"METHOD DEVELOPMENT AND VALIDATION OF ESOMEPRAZOLE AND DOMPERIDONE IN CAPSULE DOSAGE FORM BY RP-HPLC METHOD"

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Abstract: This study has been undertaken to investigate and evaluate the values by proposed HPLC method provides simple, precise, rapid and robust quantitative analytical method for determination of Esomeprazole and Domperidone in combined dosage form.

I. INTRODUCTION

Analytical chemistry is a measurement science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine. It is concerned with the chemical characterization of matter and the answer to two important questions: what is it? (qualitative) and how much is it (quantitative)?

Chemicals make up everything we use or consume, and knowledge of chemical composition of many substances is important in our daily lives. Analytical chemistry plays an important role in nearly all aspects of chemistry, for example agricultural, clinical, environmental, forensic, manufacturing, metallurgical and pharmaceutical chemistry. [A Kenneth Connors].

Analytical Chemistry involves separation, identification, and determination of relative amounts of the components making up the sample. The qualitative analysis reveals the chemical identity of the sample while quantitative analysis gives the amount of one or more of the components present in numerical terms.

The results in a quantitative analysis are computed from two measurements. First is the mass or volume of sample to be analyzed & second is the measurement of some property that is proportional to the amount of analyte in the sample. Depending on the property to be measured, the methods are classified as specific classical methods such as gravimetry, volumetry, titrimetry etc and systemic or instrumental methods like refractometry, colorimetry, absorptimetry etc.

It is necessary to develop new analytical methods for drugs. In brief the reasons for the development of newer methods of drugs analysis are

- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations. Analytical methods may not be available for the drug in the form of formulation excipients.
- Analytical methods for a drug in combination with other drugs & quantitation of the drug in biological fluids may not be available.
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedure and these may not be reliable.

Advantages of instrumental methods

- Small samples can be used.
- High sensitivity is obtained.
- Measurements obtained are reliable.

- Determination is very fast.
- Even complex samples can be handled easily.

For convenience and better understanding introduction is divided into four parts,

A. UV-Visible Spectrophotometry.
B. High Performance Liquid Chromatography.
C. Method Development.
D. Method Validation.

UV – VISIBLE SPECTROPHOTOMETRY

Spectrophotometry, one of the valuable techniques in pharmaceutical analysis is defined as the method of analysis, which deals with the measurement of spectra. Spectrophotometry is a branch, which embraces the measurement of absorption of radiation energy of definite and narrow wavelength approximating monochromatic radiations by chemical species. Absorption Spectrophotometry is the measurement of the absorption of electromagnetic radiation of definite and narrow wavelength range by molecules, ions and atoms of a chemical substance. Technique most commonly employed in analytical field includes ultraviolet, visible, infrared and atomic absorption spectroscopy.

This deals with the absorption of electromagnetic radiation in the wavelength region of 160 to 780 nm. UV absorption spectroscopy deals with absorption of light by a sample in the Ultra Violet (UV) region (190-380 nm), while Visible region (380-780 nm) absorption spectroscopy (colorimetry) deals with absorption of light by a sample in the visible region (380-780 nm). Absorption of UV – Visible light causes promotion of a valence electron from bonding to antibonding orbitals.

There are 4 types of transitions observed in UV visible spectroscopy, $\sigma \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, n $\rightarrow \sigma^*$, and n $\rightarrow \pi^*$. [HobartH.Willardet.al].

TERMS USED IN ABSORPTION SPECTROSCOPY

- **TRANSMITTANCE (T)**

  It is the ratio of intensity of transmitted light to that of incident light.

  $T = It / Io$

- **ABSORBANCE (A)**

  It is the negative logarithm of transmittance to the base 10.

  $A = - \log_{10} T = \log_{10} Io/It$

$A = abc$

- **MOLAR ABSORPTIVITY (ε)**

  When concentration ‘c’ in equation A= abc is expressed in mole/lit and cell length in ‘cm’ then Absorptivity is called as molar absorptivity.

  $\varepsilon = A/bc$

- **BEER LAMBERT’S LAW**

  It can be stated that as the intensity of beam of monochromatic light when passed through transparent medium decreases exponentially as the thickness and concentration of absorbing media increases arithmetically.

$A = \log Io/It = abc$

Where,

A = Absorbance of the solution at particular wavelength of the light.

I_0 = Intensity of incident light beam.

I_t = 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.

Beer's law is said to be obeyed over a concentration range if a plot of concentration against absorbance passes through origin and is a straight line.

CHROMATOGRAPHY

Introduction

Chromatography (from Greek: chroma, colour and: "grafein" to write) is the collective term for a family of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated. [P.D.Sethi].

Chromatography may be preparative or analytical. Preparative chromatography seeks to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography normally operates with smaller amounts of material and seeks to measure the relative proportions of analytes in a mixture. The two are not mutually exclusive.

TYPES OF CHROMATOGRAPHY

- Gas Chromatography
- Liquid Chromatography
- Thin Layer Chromatography
- Paper Chromatography
- Ion Exchange Chromatography
- Affinity Chromatography

Gas Chromatography

Gas chromatography makes use of a pressurized cylinder and a carrier gas, such as helium, to carry the solute through the column. The most common detectors used in this type of chromatography are thermal conductivity and flame ionization detectors. There three types of gas chromatography are gas adsorption, gas-liquid and capillary gas chromatography.

Liquid Chromatography

There are a variety of types of liquid chromatography. There is liquid adsorption chromatography in which an adsorbent is used. There is also liquid-liquid chromatography which is analogous to gas-liquid chromatography. The three types that will be considered here fall under the category of modern liquid chromatography. They are

- Reverse phase chromatography
- Size exclusion chromatography
- Supercritical fluid chromatography

Reverse phase chromatography

It is a powerful tool and involves a hydrophobic, low polarity stationary phase which is chemically bonded to an inert solid such as silica. The separation is essentially an extraction operation and is useful for separating non-volatile components.

High Performance liquid Chromatography (HPLC)

It is similar to reverse phase, only in this method, the process is conducted at a high velocity and pressure drop. The column is shorter and has a small diameter, but it is equivalent to possessing a large number of equilibrium stages.

Size exclusion chromatography

It is a chromatographic method in which particles are separated based on their size, or in more technical terms, their hydrodynamic volume. It is usually applied to large molecules or macromolecular complexes such as proteins and through the column, the technique is known as gel filtration chromatography. The name gel permeation chromatography is used when an organic solvent is used as a mobile phase. The main application of gel filtration chromatography is the fractionation of proteins

and other water-soluble polymers, while gel permeation chromatography is used to analyze the molecular weight distribution of organic-soluble polymers. Polymer chemists typically use either a silica or crosslinked polystyrene medium under a higher pressure. This media are known as the stationary phase. Supercritical fluid chromatography

It is a relatively new analytical tool. In this method, the carrier is a supercritical fluid, such as carbon dioxide mixed with a modifier. Compared to liquids, supercritical fluids have solubilities and densities have as large, and they have diffusivities and viscosities quite a bit larger. This type of chromatography has not yet been implemented on a large scale.

Thin Layer Chromatography

Thin-layer chromatography may be used with reagents such as H_2SO_4 which would react with paper and is especially useful for the separation and analysis of high molecular weight biochemical compounds. Williams carried out chromatography on adsorbent layer sandwiched between tow glass plates; one of the latter has a small hole through which solutions and developing solvents were applied to the layer. Attempts were made using adsorption chromatography on impregnated filter paper and later glass-fiber paper coated with silicic acid or alumina.

Paper Chromatography

This technique is a type of partition chromatography in which the substance are distributed between tow liquids, ie., one is the stationary liquid (usually water) which is held in the fibers of the paper and called the stationary phase; the other is the moving liquid or developing solvent and called the moving phase. The components of the mixture to be separated migrate at different rates and appear as spots at different points on the paper.

Ion Exchange Chromatography

In ion exchange chromatography the stationary phase consists of a polymeric resin matrix on the surface of ionic functional groups, e.g., carboxylic acids or quaternary amines, have been bonded chemically. As the mobile phase passes over this surface, ionic solutes are retained by forming electrostatic chemical bonds with the functional groups. The mobile phase used in this type is always liquid.

Affinity Chromatography

Affinity chromatography is a chromatographic method of separating biochemical mixtures, based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand. Affinity chromatography combines the size fractionation capability of gel permeation chromatography with the ability to design a stationary phase that reversibly binds to a known subset of molecules. Due to its interdisciplinary nature. Affinity chromatography has been the means by which many scientists from different disciplines have been introduced to the exciting fields of modern biology.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch [Hobart H.Willardet.al].

The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The technique of high performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography. Advances in column technology, high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation. [Beckett.H and Stenlake.J.B].

HISTORY OF HPLC

Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During the 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and did not achieve sufficiently high resolution to distinguish between similar compounds.

High pressure liquid chromatography was developed in the mid-1970 and quickly improved with the development of column packing materials and the additional convenience of on-line detectors. In the late 1970's new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds.

By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above those obtained using previous techniques. Computers and automation added to the convenience of HPLC column that is shorter than the typical column. A Fast HPLC column is about 3mm long and is packed with smaller particles. Currently, one has the option of selecting from a lot of columns for the separation of compounds, as well as a variety of detectors to interface with the HPLC in order to obtain optimal analysis of the compound. Although HPLC is widely.

Considered to be a technique mainly for biotechnological, biomedical, and biochemical research as well as for the pharmaceutical industry, in actual fact these fields currently comprise only about 50% of HPLC users. Currently HPLC is used in a variety of fields and industries including the cosmetics, energy, food, and environmental industries. [http:/en.wikipedia.org/HPLC].

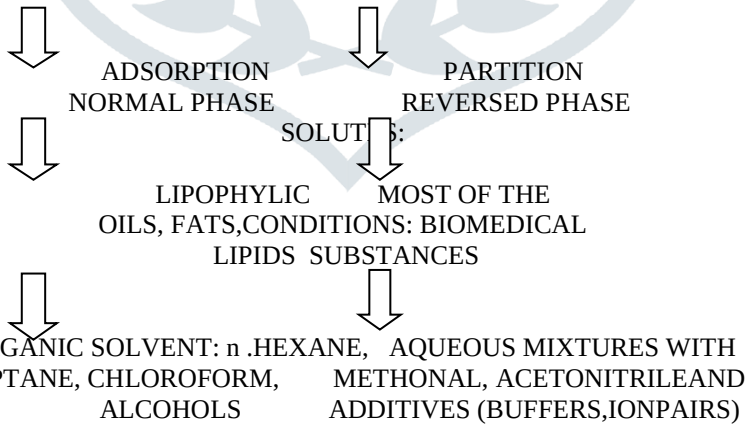
Principle

High-performance Liquid Chromatography (HPLC) is a separation technique that can be used for the analysis of organic molecules and ions .HPLC based on mechanisms of adsorption, partition, ion exchange or size exclusion, depending on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of the components of a solution results from the difference in the relative distribution ratios of the solutes between the two phases.

Based on modes of chromatography

- Normal phase chromatography
- Reversed phase chromatography

PRINCIPLE OF SEPARATION



Normal phase chromatography

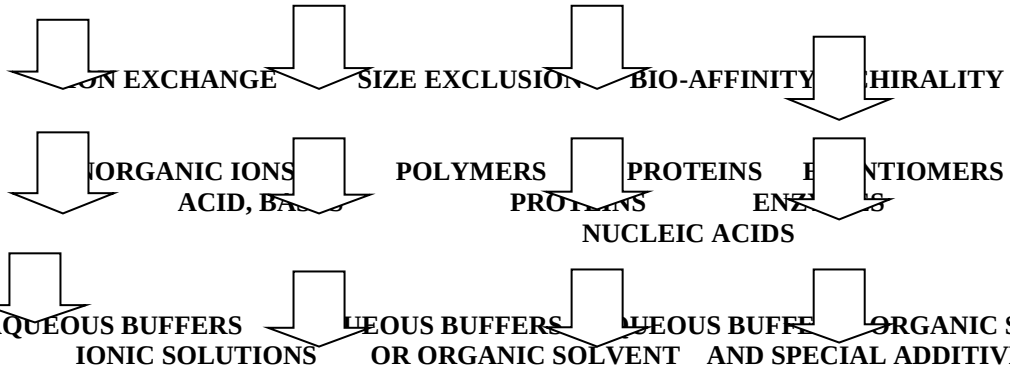
- Mechanism: Retention by interaction of the polar surface of stationary phase with polar parts of the sample molecules.
- Stationary Phase:It is a bonded siloxane with polar functional groups like SiO_2, Al_2O_3, $-NH_2$, -CN, $-NO_2$, - Diol.
- Mobile Phase: Nonpolar solvents like heptane, hexane, cyclohexane, chloroform, ethyl ether, and dioxane.
- Application: Separation of nonionic, nonpolar to medium and polar substances.

- Sample Elution Order: Least polar components are eluted first.

Reversed phase chromatograph

High performance liquid chromatography

PRINCIPLE OF SEPARATION

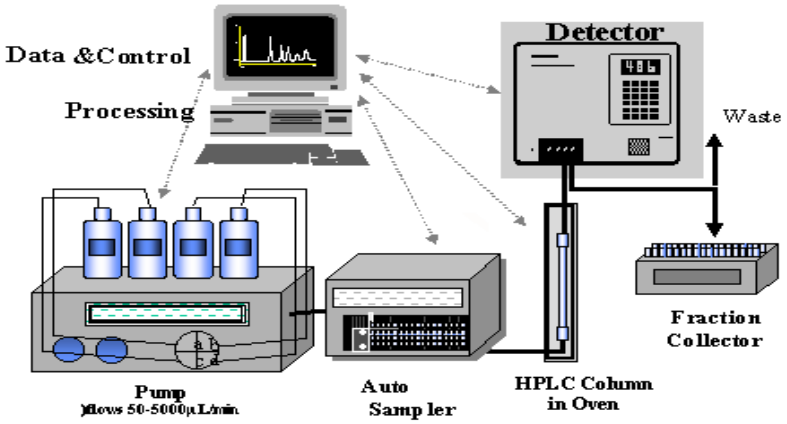


- **Mechanism**: Retention by interaction of the nonpolar hydrocarbon chain of stationary phase with nonpolar parts of sample molecules.
- **Stationary Phase**: It is bonded siloxane with nonpolar functional groups like n-octadecyl (C_{18}) or n-octyl (C_8), ethyl, phenyl, - (CH_2) n-diol, - (CH_2) n-CN.
- **Mobile Phase**: Polar solvents like methanol, acetonitrile, water or buffer (Sometimes with additives of THF or dioxane).
- **Application**: Separation of nonionic and ion forming nonpolar to medium polar substances.
- **Sample Elution Order:** Most polar components are eluted first.

INSTRUMENTATION OF HPLC

The general instrumentation for HPLC incorporates the following components.

- There is a solvent reservoir for mobile phase.
- The mobile phase must be delivered to the column by some type of pump. To obtain Separations either based on short analysis time or under optimum pressure, a wide range of pressure and flows is desirable the pumping system must be pulse-free or of pressure and flows is desirable. The pumping system must be pulse-free or else have a pulse damper to avoid to generating base line instability in the detector.
- Sampling valves or loops are used to inject the sample into the flowing mobile phase just at the head of the separation column. Sample should be dissolved in a portion of the mobile phase to eliminate an unnecessary solvent peak.
- A head of the separation column there may be a guard column or an in line filter to prevent contamination of the main column by small particles.
- To measure column inlet pressure a pressure gauge is inserted in front of the separation column.
- The separation column contains the packing needed to accomplish the desired HPLC separation. These may be silicas for adsorption chromatography, bonded Phase for liquid-liquid chromatography, ion-exchange chromatography or some other unique packing for a particular separation method.
- A detector with some type of data handling devise completes the basic Instrumentation.



HPLC BASIC INSTRUMENTATION

Figure No: 1. HPLC instrument and its components

Mobile phase:

Mobile phases used for HPLC typically are mixtures of organic solvents and water or aqueous buffers. Table given below lists the physical properties of organic solvents commonly used for HPLC. Isocratic methods are preferable to gradient methods. Gradient methods will sometimes be required when the molecules being separated have vastly different partitioning properties. When a gradient elution method is used, care must be taken to ensure that all solvents are miscible. [GurdeepChatwal, Sham k.Anand].

The following points should also be considered when choosing a mobile phase:

1. It is essential to establish that the drug is stable in the mobile phase for at least the duration of the analysis.
2. Excessive salt concentrations should be avoided. High salt concentrations can result in precipitation, which can damage HPLC equipment.
3. The mobile phase should have a pH 2.5 and pH 7.0 to maximize the lifetime of the column.
4. Reduce cost and toxicity of the mobile phase by using methanol instead of acetonitrile when possible.
5. Minimize the absorbance of buffer. Since trifluoroacetic acid, acetic acid or formic acid absorb at shorter wavelengths, they may prevent detection of products without chromophores above 220 nm. Carboxylic acid modifiers can be frequently replaced by phosphoric acid, which does not absorb above 200 nm

Table No: 1. Physical properties of common HPLC solvents

Solvent	MW	BP	RI (25°C)	UV[a] Cut-off (nm)	Density g/ml (25°C)	Viscosity cp (25°C)	Dielectric Constant
Acetonitrile	41.0	82	1.342	190	0.787	0.358	38.8
Dioxane	88.1	101	1.420	215	1.034	1.26	2.21
Ethanol	46.1	78	1.359	205	0.789	1.19	24.5
Ethyl acetate	88.1	77	1.372	256	0.901	0.450	6.02
Methanol	32.0	65	1.326	205	0.792	0.584	32.7
CH_2Cl_2	84.9	40	1.424	233	1.326	0.44	8.93
Isopropanol	60.1	82	1.375	205	0.785	2.39	19.9

	n-propanol	60.1	97	1.383	205	0.804	2.20	20.3
	THF	72.1	66	1.404	210	0.889	0.51	7.58
	Water	18.0	100	1.333	170	0.998	1.00	78.5

HPLC basically system is composed of

- Pump
- Injector
- Column
- Column oven
- Detector

PUMPS:

The HPLC pump is very important component of the system. The pump delivers the constant flow of the mobile phase or phase so that the separation of the components of the mixture occurs in a reasonable time. There are two types of pumping systems Isocratic and Gradient.

Isocratic

In the system the things are kept constant throughout the run. In case of pumping Mobile phase, the mobile phase composition is kept constant throughout the run. The nominal flow rate accuracy required is ±1% of the set flow.

Gradient

There is some change purposely incorporated during the particular sample run to achieve a better or and faster separation. In case of pumping mobile phase, the composition of the mobile phase is continuously varied during the particular run for one of the above mentioned purpose. ±1% of the step gradient composition is typical.

SAMPLE INJECTOR:

Insertion of a sample onto the column must be as a narrow plug so that the peak broadening attributable to this step is negligible. The injection system itself has no dead volume.

Injection port is of two basic types:

- Those in which the sample is injected directly into the column (on column injection.
- Those in which the sample is deposited before the column inlet and then swept by a valving action into the column by the mobile phase (valve injectors).

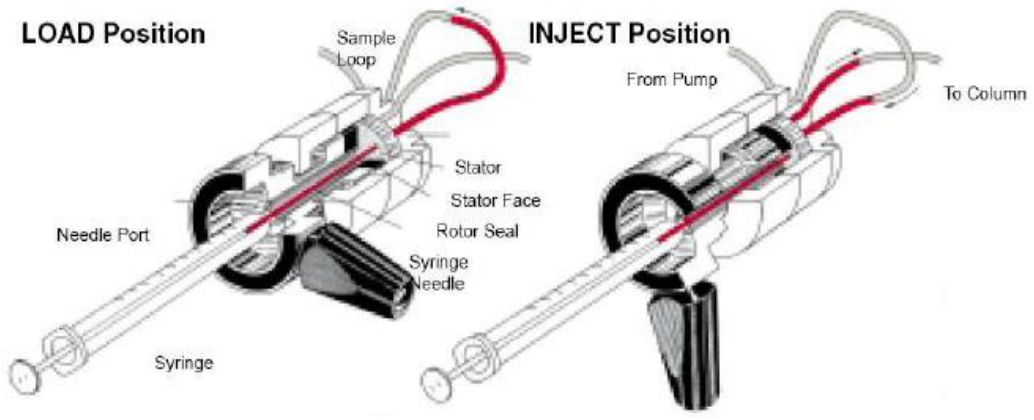


Figure No: 2.Inject positions

COLUMN

The heart of the system is the column. The choice of common packing material and mobile phases depend on the physical properties of the drug. The column is constructed from smooth bore stainless steel tubing or heavy walled glass tubing to

withstand high pressure.

- **Analytical Column**

The columns are usually 10-30 cm long and 4-10 mm inside diameter containing stationary phase at particle diameter of 5 or 10 µm.

- **Guard Column**

A short guard column is introduced before the analytical column to increase the life of the analytical column by removing not only particulate matter and contaminants from the solvents but also sample components that bind irreversibly to the stationary phase.

COLUMN PACKING

The packing used in modern HPLC consist of small, rigid particles having a narrow particle size distribution. There are three main type of column packing in HPLC.

- Porous, Polymeric Beads

Porous, polymeric beds based on styrene divinyl benzene co-polymers used for ion exchange and size exclusion chromatography. For analytical purpose these have now been replaced by silica based packing which are more efficient and more stable.

