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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NAPROXEN AND ESOMEPRAZOLE IN PHARMACEUTICAL DOSAGE FORM

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Abstract

A simple, sensitive, and precise high performance liquid chromatographic method for estimating the contents of naproxen and Esomeprazole simultaneously has been developed, validated and used in commercial pharmaceutical products. The compounds were well separated on a Symmetry C-18 reversed-phase column by use of a mobile phase consisting of 0.05 M Potassium dihydrogen Phosphate (pH 7.0), and Acetonitrile (70:30 v/v) at a flow rate of 1.0 mL/min with detection wavelength at 286 nm. The linearity ranges were 100-200 µg/L for Naproxen and 4-8 µg/L for Esomeprazole. The recovery found was more than 99 % for both the compounds. The high recovery and low relative standard deviation confirms the suitability of the method for determination of naproxen and Esomeprazole in pharmaceutical dosage forms.

Key Words: Naproxen, Esomeprazole, HPLC.

Introduction

Guidelines on pain management recommend that patients at risk of ulcers receive either a cyclo-oxygenase (COX-2) inhibitor or a non-steroidal anti-inflammatory drug (NSAID). These two treatments have similar effectiveness, but they are insufficient for protection of patients at very high risk for ulcer bleeding¹. Naproxen is chemically 2-Naphthaleneaceticacid,6-methoxy- α -methyl-,(s)-(+)-(s)-6-Methoxy- α -methyl-2-naphthalene acetic acid². 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.

Esomeprazole is 6-methoxy-2-[[[4-methoxy-3,5-dimethylpyridin-2-yl)methane] sulfinyl]-1H-1,3-benzodiazole.

It is a highly effective inhibitor of gastric acid secretion used in the therapy of stomach ulcers and Zollinger-Ellison syndrome³. There are different methods reported for individual quantitative determination of Esomeprazole and naproxen in bulk or pharmaceutical formulations include spectrophotometry, titrimetry, colorimetry and high performance liquid chromatography⁴⁻¹⁵. Hence, there was an attempt to develop a HPLC method for the simultaneous estimation of Esomeprazole and naproxen in pharmaceutical dosage forms⁽¹⁶⁻²⁰⁾.

Experimental

Equipment and chromatographic conditions

A High performance liquid chromatography equipped with auto sampler (Waters) is used for the study. The detection carried by using UV (Waters 2487) dual wavelength absorbance detector. Chromatographic separation was carried out at room temperature with Symmetry C-18 reversed-phase column by use of a mobile phase consisting of 0.05 M Potassium dihydrogen Phosphate (pH 7.0 adjusted with 1 M sodium hydroxide), and Acetonitrile (70:30 v/v) at a flow rate of 1.0 mL/min with detection wavelength at 286 nm. The mobile phase was filtered through a 0.45 µm membrane filter and degassed for 5 minutes. The injection volumes for samples and standards were 20 µL and eluted at a flow rate of 1mL/min at ambient temperature.

Materials and reagents

Acetonitrile was of HPLC grade, Potassium dihydrogen Phosphate and other reagents were of analytical-reagent grade. Water HPLC grade was obtained from a Milli-Q RO water purification system. Vimovo tablets purchased from market and were used for analysis. Each tablet was labeled to contain 500 mg Naproxen and 20 mg Esomeprazole.

Preparation of standard solutions

The standard stock solutions were prepared individually taking Naproxen and Esomeprazole. The combined standard solution was prepared by diluting the stock solutions of Naproxen and Esomeprazole proportionately to the concentration of 0.15 mg/mL and 0.006 mg/mL respectively.

Analysis of marketed formulation: The twenty tablets of Naproxen and Esomeprazole were crushed and made into powder. 586.3 mg of the powder was weighed and transferred into a 100ml clean dry volumetric flask. 70

