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Method Development And Validation By Rp-Hplc For Estimation Of Naproxen And Esomeprazole In Bulk And Pharmaceutical Dosage Form

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ABSTRACT:

A simple, precise, and accurate Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Naproxen and Esomeprazole in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a Phenomenex Luna C18 column (4.6 x 150 mm, 5µm) with a mobile phase consisting of Acetonitrile and Water in the ratio of 45:55 %v/v, delivered at a flow rate of 1.0 mL/min. The detection wavelength was set at 250 nm, with an injection volume of 10 µL and a run time of 7 minutes. The method provided well-resolved, sharp, and symmetrical peaks for both drugs, with satisfactory retention times. The developed method was validated according to ICH guidelines and was found to be linear, precise, accurate, robust, and specific for the intended analytical application. This RP-HPLC method is suitable for routine quality control analysis of Naproxen and Esomeprazole in combined dosage forms.

Keywords: RP-HPLC, Naproxen, Esomeprazole, simultaneous estimation, validation. Phenomenex Luna C18 column.

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[1-4], 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[5-7].

Naproxen[8,9] is chemically designed as (2S)-2-(6-methoxynaphthalen-2-yl) propanoic acid Naproxen is used as Anti inflammatory[10] and analgesic drug and Esomeprazole[11,12] 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[13]. Esomeprazole is cost effective in the treatment of gastric esophageal reflux diseases.

MATERIALS & METHODS:

The working standards of Esomeprazole and Naproxen were procured from Sun pharma, provided by Sura Pharma labs. Methanol and Water HPLC grade were obtained from LICHROSOLV (MERCK). Acetonitrile for HPLC obtained from Merck**.**

HPLC (WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector, pH meter (Lab India), Weighing machine (Sartorius), glassware (Borosil), Digital ultra sonicator (Labman) was used.

Preparation of standard solution: Accurately weigh and transfer 10 mg of Naproxen and Esomeprazole working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 2.25ml of the above Naproxen and 0.45ml of the Esomeprazole stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol and Water, Acetonitrile and Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and water in proportion 45:55 v/v respectively.

Optimization of Column: The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Luna C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Validation:

Preparation of mobile phase: Accurately measured 450ml (45%) of Acetonitrile and 550ml of Water (55%) were mixed and degassed in a digital ultra sonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. Mobile phase was used as the diluent.

Validation parameters:

Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Naproxen and Esomeprazole working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 2.25ml of the above Naproxen and 0.45ml of the Esomeprazole stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

System Suitability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Specificity study of drug:

Preparation of Sample Solution: Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Naproxen and Esomeprazole sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 2.25ml of Naproxen and Esomeprazole above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

Preparation of drug solutions for linearity:

Accurately weigh and transfer 10 mg of Naproxen and Esomeprazole working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I to V solutions:

Pipette out 0.15ml (15ppm), 0.3ml (30ppm), 0.45 (45ppm), 0.6ml (60ppm), 0.75ml (75ppm) of the Naproxen and 0.1ml (10ppm), 0.2ml (20ppm), 0.3ml (30ppm), 0.4ml (40ppm), 0.5ml (50ppm) of the Esomeprazole from the above stock solutions in to a 10ml of five volumetric flask and dilute the solution, sonicate for 10minutes.

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision:

Repeatability:

Further pipette 2.25ml of the above Naproxen and 0.45ml of the Esomeprazole stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions. 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.

Accuracy:

Preparation of 50% Standard stock solution: pipette out 1.12ml of the above Naproxen and 0.225ml of the Esomeprazole stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of 100% Standard stock solution: pipette out 2.25ml of the above Naproxen and 0.45ml of the Esomeprazole stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of 150% Standard stock solution: pipette out 3.37ml of the above Naproxen and 0.675ml of the Esomeprazole stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure: Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Naproxen and Esomeprazole and calculate the individual recovery and mean recovery values.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Preparation of Standard solution: pipette out 2.25ml of the above Naproxen and 0.45ml of the Esomeprazole stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions: The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Water was taken in the ratio and 60:40, 70:30 instead of 45:55, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULT AND DICUSSION:

After repeating the same conditions got proper peaks, base line is good and we can go for repeatability. Therefore, separations of two peaks, base line, peak symmetry, resolution are proper.

Optimized Chromatographic conditions:

Column	:	Phenomenex Luna C18 (4.6 x 150mm, 5µm)
Mobile phase	:	Acetonitrile and water (45:55 %v/v)
Flow rate	:	1ml/min
Wavelength	:	250 nm
Injection volume	:	10 µl
Run time	:	7 min

The linearity of the proposed method was established by least square regression analysis of the calibration curve and the constructed calibration curves were linear and concentration range of 10-50µg/ml for Esomeprazole (r = 0.9999) and 4-20µg/ml Naproxen (r = 0.9999).

The robustness was evaluated by analyzing the samples by varying few parameters like wavelength and flow rate. The validation results obtained confirm the suitability of the proposed RP-HPLC method for simple, accurate and precise analysis of Esomeprazole and Naproxen in pharmaceutical preparations.

