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A New Bioanalytical Method Development & Validation for Simultaneous Estimation of Esomeprazole and Naproxen in Human Plasma by Using RP-HPLC

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SAK and JVLNSR have designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author DGS managed the analyses of the study. Author MD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: A new reverse phase high performance liquid chromatography (RP-HPLC) method for the quantitative determination of Esomeprazole and Naproxen in human plasma was developed and validated as per US-FDA guidelines.

Methodology: The drug was spiked in the plasma and extracted with mobile phase by precipitation method. The extracted analyte was injected into Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent, maintained at ambient temperature and effluent was monitored at 285nm. The mobile phase was composed of potassium dihydrogen phosphate and acetonitrile [HPLC Grade] in the ratio of 60:40. The pH of the potassium buffer was adjusted to 3.0 by using Ortho Phosphoric Acid. The flow rate was maintained at 1.0 mL/min.

Results: The developed method shows high specificity for Esomeprazole and Naproxen. The calibration curve for Esomeprazole and Naproxen was linear from 1.0 to 6.0 ppm (r^2= 0.999) and 25.0 to 150.0 ppm (r^2= 0.999) respectively. The inter-day and intra-day precision was found to be within limits. The proposed method was adequate sensitivity, reproducibility, and specificity for the determination of esomeprazole and naproxen in plasma. The Lower limit of quantification (LLOQ) for the drug Esomeprazole and Naproxen were found to be 0.04µg/ml and 0.4µg/ml respectively. The average percent recovery for the drugs Esomeprazole and Naproxen were found to be 98.97-99.84 & 99.80-100.95 respectively and reproducibility was found to be satisfactory.

Conclusion: The proposed method was accurate, and precise for the quantification of Esomeprazole and Naproxen in the plasma. The proposed can also be used for routine analysis in quality control. The method was validated for parameters like selectivity, sensitivity, precision, intermediate precision, accuracy, linearity, recovery & stability. This RP -HPLC method is suitable for determining the concentration of Esomeprazole and Naproxen in plasma and it can applied for routine analysis for determination of the Esomeprazole and Naproxen from dosage form during pharmacokinetic study.

Keywords: Esomeprazole; naproxen; RP-HPLC; ICH; validation; human plasma; US-FDA guideline.

1. 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. Naproxen [(S)-6-methoxy-α-methyl-2-naphthaleneacetic acid], (Fig. 2) 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. The combination of both the drugs esomeprazole magnesium and naproxen is used for the treatment and control of signs and symptoms of ankylosing spondylitis, rheumatoid arthritis and osteoarthritis. The combination is also useful to reduce the risk of developing gastric ulcers [NSAID associated gastric ulcers]. Several chromatographic methods were reported for estimation of esomeprazole magnesium and naproxen in raw materials, solid dosage forms mainly tablet and blood-plasma by RP-HPLC

[1,2]. Densitometric determination of esomeprazole with domperidone was also established [3]. Spectroscopic estimation of Esomeprazole magnesium in solid dosage form with some other NSAID'S [4-7] were available in the literature. Physico-chemical characterization, UV Spectrophotometric method development and validation studies for esomeprazole magnesium trihydrate was reported in Literature [8-9]. Simultaneous estimation of esomeprazole and domperidone in pharmaceutical dosage form by using RP-HPLC was reported in the literature [10-11]. A UPLC stability indicating method for determination of impurities in esomeprazole magnesium gastro resistant tablets was also reported in the literature [12]. Although literature survey reveals that various methods were reported for esomeprazole and naproxen both for single estimation and in combination with others drugs. However, no references were found for simultaneous determination of esomeprazole and naproxen in plasma till dated. Considering the fact a successful attempt has been made to estimate both esomeprazole and naproxen in plasma by RP- HPLC with photo diode array detector.

