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Analytical Method Development and Validation for Simultaneous Estimation of Naproxen and Esomeprazole in Pharmaceutical Dosage form by RP-HPLC

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Abstract

A new, accurate, simple, rapid, inexpensive and sensitive RP-HPLC method has been developed for the quantification of esomeprazole and naproxen in tablet dosage form, the separation of these molecules is achieved on PHENOMENEX C18 – column (250 × 4.6mm, 5µm) using mobile phase Phosphate Buffer, Acetonitrile and Methanol in the ratio of 459:458:83) with by induction of 20µl sample. Wavelength is selected at 303 nm with flow rate of 01ml/min. The retention time of Esomeprazole was found to be 3.0 and for Naproxen it was 8.2min. The linearity range is 89.6-134.4µg/ml and 4.8-7.2µg/ml for Naproxen and Esomeprazole magnesium trihydrate respectively. The correlation coefficient was 0.9999 for Esomeprazole and 0.9961 for Naproxen. The method is validated for Specificity, Accuracy, Precision, LOD, LOQ, Linearity, and Robustness.

Keywords: Naproxen, Esomeprazole, RP-HPLC Method

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Introduction

The major role in the manufacture and development of drug is drug analysis. In pharmacopoeias the formulation of new drugs are not present, so It is necessary to develop simple, accurate, specific, linear analytical methods.

Drug Profile

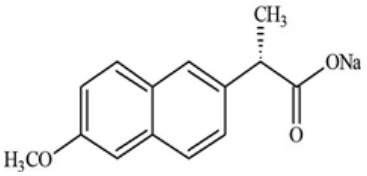
Naproxen:

Naproxen is a nonsteroidal anti-inflammatory drug. It has both Anti-inflammatory and analgesic properties, it is mostly used for fever, inflammation and to reduce moderate to severe pain, and stiffness. Its acts by inhibiting COX-1 and COX-2 enzymes. Disturbances in the gastrointestinal tract can also be occurred by using Naproxen, Like other NSAIDs.

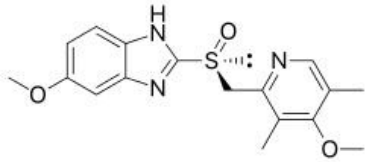
Esomeprazole:

Esomeprazole, chemically known as bis (5-methoxy-2-[(S)-[(4-methoxy-3, 5-dimethyl -2-pyridinyl) methyl] sulfinyl]-1H-benzimidazol-1-yl).Its mode of action involves proton pump inhibition, that result in inhibition of gastric acid secretion. Treatment of diseases like gastric esophageal reflux is done by Esomeprazole.

Structure



Naproxen Sodium



Esomeprazole

Materials and Methods

Chemicals and reagents:

Marketed formulation Vimovo contains 375mg of Naproxen and 20mg of Esomeprazole magnesium trihydrate, procured it from US market (Astrazeneca ltd.) The reference working standards of both drugs were used from Global Pharmaceuticals. Disodium phosphate, Potassium dihydrogen phosphate, Sodium Hydrogen Pellets, Triethylamine, Acetonitrile and Methanol (HPLC grade) were purchased from E. Merck Ltd, Pakistan.

Instrument used: A Hitachi (5110-5410), pH meter Adwa – AD 1020.

Prepration of Solution:

Diluent:

Mobile phase

Stock Solution Preparation:

Solution A (Naproxen):

Accurately weighted working standard of Naproxen equivalent to 28mg Naproxen in 25ml volumetric flask, volume was made up with diluent. This solution having concentration of 1120 µg/ml of Naproxen

Solution B (Esomeprazole):

Accurately weighted working standard of Esomeprazole equivalent to 20mg Esomeprazole in 100ml volumetric flask, volume was made up with diluent. This solution having concentration of 200 µg/ml of Esomeprazole

Standard Prepration:

10ml of solution A and 3ml of solution B then transferred in 100ml volumetric flask and volume maked up with diluent. This solution having concentration of 112 µg/ml of Naproxen and 6 µg/ml of Esomeprazole.

