Original Research Article

SIMPLE AND RAPID LIQUID CHROMATOGRAPHIC METHOD FOR REAL-TIME QUANTIFICATION OF NAPROXEN / ESOMEPRAZOLE MAGNESIUM COMBINATION TABLETS

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ABSTRACT

Purpose: In this present investigation a simple, rapid and accurate reverse phase- liquid chromatographic method for the real- time quantification of naproxen and esomeprazole magnesium in tablet dosage form has been developed and validated.
Materials and Methods: Isocratic elution mode with a mixture of phosphate buffer, pH 4.0 and acetonitrile in the ratio of 55:45 v/v was selected as the mobile phase with a universal C_{18} column (250x4.6mm, 5µm) by using waters alliance 2695 HPLC system with UV-detector. The retention time of naproxen and esomeprazole magnesium were found to be 2.229 min and 3.379 min, respectively at flow rate of 1.0ml/min with UV detector to evaluate at 306 nm.
Results: A linear relation ship was found between peak area response and concentration in the range of 18.75 µg/mL- 112.5 µg/mL and 1µg/mL- 6 µg/mL for naproxen and esomeprazole magnesium. The % RSD values were found to be less than 2 for accuracy and precision studies.
Conclusion: The method can be applied to quality control of pharmaceutical formulations containing naproxen and esomeprazole magnesium.

***Keywords*:** Naproxen, Esomeprazole magnesium, RP-HPLC

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Running Title: Real –time quantification of NAP and ESO.

INTRODUCTION

Esomeprazole magnesium trihydrate (ESO), bis (5-me thoxy-2- [(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl-1H-benzimidazole-1-yl) magnesium trihydrate [Figure 1] is proton pump inhibitors in the management of acid-related disorders.

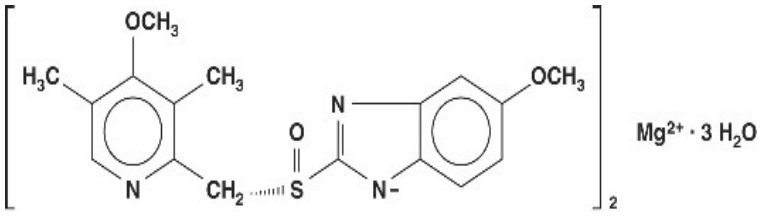

Figure 1.Chemical structure of ESO.

Naproxen [NAP] is chemically (s)-2- (6-methoxy naphthalene-2-yl) prop ionic acid (Figure 2), is a nonsteroidal 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, NAP is capable of producing disturbances in the gastrointestinal tract. [1-3]

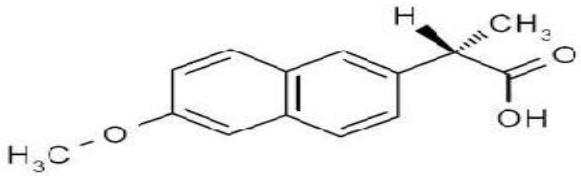

Figure 2.Chemical structure of NAP.

Combination of both NAP and ESO is used for the treatment indicated for the relief of signs and symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis and to diminish the risk of developing gastric ulcers by the usage of NSAID alone. Literature survey revealed that, four simultaneous determinations available for ESO and NAP in pharmaceutical formulations by RP-HPLC method, but that methods has some technical hitches like organic phase ratio was higher in mobile phase, flow rate was high and retention time of NAP and ESO were too low. Keeping all these points into consideration, we have undertaken the present investigation with the aim to develop and validate the rapid, simple and accurate real-time quantification of NAP and ESO in tablet dosage form. [4-7]

MATERIALS AND METHODS

Instruments

Water alliance 2695 HPLC system having empower software equipped with rheodyne injector, auto sampler, universal C_{18} column (250 x 4.6mm, 5µm) and UV-visible detector were used. Along with this Jasco UV-visible spectrophotometer, an analytical balance (Mettler Toledo MS205DU), and a p^H meter (Mettler Toledo S20-K) were also used.

Materials

Solvents such as acetonitrile and methanol were of HPLC grade, while all other chemicals and reagents such as orthophosphoric acid, potassium dihydrogen phosphate, and hydrochloric acid were of analytical reagent grade of Merck make. Double distilled water and milli-Q water was used for all the experiments appropriately. NAP and ESO drug samples gifted by Mylon Laboratories Ltd., Hyderabad, India.

Preparation of standard solutions

Stock solutions of NAP and ESO were prepared separately by dissolving 37.5 mg of NAP and 2 mg of ESO in 50 mL of mobile phase to get concentrations of 375 µg/mL NAP and 20 µg/mL ESO stock solutions. Standard solutions were further diluted with the mobile phase to get the final concentrations within the linearity range.