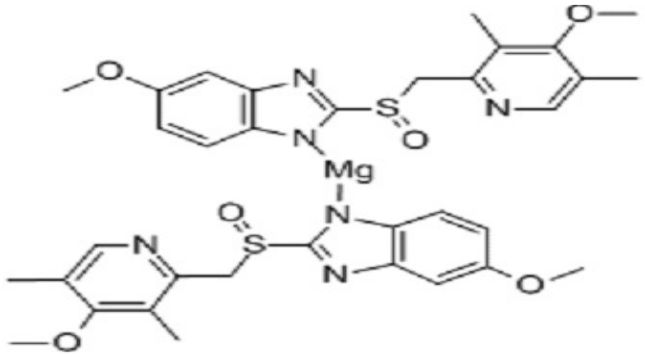

Fig. 1. Chemical structure of esomeprazole

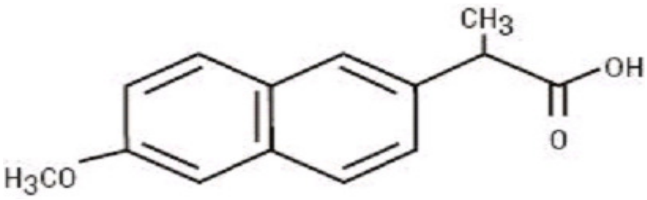

Fig. 2. Chemical structure of naproxen

2. MATERIALS AND METHODS [13-14]

2.1 Chemicals and Reagents Used

The following chemicals were used for the process: Water [HPLC Grade] Acetonitrile [HPLC Grade], Ortho phosphoric acid, Potassium dihydrogen phosphate and Esomeprazole and Naproxen [Working Standards] all these chemicals were collected as a gift sample from STANDARD SOLUTIONS. Esomeprazole (20mg) and Naproxen (500mg) tablet was collected from the Local market, Brand Name VIMOVO and the manufacturer was Belgian Pharmaceutical Company.

2.2 Apparatus and Chromatographic Conditions

The equipment used for the method was HPLC (equipped with Auto Sampler and PDA detector). The column selected for the method was XTerra Symmetry C_{18} column (Dimension: 4.6 x 150mm, 5µm). The flow rate was monitored at 1.0 mL/min and the run time was 7 min. The wavelength selected for the method was 285 nm. The volume of injection selected 20 µl. The temperature of the column oven was chosen ambient.

2.3 Preparation of Phosphate Buffer [15]

The phosphate buffer was prepared by accurately weighing and transferring 7.0 gm of KH_2PO_4 into a 1000ml clean and dry volumetric flask, dissolved and diluted with 1000ml of water [HPLC grade]. The pH was adjusted to 3.0 with Orthophosphoric Acid.

2.4 Preparation of Mobile Phase and Diluent

The mobile phase was consists of phosphate buffer and acetonitrile [HPLC Grade] at the ratio of 60:40 (v/v). The prepared mobile phase was degassing in ultrasonic water bath for 5 minutes. It was filtered through 0.45 µ filter under vacuum filtration. The mobile phase was used as diluent.

2.5 Preparation of the Esomeprazole and Naproxen Standard and Sample Solution

2.5.1 Standard solution preparation

The standard stock solution was prepared by accurately weighing and transferring 10 mg Esomeprazole and 10 mg Naproxen [Working standard] into a 100 ml and 10 ml clean and dry volumetric flask respectively. Initially about 70 ml and 7 ml of diluents were added and sonicated to dissolve the drug completely and the volume was made up to the mark with the same solvent. Further from the above prepared stock solution 0.4 ml of Esomeprazole and 1.0 ml Naproxen was pipette out into a 10 ml volumetric flask and diluted was added up to the mark.

2.5.2 Esomeprazole & naproxen spiked to plasma and extracted from plasma

The serial dilutions of analyte were prepared in mobile phase and 0.5ml of each dilution was spiked into 0.5ml of plasma in a polypropylene tubes. Then all the tubes were cyclo mix for 5 min. Further 1ml of acetonitrile was added to the mixture and centrifuged for 20 min at 3000 rpm. After centrifugation process, the supernatant liquids were collected in another eppendorf tube and 20µL supernatant was injected into the analytical column.

3. VALIDATION DEVELOPMENT [16-22]

3.1 Selectivity

Selectivity is defined as the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. An aqueous mixture of Esomeprazole and Naproxen was prepared and injected to the column and the retention

time was checked. There were no interferences found in the retention of drug extracted from plasma. The method was found to be precised and specific.

3.2 Sensitivity

To determine the sensitivity in terms of LLOQ, 'Lower Limit of Quantification' where the response of LLOQ must be at least five times greater than the response of interference in blank matrix at the retention time or mass transitions of the analyte(s).

3.2.1 Preparation of esomeprazole solution

The solution was prepared by weighing accurately and transferred 10mg of Esomeprazole [Working Standard] into a 100 mL volumetric flask and added about 70 mL of the diluent and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. From the above prepared stock solution pipette out 0.4ml into a clean and dry 10ml volumetric flask and the volume was upto the mark with the diluent.

