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Development and validation of RP-HPLC method for the simultaneous estimation of naproxen sodium and esomeprazole magnesium in pharmaceutical tablet dosage form

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ABSTRACT

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.

Keywords: Naproxen sodium, Esomeprazole magnesium trihydrate, RP-HPLC method, C_{18}Thermo eletrole, Acetonitrile, Method development and Validation.

INTRODUCTION

Naproxen sodium (NS), 6 methoxy-α-methyl-2-naphthalenneacetic acid (Fig1) is a nonsteroidal anti-inflammatory drug (NSAID).[1] Like that of other NSAIDs, it is believed to be associated with the inhibition of cyclooxygenase activity. Two unique cyclooxygenases have been described in mammals. The constitutive cyclooxygenase, COX-1, synthesizes prostaglandins necessary for normal gastrointestinal and renal function.[2] The inducible cyclooxygenase, COX-2, generates prostaglandins involved in inflammation.[3] Inhibition of COX-1 is thought to be associated with gastrointestinal and renal toxicity while inhibition of COX-2 provides anti-inflammatory activity.[4] Esomeprazole magnesium trihydrate (ES), bis (5-methoxy-2-[(s)-[(4-methoxy-3,5-dimetyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole-1-yl) magnesium trihydrate(Fig2) is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of H+/K+-ATPase in the gastric parietal cell.[5] By acting specifically on the proton pump, esomeprazole blocks the final step in acid production, thus reducing gastric acidity. This effect is dose-related up to a daily dose of 20 to 40 mg and leads to inhibition of gastric acid secretion.[6] Literature review revealed that analysis of Naproxen sodium and Esomeprazole magnesium trihydrate carried out by UV-Spectrophotometer.[7], simultaneous equation method by spectroscopy were also reported[8, 9].There are some UV-spectrophotometric methods and three RP-HPLC methods have been reported for estimation of Naproxen sodium and Esomeprazole magnesium trihydrate in Pharmaceutical tablet dosage form[10]. Stability indicating assay of Esomeprazole and Naproxen in Tablets by RP-UPLC PDA-Method were reported earlier.[11, 12]The present work describes simple, specific, rapid, accurate and precise chromatographic method based on RP-

HPLC mechanism for estimation of drugs in tablet dosage form.

MATERIALSANDMETHODS

Instruments: The chromatographic technique performed on a ShimadzuLC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software , reversed phaseC_{18}column(Thermo eletrole, ODS, 250mm × 4.6 mm i.d, 5µm) as stationary phase. Thermo Electron Corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220,Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

Reagents and Chemicals: Pharmaceutically pure samples of Naproxen sodium and Esomeprazole magnesium trihydrate were obtained as gift sample from Chandra laboratories pvt ltd, Prashanthinagar, Kukatpally, Hyderabad ,India. The purity of the drugs were evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drugs were used without further purification. Acetonitrile, methanol, Water and Potassium dihydrogen orthophosphate (HPLC-grade, were from Merck). Marketed formulation ofVimovo contains 500mg of Naproxen sodium & 20mg of Esomeprazole magnesium trihydrate. Vimovo approved by US-FDA & it is not available in India so I procured it from US market (AstraZeneca ltd).

Determination of Working Wavelength(λmax): 10 mg of Naproxen sodium standard drug was taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. 10 mg of Esomeprazole magnesium trihydrate standard drug was taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solutions were scanned in UV between 200-400 nm using methanol as blank. The λ_{max} of Naproxen sodium & Esomeprazole magnesium trihydrate were found to be 232nm & 206nm respectively. Then 220nm was selected as common wavelength for simultaneous estimation of both the drugs, as these are eluting in the same mobile phase with good absorbance. The maximum absorbance with good peak intensity, good peak shape and height was observed at 220nm (Fig. 3)

