ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NAPROXEN AND ESOMEPRAZOLE MAGNESIUM IN TABLET DOSAGE FORM BY RP-HPLC

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI

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

MASTER OF PHARMACY

(PHARMACEUTICAL ANALYSIS)

By

(REG.NO:261330351)

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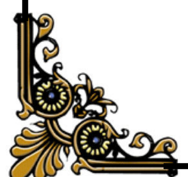
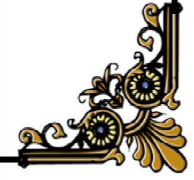

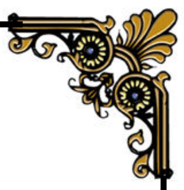

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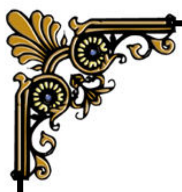
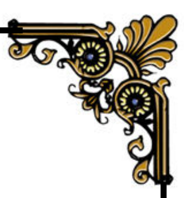

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"Success is how high you bounce when you hit bottom"

"If you can dream it, you can do it"

The completion of my M. Pharmacy is not only fulfillment of my dreams but also the dreams of my parents who have taken lots of pain for me in completion of my higher studies. I solicit my deep sense of appreciation and love to my wonderful parents and consider my self-privilege to have seen an entity of almighty in them.

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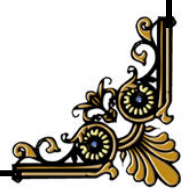
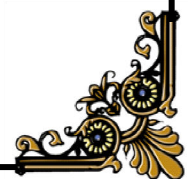
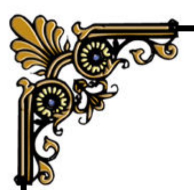


Affectionately dedicated
to
My beloved Parents





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LIST OF ABBREVATIONS

A	Absorbance
API	Active Pharmaceutical Ingredient
AR	Analytical grade
AUC	Area Under Curve
ASB	As such basis
A_s	Asymmetry Factor
BKT STD	Bracketing Standard
cm	Centimeter
V_C	Empty Column Volume
ESO	Esomeprazole
Exp	Expiry
F	Flow Rate
GLC	Gas Liquid Chromatography
GSC	Gas Solid Chromatography
GPC	Gel Permeation Chromatography
HETP	Height Equivalent to a Theoretical Plate
HPLC	High Performance Liquid Chromatography
HPLC	High performance liquid chromatography
i.d.	Internal Diameter
ICH	International conference on harmonization of technical requirements for registration of pharmaceuticals for human use.
IUPAC	International Union of Pure and Applied Chemistry
V_E	Interstitial Volume
V_0	Intraparticle Volume
L	Length of Column
LOD	Limit of Detection
LOQ	Limit of Quantitation
Mfg	Manufacture
µL	Micro liter
mcg	Microgram
µm	Micrometer
mL	Milli Liter
mg	Milligram
M	Molarity
nm	Nanometer
NAP	Naproxen
n	Noise
N	Normality
ODS	Octadecylsilane
h	Peak Height

W_b	Peak Width
%	Percentage
PDA	Photo Diode Array
N	Plate Number
RSD	Relative standard deviation
R&D	Research and Development
Rs	Resolution
k	Retention Factor or Capacity Factor
t_R	Retention Time
V_R	Retention Volume
RP-HPLC	Reverse phase high performance liquid chromatography
SPL	Sample
α	Separation Factor or Selectivity
S	Signal
STD	Standard
Std dev	Standard Deviation
STP	Standard testing procedure
T_f	Tailing Factor
TLC	Thin Layer Chromatography
UV	Ultra Violet
USP	United states pharmacopeia
USP	United States Pharmacopeia
Q2R1	Validation of analytical procedures: Text and methodology
C_p	Viscosity
t_M	Void Time
V_M	Void Volume
Wt	Weight
WS/RS	Working standard/Reference standard

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CHAPTER 1

1.0 INTRODUCTION [1-5]

Analytical chemistry, like other areas of chemistry and science, has gone through drastic changes and growth. Analytical chemistry may defined as the “Science and art of determining the composition of materials in terms of the elements or compounds contained”. In analytical chemistry it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species, that is to find out what a substance is composed of and exactly how much .

Analytical method is a specific application of a technique to solve an analytical problem. The use of instrumentation is an exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied science. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs and water. The manufacture of materials, whose composition must be known precisely such as substances used in integrated circuit chips, is monitored by analytical instruments.

Instrumental or physicochemical methods are based on the theory of relations between the content and the corresponding physicochemical and physical properties of the chemical system being analyzed. Changes in the system properties are either detected or recorded through the measurement of current, electrode potential, electrical conductivity, optical density, refractive index etc. with suitable and sensitive instruments. In instrumental analysis physical property of substance is measured to determine its chemical composition.

Measurements of physical properties of analyte such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analyses. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction, and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination.

1.1 PRINCIPAL TYPES OF CHEMICAL INSTRUMENTATION

- **Spectroscopic Techniques**
  1) Ultraviolet & visible Spectrophotometry
  2) Fluorescence & Phosphorescence Spectrophotometry
  3) Atomic Spectrometry (Emission & absorption)
  4) Infrared Spectrophotometry
  5) Raman Spectroscopy
  6) Radiochemical techniques including activation analysis
  7) Nuclear Magnetic Resonance Spectroscopy
  8) Electron Spin Resonance Spectroscopy

- **Electrochemical Techniques**
  1) Potentiometry
  2) Volta metric techniques
  3) Amperometric techniques
  4) Coulometry
  5) Electrogravimetry
  6) Conductance techniques

- **Chromatographic Techniques**
  1) High Performance Liquid Chromatography
  2) Gas chromatography

- **Miscellaneous Techniques**
  1) Thermal analysis
  2) Mass Spectrometry
  3) Kinetic techniques

- **Hyphenated Techniques**
  1) GC-MS (Gas Chromatography – Mass Spectrometry)
  2) ICP-MS (Inductively Coupled Plasma - Mass Spectrometry)
  3) GC-IR (Gas Chromatography – Infrared Spectroscopy)
  4) MS-MS (Mass Spectrometry – Mass Spectrometry)

1.2 INTORDUCTION TO CHROMATOGRAPH [6-7]

Chromatography was first invented by Michael Tswett, a Russian botanist in 1906 in Warsaw for the separation of colored substance into individual component.

Chromatography is defined as a procedure by which solutes are separated by a dynamic differential migration process in a system consisting of two or more phases, one of which moves continuously in a given direction and in which the individual substances exhibit mobilities by reason of differences in adsorption, partition, solubility, vapour pressure, molecular size or ionic charge density. The individual substances thus obtained can be identified or determined by analytical methods.

Chromatography was invented nearly 100 years ago, but it is only in the past few years that the development of the technique and associated instrumentation has reached a level that might be called the '*steady state*'.

Separation Process:

The Chromatographic method of separation, in general, involves the following steps:

- Adsorption or retention of substance or substances on the stationary phase.
- Separation of the adsorbed substances by the mobile phase.
- Recovery of the separated substances by the continuous flow of the mobile phase.
- Qualitative and quantitative analysis of the eluted substances.

1.2.1 DIFFERENT FORMS OF CHROMATOGRAPH

The different forms of chromatography are shown in Fig. 1.

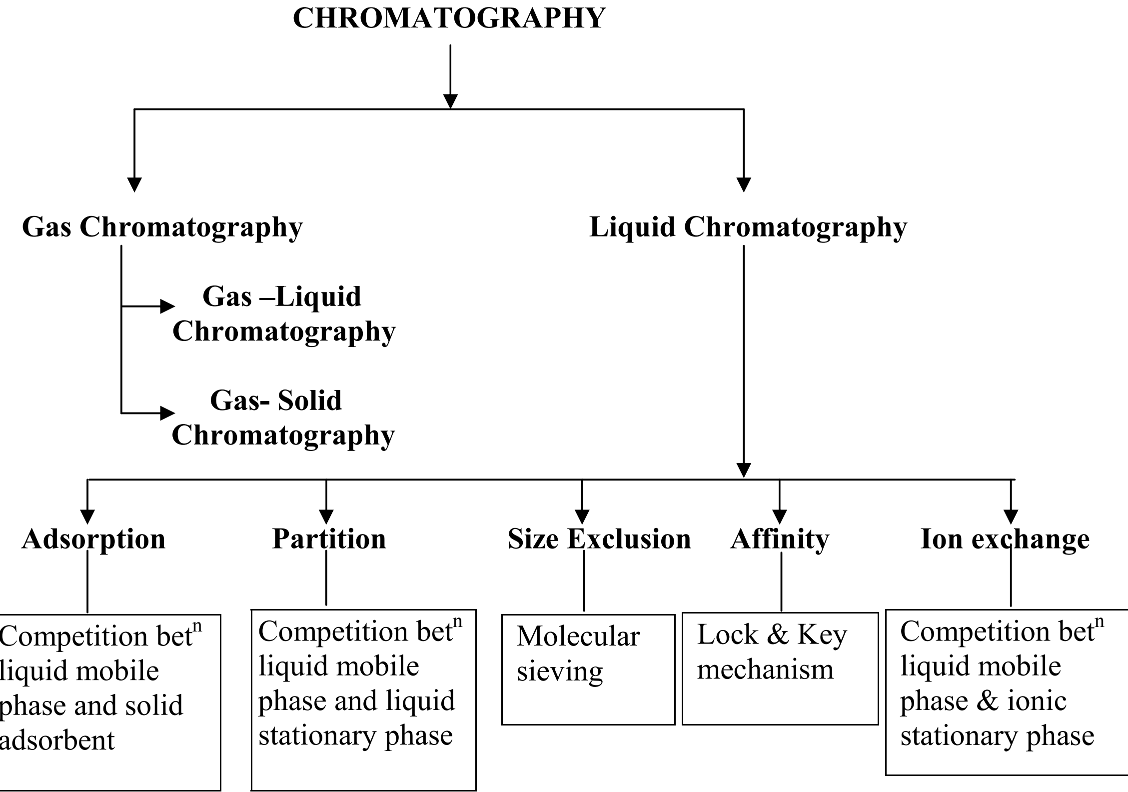


Fig 1: Different Forms Of Chromatography

1.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY [8]

High performance Liquid Chromatography (HPLC), also known as high pressure liquid chromatography, is essentially a form of column Chromatography in which the stationary phase consists of small particle (3-50 µm) packing contained in a column with a small bore (2-5mm), one end of which is attached to source of pressurized liquid eluent (Mobile phase).

In High Performance Liquid Chromatography, the mobile as well as the stationary phase compete for the distribution of the sample components. In case of HPLC, separation is based on:

- **Partition** **:** Separation due to differences in solubility characteristics between two phases.

- **Adsorption** : Separation due to difference in interaction with the surface of the stationary phase.
- **Ion Exchange**: Due to differences in the affinity of ions for the ion exchanger.
- **Size Exclusion***:* Due to differences in molecular weight and size of the molecules to be separated.

Today, HPLC is the most widely used analytical separation method. The method is popular because it is non-destructive and may be applied to thermally labile compounds (unlike GC); it is also very sensitive technique since it incorporates a wide choice of detection methods. With the use of post-column derivatization methods to improve selectivity and detection limits, HPLC can easily be extended to trace determination of compounds that do not usually provide adequate detector response. The wide applicability of HPLC as separation method makes it a valuable separation tool in scientific fields.

1.3.1 MODES OF SEPARATION IN HPLC

There are different modes of separation in HPLC. They are

- Normal Phase Chromatography (NPC);
- Reversed Phase Chromatography (RPC);
- Ion Exchange Chromatography;
- Ion Pair Chromatography;
- Size Exclusion Chromatography; and
- Affinity Chromatography

1.3.2 COMMONLY USED METHOD IN HPLC

1) Normal phase chromatography

For a polar stationary bed like silica we need to choose a relatively non polar mobile phase. This mode of operation is termed as *Normal phase chromatography.* Here the least polar component elutes first, and increasing the mobile phase polarity leads to decrease in elution time. Isopropanol, diisopropyl ether and acetic acid are used as modifiers or tail reducers. Nonpolar solvents like Pentane, Hexane, Iso Octane, Cyclohexane, etc. are more popular. It is extremely important to control the water content of the stationary phase while using silica columns. Variation in moisture content of silica leads to variation in retention times. Therefore, it is a common practice to saturate the mobile phase with water before using it as eluent in Normal phase chromatography. It is mainly used for separation nonionic, nonpolar to medium polar substances.

2) Reverse phase chromatography

For hydrocarbon type or nonpolar stationary phase, we need to choose a polar mobile phase. This mode of partition chromatography is called as *Reverse phase chromatography.* Here the most polar component elutes first. Increasing mobile phase polarity leads to decrease in elution time. Common solvents used in this mode include Methanol/Acetonitrile/Isopropanol etc. Control of pH is another way to control resolution. In the case of an aromatic acid, a low pH buffer will suppress the ionization, and will increase the capacity factor, while with a high pH buffer, the capacity factor will decrease. Small amounts of modifiers like sodium phosphate/ sodium acetate reduce peak tailing, and increase the separation efficiencies. Mostly used for separation of ionic and polar substances**.**

3) Ion- Pair Chromatography

Ion-pair and RP- HPLC have several similar features. The column and mobile phase used for both these separation techniques are similar, differing mainly in the addition of an ion-pair reagent to the mobile phase for ion-pair chromatography (IPC). If RPC method development is unable to provide an adequate separation due to poor band spacing, IPC provides selectivity option.

1.3.3 Elution Techniques

a) Isocratic elution:

A separation in which the mobile phase composition remains constant throughout the procedure is termed **isocratic** (meaning constant composition).

b) Gradient elution:

The mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution. One example is a gradient starting at 10% methanol and ending at 90% methanol after 20 minutes. The two components of the mobile phase are typically termed "A" and "B"; A is the "weak" solvent which allows the solute to elute only slowly, while B is the "strong" solvent which rapidly elutes the solutes from the column. Solvent A is often water, while B is an organic solvent miscible with water, such as acetonitrile, methanol, THF or isopropanol.

In isocratic elution, peak width increases with retention time linearly according to the equation for N, the number of theoretical plates. This leads to the disadvantage

that late-eluting peaks get very flat and broad. Their shape and width may keep them from being recognized as peaks.

Gradient elution decreases the retention of the later-eluting components so that they elute faster, giving narrower (and taller) peaks for most components. This also improves the peak shape for tailed peaks, as the increasing concentration of the organic eluent pushes the tailing part of a peak forward. This also increases the peak height (the peak looks "sharper"), which is important in trace analysis. The gradient program may include sudden "step" increases in the percentage of the organic component, or different slopes at different times - all according to the desire for optimum separation in minimum time.

In isocratic elution, the selectivity does not change if the column dimensions (length and inner diameter) change - that is, the peaks elute in the same order. In gradient elution, the elution order may change as the dimensions or flow rate change.

The driving force is originated in reversed phase chromatography in the high order of the water structure. The role of the organic mobile phase is to reduce this high order by reducing the retarding strength of the aqueous component.

1.3.4 HPLC Instrumentation

A liquid chromatography consists of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column, a detector, and a data collection device such as a computer, integrator, or recorder. Short, small bore columns containing densely packed particles of stationary phase provide for the rapid exchange of compounds between the mobile and stationary phases. In addition to receiving and reporting detector output, computers are used to control chromatographic settings and operations, thus providing for long periods of unattended operations.[9-10]

The different parts of HPLC system are schematically shown in the Fig. 2.

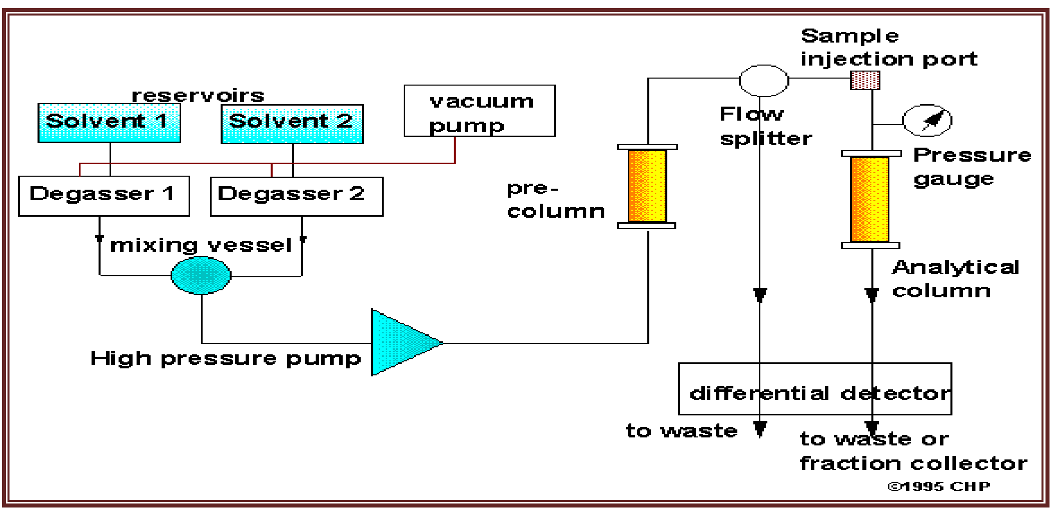


Fig. 2: High Performance Liquid Chromatography (Schematic).

1.4 HPLC METHOD DEVELOPMENT

1.4.1 METHOD DEVELOPMENT[9-10]

Today the development of a method of analysis is usually based on prior art or existing literature, using the same or quite similar instrumentation. It is rare today that an HPLC based method is developed that does not in some way relate or compare to existing, literature based approaches. The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final needs or requirements of the method.

Method development usually requires selecting the method requirements and deciding on what type of instrumentation to utilize and why. In the development stage, decisions regarding choice of column, mobile phase, detectors, and method of quantitation must be addressed. In this way, development considers all the parameters pertaining to any method.

There are several valid reasons for developing new method of analysis:

- There may not be suitable method for a particular analyte in the specific sample matrix.
- Existing method may be inaccurate, artifact , and /or contamination prone, or they may be unreliable (have poor accuracy or precision)
- Existing methods may be too expensive, time consuming or energy intensive, or they may not be easily automated.

- Existing methods may not provide adequate sensitivity or analyte selectivity in samples of interest.
- Newer instrumentation and techniques may have evolved that provide opportunities for improved methods, including improved analyte identification or detection limits, greater accuracy or precision , or better return on investment.
- There may be a need for an alternative method to confirm for legal or scientific reasons analytical data originally obtained by existing methods.

Separation Goals And Steps Involved In HPLC Method Development

The major steps involved in the methods development are shown in the Fig. 3 and the separation goals in HPLC method development are shown in Table 1.

Goal	**Comment**
Resolution	Precise and rugged quantitative analysis requires that *Rs* be greater than 1.5.
Separation time	<5-10 min is desirable for routine procedures.
Quantitation	≤2% (1 SD) for assays; ≤5% for less-demanding analyses; ≤15% for trace analyses.
Pressure	<150 bar is desirable, <200 bar is usually essential (new column assumed).
Peak height	Narrow peaks are desirable for large signal/ noise ratios.
Solvent consumption	Minimum mobile-phase use per run is desirable.

Table 1: Separation Goals In Hplc Method Development

HPLC method development is based on few basic steps includes: [11-12]

1. Information on sample, define separation goals

2. Need for special HPLC procedure, sample pre-treatment, etc?

3. Choose detector and detector setting

4. Choose LC method; preliminary run, estimate best separation conditions

5. Optimize separation conditions

6. Check for problems or requirement for special procedures

7a. Recover purified material

7b. Quantitative calibration

7c. Qualitative method

8. Validate method for release to routine laboratory

Fig 3: Steps in HPLC Method Development

Once the instrumentation has been selected, based on the criteria suggested above, it is important to determine "Analyte Parameters" of interest. To develop a method it is necessary to consider the properties of analytes of interest that may be advantageous to establish optimal ranges of analyte parameter values. It is important that method development may be performed using only analytical standards that have been well identified and characterized, and whose purity is already known. Such precautions will prevent problems in the future and will remove variables when one is trying to optimize or improve initial conditions during method development.

1.4.2 Step-by-Step HPLC method development[13-16]

Documentation starts at the very beginning of the method development process, a system for full documentation of the development studies must be established. All data relating to these studies must be established. All data relating to these studies must be recorded in laboratory notebooks or an electronic database.

Different steps used in method development are:

1. Literature collection

- ❖ Search the literature (USP, EP, JP, IP, Chromatography journals, patents, internet etc.) for available/ existing HPLC methods for the given molecule or molecules having similar structures.
- ❖ Check the suitability of reported literature methods and see if the requirements are met. If required, modify the method to meet the requirements such as resolution of possible impurities as per the synthetic process.
- ❖ Specify the components to be evaluated in the HPLC method considering all the possible impurities that can be present in drug substance which could be intermediates, raw materials, process related impurities and degradants.
- ❖ Collect samples, standards and all possible impurities of each stage, if available.

2. Chemical structure and other information

- ❖ Obtain information on analyte solubility, the pKa values (if available) and functional groups since it is a prerequisite for effective method development.
- ❖ Based on the structure and the functional group present it can be determined whether the compound is basic, acidic or neutral.

3. Polarity

- ❖ Depending on the solubility and nature (acid/base/ionic/non-ionic) of the molecule select the appropriate mode given below.
- ❖ Reversed phase chromatography - acids, bases and non-ionic sample
- ❖ Ion-pair chromatography - ionic samples.
- ❖ Ion exchange chromatography - ionic samples.
- ❖ Normal phase chromatography - isomers, non-polar/non-ionic samples, chiral molecules.
- ❖ Size exclusion chromatography - high molecular weight samples such as proteins.

4. pH/pka Value of compounds

- Based on pH/pKa values, the nature of the compound (acidic, basic or neutral in nature) and polarity of the compound can be assumed.

  Note: if the compound is acidic, acidic mobile phase is preferable. For a basic compound, low pH and basic mobile phase is preferable. For a neutral compound neutral mobile phase is suitable.

5. Solubility

- Check the solubility of the sample in solutions like mobile phase, mobile phase organic mixtures, water-organic mixtures and mixtures of acids like perchloric acid, phosphoric acid etc.

  Note: If the analyte is soluble in polar solvents like water, methanol, chloroform, acetonitrile or tetrahydrofuran, the method development should be initiated in reversed phase chromatography. If the analyte is soluble in non-polar solvents like ethyl acetate, n-hexane, dioxane etc. then the method development can be initiated in normal phase chromatography.

6. Column selection

- **Reversed phase**

  Column containing C_4, C_8, C_{18}, Cyano and phenyl, amino stationary phases comes under reversed phase category and can be used against a more polar mobile phase. The retention of non-polar compounds increases as the length of the alkyl ligand (C_4, C_8, C_{18} etc.) increases.

- **Normal phase**

  Columns containing cyano, phenyl and silica stationary phases come under normal phase category and are used against non-polar mobile phases.

  The impact of the bonded phase, internal diameter, particle surface area, pore volume and size of theoretical plates, peak symmetry, selectivity and resolution should be evaluated.

7. Selection of detector

- Select a suitable detection technique based on the structure and nature of the molecule.
- UV detectors :compounds having chromophores
- Fluorescence Detector : Compounds having Fluorescence properties
- Electrochemical Detector : Easily oxidizable compounds

- ❖ RI Detector: Universal Detector: but cannot be used with gradient elution. This detector is used for compounds which do not contain chromophores.
- ❖ ELSD: Universal Detector, but can be used with gradient analysis with higher sensitivity.

8. Mobile phase selection

❖ **Choice of solvents for reversed phase chromatography**

The solvents like Water, Methanol, Acetonitrile, Tetrahydrofuran, Isopropyl alcohol, Ethanol etc.

Note: when THF is used as mobile phase component, the content should not be more than 20%**.**

❖ **Organic modifiers**

Triethylamine, Diethyl amine, Trifluoroacetic acid, Phosphates, Perchlorates, Chlorides, Bromides, Nitrates etc.

❖ **Choice of buffering agent**

Phosphates of sodium, potassium, ammonium etc, perchlorates of ammonium, sodium, potassium etc., ammonium acetate etc. Buffering agent should be selected appropriately in their buffering range to maintain a constant pH of the mobile phase.

❖ **Choice of solvents for normal phase chromatography**

Solvents like Isopropyl alcohol, Ethanol, n-hexane, Dioxin, Chloroform etc. Organic modifiers used with normal phase chromatography: Triethylamine, Diethyl amine, Trifluoroacetic acid, Formic acid and Acetic acid.

❖ **Ionic compounds**

Compounds which are highly polar or ionic in nature do not retain on reversed phase columns and have poor peak symmetry. For such compounds the method should be developed either in ion exchange chromatography mode or ion pair chromatography is preferred because it can be run on the conventional reversed phase columns such as C_{8}, C_{18} etc. with mobile phase additives.

Ion pair reagents (sodium lauryl Sulphate, heptane Sulphonic acid, Tetra butyl ammonium hydroxide, etc.) of concentrations 0.001%-0.5% can be added to the mobile phase to get optimum retention, peak symmetry and separations.

Note: Concentrations more than specified range should be justified for acidic compounds cationic ion pair reagents like tetra butyl ammonium hydrogen sulphate, tetra

methyl ammonium bromide, sodium per chlorate, can be used as additives in the mobile phase at suitable pH.

For basic compounds, anionic ion pair reagents like sodium salt of butane, pentane, hexane or octane sulphonic acid, per chlorates etc. can be used as additives in the mobile phase at suitable pH.

9. Instrument set up and Initial Studies:

a) Set up the required instrumentation. Verify installation and operational and performance qualifications of instrumentation using laboratory standard operating procedures (SOP's).

b) Always use new consumables (e.g. solvents filters and gases). For example never start method development on an HPLC column that has been used before.

c) Prepare the analyte standard in a suitable injection / introduction solution and in known concentration and solvents. It is important to start with authentic, known standards rather than with a complex sample matrix. If the sample is extremely close to the standard (eg. Bulk drug), then it is possible to start work with actual sample.

d) Begin the analysis using the analytical conditions described in the existing literature.

e) Evaluate feasibility of method with regard to the analytical figures of merit obtained

10. Optimization

During optimization change one parameter at a time, and isolate set of conditions, rather than use a trial and error approach. . Work from an organized, methodical plan, and document every step (Keep a lab notebook) in case of dead ends.

11. Demonstration of analytical figures of merit

Document the originally determined analytical figures of merit [limit of quantitation (LOQ), limit of detection (LOD), linearity, time per analysis, cost, sample preparation etc]

12. Evaluation of method development with actual samples

Ensure that the sample solution leads to unequivocal, absolute identification of the analyte peak of interest apart from all other matrix components.

13. Determination of percent recovery of actual sample and demonstration of quantitative sample analysis

Determine percent recovery of spiked, authentic standard analyte into sample matrix that is shown to contain no analyte. Show reproducibility of recovery (average ± standard deviation) from sample to sample and whether recovery has been optimized. It is not necessary to obtain 100% recovery as long as the results are reproducible and known with a high degree of certainty.

1.4.3 Chromatographic Parameters:

a) RETENTION TIME
b) RETANTION VOLUME
c) SEPERATION FACTOR
d) CAPACITY FACTOR
e) ASYMMETRIC FACTOR
f) THEORETICAL PLATES
g) RESOLUTION

a) RETENTION TIME (Rt):

It is the difference in time between the point of injection and the appearance of peak maxima. Retention time is the time required for 50 % of a component to be eluted from a column. It is measured in minutes or seconds.

b) RETENTION VOLUME (Vr):

It is the volume of carrier gas required to elute 50% of the component from the column.

$$R.V = R.T \times \text{Flow rate}$$

c) SEPERATION FACTOR:

Separation factor is the ratio of partition - co-efficient of the two components to be separated.

$$S = \frac{Kb}{Ka} = \frac{Ka}{Kb} = \frac{(tb - t\,o)}{(ta - t\,o)}$$

Where, to = Retention time to unretained substance

Kb,Ka = Partition Coefficient of b and a

tb,ta = Retention time of substance b and a

S = depends on liquid phase, column temperature.

d) CAPACITY FACTOR:

It is generally required to be calculated by the difference in the retention time of main peak and the retention time of uracil injected to the retention time of the main peak.

$$K' = \frac{Rt-Ru}{Rt}$$

Where,

R_t : is the retention time of main peak,

R_u : is the retention time of uracil.

K' : is the capacity factor

It is one of the important factor need to be determined while performing method development of multi formulation, it should be always less than 2.

e) ASSYMETRIC FACTOR:

A chromatographic peak should be symmetrical about its center and said to follow Gaussian distribution. But due to some factors, the peak is not symmetrical and shows tailing and fronting.

FRONTING: It is due to the saturation of stationary phase and can be avoided by using less quantity of sample.

TAILING: It is due to more active adsorption sites and can be eliminated by support pretreatment, more polar mobile phase, increasing the amount of liquid phase.

f) THEORETICAL PLATES (PLATE THEORY):

A theoretical plate is an imaginary or hypothetical unit of a column where distribution of solute between stationary and mobile phase has attained equilibrium. A theoretical plate can also be called as a functional unit of the column.

g) RESOLUTION (Rs):

Resolution is measure of the extent of separation of the two components and the base line separation achieved.

1.5 METHOD VALIDATION [17-18]

Validation is defined as follows by different agencies

Food and Drug administration (FDA): Establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce a product meeting its predetermined specification and quality attributes.

World Health Organization (WHO): Action of providing that any procedure, process, equipment, material, activity, or system actually leads to the expected results.

European Committee (EC): Action of providing in accordance with the principles of good manufacturing practice, that any procedure, process, equipment material, activity or system actually lead to the expected results. In brief validation is a key process for effective Quality Assurance.

Analytical method validation

Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety, efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development.

Analytical method validation is the corner stone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do. All new analytical methods developed are validated.

Steps followed for validation procedures

1. Proposed protocols or parameters for validations are established
2. Experimental studies are conducted
3. Analytical results are evaluated
4. Statistical evaluation is carried out
5. Report is prepared documenting all the results

1.5.1 VALIDATION PARAMETERS [19-25]

Analytical methods are required for the identification, batch analysis and storage stability data for active constituents of Pharmaceutical product, and for post-registration compliance purposes. Analytical method development as a first step is carried out to ensure that the API used and the dosage forms that are developed and manufactured for human consumption are meeting the regulated quality norms. Every newly developed method must be validated prior to sample analysis. Validation must also be repeated if a parameter has been modified or if the validation was strongly performed in another laboratory, to ensure that the methods are transferable. A verification is necessary if the analyst or instrument have been changed, or if the sample type has been modified.

The objective of validation of an analytical method is to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. Method

validation is a practical process designed and experimentally carried out to ensure that an analytical methodology is accurate, specific, reproducible and rugged over the specified range of analysis. Validation provides both assurance and reliability during normal use and documented evidence that the method is 'fit for purpose'.

Method validation ensures the validity of a measurement before it is carried out and is essential part of quality assurance procedures. The process of method validation provides information on the critical factors affecting the method output, thus enabling suitable quality control procedures to be implemented to ensure data quality. The extent of method validation will vary with applications, sector and regulatory compliance.

Typical validation characteristics which should be considered are listed below

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

1.5.2 ICH VALIDATION PARAMETERS [19,20]

Type of analytical procedure	Identification	Testing for impurities		Assay-dissolution (measurement only) - content/potency
Characteristics		Quantitation	Limit	
Accuracy	-	+	-	+
Precision Repeatability Interm. Precision	 - -	 + (1)	 - -	 + (1)
Specificity (2)	+	+	+	+
Detection Limit	-	(3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

Table 2 : Data Elements Required For Validation According To ICH

- \- signifies that this characteristic is not normally evaluated

- + signifies that this characteristic is normally evaluated
- (1) In cases where reproducibility has been performed, intermediate precision is not needed
- (2) Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)
- (3) May be needed in some cases

1.5.3USP VALIDATION PARAMETERS [19,20]

Analytical Performance Characteristics	Category I	Category II		Category III	Category IV
		Quantitative	Limit Tests		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
Detection Limit	No	No	Yes	*	No
Quantitation Limit	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No
* May be required, depending on the nature of the specific test.					

Table 3: Data Elements Required for Validation According to USP

1) Accuracy

The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value. Accuracy may be measured in different ways and the method should be appropriate to the matrix. The accuracy of an analytical method may be determined by any of the following ways:

- Analyzing a sample of known concentration and comparing the measured value to the 'true' value. However, a well characterized sample (e.g. reference standard) must be used.
- Spiked-placebo (product matrix) recovery method. In the spiked-placebo recovery method, a known amount of pure active constituent is added to formulation blank (sample that contains all other ingredients except the active ingredient), the resulting mixture is assayed, and the results obtained are compared with the expected result.
- Standard addition method. In the standard addition method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed.

The difference between the results of the two assays is compared with the expected answer.

In both methods (spiked-placebo recovery and standard addition method), recovery is defined as the ratio of the observed result to the expected result expressed as a percentage.

The accuracy of a method may vary across the range of possible assay values and therefore must be determined at several different fortification levels. The accuracy should cover at least 3 concentrations (50%, 100% and 150%) in the expected range.

Accuracy may also be determined by comparing test results with those obtained using another validated test method.

Acceptance criteria:

The expected recovery depends on the sample matrix, the sample processing procedure and on the analyte concentration. The mean % recovery should be within the following ranges: For assay: 98%-102%

2) Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision may be considered at three levels:

- System Precision (Repeatability)
- Method Precision (Reproducibility)
- Intermediate precision (Ruggedness)

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. A minimum of 6 replicate sample determinations should be made together with a simple statistical assessment of the results, including the percent relative standard deviation. The following levels of precision are recommended.

Acceptance criteria: For an assay the %RSD should be NMT 2%

3) Specificity [21-23]

It signifies the instruments, ability to measure or identify the analyte without any interference from sample matrix, impurities, precursors or degradation product.

Forced degradation

Forced degradation provides useful information about the degradation pathways and degradation products during the product storage. The information thus obtained will facilitate pharmaceutical development in areas such as formulation development, manufacturing, and packaging, where knowledge of chemical behavior can be used to improve the quality of drug product.

HPLC methods should be able to separate, detect, and quantify the various drug-related degradants that can form on storage or manufacturing, plus detect and quantify any drug-related impurities that may be introduced during synthesis. Forced degradation studies (chemical and physical stress testing) of new chemical entities and drug products are essential to help develop and demonstrate the specificity of such stability-indicating methods. In addition to demonstrating specificity, forced degradation studies can be used to determine the degradation pathways and degradation products of the APIs that could form during storage, and facilitate formulation development, manufacturing, and packaging. Procedures for the preparation of specific degradation products needed for method validation often emerge from these studies.

For marketing applications, current FDA and ICH guidance recommends inclusion of the results, including chromatograms of stressed samples, demonstration of the stability indicating nature of the analytical procedures, and the degradation pathways of the API in solid state, solution, and drug product. The chemical structures of significant degradation products and the associated procedures for their isolation and/or characterization are also expected to be included in the filing. The experimental protocol for performing forced degradation studies will depend on the active ingredients and formulation involved because the chemistry of each compound is different. In general, a target of approximately 10-30% degradation of the API during forced degradation, or exposure to energy in slight excess of what is typically used in accelerated storage is recommended. In this way, the “worst-case” degradation products can be studied. The following will provide some suggestions for performing forced degradation studies based upon available guidance from the ICH and FDA.

Stress testing is likely to be carried out on a single batch of the drug substance. The testing should include the effect of temperatures [in 10°C increments (i.e., 50°C, 60°C) above that for accelerated testing], humidity (i.e., 75% relative humidity or greater) where appropriate, oxidation, and photolysis on the drug substance. The testing should also

evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension.

The stress studies should assess the stability of the drug substance in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels. The guidance does not specify pH, temperature ranges, specific oxidizing agents, or conditions to use, the number of freeze-thaw cycles, and so on. In general, values anywhere between 5% to 20% degradation of the drug substance have been considered as reasonable and acceptable for validation of chromatographic assays.

Different forced degradation conditions to be used for drug substances & products [16]

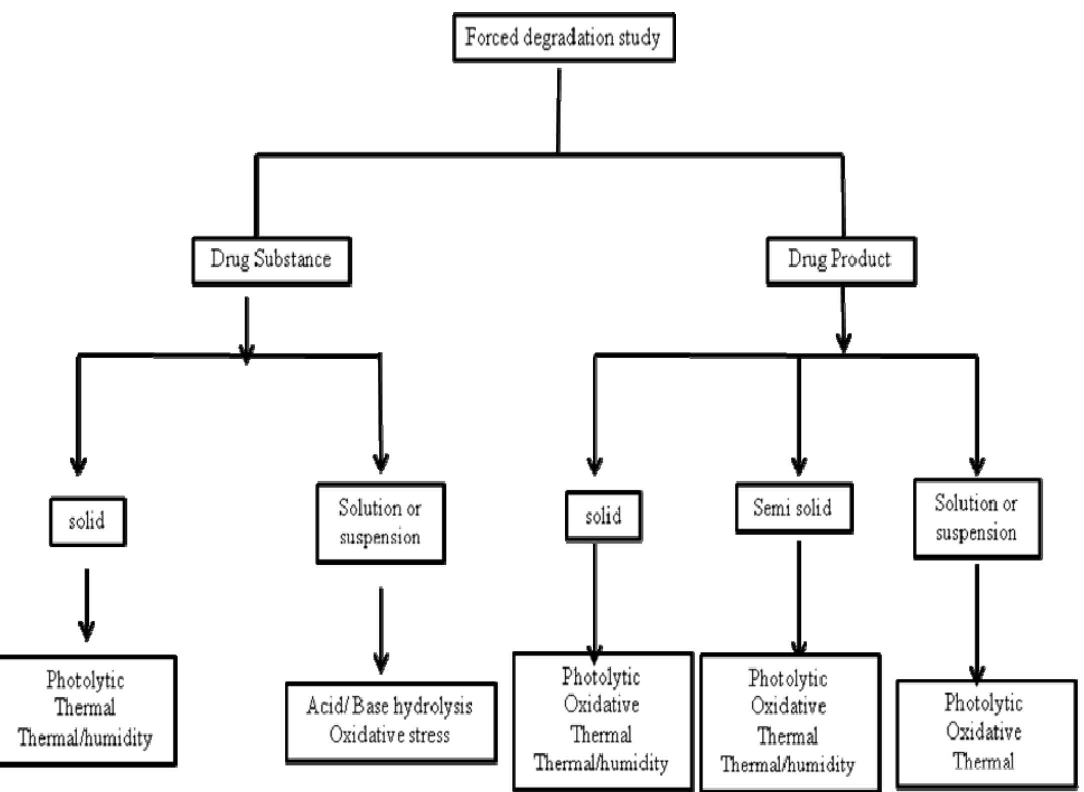


Fig 4: Different Forced Degradation Conditions To Be Used For Drug Substances & Products

However, for small pharmaceutical molecules for which acceptable stability limits of 90% of label claim is common, pharmaceutical scientists have agreed that approximately 10% degradation is optimal for use in analytical validation. In designing forced degradation studies, it must be remembered that more strenuous conditions than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) should be used.

It must be noted that a forced degradation study is a “living process” and should be done along the developmental time line as long as changes in the stability-indicating

methods, manufacturing processes, or formulation changes are ongoing. Forced degradation is only considered complete after the manufacturing process is finalized, formulations established, and test procedures developed and qualified.

At a minimum, the following conditions should be investigated:

1. Acid and base hydrolysis
2. Hydrolysis at various pH
3. Thermal degradation
4. Photolysis degradation
5. Oxidation degradation.

4) Detection limit

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

The LOD may be determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level (lowest calibration standard) at which the analyte can be reliably detected. The lowest calibration standard which produces a peak response corresponding to the analyte should be measured 'n' times (normally 6-10). The average response (X) and the standard deviation (SD) calculated.

The LOD = 3.3 * SD/ Slope of Calibration curve

SD = Standard Deviation

5) Quantitation limit

The limit of quantitation is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions. The limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products or low levels of active constituent in a product. The LOQ may be determined by preparing standard solutions at estimated LOQ concentration (based on preliminary studies). The solution should be injected and analyzed 'n' times (normally 6-10). The average response and the standard deviation (SD) of the n results should be calculated and the SD should be less than 20%. If the SD exceeds 20%, a new standard solution of higher concentration should be prepared and the above procedure repeated.

The LOQ = 10 * SD/ Slope of Calibration curve

SD = Standard Deviation

6) Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weighing of synthetic mixtures of the test product components, using the proposed procedure.

Linearity is determined by series injections of whose concentrations span 50-150 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation. A linear regression equation applied to the results should have an intercept not significantly different from zero (0). If a significant nonzero intercept is obtained, it should be demonstrated that this has no effect on the accuracy of the method.

7) Range

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

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

8) Robustness

Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method's robustness, a number of method parameters, for example, pH, flow rate, column temperature, injection volume, detection wavelength or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range.

Obtaining data on these effects helps to assess whether a method needs to be revalidated when one or more parameters are changed, for example, to compensate for column performance over time. In the ICH document, it is recommended to consider the evaluation of methods robustness during the development phase, and any results that are

critical for the method should be documented. This is not however, required as part of a registration.

9) Ruggedness

Ruggedness tests examine the effect that operational parameters have on the analysis results. The ruggedness was determined by changing the column same manufacture but different lot numbers and Analyst variability and system to system variability. In all the ruggedness conditions the method parameters shall meet the system suitability criteria.

10) Solution stability

Solution stability was determined by Preparing the samples and standard solutions at room temperature and inject at initially and after 1 day, 2day, 3day if possible Similarly Prepare the mobile phase and keep it at room temperature in well closed condition and inject into HPLC system. Evaluate the all the system suitability parameters.

Proposed Acceptance Criteria for the Different Characteristics of Validation by ICH [19-20]

Characteristics	Proposed Use Acceptance Criteria
Linearity	$r^2 \geq 0.99$, similar response ratios
Precision-System	RSD < 2 %
Precision-Method	RSD < 2 %
Precision Repeatability/Reproducibility	RSD ≤ 2 %
Accuracy	FDA 98-102 %, EPA 50-150 %
Specificity	No interference
Detection Limit	>2 times base line
Quantitative Limit	Signal to Noise ratio = 10:1
Range	Concentration where data can be reliably detected

Table 4: Proposed ICH Acceptance Criteria For The Different Characteristics Of Validation

CHAPTER 2

REVIEW OF LITERATURE

The following methods have been reported for the estimation of Naproxen and Esomeprazole magnesium individually and in combination with other drugs.

1) **Srinivas Ampati, et al**[26] Development And Validation Of RP-HPLC Method For The Simultaneous Estimation Of Naproxen Sodium And Esomeprazole magnesium In Pharmaceutical Tablet Dosage Form. An isocratic RP-HPLC method was developed and validated for the Simultaneous estimation of Naproxen sodium and Esomeprazole magnesium trihydrate in Pharmaceutical tablet dosage form. The separation was achieved by using a reversed-phase C 18 column(Thermo eletrole, ODS, 250mm × 4.6 mm i.d, 5µm) at ambient temperature with mobile phase consisting of Phosphate buffer (pH adjust to 3.8using OPA): Acetonitrile : Methanol (30:50:20v/v). The flow rate was 1.0 ml/min. Detection was carried out at a wavelength of 220 nm. Retention time of Naproxen sodium and Esomeprazole magnesium trihydrate were found tobe2.417 and 3.903min respectively. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay method was found to be linear from 75-175µg/ml and 3-7µg/ml for Naproxen sodium and Esomeprazole magnesium trihydrate respectively. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Naproxen sodium and Esomeprazole magnesium trihydrate in Pharmaceutical tablet dosage form.
2) **Chandrakant Sojitra, et al**[27] Development and Validation of RP-HPLC Methods for Simultaneous Estimation of Naproxen and Esomeprazole magnesium Trihydrate in Combined Pharmaceutical Formulation. A rapid, specific, sensitive and simple high performance liquid chromatography was developed for simultaneous estimation of Esomeprazole magnesium trihydrate and Naproxen in tablet formulation. The separation was achieved by SUPELCO 516 C 18–DB column (250× 4.6 mm, particle size 5µm) with a mobile phase consisting of 0.01M phosphate buffer pH 7.5: ACN: methanol(40:50:10v/v, addition of 0.1% triethyl amine), at a flow rate of 0.8 ml/min. Detection was carried out at 303 nm. Retention time of Esomeprazole magnesium trihydrate and Naproxen were found to be 4.6 and 3.2min, respectively. The linear dynamic range was 0.8-4.8µg/ml and 15-90µg/ml for Esomeprazole magnesium trihydrate and Naproxen, respectively.

The method is validated for Accuracy, Precision, Ruggedness and Robustness. The proposed method is successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies.

3) **Deshpande.S.V, et al**[28] Development and Validation of Esomeprazole and Naproxen in Bulk and Tablet Dosage Form by RP-HPLC Method. A simple, rapid, reproducible, accurate and precise RP-HPLC method was developed for the quantitative simultaneous estimation of Esomeprazole and Naproxen in combined tablet dosage form. Esomeprazole is used as anti ulcerative and naproxen has non steroidal anti-inflammatory activity. The chromatographic separation of both drugs was achieved with 250x4.6 mm, 5 µm c-18 column using acetonitrile:0.01 m potassium dihydrogen phosphate buffer (60:40 v/v) at the flow rate of 1.0 ml/min at 244.0 nm. The linearity range was found to be 2-30 µg/ml for esomeprazole and 5-100 µg/ml for naproxen. The coefficient of correlation for esomeprazole and naproxen was found to be 0.9993 and 0.9989 respectively. The percent recoveries obtained for esomeprazole and naproxen were found to be 99.86 and 99.72 respectively. The method was validated for linearity, range, precision, accuracy, specificity, selectivity, intermediate precision, ruggedness, robustness, stability and suitability.

4) **Kumar, et al**[29] Simultaneous Estimation of Esomeprazole and Naproxen in Bulk As well As in Pharmaceutical Formulations by Using RP-HPLC. Esomeprazole is used to treat gastro esophageal reflux disease. Naproxen is a Non-steroidal anti-inflammatory drug (NSAID) used in the treatment of pain or inflammation caused by conditions such as arthritis, ankylosing spondylitis, tendinitis, bursitis, gout, or menstrual cramps. A simple, precise, cost effective RP-HPLC method was developed and validated for the determination of both Esomeprazole and Naproxen in Pharmaceutical compositions. The chromatographic separation was achieved on a Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) using a mobile phase consist of a mixture of phosphate buffer (pH 3) and Acetonitrile [60:40]. The flow rate of mobile phase was maintained 1.0 mL per minute. The wavelength chosen for detection was 285 nm. The retention times of Esomeprazole and Naproxen peaks were around 2.105 and 3.555 mins respectively. The Accuracy was calculated for 50%, 100% and 150% and the % recovery was found to be 98.0%-100.4%. The method was found to be linear over the range of 5ppm to 9ppm per mL for

Esomeprazole 125ppm to 225ppm per mL for Naproxen. The proposed method was validated as per the ICH and USP guidelines.

5) **T. Manikya Rao, et al**[30] Stability indicating assay of Esomeprazole and Naproxen in Tablets by RP-UPLC PDA-Method. A simple fast, accurate, precise and cost effective isocratic RP-UPLC method is developed for simultaneous determination of Esomeprazole and Naproxen in tablet formulation. The retention times of Esomeprazole and Naproxen were found to be 0.7 and 1.2 minutes respectively. The method was linear over the range of 5 to 25 ppm with correlation of 0.999 for Esomeprazole and 125 to 625 ppm with correlation of 0.998 for Naproxen. Mean recovery for Esomeprazole and Naproxen were 99.82% and 99.74% respectively. The method found simple, accurate, precise, and linear over the given range, rugged and robust.

6) **Palavai Sripal Reddy, et al**[31] Stability indicating simultaneous estimation of assay method for naproxen and esomeprazole in pharmaceutical formulations by RP-HPLC. Naproxen is a Non steroidal simultaneous anti inflammatory drug (NSAID) used in the treatment of pain or inflammation caused by conditions such as arthritis, ankylosing spondylitis, tendinitis, bursitis, gout, or menstrual cramps. Esomeprazole is used to treat gastro esophageal reflux disease. A simple, precise cost effective and stability indicating RP-HPLC method has been developed and validated for the determination of both Naproxen and Esomeprazole in pharmaceutical compositions. Separation of Naproxen and Esomeprazole from its potentional degradants were achieved within a shorter run time with required resolution, accuracy and precision thus enabling the utility of the method for routine analysis. 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 milliQ grade water with a pH of 8.7 which is mixed with Acetonitrile and Methanol at a flow rate of 1.5 mL per minute. Wavelength chosen for detection is 305 nm. The retention times of Naproxen and Esomeprazole peaks are around 3 and 6 minute respectively. The method was found to be linear over the range of 100.28 to 902.520 µg per mL for Naproxen and 9.6 to 45.6 µg per mL for Esomeprazole. The proposed method is validated as per the ICH and USP guidelines.

7) **Jain, et al**[32] The RP-HPLC method for simultaneous estimation of esomeprazole and naproxen in binary combination. A simple, precise, reliable, rapid, sensitive and

validated RP-HPLC method has been developed to determine Esomeprazole magnesium trihydrate (ESO) and naproxen (NAP) in synthetic mixture form. Materials and Methods: Chromatographic separation achieved isocratically on Phenomenex, Luna C18 column (150 × 4.6 mm, 5µ) and acetonitrile: phosphate buffer (pH 7.0) in the ratio of 50:50 (v/v) as the mobile phase, at a flow rate of 0.5 ml/min. Detection was carried out at 300 nm. The retention times for NAP and ESO was found to be 2.67 and 5.65 min respectively. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. Results: The method was linear in the concentration range of 50-250µg/ml for NAP and 2-10µg/ml for ESO with correlation coefficient of 0.999 and 0.998 respectively. The mean recoveries obtained for NAP and ESO were 100.01% and 97.76 % respectively and RSD was less than 2. The correlation coefficients for all components are close to 1. Conclusions: Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of NAP and ESO.

8) **Muhammad Ashfaq, et al**[33] Development and Validation of Liquid Chromatographic Method for Naproxen and Esomeprazole in Binary Combination. The present study reports the development and validation of a simple, economic and sensitive HPLC method for the concurrent determination of naproxen and esomeprazole in pharmaceutical formulations. Isocratic chromatography was performed with C-18 column and mixture of phosphate buffer (pH 6.1) and acetonitrile in ratio of (40:60, v/v) at 1.5 mlmin-1. The eluents were monitored at 302 nm using UV detector. The method was isocratic in the range of 9.38 to 300 µgml-1 for naproxen and 0.5 to 16 µgml-1 for esomeprazole. Validation of the method was performed by testing parameters like linearity, accuracy, precision, robustness, specificity, LOD and LOQ values. In the specificity the drugs were subjected to forced degradation studies like acidic, basic, oxidative and thermal stresses. Both the analytes were separated within three minutes. As the method separates the degradation products produced during forced degradation studies from the active analytes so it can be used not only for regular determination of naproxen and Esomeprazole but also for their stability studies.

9) **Khagga Bhavyasri.V, et al**[34] Rapid Simultaneous Determination of Naproxen and Esomeprozole Magnesium in Combined Tablets by Validated Ultra Performance Liquid Chromatographic Method. A stability- indicating ultra Performance liquid

chromatography (UPLC) method has been developed and validated for the simultaneous determination of Naproxen and Esomeprazole magnesium in pharmaceutical preparations. An Agilent Zorbax SB Phenyl column (50X4.6mm i.d., 1.8µm particle size) was used. The mobile phase consisted of a mixture of 10 mm Ammonium Bicarbonate (adjusted to pH 7.0 with Phosphoric acid) and acetonitrile in the ratio 50:50 Ultraviolet(UV) detection was performed at 215 nm. Total run time was 5 min; these two drugs were eluted at the retention times of 0.766 and 1.484 min for Naproxen and Esomeprazole magnesium respectively. The method was validated in terms of linearity, range, specificity, accuracy, and precision, limit of detection (LOD) and limit of quantitation (LOQ). The linearity for both the drugs was found in the range of 18.7-150 µg mL-1of Nap and 1-8 µg/mL-1. The % recoveries of Naproxen were found to be 98.2-100.2% and Esomeprazole magnesium were found to be 99.8-101.6%. The utility of the procedure is verified by its application to marketed formulations that were subjected to accelerated degradation studies. The method distinctly separated the drug and degradation products even in actual samples. The products formed in marketed tablet dosage forms are similar to those formed during stress studies.

CHAPTER 3

DRUG PROFILE [35]

A) NAPROXEN

S.No	Parameters	Details
1	Description	White or almost white, hygroscopic, crystalline powder.
2	CAS No	22204-53-1
3	Chemical Structure	
4	Molecular Formula	$C_{14}H_{13}NaO_3$
5	Molecular Mass	252.24 g/mol
6	Chemical Name	2-Naphthaleneaceticacid,6-methoxy-α-methyl-(*S*)-(+)-(*S*)-6-Methoxy-α-methyl-2-naphthaleneacetic acid
7	Solubility	Freely soluble in water, freely soluble or soluble in methanol, sparingly soluble in ethanol (96%).
8	Half Life	12 to 24 hour
9	Therapeutic Uses	Naproxen is commonly used for the reduction of pain, fever, inflammation, and stiffness caused by conditions including migraine, osteoarthritis, stones, rheumatoid, psoriatic arthritis, gout, ankylosing spondylitis, menstrual cramps, tendinitis, and bursitis, among others. It is also used for the treatment of primary dysmenorrhea.
10	Mechanism of Action	Naproxen works by reversibly inhibiting both the COX-1 and COX-2 enzymes.

B) ESOMEPRAZOLE MAGNESIUM

S.No	Parameters	Details
1	Description	White to slightly coloured powder.
2	CAS No	0119141-88-7
3	Chemical Structure	
4	Molecular Formula	$C_{17}H_{19}N_3O_3S$
5	Molecular Mass	345.415 g/mol
6	Chemical Name	(*S*)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)-methylsulfinyl]-3*H*-benzoimidazole
7	Solubility	Esomeprazole drug is soluble in methanol, slightly soluble in water, insoluble in heptane.
8	Half Life	1-1.5 Hourrs
9	Therapeutic Uses	A highly effective inhibitor of gastric acid secretion used in the therapy of stomach ulcers and zollinger-ellison syndrome. The drug inhibits the H^+/K^+-ATPase (H^+/K^+-exchanging ATPase) in the proton pump of gastric parietal cells.
10	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 blocks the final step in acid production, thus reducing gastric acidity.

CHAPTER 4
AIM OBJECTIVE AND PLAN OF WORK

AIM

To develop and validate the method for simultaneous estimation of Naproxen and Esomeprazole magnesium by RP-HPLC.

OBJECTIVES

On literature survey it was found that Naproxen and Esomeprazole magnesium is estimated by HPLC and UV Spectrophotometric methods, in combination with other drugs in tablet and bulk dosage form.

However no method could be found for simultaneous estimation of Naproxen and Esomeprazole magnesium, and also no method was available for such estimation in the pharmacopoeia. In view of the need for a suitable method for routine analysis of Naproxen and Esomeprazole magnesium in formulations, attempts are being made to develop simple, precise and accurate analytical methods for simultaneous estimation of Naproxen and Esomeprazole magnesium and extend it for their determination in formulations.

The utility of the developed methods to determine the content of both drugs in commercial tablet is also demonstrated. Validation of the method was done in accordance with USP and ICH guidelines for the assay of active ingredients. The methods were validated for parameters like accuracy, linearity, precision, specificity, ruggedness, robustness, and system suitability. These methods provide means to separate the individual components of a mixture and simultaneously characterize and quantify the components. These proposed methods are suitable for the pharmaceutical analysis in analytical laboratories.

The Chromatographic methods proposed in this presume that there is a linear relationship between absorbance and component concentration. These methods have a calibration step followed by the prediction step, in which the results of the calibration step are used to estimate the component concentration from an unknown sample spectrum. The Chromatographic methods have many of full spectrum advantages. These methods are known to provide additional advantages that calibration can be performed by ignoring the concentration of all other components except the analyte of interest. These methods have

been successfully applied to the quantitative analysis in spectrophotometric, chromatographic and electrochemical data.

In summary, the primary objective of proposed work is to:-

- Develop new, simple, sensitive, accurate, and economical analytical methods for the simultaneous estimation of Naproxen and Esomeprazole magnesium.
- Validate the proposed methods in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of these drugs in their combined dosage form.

PLAN OF WORK

1. Selection of drug sample.
2. Procurement of drug & other chemicals.
3. UV-Visible Spectrophotometry
    - Determination of λ max by UV Spectrophotometry.
4. HPLC Method Development:
    - Selection of preliminary HPLC conditions
    - Selection of mobile phase
    - Selection of column
    - Selection of wavelength
    - Analysis of laboratory mixture.

The steps in Method development will be as follows-

- Analysis of standard laboratory mixture to see feasibility of the proposed methods.
- To adopt the selected methods on marketed formulation.
- Validation of the proposed methods.

CHAPTER 5

5.1 METHOD DEVELOPMENT

MATERIAL AND CHEMICALS:

S.No.	Name	Grade	Make	Lot Number	Purity	Valid up to
01	Monobasic sodium phosphate	AR	Himedia	0000194436	98.0 %	02/2016
02	Dibasic sodium phosphate	AR	SRL	T8380795	99.0 %	07/2016
03	Acetonitrile-HPLC grade	AR	Finar	118381004JN	99.8 %	09/2017
04	Orthophosphoric acid	AR	Merck	CF4C640314	85.0 %	08/2017
05	Sodium Hydroxide	AR	SRL	T-8382161	98.0%	12/2017
06	Purified Water	Not Applicable				

Table No. 5: Detail of Material and Chemicals

INSTRUMENTS:

S. No.	Name	Make & Model	Date of Calibration	Due date of Calibration
01	Analytical Balance	Mettler Toledo XS105 (Digital Analytical Balance)	Daily calibration	
02	pH	Hanna (Digital pH Meter)	Daily calibration	
03	Sonicator	PCI India	15.09.2014	14.03.2014
04	Filter	Nylon & PVDF 0.45 µm (Millipore)	Not Applicable	
05	HPLC	Agilent 1260 & 1290 series (Software-Open Lab)	08.10.2014	02.04.2015
06	UV	Shimadzu UV1700 series (Software-UV Probe)		

Table No. 6: Detail of Instruments

COLUMN:

S.No.	Make	Column Name	Serial No
01	Reliant	C18, 4.6-mm X 25cm; 5 µm	272I340281400 3

Table No. 7: Detail of Column

All the glassware's used were of borosilicate glass of class A and all the solvents and prepared solutions were filtered through Nylon filter 0.45u.

Drugs	Naproxen and Esomeprazole magnesium
Label Claim	Naproxen-500 mg Esomeprazole magnesium-20 mg
Manufactured by	Caplin Point Unit-II, India.

Table No. 8: Detail of Marketed Formulation

S. No.	Name	Purity	Valid Up to
01	Esomeprazole magnesium	92.46 %	05/2015
02	Naproxen Sodium	98.93%	12/2018

Table No. 9 : Details Of Working Standard

5.2 Study of Spectra and Selection of Wavelength:

Preparation of Diluent:

Methanol: Water (50:50) v/v.

Naproxen Standard Solution (25 mcg/mL of Naproxen):

An accurately weighed 137.0 mg of Naproxen Sodium WS (Equivalent to 125 mg of Naproxen) and transfer into a 50 mL volumetric flask. Add 25 mL of diluents and sonicate for 5 minutes to dissolve. Cool and dilute up to the volume with diluents. With draw 5 ml from this solution and transfer in 100 mL volumetric flask dilute up to the volume with mobile phase. With draw 5 ml from this solution and transfer in 25 mL volumetric flask dilute up to the volume with mobile phase.

Esomeprazole Standard Solution (20 mcg/mL of Esomeprazole):

An accurately weighed 20.65 mg of Esomeprazole magnesium WS (Equivalent to 20 mg of Esomeprazole) and transfer into a 200 mL volumetric flask. Add 125 mL of diluents and sonicate for 5 minutes to dissolve. Cool and dilute up to the volume with diluents. With draw 5 ml from this solution and transfer in 25 mL volumetric flask dilute up to the volume with mobile phase.

The solutions were scanned in the range of 600-200 nm in 1 cm cell against blank. The individual UV absorbance spectrum of Blank and NAP and ESO are shown in Fig.No.5, 6, 7 & 8.

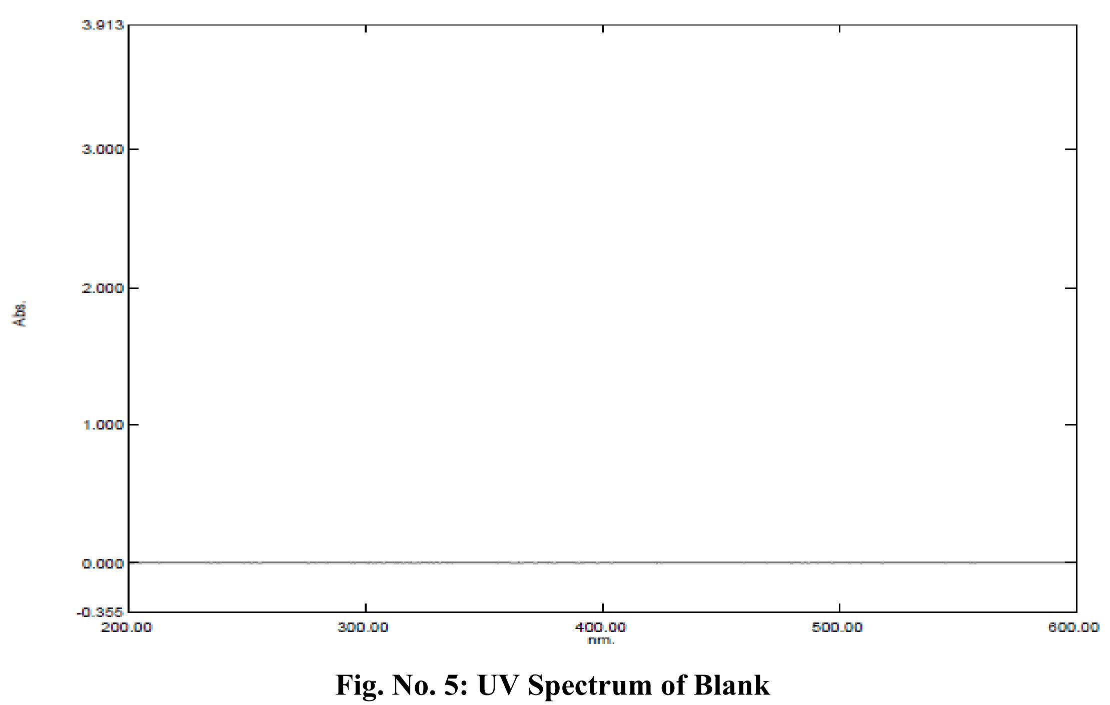


Fig. No. 5: UV Spectrum of Blank

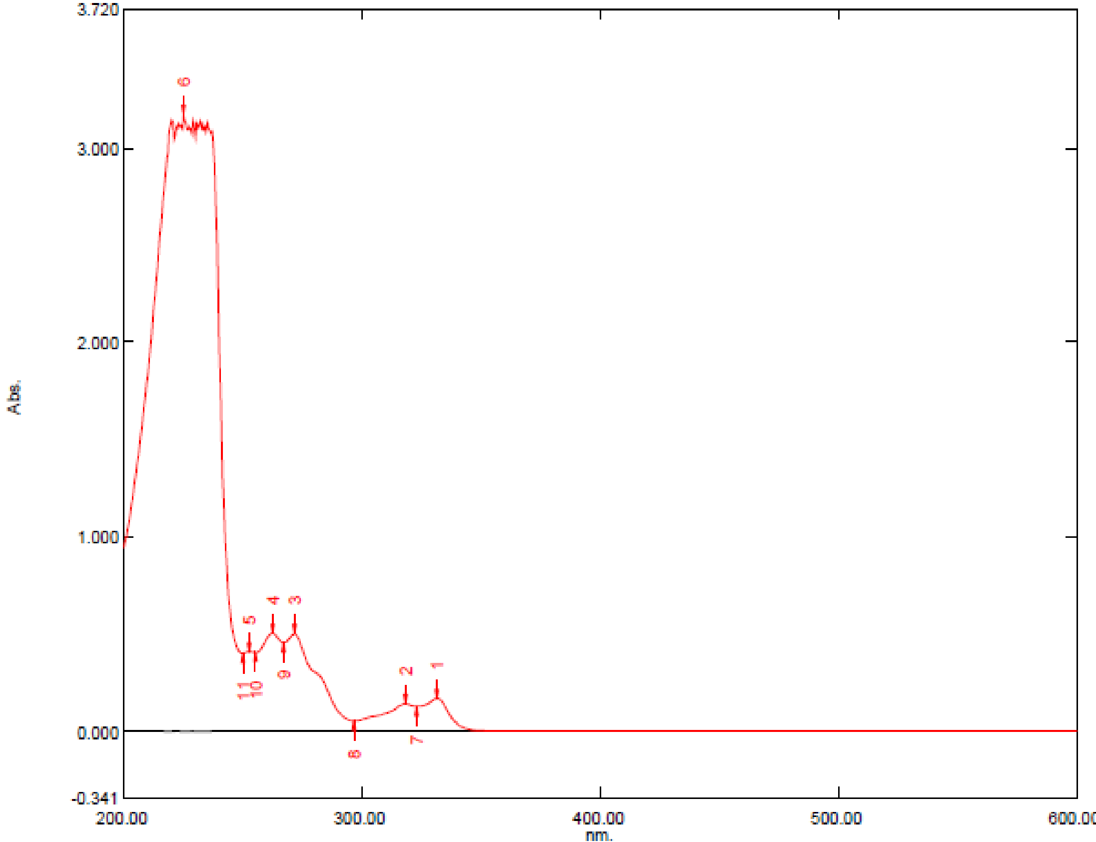


Fig. No.6: UV Spectrum of Naproxen

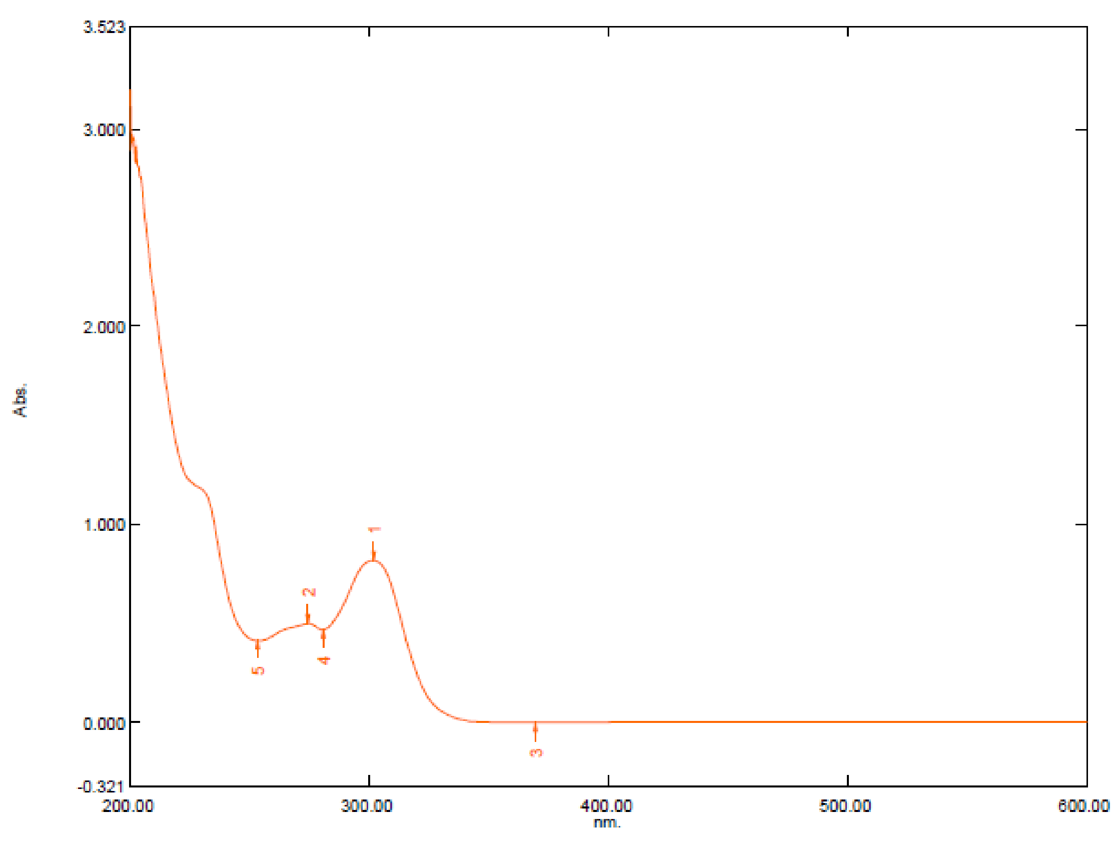


Fig.No.7: UV Spectrum of Esomeprazole

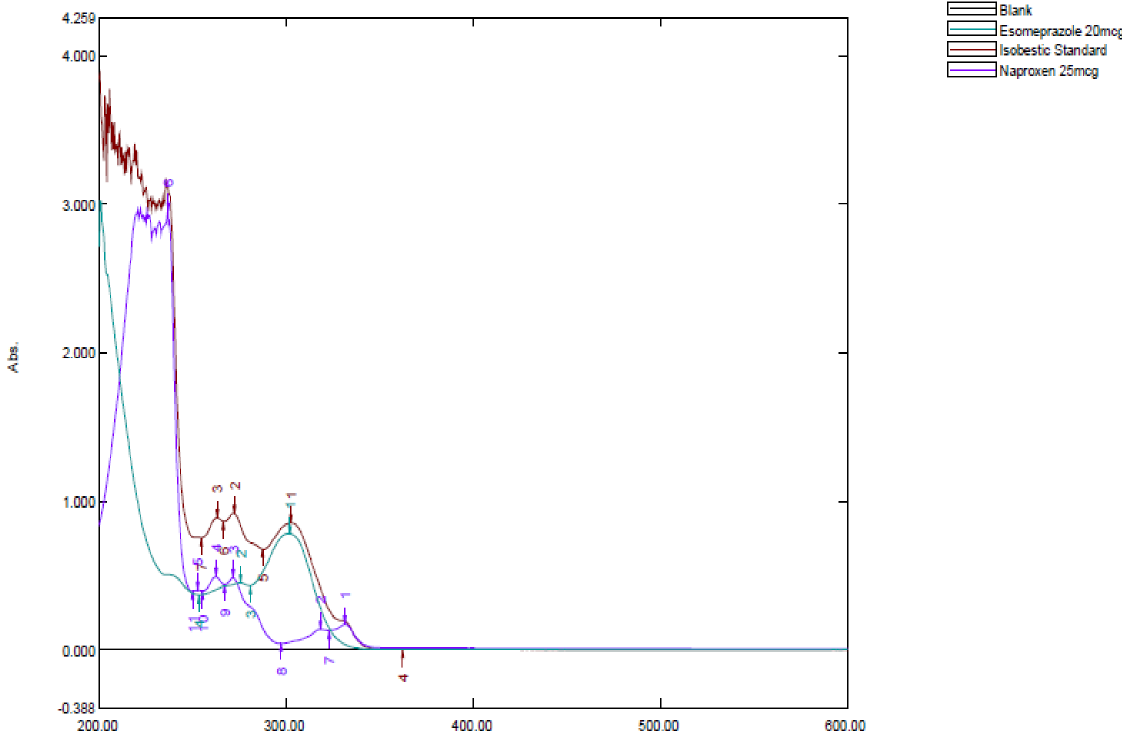


Fig.No.8: UV Spectrum of Isobestic Standard & Individual Standard

Beers - Lamberts Law

Beers Law:- It states that a similar relationship holds between transmittance and the concentration of solution i.e. the intensity of abeam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substance arithmetically. There exist a relationship between the absorbance (A), transmittance (T) and the molar absorption coefficient (ε) i.e.

$$A= \varepsilon ct = \log I_0/It = \log 1/T = -\log T$$

Lamberts law:- It states that when a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light.

Mathematical lamberts law stated as:- $-\frac{dI}{dt} = kI$

Where,

I - Intensity of incident light of wavelength

T - Thickness of the medium

K - Proportionality factor

Resemblance of Beers law to Naproxen:

Concentration(ppm)	Absorbance at 272 nm
0	0
15	0.278
20	0.372
25	0.472
30	0.562
40	0.745

Table No. 10 : Linearity of Naproxen

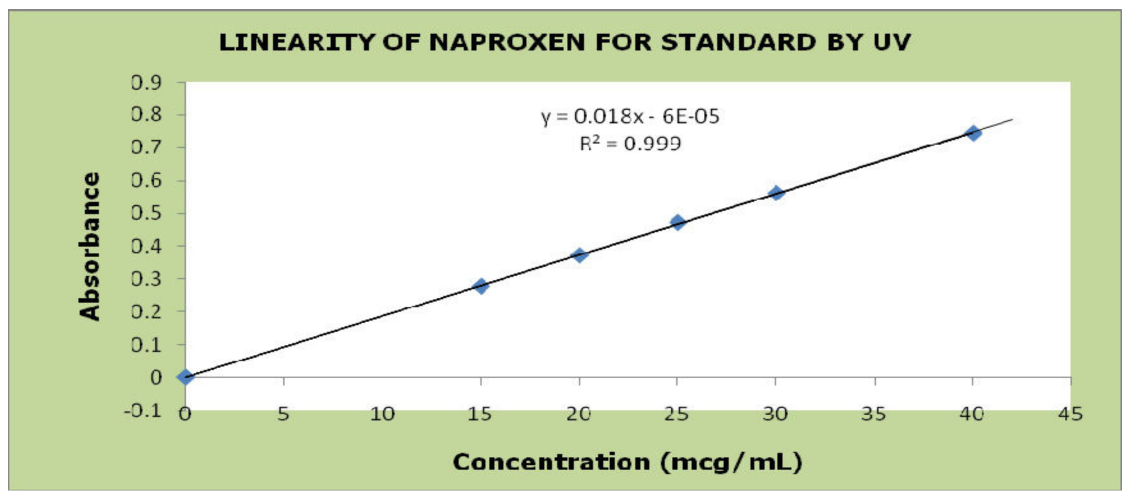


Fig.No.9: Linearity of Naproxen

Resemblance of Beers law to Esomeprazole:

Concentration(ppm)	Absorbance at 302 nm
0	0
12	0.493
16	0.652
20	0.809
24	0.979
32	1.309

Table No. 11 : Linearity of Esomeprazole magnesium

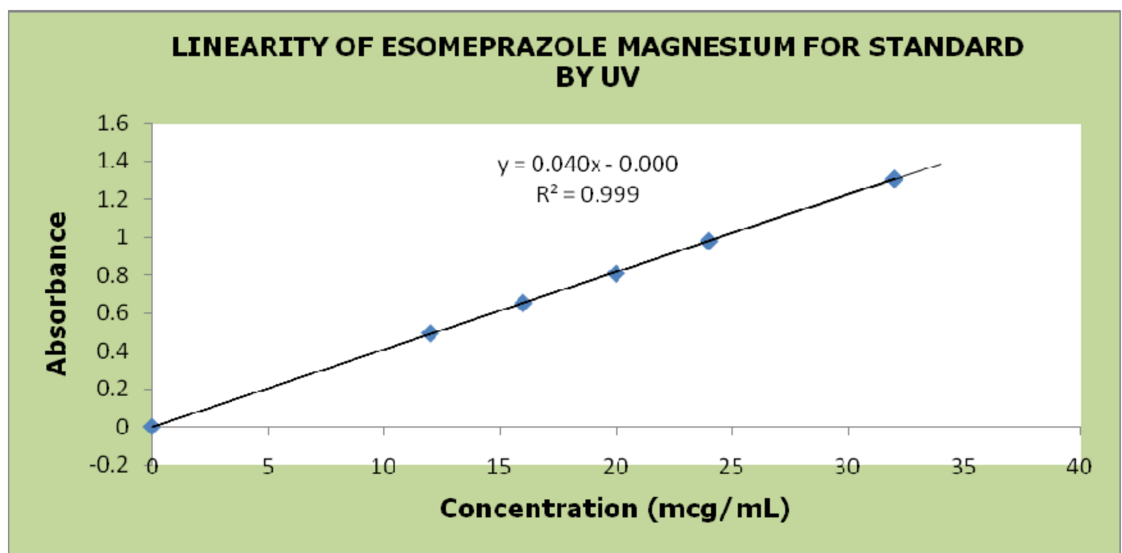


Fig.No.10: Linearity of Esomeprazole magnesium

Naproxen obeys Beers law in the range of 0-40 ppm and shows the $R^2 = 0.999$.

Esomeprazole magnesium obeys Beers law in the range of 0-32 ppm and shows the $R^2 = 0.999$.

5.3 Estimation of Naproxen and Esomeprazole magnesium in Tablet Formulation Form by RP-HPLC:

Based on sample solubility, stability and suitability, various mobile phases compositions were tried to get a good resolution and sharp peak.

The standard solution containing mixture of NAP and ESO as well as individual drugs were run in different mobile phases.

The following trail were performed

Preparation of Phosphate Buffer

Preparation of 1.0 M Monobasic Sodium Phosphate Buffer:

Weigh accurately about 11.998 g of Monobasic Sodium Phosphate and transfer into 100 mL beaker and dilute up to the volume with water, and mix well.

Preparation of 0.5 M Dibasic Sodium Phosphate Buffer:

Weigh accurately about 8.90 g of Dibasic Sodium Phosphate and transfer into 100 mL beaker and dilute up to the volume with water, and mix well.

Preparation Buffer solution:

Prepare a Phosphate buffer by mixing 10.5 mL of 1.0 M monobasic sodium phosphate buffer and 60 mL of 0.5 M dibasic sodium phosphate buffer transfer into 1000 mL beaker, and dilute up to the volume with water. Mixed and adjust the required pH with Ortho Phosphoric acid or Sodium Hydroxide.

Trail-1

Chromatographic Conditions:

Parameter/Conditions	Description/Values
Column Name	C18column(ODS,150mm×4.6 mm×5µm)
Flow rate	1 mL
Injection volume	20 µL
Wavelength	302 nm
Mobile phase	pH 3.8PO_4Buffer:ACN:Water (50:35:25)

Table No.12:Chromatograpic Condition of Trail-1

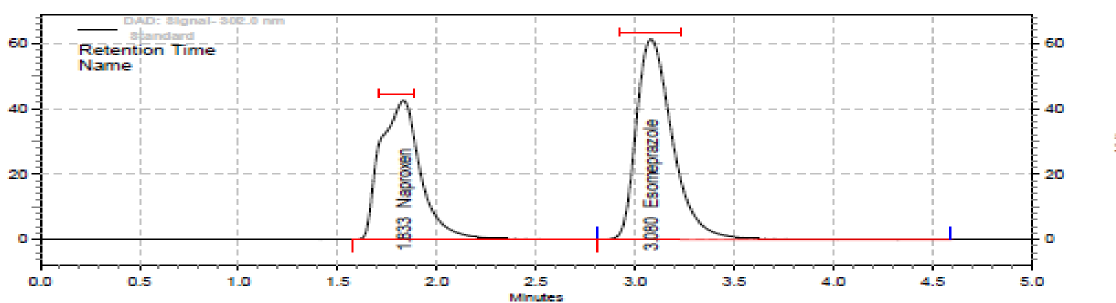


Fig.No.11: Chromatogram of Trail-1

Observation:

Peak Number	Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry	Resolution (USP)
1	Naproxen	1.833	80715140	480	1.20	0.00
2	Esomeprazole	3.080	104442531	1448	1.48	3.79
Totals			185157671			

Conclusion: Peak shape is not sharp.USP Plate count of Naproxen & Esomeprazole is also below the limit. (i.e. 2000).

Trail - 2

Chromatographic Conditions:

Parameter/Conditions	Description/Values
Column Name	C18column(ODS,150mm×4.6 mm×5µm)
Flow rate	1 mL
Injection volume	20 µL
Wavelength	302 nm
Mobile phase	pH 4.2Po_4 Buffer:ACN:Water (50:35:15)

Table No.13:Chromatograpic Condition of Trail-2

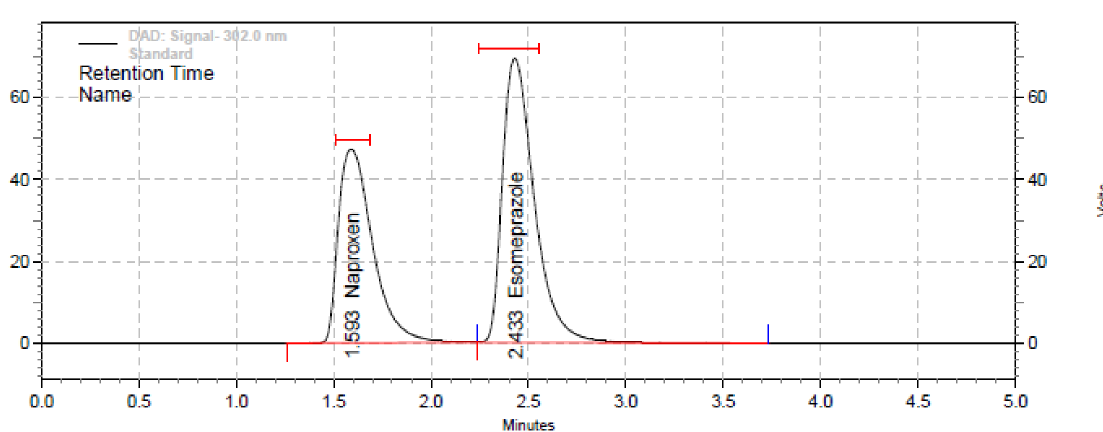


Fig.No.12: Chromatogram of Trail-2

Observation:

Peak Number	Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry	Resolution (USP)
1	Naproxen	1.593	77979956	442	1.71	0.00
2	Esomeprazole	2.433	106130574	1146	1.57	2.84
Totals			184110530			

Conclusion: USP Plate count of Naproxen & Esomeprazole is also below the limit. (i.e.2000).

Trail - 3

Chromatographic Conditions:

Parameter/Conditions	Description/Values
Column Name	C18column(ODS,250mm×4.6 mm×5µm)
Flow rate	1 mL
Injection volume	20 µL
Wavelength	302 nm
Mobile phase	pH 5.3Po$_4$ Buffer:ACN:Water (40:50:10)

Table No.14:Chromatograpic Condition of Trail-3

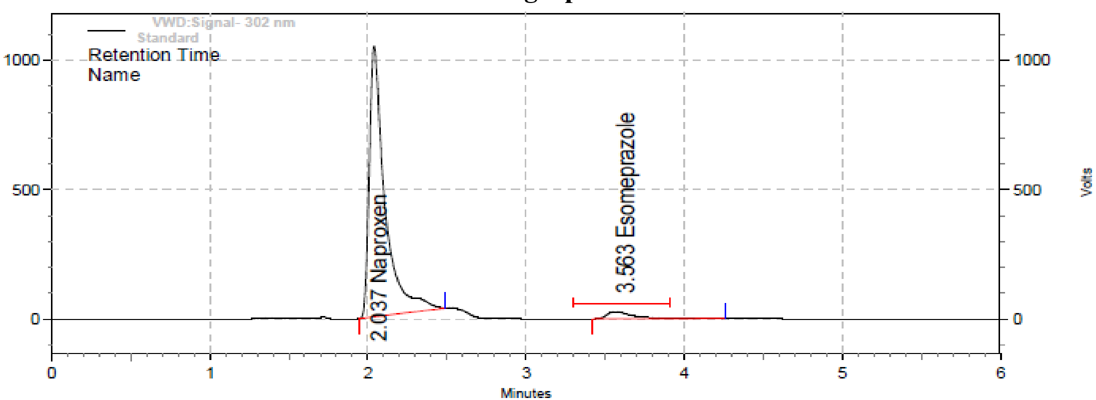


Fig.No.13: Chromatogram of Trail-3

Observation:

Peak Number	Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry	Resolution (USP)
1	Naproxen	2.037	121217201	2647	2.72	0.00000
2	Esomeprazole	3.563	5138866	2717	2.37	7.07148
Totals			126356067			

Conclusion: USP tailing factor of Naproxen & Esomeprazole is also above the limit. (i.e. <2.0).

Trail - 4

Chromatographic Conditions:

Parameter/Conditions	Description/Values
Column Name	C18column(ODS,250mm×4.6 mm×5µm)
Flow rate	1 mL
Injection volume	20 µL
Wavelength	302 nm
Mobile phase	pH 4.2Po$_4$ Buffer:ACN:Water (30:50:20)

Table No.15:Chromatograpic Condition of Trail-4

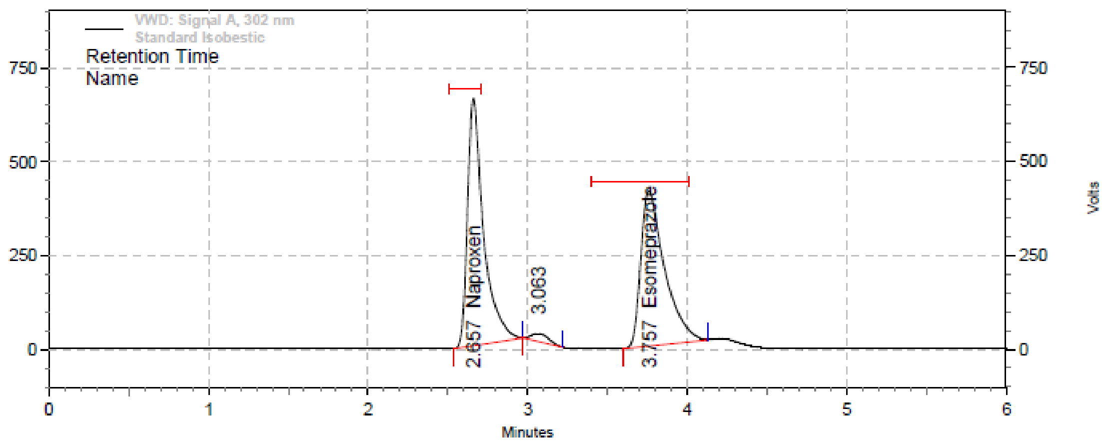


Fig.No.14: Chromatogram of Trail-4

Observation:

Peak Number	Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry	Resolution (USP)
1	Naproxen	2.657	77183939	3839	1.85	0.00000
2		3.063	2726709	3609	1.28	2.16622
3	Esomeprazole	3.757	71909828	3206	1.83	2.95443
4		8.010	105925228	18747	2.15	17.03382
Totals			257745704			

Conclusion: Peak Elution is not proper. Also Peak shape is not proper.

Trail - 5

Chromatographic Conditions:

Parameter/Conditions	Description/Values
Column Name	C18column(ODS,250mm×4.6 mm×10µm)
Flow rate	1 mL
Injection volume	20 µL
Wavelength	302 nm
Mobile phase	pH 7.0PO_4Buffer:ACN:Water (50:35:15)

Table No.16:Chromatograpic Condition of Trail-5

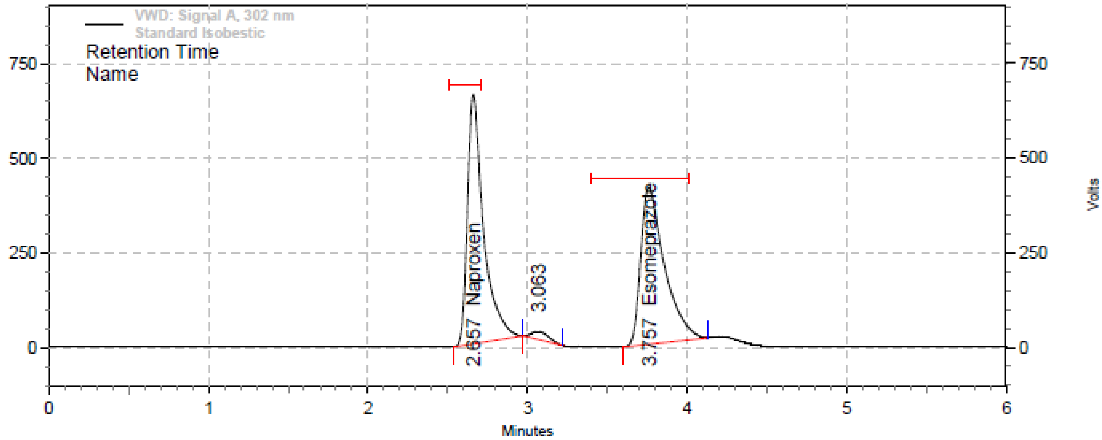


Fig.No.15: Chromatogram of Trail-5

Observation:

Peak Number	Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry	Resolution (USP)
1	Naproxen	2.657	77183939	3839	1.85	**0.00000**
2		3.063	2726709	3609	1.28	**2.16622**
3	Esomeprazole	3.757	71909828	3206	1.83	**2.95443**
4		8.010	105925228	18747	2.15	**17.03382**
Totals			257745704			

Conclusion: Peak Elution is not proper. Also Peak shape is not proper.

Trail - 6

Chromatographic Conditions:

Parameter/Conditions	Description/Values
Column Name	C18column(300mm×4.6 mm×5µm)
Flow rate	1 mL
Injection volume	20 µL
Wavelength	302 nm
Mobile phase	pH 7.3Po_4Buffer:ACN:Water (40:45:15)

Table No.17:Chromatograpic Condition of Trail-6

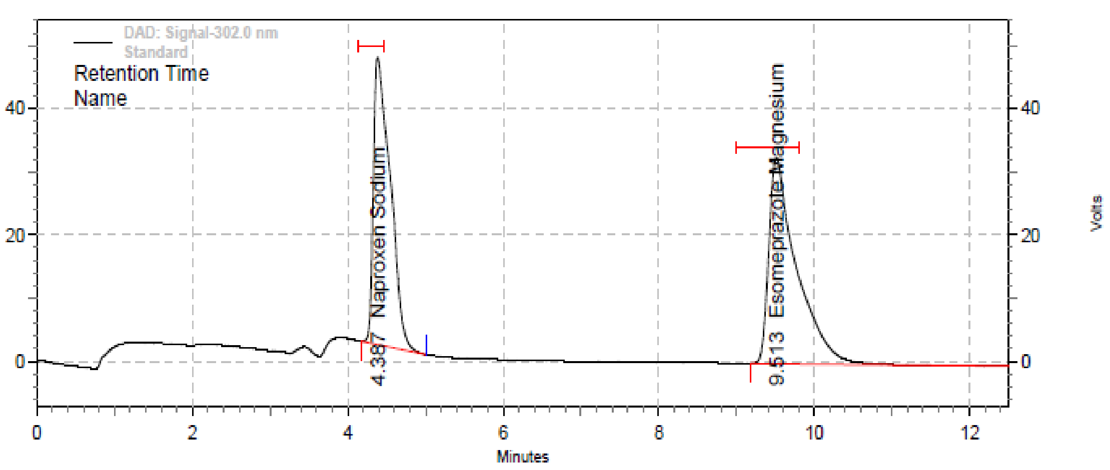


Fig.No.16: Chromatogram of Trail-6

Observation:

Peak Number	Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry	Resolution (USP)
1	Naproxen Sodium	4.387	92473131	1726	2.06	0.00
2	Esomeprazole Magnesium	9.513	109132964	4227	2.43	10.17
Totals			201606095			

Conclusion: USP tailing factor of Naproxen & Esomeprazole is also above the limit. (i.e.<2.0).

Trail - 7

Chromatographic Conditions:

Parameter/Conditions	Description/Values
Column Name	C18column(300mm×4.6 mm×5µm)
Flow rate	1 mL
Injection volume	20 µL
Wavelength	302 nm
Mobile phase	pH 7.3PO_4Buffer:ACN:Water (50:35:15)

Table No.18:Chromatograpic Condition of Trail-7

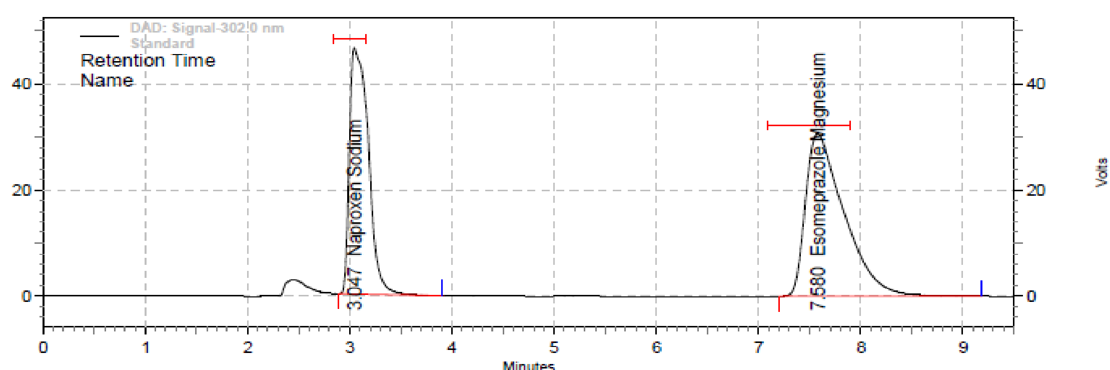


Fig.No.17: Chromatogram of Trail-7

Observation:

Peak Number	Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry	Resolution (USP)
1	Naproxen Sodium	3.047	80113929	1594	1.89	0.00
2	Esomeprazole Magnesium	7.580	108089913	1859	1.94	8.99
Totals			188203842			

Conclusion: Peak Elution is not proper. Also Peak shape is not proper.

Trail - 8

Chromatographic Conditions:

Parameter/Conditions	Description/Values
Column Name	C18column(250mm×4.6 mm×5µm)
Flow rate	1 mL
Injection volume	20 µL
Wavelength	302 nm
Mobile phase	pH 7.3PO_4Buffer:ACN:Water (50:35:15)

Table No.19:Chromatograpic Condition of Trail-8

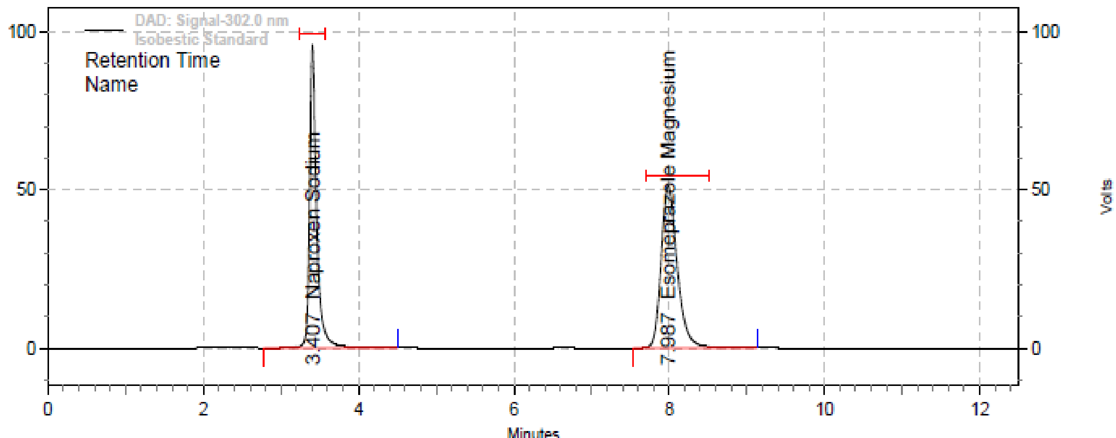


Fig.No.18: Chromatogram of Trail-8

Observation:

Peak Number	Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry	Resolution (USP)
1	Naproxen Sodium	3.407	81256051	8270	1.49	0.00
2	Esomeprazole Magnesium	7.987	99438375	7346	1.29	17.53

Totals		
		180694426

Conclusion: Tailing factor was <2.0, Resolution was optimum and Plate count was >2000, so this method is considered as the optimized method.

CHAPTER 6

6.1 METHOD VALIDATION

Preparation of 1M monobasic sodium phosphate buffer:

Weighed accurately 11.998 g of monobasic sodium phosphate anhydrous and transferred into 100 mL volumetric flask, and dilute up to the volume with water.

Preparation of 0.5M dibasic sodium phosphate buffer:

Weighed accurately 7.10 g of dibasic sodium phosphate anhydrous and transferred into 100 mL volumetric flask, and dilute up to the volume with water.

Prepare of 1M sodium hydroxide:

Weighed accurately 4.0 g of sodium hydroxide and transferred into 100 mL volumetric flask, and dilute up to the volume with water.

Prepare of 1M phosphoric acid:

Pipette out 6.4 mL of ortho phosphoric acid and transferred into 100 mL volumetric flask, and dilute up to the volume with water.

Prepare of pH 7.3 phosphate buffer for mobile phase:

Prepared a mixture 10.5 mL of 1M mono basic sodium phosphate buffer and 60 mL of 0.5M dibasic sodium phosphate buffer, and dilute with water to 1000 mL Adjusted pH to 7.3 with 1M sodium hydroxide or 1M phosphoric acid.

Preparation of Mobile Phase:

Prepared a mixture 350 volume of Acetonitrile, 500 volume of pH 7.3 buffer solution and 150 volume of water. Filtered the solution 0.45 µm nylon filter and sonication was done for 10 minutes.

Preparation of diluents:

Water: Methanol (50:50) v/v.

Procedure for preparation of analytical solutions:

Preparation of standard stock solution A (100 mcg/mL of Esomeprazole):

Weighed accurately 20.65 mg of Esomeprazole magnesium WS (Equivalent to 20 mg Esomeprazole) and transferred into a 200 mL volumetric flask. Added 100 mL of diluent and sonication was done for 5 minutes to dissolve. Cooled and diluted up to the volume with diluent.

Preparation of standard stock solution B (2500 mcg/mL of Naproxen):

Weighed accurately 137.0 mg of Naproxen Sodium WS (Equivalent to 125 mg of Naproxen) and transferred into a 50 mL volumetric flask. Added 25 mL of diluent and sonication was done for 5 minutes to dissolve. Cooled and diluted up to the volume with diluent.

Preparation of standard solution (20 mcg/mL of Esomeprazole and 500 mcg/mL of Naproxen):

Transferred 5 mL of the each stock solution A and 5 mL of the each stock solution B through pipette into a 25 mL volumetric flask and diluted up to the volume with mobile phase and mixed well. Filtered the solution through 0.45 µm Nylon filter and collected the solution in an HPLC vial after discarded the first 2 mL of filtrate.

Sample solution (20 mcg/mL of Esomeprazole and 500 mcg/mL of Naproxen):

Weighed and finely powder not fewer than 20 tablets. Transferred an accurately weighed portion of the powder, equivalent to about 20 mg of Esomeprazole and 500 mg of Naproxen into a 100 mL volumetric flask. Added 50 mL of diluent and sonication was for 15 minutes to dissolve. Cooled and diluted up to the volume with diluent. Transferred 5 mL of this above solution through pipette into a 50 mL volumetric flask and diluted up to the volume with mobile phase and mix. Filtered the solution through 0.45 µm Nylon filter and collected the solution in an HPLC vial after discarded the first 2 mL of filtrate.

Placebo solution:

Weighed accurately 587 mg of Esomeprazole magnesium and Naproxen tablet placebo and transferred into 100 mL volumetric flask. Added 50 mL of diluent and sonication was done for 15 minutes to dissolve. Cooled and diluted up to the volume with diluent. Transferred 5 mL of this above solution through pipette into a 50 mL volumetric flask and diluted up to the volume with mobile phase and mixed well. Filtered the solution through 0.45 µm Nylon filter and collected the solution in an HPLC vial after discarded the first 2 mL of filtrate.

CALCULATIONS:

Content of Esomeprazole in mg:

$$= \frac{\text{SPL Area}}{\text{STD Area}} \times \frac{\text{STD wt in mg}}{200} \times \frac{5}{25} \times \frac{100}{\text{SPL Wt}} \times \frac{50}{5} \times \frac{\text{STD Purity in ASB}}{100} \times \frac{690.83}{713.12} \times \text{Avg .Wt.}$$

=690.83 is a molecular weight of Esomeprazole.

=713.12 is a molecular weight of Esomeprazole magnesium Anhydrous.

Content of Esomeprazole in %:

$$= \frac{\text{Esomeprazole in mg}}{\text{Label claim of Esomeprazole tablet in mg}} \times 100$$

Acceptance Criteria:

18.0 mg to 22.0 mg (90.0 % to 110.0 % of label claim)

Content of Naproxen in mg:

$$= \frac{\text{SPL Area}}{\text{STD Area}} \times \frac{\text{STD wt in mg}}{50} \times \frac{5}{25} \times \frac{100}{\text{SPL Wt.}} \times \frac{50}{5} \times \frac{\text{STD Purity in ASB}}{100} \times \frac{230.26}{252.24} \times \text{Avg. Wt.}$$

= 230.26 is a molecular weight of Naproxen.

= 252.24 is a molecular weight of Naproxen Sodium.

Content of Naproxen in %:

$$= \frac{\text{Naproxen in mg}}{\text{Label claim of Naproxen in tablet in mg}} \times 100$$

Acceptance Criteria:

450.0 mg to 550.0 (90.0 % to 110.0 % of label claim)

Chromatographic Conditions:

Parameter/Conditions	Description/Values
Column Name	C18column(250mm×4.6 mm×5µm)
Flow rate	1 mL
Injection volume	20 µL
Wavelength	302 nm
Mobile phase	pH 7.3Po4Buffer:ACN:Water (50:35:15)

Table No.20:Chromatograpic Condition of Optimized method

System Suitability requirement:

1) The resolution of the peaks obtained for six replicates standard solution injections between Naproxen and Esomeprazole should be not less than 3.0
2) The tailing factor of the peaks obtained for six replicates standard solution injections of Naproxen and Esomeprazole should be not more than 2.0.
3) The relative standard deviation of the area obtained for six replicates standard solution of Naproxen and Esomeprazole should be not more than 2.0 %.
4) Theoretical plates of the peaks obtained for six replicates standard solution injections of Naproxen and Esomeprazole should be not less than 2000.

1. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

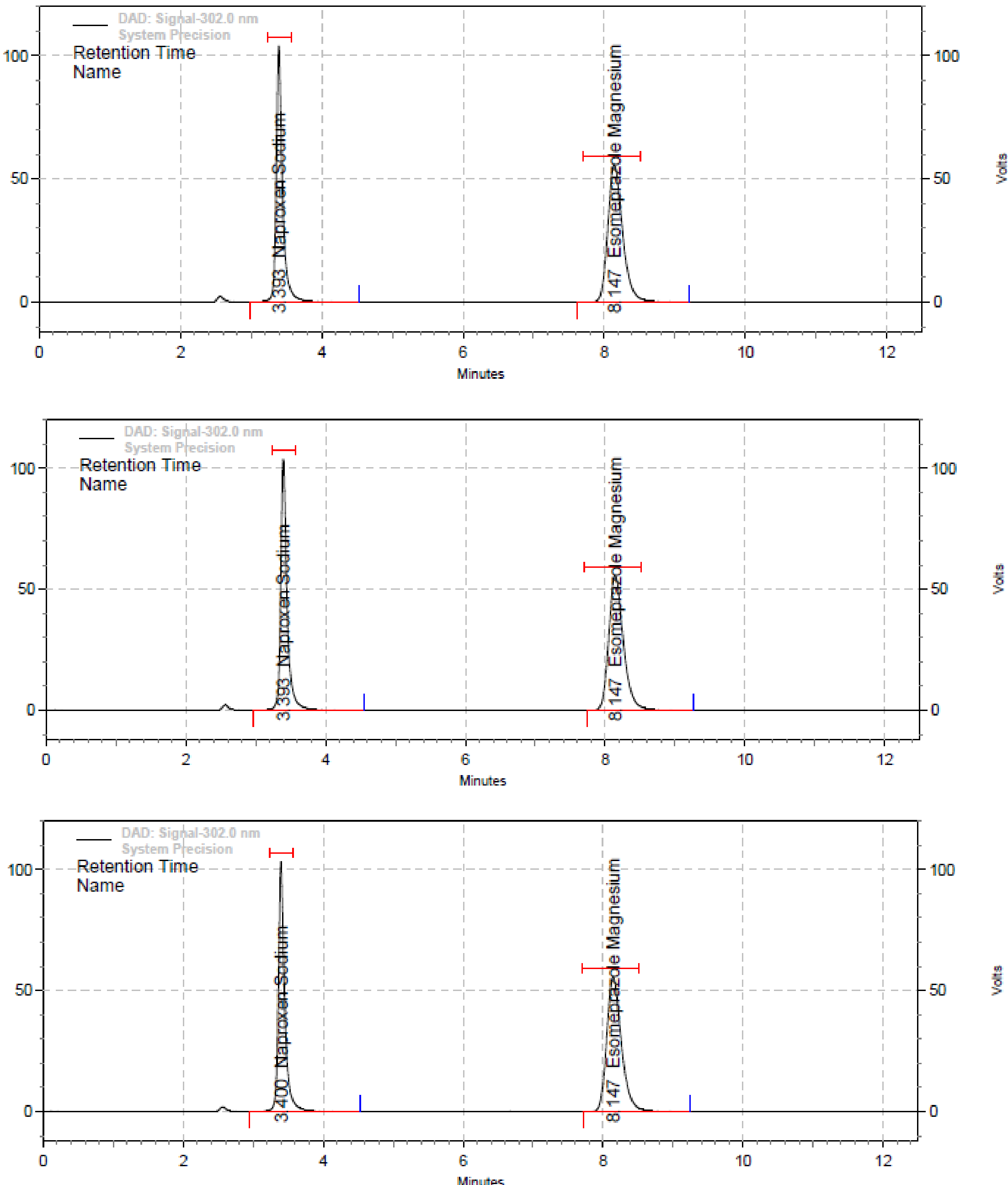
Precision may be considered at three levels:

a) System Precision (Repeatability)
b) Method Precision (Reproducibility)
c) Intermediate precision (Ruggedness)

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. A minimum of 6 replicate sample determinations should be made together with a simple statistical assessment of the results, including the percent relative standard deviation. The following levels of precision are recommended.

a) System Precision (Repeatability)

Determine the closeness of agreement of the same homogenous standard preparations under the prescribed conditions. Six replicate injections were injected into the HPLC system. The % RSD for the peak responses of six replicate injections should be ***NMT 2.0***.



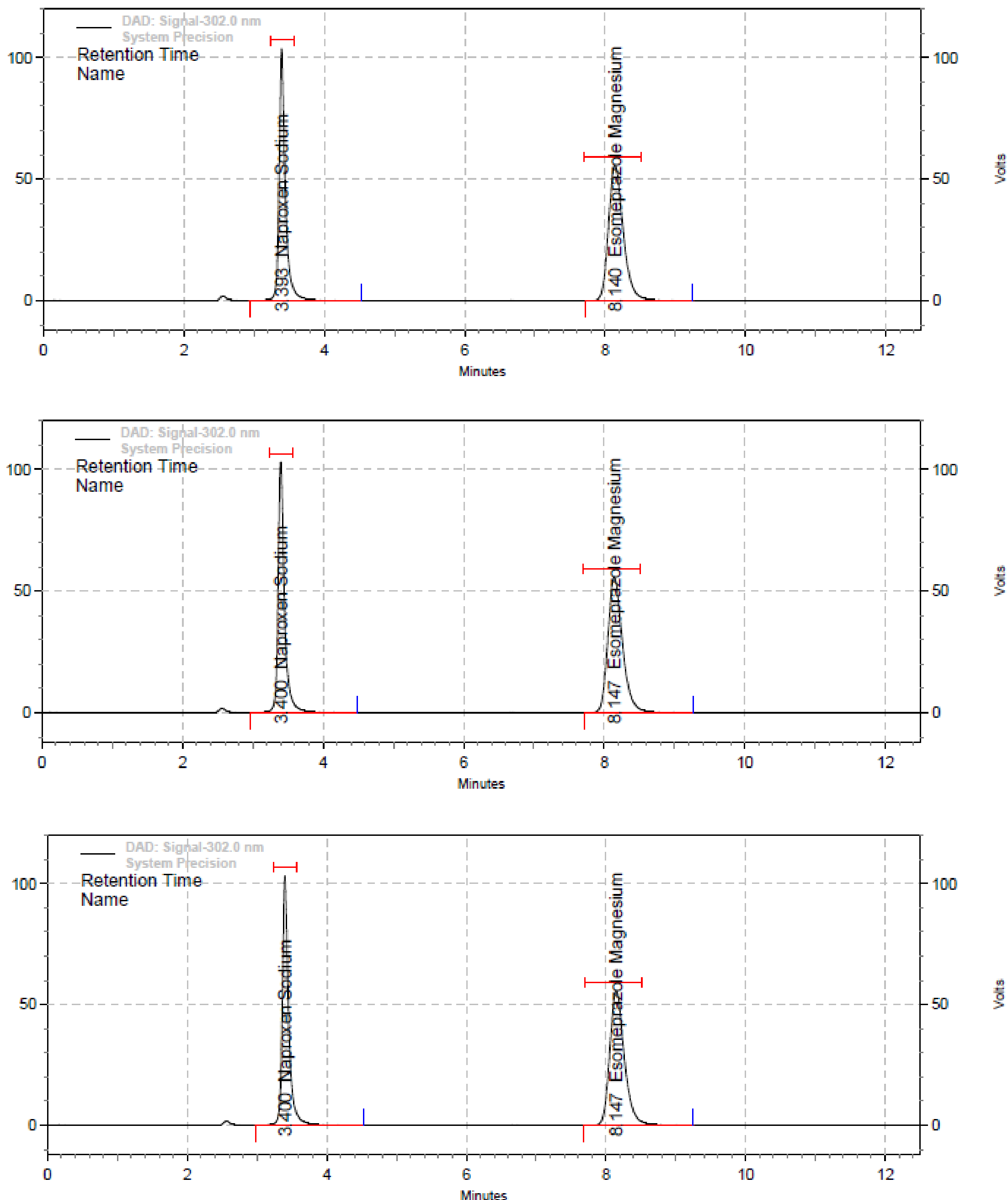


Fig.No.19: Chromatogram of System precision

Peak Results for System Precision

Naproxen			Esomeprazole magnesium		
Inj.	**RT (min)**	**Area (µV*sec)**	**Inj.**	**RT (min)**	**Area (µV*sec)**
1	3.393	89104756	1	8.147	109097952
2	3.393	89041992	2	8.147	108993605
3	3.400	89123222	3	8.147	109015747
4	3.393	89145254	4	8.140	109024679
5	3.400	89122794	5	8.147	109029797
6	3.400	89138970	6	8.147	108908594
Mean		**89112831**	Mean		**109011729**
% RSD		**0.042**	% RSD		**0.056**

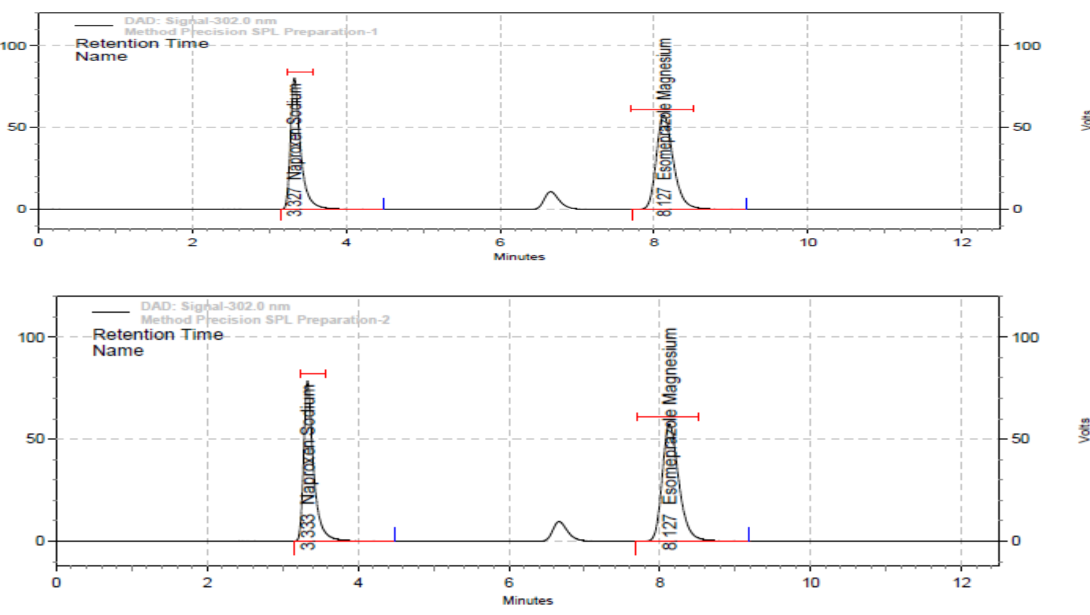
Table No.21:Peak Result for System Precision

Data Interpretation

It is observed from the data tabulated above, that the % RSD of the peak responses as peak area was found to be within acceptance criteria indicating an acceptance level of precision for system precision studies.

b) Method Precision (Reproducibility)

In method precision, a homogenous sample of a single batch should be analysed six times. This indicates whether a method is giving consistent results for a single batch. The % RSD for the six determinations should be ***NMT 2.0***.



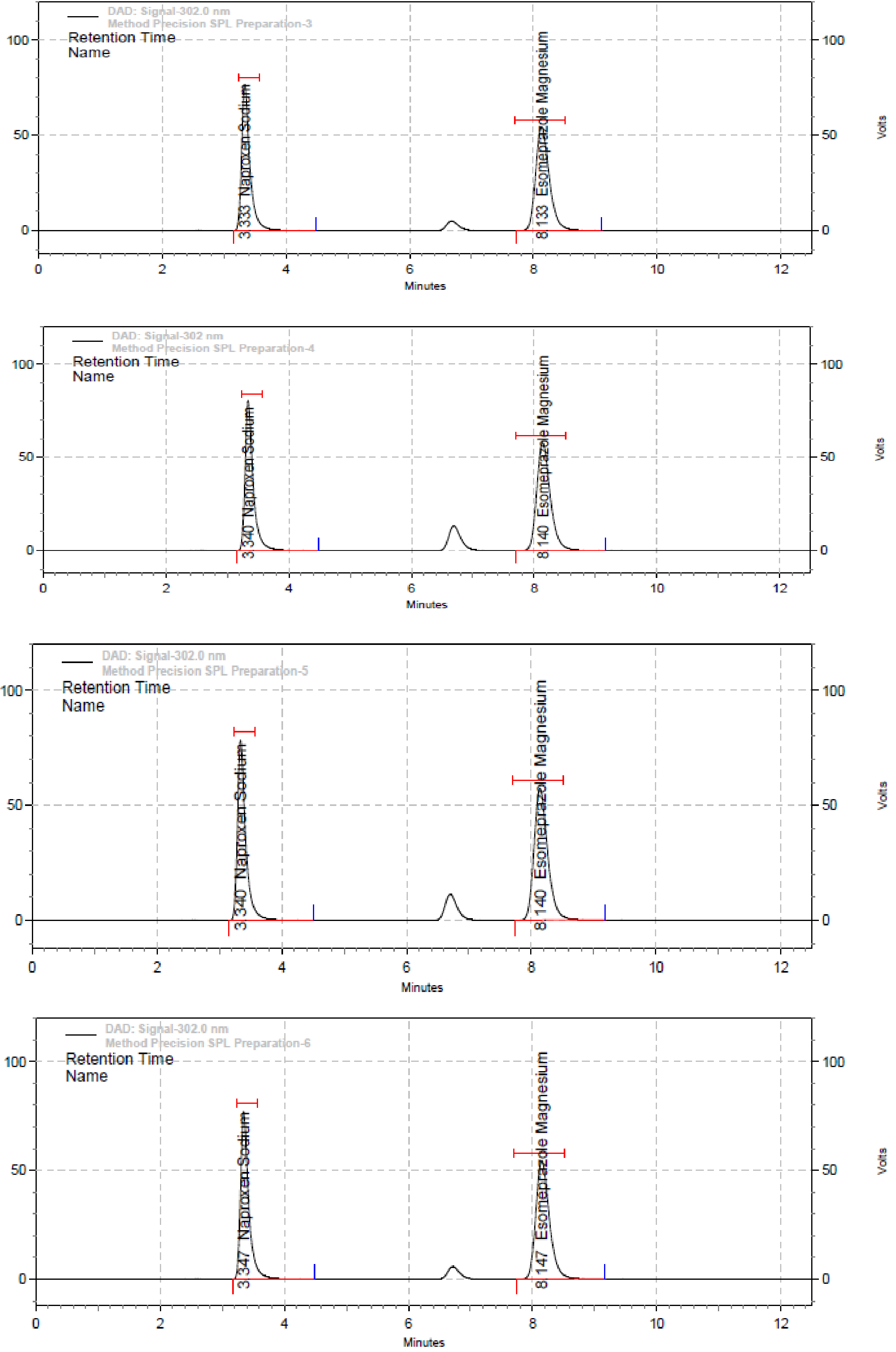


Fig.No.20: Chromatogram of Method precision

Method Precision Results for Naproxen

Test No	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6
Avg.wt (in mg)	1156.37					
Wt.taken (in mg)	1159.21	1158.48	1159.42	1159.06	1159.36	1158.27
Area(Injection-1)	87178270	87240756	87462405	87366231	87772791	87344466
Area(Injection-2)	87178986	87254969	87475855	87398182	87954872	87285843
Mean	87178628	87247863	87469130	87382207	87863832	87315155
STDV	1.354					
%RSD	0.262					

Table No.22:Method Precision Result for Naproxen

Method Precision Results for Esomeprazole magnesium

Test No	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6
Avg.wt (in mg)	1156.37					
Wt.taken (in mg)	1159.21	1158.48	1159.42	1159.06	1159.36	1158.27
Area(Injection-1)	119867586	119930122	119828973	119872459	119812111	119775234
Area(Injection-2)	119884786	119834075	119895431	119002620	119853347	119770953
Mean	119876186	119882099	119862202	119437540	119832729	119773094
STDV	0.032					
%RSD	0.157					

Table No.23:Method Precision Result for Esomeprazole magnesium

Data Interpretation: From the above results, it was concluded that the method is precise.

2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. A series of standard concentrations were prepared from 60%, 80%, 100%, 120% &160% of the targeted concentration of both Naproxen and Esomeprazole magnesium. A linearity graph of concentration (µg/ml) versus average area response was plotted for Naproxen and Esomeprazole magnesium peaks and the correlation coefficient was calculated. ***The correlation coefficient should be NLT 0.999***.

Preparation of standard stock solution A (100 mcg/mL of Esomeprazole):

Weigh accurately about 20.65 mg of Esomeprazole magnesium WS (Equivalent to 20 mg Esomeprazole) and transfer into a 200 mL volumetric flask. Add about 100 mL of

diluent and sonicate for 5 minutes to dissolve. Cool and dilute up to the volume with diluent.

Preparation of standard stock solution B (2500 mcg/mL of Naproxen):

Weigh accurately about 137.0 mg of Naproxen Sodium WS (Equivalent to 125 mg of Naproxen) and transfer into a 50 mL volumetric flask. Add about 25 mL of diluent and sonicate for 5 minutes to dissolve. Cool and dilute up to the volume with diluent.

Preparation of Linearity Dilution for Naproxen and Esomeprazole magnesium:

Linearity Level(%)	Volume of Stock Taken (mL)	Diluted to (mL)	Naproxen Conc.(mcg/mL)	Esomeprazole magnesium Conc.(mcg/mL)
60	3	25	12	300
80	4	25	16	400
100	5	25	20	500
120	6	25	24	600
160	8	25	32	800

Table No.24:Linearity dilution for Naproxen and Esomeprazole magnesium

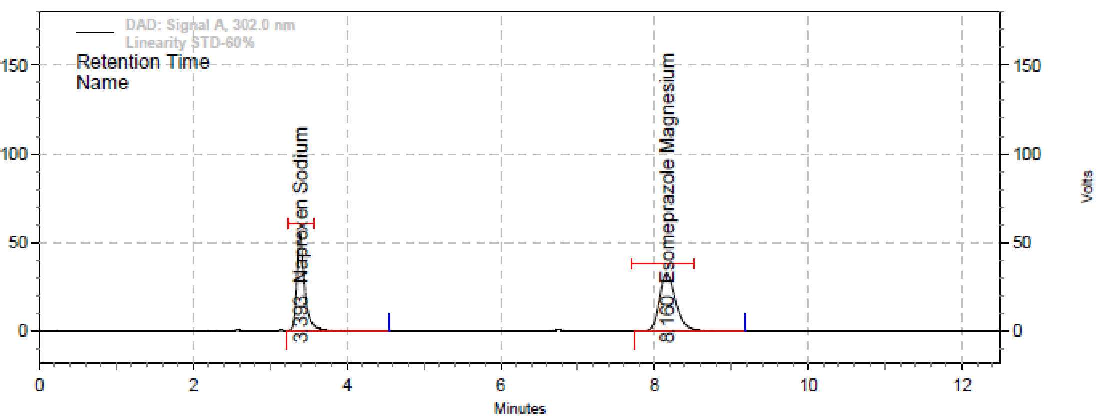


Fig.No.21: Chromatogram for Linearity at 60%

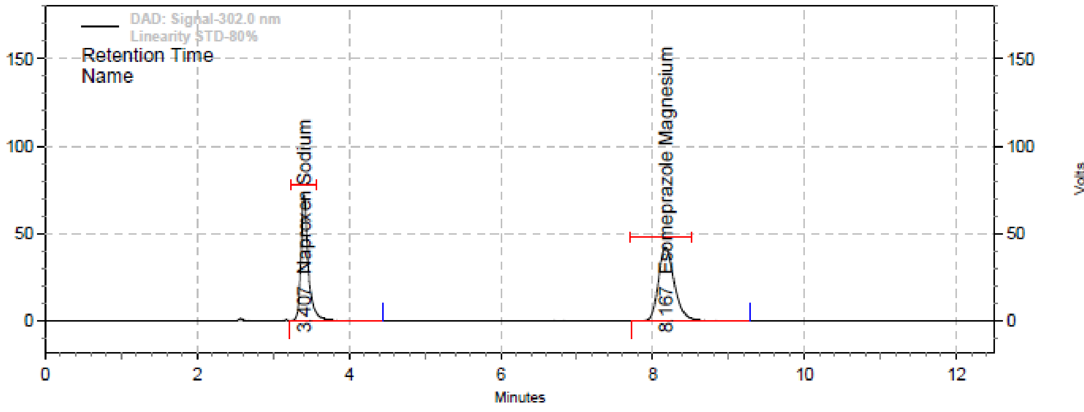


Fig.No.22: Chromatogram for Linearity at 80%

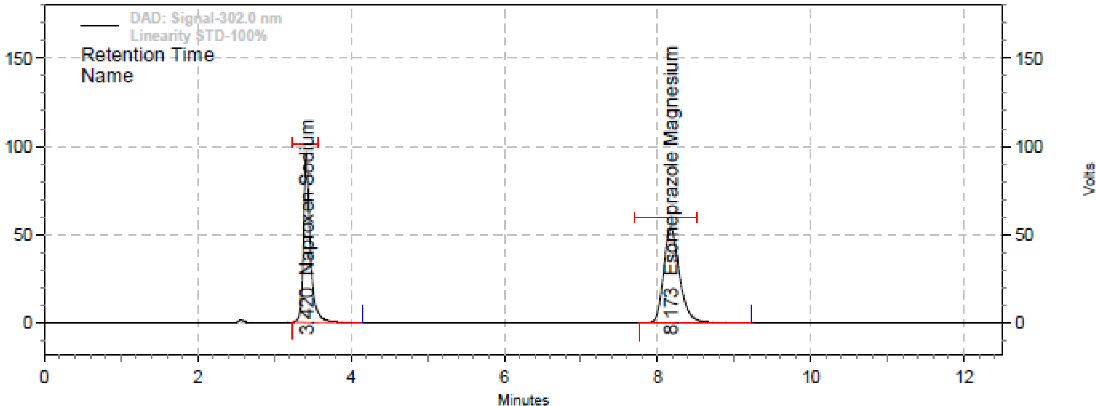


Fig.No.23: Chromatogram for Linearity at 100%

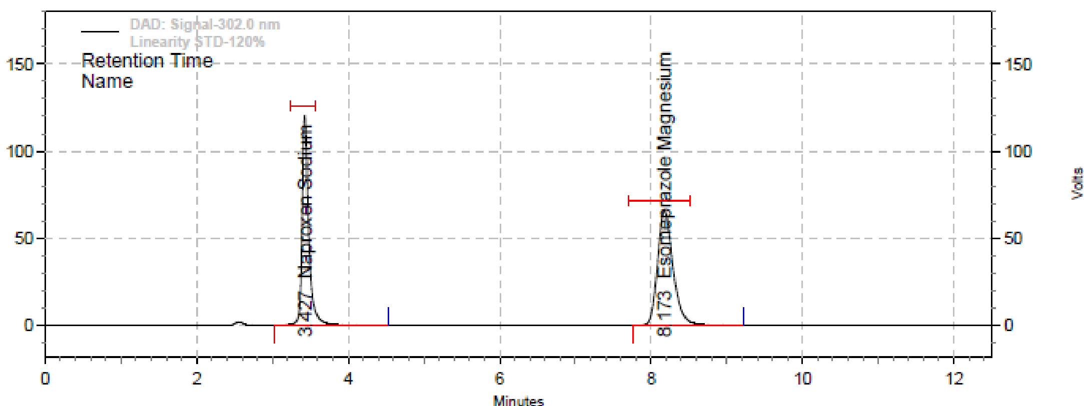


Fig.No.24: Chromatogram for Linearity at 120%

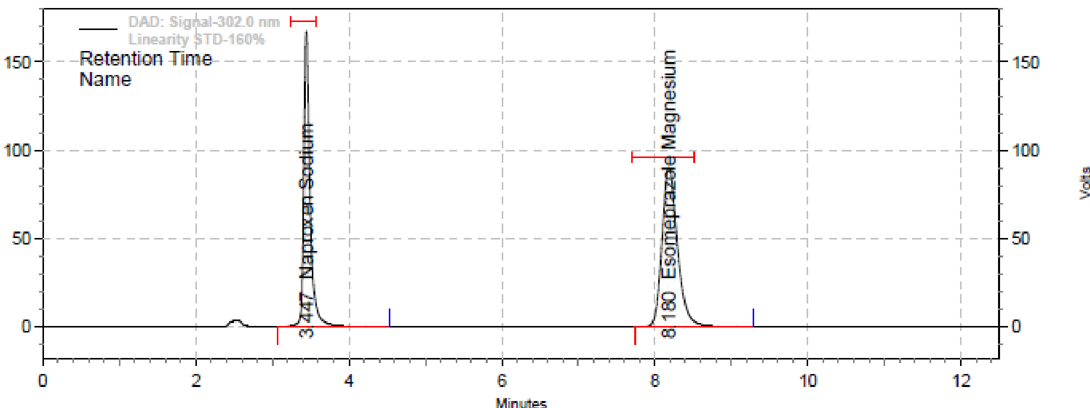


Fig.No.25: Chromatogram for Linearity at 160%

Calculation for Linearity of Naproxen

Linearity Level	Conc(mcg/mL)	Area (Injection-1)	Area (Injection-2)	Average
60	300	53087978	53120909	53104444
80	400	70853430	70938599	70896015
100	500	90124646	90341412	90233029
120	600	107228155	107404925	107316540
160	800	140669395	141002883	140836139
Correlation coefficient		0.999		
Slope		175839.7448		
Y Intercept		1040565.905		
% Y-intercept		0.969623047		

Table No.25:Linearity Calculation for Naproxen

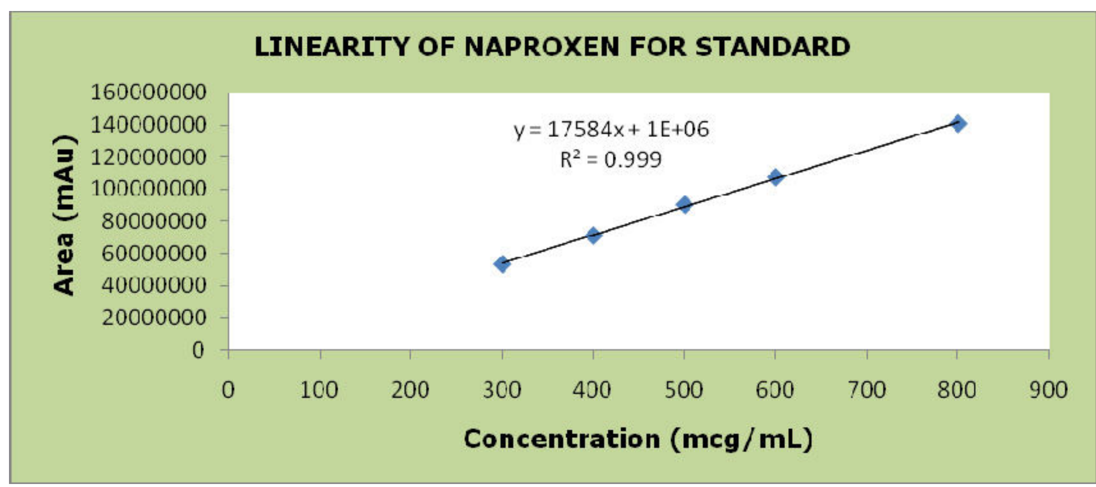


Fig.No.26: Linearity Plot of Naproxen

Linearity Calculation for Esomeprazole magnesium

Linearity Level	Conc(mcg/mL)	Area (Injection-1)	Area (Injection-2)	Average
60	12	64891655	64972888	64932272
80	16	83209664	83151599	83180632
100	20	104922648	105028486	104975567
120	24	126580317	126694766	126637542
160	32	169350156	169364758	169357457
Correlation coefficient		0.999		
Slope		5267703.451		
Y Intercept		248461.9189		
% Y-intercept		0.19619926		

Table No.26:Linearity Calculation for Esomeprazole magnesium

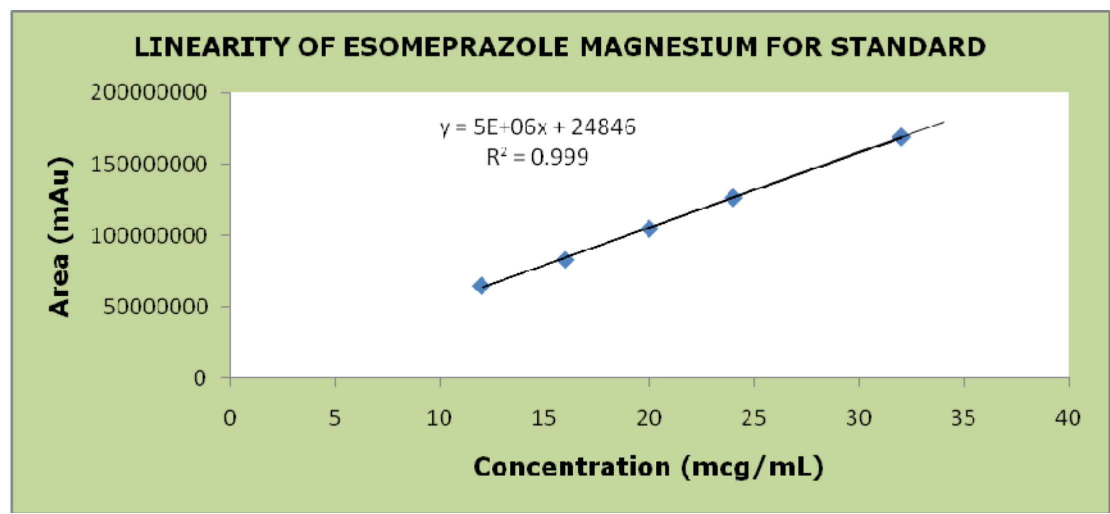


Fig.No.27: Linearity Plot of Esomeprazole magnesium

Data Interpretation

The *Correlation coefficient* for Naproxen and Esomeprazole magnesium was found to be **0.999** and **0.999** respectively, which indicates that the peak responses are linear. This concluded that the method was linear throughout the range selected.

3. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy study was conducted by spiking the known amount of active ingredients into the placebo at three different levels (50%, 100% and 150% of target concentration). The samples were analysed as per the proposed test procedure and the % recovery for each spiked level was calculated.

The % RSD at each spike level should be NMT 2.0. The overall % RSD for % recovery for all spike level should be NMT 2.0. The % recovery at each spike level should be NLT 98.0 and NMT 102.0 of the added amount.

Procedure for Accuracy

Placebo spiked with 50% standard solution preparation (10 mcg/mL of Esomeprazole and 250 mcg/mL of Naproxen):

Weigh accurately about 10.33 mg of Esomeprazole magnesium WS (Equivalent to 10 mg Esomeprazole), and 273.86 mg of Naproxen Sodium (Equivalent to 250 mg of Naproxen), and transfer into a 100 mL volumetric flask. Add about 293.5 mg of Naproxen

and Esomeprazole magnesium Tablet Placebo and 50 mL of diluent, and sonicate for 15 minutes to dissolve. Cool and dilute up to the volume with diluent. Transfer 5 mL of this above solution through pipette into a 50 mL volumetric flask and dilute up to the volume with mobile phase and mix. Filter the solution through 0.45 µm Nylon filter and collect the solution in an HPLC vial after discarding about first 2 mL of filtrate.

Placebo spiked with 100% standard solution preparation (20 mcg/mL of Esomeprazole and 500 mcg/mL of Naproxen):

Weigh accurately about 20.65 mg of Esomeprazole magnesium WS (Equivalent to 20 mg Esomeprazole), and 547.73 mg of Naproxen Sodium (Equivalent to 500 mg of Naproxen), and transfer into a 100 mL volumetric flask. Add about 587 mg of Naproxen and Esomeprazole magnesium Tablet Placebo and 50 mL of diluent, and sonicate for 15 minutes to dissolve. Cool and dilute up to the volume with diluent. Transfer 5 mL of this above solution through pipette into a 50 mL volumetric flask and dilute up to the volume with mobile phase and mix. Filter the solution through 0.45 µm Nylon filter and collect the solution in an HPLC vial after discarding about first 2 mL of filtrate.

Placebo spiked with 150 % standard solution preparation (30 mcg/mL of Esomeprazole and 750 mcg/mL of Naproxen):

Weigh accurately about 30.97 mg of Esomeprazole magnesium WS (Equivalent to 30 mg Esomeprazole), and 821.60 mg of Naproxen Sodium (Equivalent to 750 mg of Naproxen), and transfer into a 100 mL volumetric flask. Add about 880.5 mg of Naproxen and Esomeprazole magnesium Tablet Placebo and 50 mL of diluent, and sonicate for 15 minutes to dissolve. Cool and dilute up to the volume with diluent. Transfer 5 mL of this above solution through pipette into a 50 mL volumetric flask and dilute up to the volume with mobile phase and mix. Filter the solution through 0.45 µm Nylon filter and collect the solution in an HPLC vial after discarding about first 2 mL of filtrate.

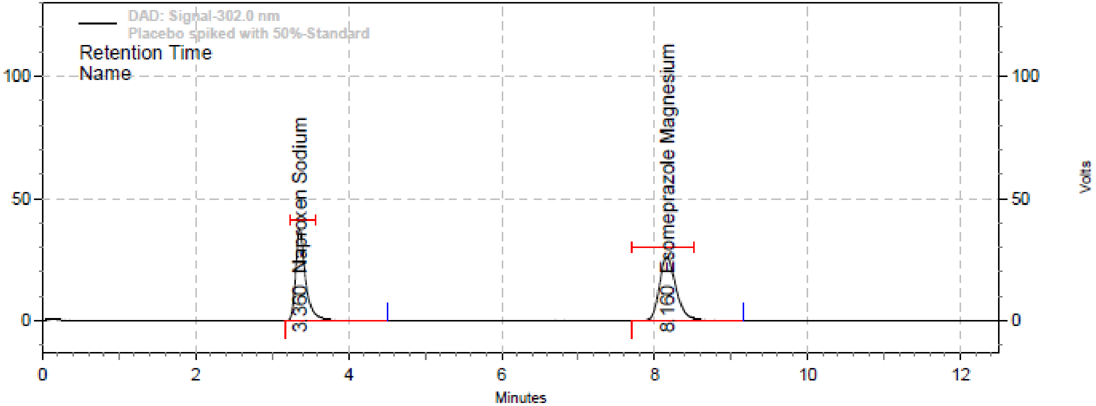


Fig.No.28: Chromatogram for Accuracy at 50% level

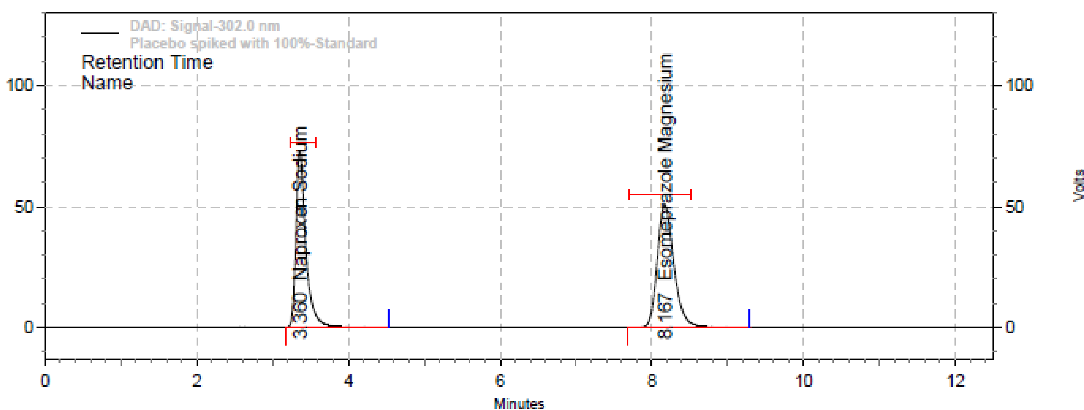


Fig.No.29: Chromatogram for Accuracy at 100% level

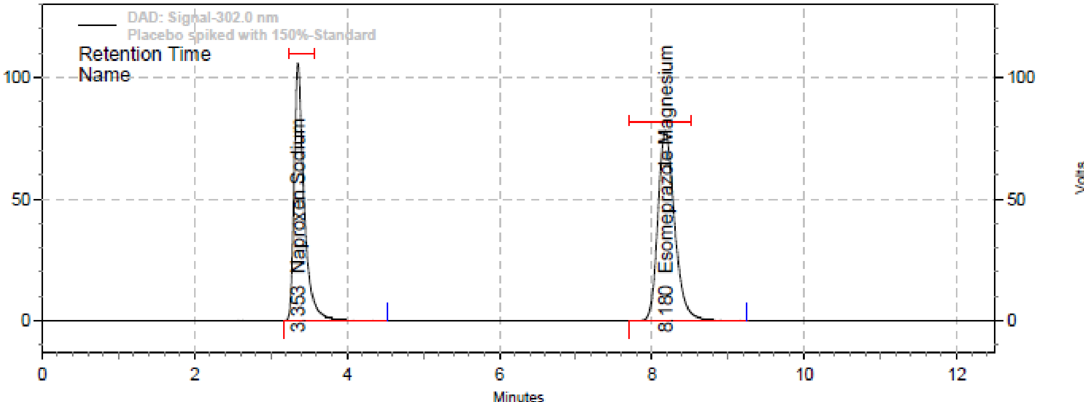


Fig.No.30: Chromatogram for Accuracy at 150% level

Results for Accuracy of Naproxen

Concentration	Spiked Std (mg)	Area Inj-1	Area Inj-2	Avg. Area	Recovered Std (mg)	Amt Recovered (%)	Mean of % Recovered
50	273.93	44962664	44025661	44494163	273.230	99.74	99.83
	274.06	44871445	44303910	44587678	273.804	99.91	
100	547.91	88907136	88978636	88942886	546.180	99.68	99.68
	548.13	88960179	88970438	88965309	546.318	99.67	
150	821.76	132885170	132946970	132916070	816.211	99.32	99.33
	821.64	132924809	132920805	132922807	816.252	99.34	
						Overall Recovery	99.61

Table No.27: Peak Results for Accuracy of Naproxen

Results for Accuracy of Esomeprazole magnesium

Concentration	Spiked Std (mg)	Area Inj-1	Area Inj-2	Avg. Area	Recovered Std (mg)	Amt Recovered (%)	Mean of % Recovered
50	10.50	54598780	54596647	54597714	10.436	99.39	99.83
	10.40	54542550	54565139	54553845	10.428	100.27	
100	20.71	108351302	108354041	108352672	20.712	100.01	99.90
	20.75	108326424	108312606	108319515	20.706	99.79	
150	31.03	162861284	161164468	162012876	30.969	99.80	99.75
	31.07	161970148	162105020	162037584	30.974	99.69	
						Overall Recovery	99.83

Table No.28: Peak Results for Accuracy of Esomeprazole magnesium

Data Interpretation

The results were found within acceptance criteria. Hence the method is accurate throughout the selected range.

4. Specificity

Blank & Placebo Interference: Placebo was injected by weighing the equivalent amount present in the finished drug product and analysed for interference due to placebo.

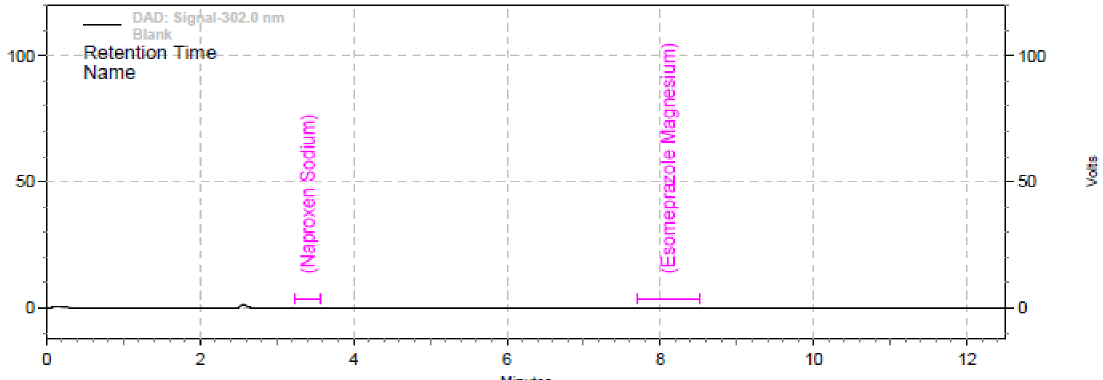


Fig.No.31: Chromatogram of Blank

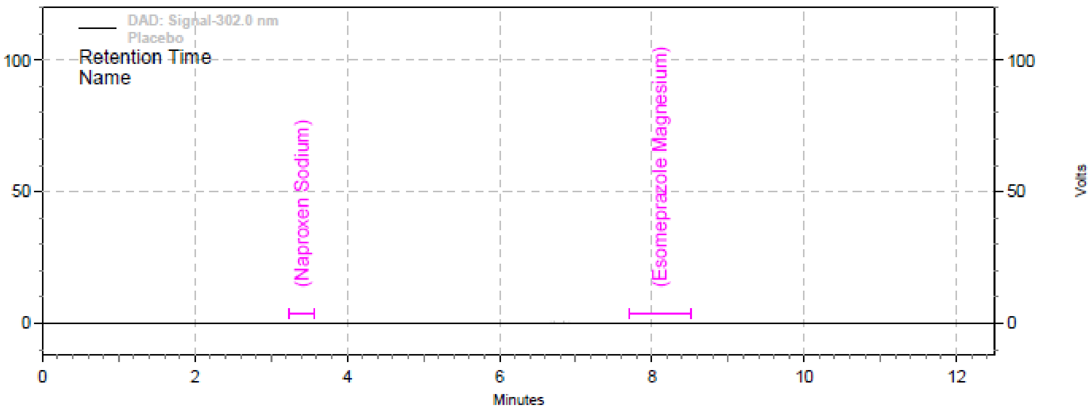


Fig.No.32: Chromatogram of Placebo

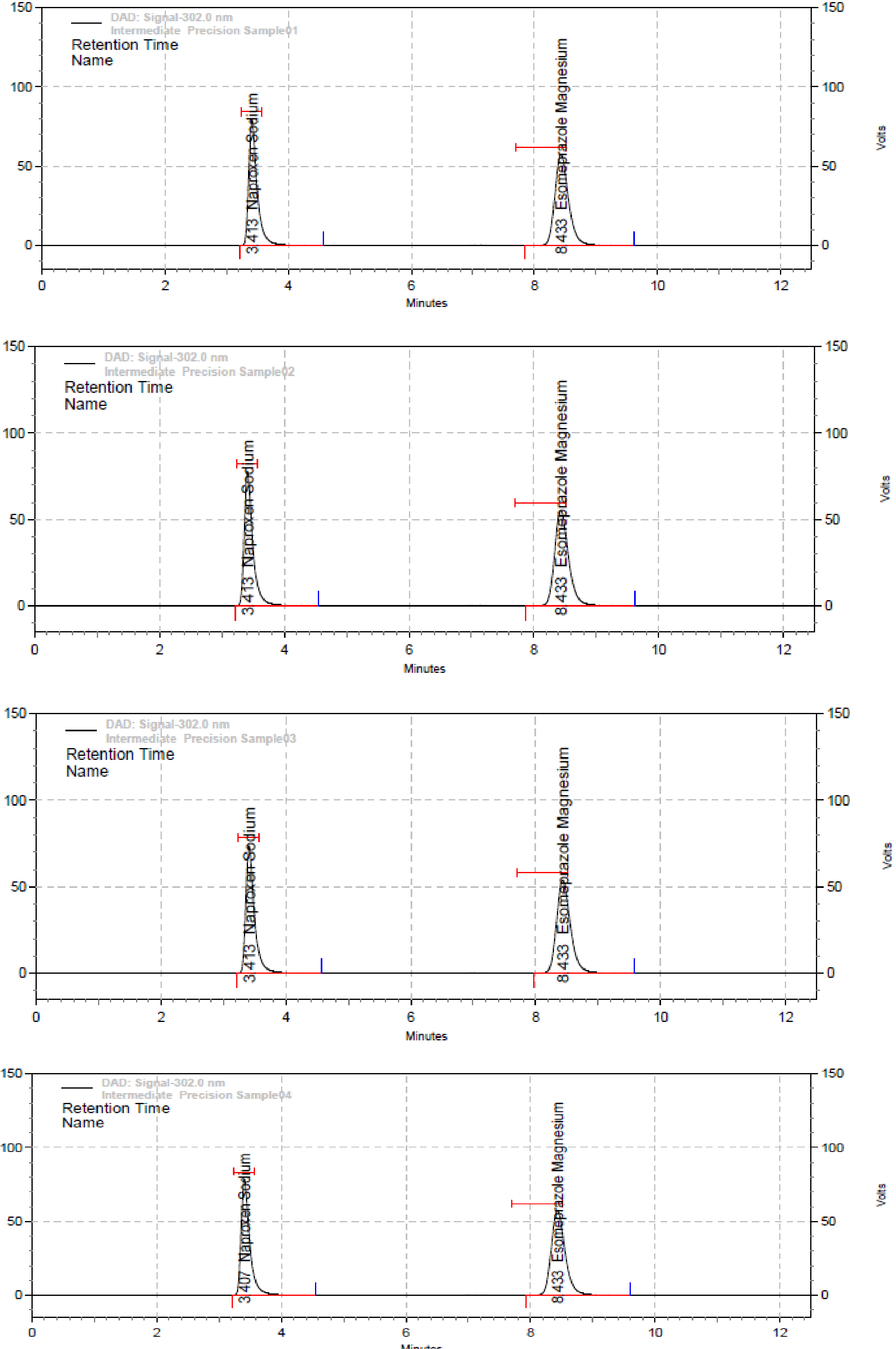
Data Interpretation

On the basis of these chromatograms we can say that there is no interference of blank and placebo at the retention time of Naproxen and Esomeprazole magnesium. Hence the method is specific.

5. Intermediate Precision (Ruggedness)

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Intermediate precision is to be performed by different analyst coupled with different day. The % RSD for the six determinations should be ***NMT 2.0***.


DAD: Signal-302.0 nm
Intermediate Precision Sample01
Retention Time
Name
3.413 Naproxen Sodium
8.433 Esomeprazole Magnesium
Minutes
Volts
DAD: Signal-302.0 nm
Intermediate Precision Sample02
Retention Time
Name
3.413 Naproxen Sodium
8.433 Esomeprazole Magnesium
Minutes
Volts
DAD: Signal-302.0 nm
Intermediate Precision Sample03
Retention Time
Name
3.413 Naproxen Sodium
8.433 Esomeprazole Magnesium
Minutes
Volts
DAD: Signal-302.0 nm
Intermediate Precision Sample04
Retention Time
Name
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8.433 Esomeprazole Magnesium
Minutes
Volts

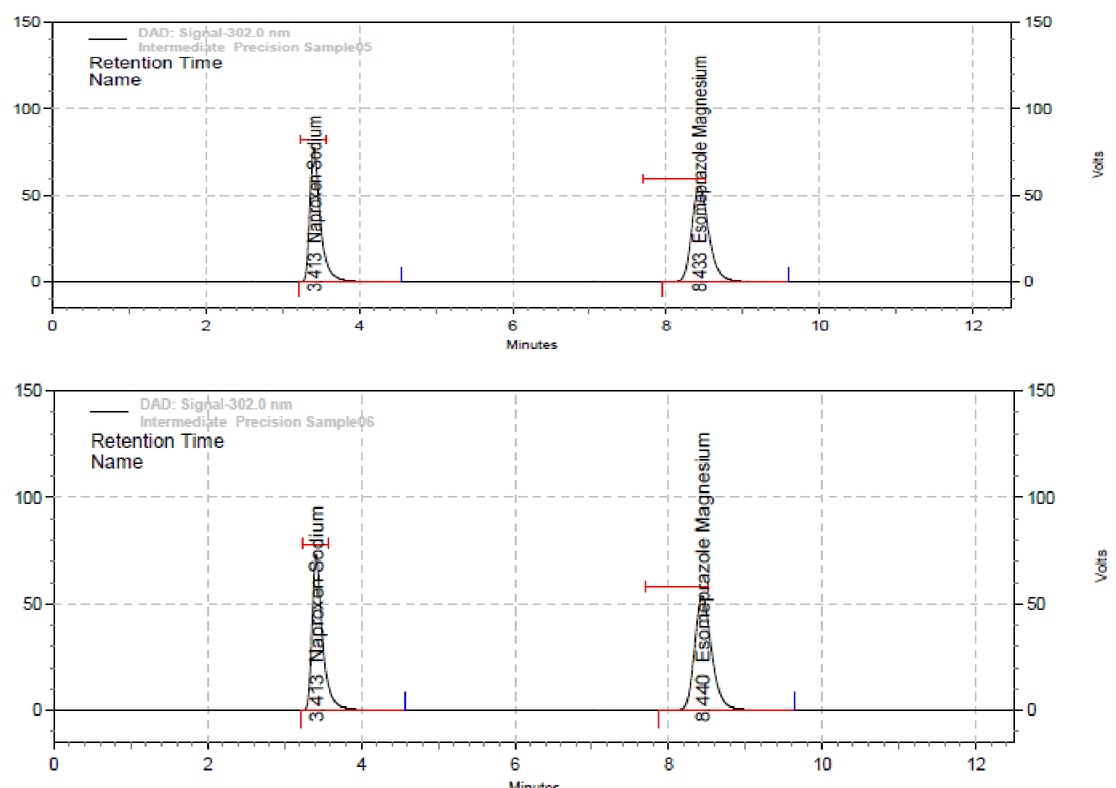

Fig.No.33: Chromatogram of Intermediate precision

Intermediate Precision Results for Naproxen

Test No	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6
Avg.wt (in mg)	1156.37					
Wt.taken (in mg)	1159.16	1159.24	1159.36	1158.09	1157.36	1156.13
Area(Injection-1)	87097682	87154613	87096407	87098788	87092407	87076030
Area(Injection-2)	87097891	87095231	87093570	87092879	87091153	87076945
Mean	**87097787**	**87124922**	**87094989**	**87095834**	**87091780**	**87076488**
%RSD	**0.099**					

Table No.29: Intermediate Precision result for Naproxen

Intermediate Precision Results for Esomeprazole magnesium

Test No	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6
Avg.wt (in mg)	1156.37					
Wt.taken (in mg)	1159.16	1159.24	1159.36	1158.09	1157.36	1156.13
Area(Injection-1)	113630040	113102780	113957873	113736070	113702174	113817901
Area(Injection-2)	113514693	113234171	113971749	113840069	113662524	113785272
Mean	**113572367**	**113168476**	**113964811**	**113788070**	**113682349**	**113801587**
%RSD	**0.298**					

Table No.30: Intermediate Precision result for Esomeprazole magnesium

Data Interpretation: System suitability result passes and the results obtained for Intermediate precision are found within the acceptance criteria.

6. ROBUSTNESS:

The Robustness for the analytical procedure expresses a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during analysis.

a) Change in flow rate plus (1.2 mL/minute):

For Chromatographic conditions, follow the method of analysis given in the Table No.20 except by changing the flow to 1.2 mL / minute instead of 1.0 mL / minute.

b) Change in flow rate minus (0.8 mL/minute):

For Chromatographic conditions, follow the method of analysis given in the Table No.20 except by changing the flow to 0.8 mL / minute instead of 1.0 mL / minute.

c) Change in wavelength plus (304 nm):

For Chromatographic conditions, follow the method of analysis given in the Table No.20 except by changing the wavelength to 304 nm instead of 302 nm.

d) Change in wavelength minus (300 nm):

For Chromatographic conditions, follow the method of analysis given in the Table No.20 except by changing the wavelength to 300 nm instead of 302 nm.

e) Change in mobile phase organic content plus (+5%):

Change the least organic solvents ratio to 36.5 volumes instead of 35 volume, and adjust the quantity variation from Buffer concentration to maintain 100 %. Maintain other chromatographic condition remains same as given in the Table No.20.

f) Change in mobile phase organic content minus (-5%):

Change the least organic solvents ratio to 33.25 volumes instead of 35 volumes, and adjust the quantity variation from Buffer concentration to maintain 100%. Maintain other chromatographic condition remains same as given in the Table No.20.

System suitability results for Naproxen:

S. No.	Parameter Name	Naproxen Results obtained		
		Tailing factor	**Area (RSD)**	**Theoretical Plates**
01	Change in wavelength Plus 304nm	1.65	0.451	8331
02	Change in wavelength Minus 300 nm	1.57	0.049	8174
03	Change in Flow rate 0.80 mL	1.56	0.318	10625
04	Change in Flow rate 1.20 mL	1.84	0.026	6992
05	Change in Mobile phase plus	1.10	0.304	12060
06	Change in Mobile phase minus	1.34	0.161	5801
	Acceptance criteria	**NMT 2.0**	**NMT 2.0%**	**NLT 1000**

Table No.31: System Suitability result for Naproxen

System suitability results for Esomeprazole magnesium:

S. No.	Parameter Name	Esomeprazole magnesium Results obtained		
		Tailing factor	**Area (RSD)**	**Theoretical Plates**
01	Change in wavelength Plus 304nm	1.06	0.094	7329
02	Change in wavelength Minus 300 nm	1.06	0.101	7328
03	Change in Flow rate 0.80 mL	0.83	0.135	8564
04	Change in Flow rate 1.20 mL	1.00	0.980	6696
05	Change in Mobile phase plus	1.74	0.161	6585
06	Change in Mobile phase minus	0.98	0.071	8008
	Acceptance criteria	**NMT 2.0**	**NMT 2.0%**	**NLT 1000**

Table No.32: System Suitability result for Esomeprazole magnesium

Robustness results obtained for Naproxen:

S. No.	Parameter Name	Results obtained		Acceptance criteria
		Naproxen drug in mg	**Naproxen drug in %**	
1	Robust Wavelength 304 nm	**499.76**	**99.95**	**90.0% - 110.0%**
2	Robust Wavelength 300 nm	**503.12**	**100.62**	
3	Robust flow rate 0.8 mL	**499.75**	**99.95**	
4	Robust flow rate 1.2 mL	**501.91**	**100.38**	
5	Robust mobile phase composition +5 %	**501.48**	**100.38**	
6	Robust mobile phase composition -5 %	**501.40**	**100.28**	

Table No.33: Robustness result for Naproxen

Robustness results obtained for Esomeprazole:

S. No.	Parameter Name	Results obtained		Acceptance criteria
		Esomeprazole drug in mg	**Esomeprazole drug in %**	
1	Robust Wavelength 304 nm	**19.90**	**99.50**	**90.0% - 110.0%**
2	Robust Wavelength 300 nm	**19.93**	**99.65**	
3	Robust flow rate 0.8 mL	**19.99**	**99.95**	
4	Robust flow rate 1.2 mL	**19.84**	**99.20**	
5	Robust mobile phase composition +5 %	**20.10**	**100.50**	
6	Robust mobile phase composition -5 %	**19.90**	**99.50**	

Table No.34: Robustness result for Esomeprazole magnesium

Combined Method precision and Robustness results obtained for Naproxen:

S. No.	Parameter Name	Results obtained		
		Drug in mg	Drug in %	Acceptance criteria
01	Method precision – 1	509.53	101.91	90.0%-110.0%
02	Method precision – 2	509.67	101.93	
03	Method precision – 3	509.38	101.88	
04	Method precision – 4	509.62	101.92	
05	Method precision – 5	509.38	101.88	
06	Method precision – 6	509.87	101.97	
07	Robust Wavelength 304 nm	499.76	99.95	
08	Robust Wavelength 300 nm	503.12	100.62	
09	Robust flow rate 0.8 mL	499.75	99.95	
10	Robust flow rate 1.2 mL	501.91	100.38	
11	Robust mobile phase composition +5 %	501.48	100.30	
12	Robust mobile phase composition -5 %	501.40	100.28	
Mean			**101.08**	
Std Dev			**0.889**	**NMT 2.0%**
% RSD			**0.879**	

Table No.35: Combined Method precision and Robustness result for Naproxen

Combined method precision and Robustness results obtained for Esomeprazole magnesium:

<table>
<tr><th rowspan="2">S. No.</th><th rowspan="2">Parameter Name</th><th colspan="3">Results obtained</th></tr>
<tr><th>Drug in mg</th><th>Drug in %</th><th>Acceptance criteria</th></tr>
<tr><td>01</td><td>Method precision – 1</td><td>20.23</td><td>101.15</td><td rowspan="13">90.0%-110.0%</td></tr>
<tr><td>02</td><td>Method precision – 2</td><td>20.15</td><td>100.75</td></tr>
<tr><td>03</td><td>Method precision – 3</td><td>20.21</td><td>101.05</td></tr>
<tr><td>04</td><td>Method precision – 4</td><td>20.12</td><td>100.60</td></tr>
<tr><td>05</td><td>Method precision – 5</td><td>20.24</td><td>101.20</td></tr>
<tr><td>06</td><td>Method precision – 6</td><td>20.14</td><td>100.70</td></tr>
<tr><td>07</td><td>Robust Wavelength 304 nm</td><td>19.90</td><td>99.50</td></tr>
<tr><td>08</td><td>Robust Wavelength 300 nm</td><td>19.93</td><td>99.65</td></tr>
<tr><td>09</td><td>Robust flow rate 0.8 mL</td><td>19.99</td><td>99.95</td></tr>
<tr><td>10</td><td>Robust flow rate 1.2 mL</td><td>19.84</td><td>99.20</td></tr>
<tr><td>11</td><td>Robust mobile phase composition +5 %</td><td>20.10</td><td>100.50</td></tr>
<tr><td>12</td><td>Robust mobile phase composition -5 %</td><td>19.90</td><td>99.50</td></tr>
<tr><td colspan="3">Mean</td><td>100.31</td></tr>
<tr><td colspan="3">Std Dev</td><td>0.715</td><td rowspan="2">NMT 2.0%</td></tr>
<tr><td colspan="3">% RSD</td><td>0.713</td></tr>
</table>

Table No.36: Combined Method precision and Robustness result for Esomeprazole magnesium

Data Interpretation

System suitability result passes in all the deliberately changed methods and the results obtained for all deliberately changed methods are found within the acceptance criteria. Combined deliberately changed methods results and method precision results are found within the desirable limit. It is concluded that the deliberately changed assay methods results are remains unaffected in small variations.

7. Solution Stability

Solution stability for Naproxen

Standard				Sample		
Time	**Area**	**Average**	**% RSD**	**Area**	**Average**	**% RSD**
0 hrs	89227132	NA	NA	87348145	NA	NA
3 hrs	89077564	89152348	0.119	87232593	87290369	0.094
6 hrs	89044890	89116529	0.109	87254199	87278312	0.070
9 hrs	89950958	89325136	0.475	87378416	87303338	0.081
12 hrs	89056712	89271451	0.433	87288925	87300456	0.071
15 hrs	89272542	89271633	0.388	87565638	87344653	0.139
18 hrs	89272580	89271768	0.354	87670265	87391169	0.190
21 hrs	89685749	89323516	0.366	87930200	87458548	0.280
24 hrs	89932554	89391187	0.411	87851935	87502257	0.301

Table No.37: Solution Stability for Naproxen

Solution stability for Esomeprazole magnesium

Standard				Sample		
Time	**Area**	**Average**	**% RSD**	**Area**	**Average**	**% RSD**
0 hrs	104820179	NA	NA	118471614	NA	NA
3 hrs	104739579	104779879	0.054	118311525	118391570	0.096
6 hrs	104619981	104726580	0.096	118372713	118385284	0.068
9 hrs	104557597	104684334	0.113	118414551	118392601	0.057
12 hrs	104670188	104681505	0.098	118198942	118353869	0.088
15 hrs	104614098	104670270	0.091	118098321	118311278	0.118
18 hrs	104119947	104591653	0.216	117779367	118235290	0.202
21 hrs	104336847	104559802	0.218	117791135	118179771	0.229
24 hrs	104344670	104535898	0.215	117402039	118093356	0.307

Table No.38: Solution Stability for Esomeprazole magnesium

Data Interpretation

System suitability result passes and the results obtained for stability of standard solution and sample solution are found within the acceptance criteria for the minimum period of 24 hours study. Hence, it is concluded that the standard solution and sample solution as mentioned in the assay method are capable to inject into the chromatography within 24 hours from the time of preparations.

CHAPTER 7

RESULT AND DISCUSSION

The objective of the proposed work was method development for the simultaneous estimation of Naproxen and Esomeprazole magnesium in tablets by RP-HPLC and to validate the developed method according to USP and ICH guidelines and applying the same for use in the quality control samples in pharmaceutical industry.

As there is no official method for the simultaneous estimation of Naproxen and Esomeprazole magnesium in this selective combination, so we tried to develop a method by which we can quantify the amount of drug present in the given sample.

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate titled ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with pH7.3 Phosphate Buffer: ACN: Water (50:35:15) at flow rate of 1.0 ml/min was found to be quite robust.

The optimum wavelength for detection was 302 nm at which better detector response for both the drugs was obtained. The average retention times for Naproxen and Esomeprazole magnesium was found to be 3.397 and 8.146 min, respectively. According to United States Pharmacopeia, system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in various concentration range with correlation.

The low values of RSD indicate that the method was precise and accurate. The mean recoveries were found in the range of 98 – 102 %. System precision is evaluated by injecting 6 injections of standard solution and low value of % RSD shows that system is precise. Precision for method is evaluated by analyzing a sample of homogenous batch six times and the low % RSD value shows the method is precise.

Method robustness was evaluated by alteration of flow rate (±0.2 mL), Wavelength (± 2 nm), Mobile phase Organic Content (±5%) and it was found robust as % RSD was below 2.0%.

Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slot in different laboratories, by different analysts, different column, different system using similar environmental conditions, the % R.S.D. reported was found to be less

than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

Both sample solution and standard solution are stable at 25°C for 24 hrs. as the % difference in the RSD was found to be less than 2.0%.

Finally, it can be concluded that the assay values of formulation were the same as mentioned in the label claim with the RSD of ≤ 2.0%.

The proposed method was found to be accurate, precise, reproducible and stable, and can be successfully applied for the routine analysis of both the drugs in combined tablet dosage forms.

CHAPTER 8

CONCLUSION

An efficient high performance liquid chromatographic method was developed and validated for the simultaneous estimation of Naproxen and Esomeprazole magnesium. In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate titled ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with pH 7.3 Phosphate buffer: ACN: Water at flow rate of 1.0 mL/min was found to be quite robust.

The low values of RSD indicate that the method was precise and accurate. The mean recoveries were found in the range of 98 – 102 %. System precision is evaluated by injecting 6 injections of standard solution and low value of % RSD shows that system is precise. Precision for method is evaluated by analysing a sample of homogenous batch six times and the low % RSD value shows the method precise.

CHAPTER 9

SUMMARY

Validation Parameter		Acceptance Criteria	Result	
			Naproxen	**Esomeprazole magnesium**
Precision	**System**	The %RSD of 6 replicate injections should be **≤ 2.0%**	**0.042**	**0.056**
	Method	The% RSD calculated on 6 determinations of assay value should be **≤ 2.0%**	**0.262**	**0.157**
Linearity		The correlation coefficient should be ≥**1.0**	**0.99960**	**0.99964**
Accuracy		The Overall % recovery for all spike level should be **NLT 98.0 and NMT 102.0** of the added amount.	**99.61**	**99.83**
Specificity		The peaks of diluents and placebo should not interfere with the main peaks	The peaks of diluents and placebo did not interfering with the peaks of Naproxen and Esomeprazole magnesium.	
Intermediate Precision (Ruggedness)		The% RSD calculated on 6 determinations of assay value should be **≤ 2.0%**	**0.099**	**0.298**
Robustness		Flow rate plus (1.2 mL)	**0.026**	**0.980**
		Flow rate minus (0.8 mL)	**0.318**	**0.135**
		Wavelength plus (304 nm)	**0.451**	**0.094**
		Wavelength minus (300 nm)	**0.049**	**0.101**
		Mobile phase organic content plus (+5%)	**0.304**	**0.161**
		Mobile phase organic content minus (-5%)	**0.161**	**0.071**
Solution Stability		**RSD ≤ 2.0%**	**0.301**	**0.307**

Table No.39: Summery

CHAPTER 10

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