3.2.2 Preparation of naproxen solution

The solution was prepared by weighing accurately and transferred 10mg of Naproxen [Working Standard] into a 100 mL volumetric flask and added about 70 mL of the diluent and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. From the above prepared stock solution pipette out 0.1ml into a clean and dry 10ml volumetric flask and the volume was upto the mark with the diluent.

3.2.3 Preparation of 0.04 µg/ml of esomeprazole and 0.4 µg/ml of naproxen

From the above prepared solutions pipette out 0.1 ml [from Esomeprazole stock solution] and 0.4ml [from Naproxen stock solution] and transferred into a clean and dry 10 ml volumetric flask and the volume was made upto the mark with the diluent. The resultant solution was mixed well and filtered through 0.45µm filter.

3.2.4 Calculation of S/N ratio for esomeprazole

Average Baseline Noise obtained from Blank:43µV
Signal Obtained from LOQ solution (0.5% of target assay concentration):433µV
S/N=433/43=10.07

3.2.5 Calculation of S/N ratio for naproxen

Average Baseline Noise obtained from Blank: 43µV
Signal Obtained from LOQ solution (0.5% of target assay concentration): 435 µV
S/N=435/43=10.12

3.2.6 Acceptance criteria

The S/N Ratio value should be ≥ 5 for LOQ solution.

3.3 Precision

The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. The precision of the analytical method was determined by analyzing the drug in the specific concentrations spiked to plasma in six replicates. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits (Tables 1 and 2).

Table 1. The precision result for esomeprazole

Injection	Retention time	Area
Injection-1	2.375	324758
Injection-2	2.381	328695
Injection-3	2.385	327458
Injection-4	2.394	330147
Injection-5	2.379	327542
Injection-6	2.376	326475
Average	2.382	327513
Standard deviation	0.007	1844.26
%RSD	0.295	0.56

Table 2. The precision result for naproxen

Injection	Retention time	Area
Injection-1	3.741	1261425
Injection-2	3.759	1247251
Injection-3	3.744	1266895
Injection-4	3.753	1247585
Injection-5	3.756	1265214
Injection-6	3.744	1254758
Average	3.750	1257188
Standard deviation	0.007	8642.47
%RSD	0.20	0.69

3.3.1 Acceptance criteria

The % RSD for the area and retention time for standard injection results should not be more than 2%.

3.4 Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by using same conditions. The % RSD for the area and retention time of six replicate injections was found to be within the specified limits (Tables 3 and 4).

Table 3. The intermediate precision result for esomeprazole

Injection	Retention time	Area
Injection-1	2.334	330147
Injection-2	2.376	329425
Injection-3	2.368	327458
Injection-4	2.375	328541
Injection-5	2.379	321475
Injection-6	2.356	328475
Average	2.365	327587
Standard deviation	0.017	3130.613
% RSD	0.724	0.96

Table 4. The intermediate precision result for naproxen

Injection	Retention time	Area
Injection-1	3.738	1271472
Injection-2	3.741	1297585
Injection-3	3.753	1279854
Injection-4	3.747	1286544
Injection-5	3.725	1287582
Injection-6	3.741	1274755
Average	3.741	1282965
Standard deviation	0.009	9560.77
%RSD	0.25	0.75

3.4.1 Acceptance criteria

The % RSD for the area and retention time of standard injection results should not be more than 2%.

3.5 Accuracy

The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Precision and accuracy is determined by replicate analysis of samples containing known amounts of the analyte. As in the standard preparation, the samples were spiked to the plasma and it was extracted and collected in vials and injected into HPLC system. The amount of the drug Esomeprazole & Naproxen to be added was calculated as well as the amount found was calculated. Further the individual recovery and mean recovery values were also calculated by using suitable formula (Tables 5 and 6).

3.5.1 Acceptance criteria

The % recovery for each accuracy level should be between 98.0 to 102.0%.

3.6 Linearity

It is the relationship between instrument response and known concentrations of the analyte. A linearity curve should be generated for each analyte in the sample and should be prepared

in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte (Tables 7 and 8). The standard curves are represented in Figs. 3 and 4.