- Porous Layer Beds

This consisting of a thin shell (1-3 µm) of silica or modified silica on a spherical inert core (e.g. glass). After the development of totally porous micro particulate packing, these have not been used in HPLC.

- Totally Porous Silica Particles (dia. <10 µm)

Particles of diameter >20µm are usually dry packed. While particles of diameter <20µm are slurry packed in which particles are suspended on a suitable solvent and the slurry is driven into the column under pressure.

DETECTOR:

The function of the detector in HPLC is to monitor the mobile phase as it merges from the column.

Detectors are of usually two types:

- Bulk Property Detectors

It responds to bulk property of mobile phase which is modulated by the presence of an eluting solute.
E.g. refractive index, dielectric constant or density.

- Solute Property Detectors

It responds to physical property of mobile phase which is not exhibited by pure mobile phase.
E.g. UV absorbance, fluorescence or diffusion current detector.

HPLC Detectors

- Universal detector-Refractive Index Detector
- UV-Visible Detector
- Electrochemical Detector
- Fluorescence Detector
- Conductivity detector
- Mass Detector (MS)
- Evaporative Light Scattering Detector (ELSD)

Basic detector requirements

An ideal LC detector should have the following properties: Low drift and noise level (particularly crucial in trace analysis).

- High sensitivity.
- Fast response.
- Wide linear dynamic range (this simplifies quantitation).
- Low dead volume (minimal peak broadening).
- Cell design which eliminates remixing of the separated bands.
- Insensitivity to changes in type of solvent, flow rate, and temperature.
- Operational simplicity and reliability, It should be non-destructive.
- It should be tunable so that detection can be optimized for differentCompounds.

VARIOUS METHODS OF QUNTITATIVE ANALYSIS IN HPLC

The sample or solute is analysed quantitative in HPLC by either peak height or peak area Measurements. Peak areas are proportional to the amount of constant rate Peak heights are proportional to the amount of material only when peak width are constant and are strongly affected by the sample injection techniques. Once the peak height or the peak areas are measured, there are five principle evaluation methods for quantifying the solute.

a. Calibration by standards

Calibration curves for each component are prepared from pure standards, using identical injection volumes of operating conditions for standards and samples. The Concentration of solute is read from its curve if curve is linear.

$$X = K \times \text{Area}$$

Where, X = Concentration of solute.
K= Proportionality constant (slop of curve)

b. Internal Standard Method

In this technique a known quantity of the internal standard is chromatographed and area versus concentration is ascertained. Then a quantity of the internal standard is added to the raw sample prior to any sample pretreatment or separation operations.

The peak area of the standard in the sample run is compared with the peak area when the standard is run separately. This ratio serves as a correction factor for variation in Sample size, for losses in any preliminary pretreatment operations, or for incomplete elution of the sample. The material selected for the internal standard must be completely resolved from adjacent sample components, must not interfere with the sample components and must never be present in sample.

$$\text{Area ratio} = \frac{\text{Area of sample}}{\text{Area of internal standard}}$$

$$\text{Sample concentration} = \frac{\text{Area of sample}}{\text{Area of internal standard}} \times \text{Concentration of standard}$$

c. Area Normalization

The technique is often used for the sample having identical components. It is used to evaluate the absolute purity of the sample. The procedure is to total up the areas under all peaks and then calculate the percentage of the total area that is contributed by the compound of interest. For this method the entire sample must be eluted, all components must be separated and each peak must be completely resolved.

d. Standard Addition Method

If only few samples are to be chromatographed, it is possible to employ the method of standard addition (s). The chromatogram of the unknown analyte is recorded, then a known amount of analyte (s) is added and chromatogram is repeated using same reagents, instruments and same conditions. From the increase in the peak area (or) peak height, the original concentration can be computed by interpolation.

The detector response must be a linear function of analyte concentration and yield no signal at zero concentration of the analyte. Sufficient time must elapse between addition of the standard and actual analysis to allow equilibrium of added standard with any matrix interferon.

If an instrumental reading (area/height) 'R_x' is obtained, from a sample of unknown 'X' and a reading 'R_t' is obtained from the sample to which a known concentration 'a' of analyte has been added, then 'X' can be calculated from.

$$\frac{X}{X + a} = \frac{R_x}{R}$$

e. External Standard Method

It employs a separate injection of a fixed volume of sample and standard solution.

The peaks are integrated and concentration is calculated.

$$\text{Sample concentration} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{Concentration of standard}$$

STEPS INVOLVED IN DEVELOPMENT OF HPLC METHOD

Selection of chromatographic method

- First reversed phase should be tried.
- If not successful than normal phase should be taken into consideration.
- For ion exchange or ion pair chromatography, first ion suppression by P^H control and reversed phase chromatography should be tried.
- Selection of stationary phase

  Matching the polarity of sample and stationary phase and using a mobile phase of different polarity achieve a successful separation.
- Selection of mobile phase

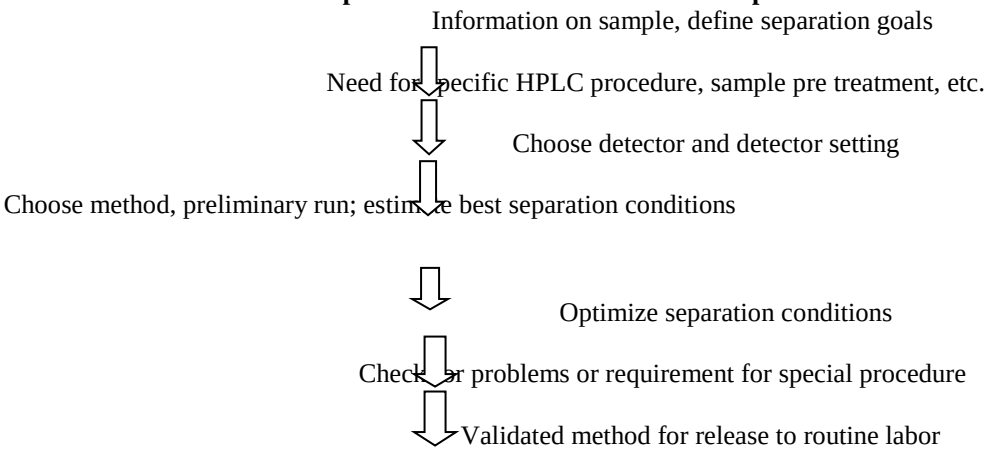
  Reversed phase bonded packing, when used in conjunction with highly polar solvents; approach is ideal and is a universal system for liquid chromatography. Mobile phase may be either single liquid or combination of liquids, which are compatible with sample, column and instrument.
- Selection of suitable detector

  Detector is the eye of HPLC system that measures the compounds after their separation on the column. There are basically two types of detectors- the bulk property detectors and solute property detectors. Detectors, in order of their popularity are UV, fluorescent, conductivity, polarimeter and refractive index detectors. UV detector is the first choice because of its convenience and applicability in case of most of the samples. The latest versions of equipments are available with photo diode- array detectors (PAD or DAD).

METHOD DEVELOPMENT AND OPTIMIZATION

During the optimization stage, the initial sets of conditions that have evolved from the First stages of development are improved or maximized in terms of resolution and shape, plate Counts asymmetry, capacity, elution time, detection limits, limit of quantization, and overall ability to quantify the specific analyte of interest.

Steps involved in HPLC method development



METHOD VALIDATION

Method validation is the process by which it is established that performance characteristics of the method meet the requirement for the intended analytical applications. Methods need to be validated or revalidated before their introduction into routine use. The International conference on Harmonization (ICH) of technical requirements for the registration of pharmaceuticals for human use has developed a text on the validation of analytical procedures.

TYPES OF VALIDATION

- Retrospective validation
- Prospective validation
- Concurrent validation
- Revalidation

1.Retrospective validation

Retrospective validation is the most pertinent for use by most pharmaceutical companies; establishing documented evidence that a system does it purports to do based on review and Analysis of historical information.

2.Prospective validation

Prospective validation is a validation conducted prior to distribution either of new Product, or a product made under a revised manufacturing process. Validation is completed and the results are approved prior to any product release.

3.Concurrent validation

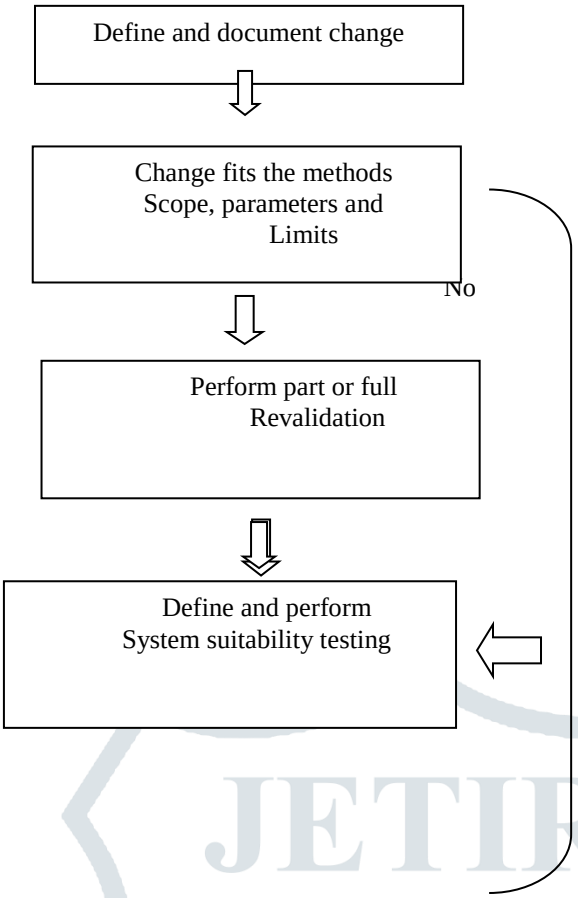
This is performed in two instances i.e. for existing equipment; verification of proper Installation along with specific operation tests is done. In case of an existing, infrequently made product, data is gathered from at least three successful trails.

4.Revalidation

Most likely method parameters have to be changed or adjusted during the life of the Method if the method performance criteria fall outside their acceptance criteria. The question is whether such changed requires revalidation. In order to clarify these questions upfront, operating should be defined for each method, either based on experience with similar methods or else Investigated during method development. These ranges should be verified during method validation in robustness studies and should be part of the method characteristics. Availability of such operating ranges makes it easier when a method should be revalidated.

A revalidation is necessary whenever a method is changed, and the new parameter lies outside the operating range. If for example, the operating range of the column temperature has been specified to be Between 30 and 40^{o}C, the method should be revalidated if, for whatever reason, the new operating parameter is 41^{o}C.

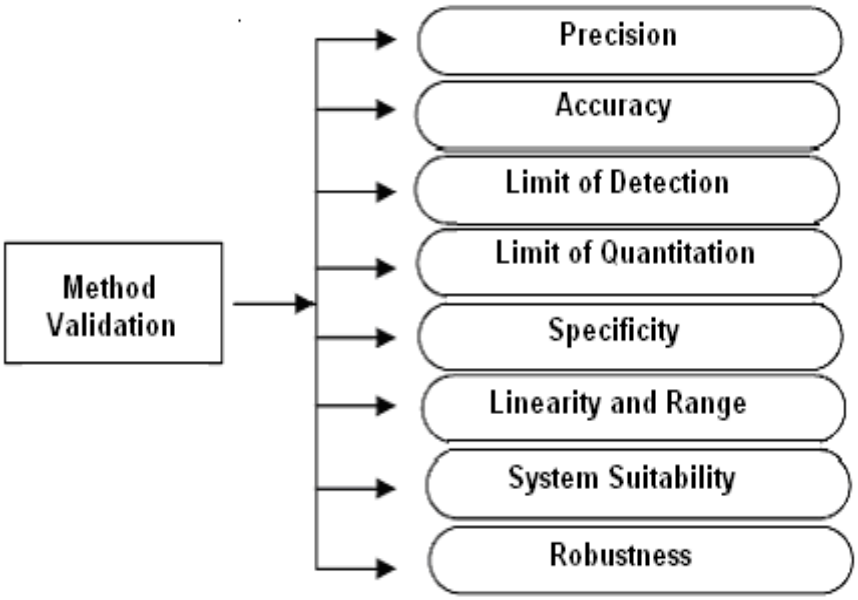
Revalidation is also required if the scope of the method has been changed or extended, for example, if the sample matrix changed or if operating conditions change. Furthermore, revalidation is necessary if the intention is to use instruments with different characteristics, and these new characteristics have not been covered by the initial validation. For example, an HPLC method may have been developed and validated on a pump with a delay volume of 5 ml, but the new pump has a delay volume of only 0.5 ml.



Flow diagram for revalidation

Part of full revalidation may also be considered if system suitability tests, or the results of QC Sample analysis, lie outside preset acceptance criteria and where the source of the error cannot be traced back to instruments or any other cause.

ICH METHOD VALIDATION



The objective of the analytical procedure should be clearly understood since this will Govern the validation characteristis which need to be evaluated. Typical validation Characteristics which should be considered are listed below.

PARAMETERS OF METHOD VALIDATION

The following are typical analytical performance characteristics which may be tested during method validation.

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

ANALYTICAL METHOD VALIDATION

ACCURACY

The accuracy of the analytical method is a measure of the systematic error or bias and is defined as the agreement between the measured value and the true value. Accuracy is the best reported as percentage bias, which is calculated from the expression.

$$\% \text{ Bias} = \frac{(\text{Measures value - true value})}{\text{True value}} \times 100$$

Since the true value is not known for real samples, an approximation is obtained, based on spiking drug-free matrix to a nominal concentration. The accuracy of analytical method is then determined at each concentration by assessing the agreement between the measured and nominal concentration of the analytes in the spiked drug-free matrix samples. For the validation of a new analytical method for the use, the measured concentrations will be those obtained during the estimation of precision i.e. from the 4 × 6 × 4 experiment. All results other then those rejected for analytical reasons, i.e. poor chromatography, should be used in the calculation and accuracy of the method should be within ±15% at all concentrations.

PRECISION

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility.

i. Repeatability

- Injection Repeatability

Sensitivity is the ability to detect small changes in the concentration of the analyte in the sample. Sensitivity can be partially controlled by monitoring the specification for injection reproducibility (system suitability testing).

The sensitivity or precision as measured by multiple injections of a homogeneous sample (prepared solution) indicates the performance of the HPLC instrument under the chromatographic conditions and day tested. The information is provided as part of the validation data and as a system suitability test. The specification, as the coefficient of variation in % or relative standard deviation (RSD), set here will determine the variation limit of the analysis. The tighter the value, the more precise or sensitive to variation one can expect the results. This assumes that the chromatograph does not malfunction after the system suitability testing has been performed. Keep in mind, however, that it does not consider variations due to the drug product manufacturing and laboratory sample preparation procedures. The set of four duplicate samples were injected sequentially. Variations in peak area and drift of retention times are noted.

Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its central value. The term 'set' is defined as referring to a number (N) of independent replicate measurements of some property. One of the most common statistical terms employed is the standard deviation of a population of observation. Standard deviation is the square root of the sum of squares of deviations of individual results for the mean, divided by one less than the number of results in the set.

The standard deviation S, is given by

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2},$$

Standard deviation has the same units as the property being measured.

The square of standard deviation is called variance (S^2). Relative standard deviation is the standard deviation expressed as a fraction of the mean, i.e., S/x. It is sometimes multiplied by 100 and expressed as a percent relative standard deviation. It becomes a more reliable expression of precision.

$$\% \text{ Relative standard deviation} = S \times 100 / x$$

- Analysis Repeatability

Determination, expressed as the RSD, consists of multiple measurements of a sample by the same analyst under the same analytical conditions. For practical purpose, it is often combined with accuracy and carried out as a single study.

(ii) Intermediate Precision

Intermediate precision was previously known as part of ruggedness. The attribute evaluates the reliability of the method in a different environment other than that used during development of the method. The objective is to ensure that the method will provide the same results when similar samples are analyzed once the method development phase is over. Depending on time and resources, the method can be tested on multiple days, analysts, instruments, etc.

Intermediate precision in the test method can be partly assured by good system suitability specifications. Thus, it is important to set tight, but realistic, system suitability specifications.

(iii) Reproducibility

As defined by ICH, reproducibility expresses the precision between laboratories as in collaborative studies. Multiple laboratories are desirable but not always attainable because of the size of the firm.

RANGE

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to determine with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the results (e.g. percentage, parts per million) obtained by the analytical method.

For assay, tests, the ICH(5) requires the minimum specified range to be 80% to 120% of the test concentration, and for the determination of an impurity, the range to extend from the limit of quantification, or from 50% of the specification of each impurity, whichever is greater, to 120% of the specification.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

In case of assay, demonstration of specificity requires to be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substances or product with appropriate levels of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials.

LINEARITY

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. A linearity correlation coefficient above 0.999 is acceptable for most methods, especially for major components in assay methods. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

DETECTION LIMIT

The limit of detection is the parameter of limit of tests. It is the lowest level of analyt that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The limit test thus merely substantiates that analyte concentration is above or below a certain level.

The determination of limit of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted.

Limit of detection is calculated by taking the concentration of peak of interest divided by three times the signal-to-noise ratio.

$$LOD = 3.3 \text{ S.D /slope}$$

QUANTITATION OF LIMIT

Limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantitation is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied.

It is measured by analyzing sample containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable. Where the final assessment is based on an instrumental reading, the magnitude of background response by analyzing a number of blank samples and calculating the standard deviation of this response. The standard deviation multiplied by a factor (usually 10) provides an estimation of the limit of quantitation. In many cases, the limit of quantitation is approximately twice the limit of detection.

LOQ = 10 S.D /slope

ROBUSTNESS

ICH defines robustness as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. Robustness can be partly assured by good system suitability specifications. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used. [ICH-2005].

RUGGEDNESS

Ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of same samples under a variety of normal test conditions such as different laboratories, different analysis using operational and environmental conditions that may differ but still within specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of influence on the test results of operational and environmental variable of analytical method.

For the determination of ruggedness the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to precision of assay under normal condition to obtain a measure of the ruggedness of the analytical method.

SYSTEM SUITABILITY PARAMETERS

System suitability test are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronic, analytical operation and sample constant an integral system that can be evaluated as a whole. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

The parameters that effected by the changes in chromatographic conditions are,

- Retention or Capacity factor (K_A)
- Resolution (R_S)
- Selectivity (α)
- Column efficiency factor (A_S)
- Peak asymmetry factor(A_S)

➢ **Retention**

The retention time of drug with a given packaging material and elute can be expressed as retention time or retention volume, but both of these are dependent on flow rate, column length and column diameter. The retention is best described as a column capacity ratio (k), which is independent of these factors.

The column capacity ratio of a compound (A) is defined as

$$K_A = \frac{V_A - V_O}{V_O}$$

Where

V_A = Elution volume of A

V_O = Elution time of a non retained compound (Void volume)

At constant flow rate, retention time ($t_A$and t_o) can be used instead of retention volumes.

➢ **Resolution (R_S)**

The aim of chromatography is to separate components in a mixture into bonds or peaks as they migrate through the column. Resolution is equal to the distance between the peak centre divided by the average band width to increase resolution. Resolution, R provides quantitative measurements of the ability of a column to separate two analytes.

$$R_s = \frac{Rt_2 - Rt_1}{0.5\,(W_1 + W_2)}$$

Where,

Rt_1 and Rt_2 are the retention times of components 1 and 2

W_1 and W_2 are peak widths of components 1 and 2.

For tow peaks to be recognized as separate the resolution should be at least 0.5. Two peaks are seen more completely separate if R is greater than 1.5.Resolution can be improved by lengthening column but this will also increase the analysis.

- **Selectivity (α)**

The selectivity is a measure of relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula

$$A = \frac{V_2 - V_O}{V_1 - V_O}$$

Where,

V_O is the void volume of the column and

V_1 and V_2 are the retention volumes of 2 and 1 peak.

- **Column efficiency (N)**

Efficiency N, of a column is the number of theoretical plates per meter. It is the Measure of band spreading of a peak. Smaller the band spread higher is the number of Theoretical plates, indicating good column and system performance. Columns with N ranging from 5000 to 10,000 plates/meter are ideal for good system. Efficacy is calculated by using the formula.

$$N = 16 \frac{R_t^2}{W^2}$$

Where;

R_t is the retention time and W is peak width.

- **Peak asymmetry factor (A_S)**

Peak asymmetry factor, A_S can be used as a criterion of column performance. The peak Half width, b of a peak at 10% of peak height, divided by the corresponding front half width, a Gives the asymmetry.

$$A_s = b/a$$

For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

Table No: 2.System Suitability Parameters and Recommendations

Parameter	Recommendation
Capacity Factor (k')	The peak should be well-resolved from other peaks and the void volume, generally k'>2.0
Repeatability	RSD $\leq$ 1% for N $\geq$ 5 is desirable.
Relative retention	Not essential as long as the resolution is stated.
Resolution (R_s)	R_s of > 2 between the peak of interest and the closest eluting potential interferent (impurity,excipient, degradation product, internal standard, etc.
Tailing Factor (T)	T of $\leq$ 2
Theoretical Plates (N)	N > 2000

STATISTICAL PARAMETERS

- Regression equation

The linear relationship is characterized by attendency of the points of the scattered diagram to cluster along a straight line, known as the regression line.

$$Y = a + b\,x$$

It is used to describe the dependent of one characteristic (Y) up on the other characteristic (X), both X,Y represent values of two characters, a, b are two constants it will be evident that two regression lines can be computed for every set of data-one each to describe the dependence of one character. B is known as regressive coefficients which show change expected in Y for unit change in X, it is dependence of Y & X; b is the regressive coefficient of Y&X.