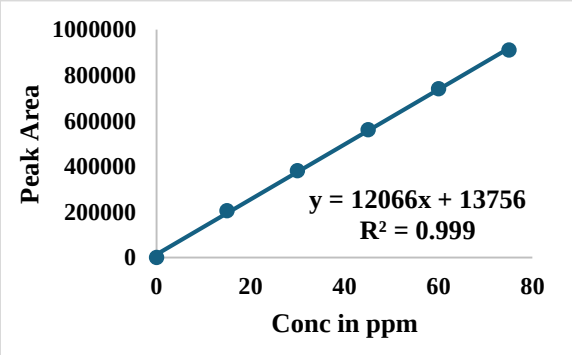

Fig 1: Calibration graph for Naproxen

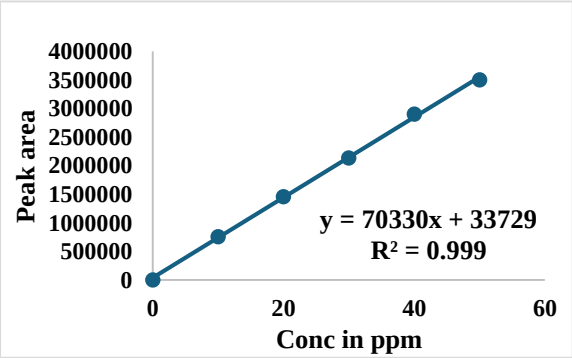

Fig 2: Calibration graph for Esomeprazole

Table 1: Validation parameters of Esomeprazole and Naproxen.

Parameters	Esomeprazole	Naproxen
System suitability	0.046678	0.060932
Repeatability	0.595695	0.177123
Intermediate Precision	0.197436	0.176616
Accuracy %	100.93	100.351
Limit of Detection (LOD) (µg/ml)	0.8	0.6
Limit of Quantification (LOQ) (µg/ml)	2.4	1.8

Table 2: Accuracy results of Naproxen& Esomeprazole

Drug	% Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
Naproxen	50%	392891.7	5	5.027	100.540%	100.351%
	100%	781996	10	10.026	100.260%	
	150%	1171988	15	15.038	100.253%	
Esomeprazole	50%	204962	15	15.156	101.040%	100.93%
	100%	365018	30	30.378	101.260%	
	150%	521064.3	45	45.218	100.484%	

CONCLUSION:

A robust and precise Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Naproxen and Esomeprazole in bulk and pharmaceutical dosage forms. The separation was achieved using a Phenomenex Luna C18 column (4.6 x 150 mm, 5 µm) with a mobile phase consisting of Acetonitrile and Water (45:55 %v/v). The mobile phase was delivered at a flow rate of 1 mL/min, and detection was carried out at a wavelength of 250 nm. The injection volume was 10 µL, and the total run time was 7 minutes. Both Naproxen and Esomeprazole were well resolved under these chromatographic conditions with satisfactory retention times and sharp, symmetric peaks. The method was validated as per ICH guidelines for parameters such as linearity, precision, accuracy, specificity, robustness, and system suitability. All validation results were found to be within acceptable limits, indicating the reliability of the method.

The developed RP-HPLC method is simple, accurate, specific, and reproducible for the simultaneous estimation of Naproxen and Esomeprazole in both bulk and formulated products. The optimized chromatographic conditions ensure effective separation within a short run time of 7 minutes, making the method suitable for routine quality control analysis in pharmaceutical industries.

REFERENCES:

1. Martin M, Guiochon G. Effects of high pressures in liquid chromatography. J.Chromatogr. Anal. 2005;(1-2) 7:16-382.
2. Liu Y, Lee ML. Ultra high pressure liquid chromatography using elevated temperature. J.Chromatogr.2006;1104(1-2):198–2023.
3. Abidi SL. High-performance liquid chromatography of phosphatidic acids and related polar lipids. J. Chromatogr.1991; 587:193-2034.
4. Hearn MT. Ion-pair chromatograph on normal and reversed-phase systems. Adv. Chromatogr.1980; 18:59–100.
5. Harmita, et al. Optimation and validation of analytical method of cotrimoxazole in tablet and plasma in vitro by high-performance liquid chromatography. J Bioanal Biomed. 2012; 4:26-9.
6. Nardulli P, et al. A combined HPLC and LCMS approach for evaluating drug stability in elastomeric devices: a challenge for the sustainability in pharmaco-economics. J Pharmacovigilance. 2014; 2:157.
7. Hafez HM, et al. Development of a stability indicating HPLC method for simultaneous determination of amlodipine besylate and atorvastatin calcium in bulk and pharmaceutical dosage form. Pharm Anal Acta. 2014; 5:316.
8. Shintani H. Immobilized enzyme column combined with HPLC and column switching method for the analysis of complicated matrix such as body fluids. Pharmaceutical Regulatory Affairs. 2014; 03(5): e142. doi: 10.4172/2167- 7689.1000e142.

9. Suresh Babu VV, et al. Validated HPLC method for determining related substances in compatibility studies and novel extended release formulation for ranolazine. J Chromatogr Separate Technique. 2014; 5:209.
10. Arayne MS, et al. Monitoring of pregabalin in pharmaceutical formulations and human serum using UV and RPHPLC techniques: application to dissolution test method. Pharm Anal Acta. 2014; 5:287
11. Abdulla SA, et al. Validated HPLC method for the determination of nisoldipine. Pharm Anal Acta. 2013; S1:004.
12. Sawsan Mohammed AH, et al. Effects of blood collection tubes on determination vitamin-A by HPLC. J Chromatogr Sep Tech. 2013; 4:184.
13. Subbaiah PR, Kumudhavalli MV, Saravanan C, Kumar M, Chandira RM. Method development and validation for estimation of moxifloxacin HCl in tablet dosage form by RP-HPLC method. Pharm Anal Acta. 2010;01(2):109. doi: 10.4172/2153- 2435.1000109.

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