Table 5. The accuracy result for esomeprazole

Accuracy level	% recovery	Avg.% recovery	Amount recovered	SD	%RSD
80%	99.18	99.03	7.93	0.15	0.15
100%	98.88	99.84	7.91	0.10	0.10
120%	99.04	98.97	7.92	0.86	0.87
	99.73		9.97		
	99.93		9.99		
	99.86		9.99		
	98.86		11.86		
	99.88		11.99		
	98.17		11.78		

Table 6. The accuracy result for naproxen

Accuracy level	% recovery	Avg.% recovery	Amount recovered	SD	%RSD
80%	100.52	100.33	8.04	0.34	0.34
100%	100.53	99.80	8.04	0.10	0.10
120%	99.94	100.95	8	0.37	0.37
	99.78		9.98		
	99.91		9.99		
	99.71		9.97		
	101.24		12.15		
	101.07		12.13		
	100.53		12.06		

Table 7. The linearity result for esomeprazole

Sr. no.	Linearity level	Concentration	Area
1	I	1ppm	83760
2	II	2ppm	158962
3	III	3ppm	238443
4	IV	4ppm	321452
5	V	5ppm	432536
6	VI	6ppm	475856
Correlation coefficient			0.998

Table 8. The linearity result for naproxen

S. no	Linearity level	Concentration	Area
1	I	25ppm	318922
2	II	50ppm	637845
3	III	75ppm	956767
4	IV	100ppm	1275690
5	V	125ppm	1594612
6	VI	150ppm	1842525
Correlation coefficient			0.999

3.6.1 Acceptance criteria

The correlation coefficient should be not less than 0.999.