Preparation of sample solution

Twenty tablets, (Vimovo, Astrazeneca) each containing 20mg of ESO, 500 mg of NAP were weighed and finely powdered. A quantity of powder equivalent to 20mg of ESO and 500 mg of

NAP were weighed and transferred in to 100 mL of standard volumetric flask and diluted by using mobile phase. The sample was kept in an ultrasonic bath for 10min.Then it was filtered through 0.22µ membrane filter paper. This solution was further diluted with mobile phase to get concentrations of 4 µg/mL of ESO and 75 µg/mL of NAP. 20µl of this solution was injected in to HPLC system and chromatograms were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the chromatograms were recorded. The amount of ESO and NAP present in each tablet were calculated by comparing the peak area of the standard solution and sample.

Method Validation

This optimised HPLC method was validated for the parameters listed in the International Conference on Harmonisation (ICH) guidelines. [8,9]

Linearity

Appropriate aliquots of the NAP and ESO were pipette out from the standard stock solution into a series of 10 ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain a set of solutions for two drugs were found to be in the range of 18.75-112.5 µg/mL for NAP and 1-6 µg/mL for ESO. Triplicate dilutions of each concentration of the drug were prepared separately and evaluation of the drugs was performed with the UV detector set at 306 nm and the peak areas were recorded. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation co-efficient.

Precision

Intraday and interday precision were evaluated by determining the corresponding responses of standard solutions in triplicate on same day (repeatability) and on different days (intermediate precision) at 75 µg/mL of NAP and 4µg/mL of ESO. The results were reported in terms of % RSD (Relative standard deviation).

Accuracy

Accuracy was determined by calculating recovery of NAP and ESO by the standard addition method. The concentrations such as 50%, 100%, and 150% level of bulk drugs were added to the pre quantified test solutions of NAP and ESO. Each solution was injected in replicate, and the recovery was calculated by measuring peak areas and fitting these values into the regression equation of the calibration curve.

Sensitivity

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of Y-intercept of regression lines and slope of the calibration curves were used to calculate the LOD and LOQ.

System suitability

The system suitability parameters like theoretical plates (N), resolution (R) and tailing factor (T) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of NAP and ESO was validated or not.

RESULTS AND DISCUSSION

Chromatographic conditions

The mobile phase was chosen after several trials with methanol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of acetonitrile: phosphate buffer (45:55 v/v) as selected to achieve good separation and resolution. A flow rate of 1mL/min gave an optimal signal to noise ratio with a reasonable separation time and run time of 5 min, using a reverse phase C_{18} column. The retention times for NAP and ESO were observed to be 2.229 and 3.379 min respectively. The wavelength fixed at 306 nm which gave good absorbance both for NAP and ESO. The optimized chromatogram and system suitability parameters of proposed method were mentioned in Figure-3 and Table-1.

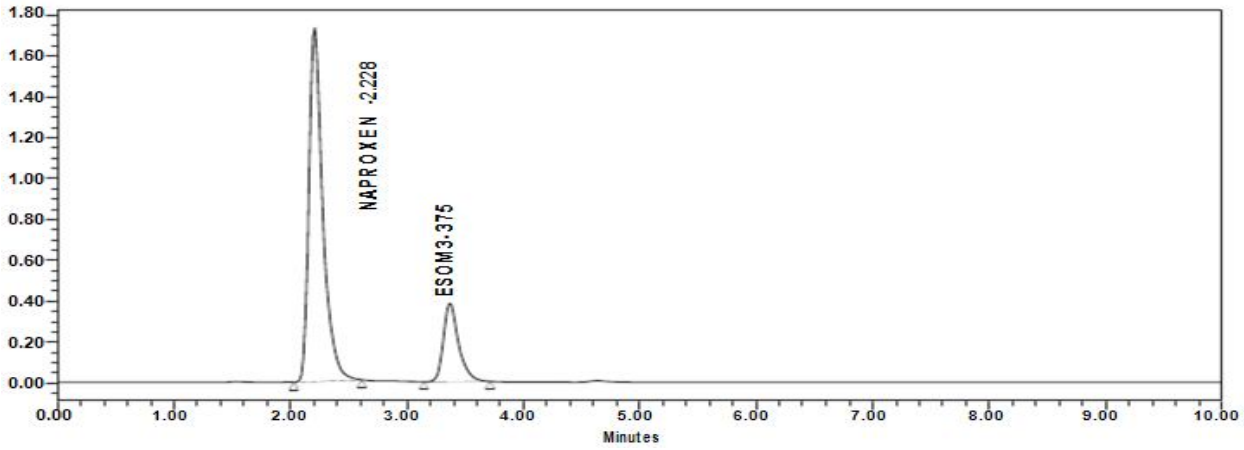

Figure 3: Chromatogram of NAP (2.229) and ESO (3.375)