The regressive coefficient of b is estimated,

$$B = \frac{\sum [x - x]\,[y - y]}{\sum [x - x]^2}$$

b = The slope of the regression line and is calculated by this formula

x = An arbitrarily chosen value of the predictor variable for which the corresponding value of the criterion variable is desired. Correlation coefficient

A measure of the strength of the relationship between tow variables is provided by the coefficient of correlation, denoted by r, if the relationship between the two variables is of the linear form. It is also called the coefficient of liner correlation.

Pearson's correlation

The correlation coefficient calculation for data values should be +1 or -1 where the values of correlation coefficient is +1 -1 positive.

Correlation coefficient is -1- negative

$$r = \frac{\left[\sum_{i=1}^{n}\left(X_i - \overline{X}\right)\left(Y_i - \overline{Y}\right)\right]}{\frac{(n-1)}{\left[\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2 \sum_{i=1}^{n}\left(Y_i - \overline{Y}\right)^2\right]}{(n-1)^2}}$$

Where, X = value of one character; Y = value of another character

- **Standard deviation**

It is the square root of the average of the squared deviations of the observations. From the arithmetic mean, it is used for measures of dispersion. It is denoted by

$$\text{Standard Deviation} = \frac{\sqrt{\sum [x - \bar{x}]^2}}{\sqrt{n-1}}$$

$$\text{R.S.D (\%)} = \frac{S.D \times 100}{X}$$

Where,

$\sum$ = Sum of observations

$\bar{X}$ = Mean or arithmetic average

X = Individual observed value

$X - \bar{X}$ = Deviation of a value from the mean

n = Number of observations

- **Standard error of mean**

The population of standard deviation is not given, but the size of is large, so the sample standard deviation is representing the population of standard deviation.

$$S.E = \frac{S.D}{\sqrt{n}}$$

Where,

S.D = Standard deviation

n = no. of observations

3 . LITERATURE REVIEW

1. Ramesh Sawant,et al.,simultaneous estimation of paracetamol, domperidone and tramadol HCl in pure and tablet dosage form by using 0.1N NaOH as a solvent and absorbance maximums at 256 nm, 289.6 nm and 218.4 nm respectively. Validation study reveals that the methods are specific, accurate, precise, and reproducible. Validation studies are statistically significant as all the statistical parameters are within the acceptance range (% COV< 2.0 and S.D. < 2.0) for both accuracy and precision study. High recovery and low % COV reveals the reliability of the method for quantitative study of three drugs in tablet formulation.

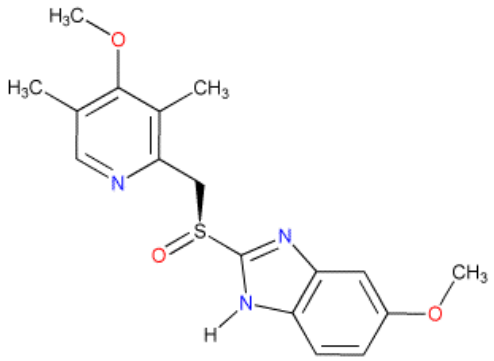
2. Dilip G Maheshwari, et al., HPLC method was developed for the simultaneous determination of esomeprazole and domperidone in combined dosage forms. The method employed C18 phenomenex column, acetate buffer: acetonitrile: methanol (55:35:10) as mobile phase and detection was made at 290nm. The retention times were found to be 6.76 and 4.42 The value for LOD was found to be 0.3 µg/mL and 0.4 µg/mL and LOQ was found to be 1.5 µg/mL and 2.5 µg/mL for ESO and DOMPE respectively The method was suitable for routine analysis of ESO and DOMPE both individually and in combined dosage forms.
3. Md. AhsanulHaque, et al., RP-HPLC method has been developed and validated for the quantification of ranitidine hydrochloride, domperidone and naproxen in solid dosage form. A shim-pack CLC-ODS column (250 mm X 4.6 mm, 5_ and a mobile phase constituting 0.1 M orthophosphoric acid solution (pH 3.0): methanol (35:65 v/v) were used. The retention times is 2.702 min, 3.666 min and 9.842 min respectively The calibration curves were linear over the concentration range of 80% to 120% of target concentration The method is accurate with 99.5% to 100.04% recovery.
4. Kaur Pardeep, et al., The use of first order derivative spectrophotometry allowed simultaneous determination of domperidone and esomeprazole, in fixed dose combination products. The absorbance values at 285nm and 301. This method obeyed Beer's law in the concentration range of 8-30 µg/ml and 5-20 µg/ml for both domperidone and esomeprazole, respectively. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method.
5. Bhavna Patel, et al.,,A RP-HPLC method has developed for simultaneous estimation of lansoprazole and domperidone in combined capsule dosage form. mobile phase is Acetonitrile:
Methanol (81:19). The detection of the combined dosage form was carried out at 280 nm and a Linearity was obtained in the concentration range of 8-24 µg/ml of lansoprazole and 8-24 µg/ml of domperidone with a correlation coefficient of 0.9977 and 0.9992. the column used RP-C18.
6. Sohan S. Chitlange, et al.,RP-HPLC method has been developed for the simultaneous determination of Dexrabeprazole and Domperidone in combined dosage form. Chromatography was carried out on a C-18 column (4.6 mm × 250 mm, 5 µm) using Acetonitrile: 0.025 M potassium dihydrogen orthophosphate buffer (pH adjusted to 5.1 with triethylamine) in the ratio of 30:70 (v/v).
7. R. Kalirajan, et al.,RP-HPLC has been developed for the simultaneous determination of rabeprazole and domperidone in pharmaceutical dosage forms. Chromatography was carried out on a C-18 column (4.6 mm × 250 mm, 5 µm) using a mixture of phosphate buffer (pH 7.4) and acetonitrile in the ratio of 65:35 (v/v) as the mobile phase at a flow rate of 1.5 mL/min and eluents are monitored at 290nm. Retention time of rabeprazole and domperidone was found to be 5.45 and 11.07 respectively. The % recovery value for rabeprazole is 100.2% and for domperidone is 102.
8. T. Sivakumar, et al., RP-HPLC method has been developed and validated for simultaneous determination of domperidone and pantoprazole in capsules. The com-pounds were separated on an ODS analytical column with a mixture of methanol, acetonitrile, and triethylamine solution (10 mM, pH 7.0 ± 0.05adjustedwith85%phosphoricacid) in the ratio 20:33:47 (v/v) . UV detection was performed at 285 nm.
9. Girish G. Rathi, et al., RP-HPLC method was developed for the determination of Esomeprazole magnesium present in bulk and pharmaceutical dosage forms. Efficient chromatographic separation was achieved on Kromasil100-C18 stationary phase (250 X 4.6 mm i.d., 5µ particle size) .mobile phase combination of acetonitrile: phosphate buffer 55: 45 (V/V).
10. Priti D Trivedi, et al., Absorption ratio method was developed and validated for the determination of Esomeprazole and Domperidone in capsules. The method involved Q-absorption analysis based on the measurement of absorbance at two wavelengths, i.eλmax of Esomeprazole (301 nm) and Iso-absorptive point of both drugs (290 nm). Beer's law was obeyed in the concentration range between 1-11µg for both Esomeprazole and Domperidone.
11. Palavai Sripal Reddy et al., RP-HPLC method and validated for the determination of both Naproxen and Esomeprazole in pharmaceutical compositions. The chromatographic separation was achieved on a Xterra RP-18 column (150 × 4.6 mm, 5µ) using a mobile phase consisting buffer prepared with 0.005 mole of sodium perchlorate, 5 mL N-butyl amine in

milli-Q grade water with a pH of 8.7 which is mixed with Acetonitrile and Methanol at a flow rate of 1.5mL per minute. Wavelength chosen for detection is 305 nm.

4. DRUG PROFILE

1.ESOMEPRAZOLE

MOLECUALR STRUCTURE:



CHEMISTRY:

6-methoxy-2-{[(4-methoxy-3,5-dimethylpyridin-2-yl)methane]sulfinyl}-1H-1,3-benzodiazole.
a)Molecular for : $C_{17}H_{19}N_3O_3S$

b) Molecular weight: 345.416g/mol

APPEARANCE :

It is odorless, white crystalline powder

SOLUBILITY :

It is slightly soluble in water and sparingly soluble in ethanol, soluble in methanol,

and acetonitrile.

PK_a :18.29

CATEGORY:

- Anti – Ulcer Agents
- Enzyme Inhibtors
- Proton – Pump Inhibitors
- Antihistamines

MECHANISM OF ACTION:

Esomeprazole is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the H^+/K^+ -ATPase in the gastric parietal cell. By acting specifically on the proton pump, esomeprazole bloks the final step in acid production, thus reducing gastric acidity.

PHARMACOKINETES:

It delayed-release formulations and are effective orally. Metabolites of these agents are excreted in urine and feaces.

DOSE : 10mg, 20mg, 40mg.

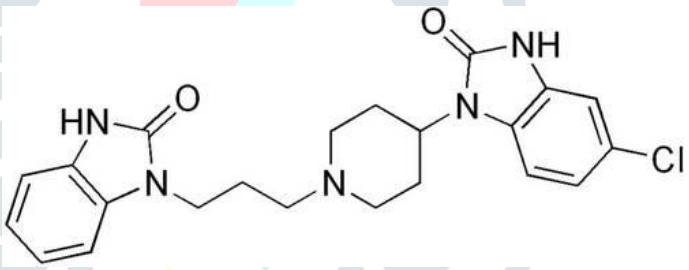
BRAND NAMES: NEXIUM

ADVERSE EFFECTS:

- Diarrhea
- Headache
- Nausea
- Stomach ache
- Constipation

2.DOMPERIDONE

MOLECULAR STRUCTURE:



CHEMISTRY:

5-chloro-1-{1-[3-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)propyl]piperidin-4-yl}- 2,3-dihydro-1H-1,3-benzodiazol-2-one

Molecular formula: $C_{22}H_{24}ClN_5O_2$

Molecular weight: 425.911g\mol

MELTING POINT: 242.5 Oc

SOLUBILITY: It is insoluble in water, methanol, Acetonitrile.

PKa : 7.9

CATEGORY: Antiemetics, Dopamine Antagonists

MECHANISM OF ACTION:

Domperidone acts as a gastrointestional emptying (delayed) adjunct and peristaltic Stimulant. The gastroprokinetc properties of domperidone are related to its peripheral dopamine receptor blocking properties. Domperidone facilitates gastric emptying and decreases small bowel transit time by increasing esophageal and gastric peristalsis and by lowering esophageal sphincter pressure. Antiemetic: The antiemetic properties of domperidone are related to its dopamine receptor blocking activity at both the chemoreceptor trigger zone and at the gastric level. It has strong affinities for the D_2 and D_3 dopamine receptors, which are found

in the chemoreceptor trigger zone, located just outside the blood brain barrier, which- among others- regulates nausea and vomiting.

DOSE: 30mg.

BRAND NAMES: Lopac –D-122, Motilium, Nauzelin

ADVERSE EFFECTS: Galactorrhea, Gynecomastia, Dry mouth, Headache

5. EXPERIMENTAL INVESTIGATION

5.1. MATERIALS AND INSTRUMENTS

5.1.a. Instruments used:

System : HPLC Shimadzu LC 10AT (Isocratic system)

Pump : I80 (LC – 10 AT Vp series)

Detector : UV-visible

Column : Xteera RP8 C18 150 X 4.6mm.5u

Injector : Rheodyne(U.S.A)

Elico pH meter

LABINDIA 3000 – Double beam UV-VISIBLE spectrophotometer

Gelman science vaccum pump

A & D – Digital analytical balance

5.1. b. Reagents and Chemicals:

Sodium dihydrogen phosphate A.R	:	Merck
Methanol	:	HPLC Grade, (Merck)
Water	:	HPLC Grade

5.1. c. Reference Standards:

1. Esomeprazole % purity - 99.6

2. Domperidone % purity –99.53

5.1. d. Tablet Brand Used: Izra* D 40

Label claim:
Esomeprazole : 40mg

Domperidone : 30mg

5.2 METHOD DEVELOPMENT AND OPTIMIZATION BY HPLC

Solubility:-

According to literature studies, it was found that Esomeprazole is slightly Water soluble and Domperidone is soluble in methanol. The solubility of both the drugs in methanol, and water was checked and it was found that both the drugs were soluble in the above mentioned solvents. Finally methanol were chosen as a component of mobile phase for the present work.

Selection of chromatographic condition:-

Proper selection of the method depends upon the nature of the sample (ionic / ionisable / neutral molecule), its molecular weight and solubility. The drugs selected in the present study are polar in nature and hence reversed phase HPLC method may be the most suitable method. The reversed phase HPLC was selected for the separation because of its simplicity and suitability.

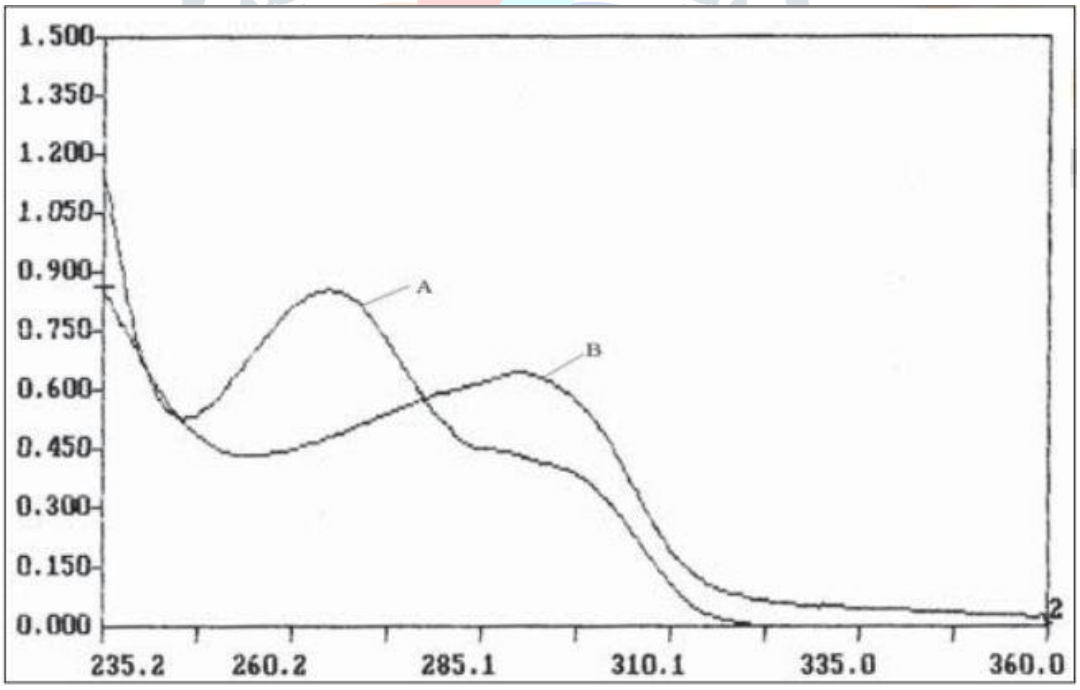
Preparation of Standard Stock solutions:-

About 40 and 30 mg of and Esomeprazole and Domperidone were accurately weighed and transferred into 25 ml volumetric flasks and dissolved in 10ml of Methanol and made up to 25 ml with Methanol.

Selection of maximum wavelength:-

The standard stock solutions were further diluted with Methanol to get desired concentration. These solutions were scanned separately between 200-400 nm range using Methanol as blank. From UV spectra, 210 nm and 280 nm were found to be the maximum absorption wavelengthsof Esomeprazole and Domperidone respectively. Absorbance of combined stock solutions was also determined and the maximum wavelength for the analysis of both the drugs was determined at 210 nm.

Figure:- 3 Combined U.V.Spectra of Esomeprazole and Domperidone.



Optimization of Chromatographic conditions:-

Different initial chromatographic conditions like the type of the column, the detector used, injection volume, column temperature, flow rate were selected and tried with different mobile phase compositions depending upon the type of the analyte. Further the most suitable chromatographic conditions for the analysis of Esomeprazole and Domperidone were optimized for carrying out further work.

5.2.a. METHOD DEVELOPMENT TRIALS

Trial-I

Objective:

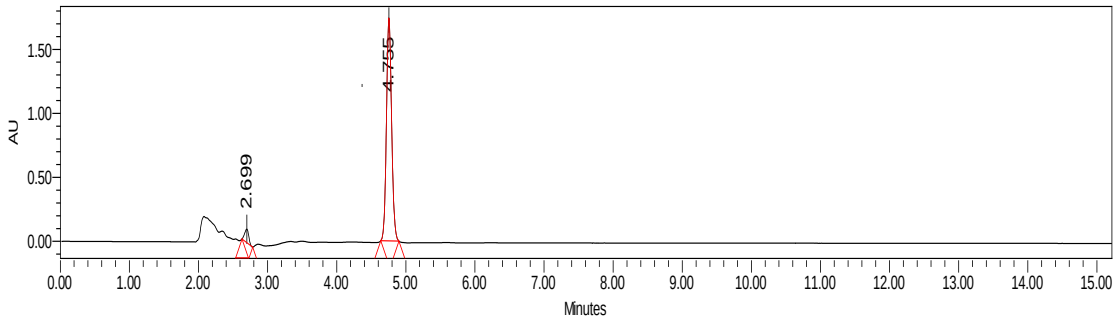
To develop a method for the determination of Esomeprazole and Domperidone by HPLC.

Chromatographic conditions:

Column	:	Xteera RP8 C18,150x 4.6 mm column ,5 µm.
Column temperature	:	30◦c
Flow rate	:	1.0 ml/min.
Injection volume	:	10 µl.
Wavelength	:	210 nm
Mobile phase composition	:	Water : methanol (50:50 %v/v)

Trial 1

Chromatogram no. 1



Observation:

In this trail, peak of only one drug was observed. Hence gone for further trail.

Trial-II

Objective:

To develop a method for the determination of Esomeprazole and Domperidone by HPLC.

Chromatographic conditions:

Column : Xteera RP8 C18,150x 4.6 mm column ,5 µm.

Column temperature : 30◦c.

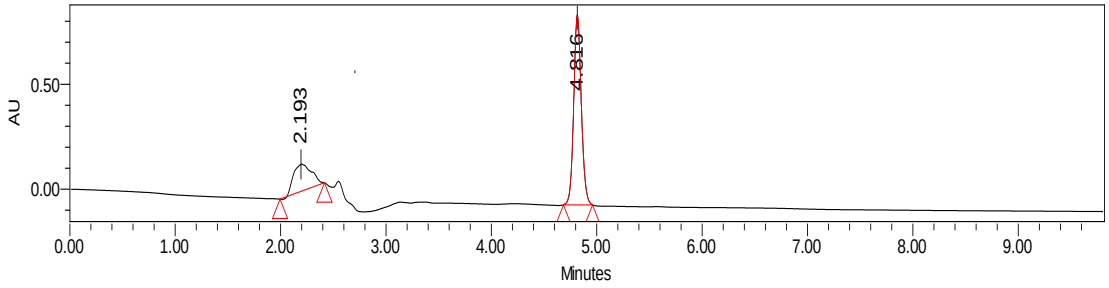
Flow rate : 1.0 ml/min.

Injection volume : 10 µl.

Wavelength : 210 nm

Mobile phase composition : Water : Methanol (30:70)

Chromatogram no. 2



Observation:

In this trail, peak of only one drug was observed. Hence gone for further trail.

Trial-III

Objective:

To develop a method for the determination of Esomeprazole and Domperidone

By HPLC.

Chromatographic conditions:

Column : Xteera RP8 C18,150x 4.6 mm column ,5 µm.

Column temperature : 30◦c.

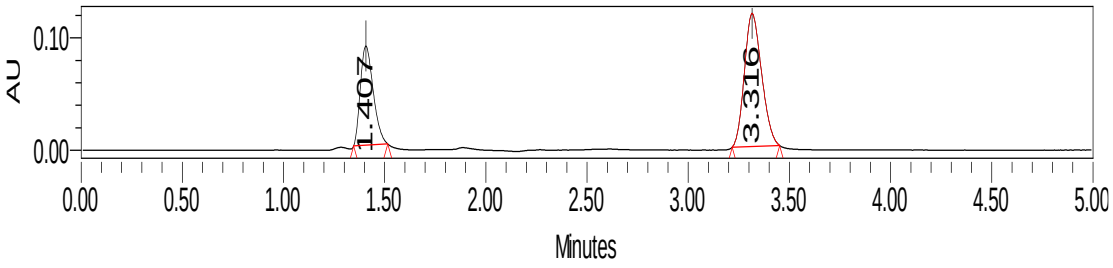
Flow rate : 1.0 ml/min.

Injection volume : 10 µl.

Wavelength : 210nm

Mobile phase composition : sodium dihydrogen Phosphate(0.05M):methanol (%50:50)

Chromatogram no. 3



Observation:

In this trail, two drugs wer eluted but resolution was not satisfactory and also baseline linearity was not good. Hence another trial was carried out.

