular weight, stability, pKa value, and type (ionic, ionisable, or neutral molecules) all influence the process choice. Ion exchange chromatography or reversed phase chromatography can be utilised because the medicines chosen for this study are polar. Due to its ease of use and the presence of Esomprazole sodium, reverse phase HPLC was chosen for the initial separation. Phenomenex Gemini C18 (250 x 4.6mm) 5 μ column was selected as the stationary for the literature review based on understanding of the characteristics of the chosen medications. phase and mobile phase of various compositions, including acetonitrile, were employed. The use of buffer was decided upon as the separations were not noticed.

To get around the original separation condition, all available, observed, and collected data were included.

Initial separation condition

The following chromatographic conditions were fixed initially to improve the separation of both drugs.

Instrument: Shimadzu prominence

Column: Phenomenex Gemini C18 (250 mm x 4.6mm), 5 μ .

Column oven temperature: Ambient

Wavelength: 280 nm

Flow rate: 1.5 ml/min

Injection volume: 20 μ l

Run time: 10 min

Mobile phase: 40:60

Solvent B - Methanol

Solvent C - Acetonitrile

Solvent Ratio: 40:60% v/v

Trail-1

The trail was performed using Mobile phase in the ratio 40:60 using Phenomenex C8 (250mm x 4.6 mm, 5 μ) with flow rate of 1.5ml/min.

In this trail, the retention time of peak was found to be 0.9 and 4.0 min respectively.

Trail-2

The trail 2 was performed using Mobile phase in the ratio 40:60 using Phenomenex C8 (250x 4.6 mm, 5µm) with flow rate of 1.5 ml/min.

In this trail, the retention time of Esomprazole sodium and peak was found to be 0.7 and 2.4 min respectively.

Trial-4:

The trail 4 was performed using Mobile phase in the ratio of 40:40:20 using Phenomenex (250 x 4.6 mm, 5µ) with flow rate of 1 ml/min.

In this trail, only two peaks were shown at 2 and 3.2 min.

Out of 4 trails made in the lab, the 3rd trail was selected for further studies because when compared to other trails, the 3rd trail was found to be having less retention time and within the acceptance criteria.

Effect of ratio of mobile phase

Under the chromatographic conditions mentioned above, the different ratios of mobile phase were tried.

The chromatograms were observed for each of the trials, out of which 30:35:35

i.e., 30 Buffer: 35 Methanol: 35 Acetonitrile was selected as the separation was achieved in minimum retention time.

Preparation of Buffer Solution

Dissolve 6.8 g of potassium dihydrogen orthophosphate & 1 gm NaOH in 1000 ml of water, adjusted to pH 7.0 with orthophosphoric acid or sodium hydroxide solution.

Mobile phase

40 volume of buffer solution: 60 volume of methanol.

Reference Solution

igh about 150 mg of Esomeprazole Sodium Sterile RS/WS in 100 ml volumetric flask, add about 60 ml methanol sonicate to dissolve and make up the volume up to 100 ml with methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.

Test Solution

Weigh about 150 mg of sample in 100 ml volumetric flask, add about 60 ml methanol sonicate to dissolve and make up the volume up to 100 ml with methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.

System Suitability Solution: Reference Solution

S. No.	System Suitability Parameter	Acceptance Criteria	Result
1.0	Theoretical plates	NLT 2000	OK
2.0	Tailing factor	NMT 2.0	OK
3.0	Relative standard deviation	NMT 2.0%	OK

Table 5: List of System Suitability Parameter.

Specificity:

Specificity of an analytical method is its ability to measure accurately and specifically the analyte in the presence of placebo that are present in the sample matrix.

Placebo solution – Use water for injection as placebo.

Standard solution – Prepare the Standard solution as given in the Assay methodology.

Procedure

Inject single injection (20µl) of Blank (diluent) solution, Placebo solution, Standard solution and the Test solution in the sequence and record the chromatograms. Record the observation in the format

Observation

Sr. No.	Particulars	Area	Result
1.	Blank	0	OK
2.	Placebo solution	0	OK
3.	Standard solution	974556	OK
4.	Test solution	985006	OK

Table 6: List of solutions for specificity test.

Conclusion:

Based on the obtained result it is concluded that there is no interference observed due to blank & placebo solution at the retention time of tranexamic acid solution and standard solution. Hence, the method for the determination % assay of tranexamic acid is specific.

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.

System Precision

Inject the six replicates of the standard solution and record the chromatogram.

Observation:

Sr. No.	Particulars	Retention Time	Area
1.	Standard solution 1	5.950	976092
2.	Standard solution 2	5.982	975848
3.	Standard solution 3	5.980	974371
4.	Standard solution 4	5.982	976808
5.	Standard solution 5	5.992	977640
6.	Standard solution 6	5.988	978912
Average		5.979	976612
Standard deviation		0.021	1566
RSD (%)		0.346 %	0.160
Acceptance criteria: The % RSD of the area of the principal peak from standard solution should not be more than 2.0% and for the retention time should not be more than 1.0%.			

Table 7: List of standard solutions for chromatogram.

Conclusion: The % RSD for the retention time found 0.346 % and for the Area found 0.160 %; hence the system precision study is satisfactory for this method

Method Precision

Prepare six homogenous samples from a single lot of the Injection. Prepare the Test solution and Standard solution as given in the methodology and determine the Assay results.

Inject the solutions as given in the sequence below and record the chromatograms.

Standard solution – Standard solution prepared in specificity test was used.

Test Solution: Mix the content of minimum 3 vials and transfer 2 ml of Esomeprazole for Injection in 200 ml volumetric flask, dilute and make up the volume up to 200 ml with water.

Standard weight: 150.3 mg

Particular	Sample Wt. (mg)	Mean Area Test solution	Mean Area (Standard solution)	Assay (mg)	Assay (%)
Test solution 1	150.5	987518	987045	40.29	
Test solution 2		990644		40.48	
Test solution 3		988377		40.75	
Test solution 4		987400		40.32	
Test solution 5		983968		40.05	

Table 8: List of test solutions

Remarks: The six assay results are found within the specification limit and the % RSD of six Assay results is found within the limit; hence the method is precise in this study.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness; Accuracy should be established across the specified range of the analytical procedure.

Accuracy is usually demonstrated by adding known amounts of analyte(s) to the sample matrix and determining the measured result using the analytical procedure. The recovery of measured against actual amounts is then calculated. Usually a minimum of three determinations at each of three concentrations across the intended range, is recommended.

Spike the amount of the active ingredient Esomeprazole for Injection with the placebo in the proportion given below. Prepare the samples (about 10 units) for 50%, 100% and 150% of label claim of Tranexamic acid in the injection.

Spike the Working Standard with placebo in the following manner:

- 50% - 75 mg of Esomeprazole Sodium with 100 ml of methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.
- 100% - 150 mg of Esomeprazole Sodium with 100 ml of methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.
- 150% - 225 mg of Esomeprazole Sodium with 100 ml of methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.

