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

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ABSTRACT

The objective of the study was to develop a simple, accurate, precise RP-HPLC method for the determination of Esomeprazole and Naproxen using mobile phase (A mixture of Acetonitrile and Methanol in the ratio of 60:40 was considered to be the optimal composition of solvent) as the solvent. The proposed method was involves the measurement of retention time at selected analytical wavelength 260.0 nm was selected as the analytical wavelength. The retention time of Esomeprazole and Naproxen was found to be 3.425 and 4.352. The linearity of the proposed method was in the range of r = 0.9999 for Esomeprazole and r = 0.9999 for Naproxen. The method was statistically validated for its linearity, accuracy and precision of the formulation.

KEYWORDS: Esomeprazole, Naproxen, RP-HPLC method.

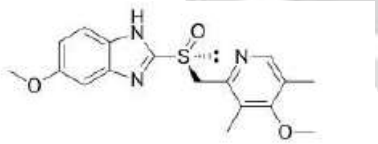
1. INTRODUCTION:

The drug analysis plays an important role in the development, manufacture and therapeutic use of drug. Standard analytical procedure for newer drugs or formulation may not be available in pharmacopoeias, it is essential for the develop a newer analytical methods which are accurate, precise, specific, linear, simple and rapid. Many studies have been reported for the determination of Esomeprazole and Naproxen in Pharmaceutical formulations.

Naproxen is chemically designed as (2S)-2-(6-methoxynaphthalen-2-yl) propanoic acid Naproxen is used as Anti inflammatory and analgesic drug and Esomeprazole is a chemically bis (5-methoxy-2-[(S)-[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazol-1-yl) a compound that inhibits gastric acid secretion. Esomeprazole is cost effective in the treatment of gastric esophageal reflux diseases.

DRUG PROFILE:

Esomeprazole



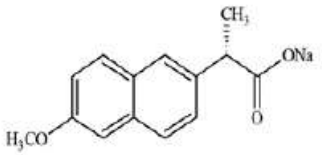
Structure: Esomeprazole

Chemical name: 5-methoxy-2-{(S)-[(4-methoxy-3, 5-dimethyl-2-Pyridinyl) methyl] sulfinyl} benzimidazole.

Molecular formulae : $C_{17}H_{19}N_3O_3S$

Molecular Weight : 345.4

Naproxen:



Naproxen Sodium

Structure:

Chemical name: (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid
Molecular formulae: $C_{14}H_{14}O_3$
Molecular Weight: 230.3

METHOD VALIDATION :

Chemicals and reagents:

The working standards of Esomeprazole and Naproxen were gifted from Pharma Tech labs, Hyderabad. Acetonitrile and Methanol (HPLC grade) were obtained from E. Merck Ltd Mumbai, India.

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

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

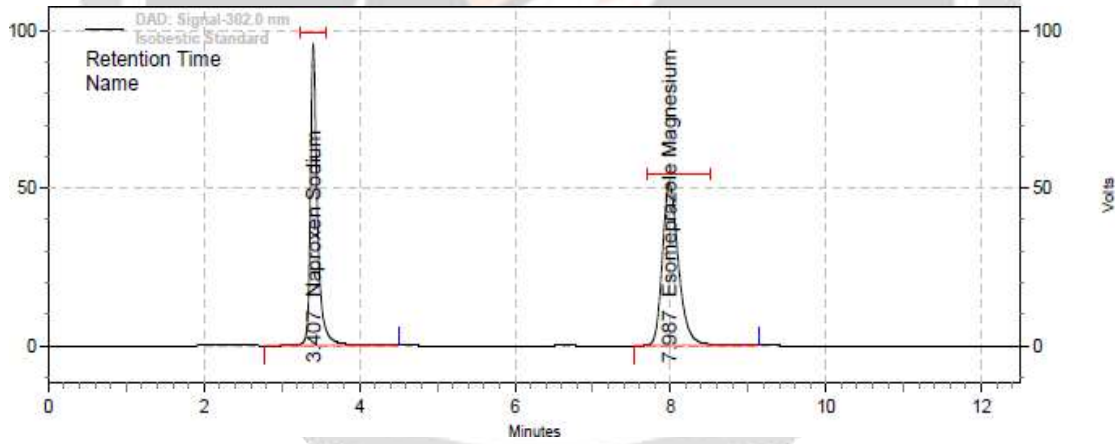


Fig.No.1: Chromatogram of optimized trail

Tailing factor was <2.0, Resolution was optimum and Plate count was >2000, so this method is considered as the optimized method. Procedure for preparation of analytical solutions:

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

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

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

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

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

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

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

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

Placebo solution:

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

Chromatographic Conditions:

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

Table No.1:Chromatograpic Condition of Optimized method

1. Precision

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

a) System Precision (Repeatability)

b) Method Precision (Reproducibility)

c) Intermediate precision (Ruggedness)

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

a) System Precision (Repeatability)

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

Peak Results for System Precision

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

Table No.2:Peak Result for System Precision

Data Interpretation

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

b) Method Precision (Reproducibility)

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

Method Precision Results for Naproxen

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

Table No.3:Method Precision Result for Naproxen

Method Precision Results for Esomeprazole magnesium

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

STDV	0.032
%RSD	0.157

Table No.4:Method Precision Result for Esomeprazole magnesium

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

2. Linearity

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

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

Weigh accurately about 20.65 mg of Esomeprazole magnesium WS (Equivalent to 20 mg Esomeprazole) and transfer into a 200 mL volumetric flask. Add about 100 mL of diluent and sonicate for 5 minutes to dissolve. Cool and dilute up to the volume with diluent.

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

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

Preparation of Linearity Dilution for Naproxen and Esomeprazole magnesium:

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

Table No.5:Linearity dilution for Naproxen and Esomeprazole magnesium

Calculation for Linearity of Naproxen

Linearity Level	Conc(mcg/mL)	Area (Injection-1)	Area (Injection-2)	Average
60	300	53087978	53120909	53104444
80	400	70853430	70938599	70896015
100	500	90124646	90341412	90233029
120	600	107228155	107404925	107316540

160	800	140669395	141002883	140836139
Correlation coefficient		0.999		
Slope		175839.7448		
Y Intercept		1040565.905		
% Y-intercept		0.969623047		