Trial-IV

Objective:

To develop a method for the determination of Esomeprazole and Domperidone

By HPLC.

Chromatographic conditions:

Column : Xteera RP8 C18,150x 4.6 mm column ,5 µm.

Column temperature : 30◦c.

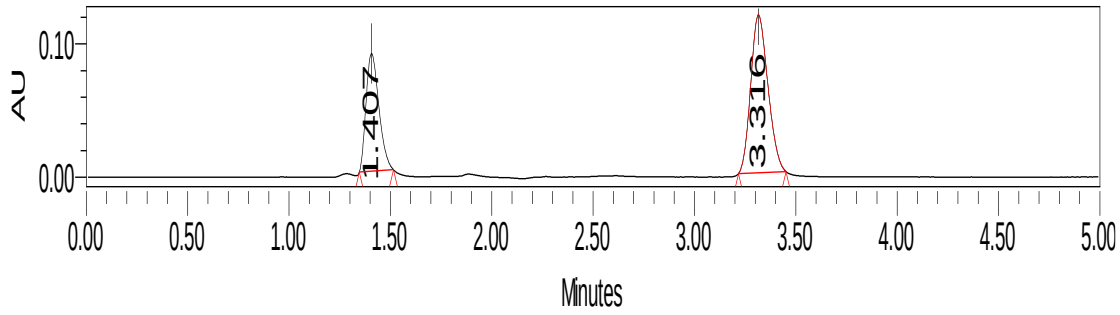
Flow rate : 1.0 ml/min.

Injection volume : 10µl.

Wavelength : 210nm

Mobile phase composition : sodium dihydrogen Phosphate(0.05M): methanol(90:10)

Chromatogram no. 4



Observation:

In this trial, two drugs was eluted but further trial was perform to reduce retention time.

Trial-V (OPTIMISED and PROPOSED METHOD)

Objective:

To develop a method for the determination of Esomeprazole and Domperidone by HPLC.

Chromatographic conditions:

Column : Xteera RP8 C18,150x 4.6 mm column ,5 µm.

Column temperature : 30◦c.

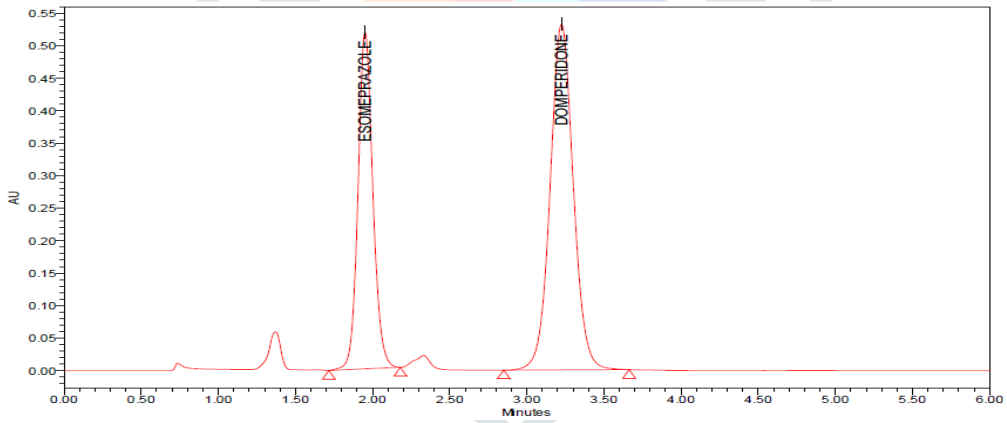
Flow rate : 1.0 ml/min.

Injection volume : 10 µl.

Wavelength : 210nm

Mobile phase composition : sodium dihdrogen Phosphate(0.05M): methanol(80:20)

Chromatogram no. 5



Observation:

In this trial, both peaks are very good and less tailing effect is observed. Peaks are with good resolution and the retention times are also satisfactory. Hence this method is taken for validation.

Preparation of mobile phase:

HPLC grade methanol and the prepared sodium dihydrogen phosphate buffer (0.05M) were taken in 80:20 (%v/v) ratio. The mobile phase was then degassed first in vaccum filter and then in sonicator for about 30 minutes.

Preparation of phosphate buffer:

7.20gm of NaH_2PO_4. (sodium dihydrogen phosphate) was taken in 1000ml of beaker. 500 ml of water was added and degassed first in vaccum filter. The solution was sonicated for about 30 minutes and made up to the volume with water.

Standard preparation:

Weighed accurately about 40mg Eomeprazole working standard and 30mg Domperidone of Working standard in to a 25ml of volumetric flask and add 10ml of Methanol and sonicate 20minutes and make up with Methanol. Transfer above solution in to 2ml in to 50ml volumetric flask dilute to volume with Methanol.

Sample preparation:

20 capsules were taken, weighed, opened and the granules were powdered. From the powdered mass, weighed accurately about 223.2mg of powder Equivalent to the label claim and transferred to a 25 ml volumetric standard flask and add 10ml of Methanol and sonicate 20minutes and make up with Methanol.

Transfer above solution in to 2ml in to 50ml volumetric flask dilute to volume with Methanol to get the concentration of 64ug/ml, 48ug/ml of Esomeprazole and Domperidonerespectvily.

ASSAY RESULTS OBTAINED:

Separately injected blank, standard (3 injections) and sample preparations (3injections) into the chromatograph and the peak area responses were recorded.

Calculation:

The amount of Esomeprazole and Domperidone was calculated by using the following formula,

$$\frac{\text{Sample area} \times \text{sample dilution} \times \text{purity of working standard} \times \text{Average weight} \times 100}{\text{Standard area} \times \text{standard dilution} \times \text{Label claim}}$$

Table No: 3 Assay result for Replicate injections of Standards

Inj .No	Area of Eomeprazole	Area of Domperidone	% of Esomeprazole	% of Domperidone
1	4148026	6814075	100	100
2	4201027	6912429	101	102
3	4121296	6798317	99	100
4	4244775	6930616	102	102
5	4086445	6736939	98	99
6	4053389	6682350	97	98
Mean	4040943	6633213	100	100
S.D	71342	96736	1.70	1.46
%R.S.D	1.7	1.4	1.72	1.46

Conclusion:

The mean recovery for assay result was found to be 100% and 100% for Esomeprazole and Domperidone respectively.

5.2.b. METHOD VALIDATION

The method development was followed by the method validation step which involves various parameters like Selectivity (Specificity), Precision, Accuracy, Linearity, Range, Limit of detection, Limit of quantification, Ruggedness, Stability, Robustness.

1. System suitability parameters

System suitability test provides the assurance that on a specific occasion the method is giving, accurate and precise results. System suitability tests are run every time a method were used either before or during analysis.

Esomeprazole and Domperidone standard solutions as per test method were prepared and five replicate injections were given. Then system suitability parameters like Theoretical plate number (N), Peak asymmetry factor (As) were studied with the help of standard chromatograms.

Table No:4System suitability Parameters

System Suitability Parameters	Esomeprazole	Domperidone
Resolution	5.33	
Tailing Factor	1.1	1
Number of theoretical Plates	2293	2609
Retention time	2.3	3.6

Linearity and Range:

Esomeprazole and Domperidone showed linearity in the range of 32-96 µg/ml and 24-72 µg/ml. The calibration graph was plotted taking peak area on Y-axis and concentration of standard solution on X-axis and the degree of linearity was estimated by calculating the correlation coefficient, slope of regression line and Y- intercept.

The slope, intercept and correlation coefficient values for Esomeprazole were found to be 55773, 197.1 and 1 respectively.

The slope, intercept and correlation coefficient values for Domperidone were found to be 11316, -10327 and 1 respectively.

Table- 5 : Linearity Data for Esomeprazole and Domperidone

Concentration of Esomeprazole (µg/ml)	Peak Area of Esomeprazole	Concentration of Domperidone (µg/ml)	Peak Area of Domperidone
32	1789722	24	2703946
48	2660326	36	4055692
64	3579445	48	5410454
80	4475206	60	6769389
96	5343689	72	8157485

Figure no.2. Linearity plot for Esomeprazole

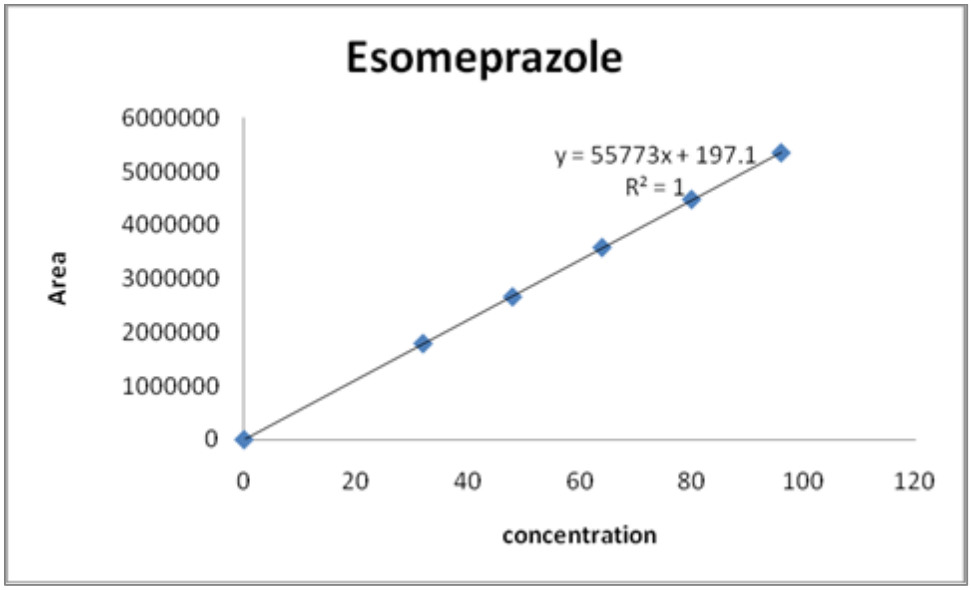


Figure no. 3. Linearity plot for Domperidone

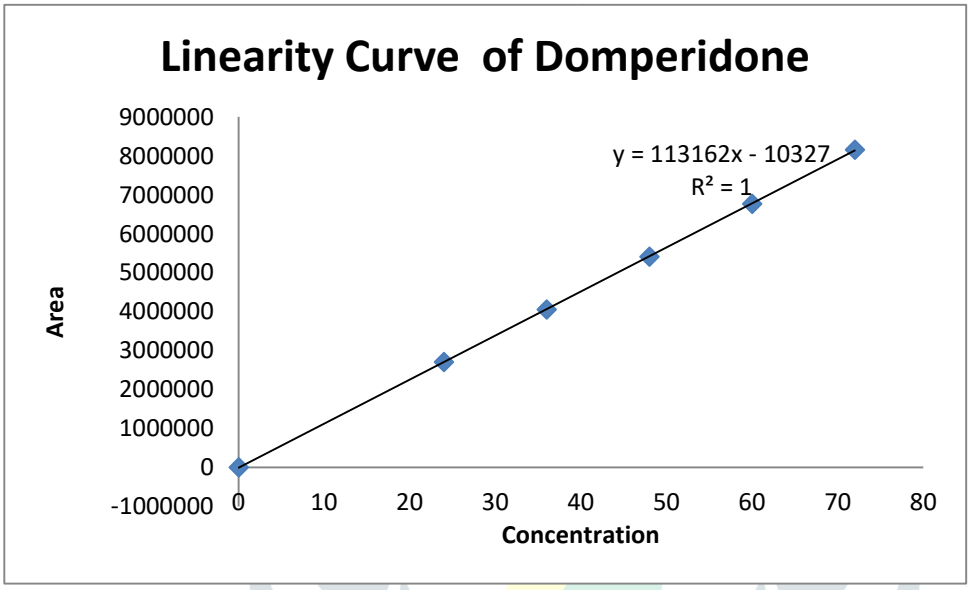


Table :6 Analytical performance Parameters for Esomeprazole and Domperidone

Parameters	Esomeprazole	Domperidone
Linearity range	32-96 µg/ml	24-72 µg/ml
Correlation Coefficient	1	1
Slope (m)	55773	11316
Intercept	197.1	-10327

ACCURACY

The accuracy of a measurement is defined as the closeness of the measured value to the true value and in a method said to be with high accuracy, a sample (whose "true value" is known) is analyzed and the measured value should ideally be identical to the true value.

Recovery studies were carried out, by adding a known quantity of the standard drug to the sample before analysis and recovery studies were carried out at 50%, 100%, 150% level and the contents were determined from the respective chromatograms. From the results obtained the accuracy of the method was confirmed.

Table No: 7: Recovery studies for Esomeprazole and Domperidone

S.I No:	Inj.Sample	Spike level	Amount Present	Amount Recovered	% Recovered
1	Esomeprazole	50 %	29.4944	29.5167	101 %
2		100 %	64.0286	62.4357	98%
3		150 %	91.6331	89.7215	99%
4.	Domperidone	50 %	22.1423	22.3102	102%
5		100 %	48.0215	47.1615	98%
6		150 %	68.7248	68.1794	100%

Table No: 8 Mean Average Recovery of Esomrprazole and Domperidone for Accuracy

Accuracy level	Mean Recovery of Esomeprazole	Mean Recovery of Domperidone
Accuracy 50%	101	102
Accuracy 100%	98	98
Accuracy 150%	99	100

PRECISION:

REPEATABILITY OF INJECTION

Precision can be defined according to ICH as "the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample". The Precision of test method was done by performing assay on six replicate determination of sample preparation at test concentration level (as per method of analysis) and relative standard deviation of assay results was then calculated accordingly.

From the six standard solutions six injections were taken and injected. The peak areas were obtained and %RSD was calculated. By the values obtained, system precision and method precision were determined.

Table No: 9 System Precision ofEsomeprazole andDomperidone

S.No.	Area of Esomeprazole	Area of Domperidone
1	4148026	6814075
2	4201027	6912429
3	4121296	6798317
4	4244775	6930616
5	4086445	6736939
6	4053389	6682350
Mean	4142493	6812454
S.D	71342	96736
%R.S.D	1.72	1.42

Acceptance Criteria: The RSD should be NMT 2.0%

Table No: 10 Method Precision of Esomeprazole andDomperidone

Sample No:	Area of Esomeprazole (mV)	Area of Domperidone (mV)
1	4147132	6804075
2	4201127	6922429
3	4121257	6790317

4	4244785	6930016
5	4086346	6730939
6	4053482	6680350
MEAN	4142355	6809688
S.D	65127.33	91765.84
% R.S.D	1.57	1.34

Acceptance Criteria: The RSD should be NMT 2.0%

ROBUSTNESS

Robustness can be defined according to ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". To demonstrate the robustness of the method developed, slight significant changes in some of the method parameters (like flow rate, pH and Column temperature) were made. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample. The effect of flow rate and change in wavelength were studied to determine the robustness of the proposed method here.

Table no.11 :- Robustness Esomeprazole and Domperidone.

Effect	Retention time of Esomeprazole	Retention time of Domperidone
Column Temperature 25◦c	2.347	3.895
Column Temperature 35◦c	2.311	3.784
Flow rate (0.8ml/min)	2.887	4.550
Flow rate (1.2ml/min)	1.984	3.209

RUGGEDNESS

Ruggedness is defined by USP as "the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials". Method ruggedness is in short told, the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, source of reagents, chemical, solvents and so on.

Table no: 12 Ruggedness

Analysts	Area of Esomeprazole	Area of Domperidone
Analyst 1	4101047	6749484
Analyst 2	4064094	6718904

LIMIT OF DETECTION :

The definition of Limit of detection can be given as "the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantified by the method, under the stated experimental conditions". It is the smallest level of analyte that gives a response which can be measured. The LOD is often based on a certain signal-to-noise (S/N') ratio, typically 2 or 3. From the linearity curve the minimum concentration at which the analyte can be detected (LOD) is determined by applying the following formula.

LOD = 3.3 (SD/slope)

The lowest concentration of Esomeprazole that can be detected was determined from standard curve 0.136µg/ml.

The lowest concentration of Domperidone that can be detected was determined from standard curve was 0.915µg/ml.

LIMIT OF QUANTITATION:

The definition of Limit of quantitation can be given as "the lowest concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug with acceptable precision and accuracy under the stated experimental conditions of the method". The limit of Quantitation (LOQ) is the smallest concentration of analyte which gives a response that can be accurately quantified. From the linearity curve the minimum amount of analyte that can be quantified (LOQ) can be calculated by applying the following formula.

LOQ = 10 (SD/ slope).

The lowest concentration at which peak can be quantified is called LOQ. It was found to be 0.4132µg/ml for Esomeprazole and for Domperidone was found to be 0.2775µg/ml.

Table No: 13 LOD and LOQ values

Sample	LOD	LOQ
Esomeprazole	0.136 µg/ml	0. 4132µg/ml
Domperidone	0.915 µg/ml	0.2775 µg/ml

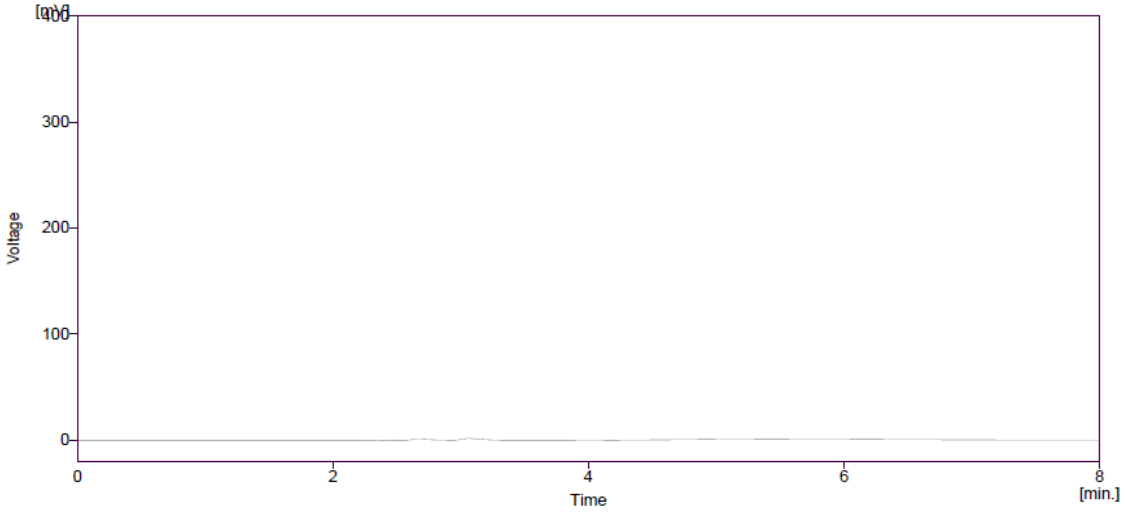
STABILITY STUDIES:

The sample and standard used in HPLC method should be stable for a time sufficient to generate reproducible and reliable results. The stability of the sample spiked with drug was subjected to short term stability at room temperature (Initial & after 8 hours) and the stability of the respective solution was determined.

Table No: 14 Solution Stability after 8 hours.

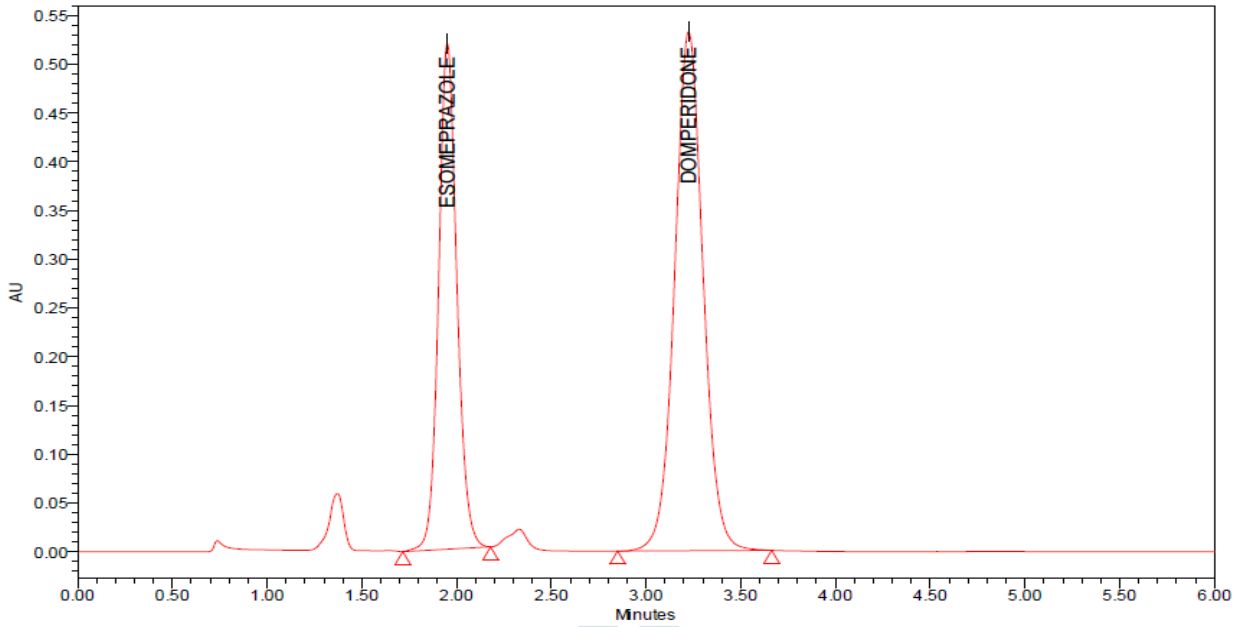
Time	Area of Esomeprazole	Area of Domperidone
Initial	4101047	6749484
After 8 hours	4148026	6772833
Deviation	46979	23349

CHROMATOGRAMS
Chromatogram No : 6 BLANK


[mV]
400
300
200
100
0
Voltage
0
2
4
6
8
Time
[min.]