Accuracy Solution preparation

- Prepare the Standard solution as given in the methodology. Prepare the test solutions at each accuracy level i.e. 50%, 100% and 150% as given in the methodology. Carry out the recovered amount as per methodology and % recovery of spiked active amount as given below:
- **Calculations:**

Calculate the content of C₁₇H₁₈N₃O₃S. Na in the injection.

$$\frac{\text{Test Sol area} \times \text{Std. Wt.} \times \frac{5}{100} \times \frac{50}{100} \times \frac{\text{Purity of WS (OSB)}}{100}}{\text{Filled wt.}} \times \text{Avg}$$

$$\frac{\text{Ref. Sol. area} \times 100 \times 50 \times \text{Test Wt.} \times 5 \times 100}{\text{Filled wt.}}$$

$$= \text{-----mg/mg}$$

$$\text{Assay\%} = \text{-----} \times 100$$

Label Claim

Observation:

Particular	Spiked amount of Esomeprazole Sodium (mg)	Mean Area (Standard solution)	Recovered amount (mg)	Recovery (%)	Mean Recovery (%)
50% Accuracy solution 1	74.5	1002848	39.05	97.63	97.76
50% Accuracy solution 2			38.99	97.47	
50% Accuracy solution 3			39.27	98.18	
100% Accuracy solution 1	149.8		38.83	97.08	97.07
100% Accuracy solution 2			38.83	97.08	
100% Accuracy solution 3			38.82	97.05	
150% Accuracy solution 1	224.6		39.41	98.52	97.94
150% Accuracy solution 2			39.41	98.52	
150% Accuracy solution 3			38.72	96.80	
Acceptance criteria: The mean recovery % at each accuracy level should be between 95% to 105%					

Table 9: Observation table for accuracy of solutions.

Remarks: The accuracy study found within the acceptance criteria

Linearity & Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Linearity is usually demonstrated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares.

Standard stock solution preparation

- ◆ 50% - 75 mg of Esomeprazole Sodium with 100 ml of methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.
- ◆ 80% - 120 mg of Esomeprazole Sodium with 100 ml of methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.
- ◆ 100% - 150 mg of Esomeprazole Sodium with 100 ml of methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.
- ◆ 120% - 180 mg of Esomeprazole Sodium with 100 ml of methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.
- ◆ 150% - 225 mg of Esomeprazole Sodium with 100 ml of methanol. Further dilute 5 ml of this solution to 50 ml with mobile phase.

Linearity Level	Actual Concentration (ppm)	Area	Standard weight (mg)	Correlation coefficient
50%	75	485684	150.2	0.9999
80%	120	775048		
100%	150	989526		
120%	180	1156908		
150%	225	1441502		
Acceptance criteria: The correlation coefficient value should be greater than or equal to 0.999				

Table 10: List of Linearity level.

Linearity Graph attached.

Range

The range of an analytical method is the range of analyte concentrations (amounts) between the upper and lower limits for which it has been shown that the analytical process is sufficiently linear. The required range is often determined via linearity studies and is contingent upon the procedure's intended use.

It is determined by verifying that, when applied to samples that contain analyte quantities within or at the extremes of the analytical method's stated range, the analytical procedure offers an acceptable degree of linearity.

Graph Attached for Range in 80% to 120% of concentration level and plot line of regression.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness is usually demonstrated by making small deliberate changes to one of the operating parameters of the method, analysing samples and comparing the results to those obtained using the prescribed method. The following variations shall be made in the method parameters and inject the Standard solution and record the chromatograms.

1.Flow rate – 1.5 ± 0.1 ml/minute

2.pH – 7.0 ± 0.5

3.Mobile Phase Composition - 600: 400 to 680:392 & 592:408 (Methanol: Buffer)

Record the observation in the format given below

Robustness (Original method parameters for Flow Rate)

	Area	Assay (mg)	Assay (%)
Standard solution	971746	41.38 mg	103.44
Test solution	1021591		

Table 11: Robustness (Original method parameters for Flow Rate)

Robustness (After change in Flow rate -0.1 ml/minute i.e. 1.4ml/min)

	Area	Assay (mg)	Assay (%)
Standard solution	1041415	41.14	102.86
Test solution	1087246		

Table 12: Robustness (After change in Flow rate -0.1 ml/minute i.e. 1.4ml/min)

	Area	Assay (mg)	Assay (%)
Standard solution	906223	40.62	101.55
Test solution	934037		

Result of Robustness

	Assay (%)
Without variation	103.44
Flow change (1.4 ml/min)	102.86
Flow change (1.6 ml/min)	101.55

Table 13: Result of Robustness

	Area	Assay (mg)	Assay (%)
Standard solution	960834	42.30	105.75
Test solution	1011704		

Table 14: Robustness (Original method parameters for pH)

	Area	Assay (mg)	Assay (%)
Standard solution	964544	42.20	105.49
Test solution	1015131		

Table 15: Robustness (After change in pH -0.5 ml i.e 6.95)

	Area	Assay (mg)	Assay (%)
Standard solution	963521	43.81	109.51
Test solution	1100017		

Table 16: Robustness (After change in pH +0.5 ml i.e 7.05)

Result of Robustness

	Assay (%)
Without variation	105.75
pH 6.95	105.49
pH 7.05	109.51

Table 17: Robustness (Original method parameters for Mobile Phase, 600: 400)

	Area	Assay (mg)	Assay (%)
Standard solution	966502	41.19	102.98
Test solution	1017698		

Table 18: Robustness (After change in Mobile Phase, 608: 392)

1.	Area	Assay (mg)	Assay (%)
Standard solution	964287	41.14	102.86
Test solution	1018891		

Table 19: Robustness (After change in Mobile Phase, 592: 408)

2.	Area	Assay (mg)	Assay (%)
Standard solution	965225	40.74	101.84
Test solution	1016490		

Result of Robustness

	Assay (%)	% variation from original
Without variation	102.98	
pH 6.95	102.86	0.12
pH 7.05	102.72	

Acceptance criteria: The system suitability parameter should meet after each variation and the % RSD of Assay should be not more than 1.0% from original parameters.

Remarks: The robustness study found within the acceptance criteria.

Solution Stability

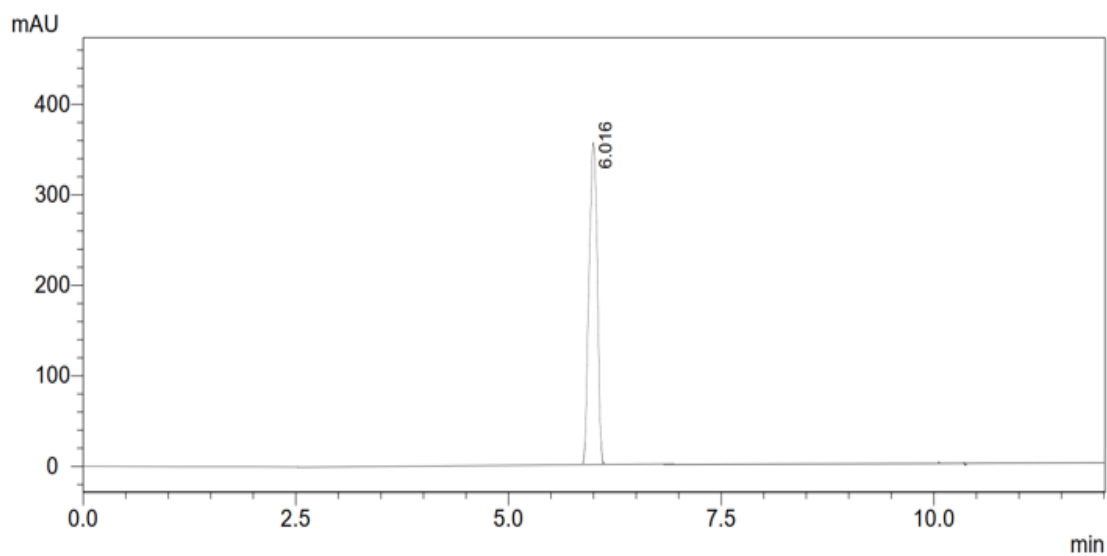
The stability of analyte in the solution should be demonstrated to show the solution is stable for the required time period at specific condition. Stability of solution are carried out as that the 100 % concentration of assay solution in different intervals 0hr, 4hr, 8 hr, 24hr, 30 hr, 36hr & 48hr at room temperature.

