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Stability indicating simultaneous estimation of assay method for naproxen and esomeprazole in pharmaceutical formulations by RP-HPLC

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ABSTRACT

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 milli-Q 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.

Key words: Naproxen, Esomeprazole magnesium HPLC and validation.

INTRODUCTION

Esomeprazole magnesium which has a chemical name of *bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl]-1-H-benzimidazole-1-yl)* magnesium salt (Fig.1) is a compound that inhibits gastric acid secretion. Esomeprazole 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. Several chromatographic methods have been reported for determination of Esomeprazole Magnesium in raw material [1],tablets [2-5], plasma [6-8].

Naproxen which has a chemical name of *(S)-6-methoxy-α-methyl-2-naphthaleneacetic acid*, (Fig.2). Naproxen is a non-steroidal anti-inflammatory drug (NSAID)commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness. It works by inhibiting both the COX-1 and COX-2 enzymes. Like other NSAIDs, Naproxen is capable of producing disturbances in the gastrointestinal tract. Several chromatographic methods have been reported for determination of Naproxen in tablets [9-12], plasma [13-15], urine [16], intestinal perfusion samples [17] and pharmaceutical preparations [18,19], combination formulations [20-23].

Combination of both Naproxen and Esomeprazole magnesium is used for the treatment indicated for the relief of signs and symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis and to decrease the risk of developing gastric ulcers in patients at risk of developing NSAID associated gastric ulcers. However, no references have been found for simultaneous determination of Esomeprazole and Naproxen in pharmaceutical formulations. A successful attempt has been made to estimate both the drugs simultaneously by HPLC with VU detection.

MATERIALS AND METHODS

I. Chemicals and Reagents

Working standards of Naproxen and Esomeprazole Magnesium and tested pharmaceutical compositions were obtained from Dr. Reddy's Laboratories. N-Butyl amine, acetonitrile, methanol were of suitable analytical grade.

II. Apparatus and Chromatographic Conditions

HPLC analysis was performed on waters HPLC system with diode array detector. Separations were carried on a L1 Column(Xterra RP18 150 × 4.6 mm, i.d., 5 µm particle size) using gradient elution. The flow rate was 1.5 mL/min. UV detection was performed at 305 nm. HPLC Column maintained at a temperature of 40°C. Peaks identity was confirmed by retention time comparison and the HPLC was operated at room temperature.

Preparation of Mobile Phases

Preparation of buffer solution: Buffer is prepared by dissolving 0.71 g (0.005M) of Sodium perchlorate in 1000 mL of milli-Q water, added with 5 mL of N butyl amine. The pH of the buffer is adjusted to pH to 8.7 using diluted solution of Perchloric acid.

Mobile Phase-A: Mobile phase-A is prepared by mixing Buffer, Acetonitrile and Methanol in the ratio of (70:20:10) v/v/v which is filtered through a 0.45µm nylon filter (Millipore, USA) and degassed by sonication prior to use.

Mobile Phase-B: Mobile phase-B is prepared by mixing Buffer, Acetonitrile in the ratio of (20:80) v/v which is filtered through a 0.45µm nylon filter (Millipore, USA) and degassed by sonication prior to use.

The chromatographic elution mode is in Gradient mode with the following program.

Time(Minute)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
8	100	0
12	0	100
15	0	100
17	100	0
20	100	0

Preparation of diluents:
Diluent 1: Dilueent-1 is a volumetric mixture of 800 mL of Methanol and 200 mL milli-Q grade water and 4 mL of Triethyl amine.

Diluent 2: 0.25 N Sodium hydroxide.

Diluent 3: Mobile phase-A.

Preparation of Standard stock Solutions
The standard stock solution of Naproxen was prepared by dissolving accurate known quantity of Naproxen working standard in the diluent-1 and diluent-2 at a concentration of 5040 ppm.