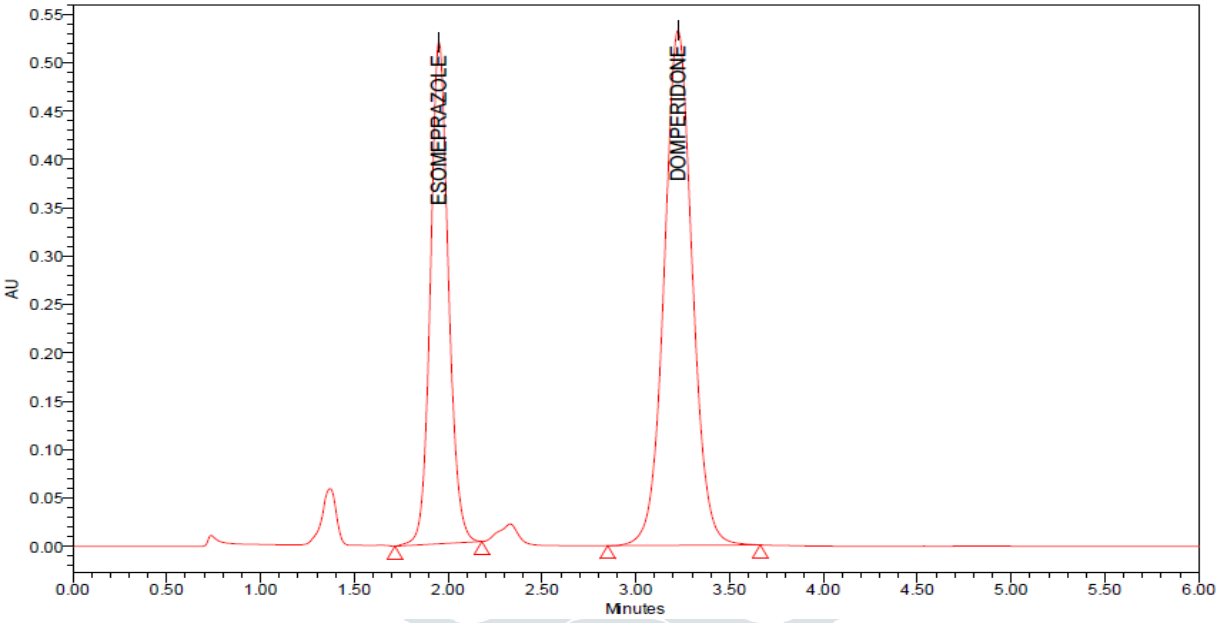
Chromatogram No : 7

Standard Chromatogram : Esomiprazole and Domperidone



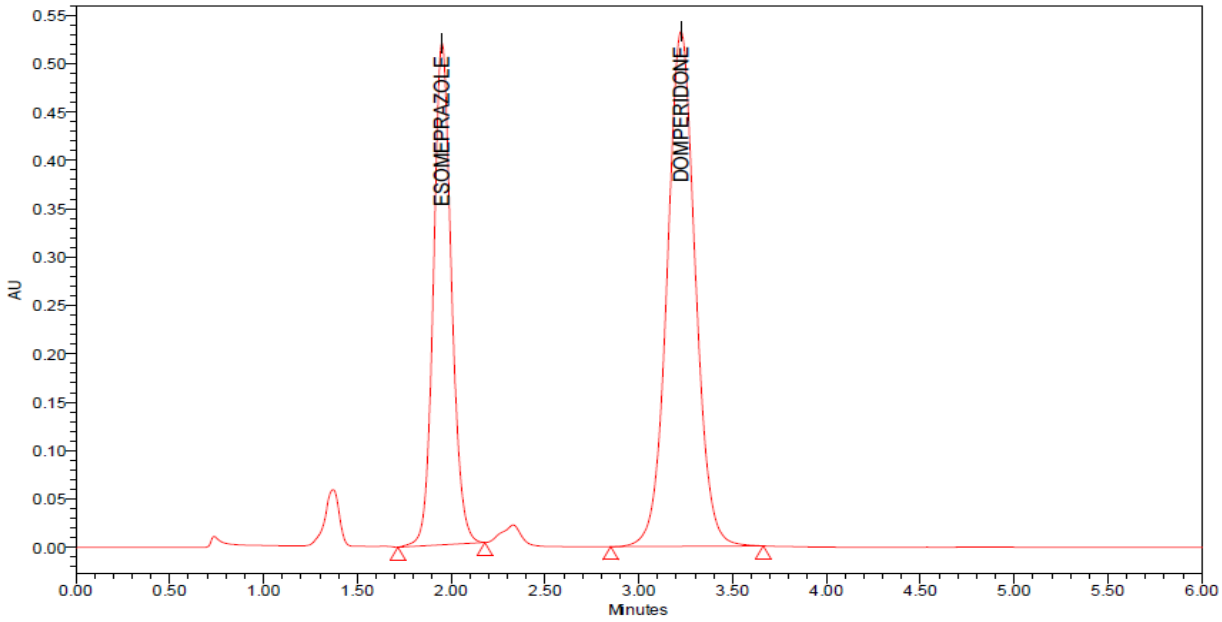
Chromatogram No : 8

Assay



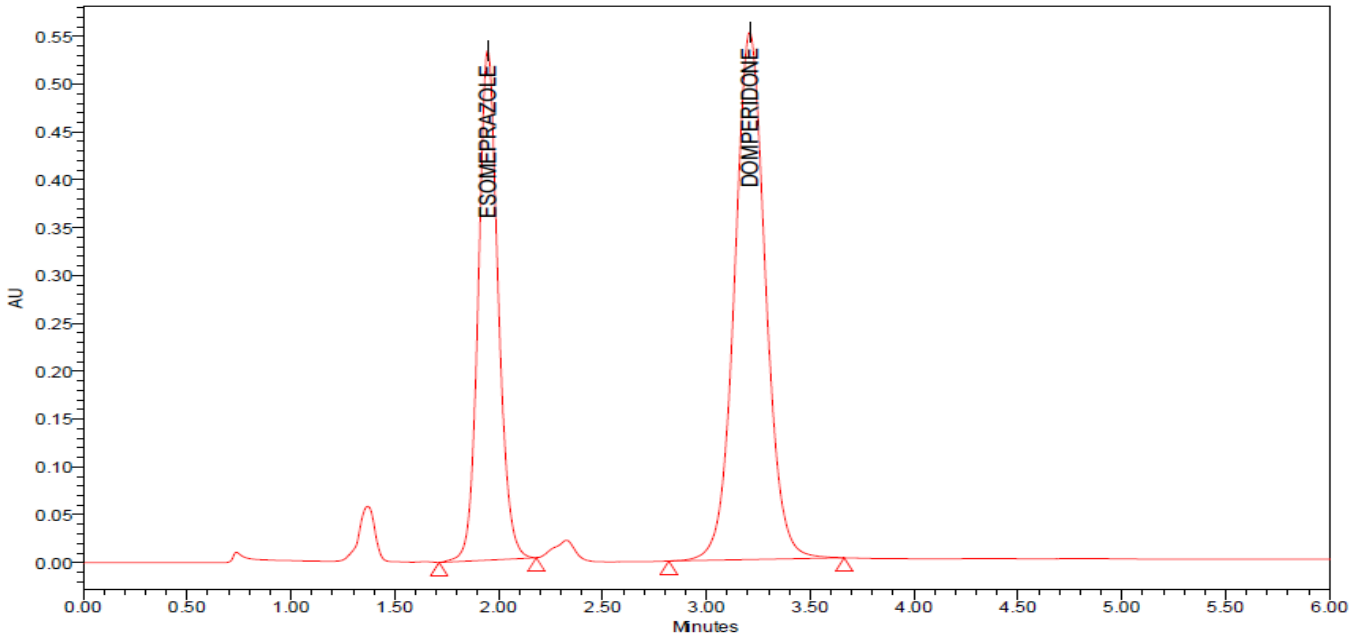
Chromatogram No : 9

System Suitability



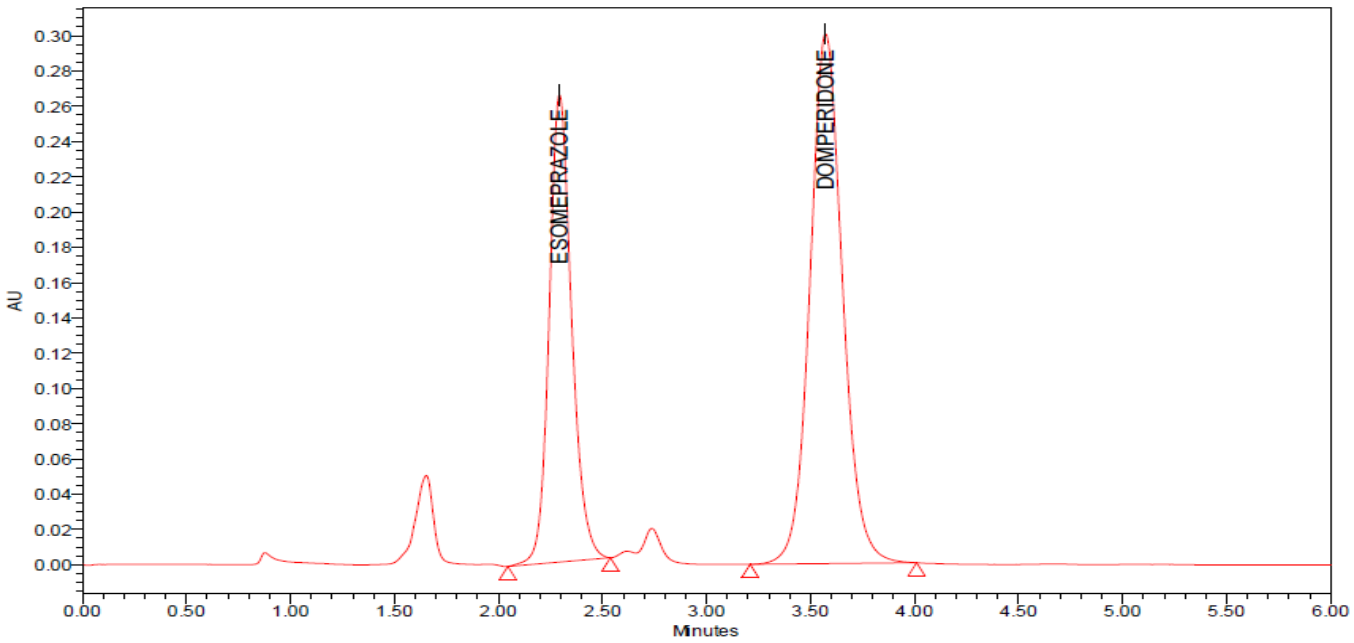
Chromatogram No : 10

Linearity 1



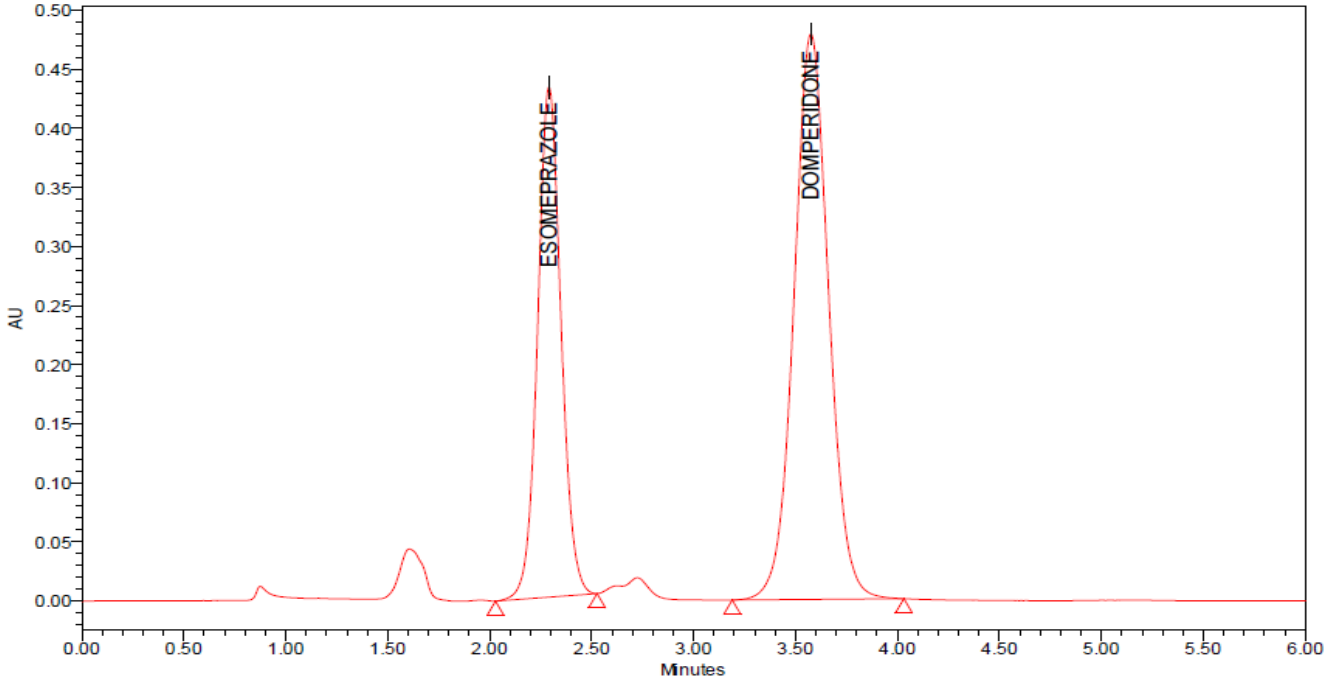
Chromatogram No : 11

Linearity 2



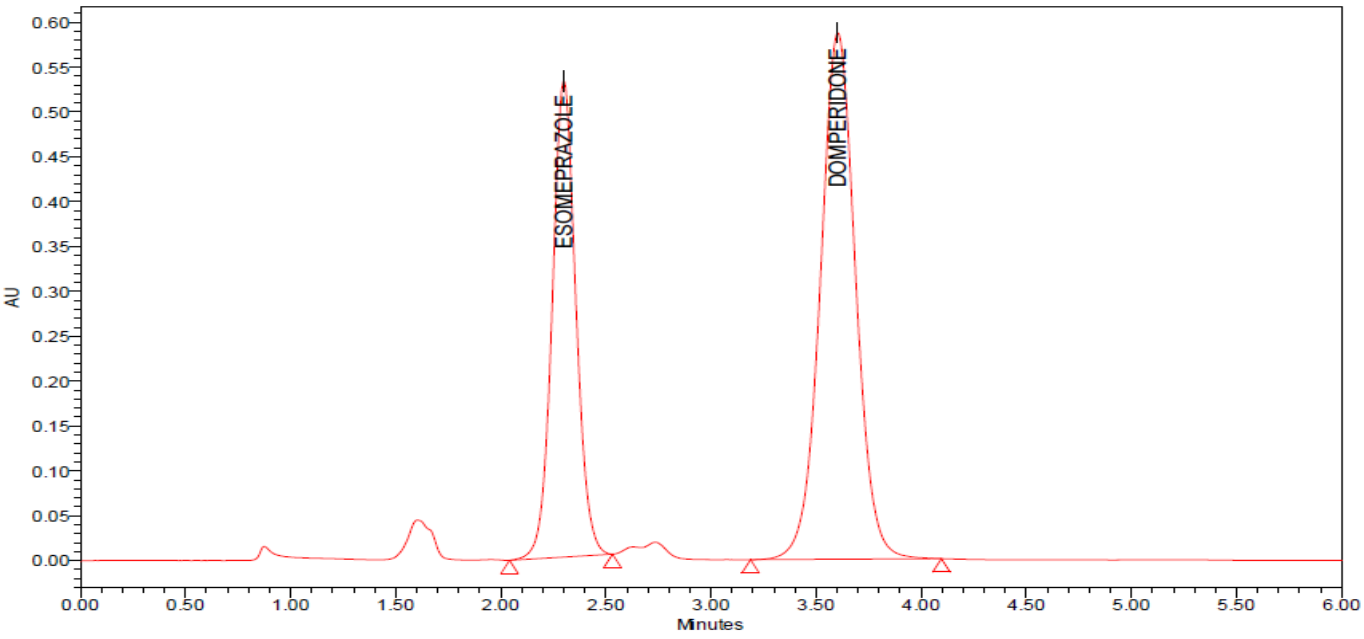
Chromatogram No : 12

Linearity 3



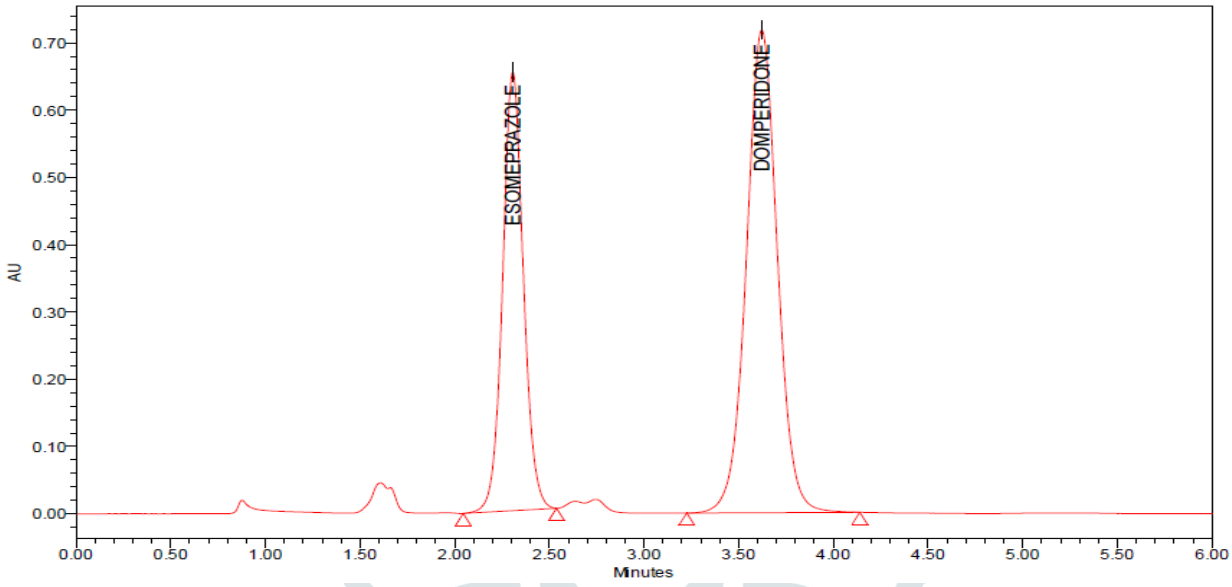
Chromatogram No : 13

Linearity 4



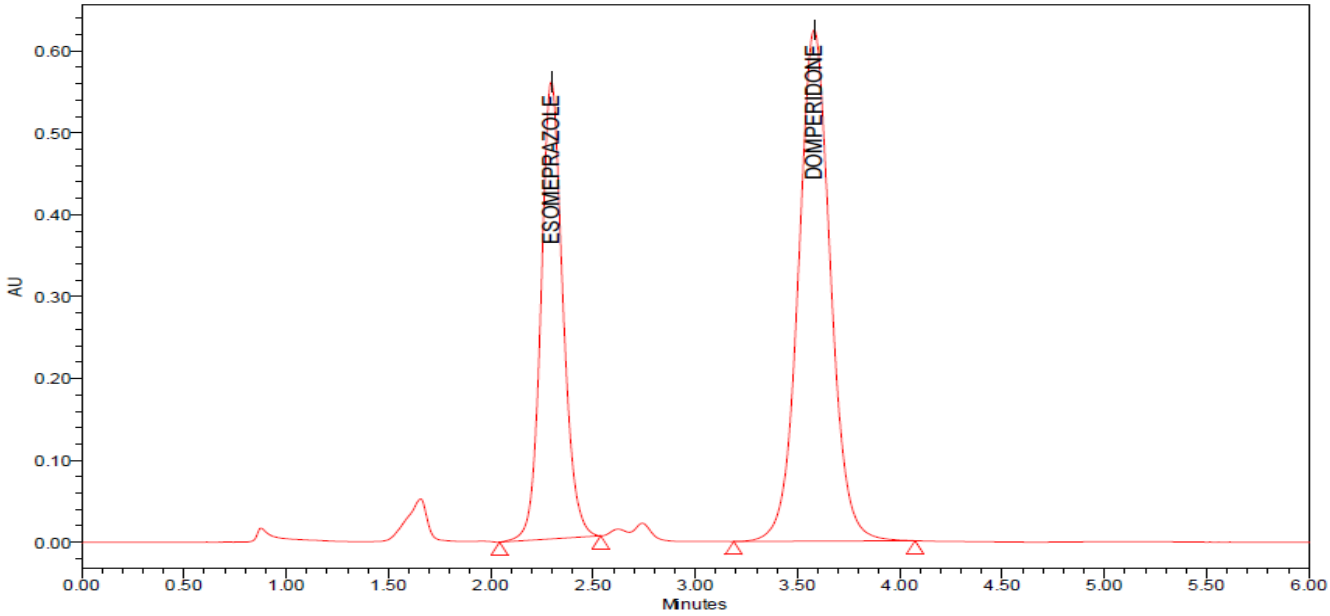
Chromatogram No : 14

Linearity 5