CHROMATOGRAM -1

SAMPLE NAME : STANDARD

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA



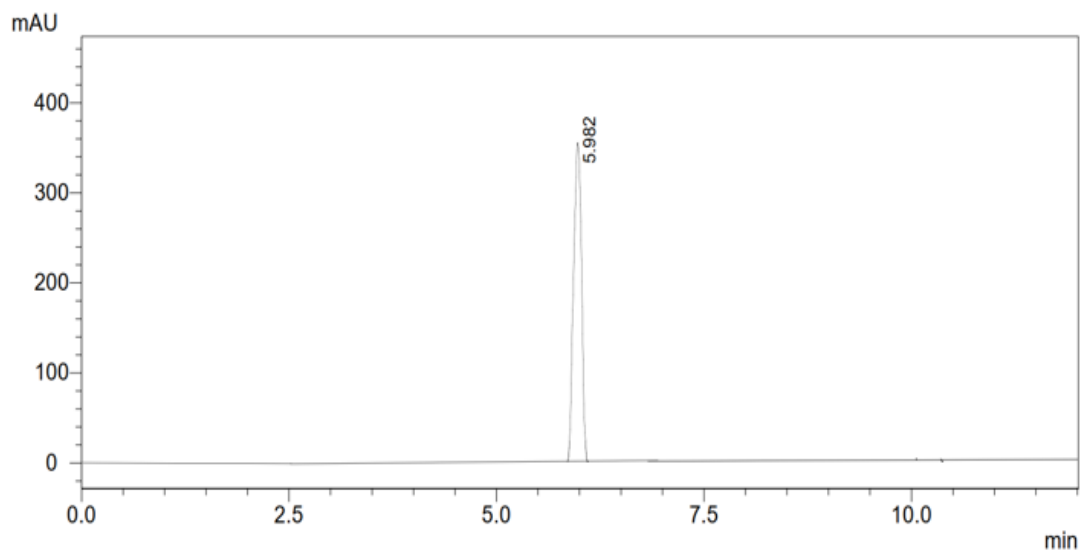
S.NO	Reten time	Area(mv)	Area(%)
1	6.016	987518	100

CHROMATOGRAM -2

SAMPLE NAME : ASSAY

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA



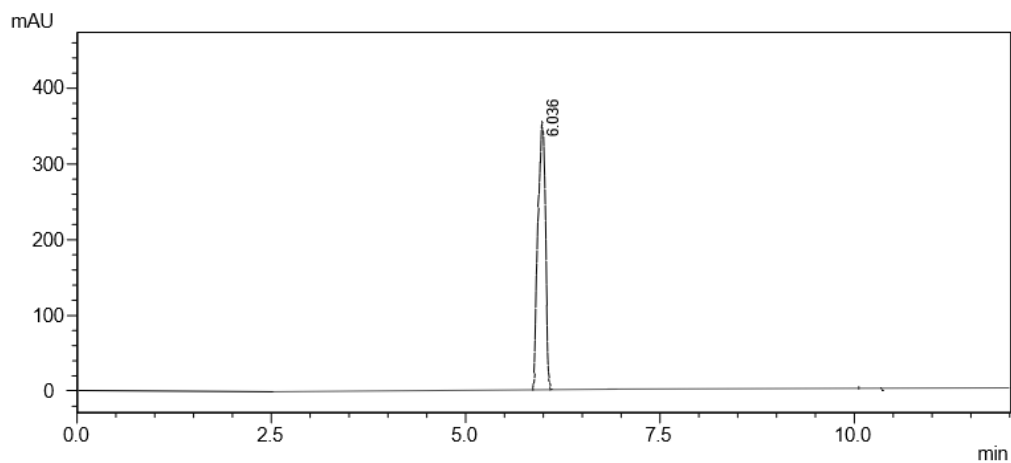
S.NO	Reten time	Area(mv)	Area(%)
1	5.982	987518	100

CHROMATOGRAM -3

SAMPLE NAME : SYSTEM PRECISION 1

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA



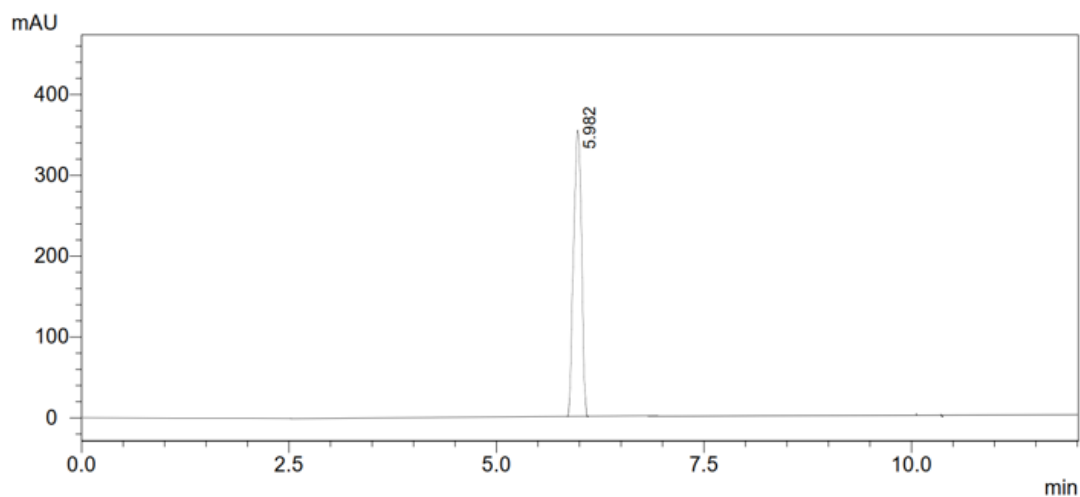
S.NO	Reten time	Area(mv)	Area(%)
1	6.026	976092	100