Table No.6:Linearity Calculation for Naproxen

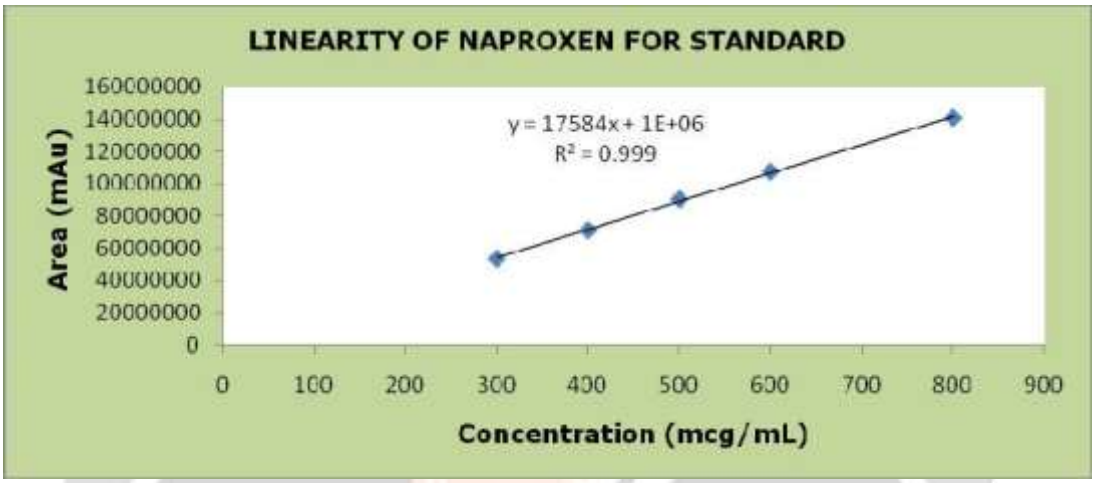


Fig.No.2: Linearity Plot of Naproxen

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

Table No.7:Linearity Calculation for Esomeprazole magnesium

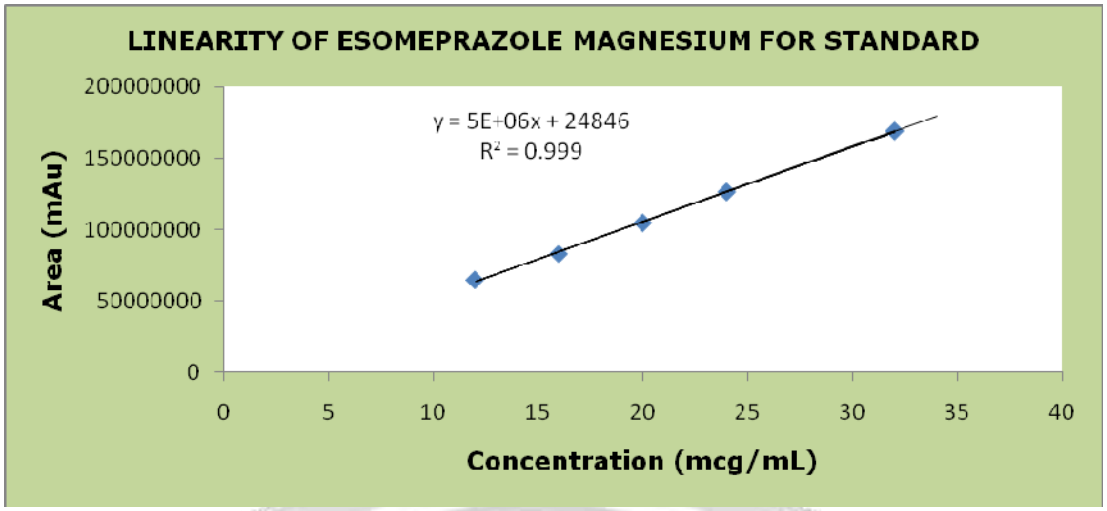


Fig.No.3: Linearity Plot of Esomeprazole magnesium

Data Interpretation

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

3. Accuracy

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

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

Procedure for Accuracy

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

Weigh accurately about 10.33 mg of Esomeprazole magnesium WS (Equivalent to 10 mg Esomeprazole), and 273.86 mg of Naproxen Sodium (Equivalent to 250 mg of Naproxen), and transfer into a 100 mL volumetric flask. Add about 293.5 mg of Naproxen and Esomeprazole magnesium Tablet Placebo and 50 mL of diluent, and sonicate for 15 minutes to dissolve. Cool and dilute up to the volume with diluent. Transfer 5 mL of this above solution through pipette into a 50 mL volumetric flask and dilute up to the volume with mobile phase and mix. Filter the solution through 0.45 µm Nylon filter and collect the solution in an HPLC vial after discarding about first 2 mL of filtrate.

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

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

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

Weigh accurately about 30.97 mg of Esomeprazole magnesium WS (Equivalent to 30 mg Esomeprazole), and 821.60 mg of Naproxen Sodium (Equivalent to 750 mg of Naproxen), and transfer into a 100 mL volumetric flask.

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

Results for Accuracy of Naproxen

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

Table No.8 : Peak Results for Accuracy of Naproxen

Results for Accuracy of Esomeprazole magnesium

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

Table No.9 : Peak Results for Accuracy of Esomeprazole magnesium

Data Interpretation

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

Specificity

Blank & Placebo Interference: Placebo was injected by weighing the equivalent amount

present in the finished drug product and analysed for interference due to placebo.