Analysis of formulation: 125mgof Naproxen sodium & 5mg of Esomeprazole magnesium trihydrate standard drugs were weighed and transferred to 100 ml of volumetric flask and dissolved in mobile phase. The flask was shaken and volume was made-up to mark with mobile phase to givea primary stock solution containing 1250ppm of Naproxen sodium& 50ppm of Esomeprazole magnesium trihydrate. From the above solution 10ml of solution is pipette out into a 100 ml volumetric flask and volume was made up to mark with mobile phase to give a solution containing 125ppm of Naproxen sodium& 5ppm of Esomeprazole magnesium trihydrate. For the estimation of the drugs in tablet formulation twenty tablets, (Vimovo, US-AstraZeneca)wereweighedandtheiraverageweightwasdetermined.Thetabletswerethenfinelypowdered.Appropriatequantityequivalentto 500mg of NS & 20mg ESO Mg $3H_2O$ was accurately weighed and The powder was transferred to 100mlvolumetric flask and shaken vigorously with mobile phase and sonicatedfor15minandvolume made up to the mark with mobile phase. The solution was shaken vigorously and filtered by using whatmann filter no.41. from the above filtered clear solution 10ml of sample pipette out into a 100 ml volumetric flask volume made up to the mark with mobile phase to give a solution containing 125ppm of Naproxen sodium& 5ppm of Esomeprazole magnesium trihydrate.

Calculation: 5 replicates of each of sample and standard solutions were injected and their average peak areas were taken. The amount of Naproxen sodium & Esomeprazole magnesium trihydrate present in the formulation was determined by using the formula given below, and results shown in table 1

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

…… (1)

Where,

AS: Average peak area due to standard preparation
AT: Average Peak area due to assay preparation
WS: Weight of standard drug taken
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation
DS: Dilution of standard preparation
AW: Average weight of 20 tablets
LC: Label claim
P: Purity of standard drug

Optimized HPLC conditions: Thermo eletrole, ODS (250mm × 4.6 mm i.d, 5µm) column maintained at ambient temperature was used as stationary phase. Isocratic mobile phase consisting phosphate buffer P^H3.8, Acetonitrile & Methanol in ratio 30:50:20v/v, at a flow rate of 1ml/min was used. The mobile phase was filtered using 0.45µm filter paper and degassed for 10min by sonication. Samples of 20µl were injected into the HPLC system with the runtime of 5min. Retention time of the drugs obtained under these conditions were 2.417 and 3.903 for NS and ESO Mg $3H_2O$ respectively. For the quantitative analytical purposes the wavelength was set at 220nm. The typical chromatogram of the mixture was shown in Fig.6

METHODVALIDATION[13]

Linearity[14]: Linearitywasstudiedbyanalyzingfivesamplesolutionscoveringtherange of 75-175 µg/ml of NS & 3-7µg/ml of ES. From the primary stock solution which containing concentration of 1250ppm of NS & 50ppm of ES, from that0.6ml,0.8ml,1.0ml,1.2ml,1.4 ml of aliquots are pipette out into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations75-175 µg/ml of NS & 3-7µg/ml of ESO Mg $3H_2O$. Overlay chromatogram of NS & ES is shown in Fig. 7. Calibration curve(Fig. 8)with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least quare method.

Method precision(Repeatability): The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n=6), without changing the parameter of the proposed chromatographic method. The % RSD in all cases was within the acceptable limit (≤ 2%).

Limit of detection and limit of quantification: The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations(2) and(3), respectively.

$$LOD = 3.3\delta / S \quad \ldots\ldots\ldots (2)$$

$$LOQ = 10\delta / S \quad \ldots\ldots\ldots (3)$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve
The slope" S" estimated from the calibration curves of the analytes.