CHROMATOGRAM -4

SAMPLE NAME : SYSTEM PRECISION-2

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

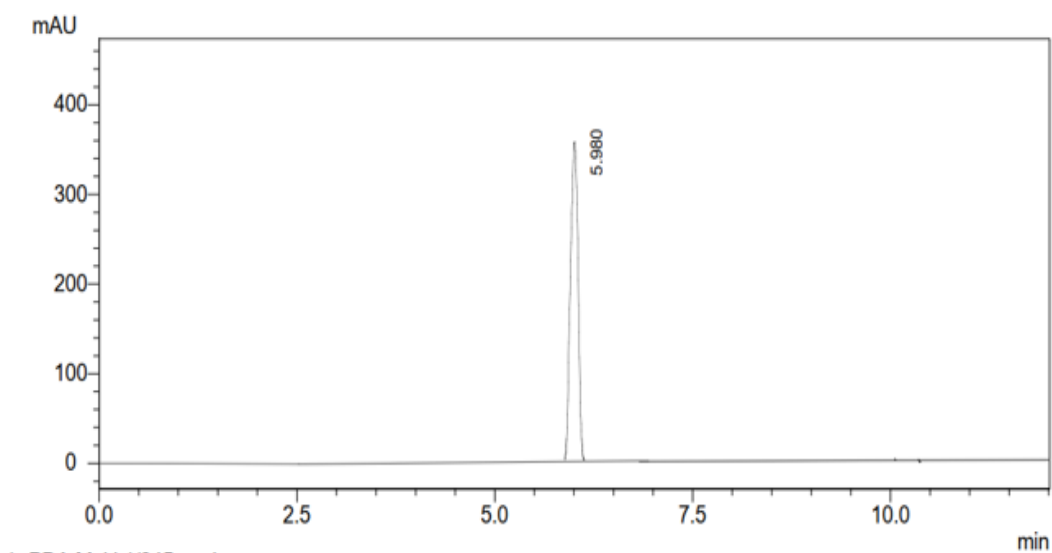


S.NO	Reten time	Area(mv)	Area(%)
1	5.982	975848	100

SAMPLE NAME : SYSTEM PRECISION-3

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

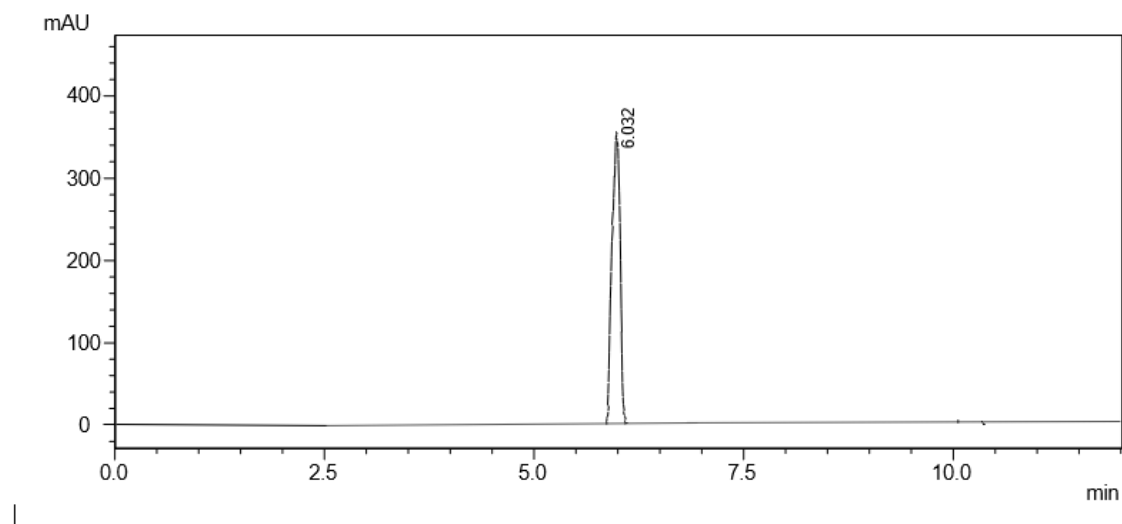


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	5.980	974371	100

SAMPLE NAME : SYSTEM PRECISION-4

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

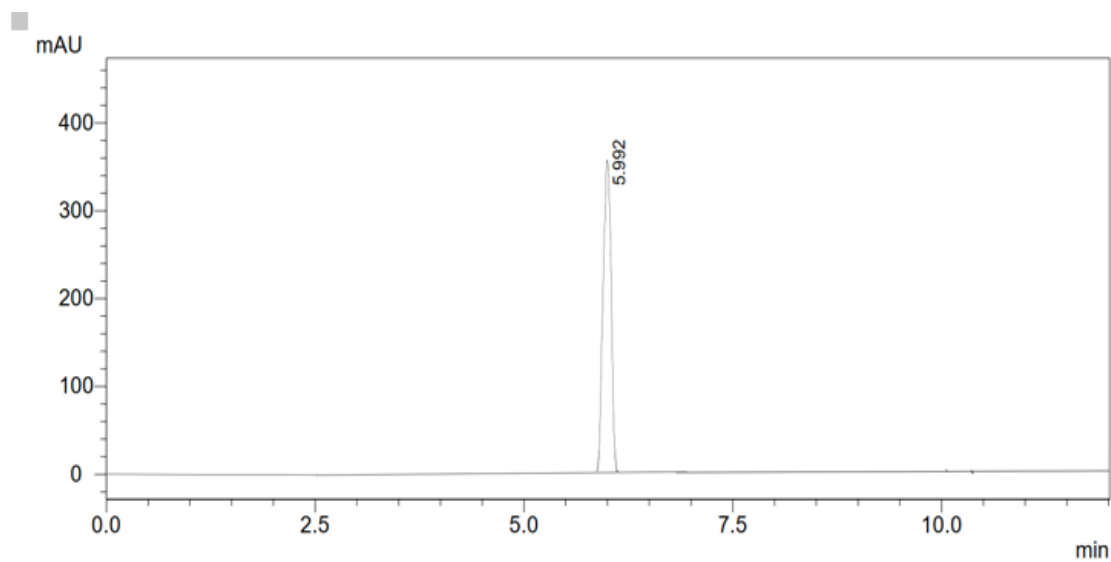


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.032	976808	100

SAMPLE NAME : SYSTEM PRECISION-5

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

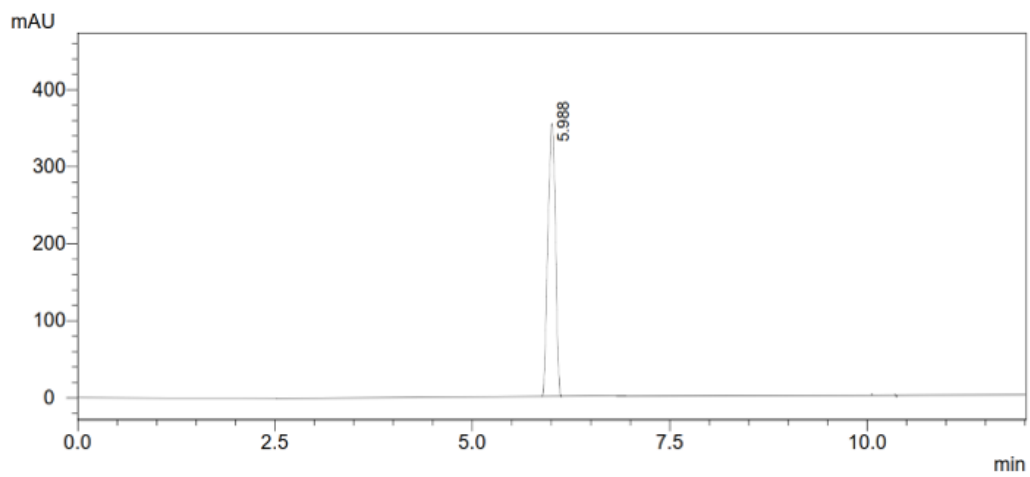


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	5.958	977640	100

SAMPLE NAME : SYSTEM PRECISION-6

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

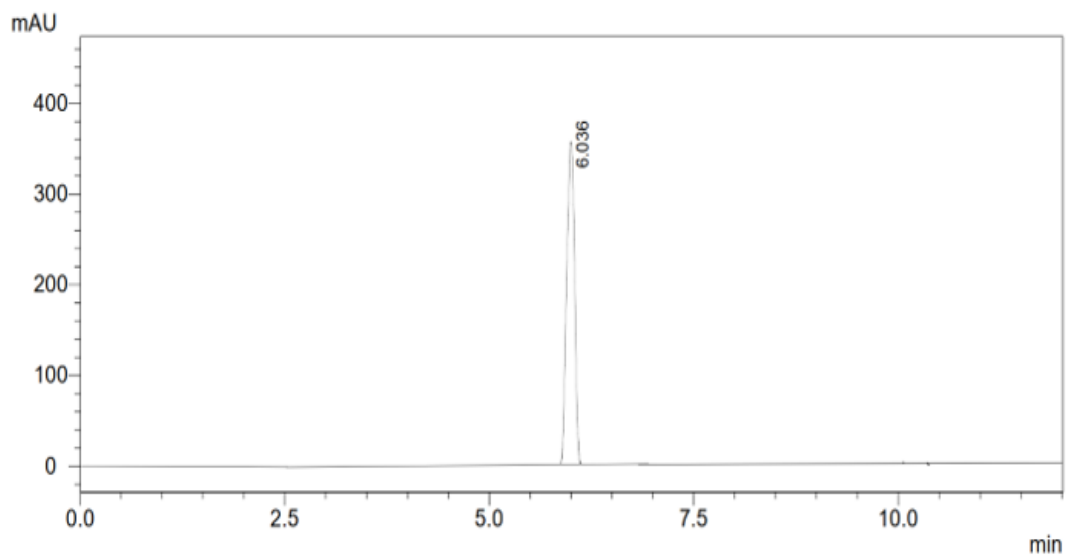


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	5.988	978912	100

SAMPLE NAME : LINEARITY 1

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

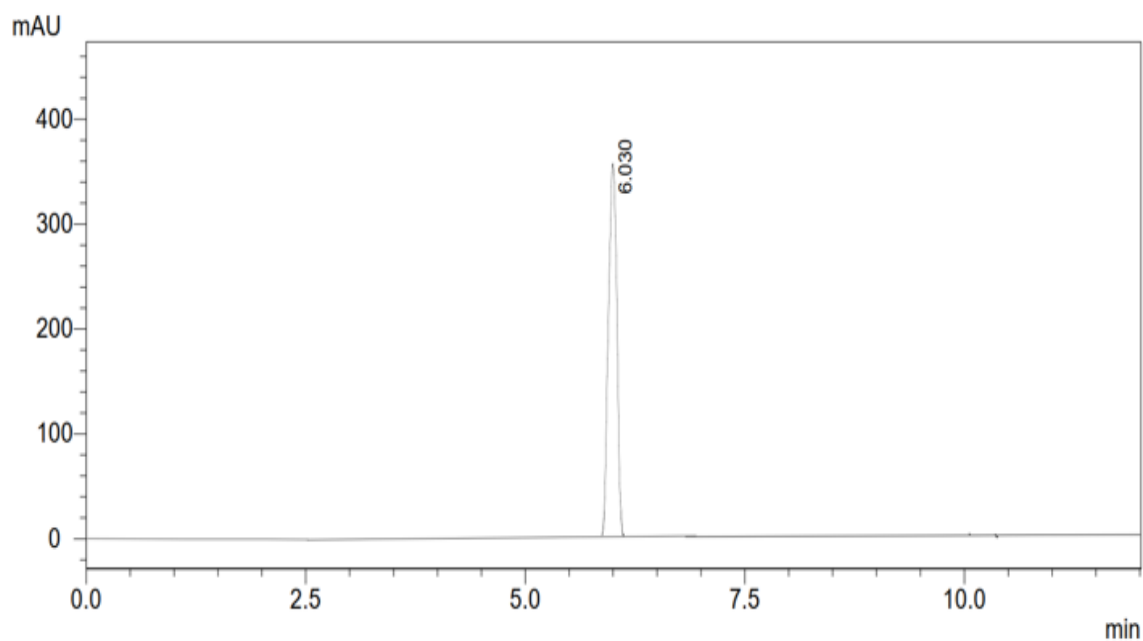


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.036	987518	100

SAMPLE NAME : LINEARITY 2

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

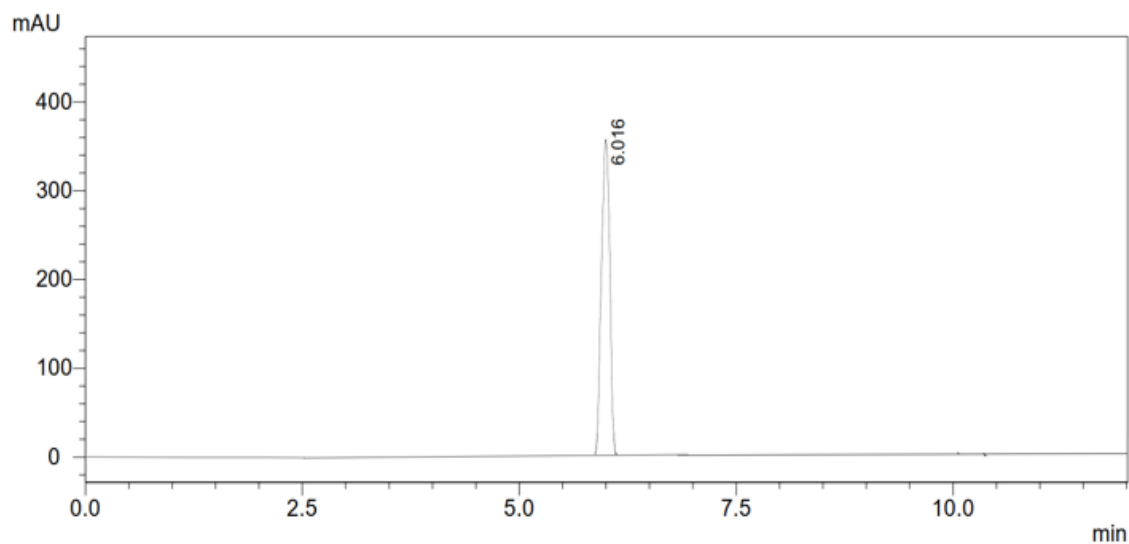