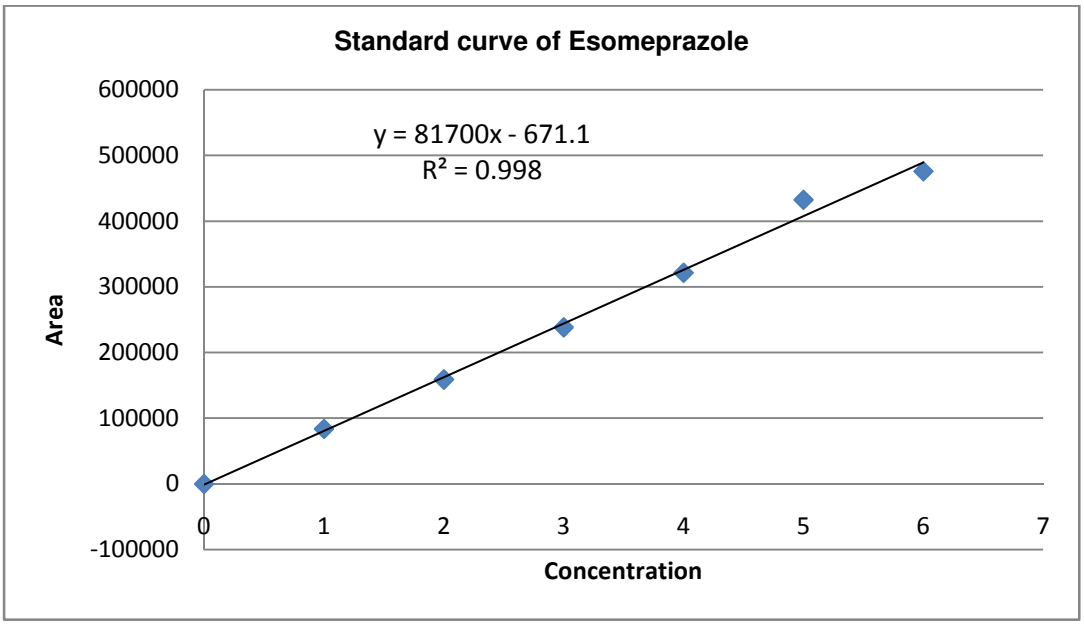

Fig. 3. Standard curve of esomeprazole

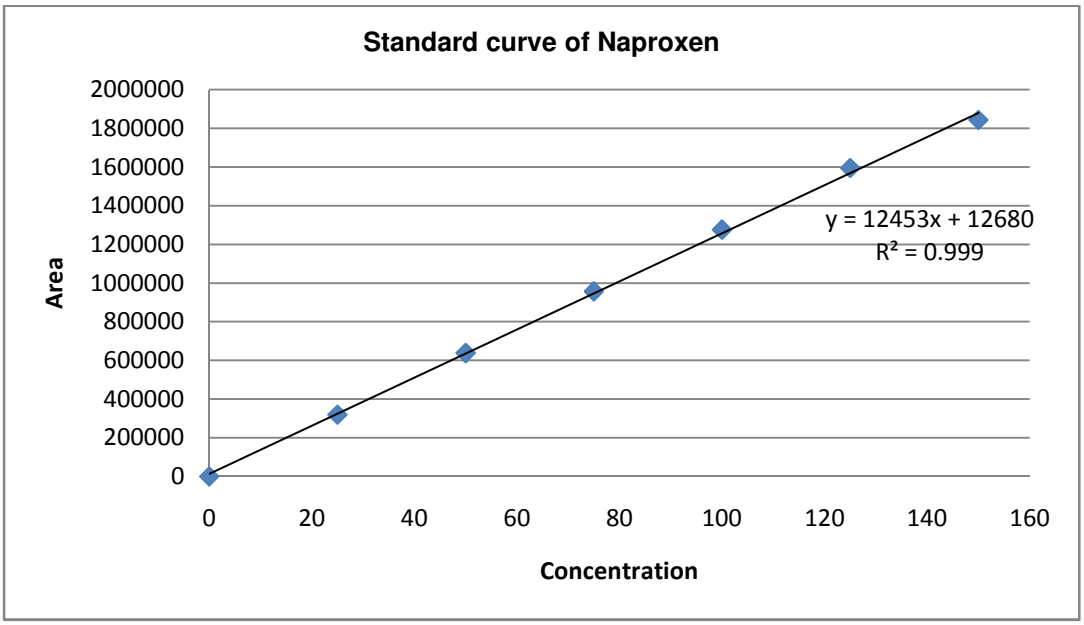

Fig. 4. Standard curve of naproxen

3.7 Stability [23-30]

Drug stability in a biological fluid depends on the storage conditions, the chemical properties of the drug, the matrix, and the container system. All stability determinations should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix. Stock solutions of the analyte for stability evaluation should be prepared in an appropriate solvent at known concentrations. Stability procedures should evaluate the stability of the analytes during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term (bench top, room temperature) storage, and after going through freeze and thaw cycles and the analytical process. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. The stability of the drug extracted, was subjected to freeze and thaw stability at -20℃±2℃, short term stability for period of 24 hours stored at room temperature, long term stability for period of 15 days stored at 4℃. All the stability samples compared against the standard stock solution assessed for stability. The results are represented in Tables 9 and 10.

Table 9. The stability result for esomeprazole

Sr. no.	Standard sample	Freeze & thaw sample	Short term stability	Long term stability
1	325852	295852	291475	264712
2	324715	301475	295862	263658
3	319854	298754	297451	257548
Mean	323474	298694	294929	261973
SD	3186	2812	3095	3868
% RSD	0.98	0.94	1.05	1.48
% Assay		92.34	91.18	80.99

Table. 10. The stability result for naproxen

Sr. no.	Standard sample	Freeze & thaw sample	Short term stability	Long term stability
1	1274859	1168952	1074852	1014755
2	1278545	1187452	1073658	1019856
3	1270475	1142586	1069874	1087425
Mean	1274626	1166330	1072795	1040679
SD	4040	22548	2599	40564
% RSD	0.32	1.93	0.24	3.90
% Assay		91.50	84.17	81.65

4. RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of the drug esomeprazole and naproxen in plasma. In order to method development under isocratic conditions, mixtures of potassium dihydrogen phosphate buffer [pH adjusted to 3.0 with Ortho Phosphoric Acid] and Acetonitrile [HPLC grade] in different combinations were tested as mobile phase on a Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) column. A binary mixture of potassium dihydrogen phosphate buffer [pH 3.0] and Acetonitrile [HPLC Grade] 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 the tailing. The retention times obtained for esomeprazole & naproxen were around 2.347 & 3.714 min. respectively. A model chromatogram was shown in (Figs. 5, 6 and 7) for the sample drug, standard drug as well as for the blank. The standard stock solution was prepared by weighing accurately and transferred 10 mg esomeprazole and 10mg naproxen [working standard] into a 100ml & 10ml clean dry volumetric flask respectively. About 70ml & 7ml of diluent were added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above prepared Stock Solution pipette out 0.4ml of esomeprazole & 1.0ml naproxen into a 10ml volumetric flask and diluted up to the mark with diluent. Serial dilutions of analyte were prepared in mobile phase and 0.5ml of each dilution was spiked into 0.5ml of plasma in a polypropylene tubes. Then all the tubes were cyclo mix for 5 min. Further 1ml of acetonitrile was added to the mixture and centrifuged for 20 min at 3000 rpm. After centrifugation process, the supernatant liquids were collected in another eppendorf tube and 20µL supernatant was injected into the analytical column.