The standard stock solution of Esomeprazole was prepared by dissolving accurate known quantity of Esomeprazole Magnesium working standard in the diluent-1 and diluent-2 at a concentration of 240 ppm of Esomeprazole Magnesium.

Preparation of Standard solution:
Above stock preparations are further diluted to obtain a solution of 504 ppm of Naproxen and 24 ppm of Esomeprazole Magnesium in diluent-3 (Mobile phase-A).

Preparation of Sample preparation
The sample solution of Naproxen (500 mg) and Esomeprazole (20 mg) tablets was prepared by transferring 10 tablets into 1000 mL of volumetric flask, followed by an addition of 600 ml of diluent-1 and sonication for 30 min with intermediate shaking. After the Sonication, the volume was made up with diluent-2.
A portion of this solution is centrifuged at 4000 rpm for 10 min. The supernatant clear solution is further diluted to obtain a solution of 500 ppm of Naproxen and 20 ppm of Esomeprazole in diluent-3 (Mobile phase-A).

RESULTS AND DISCUSSION

Method Development
Drug quality control, stability, metabolism, pharmacokinetics, and toxicity studies all require the determination of drugs in pharmaceutical formulations and biological samples. Correspondingly, efficient and validated analytical methods are very critical requirements for all these investigations.

Esomeprazole is a sensitive molecule, which is prone to degrade under acidic conditions. Thus method required to be designed to prevent to degradation of Esomerazole due to Naproxen which

is weekly acidic in nature. Based on this requirement, diluents selected are of basic nature with higher pH values.

Chromatographic parameters were preliminary optimized to develop a stability indicating assay method for Naproxen and Esomeprazole with a short analyses time (20 min). Since Esomeprazole is highly sensitive to at acidic pH, heat, humidity and oxidation, it tends to degrade while storage for long time. So these degradants need to be separated from main analytes to make the method stability indicating. To separate the degradants from main analytes developed gradient method to elute out non-polar impurities of the Naproxen, thus capturing all the possible degradants of both the components.

Retention of analytes increases with increased column length. Hence, a shorter column (150 x 4.6 mm i.d.5µm) was selected to have a shortest possible runtime not compromising on the resolution.

In order to identify a suitable organic modifier, various organic solvents like acetonitrile and methanol were tested. A solvent combination of Methanol and Acetonitrile produced better selectivity with low column backpressures.

Various buffers at different pH was verified only n butyl amine is gives sharp peaks of both the components compared to other buffers, thus n butyl amine was selected.

Screening and optimization of diluents for the preparation standard and sample solutions was based on the extraction and stability of both the drugs. Esomeprazole is highly sensitive to acidic environment. To prevent the degradation in presence of Naproxen, which also is weakly acidic, alkaline chemicals like triethyl amine and Sodium hydroxide solutions were used in the diluents.

Different gradient programs were tried to separate all the impurities from main analyte with high resolution, optimized the gradient program by choosing initial eight minutes isocratic mode followed by linear gradient and initial stabilization mode.

Method Validation

The above method was validated according to ICH and USP guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method.

System Suitability

In order to determine the adequate resolution and reproducibility of the proposed methodology, suitability parameters including retention time, asymmetry factor, %RSD of retention time and peak areas were investigated. The results are summarized in Table 1.

Specificity

The specificity of an analytical method is defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products and matrix. Specificity was evaluated by preparing the analytical placebo and it was confirmed that the signal measured was caused only by the analytes.

A solution of analytical placebo (containing all the tablet excipients except Naproxen and esomeprazole was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients , Blank, a mixture of inactive ingredients (placebo), standard solutions, and the Test pharmaceutical preparations were analyzed by the developed method. The representative chromatograms did not show any other peaks, which confirmed the specificity of the method. The Blank ,Placebo, Standard ,sample chromatogram is shown in Figure-3,4,5,6.