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.030	987518	100

SAMPLE NAME : LINEARITY 3

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

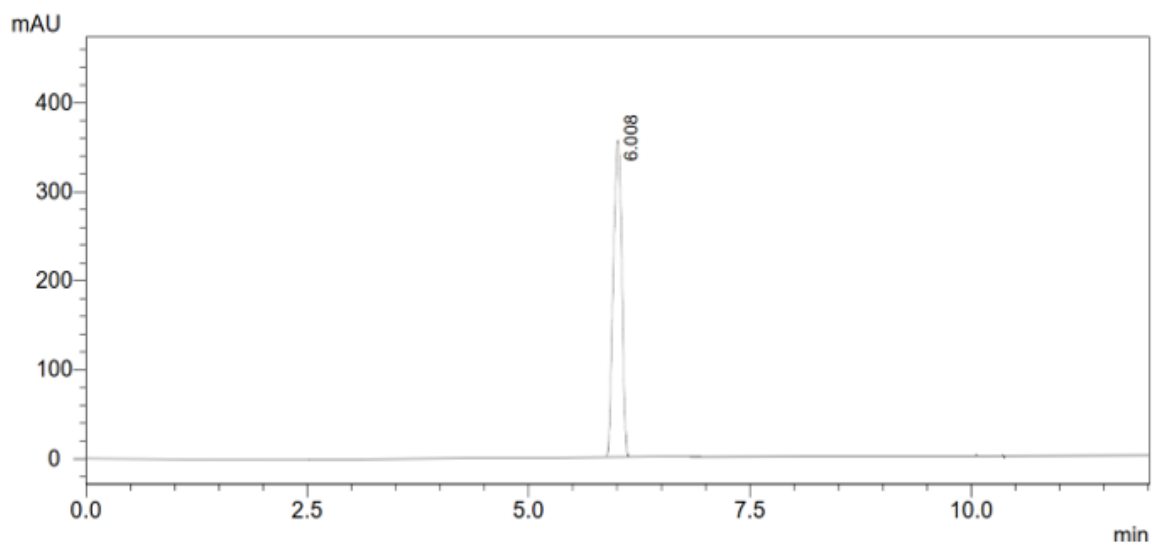


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.016	987518	100

SAMPLE NAME : LINEARITY 4

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

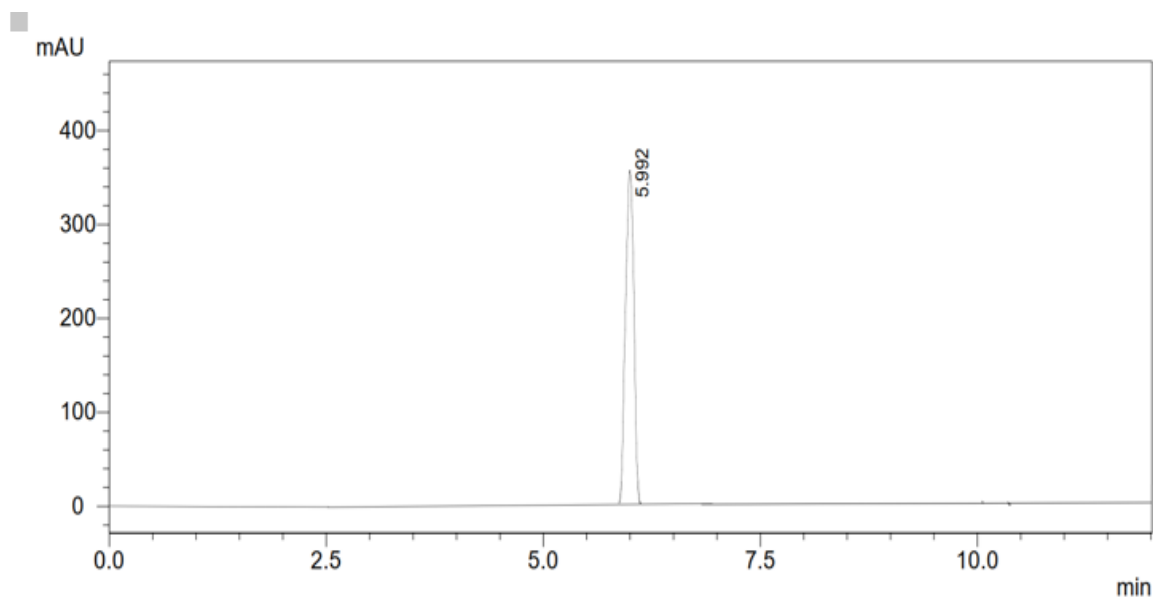


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.008	987518	100

SAMPLE NAME : LINEARITY 5

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

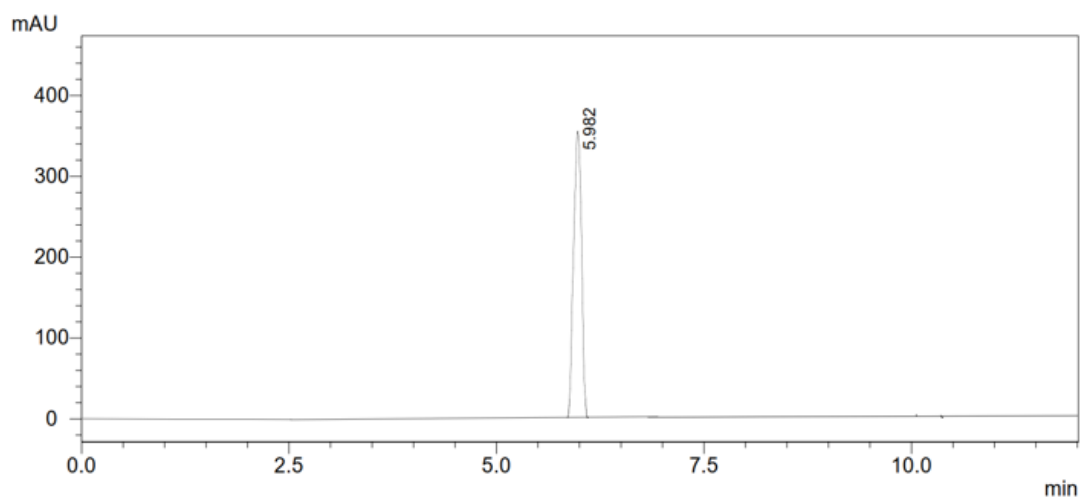


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	5.992	987518	100

SAMPLE NAME : LINEARITY 6

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

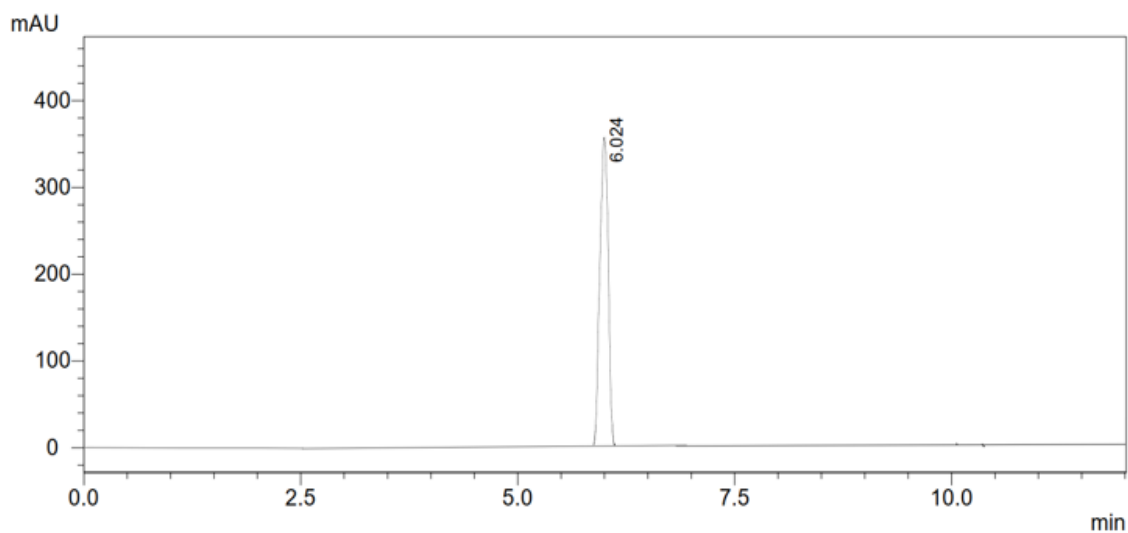


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	5.982	987518	100

SAMPLE NAME : METHOD PRECISION 1

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

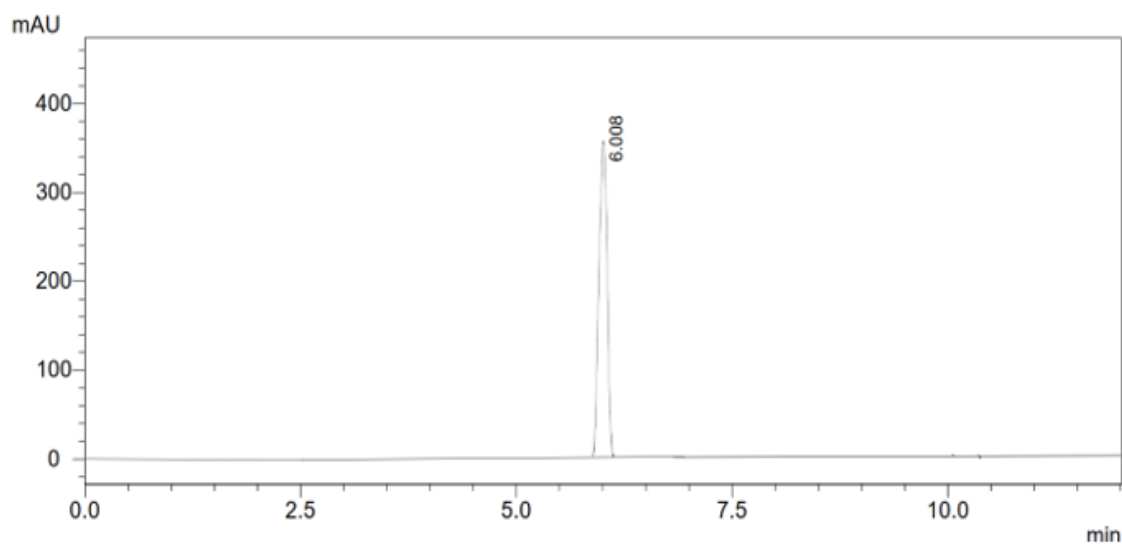


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.024	987518	100

SAMPLE NAME : METHOD PRECISION 2

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

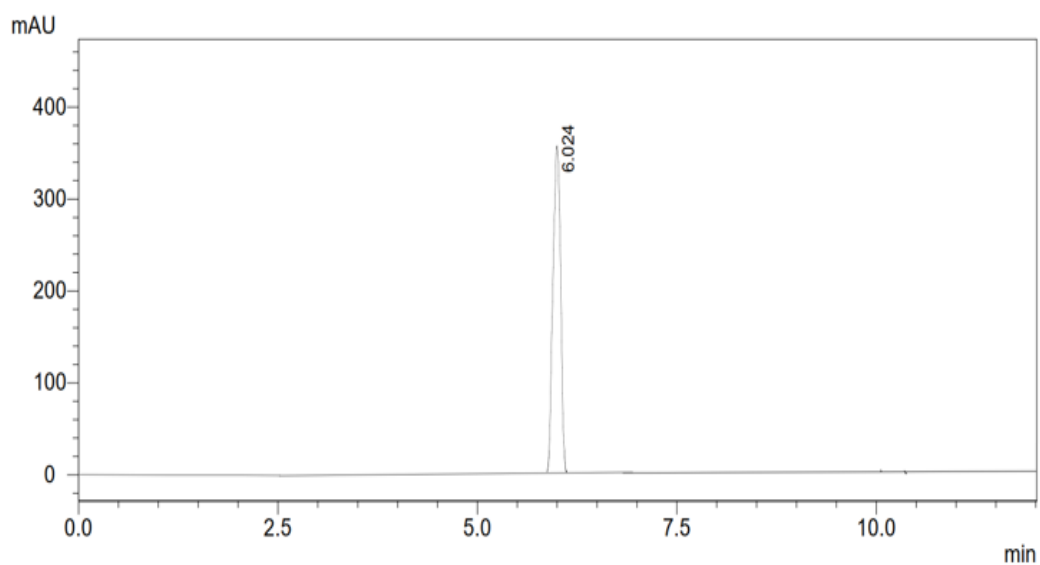


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.008	987518	100

SAMPLE NAME : METHOD PRECISION 3

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