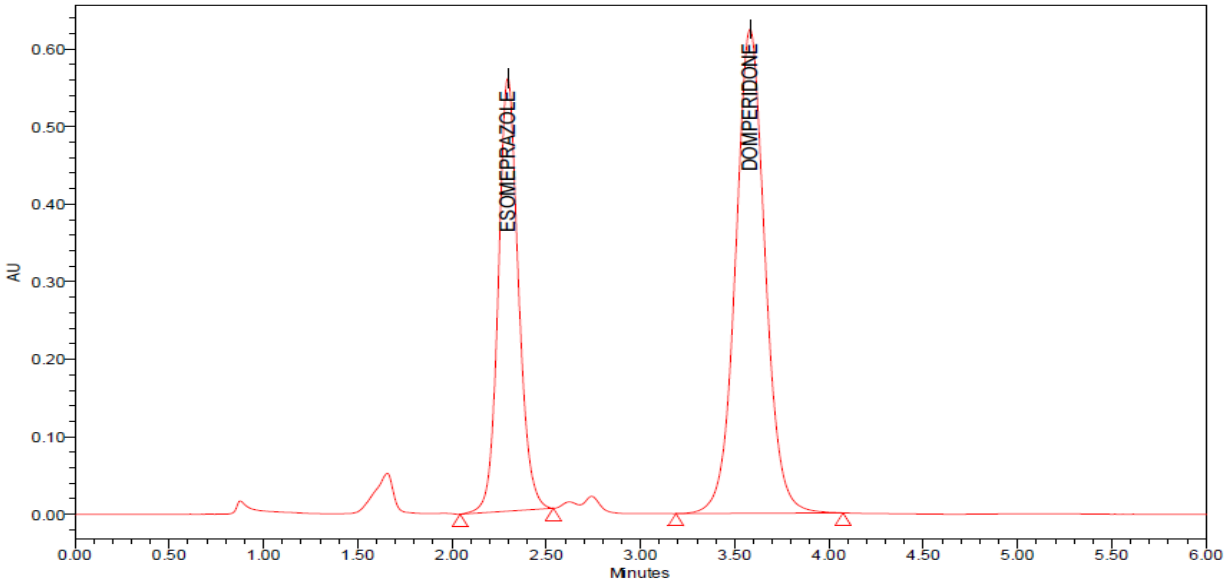
Chromatogram No :15

System precision -1



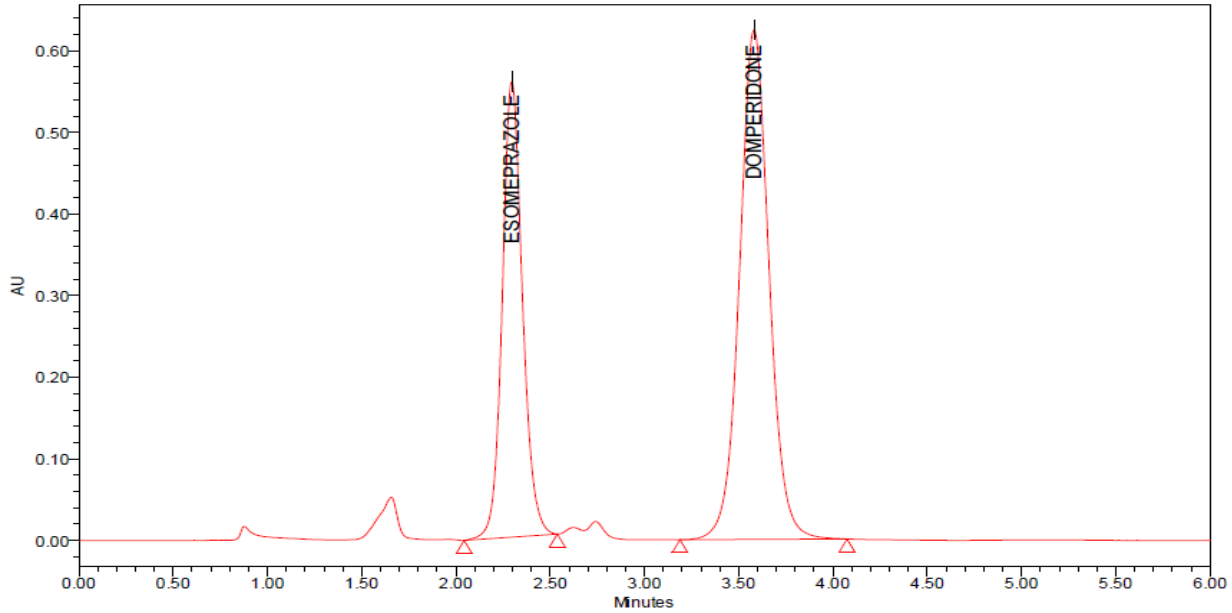
Chromatogram No :16

System Precision -2



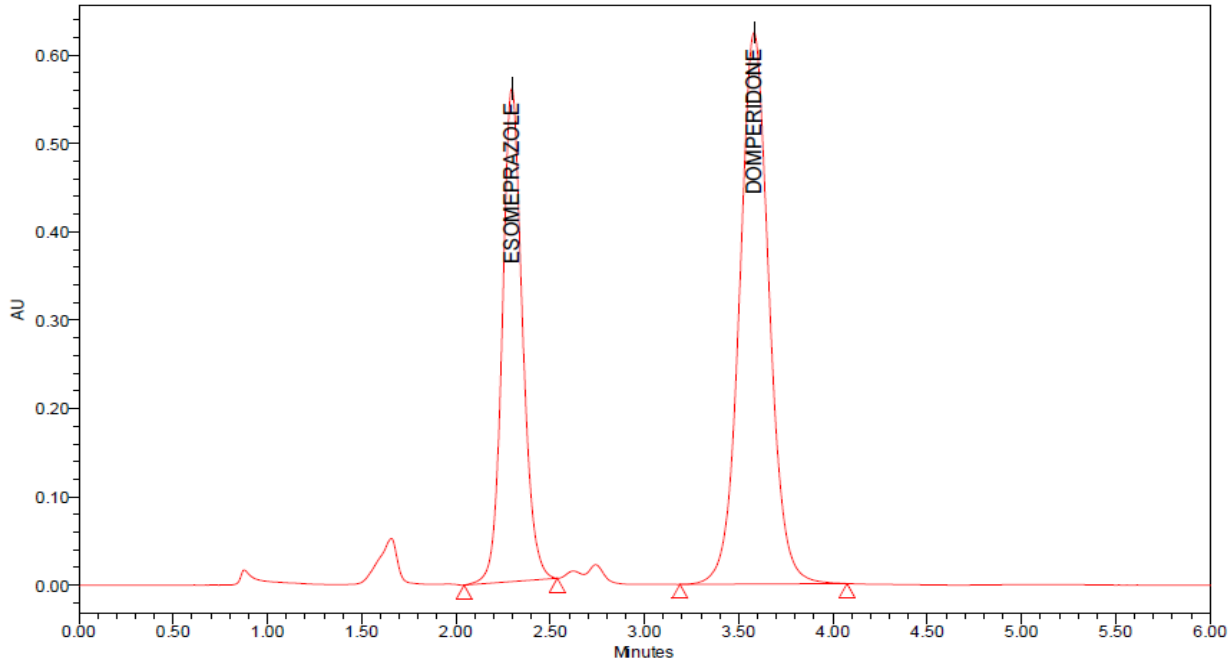
Chromatogram No :17

System Precision – 3



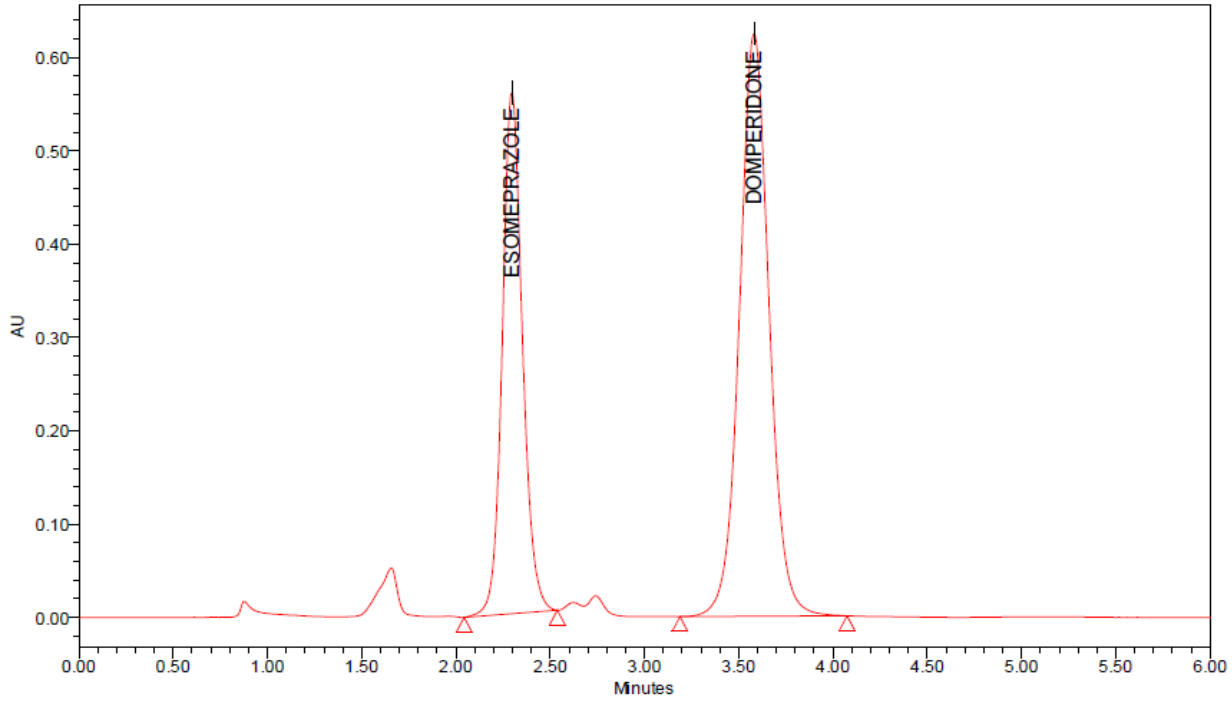
Chromatogram No :18

System Precision - 4



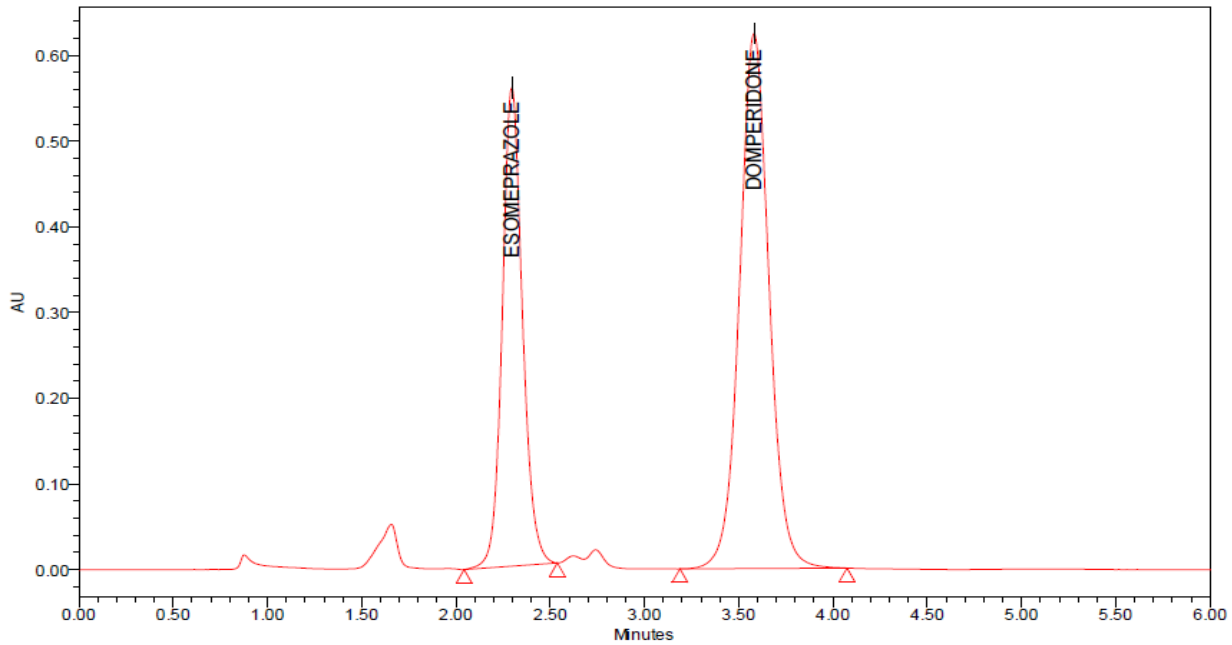
Chromatogram No :19

System Precision - 5



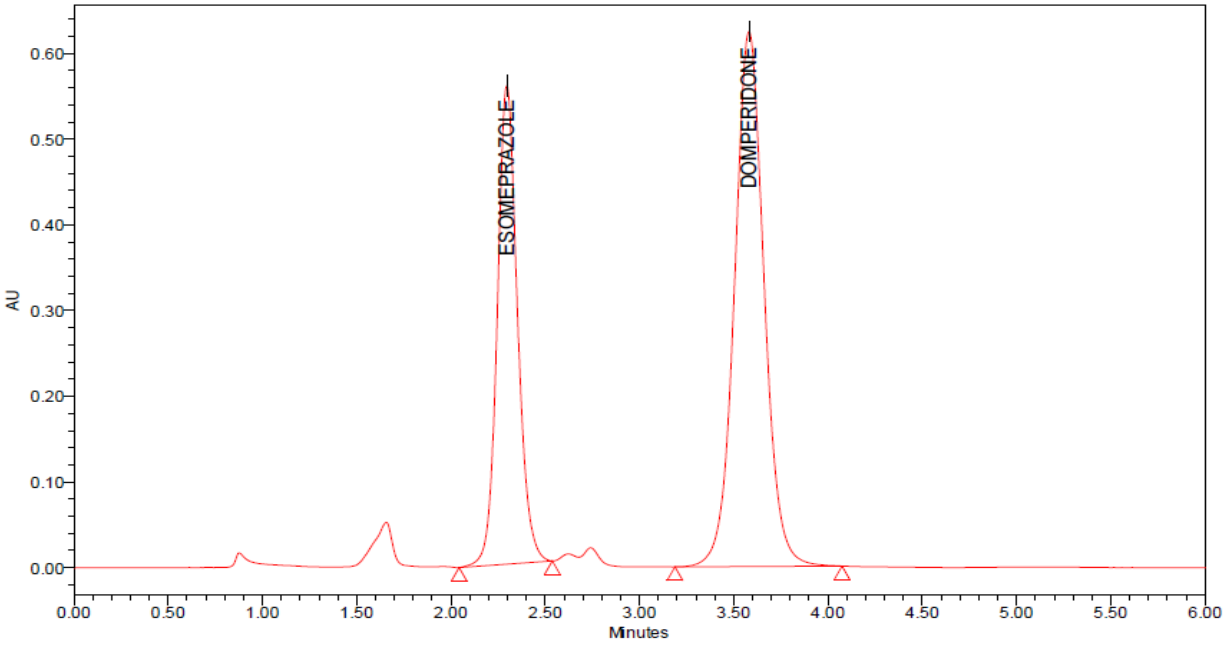
Chromatogram No :20

System Precision -6



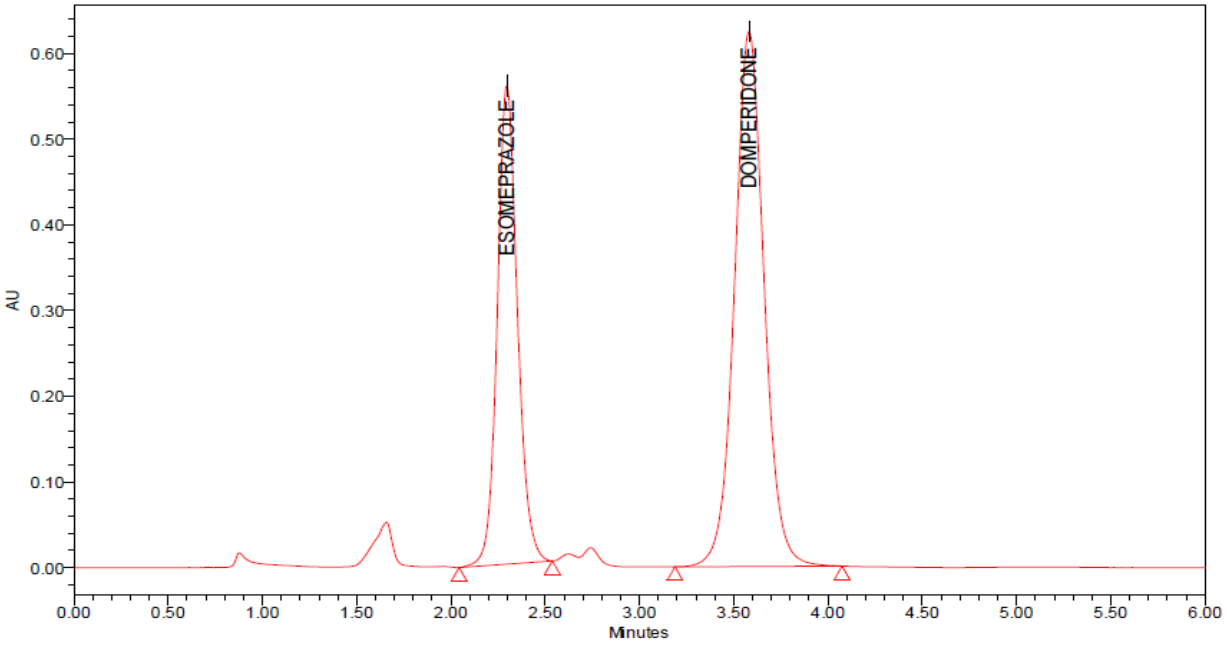
Chromatogram No :21

Method Precision No - 1



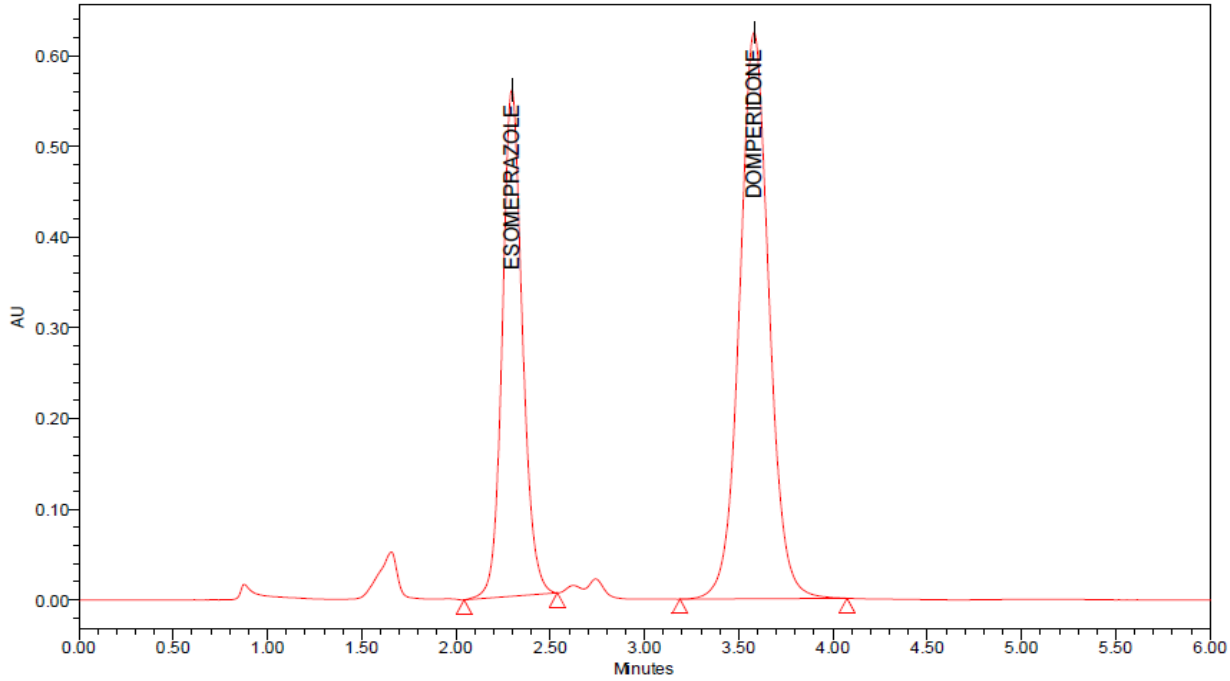
Chromatogram No :22

Method Precision -2