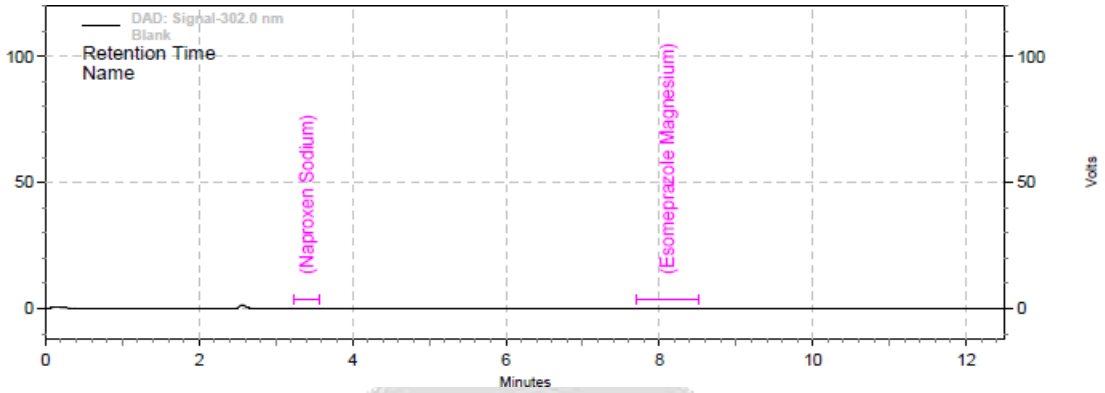


Fig.No.4: Chromatogram of Blank

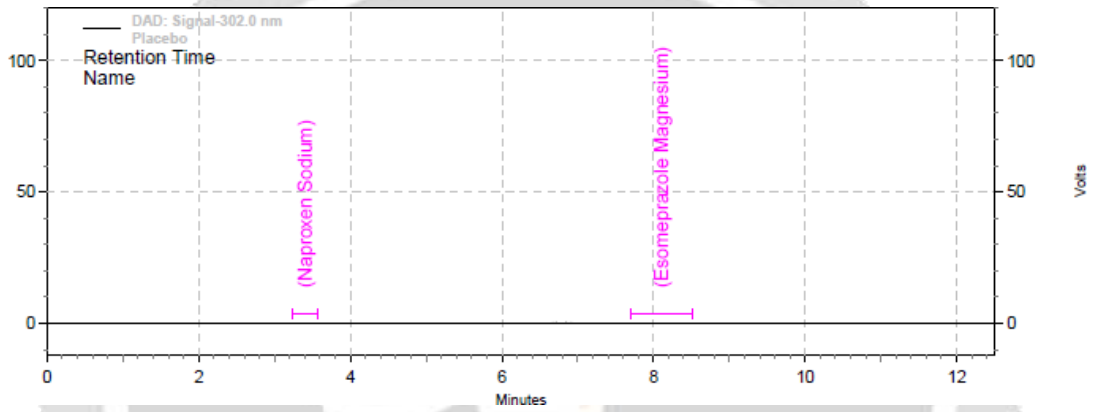


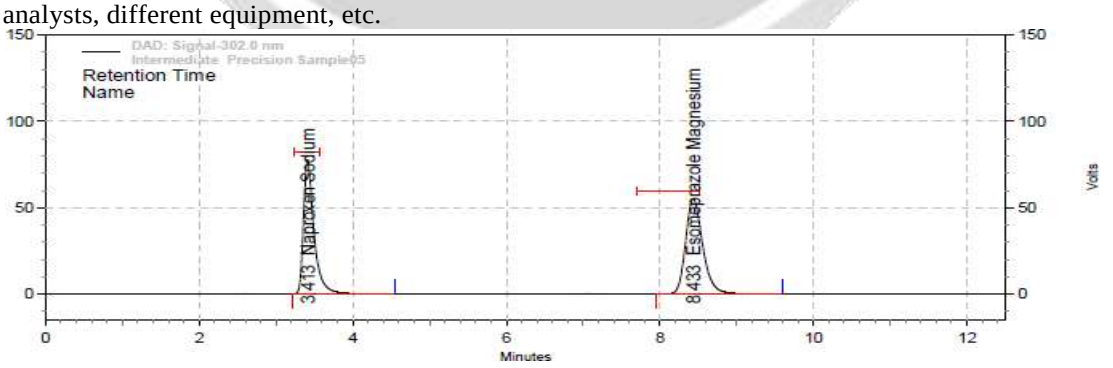
Fig.No.5: Chromatogram of Placebo

Data Interpretation

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

4. Intermediate Precision (Ruggedness)

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



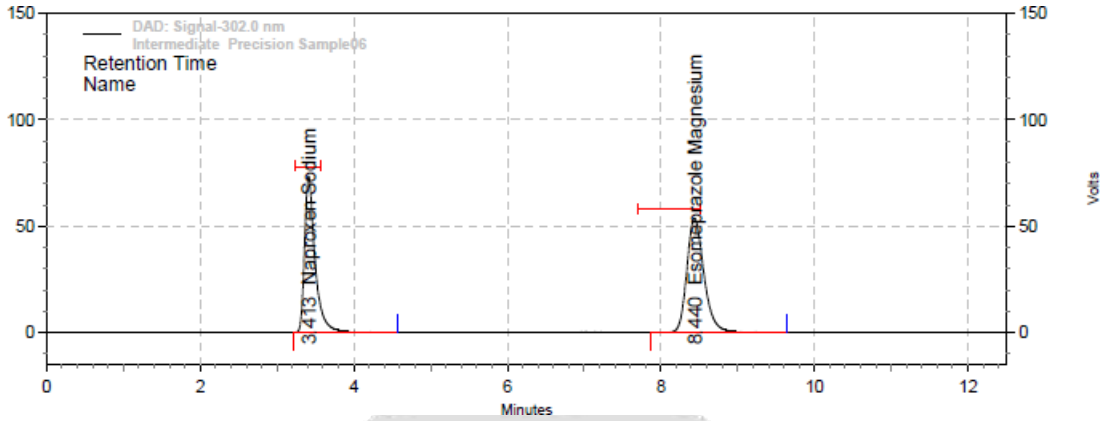


Fig.No.6: Chromatogram of Intermediate precision

Intermediate Precision Results for Naproxen

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

Table No.10: Intermediate Precision result for Naproxen

Intermediate Precision Results for Esomeprazole magnesium

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

Table No.11: Intermediate Precision result for Esomeprazole magnesium

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

5. ROBUSTNESS:

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

System suitability results for Naproxen:

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

Table No.12: System Suitability result for Naproxen

System suitability results for Esomeprazole magnesium:

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

Table No.13: System Suitability result for Esomeprazole magnesium

Robustness results obtained for Naproxen:

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

Table No.14: Robustness result for Naproxen

Robustness results obtained for Esomeprazole:

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

Table No.15: Robustness result for Esomeprazole magnesium

RESULT AND DISCUSSION

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

Combined Method precision and Robustness results obtained for Naproxen:

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

Table No.16: Combined Method precision and Robustness result for Naproxen