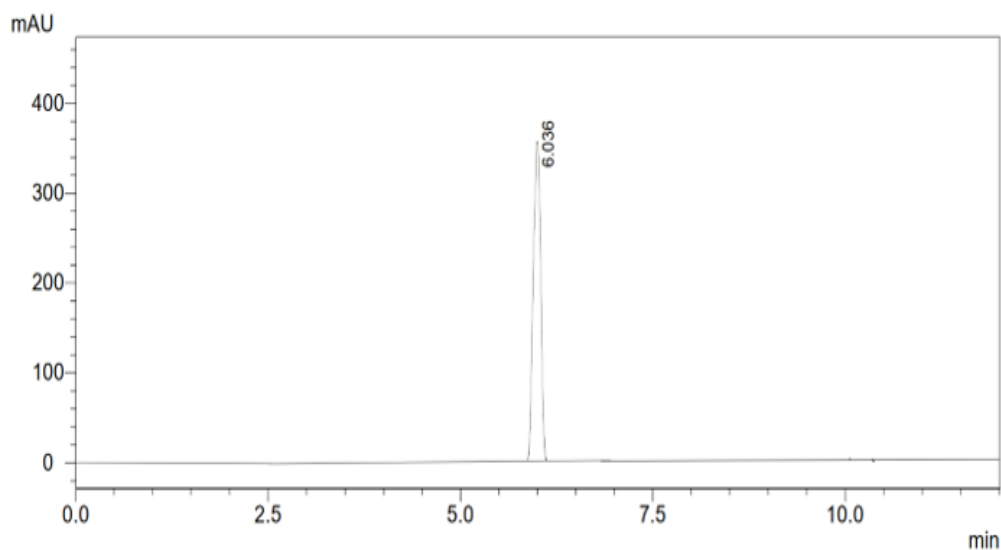


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.024	987518	100

SAMPLE NAME : METHOD PRECISION 4

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

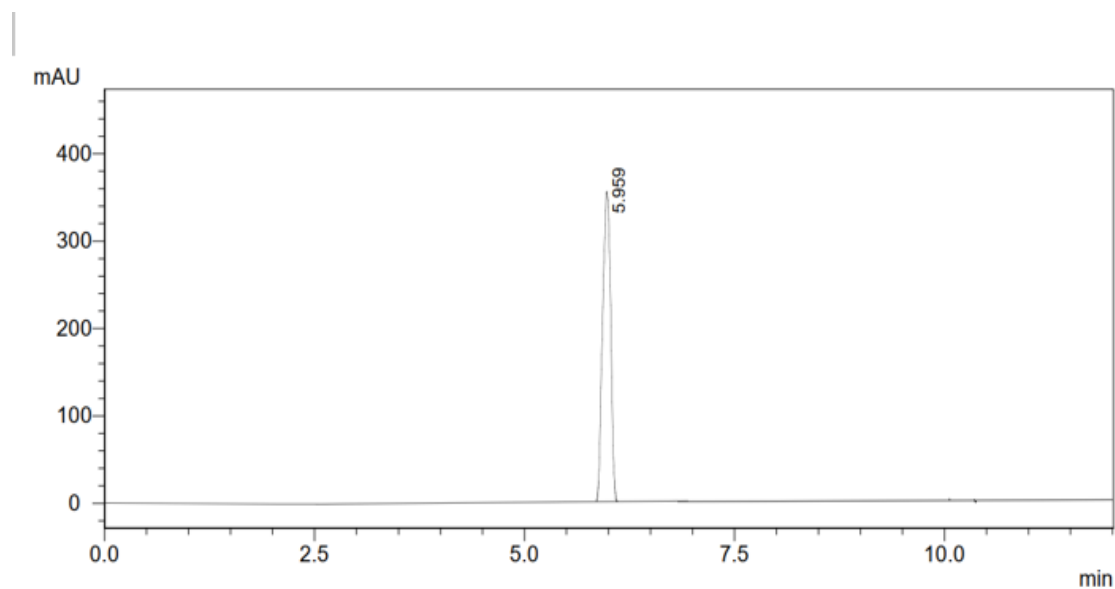


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.036	987518	100

SAMPLE NAME : METHOD PRECISION 5

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

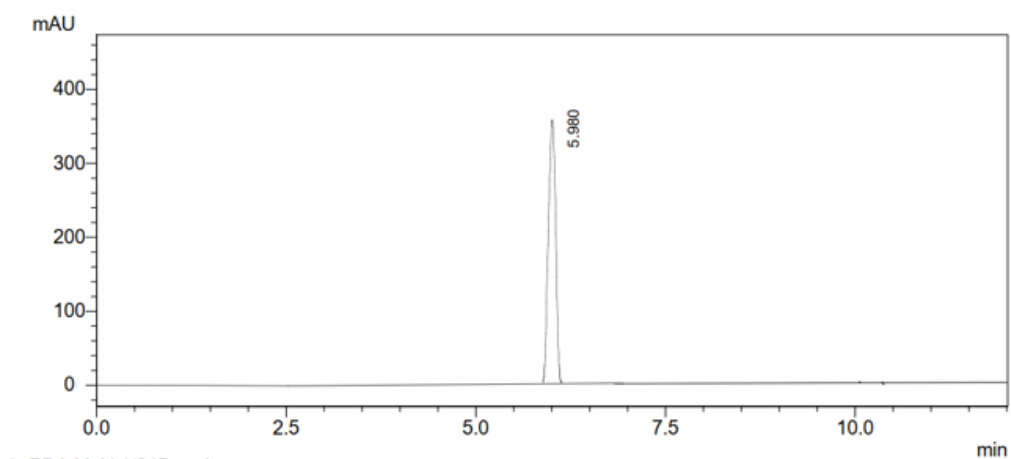


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	5.959	987518	100

SAMPLE NAME : ACCURACY-50%

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA



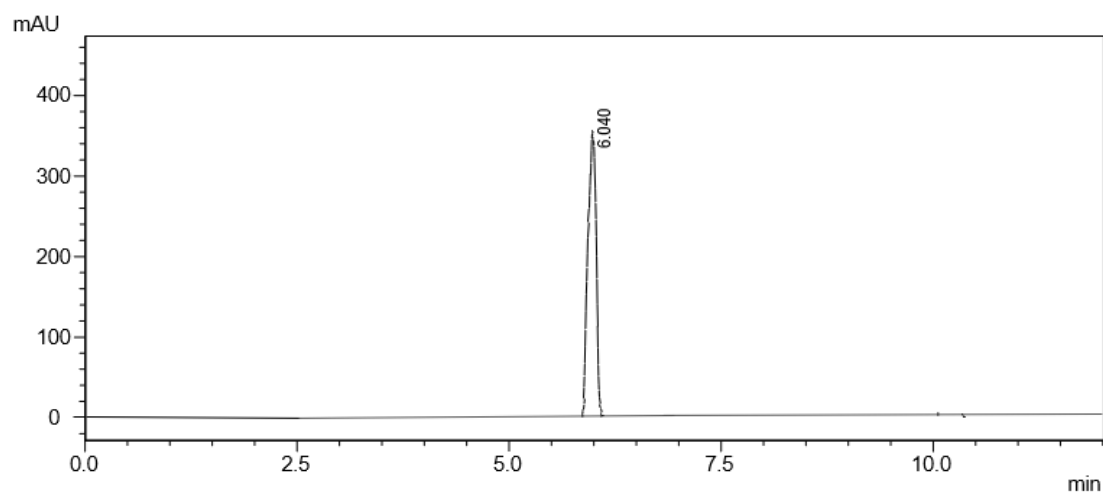
S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	5.980	485684	100

CHROMATOGRAM - 21

SAMPLE NAME : ACCURACY-100 %

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA

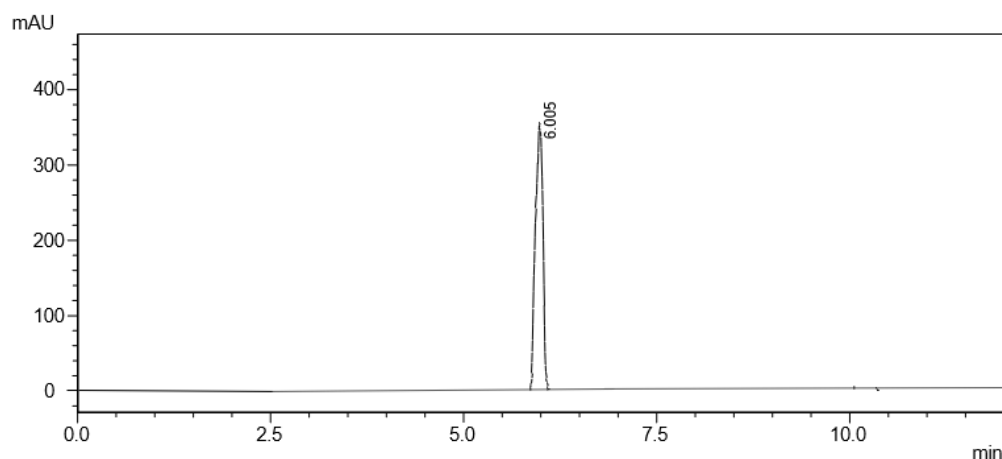


S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.040	989526	100

SAMPLE NAME : ACCURACY-120 %

SYSTEM : HPLC

DETECTOR : PERCENT ON AREA



S.NO.	RETEN TIME	AREA(%)	AREA(%)
1.	6.005	1156908	100

RESULTS AND DISCUSSION

A simple and effective reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of esomeprazole sodium in pharmaceutical dosage forms. Chromatographic separation was achieved using a Phenomenex Gemini C8 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of 