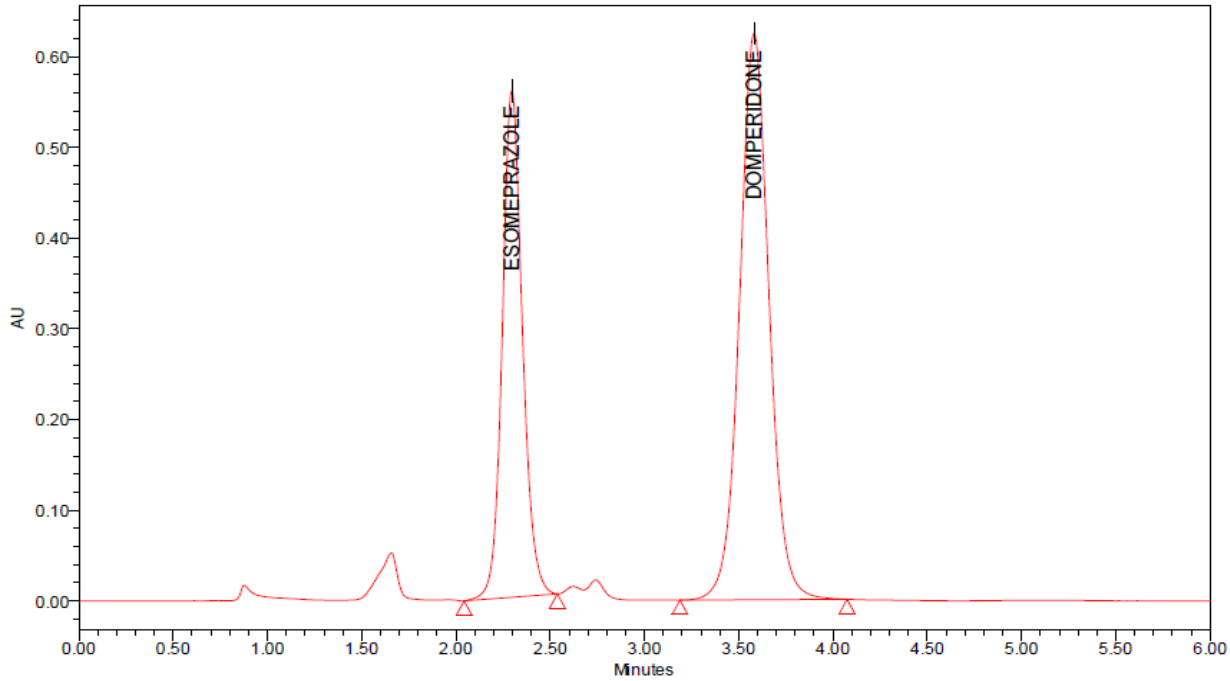
Chromatogram No :23

Method Precision - 3



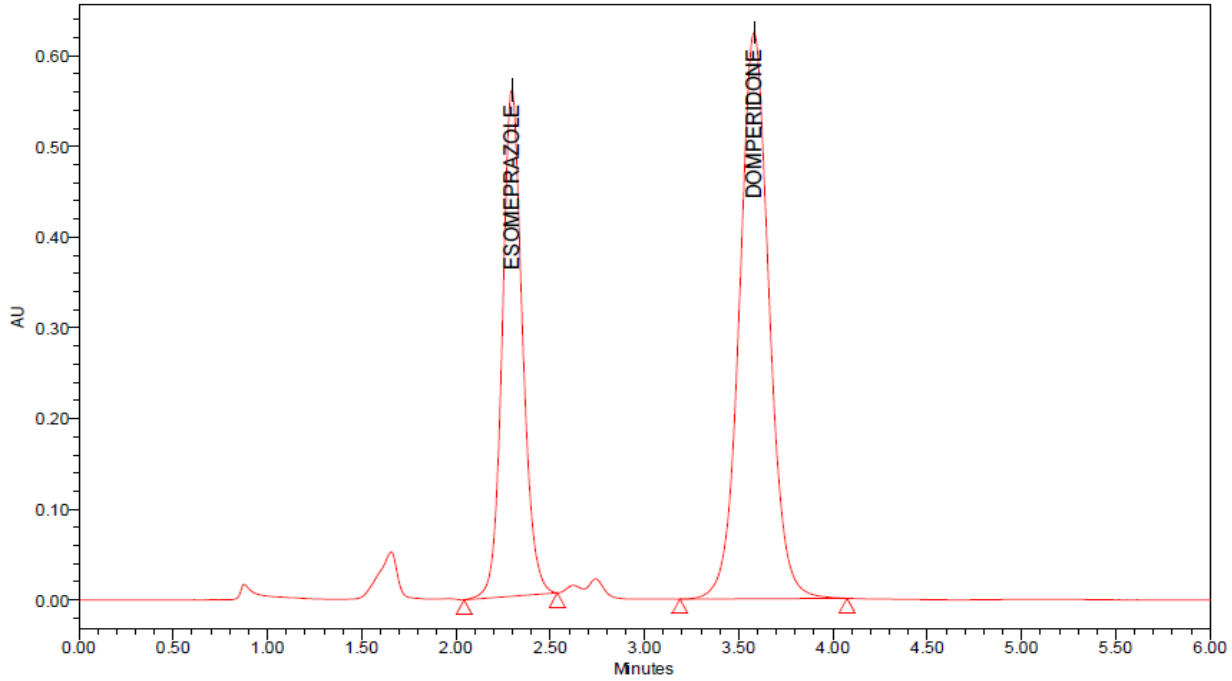
Chromatogram No :24

Method Precision -4



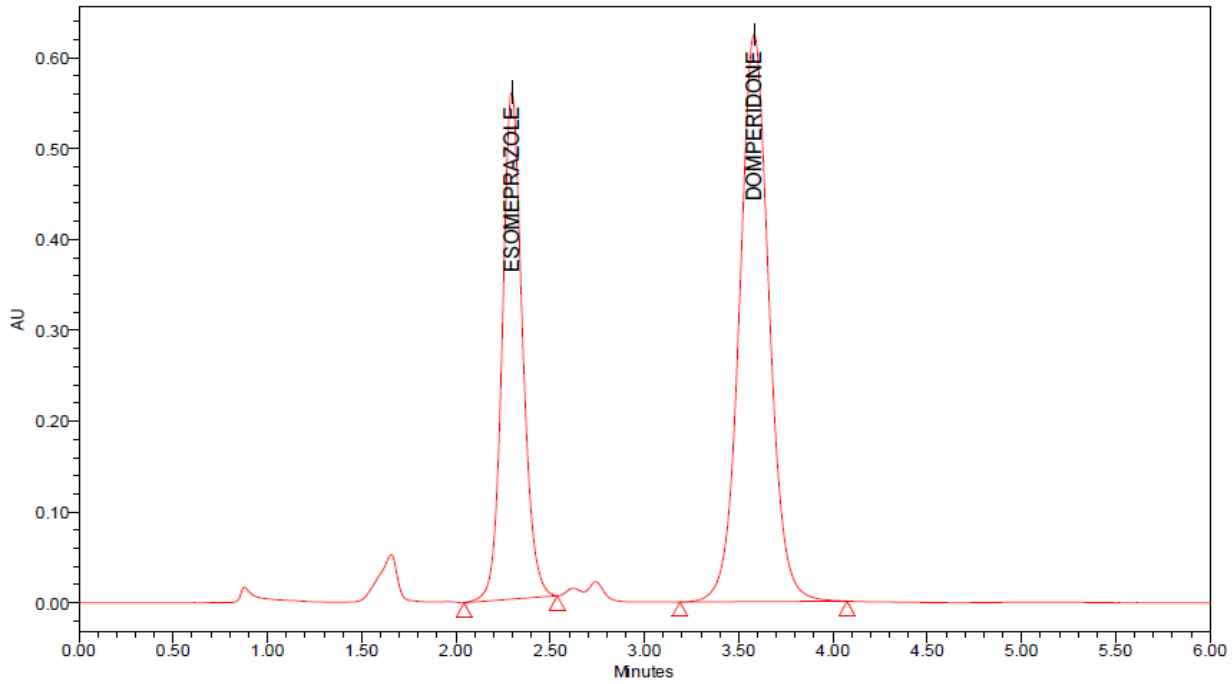
Chromatogram No –25

Method Precision - 5



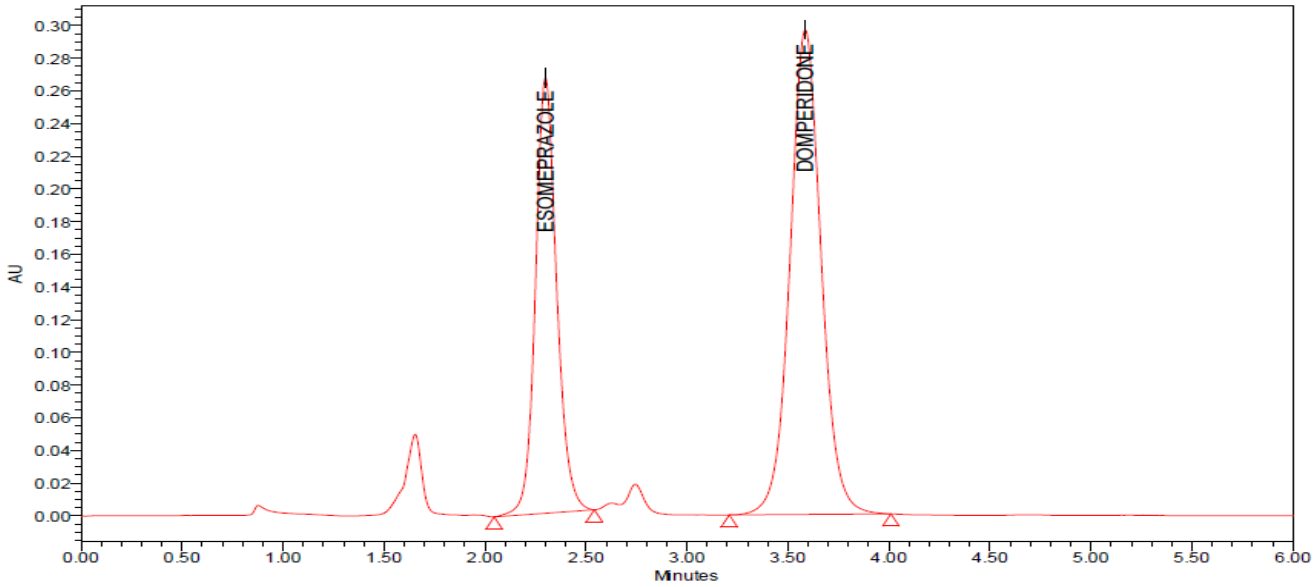
Chromatogram No –26

Method Precision - 6

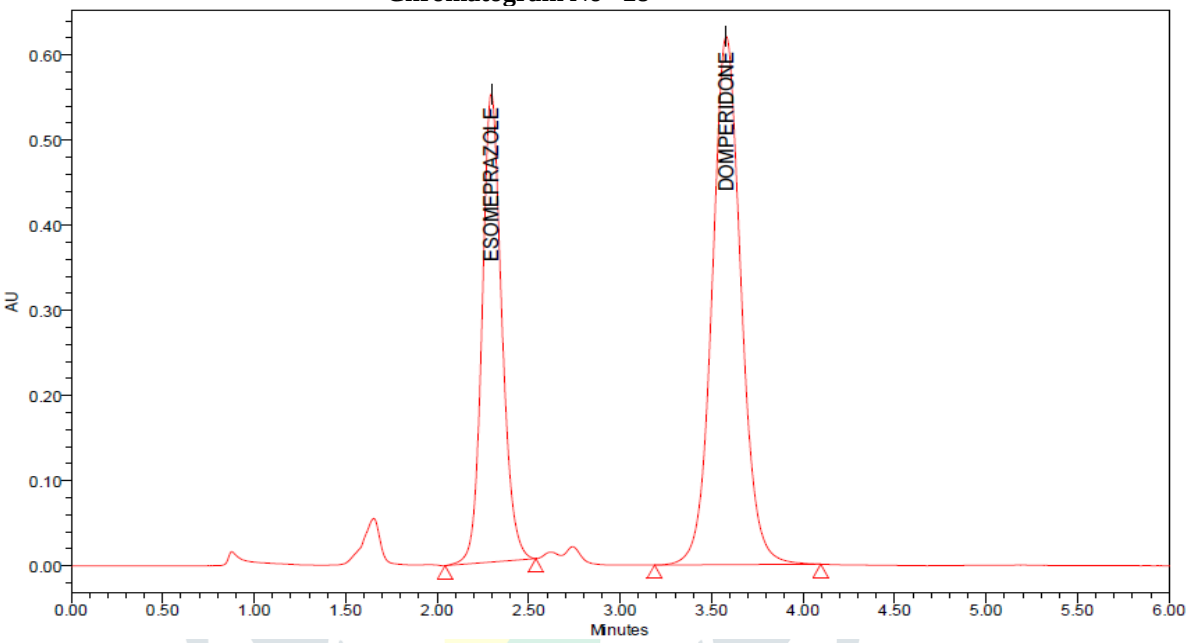


Chromatogram No –27

Accuracy - 1



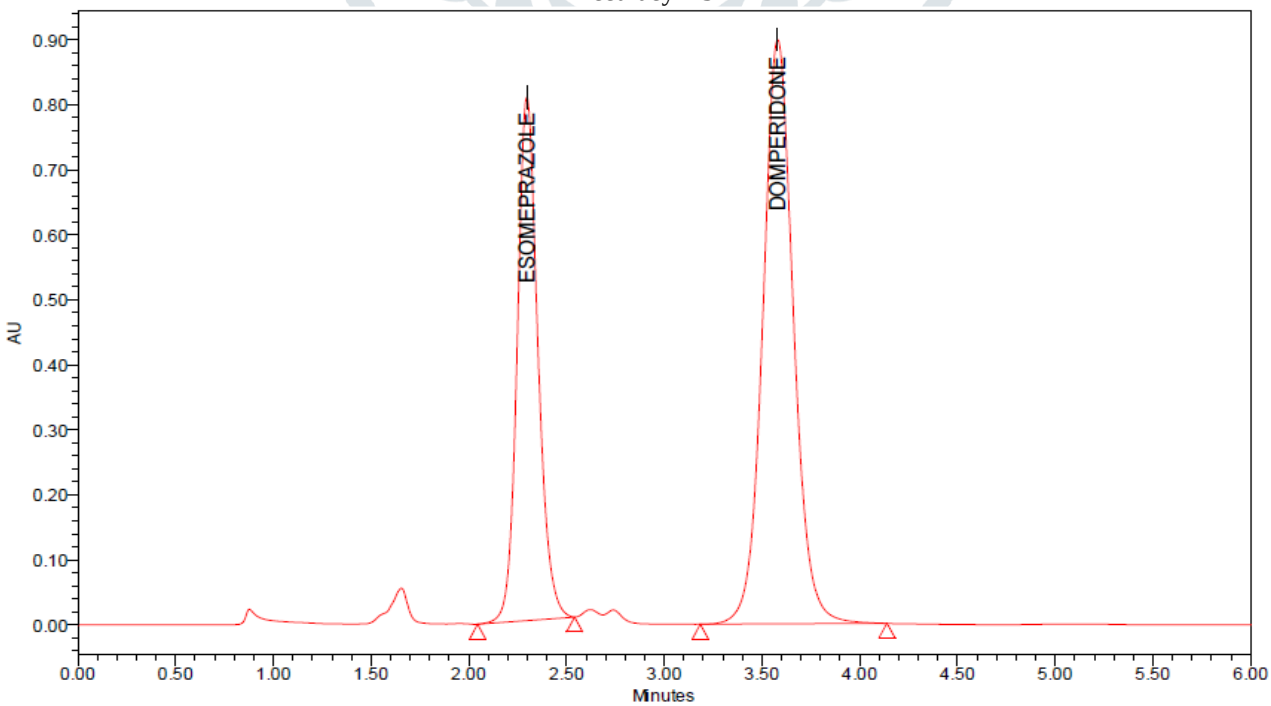
Chromatogram No –28



Accuracy - 2

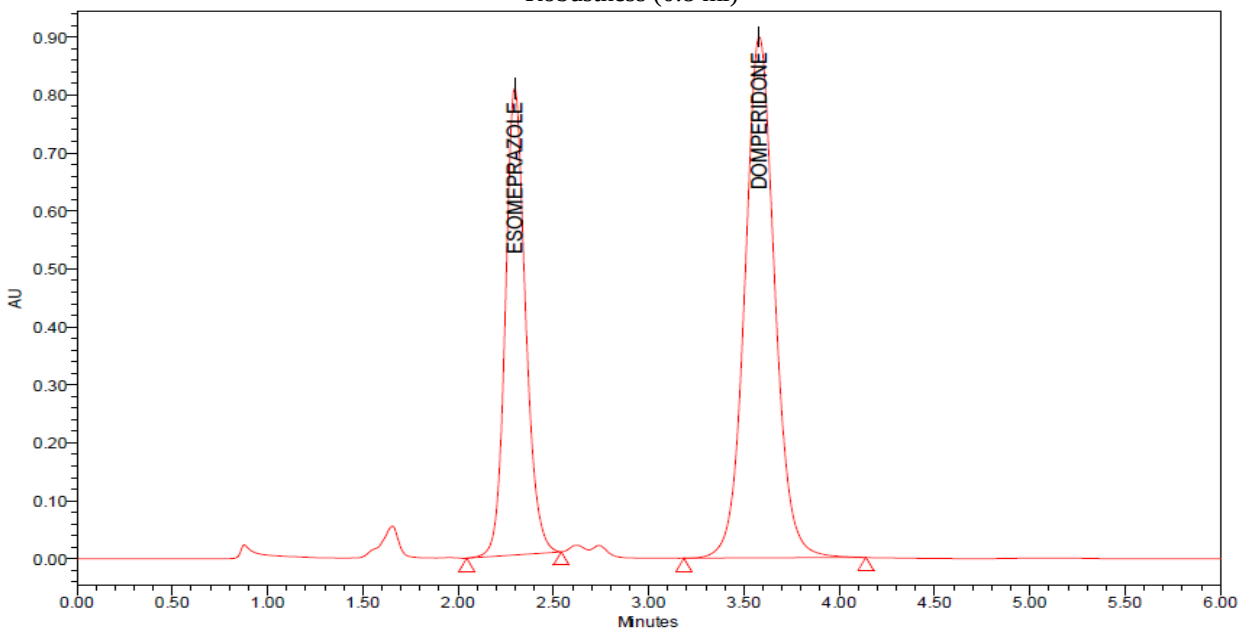
Chromatogram No –29

Accuracy – 3



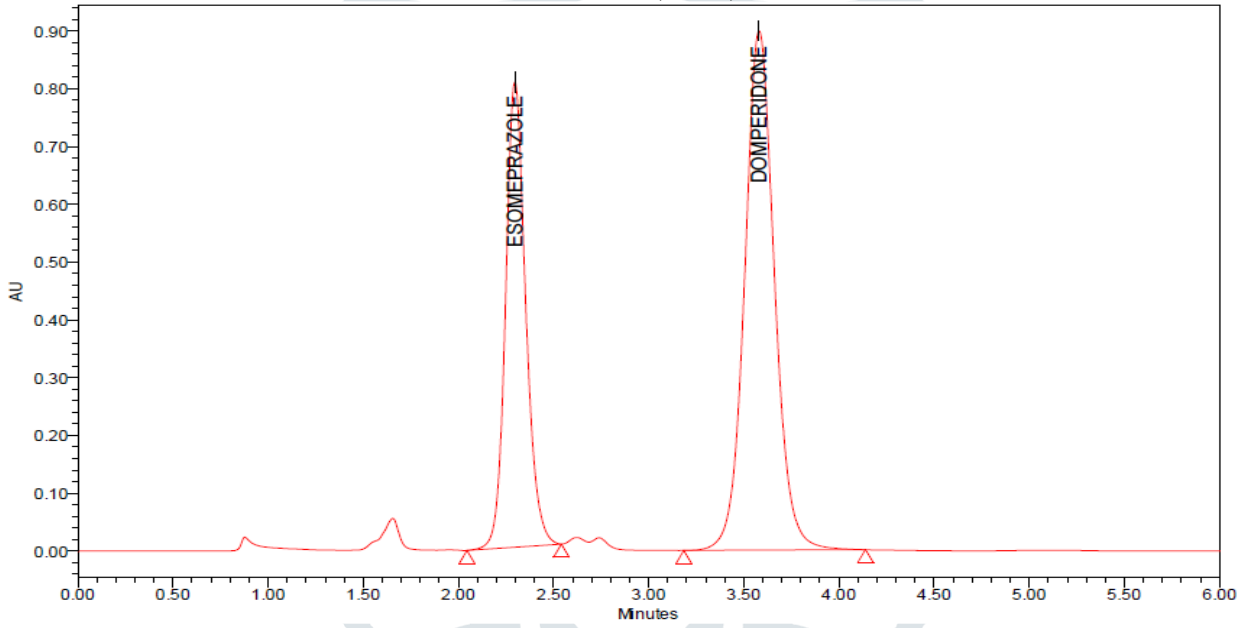
Chromatogram No –30

Robustness (0.8 ml)