Sample Preparation:

20 tablets of Vimovo was crushed in to fine powder, Accurately Weighted powder equivalent to 375mg Naproxen and 20mg Esomeprazole in 200ml volumetric flask, added 30ml diluent, stirred for 10-15 minutes and maked up to the mark with diluent. from above solution 3 ml was transferred in 50ml volumetric flask and volume maked to the mark with diluent. having concentration of 112 µg/ml of Naproxen and 6 µg/ml of Esomeprazole.

Preparation of Mobile phase:

Mixture of Phosphate Buffer, Acetonitrile and Methanol in the ratio of 459:458:83 are used as Mobile Phase. 0.4 µm membrane filter paper were used for mobile phase filtration than mobile phase was ultrasonicated for 10 min.

Chromatographic condition: Mobile phase having mixture of Phosphate Buffer, Acetonitrile and Methanol in the ratio of 459:458:83 was used to separate NAP and ESO from 20µl sample by using PHENOMENEX C18 – column (250 × 4.6mm x 5µm) as stationary phase, having wavelength of 303nm with flow rate of 1ml/min.

Result and Discussions

Method Development

Separation of two drugs having sharp peaks of Naproxen and Esomeprazole was achieved by using mixture of Phosphate Buffer, Acetonitrile and Methanol in the ratio of 459:458:83 on PHENOMENEX C18 – HPLC column. Mobile phase was selected on the basis of good resolution among Naproxen and Esomeprazole.

Method validation: USP and ICH guidelines are followed for the validation of proposed method, in terms of system suitability, specificity, linearity, precision, LOD, LOQ, and robustness.

System Suitability Test

Before running the sample for initial evaluation, System suitability was performed by 6 replicate injections of Naproxen and Esomeprazole in HPLC system. The results of suitability are given in Table 1

Replicate sample	Peak Area Ref	Tailing Factor	Theoretical Plates
1	337.598	1.862	9313
2	338.718	1.833	8894
3	336.939	1.800	8904
4	336.705	1.800	8894
5	333.400	1.833	8494
6	336.568	1.833	8894
Average	336.654		
Standard Dev	1.78		
%RSD (<2.0)	0.53		

Table 1(a): System Suitability of Naproxen

Replicate sample	Peak Area Ref	Tailing Factor	Theoretical Plates
1	259.645	1.115	8847
2	257.076	1.153	9000
3	257.732	1.134	9000
4	258.895	1.124	8857
5	256.543	1.153	8986
6	257.329	1.124	8833
Average	257.87		
Standard Dev	1.78		
%RSD (<2.0)	0.46		

Table 1(b): System Suitability of Esomeprazole

Specificity:

The ability to determine analyte in the presence of additional compounds, Specificity shows that there is no interference of analyte with mobile phase and placebo, which indicts that analytical method is specified. The chromatogram shown in Figure 1.