Accuracy(Recovery study): The accuracy of the method was determined by calculating the recoveries of NS & ES, by the standard addition method. Known amounts of standard solutions of NS & ES were added at 20 % (25mcg NS, 1mcg ES) concentration to pre quantified sample solutions of NS &ES(100,125,150µg/ml& 4, 5,6µg/ml respectively). The amount of NS & ES recovered was estimated by using the following formulas.

$$\%Recovery = \frac{amount found}{Amount added} \times 100$$

$$Amount Found (mcg/ml) = \frac{\text{Mean test area}}{\text{Mean standard area}} \times \text{Standard concentration}$$

Specificity: In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are un affected by the presence of these extraneous materials. There should be no interference of the diluents, placebo at retention time of drug substances. (Fig. 9)

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analytes of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in Table. No. 5

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD assay values between two analysts were calculated i.e. (limit ≤ 2%). This indicates the method was rugged. The results were shown in Table. No. 6

RESULTS ANDDISCUSSION

In RP HPLC method, the primary requirement for developing a method for analysis that the using different solvents and buffers and columns to get better retention time and theoretical plates, and

better cost effective and time saving method than the previously developed methods. The λmax (working wave length) of the NS &ES in methanol was found to be 220nm (Figure no. 3) by scanning in uv region.The chromatographic method was optimised with mobile phase consisting of Phosphate buffer (pH adjust to 3.8using OPA) : Acetonitrile : Methanol (30:50:20v/v)and Thermo electrode ODS column. All the validation parameters were studied at a wavelength 220nm. Accuracy was determined by calculating the recovery (Table.No.4) and the results were in acceptable range (limit 98-102%). The method was successfully used to determine the amount of NS &ESpresentintheTablet.Theresultsobtainedwereingo odagreementwiththecorrespondinglabeled amount (TableNo.1). The method was linear in the concentration range of75-175 µg/ml of NS & 3-7µg/ml of ES (Fig.7&8, Table No.2).Precision was calculated as repeatability(Table No.7).Robustness and ruggedness results were in acceptable range (Table No.5 and Table No.6).Summary of all validationparametersformethodisgiveninTableNo.8.

Byobservingthevalidationparameters,themethodwasf oundtobesimple,slective,accurateandprecise.Henceth emethodcanbeemployedfortheroutineanalysis of Naproxen sodium and Esomeprazole magnesium trihydrate in Pharmaceutical dosage form.

CONCLUSION

The proposed RP-HPLC method was found to be simple, selective, accurate and precise for determination of Naproxen Sodium and Esomeprazole magnesium trihydrate in tablet. The method utilizes easily available and cheap solvent for analysis of vimovo, hence the method was also economic for estimation ofNaproxen Sodium and Esomeprazole magnesium trihydrate from Tablet. Thecommonexcipientsandotheradditivesareusuallypr esentintheTabletmixture was not interfere in the analysis of vimovo; hence it can be conveniently adopted for routine quality control analysis of the drug in pharmaceutical formulation.