Table-1: System suitable parameters for proposed method

S.No	Parameter	NAP	ESO
1	Retention time (min)	2.229	3.375
2	Peak area response	11078391	2576205
3	Theoritical plates	2048	3658
4	Symmetric factor	1.45	1.30
5	Calibration range (µg/mL)	18-112	1-6
6	LOD (µg/mL)	0.77	0.19
7	LOQ (µg/mL)	2.56	0.576
8	%RSD	0.12	0.58

The calibration curves shows that the developed method was linear in the concentration range of 18.5-112.5 µg/mL NAP and 1-6 µg/mL of ESO (Figure 4 &5). No significant difference between intra-day and inter-day precision values, revealed that the method was reproducible. The % recovery was within the range between 99-100 (Table-2) and % RSD values for commercial tablets were shown less than 2 (Table-3). This indicates that the method is accurate and reliable. The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions (Table-4 &5).

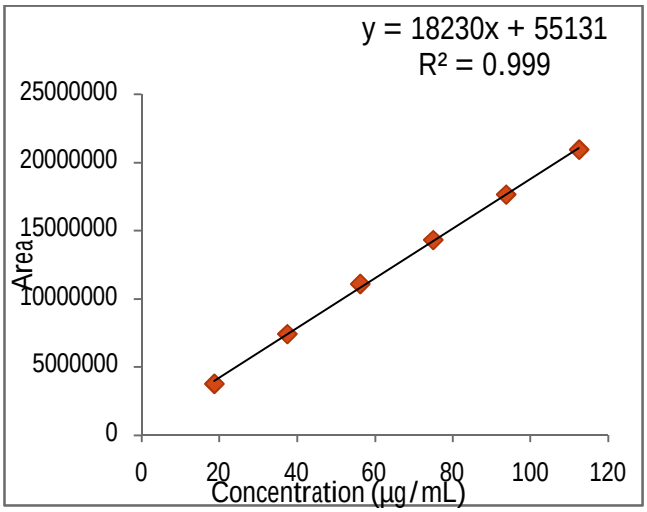

Figure 4: Calibration plot for NAP

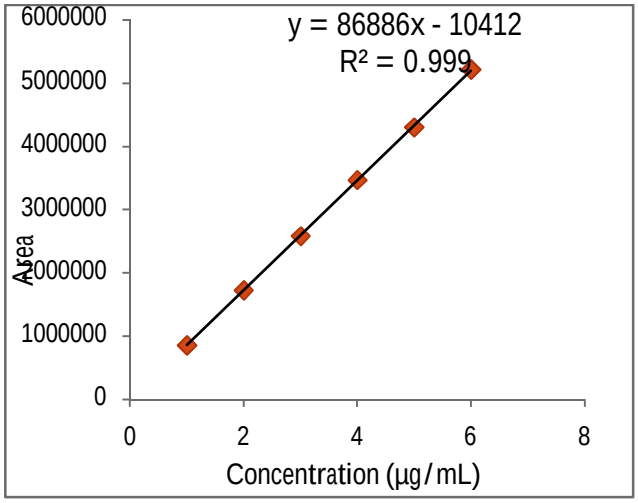

Figure 5: Calibration plot for ESO

Table 2: Recovery studies of the proposed method

%Level	% of NAP Recovered	% RSD	% of ESO Recovered	% Recovery
50%	99.75	0.152	99.56	0.342
100%	99.91	1.242	99.63	0.845
150%	99.99	0.698	99.93	0.572

Table 3: Analysis of commercial tablets (Vimovo)

S.No	Analyte	Lable claim (mg)	Amount found (mg/tablet)	Mean	%RSD
1	NAP	500	502.3	504.2	0.21
2	ESO	20	20.2	20.5	0.65

Table 4: Robustness of proposed method (flow rate variation)

Flow rate variation (ml/min)	NAP		ESO	
	Retention time	Theoretical plates	Retention time	Theoretical plates
0.9	2.483	2062	3.745	3542
1	2.229	2048	3.379	3658
1.1	2.116	2054	3.146	3652

Table 5: Robustness of proposed method (change in organic composition of mobile phase)

Level	NAP		ESO	
	Retention time	Theoretical plates	Retention time	Theoretical plates
2% less	2.342	2045	3.369	3524
Actual	2.229	2048	3.379	3658
2%more	2.214	2012	3.373	3454

CONCLUSION

A simple, precise and accurate method was developed for the real-time estimation of NAP and ESO in the presence of less organic solvent with high resolution, more sensitive and low run time. This RP-HPLC method is also validated for various parameters as per ICH guidelines and values obtained from validation proved that the method was scientifically sound. The assay values were in good agreement with their respective labeled claim, which suggested no interference of formulation excipients in the estimation. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine quality control analysis of real-time estimation of NAP and ESO.

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