0.02 M dipotassium hydrogen phosphate and methanol (40:60 v/v). The mobile phase was delivered at a flow rate of 1.5 mL/min, and detection was carried out using a UV detector at 280 nm (SPD-20A).

The retention time of esomeprazole sodium was found to be 6.009 minutes, indicating a well-resolved and symmetrical peak. The method demonstrated excellent column performance, with theoretical plates calculated as 966,502, confirming column efficiency.

Linearity was established over the range of 80% to 120% of the target concentration, showing a strong correlation with a correlation coefficient (r^2) of 0.999, indicating the method's reliability.

The method was validated according to ICH guidelines, including accuracy (recovery studies), which showed satisfactory percentage recovery, robustness (evaluated by small deliberate changes in pH and wavelength), and ruggedness (verified across different analysts and instruments).

Overall, the developed RP-HPLC method was proven to be precise, accurate, robust, and suitable for routine quality control of esomeprazole sodium in pharmaceutical formulations.

SUMMARY OF VALIDATION

A summarized report of the Validation data results should prepare as given below:

Sr. No.	Particular	Acceptance criteria	Observation
1.	Specificity	No interference should obtain at the retention time of principal peak.	Complies
2.	Precision		
	• System precision	The % RSD for Retention time and Area should not be more than 1.0%	RT-0.346 % RSD- 0.160%
	• Method precision (Repeatability)	The % RSD for six assay results should be not more than 1.0%	0.619 %
3.	Accuracy	95% to 105% at concentration level 50%, 100% & 150%	97.76 % 97.07 % 97.94 %
4.	Linearity & Range	Correlation coefficient should be ≥ 0.999	0.9999
5.	Robustness	System suitability criteria should meet	Complies
6.	Solution Stability	Cumulative % RSD of Area of standard solution among each time interval of stability study should not be more than 2.0%.	Complies

ANALYTICAL PERFORMANCE PARAMETERS:

Method was verified for Specificity, Accuracy, Precision, Linearity, Robustness , Solution Stability, method was applied to this formulation and results were compared with reference method.

OVERALL CONCLUSION

Esomeprazole was subjected to various studies as per ICH Q2R2. Purposed Method was validated for Specificity, Accuracy, Precision, Linearity, Range & robustness, Solution Stability method was applied to this formulation and results were compared with reference method which showed that there were no significant changes in the result. Show method can utilized for determination of Assay of drug.

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