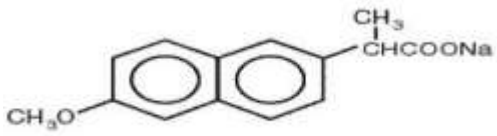

Fig.1: Naproxen Sodium

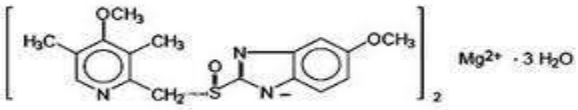

Fig. 2: Esomeprazole magnesium trihydrate

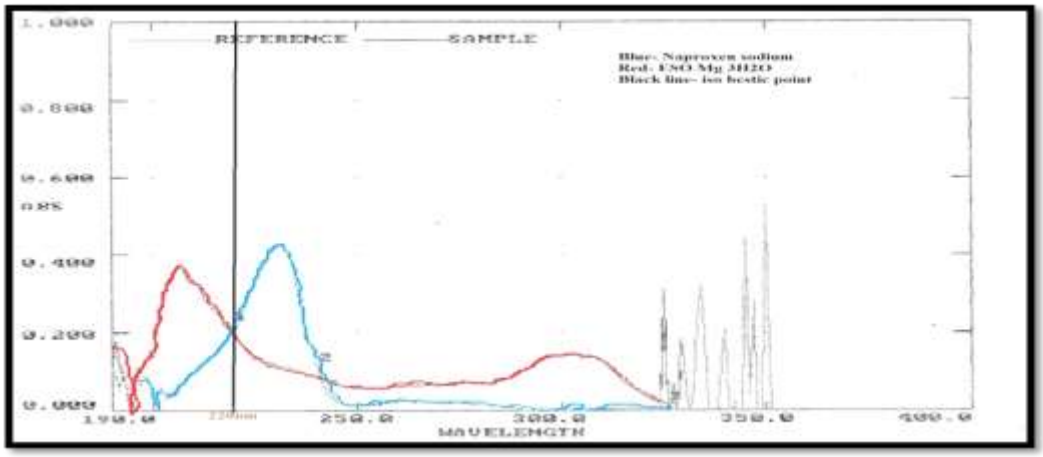

Fig. 3 Determination of working wavelength

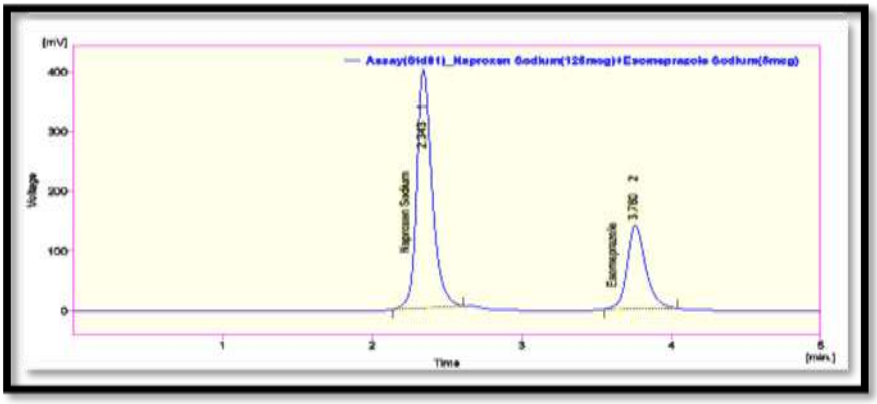

Fig.4chromatogram of Assay standard preparation

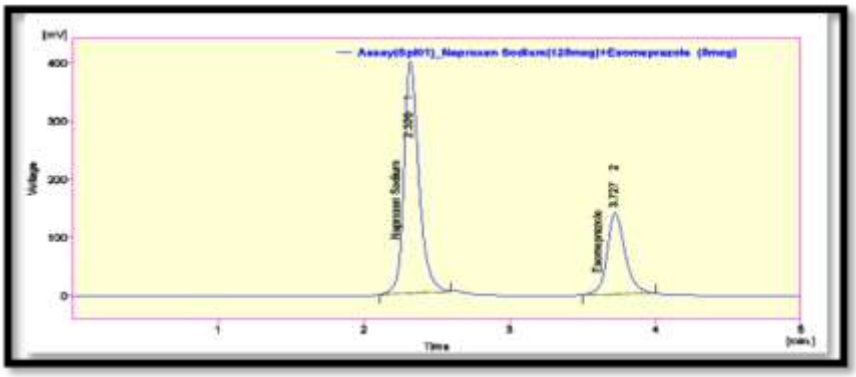

Fig.5chromatogram of Assay sample preparation

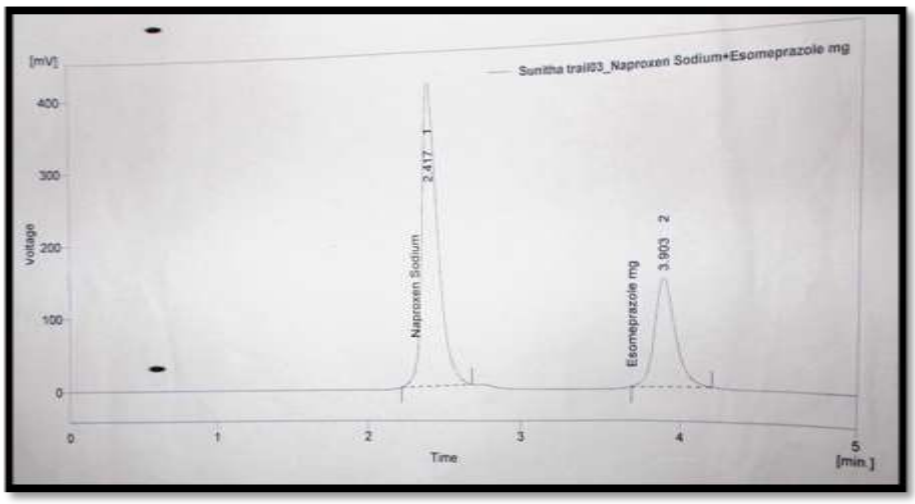

Fig. 6: chromatogram of the mixture (NS &ES)

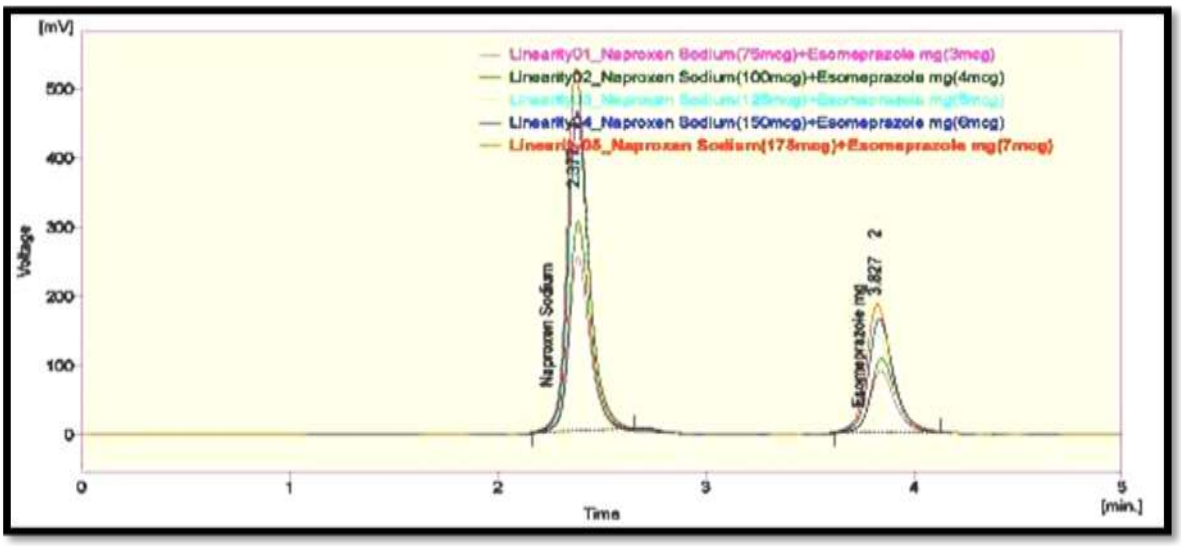

Fig. 7Overlay chromatogram (NS 75-175µg/ml&ES3-7µg/ml)