Peak purity of Naproxen and Esomeprazole was also evaluated for confirming the purity of the individual peaks of Naproxen and Esomeprazole.In all the samples Peak purity is more than the acceptance limits

(Purity angle should be less than purity threshold. Naproxen and Esomeprazole peak should not have any flag in purity results table (For Waters Empower-2 software).

Interference from Impurities:
All the impurities are injected individually, spiked into sample solution at around 1.0% of test concentration, and injected in to the system. All the impurities are well separated from each other and from main analyte. The Spiked chromatogram is shown in Figure- 7.

Forced degradation Studies:
Drug product and placebo were subjected to forced degradation at various stressed conditions like acid, base, hydrolysis, peroxide, heat, photo light, U.V light and Humidity. All the samples were analyzed for peak purity of Naproxen and Esomeprazole peak. In all the samples, Peak purity meet the acceptance limits.

(Purity angle should be less than purity threshold. Naproxen and Esomeprazole peak should not have any flag in purity results table (For Waters Empower-2 software). The results are summarized in Table 2a,2b.

Linearity:
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of detector response for Naproxen was established by analyzing serial dilutions of a stock solution of the working standard. Solutions of five different concentrations in the range of 100.28 to 902.520 µg per mL of Naproxen and 9.6 to 45.6 µg per mL of Esomeprazole were prepared and analyzed. The final concentration of each solution in µg per mL was plotted against peak area response. The details of Slope, correlation coefficient (R) and intercept were found to be satisfactory . The linear graphs were shown in Figure-8 and 9.

Precision:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Six replicate samples were prepared and analyzed as per the sample preparation procedure. Assay of each replicate, the average of 6 replicates, its standard deviation, %RSD and the 95% confidence interval were calculated. . The results are shown in Table 3.

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

Recovery study was performed at 50%, 70%, 100%, 120%, and 150% of the target assay concentration of the Naproxen and Esomeprazole by directly dropping the tablets. Drugs extracted in these preparations were analysed. The amount spiked, amount recovered, percent recovery and its mean were calculated. The results are shown in Table 4a and 4b.

Figure 1: Chemical Structure of Naproxen

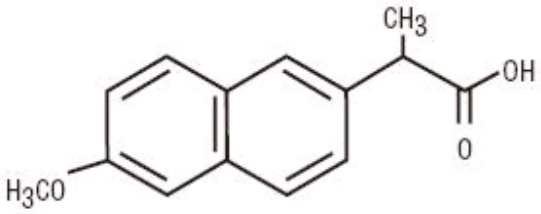
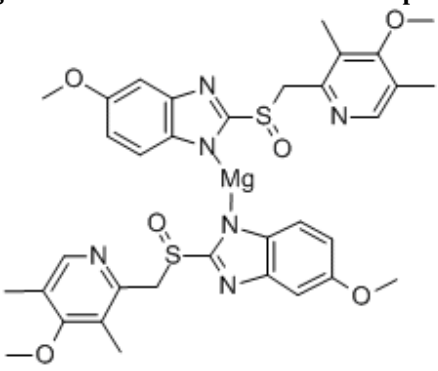


Figure 2: Chemical Structure of Esomeprazole



Range:
The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The results are shown in Table 5.

Robustness:
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The variations like flow rate of mobile phase, column temperature, does not have any significant effect on the method performance.