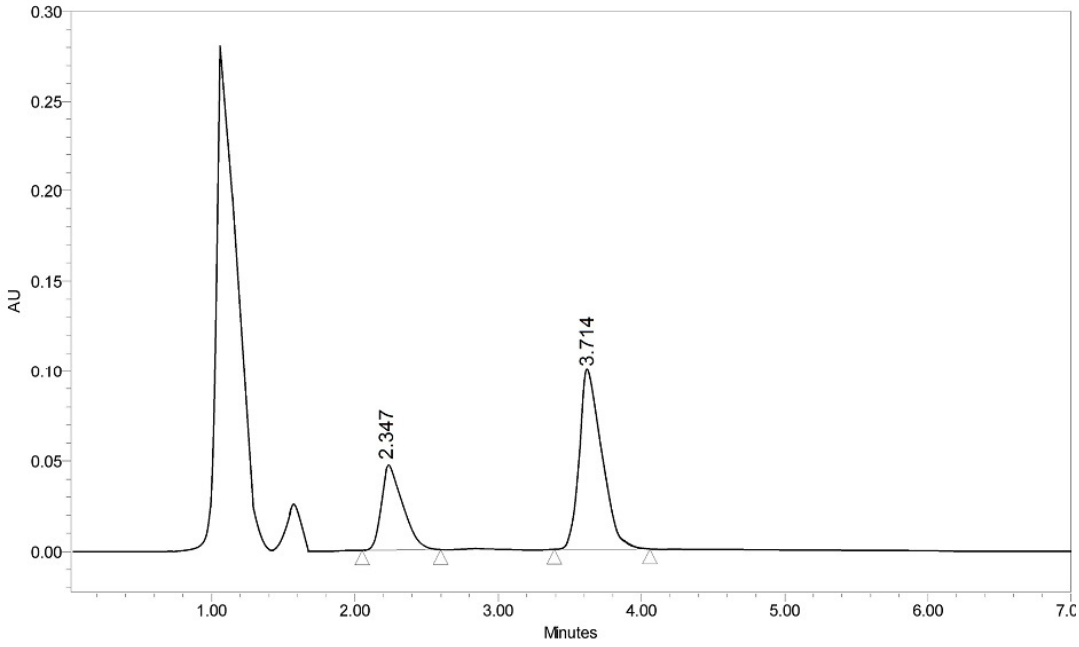

Fig. 5. Typical chromatogram for esomeprazole & naproxen in plasma (sample)

The Precision data was represented in (Tables 1 and 2). The % RSD for the area and retention time of standard injection for esomeprazole was found to be 0.295 & 0.56 respectively. The % RSD for the area and retention time of standard injection for naproxen was found to be 0.20 & 0.69 respectively. When the drug esomeprazole & naproxen was analyzed by the proposed method in the intra and inter-day (ruggedness) variation results, a low coefficient of variation was observed it was represented in (Tables 3 and 4). The % RSD for the area and retention time of standard injection for esomeprazole was found to be 0.724 & 0.96 respectively. The % RSD for the area and retention time of standard injection for naproxen was found to be 0.25 & 0.75 respectively. This shows that the present HPLC method is highly precise and it was shown in (Fig. 8).