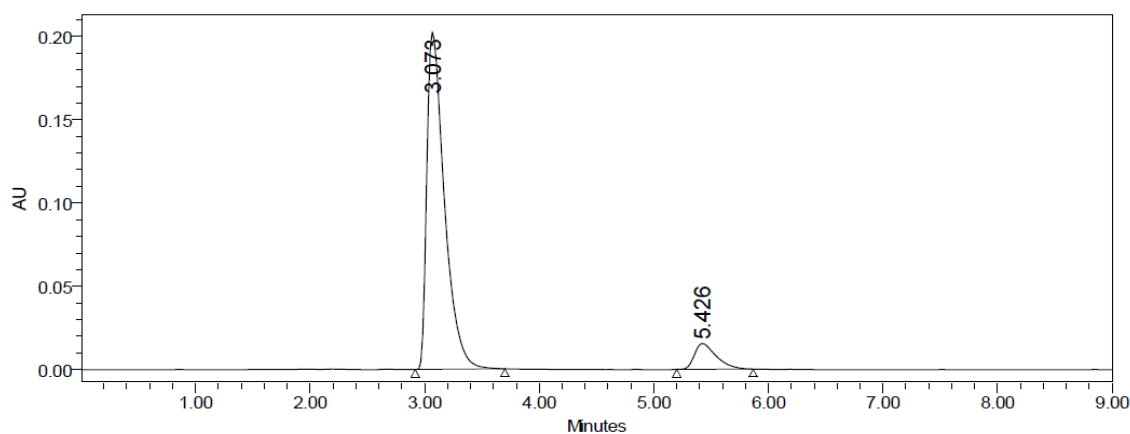
mL of diluent was added to the flask and sonicated to dissolve it completely and made up to the volume with the same (Stock solution). 0.3 mL of the stock solution was transferred into a 10ml volumetric flask and diluted up to the mark with diluent. 20 μ L of the standard, sample were injected into the chromatographic system and the areas were measured for the Naproxen and Esomeprazole peaks. The content of Naproxen and Esomeprazole were calculated and found to be 99.5 and 100.3% respectively.

Result and Discussion

Optimization of Chromatographic Conditions

Naproxen and Esomeprazole have maximum UV absorbance at 272 nm and 302 nm respectively obtained by spectroscopic analysis. The chromatographic detection was performed at 286 nm. Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in the mobile phase. Different experiments were performed to optimize the mobile phase but adequate separation of drugs could not be achieved. By altering the pH of mobile phase a good separation was achieved. The optimized mobile phase consisting of 0.05 M Potassium dihydrogen Phosphate (pH 7.0 adjusted with 1 M sodium hydroxide), and Acetonitrile mixed in the ratio of 70:30 v/v at a flow rate of 1.0 mL/min. Naproxen and Esomeprazole were eluted at 2.46 and 4.31 minutes respectively with a run time of 9 min under the above optimised chromatographic conditions. A typical chromatogram for simultaneous estimation of Naproxen and Esomeprazole is shown in Figure 1.

Figure 1: Chromatogram of Vimovo Tablet (Peak of Naproxen and Esomeprazole at 3.07 & 5.43 min respectively)



Method Validation

System Suitability Results:

For Naproxen and Esomeprazole peaks the tailing factor were found to be 1.7 & 1.6 respectively and the theoretical Plates obtained were found to be 2882.9 & 3675.1 respectively.

Linearity

The calibration curves obtained by plotting peak area against concentration for Naproxen and Esomeprazole were showed in the Figure 2 (A) & (B) respectively. The linearity was obtained in the concentration range of 100–200 $\mu\text{g mL}^{-1}$ for naproxen, and 4–8 $\mu\text{g mL}^{-1}$ for Esomeprazole. The regression coefficient values (R^2) for Naproxen and Esomeprazole found to be 0.999 and 0.999 respectively. The average retention time for Naproxen and Esomeprazole was found to be 3.06 ± 0.04 and 5.42 ± 0.05 min, respectively.

Figure 2A: Calibration curve of Naproxen.

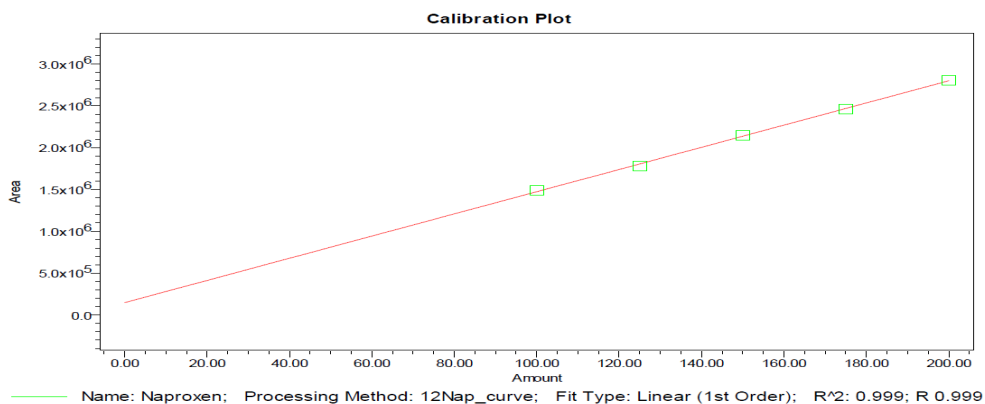
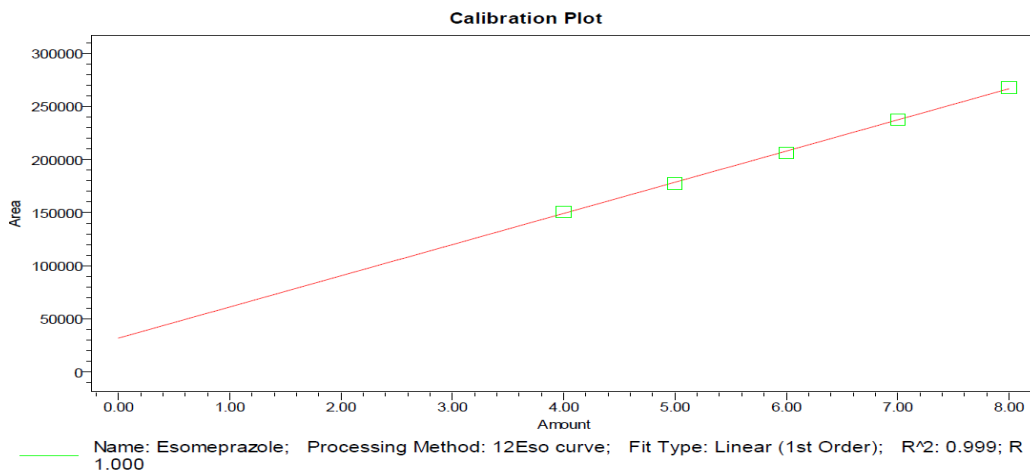