The optimum wavelength for detection was 302 nm at which better detector response for both the drugs was obtained. The average retention times for Naproxen and Esomeprazole magnesium was found to be 3.397 and 8.146 min, respectively. According to United States Pharmacopeia, system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in various concentration range with correlation. The low values of RSD indicate that the method was precise and accurate. The mean recoveries were found in the range of 98 – 102 %. System precision is evaluated by injecting 6 injections of standard solution and low value of % RSD shows that system is precise.

Combined method precision and Robustness results obtained for Esomeprazole magnesium:

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

Table No.17 : Combined Method precision and Robustness result for Esomeprazole magnesium

Precision for method is evaluated by analyzing a sample of homogenous batch six times and the low % RSD value shows the method is precise. Method robustness was evaluated by alteration of flow rate (±0.2 mL), Wavelength (± 2 nm), Mobile phase Organic Content (±5%) and it was found robust as % RSD was below 2.0%.

6. Solution Stability

Solution stability for Naproxen

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

Table No.18: Solution Stability for Naproxen

Solution stability for Esomeprazole magnesium

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

Table No.19: Solution Stability for Esomeprazole magnesium

Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slot in different laboratories, by different analysts, different column, different system using similar environmental conditions, the % R.S.D. reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations. Both sample solution and standard solution are stable at 25°C for 24 hrs. as the % difference in the RSD was found to be less than 2.0%.

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

CONCLUSION

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

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

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