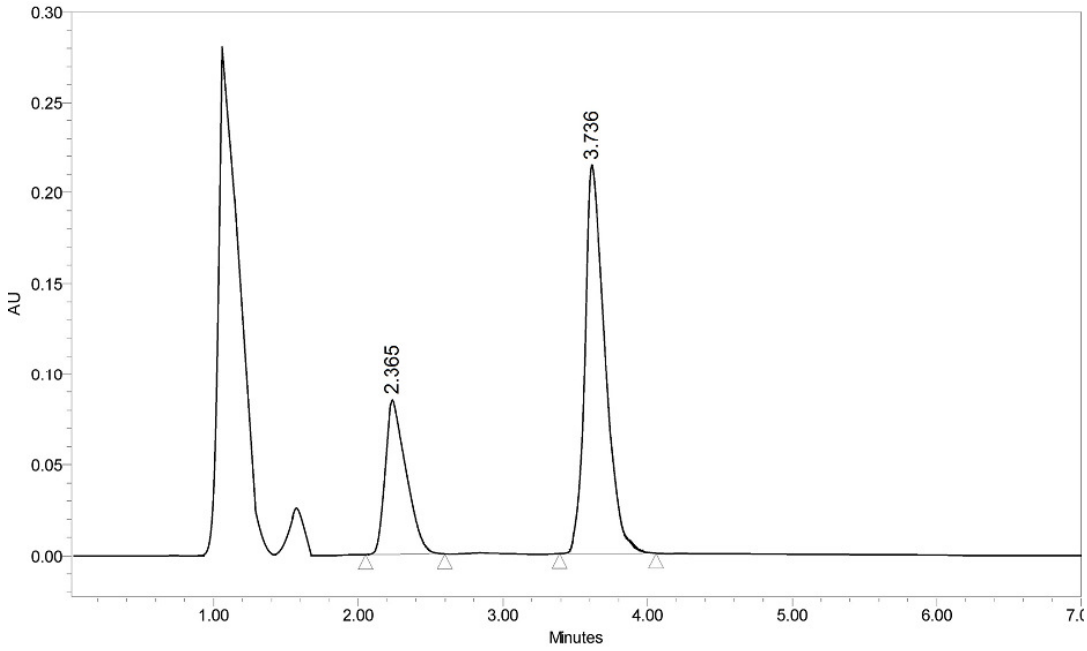

Fig. 6. Typical chromatogram for esomeprazole & naproxen in plasma (standard)