Figure 2B: Calibration curve of Esomeprazole.



Accuracy and precision

The accuracy of the RP-HPLC method was determined by calculating recoveries of Naproxen and Esomeprazole for 50%, 100% and 150% with respect to target concentration and results are tabulated in Table 1A & 1B respectively. The System precision of the proposed method was determined by injecting standard solution for five times and measured the area for them into HPLC. The Method Precision of the proposed method was determined by injecting six sample solutions into HPLC prepared individually. The %RSD for the areas of system precision was given in the Table 2. The % assay calculated from the method precision data and the % RSD calculated for % assay values were given in Table 2.

Table 1A: Recovery Results for Naproxen.

%Concentration (w.r.t. specification Level)	Mean Amount Added (mg)	Mean Amount Found (mg)	% Recovery	Mean % Recovery
50%	36.5	36.0	98.7	99.2
100%	47.5	47.8	100.6	
150%	58.0	56.9	98.1	

Table 1B: Recovery Results for Esomeprazole.

%Concentration (w.r.t. specification Level)	Mean Amount Added (mg)	Mean Amount Found (mg)	% Recovery	Mean % Recovery
50%	14.8	15.1	101.7	99.4
100%	19.3	18.9	98.1	
150%	25.0	24.6	98.5	

Table 2: Precision of Naproxen and Esomeprazole.

Method Precision	Naproxen	Esomeprazole
System Precision (%RSD for the area)	0.18	0.51
Method Precision (%assay)	99.49	100.25
%RSD	0.55	0.46

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. In the Intermediate precision, The Intermediate Precision of the proposed method was determined by injecting six sample solutions into HPLC prepared individually. The %RSD for the areas of system precision was given in the Table 3. The % assay calculated from the intermediate precision data and the % RSD calculated for % assay values were given in Table 3.

Table 3: Intermediate Precision of Naproxen and Esomeprazole.

Intermediate Precision	Naproxen	Esomeprazole
System Precision (%RSD for the area)	0.19	1.00
Intermediate Precision (%assay)	99.92	100.36
%RSD	0.41	0.52

Limits of Detection and Quantitation

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the standard deviation and slope method was adopted. The LOD for Naproxen and Esomeprazole was 0.10µg/mL and 0.05 µg/mL respectively and LOQ was 0.35 µg/ mL and 0.17 µg/mL respectively.

Robustness

As part of the Robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method. The results reveal that the method is robust enough. The results are summarized in Table 4A & 4B.

Table 4A: Robustness results with change in the flow rate.

S.No	Flow Rate (ml/min)	System Suitability Results					
		Naproxen			Esomeprazole		
		USP Plate Count	USP Tailing	% RSD	USP Plate Count	USP Tailing	% RSD
1	0.8	2933.4	1.6	0.22	3727.3	1.6	0.65
2	1.0	2882.9	1.7	0.18	3675.1	1.6	0.51
3	1.2	2910.0	1.6	0.20	3715.5	1.5	0.90

Table 4B: Robustness results with change in mobile phase composition.

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results					
		Naproxen			Esomeprazole		
		USP Plate Count	USP Tailing	% RSD	USP Plate Count	USP Tailing	% RSD
1	10% less	2976.4	1.7	0.20	5553.6	1.4	0.80
2	*Actual	2882.9	1.7	0.18	3675.1	1.6	0.51
3	10% more	2848.9	1.8	0.25	3029.9	1.6	0.95

Conclusion

The new HPLC method developed and validated for simultaneous determination of Naproxen and Esomeprazole in combined pharmaceutical dosage form and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid dosage form. The method was found to be simple,

accurate, economical, and rapid with a very short run time of 9 minutes. The proposed method can be used for the routine analysis of the drugs in the quality control.

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