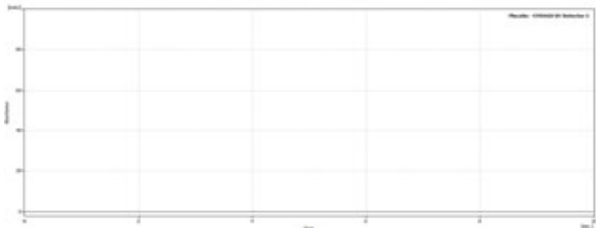


Figure 1(a): Chromatogram of Placebo

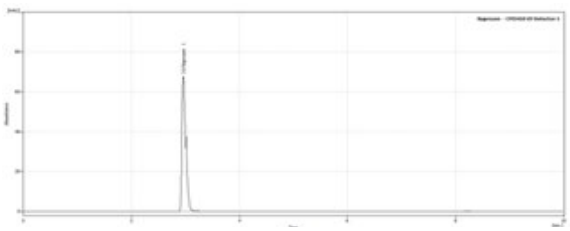
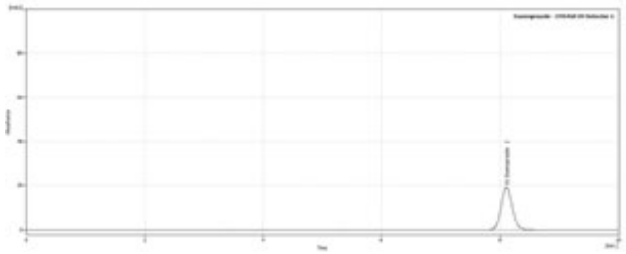
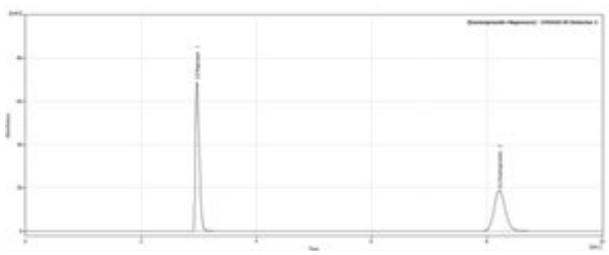


Figure 1(b): Chromatogram of Naproxen



(c): Chromatogram of Esomeprazole



(d): Chromatogram of both drugs

Linearity:

The linearity between the peak area and drug concentration was obtained by ploting X-axis and Y-axis graph, having the concentration of 80ug/ml to 140ug/ml of Naproxen and 200ug/ml to 310ug/ml of Esomeprazole. LOD and LOQ for Naproxen are 9.45ug/ml and 28.6ug/ml, and 0.186ug/ml and 0.565ug/ml for Esomeprazole respectively. The Results obtained are shown in Figure 2(a) and (b).

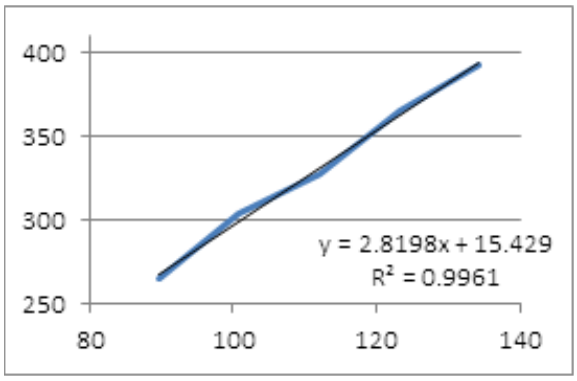


Figure 2 (a): Linearity plot of Naproxen

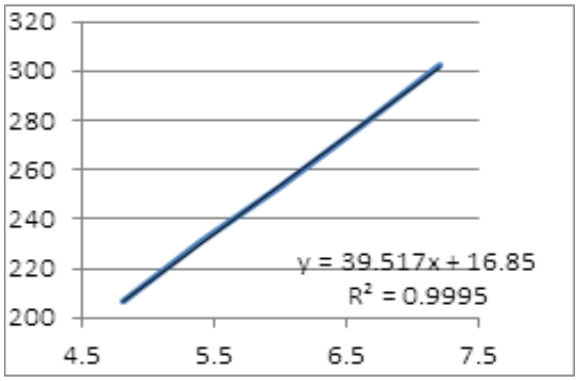


Figure 2 (b) : Linearity plot of Esomeprazole

Recovery

Recovery was performed by spiking Esomeprazole and Naproxen at the level of 80%, 100% and 120% in Placebo and recovered by injecting replicate sample. Spiked and recovered values are shown in Table 4.