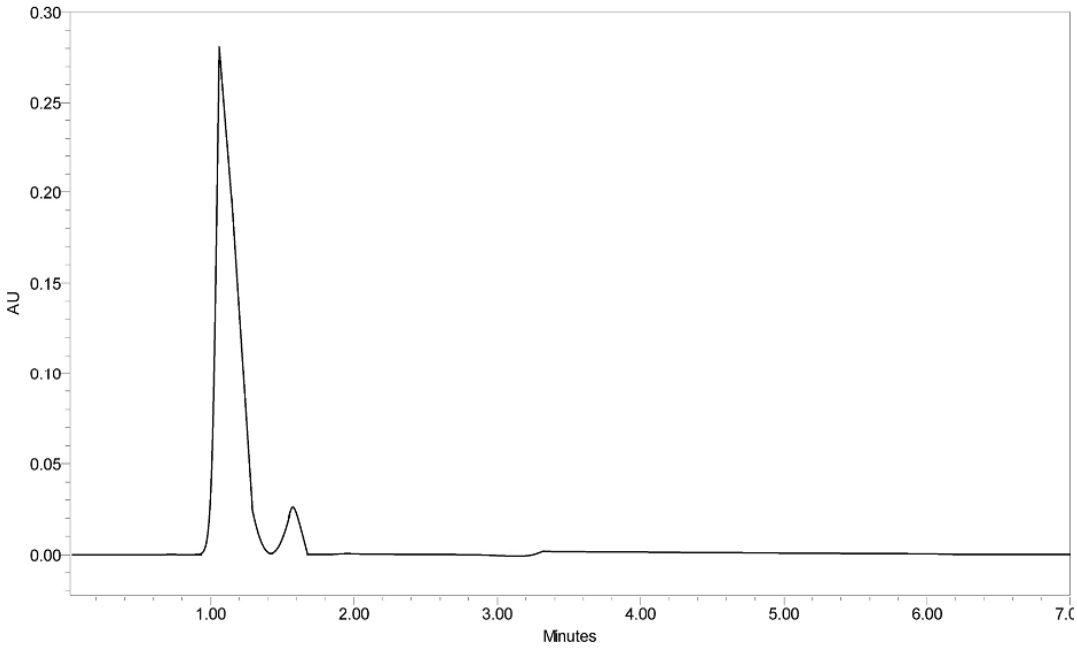

Fig. 7. Typical chromatogram for the blank plasma

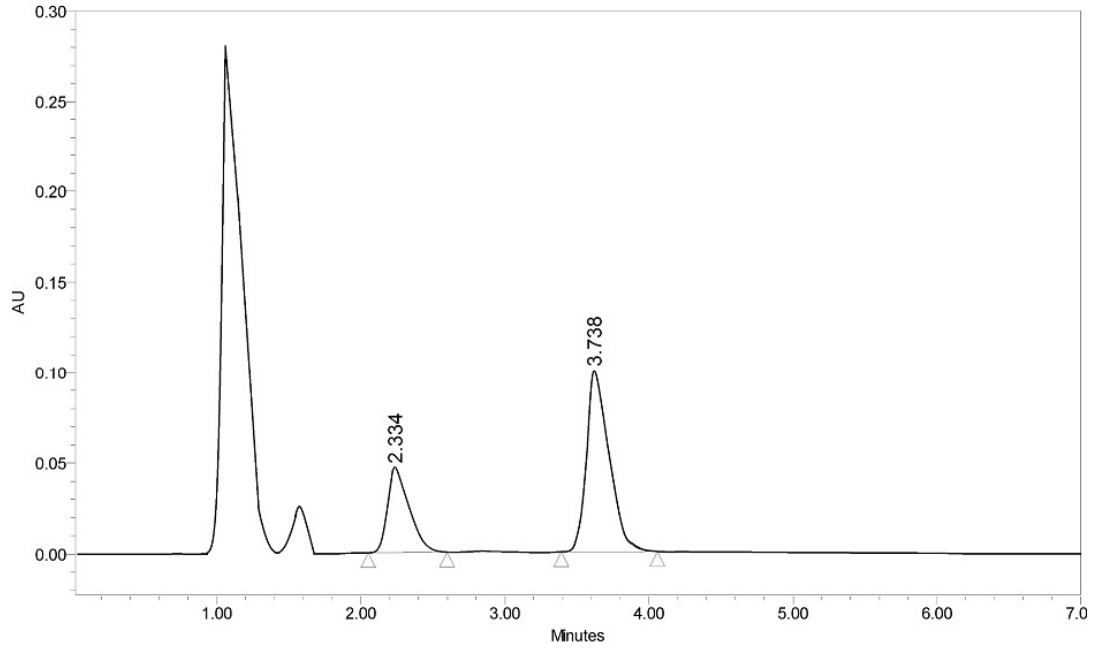

Fig. 8. Typical chromatogram for esomeprazole & naproxen for ruggedness study

The Accuracy recoveries were 98.97-99.84% [esomeprazole] & 99.80–100.95% [naproxen] and reproducibility was found to be satisfactory The Accuracy data was summarized in (Tables 5 and 6). In order to test the linearity of the method, six dilutions of the working standard solutions of the drug in the range of 1 to 6 ppm [esomeprazole] & 25 to 150 ppm [naproxen] were prepared. The data was represented in (Tables 7 and 8). Each of the dilutions was injected into the column and the graph for the linearity curve was represented in (Figs. 3 and 4). The method was duly validated by evaluation of the required parameters. The Lower limit of quantification (LLOQ) for esomeprazole & naproxen were found to be 0.04µg/ml and 0.4µg/ml respectively.

5. CONCLUSION

A simple Bioanalytical method is developed to quantify esomeprazole & naproxen in human plasma. The validated method covers the wide range of linearity over 1 to 6 ppm [esomeprazole] & 25 to 150 ppm [naproxen] and is therefore suitable for the determination of esomeprazole & naproxen in plasma at different therapeutic dose levels. Samples were prepared by using protein precipitation method for analysis. The mobile phase used is a binary mixture of potassium dihydrogen phosphate buffer [pH 3.0] and acetonitrile [HPLC Grade] in 60:40 (v/v) proportions. The % mean recovery was found to be in the range of 98.97-99.84% [esomeprazole] & 99.80–100.95% [naproxen]. The developed method is simple, selective, precise, accurate and rapid. Esomeprazole & naproxen is found to be stable when subjected under different stability conditions. The proposed method can be applied to monitor plasma concentrations of esomeprazole & naproxen in pharmacokinetic studies. It can also be used for therapeutic drug monitoring in order to optimize drug dosage on an individual basis. The mobile phase used for the developing the method 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 with plasma for their estimation. Hence the method can be easily adopted as an alternative method for the routine determination of esomeprazole & naproxen in plasma depending upon the availability of chemicals and nature of other ingredients present in the sample. Finally the simplicity of sample preparation and the shorter chromatographic runtime gives the method capability for high sample throughout. From the results of all the validation parameters we can conclude that the present method can be useful for pharmacokinetic/bioequivalence studies with desired precision and accuracy.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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