Figure 3: Chromatogram of Blank

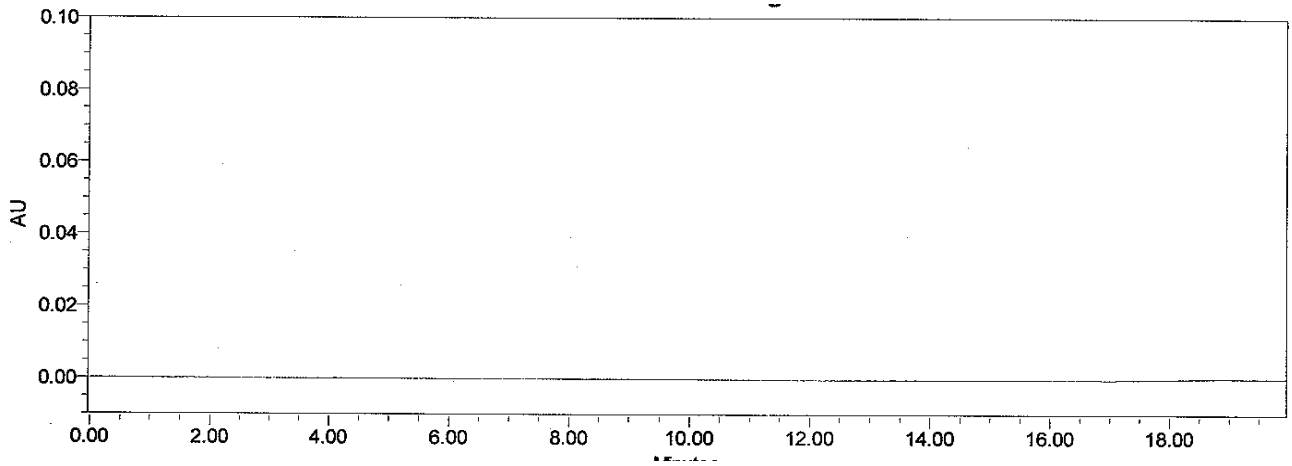


Figure 4: Chromatogram of Placebo

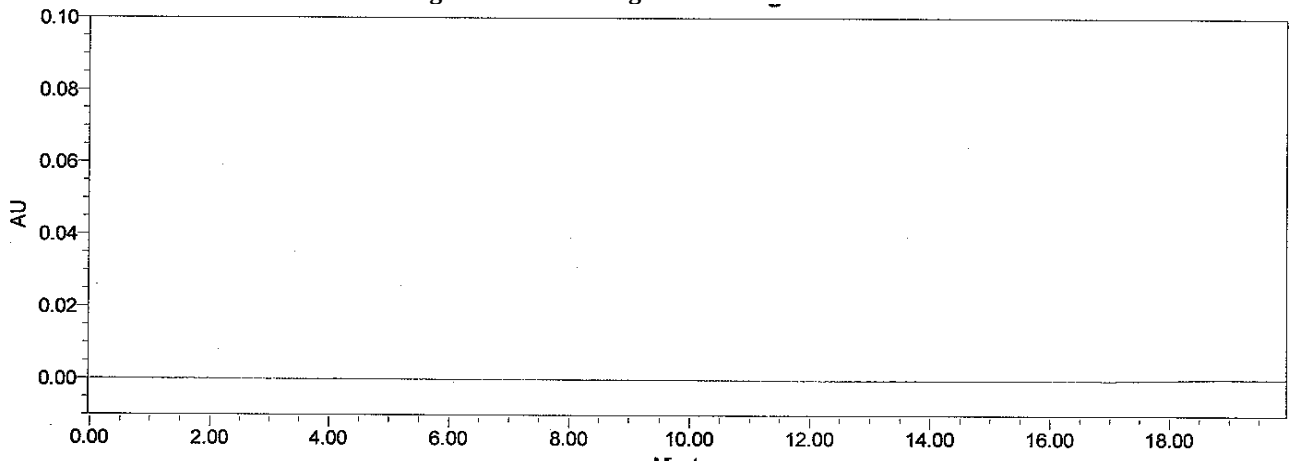


Figure 5: Chromatogram of Standard

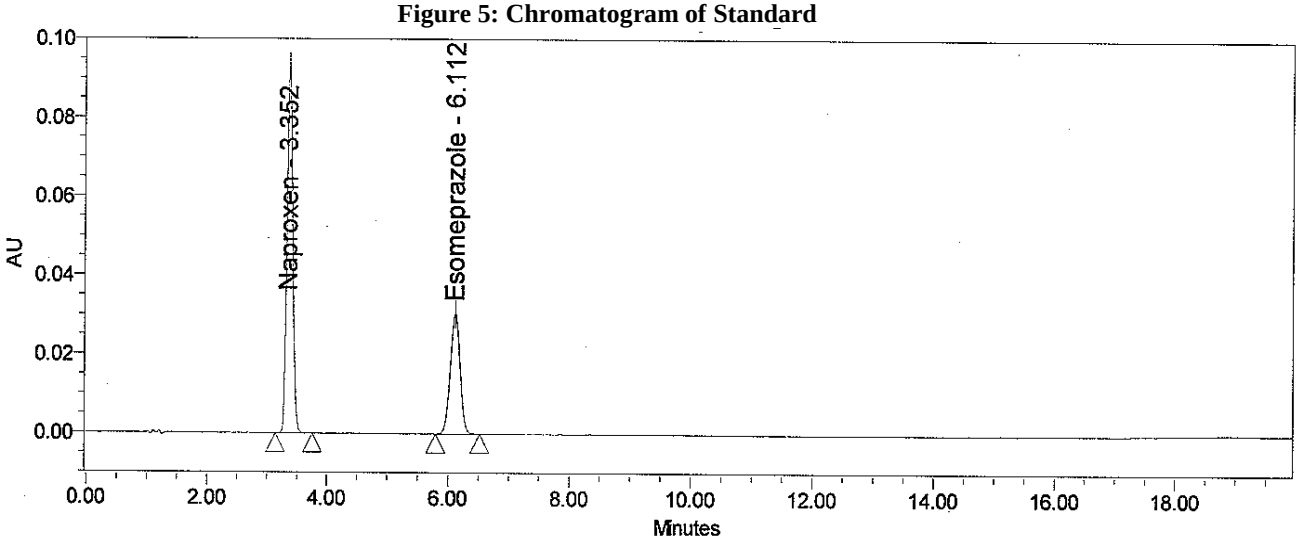


Figure 6: Chromatogram of Sample

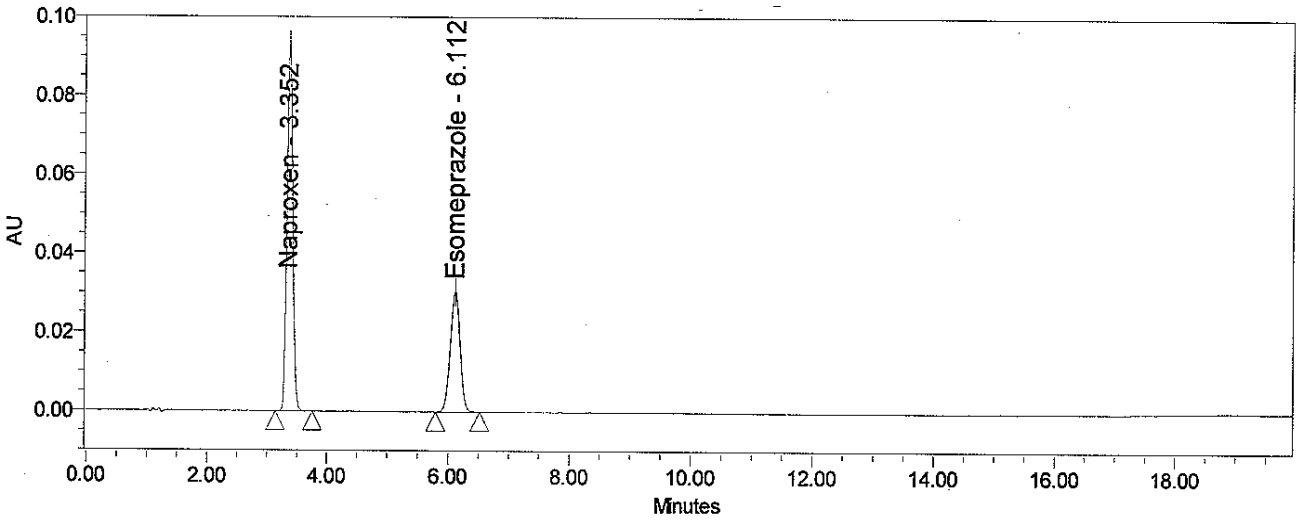


Figure 7: Interference from impurities

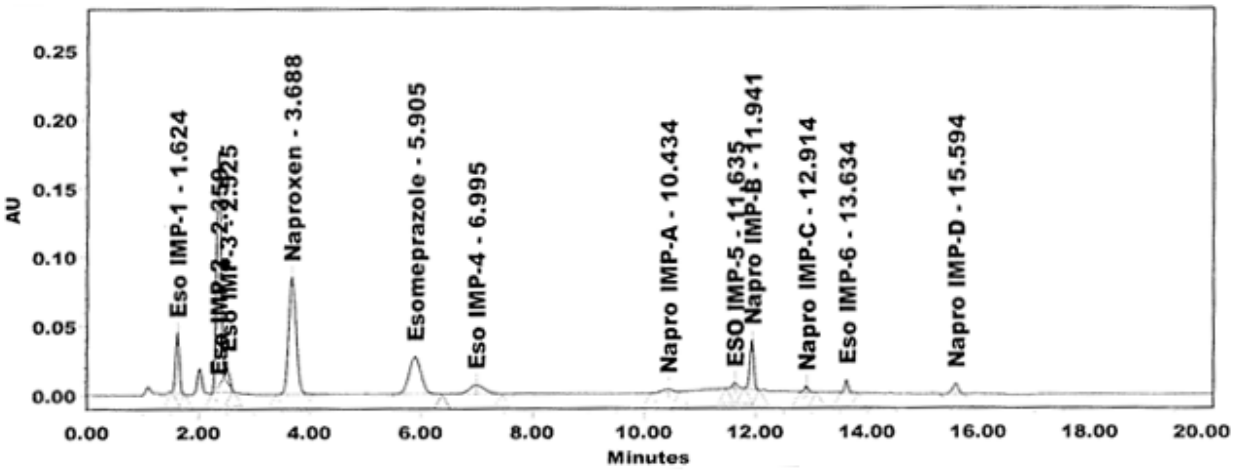


Figure 8. Linearity graph for Naproxen

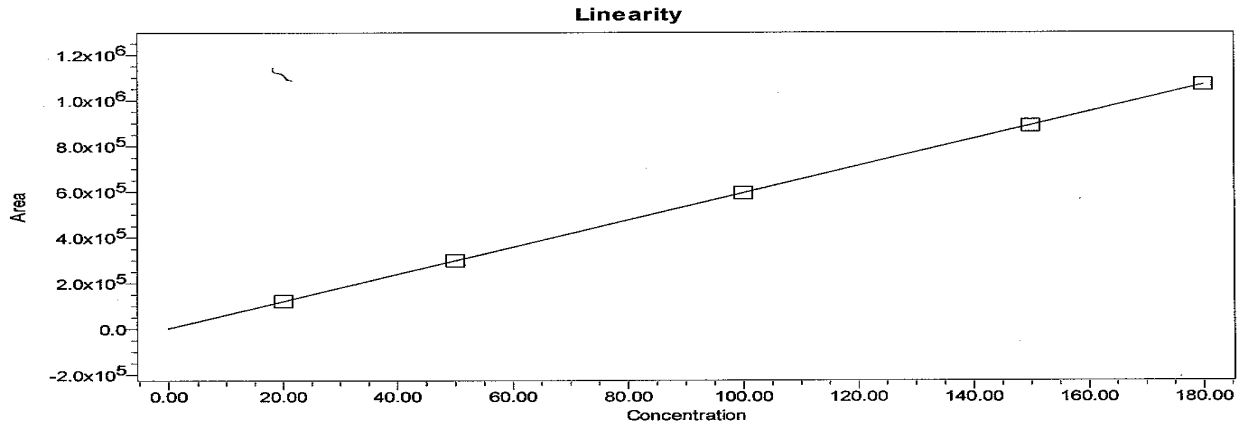


Figure 9. Linearity graph for Esomeprazole

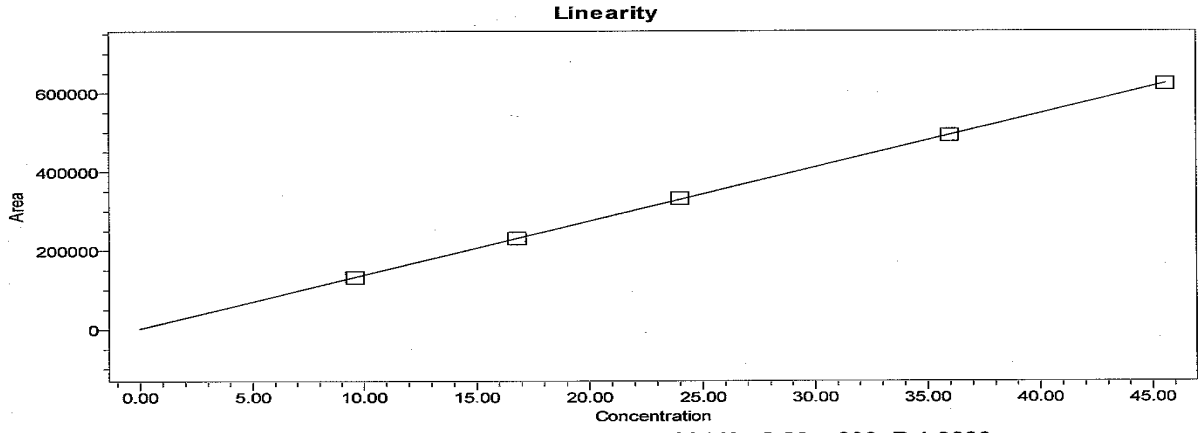


Table: 1 System Suitability

Parameter	Result	Acceptance Criteria
Tailing Factor For Naproxen peak	1.0	NMT 2.0
%RSD of Peak Area of Naproxen	0.16	NMT 2.0%
Tailing Factor For Esomeprazole peak	1.3	NMT 2.0
%RSD of Peak Area of Esomeprazole	0.3	NMT 2.0%

Table: 2a.Forced Degradation Data For Naproxen

S. No.	Sample condition	Procedure	% Net degradation	Purity flag
1.	Acid degradation	2N HCl at 60°C 15minutes	1.0	NO
2.	Alkali degradation	5N NaOH at 60°C 4Hours	0.2	NO
3.	Peroxide degradation	5.0% H_2O_2 on bench top for 2Hours	0.1	NO
4.	Water degradation	60°C for 4Hours	1.5	NO
5.	UV degradation	200 W /m^2/hours	1.5	NO
6.	Photolight	200 million Lux Hours	1.0	NO
7.	Thermal degradation	105°C for 24hrs	0.2	NO
8.	Humidity degradation	90% RH at 25°C for 7 days	0.1	NO

Table: 2b. Forced Degradation Data For Esomeprazole

S. No.	Sample condition	Procedure	% Net degradation	Purity flag
	Acid degradation	2N HCl at 60°C 15minutes	4.7	NO
2.	Alkali degradation	5N NaOH at 60°C 4Hours	1.2	NO
3.	Peroxide degradation	5.0% H_2O_2 on bench top for 2Hours	4.0	NO
4.	Water degradation	60°C for 4Hours	1.1	NO
5.	UV degradation	200 W /m^2/hours	2.0	NO
6.	Photolight	200 million Lux Hours	2.9	NO
7.	Thermal degradation	105°C for 24hrs	32.6	NO
8.	Humidity degradation	90% RH at 25°C for 7 days	1.6	NO

Table: 3 Precision

Sample. No.	% Assay	
	Naproxen	Esomeprazole
1	99.8	101.0
2	100.3	101.4
3	100.1	101.6
4	100.7	101.9
5	100.2	101.5
6	100.6	101.4
MEAN($\overline{X}$)	100.3	101.5
%RSD	0.3	0.3

Table: 4a.Recovery for Naproxen

Sample No.	Spike level	"Mg" added	"Mg" found	% Recovery	Mean % Recovery
1	50%	2500	2555.0	102.2	101.9
2	50%	2500	2546.0	101.6	
3	50%	2500	2542.5	101.7	
4	50%	2500	2540.0	101.6	
5	50%	2500	2550.0	102.0	
6	50%	2500	2557.5	102.3	
1	70%	3500	3524.5	100.7	100.7
2	70%	3500	3524.5	100.7	
3	70%	3500	3521.5	100.6	
1	100%	5000	5075.0	101.5	101.1
2	100%	5000	5055.0	101.1	
3	100%	5000	5040.0	100.8	
1	120%	6000	6048.0	100.8	100.4
2	120%	6000	6006.0	100.1	
3	120%	6000	6018.0	100.3	
1	150%	7500	7545	100.6	100.8
2	150%	7500	7552.5	100.7	
3	150%	7500	7545	100.6	
4	150%	7500	7575	101.0	
5	150%	7500	7560	100.8	
6	150%	7500	7560	100.8	

Table: 4b. Recovery for Esomeprazole

Sample No.	Spike level	"Mg" added	"Mg" found	% Recovery	Mean % Recovery
1	50%	115.200	117.158	101.7	101.3
2	50%	115.200	116.467	101.1	
3	50%	115.300	116.483	101.0	
4	50%	115.300	116.338	100.9	
5	50%	115.100	116.136	100.9	
6	50%	115.300	117.260	101.7	
1	70%	161.300	162.590	100.8	100.6
2	70%	161.200	162.167	100.6	

3	70%	161.100	161.744	100.4	
1	100%	230.500	234.419	101.7	101.3
2	100%	230.300	232.603	101.0	
3	100%	230.200	232.962	101.2	
1	120%	276.300	279.616	101.2	100.8
2	120%	276.500	277.863	100.5	
3	120%	276.500	278.159	100.6	
1	150%	345.700	349.157	101.0	101.1
2	150%	345.500	349.646	101.2	
3	150%	345.400	348.854	101.0	
4	150%	345.600	350.438	101.4	
5	150%	345.500	349.992	101.3	
6	150%	345.500	349.646	101.2	

Table 5: Range

Parameter	Acceptance Criteria	Result Naproxen	Result Esomeprazole
Linearity	R ≥ 0.999	1.0000	0.9999
Precision	%RSD of 6 Replicates NMT 2.0%	0.3% to 0.15%	0.38% to 0.16%
Accuracy	Recovery 97.0% to 103.0%	100.4%-101.9%	100.1%-101.3%

CONCLUSION

The stability indicating assay method developed is found to be simple, rapid, cost effective, accurate for the quantification of Naproxen and Esomeprazole in formulations.

The analytical conditions and the solvent system developed provides good resolution between Naproxen, Esomeprazole and all its potential degradants within a short run time. The HPLC method was validated and demonstrated good linearity, precision, accuracy, specificity. As the major impurities and degradants are well separated from each other, method can be used for quantification of impurities. The method can also be adopted for the quantifying the drug release during dissolution experiments. Thus, the developed HPLC method can be utilized for routine analysis, stability studies for Naproxen and Esomeprazole tablets.

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