Recovery Level	Drugs	Amount Spiked (mg/ml)	Amount Recovered (mg/ml)	Recovery (%age)	Standard Deviation	RSD (%)
80%	Naproxen	300 300 300	303.6 300.86 303.56	100.89	1.57	0.51
	Esomeprazole	16.0 16.0 16.0	16.07 15.85 16.00	99.83	0.11	0.70
100%	Naproxen	375 375 375	373.35 375.07 379.01	100.21	2.90	0.77
	Esomeprazole	20 20 20	20.11 19.99 20.20	100.50	0.10	0.52
120%	Naproxen	450 450 450	452.47 454.12 453.15	100.72	0.82	0.18
	Esomeprazole	24 24 24	23.93 23.99 23.92	99.77	0.37	0.15
			Naproxen	Esomeprazole		
Overall Mean			100.60	100.03%		
Overall Standard Deviation			1.76	0.19		
Overall % RSD			0.48	0.45		

Table 4: Percent recovery of Naproxen and Esomeprazole.

Precision: The degree of scatter between samples is shown by Precision of analytical method. Precision of proposed by analyzing the six replicate sample. Assay of each replicate and %RSD were calculated. The obtained Results are shown in table 5

Drugs	Peak Areas of Replicate	Average Peak Areas of each replicate	Assay %	Average	Standard Deviation	%RSD
Naproxen	**325.868**	**325.697**	**100.64**	**100.93**	0.25	0.257
	325.526					
	324.836	**327.2805**	**101.125**			
	329.725					
	325.324	**326.950**	**101.025**			
	328.576					
Esomeprazole	**252.944**	**252.928**	**100.09**	**100.28**	**0.16**	0.1615
	252.912					
	253.624	**253.662**	**100.38**			
	253.701					
	253.050	**253.617**	**100.36**			
	254.184					

Table 5: Precision of Naproxen and Esomeprazole

Robustness

Robustness is the capacity of sample to remain unchanged in the result of variation of method parameters. Variation like flow rate and Column temperature have no effect on method performance. The Results obtained are shown in table 6.

Naproxen			**Peak Areas of Replicate**	**Average Peak Areas of each replicate**	**Assay %**	**Average**	**Standard Deviation**	**%RSD**
100% Conc.	Change in Flow rate	0.95ml/min	336.729	**337.0945**	**101.98**	**99.74**	**1.98**	**1.9851**
			336.460					
		1.0ml/min	335.941	**336.901**	**99.25**			
			337.861					
		1.05ml/min	333.046	**332.683**	**98.01**			
			332.320					
	Change in column Temp.	25°C	338.214	**337.191**	**100.68**	**100.96**	**0.29**	**0.2874**
			338.168					
		30°C	334.988	**338.076**	**100.95**			
			341.164					
		35°C	339.531	**339.137**	**101.26**			
			338.743					

Table 6 (a): Robustness result of Naproxen

Esomeprazole			Peak Areas of Replicate	Average Peak Areas of each replicate	Assay %	Average	Standard Deviation	%RSD
100% Conc.	Change in Flow rate	0.95ml/min	256.796	**257.485**	**99.35**	**99.42**	**0.185**	**0.1862**
			258.174					
		1.0ml/min	255.982	**256.558**	**99.98**			
			257.194					
		1.05ml/min	255.755	**255.705**	**99.63**			
			255.655					
	Change in column Temp.	25°C	256.995	**257.981**	**100.89**	**101.02**	**0.32**	**0.3181**
			258.967					
		30°C	255.717	**257.716**	**100.79**			
			259.715					
		35°C	259.465	**259.253**	**101.39**			
			259.041					

Table 6 (b): Robustness result of Esomeprazole

Conclusion

In this study, analytical method is developed for quantitative analysis of Naproxen and Esomeprazole in tablet dosage form. The good resolution between Naproxen and Esomeprazole give good results. From the above experimental results, the method was validated according to USP and ICH guideline. So the method can be used for quantitative analysis of naproxen and esomeprazole in pharmaceutical tablets dosage forms.

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