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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHODS FOR SIMULTANEOUS ESTIMATION OF NAPROXEN AND ESOMEPRAZOLE MAGNESIUM TRIHYDRATE IN COMBINED PHARMACEUTICAL FORMULATION

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ABSTRACT

A rapid, specific, sensitive and simple high performance liquid chromatography was developed for simultaneous estimation of Esomeprazole magnesium trihydrate and Naproxen in tablet formulation. The separation was achieved by SUPELCO 516 C_{18} –DB column (250× 4.6 mm, particle size 5µm) with a mobile phase consisting of 0.01M phosphate buffer pH 7.5: ACN: methanol(40:50:10v/v, addition of 0.1% triethyl amine), at a flow rate of 0.8 ml/min. Detection was carried out at 303 nm. Retention time of Esomeprazole magnesium trihydrate and Naproxen were found to be 4.6 and 3.2min, respectively. The linear dynamic range was 0.8-4.8µg/ml and 15-90µg/ml for Esomeprazole magnesium trihydrate and Naproxen, respectively. The method is validated for Accuracy, Precision, Ruggedness and Robustness. The proposed method is successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies.

Keywords: Esomeprazole magnesium trihydrate, Naproxen, High performance liquid chromatography, Simultaneous estimation.

INTRODUCTION

Esomeprazole magnesium trihydrate (ES), bis(5-me thoxy-2- [(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl-1H-benzimidazole-1-yl) magnesium Trihydrate(Figure 1) is the S isomer of racemic omeprazole approved in February 2001 for use as a new pharmacological entity designed to improve the clinical outcome of available proton pump inhibitors in the management of acid-related disorders. Naproxen is chemically 2-Naphthaleneacetic acid, 6-methoxy-α-methyl-,(s)-(+)-(s)-6-Methoxy-α-methyl-2-naphthaleneacetic acid(Figure 2).

Naproxen 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, Naproxen is capable of producing disturbances in the gastrointestinal tract.

Method have been reported in literature for the individual analysis of Naproxen and also in combination with Ranitidine &sumatriptan including UV, HPLC technique etc. and Esomeprazole magnesium trihydrate by first order derivative spectroscopy, HPLC etc.

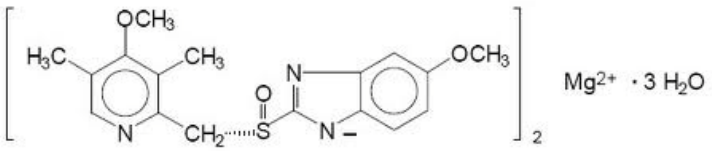

Fig. 1: Esomeprazole magnesium trihydrate

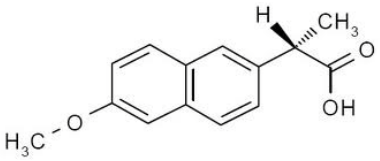

Fig. 2: Naproxen

However, to best of our knowledge no analytical method was found to be reported for simultaneous estimation of Naproxen and Esomeprazole magnesium trihydrate.

MATERIALS AND METHODS

Equipment

Chromatography was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20AT pump and Shimadzu SPD-20AV absorbance detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20µl. Data acquisition and integration was performed using Spinchrome software (Spinco biotech, Vadodara, Gujarat)

Reagents and chemicals

ACN, methanol and triethyl amine (HPLC grade, spectrochempvt ltd, Mumbai ,India), Double distilled water (Purified HPLC grade water was obtained by filtering double distilled water through nylon filter paper 0.2 µm pore size and 47 mm diameter (Pall Lifesciences, Mumbai, India)).

Disodium hydrogen orthophosphate and Potassium dihydrogen orthophosphate (AR grade- s.d. fine-chem limited, