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SIMULTANEOUS ESTIMATION OF ESOMEPRAZOLE AND NAPROXEN IN BULK AS WELL AS IN PHARMACEUTICAL FORMULATIONS BY USING RP-HPLC

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ABSTRACT: 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.

INTRODUCTION: Esomeprazole magnesium [bis (5-methoxy-2-[(S)-[(4-methoxy-3, 5- dimethyl-2-pyridinyl) methyl] sulfinyl]-1-H-benzimidazole-1-yl) magnesium salt] is a compound that inhibits gastric acid secretion. Esomeprazole (**fig. 1**) is cost effective in the treatment of gastric oesophageal reflux diseases. Esomeprazole is the S-isomer of Omeprazole, the first single optical isomer proton pump inhibitor, generally provides better acid control than racemic counterpart and has a favorable pharmacokinetic profile relative to Omeprazole.

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Naproxen [(S)-6-methoxy-α-methyl-2-naphthalene acetic acid], is a non-steroidal anti-inflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness (**fig. 2**). It works by inhibiting both the COX-1 and COX-2 enzymes.

Like other NSAIDs, combination of both Esomeprazole magnesium and Naproxen is used for the treatment and control of signs and symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis.

The combination is equally useful to decrease the risk of developing gastric ulcers [NSAID associated gastric ulcers]. Several chromatographic methods have been reported for estimation of Esomeprazole Magnesium and Naproxen in raw materials, solid dosage forms mainly tablet and blood-plasma by RP-HPLC [1, 2].

Quantitative determination of Esomeprazole with Domperidone by Densitometric method was also established [3]. Spectroscopic estimation of Esomeprazole magnesium in solid dosage form with some other NSAID'S [4-7] is available in the literature. Physico-chemical characterization, UV Spectrophotometric method development and validation studies of Esomeprazole magnesium trihydrate was reported in Literature [8-9]. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Esomeprazole and Domperidone in Pharmaceutical Dosage Form was reported in the Literature [10-11].

A validated stability indicating ultra-performance liquid chromatographic method for determination of impurities in Esomeprazole magnesium gastro resistant tablets was reported in the Literature[12]. However, no references have been found for simultaneous determination of Esomeprazole and Naproxen in pharmaceutical formulations till dated. Considering the fact a successful attempt has been made to estimate both Esomeprazole and Naproxen in bulk as well as in pharmaceutical formulations by RP- HPLC with UV detection.

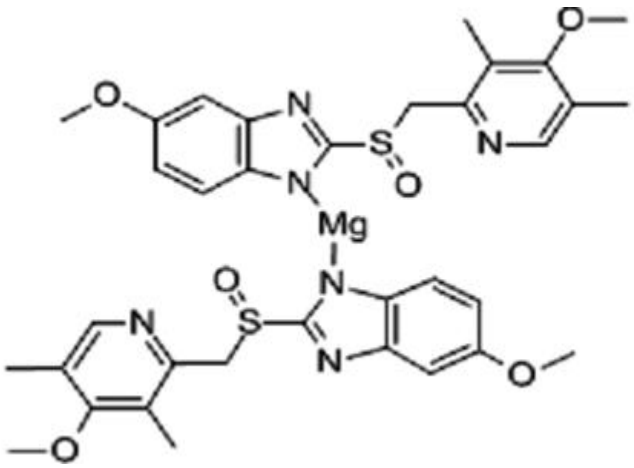

FIG. 1: CHEMICAL STRUCTURE OF ESOMEPRAZOLE

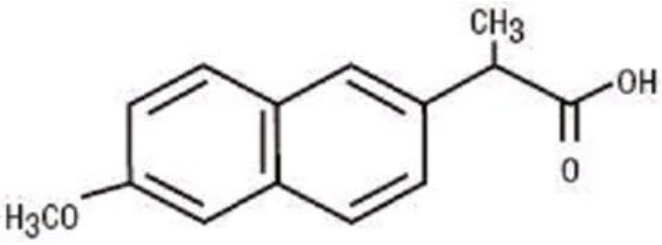

FIG. 2: CHEMICAL STRUCTURE OF NAPROXEN

MATERIALS AND METHODS:

Chemicals and Reagents Used: The following chemicals had procured for the process Water [HPLC Grade], Esomeprazole & Naproxen **[**Working Standards], Acetonitrile [HPLC Grade], Ortho phosphoric acid, Potassium dihydrogen phosphate. All the chemicals were supplied by STANDARD SOLUTIONS. Esomeprazole (20mg) and Naproxen (500mg) tablet was collected from the Local market, Brand Name VIMOVO and the manufacturer was Belgian Pharmaceutical Company.

Apparatus and Chromatographic Conditions:

Equipment: High performance liquid chromate-graphy equipped with Auto Sampler and DAD or UV detector.

Column: Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent

Flow rate	:	1.0 mL per min
Wavelength	:	285 nm
Injection volume	:	20 µl
Column oven	:	Ambient
Run time	:	7min

Preparation of Phosphate buffer: 7.0 grams KH_2PO_4 was weighed very accurately and transferred into a 1000ml volumetric flask. About 500 ml water (HPLC grade) was added to dissolve KH_2PO_4. After complete dissolution final volume was adjusted to 1000 ml with same water. pH of the buffer was checked and adjusted to 3 with Orthophosporic acid.

Preparation of mobile phase: Mobile phase was prepared by mixing 600 ml phosphate buffer (pH 3) and 400 ml Acetonitrile [HPLC grade]. Above mixture (60:40) was degassed in a ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Diluent Preparation: The Mobile phase was used as diluent.

Preparation of the Esomeprazole & Naproxen Standard & Sample Solution:

Standard Solution Preparation: The standard solutions were prepared by weighing accurately and transferred 10 mg Esomeprazole and 10mg Naproxen [working standard] into two 10mL clean dry volumetric flasks. About 7mL of diluent was added to each flask and sonicated to dissolve the powders completely. Final volumes were adjusted to the mark with the same solvent. From the Stock solutions 0.7ml Esomeprazole and 1.75ml Naproxen solutions were transferred into 10ml volumetric flasks and diluted up to the mark with same diluent.

Sample Solution Preparation: The sample solution was prepared by weighing accurately and transferred 758.9mg of Esomeprazole and Naproxen tablet powder into a 100mL clean dry volumetric flask. About 70mL of diluent was added to the powder drugs and sonicated to dissolve it completely. Finally the volume was made to the mark with the same solvent Further from the prepared solution 0.35ml of solution was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent.

Injection of Standards and Samples into the Chromatographic system: 20 µL of each standard and sample solution was injected into the chromatographic system and measured the areas of Esomeprazole and Naproxen peaks. %Assay of both the drug was calculated using the appropriate formulae.

System Suitability: The Tailing factor for the peaks due to Esomeprazole & Naproxen in Standard solution should not be more than 2.0.The Theoretical plates for the Esomeprazole & Naproxen peaks in Standard solution should not be less than 2000.

System Suitability Results (Esomeprazole):

1. The Tailing factor obtained from the standard injection was **1.7**

2. The Theoretical Plates Obtained from the standard injection was **2594.6**.

Assay Calculation for Esomeprazole and Naproxen:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt}{Label\ Claim} \times 100$$

Where: AT = average area counts of sample preparation, AS= average area counts of standard preparation, WS = Weight of working standard taken in mg. DS = Dilution of Standard solution, DT = Dilution of sample solution, P = Percentage purity of working standard

LC = Label claim of Esomeprazole/ Naproxen (mg/ml).

Assay % for Esomeprazole =

$$\frac{609743}{606862} \times \frac{10}{100} \times \frac{0.7}{10} \times \frac{100}{758.9} \times \frac{10}{0.35} \times \frac{99.8}{100} \times 100 \times \frac{758.9}{20} = 100.3\%$$

System Suitability Results (Naproxen):

1. The Tailing factor obtained from the standard injection was **1.6**

2. The Theoretical Plates obtained from the standard injection was **2389.9**

Assay % for Naproxen =

$$\frac{2245981}{2222900} \times \frac{10}{100} \times \frac{17.5}{10} \times \frac{100}{758.9} \times \frac{10}{0.35} \times \frac{99.7}{100} \times 100 \times \frac{758.9}{500} = 100.8\%$$

Validation Development [13-17]:

1. **Precision:** The precision of an analytical procedure expresses the closeness of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

- **Preparation of stock solution:** The standard solutions were prepared by weighing accurately and transferred 10 mg Esomeprazole and 10mg Naproxen **[**working standard] into two 10mL clean dry volumetric

flasks. About 7mL of diluent was added to each flask and sonicated to dissolve the powders completely. Final volumes were adjusted to the mark with the same solvent. From the Stock solutions 0.7ml Esomeprazole and 1.75ml Naproxen solutions were transferred into 10ml volumetric flasks and diluted up to the mark with same diluent.

- **Procedure:** The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits (**Table 1 & 2**).

TABLE 1: THE PRECISION RESULTS FOR ESOMEPRAZOLE

Injection	Area
Injection-1	602223
Injection-2	607748
Injection-3	607302
Injection-4	608674
Injection-5	607376
Average	606665
Standard Deviation	2542.3
%RSD	0.42

TABLE 2: THE PRECISION RESULTS FOR NAPROXEN

Injection	Area
Injection-1	2220333
Injection-2	2221573
Injection-3	2215483
Injection-4	2217379
Injection-5	2211255
Average	2217205
Standard Deviation	4100.8
%RSD	0.18

Acceptance Criteria: The %RSD for the area of five standard injections results should not be more than 2%.

2. **Intermediate Precision/Ruggedness:** To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

- **Preparation of stock solution:** The standard solutions were prepared by weighing accurately and transferred 10 mg Esomeprazole and 10mg Naproxen [working standard] into two 10mL clean dry volumetric flasks. About 7mL of diluent was added to each flask and sonicated to dissolve the powders completely. Final volumes were adjusted to the mark with the same solvent. From the Stock solutions 0.7ml Esomeprazole and 1.75ml Naproxen solutions were transferred into 10ml volumetric flasks and diluted up to the mark with same diluent.

- **Procedure:** The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits (**Table 3 & 4**).

TABLE 3: THE RUGGEDNESS RESULTS FOR ESOMEPRAZOLE

Injection	Area
Injection-1	596608
Injection-2	598959
Injection-3	595728
Injection-4	594485
Injection-5	595267
Average	596209
Standard Deviation	1718.7
%RSD	0.29

TABLE 4: THE RUGGEDNESS RESULTS FOR NAPROXEN

Injection	Area
Injection-1	2207732
Injection-2	2202266
Injection-3	2209375
Injection-4	2204037
Injection-5	2204466
Average	2205575
Standard Deviation	2899.8
%RSD	0.13

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%.

3. **Accuracy:** The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found.

- **Preparation of Standard stock solution:** The standard solutions were prepared by weighing accurately and transferred 10 mg Esomeprazole and 10mg Naproxen **[**working standard] into two 10mL clean dry volumetric flasks. About 7mL of diluent was added to each flask and sonicated to dissolve the

powders completely. Final volumes were adjusted to the mark with the same solvent. From the Stock solutions 0.7ml Esomeprazole and 1.75ml Naproxen solutions were transferred into 10ml volumetric flasks and diluted up to the mark with same diluent.

- **Preparation of Sample solutions:**

a. **Preparation of 50% solution (with respect to target Assay concentration):** The stock solutions were prepared by weighing accurately and transferred 4.86 mg Esomeprazole and 5 mg Naproxen **[**working standard] into two 10mL clean dry volumetric flasks. 7mL of diluent was added to each flask and sonicated to dissolve the powders completely. Final volumes were adjusted to the mark with the same solvent. From the Stock solutions 0.7ml Esomeprazole and 1.75ml Naproxen solutions were transferred into 10ml volumetric flasks and diluted up to the mark with same diluent.

b. **Preparation of 100% solution (with respect to target Assay concentration):** The stock solutions were prepared by weighing accurately and transferred 10 mg Esomeprazole and 10mg Naproxen **[**working standard] into two 10mL clean dry volumetric flasks. 7mL of diluent was added to each flask and sonicated to dissolve the powders completely. Final volumes were adjusted to the mark with the same solvent. From the Stock solutions 0.7ml Esomeprazole and 1.75ml Naproxen solutions were transferred into 10ml volumetric flasks and diluted up to the mark with same diluent.

c. **Preparation of 150% solution (with respect to target Assay concentration):** The stock solutions were prepared by weighing accurately and transferred 15 mg Esomeprazole and 15.8 mg Naproxen **[**working standard] into two 10mL clean dry volumetric flasks. 7mL of diluent was added to each flask and sonicated to dissolve the powders completely. Final volumes were adjusted to the mark with the same solvent. From the Stock solutions 0.7ml Esomeprazole and 1.75ml Naproxen solutions were transferred into 10ml volumetric flasks and diluted up to the mark with same diluent.

d. **Injecting the Standard Solutions to the Chromatographic System:** The standard solution was injected with Accuracy -50%, 100% and 150% solutions. The amount found was calculated and amount added for Esomeprazole & Naproxen was estimated. The individual recovery and mean recovery values were also calculated (**Table 5 & 6**).

TABLE 5: ACCURACY RESULTS FOR ESOMEPRAZOLE

%Concentration (At specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	287774	4.86	4.76	98.0%	
100%	606495	10.0	10.0	100.4%	99.2%
150%	898508	15.0	14.8	99.1%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

TABLE 6: ACCURACY RESULTS FOR NAPROXEN

%Concentration (At specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1104782	5.0	4.92	98.4%	
100%	2238655	10.0	9.97	99.7%	99.7%
150%	3577973	15.8	15.9	100.9%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

4. **Linearity:** The linearity of the analytical procedure is its ability (within a given range) to obtain the test results which are directly proportional to the concentration (amount) of analyte in the sample.

- **Preparation of stock solution:** The stock solutions were prepared by weighing accurately and transferred 10 mg Esomeprazole and 10mg Naproxen [working standard] into two 10mL clean dry volumetric flasks. About 7mL of diluent was added to each flask and sonicated to dissolve the powders completely. Final volumes were adjusted to the mark with the same solvent. From the Esomeprazole Stock Solution 1mL was pipette out into a 10 mL clean dry volumetric flask and made the volume up to the mark with the same solvent. Above prepared Stock Solutions were used for the further dilution to prepare the following Levels:

a. **Preparation of Level – I (5ppm of Esomeprazole & 125ppm of Naproxen):** 0.5ml Esomeprazole and 1.25ml Naproxen stock solution was transferred into a 10ml volumetric flask and diluted up to the mark with diluent.

b. **Preparation of Level – II (6ppm of Esomeprazole & 150ppm of Naproxen):** 0.6ml Esomeprazole and1.5ml Naproxen stock solution was pipette out into a 10ml volumetric flask and volume was made up to the mark with diluent.

c. **Preparation of Level – III (7ppm of Esomeprazole & 175ppm of Naproxen):** 0.7ml Esomeprazole &1.75ml Naproxen of stock solution was transferred into a 10ml volumetric flask and diluted up to the mark with diluent.

d. **Preparation of Level – IV (8ppm of Esomeprazole & 200ppm of Naproxen):** 0.8ml Esomeprazole &2.0ml Naproxen of stock solution was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent.

e. **Preparation of Level – V (9ppm of Esomeprazole & 225ppm of Naproxen):** 0.9ml Esomeprazole &2.25ml Naproxen of stock solution was transferred into a 10ml volumetric flask and volume was made up to the mark with diluent.

f. **Injecting the Solutions to the Chromatographic System:** Each level of solution was injected to the chromatographic system and the peak area was measured. A graph was plotted for peak area versus concentration and the correlation coefficient was calculated (**Table 7 & 8**).

TABLE 7: LINEARITY RESULT FOR ESOMEPRAZOLE

Sl. No	Linearity Level	Concentration	Area
1	I	5ppm	285035
2	II	6ppm	461239
3	III	7ppm	601128
4	IV	8ppm	740162
5	V	9ppm	899022
Correlation Coefficient 0.999			

Acceptance Criteria: The Correlation coefficient should be not less than 0.9

TABLE 8: LINEARITY RESULT FOR NAPROXEN

Sl. No	Linearity Level	Concentration	Area
1	I	125ppm	1087881
2	II	150ppm	1728941
3	III	175ppm	2232457
4	IV	200ppm	2901811
5	V	225ppm	3505573
Correlation Coefficient 0.999			

Acceptance Criteria: The Correlation coefficient should be not less than 0.999.

5. **Limit of Detection:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

Limit of Detection for Esomeprazole:

- **Preparation of 7µg/ml solution:** The Stock Solution was prepared by weighing accurately and transferred 10mg Esomeprazole [working standard] into a 10mL clean dry volumetric flask. 7mL diluent was added to the powder drug and sonicated to dissolve it completely. Final volume was made up to the mark with the same solvent. Further, from the above Stock solution 0.7ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent.

- **Preparation of 3.0% solution at Specification level (0.021µg/ml solution):** 1ml of the above solution was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. Further, 1ml of the above stock solution was transferred into a 10ml volumetric flask and diluted up to the mark with diluent. Finally, 3.0 ml of 1µg/ml solution was measured and transferred into a 10 ml of volumetric flask and dilute up to the mark with diluent.

- **Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank: 48µV

Signal Obtained from LOD solution (3.0% of target assay concentration): 142 µV

S/N = 142/48 = 2.95

Acceptance Criteria: The S/N Ratio value should be 3 for LOD solution.

Limit of Detection for Naproxen:

- **Preparation of 175µg/ml solution:** The Stock Solution was prepared by weighing accurately and transferred 10mg Naproxen [working standard] into a 10mL clean dry volumetric flask. 7mL diluent was added to the powder drug and sonicated to dissolve it completely. Final volume was made up to the mark with the same solvent. Further, from the above Stock solution 1.75ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent.

- **Preparation of 0.11% solution At Specification level (0.19µg/ml solution):** 1ml of the above stock solution was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. From this 0.11mL of 1µg/ml solution was measured and transferred into a 10 ml volumetric flask and diluted up to the mark with diluent.

- **Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank: 48 µV

Signal Obtained from LOD solution (0.11% of target assay concentration): 143 µV

S/N = 143/48 = **2.97**

Acceptance Criteria: The S/N Ratio value should be 3 for LOD solution.

6. **Limit of Quantification:** The Quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The Quantification limit 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. Several approaches for determining the Quantification limit are possible, depending on whether the procedure is a non- instrumental or instrumental.

Limit of Quantification for Esomeprazole:

- **Preparation of 7µg/ml solution:** The Stock Solution was prepared by weighing accurately and transferred 10mg Esomeprazole [working standard] into a 10mL clean dry volumetric flask. 7mL diluent was added to the powder drug and sonicated to dissolve it completely. Final volume was made up to the mark with the same solvent. Further from the above Stock solution 0.7ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent.

- **Preparation of 1.0% solution At Specification level (0.07µg/ml solution):** 1ml of the above solution was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. Further, 1ml of the above stock solution was transferred into a 10ml volumetric flask and diluted up to the mark with same diluent.

- **Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank: 48 µV

Signal Obtained from LOQ solution (1.0% of target assay concentration): 478µV

S/N = 472/46 = **9.95**

Acceptance Criteria: The S/N Ratio value should be 10 for LOQ solution.

7. **Limit of Quantification for Naproxen:**

- **Preparation of 175µg/ml solution:** The Stock Solution was prepared by weighing accurately and transferred 10mg Naproxen [working standard] into a 10mL clean dry volumetric flask. 7mL diluent was added to the powder drug and sonicated to dissolve it completely. Final volume was made up to the mark with the same solvent. Further, from the above Stock solution 1.75ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent.

- **Preparation of 0.35% solution At Specification level (0.64µg/ml solution):** 1ml of the above solution was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. Further 0.35ml of the above stock solution (1µg/ml) was transferred into a 10ml volumetric flask and diluted up to the mark with same diluent.

- **Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank: 48 µV

Signal Obtained from LOQ solution (0.35% of target assay concentration)

S/N = 476/48 = **9.91**

Acceptance Criteria: The S/N Ratio value should be 10 for LOQ solution.

8. **Robustness:** The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameter and provides an indication of its reliability during normal usage. As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a. **Variation at flow rate (0.8 ml/min to 1.2ml/min)**: The Standard solution 7ppm of Esomeprazole & 175ppm of Naproxen was prepared and analysed using the various flow rates along with actual flow rate (**Table 9 & 10**). On evaluation of the obtained results, it was concluded that the variation in flow rate did not affected the method significantly. Hence, it indicated that the method was robust even by change in the flow rate ±10%.

TABLE 9: SYSTEM SUITABILITY RESULTS OF ESOMEPRAZOLE

Sl. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2673.2	1.7
2	1.0	2594.6	1.7
3	1.2	2582.2	1.4

TABLE 10: SYSTEM SUITABILITY RESULTS FOR NAPROXEN

Sl. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2522.7	1.7
2	1.0	2389.9	1.6
3	1.2	2452.3	1.3

b. **The Organic composition in the Mobile phase varied from 45% to 35%.** The Standard solution 7 µg/ml of Esomeprazole & 175µg/ml of Naproxen was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase did not affect the method significantly. Hence, it indicates that the method is robust even by change in the Mobile phase ±10. The System suitability results are summarized in **Table 11 & 12**.

TABLE 11: SYSTEM SUITABILITY RESULTS FOR ESOMEPRAZOLE AFTER CHANGING THE ORGANIC COMPOSITION

Sl. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2642.0	1.4
2	Actual	2594.6	1.7
3	10% more	2599.4	1.5

TABLE 12: SYSTEM SUITABILITY RESULTS FOR NAPROXEN AFTER CHANGING THE ORGANIC COMPOSITION

Sl. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2310.5	1.2
2	Actual	2389.9	1.6
3	10% more	2299.0	1.6

RESULT & DISCUSSION: The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of Esomeprazole and Naproxen in pharmaceutical dosage forms. In order to effect separation of the drug under isocratic conditions, mixtures of Phosphate Buffer with pH 3 and Acetonitrile in different combinations were tested as mobile phase on a Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) column. A binary mixture of Phosphate Buffer pH 3 and Acetonitrile in 60:40 v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention times obtained for Esomeprazole and Naproxen were around 2.105 and 3.555 min. respectively. A model chromatogram showing the separation of Esomeprazole and Naproxen is represented in **Fig. 3**.

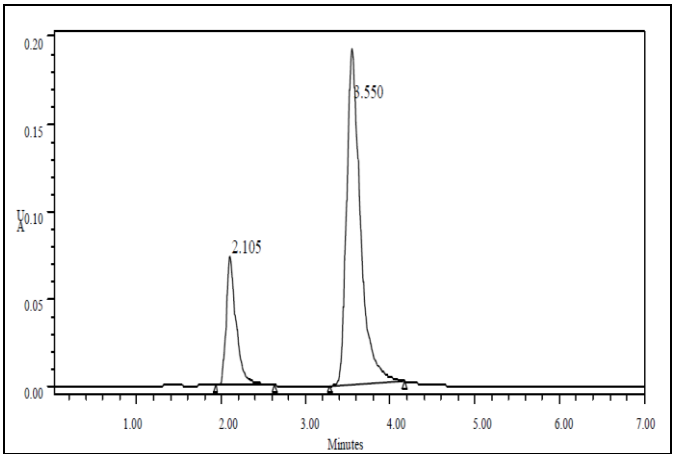

FIG. 3: THE CHROMATOGRAM REPRESENTING THE SEPARATION OF ESOMEPRAZOLE AND NAPROXEN

The Precision data for the drugs were represented in Table 1 & 2. The method was duly validated by evaluation of the required parameters. When Esomeprazole and Naproxen were analyzed by the proposed method in the intra and inter-day (Ruggedness) variation results, a low coefficient of variation was observed (Table 3 & 4). This showed that the present HPLC method was highly precise and is represented in **Fig. 4**.

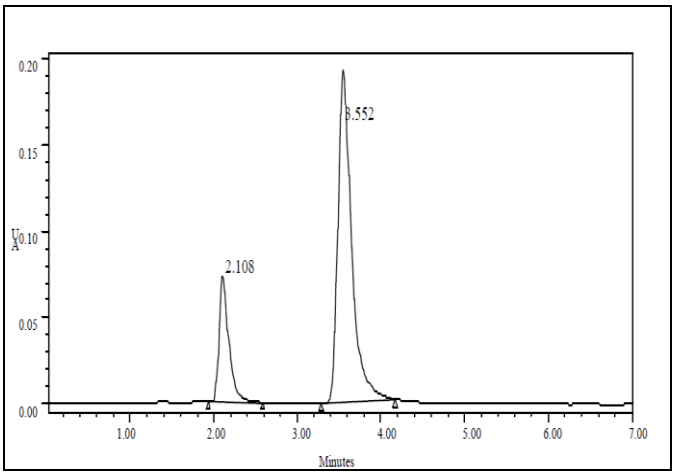

FIG. 4: THE RUGGEDNESS CHROMATOGRAPH

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. The results obtained were in specified limits and were represented in Table no.5 & 6. In order to test the linearity of the method, five dilutions of the working standard solutions of the drug in the range of 125ppm to 225ppm per mL for Naproxen and 5ppm to 9ppm per mL for Esomeprazole were prepared.

The data is represented in **Table 7 & 8**. Each of the dilutions was injected into the column and the graph for the Linearity Curve was represented in **Fig. 5 & 6**.

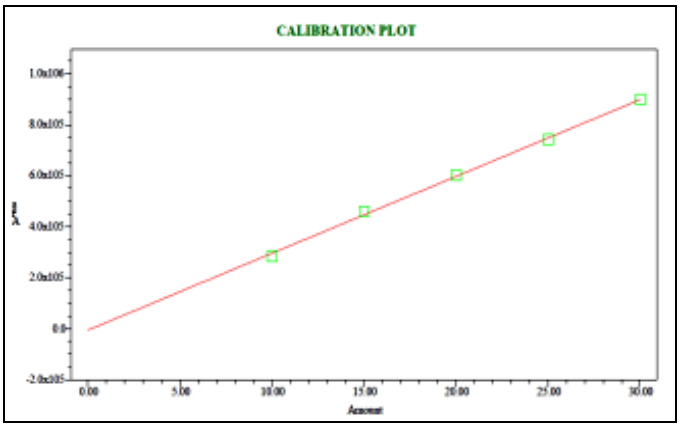

FIG. 5: THE LINEARITY CURVE OF ESOMEPRAZOLE

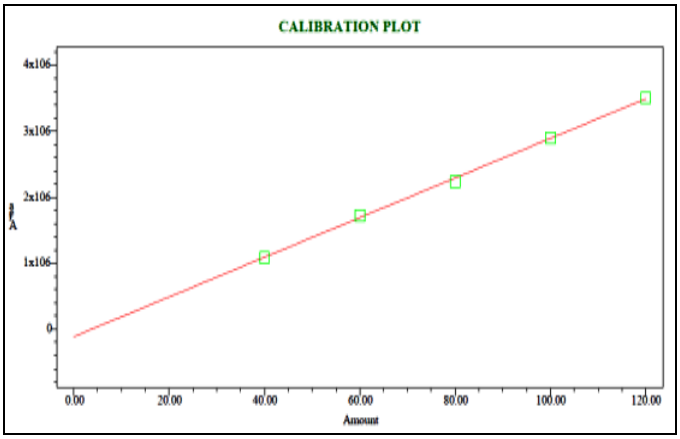

FIG. 6: THE LINEARITY CURVE OF NAPROXEN

Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate buffer pH 3 and Acetonitrile in the mobile phase.

The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The results are summarized in Table 9, 10, 11 & 12. The **Fig. 7, 8, 9 & 10** represents the robust nature of the chromatograph.

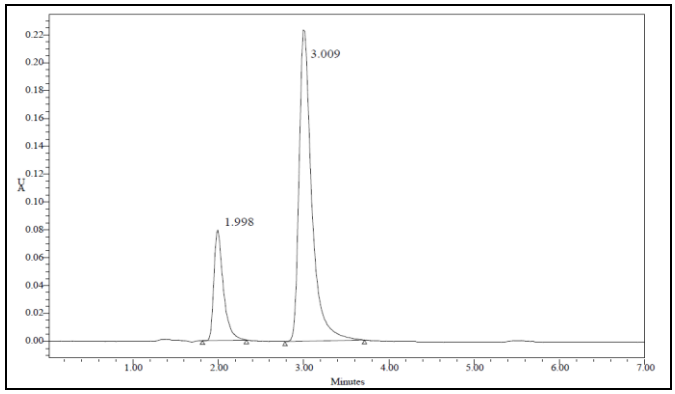

FIG. 7: THE ROBUSTNESS CHROMATOGRAPH WITH INCREASE IN COMPOSITION OF THE MOBILE PHASE

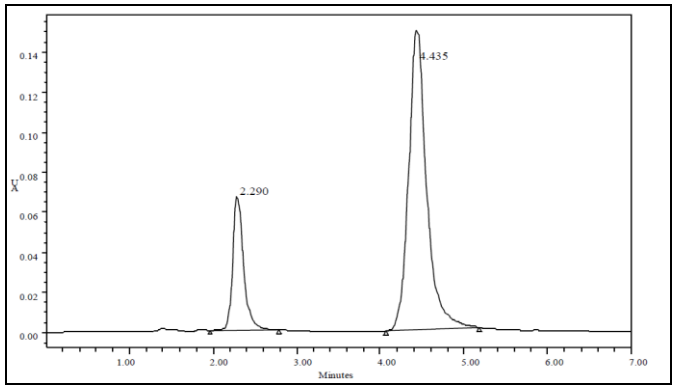

FIG. 8: ROBUSTNESS CHROMATOGRAPH WITH DECREASE IN COMPOSITION OF THE MOBILE PHASE

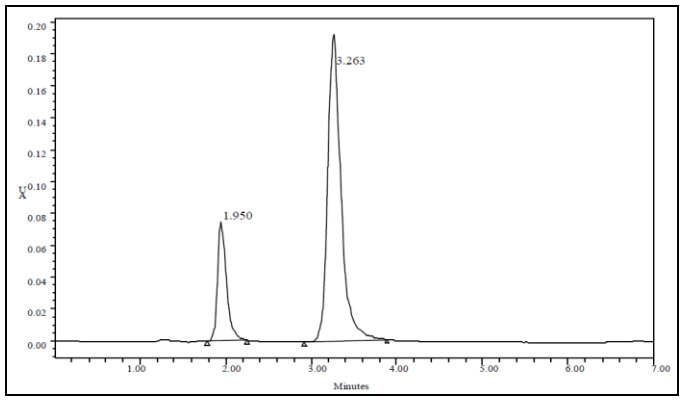

FIG. 9: ROBUSTNESS CHROMATOGRAPH WITH INCREASE IN THE FLOW RATE

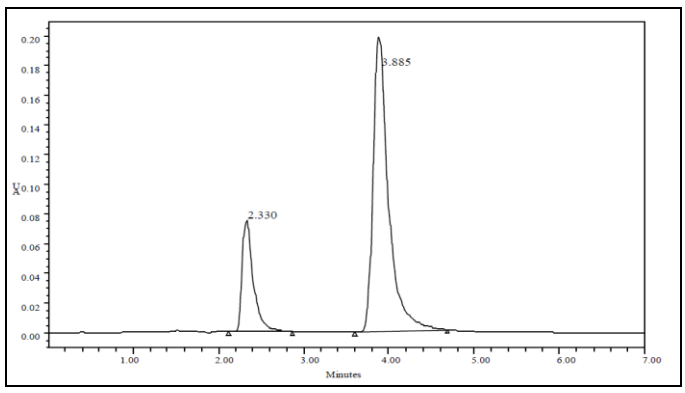

FIG. 10: ROBUSTNESS CHROMATOGRAPH WITH DECREASE IN THE FLOW RATE

The system suitability parameters were within the limits as shown in Table 9 and 10 for the respective drugs. Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD for Esomeprazole and Naproxen were found to be 0.021 and 0.19µg/mL respectively and LOQ for Esomeprazole and Naproxen were found to be 0.07 and 0.64µg/mL respectively.

The drug content formulations were quantified by using the proposed analytical method. The low coefficient of variation in the recovery data indicates the reproducibility of the method in dosage forms.

CONCLUSION: Present research work was proposed a RP-HPLC method developed for the quantitative determination of Esomeprazole and Naproxen in bulk and in pharmaceutical formulations. Developed method was simple, selective, sensitive, accurate, precise and rapid.

The proposed HPLC method was sufficiently sensitive and reproducible for the analysis of Esomeprazole and Naproxen in the Tablet formulation dosage forms within a short analysis time. The method was proved to be superior to most of the reported methods. The mobile phase was simple to prepare and economical.

The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence this method can easily adopt as an alternative method to report the routine determination of Esomeprazole and Naproxen depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also founds usefulness in clinical, biological and pharmacokinetic studies of Esomeprazole and Naproxen.

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