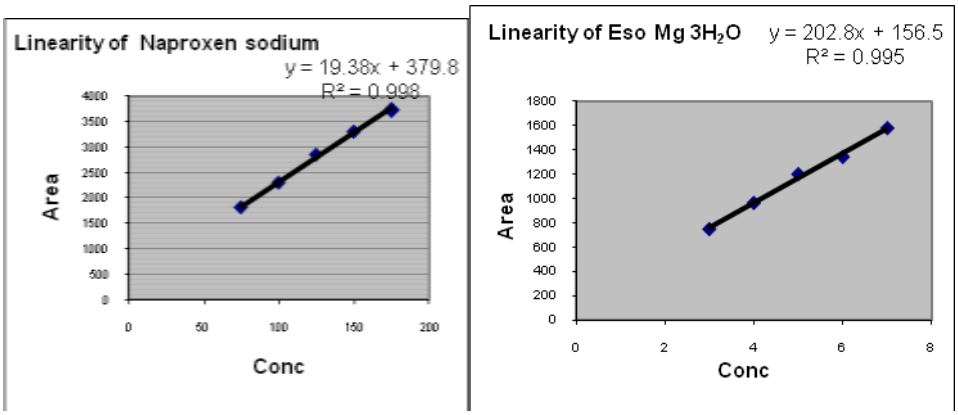

Fig.8 Calibration plot of NS & ESO Mg $3H_2O$ at 220nm

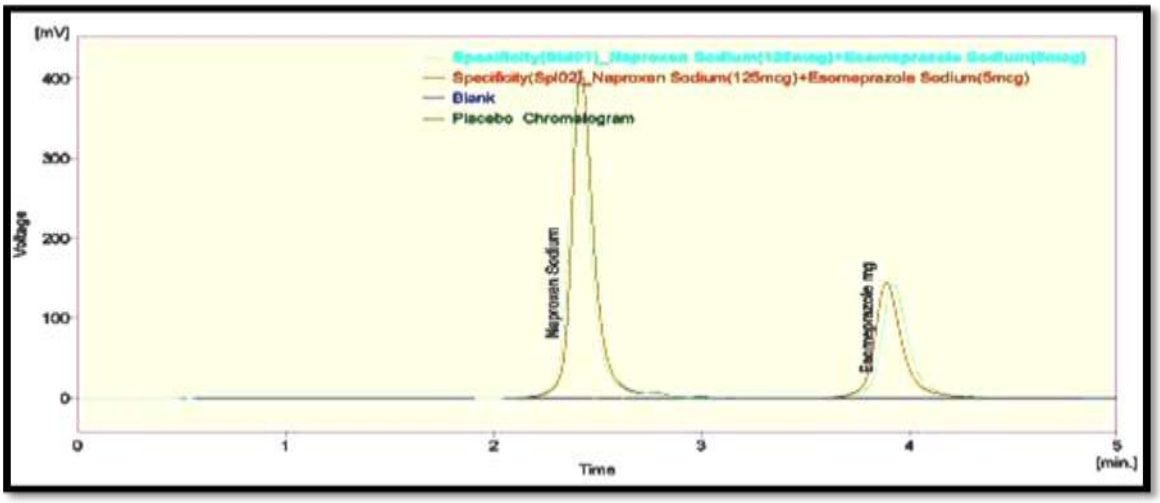

Fig.9: Overlay chromatogram of Specificity (placebo, blank, sample and standard preparations)

Table.No.1: Assay Results

S. NO.	Naproxen sodium	Esomeprazole magnesium trihydrate
	Standard area	Standard area
1	2837.746	1195.66
2	2855.063	1203.04
3	2838.024	1183.867
4	2903.175	1219.935
5	2972.393	1244.407
Average	2881.250	1209.383
	Sample area	Sample area
1	2824.845	1197.264
2	2809.438	1203.362
3	2836.387	1215.822
4	2871.637	1229.738
5	2841.086	1205.181
Average	2836.679	1210.273
Tablet averageweight	650.2mg	650.2mg
Standard weight	500mg	20mg
Sample weight	650.2mg	650.2mg
Label claim	500mg	20mg
Standard purity	99.6%	99.7%
Amount of drug present	490.29mg	19.95mg
%Assay	98.08%	99.77%

Table.No.2: Linearity results

Naproxen sodium		Esomeprazole magnesium trihydrate	
mcg	Area	mcg	Area
75	1818.747	3	754.635
100	2300.963	4	965.853
125	2850.358	5	1203.99
150	3299.764	6	1345.338
175	3741.82	7	1580.483

Table.No.3: LOD&LOQ Results

S. NO	NAPROXEN SODIUM		esomeprazole magnisium trihydrate	
	mcg	Area	mcg	Area
1.	75	1818.747	3	754.635
2	100	2300.963	4	968.853
3.	125	2850.358	5	1203.99
4.	150	3299.764	6	1345.338
5.	175	3741.82	7	1580.483
SD	39.5	767	1.5811	321
Slope	19.4		202.8	

	LOD		LOQ	
Sample	mcg/ml	Area	mcg/ml	Area
NS	6.71	130.55	20.36	395.60
ESO Mg $3H_2O$	0.025	5.23	0.077	15.85

Table.No.4: Recoverydataof NS&ESby RP-HPLC method

Level	S.No	AmountofSample (NS) Taken(µg/ml)	AmountofSample(ESO)Taken(µg/ml)	AmountofStandard Spiked(%)	%Recovery of NS	%Recovery of ESO$mg3H_2O$
I	1	100	4	20%	101.94%	101.43%
	2	100	4	20%		
	3	100	4	20%		
II	1	125	5	20%	99.82%	100.25%
	2	125	5	20%		
	3	125	5	20%		
III	1	150	6	20%	99.41%	100.83%
	2	150	6	20%		
	3	150	6	20%		

Table.No.5: Results of Robustness study

Parameter	Naproxen sodium			Esomeprazole magnesium trihydrate		
	Retention time(min)	Peak area	% Assay	Retention time(min)	Peak area	% Assay
Flow Rate						
0.8 ml/min	3.073	2895.279	99.07%	4.920	1229.887	99.91%
1.0 ml/min	2.437	2949.018	100.91%	3.973	1260.607	101.38%
1.2 ml/min	1.867	2882.906	98.64%	3.007	1218.692	98.01%
Wavelength						
218nm	2.277	2880.023	98.55%	3.677	1221.107	98.20%
220nm	2.437	2949.018	100.91%	3.973	1260.607	101.38%
222nm	2.307	2895.803	99.09%	3.713	1230.841	98.99%

Table.No.6: Ruggedness results

	Naproxen sodium				Esomeprazole magnesium trihydrate			
	Sample area	Standard area	% Assay	%RSD	Sample area	Standard area	% Assay	%RSD
Analyst 1	2838.967	2817.159	100.77%	1.24	1191.389	1196.302	99.58%	1.50
Analyst 2	2941.308	2927.662	100.46%		1274.141	1248.390	102.06%	

Table.No.7 Method precision

Naproxen sodium			Esomeprazole magnesium trihydrate	
S.No.	Retention time	Area	Retention time	Area
1.	2.437	2949.018	3.973	1240.607
2.	2.443	2942.268	3.950	1228.58
3.	2.42	2922.394	3.917	1243.396
4.	2.417	2909.010	3.903	1237.116
5.	2.423	2923.010	3.893	1222.904
6.	2.38	2923.542	3.843	1216.124
Avg	2.4200	2928.207	3.913	1231.454
SD	0.0221	14.710	0.046	10.722
%RSD	0.91	0.50	1.16	0.87

Table.No.8:Validationparameters of evaluated method

S.No.	Parameters	Values obtained for NS	Values obtained for ESOmg3H_2O
1.	Analyte wave length	220nm	220nm
2.	Accuracy (%Recovery)	99.41-101.94%	100.25-101.43%
3.	LOD(µg/ml)	6.7	0.025
4.	LOQ(µg/ml)	20.36	0.077
5.	Linearity (µg/ml)	75-175	3-7
	Regression coefficient (R2 value)	0.9984	0.9951
6.	Precision(%RSD) (Repeatability)	0.50	0.87
7	Robustness(%Assay)	98.55-101.91%	98.01-101.38%
8.	Ruggedness(%RSD analyst to analyst variation)	1.24	1.50

SD=Standard deviation, LOD=Limit of detection, LOQ=Limit of quantification,
RSD =Relative standard deviation, NS= Naproxen Sodium,
ESOmg3H_2O= Esomeprazole magnesium trihydrate.

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