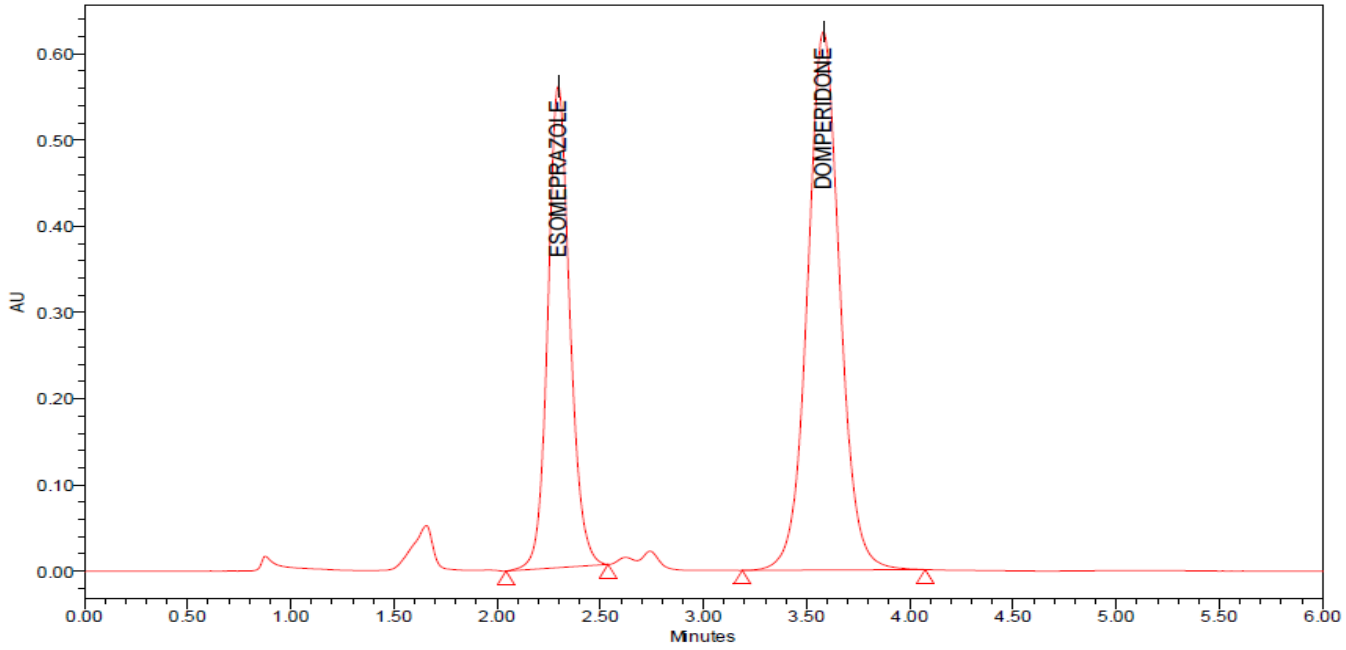
Chromatogram No –31

Robustness (1.2 ml)



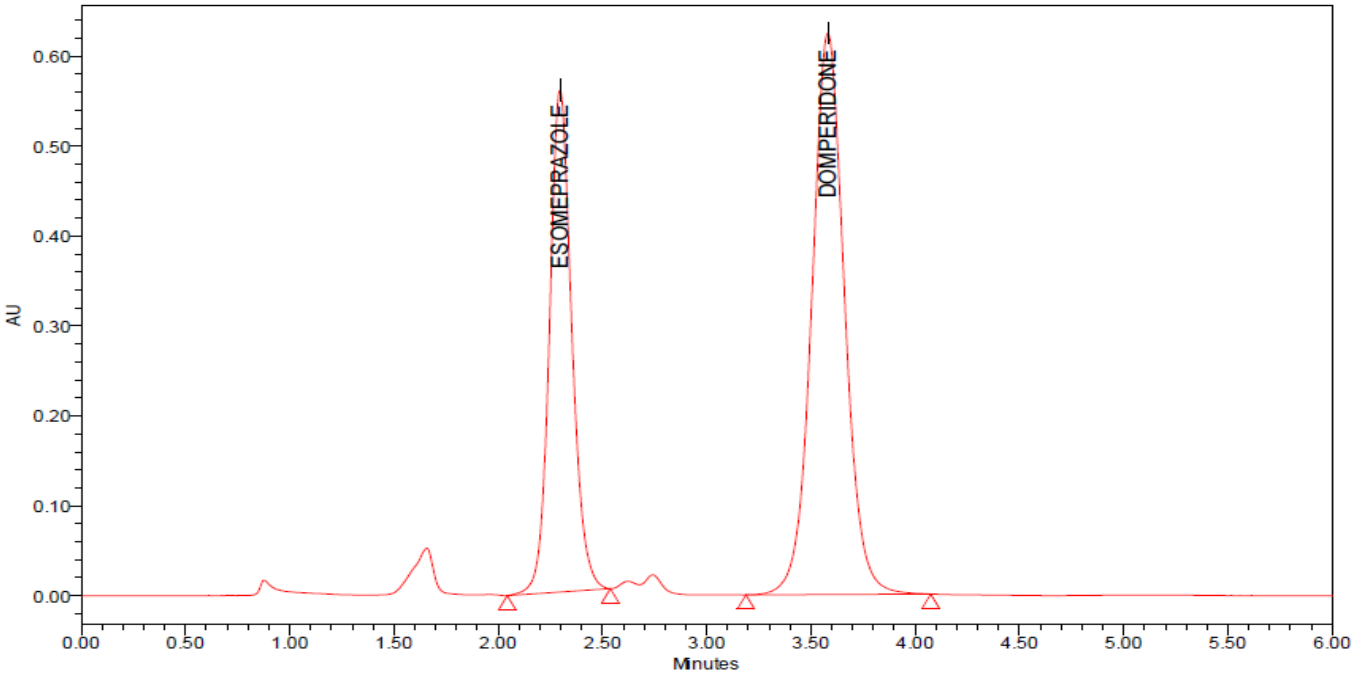
Chromatogram No-32

Column Temperature (25◦c)



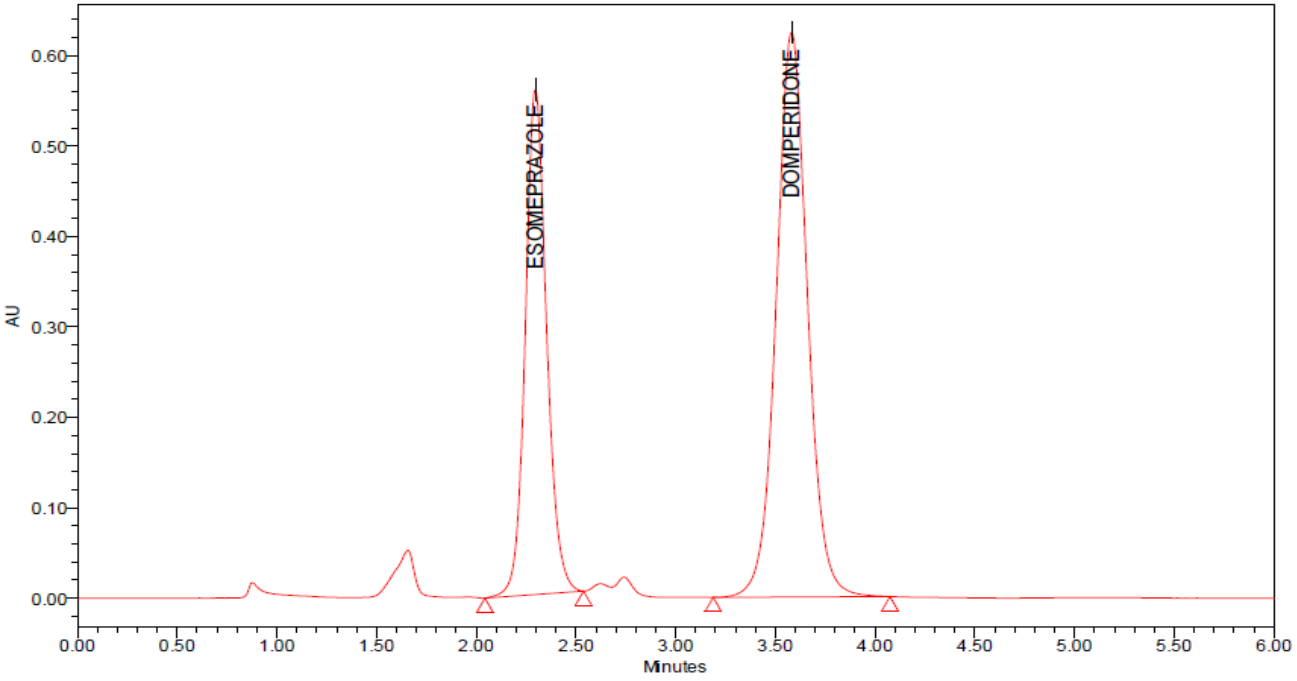
Chromatogram No –33

Column Temperature (35◦c)



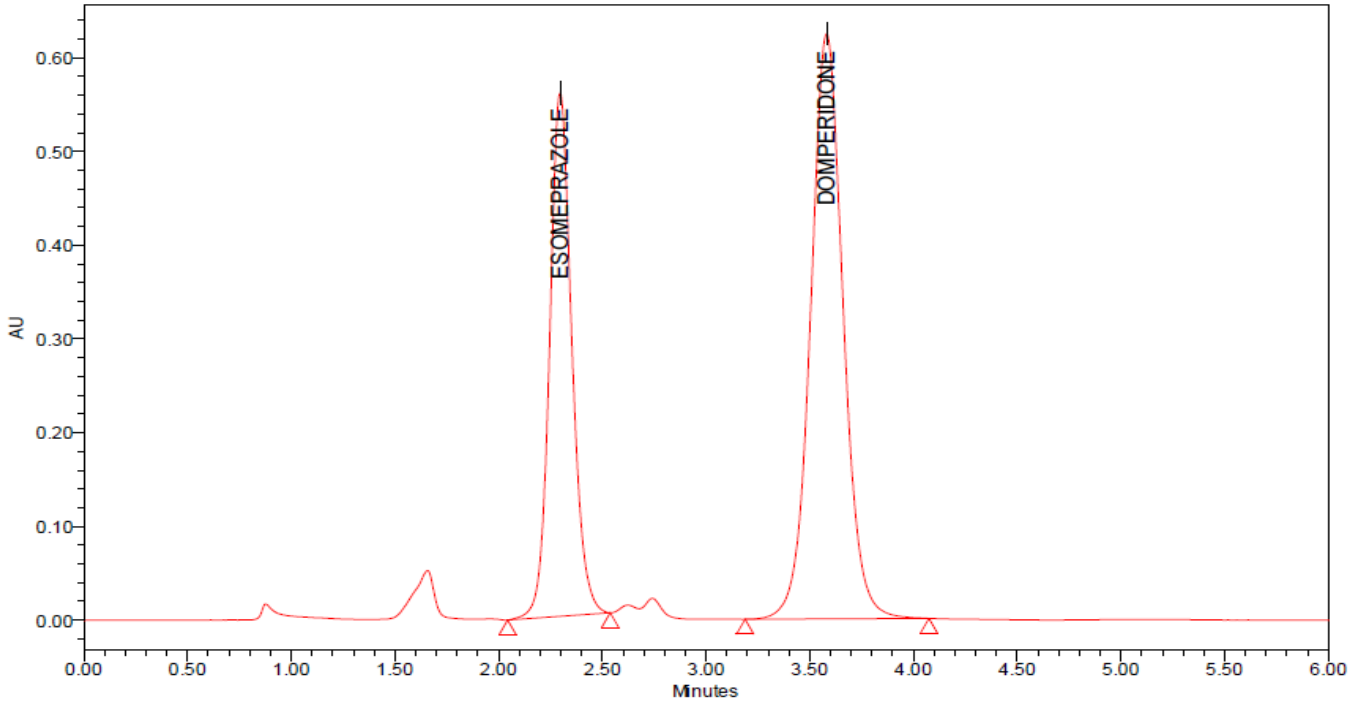
Chromatogram No –34

Ruggedness – (Analyst -1)



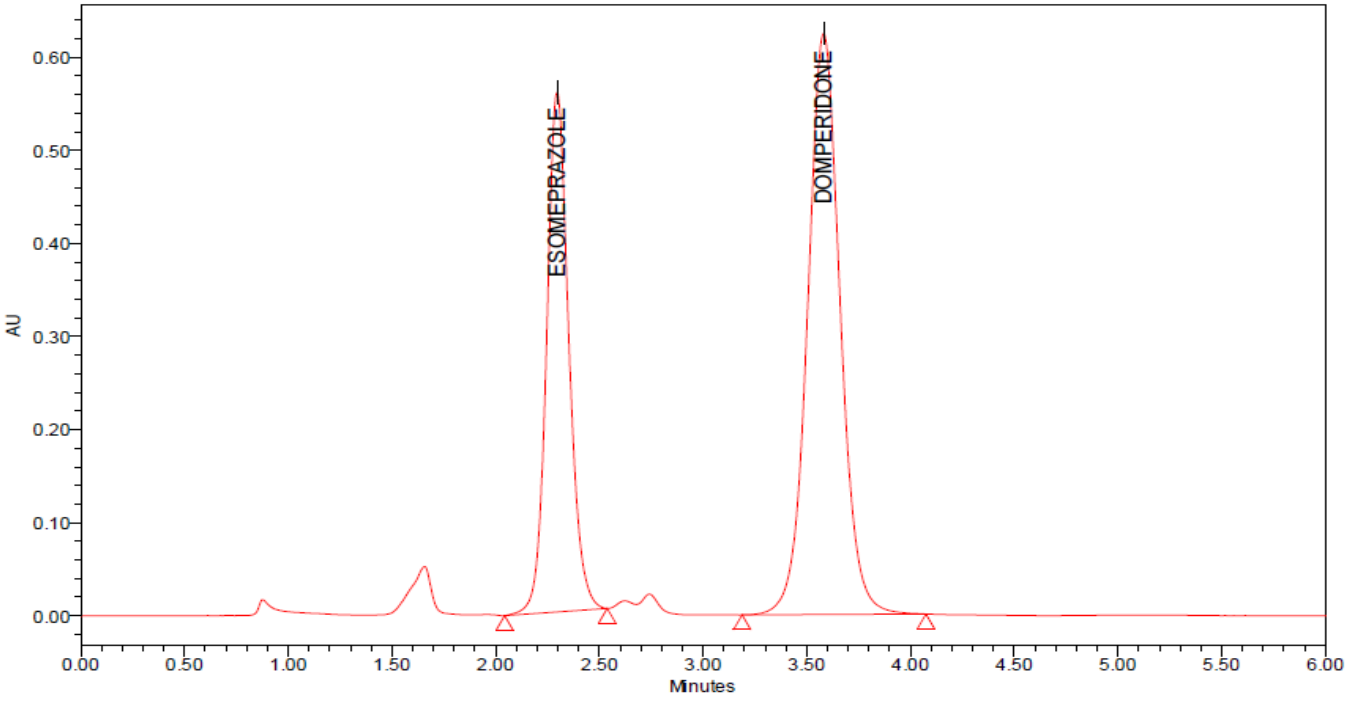
Chromatogram No – 35

Ruggedness – (Analyst – 2)



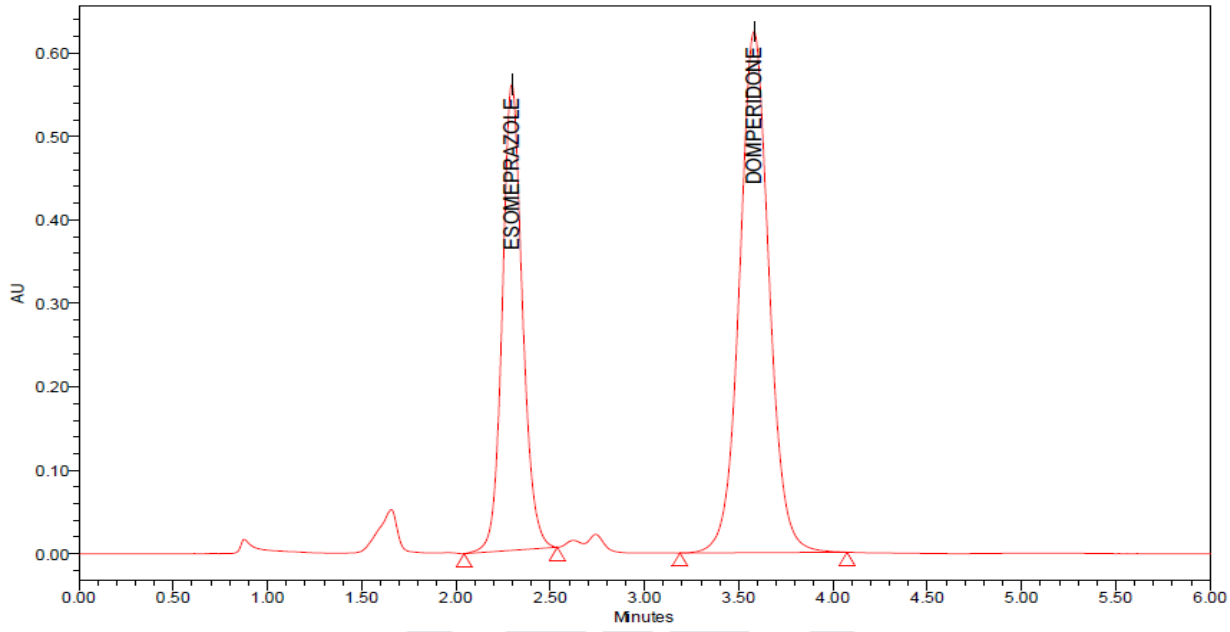
Chromatogram No- 36

Stability Studies – 0 hrs



Chromatogram No – 37

Stability Studies – 8hrs



6. RESULTS AND DISCUSSION

A simple precise and accurate HPLC method was developed for analysis of Esomeprazoleand Domperidone in combined capsule dosage form. Several mobile phase composition were tried to resolve the peaks of Esomeprazole And Domperidone. The optimum mobile phase containg Sodium dihydrogenphosphate:Methanol (80 :20 V/V) selected Because it was found ideal to resolve the analyte peaks of both the drugs. As per USP requirements system suitability studies were carried out on freshly prepared standard solutions of Esomeprazole and Domperidone. Various parameters obtained with 10 ul of injection volume.

The chromatogram of Esomeprazole and Domperidone reference standard were presented in chromatogram-5 . The retention time of Esomeprazole and Domperidone were identified 2.3 and 3.6 minutes respectively.

The method was validated as per ICH guidelines of Linearity, accuracy, specificity, precision, repeatability of measurement of peak area as repeatability of sample application and the results are shown in the below table.

Table: 15 Results

S.No	Parameters	Limit	Observations	Passes/fails

1	Specificity	No Interferences at retention time of the analyte peak	No Interferences at retention time of the analyte peak	Passes
2	Method Precision	% RSD < 2.0%	1.57	Passes
3	System Precision	% RSD < 2.0%	1.34	Passes
4	Linearity of detector response	Correlation coefficient >1.0	1.0	Passes
5	Accuracy	% Recovery range 98-102%	98 - 102	Passes
6	Limit of detection (LOD)	Signal noise ratio should be more than 3:1	Esomeprazole -0.136 Domperidone – 0.195	Passes
7	Limit of quantification (LOQ)	Signal noise ratio should be more than 10 :1	Esomeprazole -0.4132 Domperidone – 0.2775	Passes

7. SUMMARY AND CONCLUSION

The evaluation of obtained values suggests that the proposed HPLC method provides simple, precise, rapid and robust quantitative analytical method for determination of Esomeprazole and Domperidone in combined dosage form.

A good linear relation ship was observed for Esomeprazole and Domperidone in the range of 20-60mg/ml and 15-45mg/ml respectively. The Proposed HPLC method was simple and precise and validated according to the established guidelines.

High percentage of recovery shows that the method was free from the interferences of the excipients used in the formulations. The proposed method was highly accurate which showed good recovery of drug samples. The low standard deviation value and % RSD value indicate good precision. Mean percentage recovery above 95% indicates the reproducibility and accuracy of the newly developed method compared. The result of study include the proposed method is highly accurate, simple, precise and specific.

Hence the developed chromatographic method for Esomeprazole and Domperidone is said to be rapid, simple, accurate, precise and cost effective that can be effectively applied for the routine analysis in research institution, quality control departments in industries, approved drug testing laboratories, biopharmaceutical studies and in clinical pharmacokinetic studies.

II. ACKNOWLEDGMENT

My dissertation entitled "METHOD DEVELOPMENT AND VALIDATION OF ESOMEPRAZOLE AND DOMPERIDONE IN CAPSULE DOSAGE FORM BY RP-HPLC METHOD" would not have been a feasible one without the grace of the Almighty God who gave me moral till the completion of work.

I express my sincere thanks and gratitude to my respectable guide of Dr.D.Samson, M.Pharm, Ph.D, Department of Pharmaceutical Analysis, St.Anns of College Pharmacy, Chirala, Prakasam, whose scholastic guidance, keen interest and encouragement enabled me to complete this research work successfully.

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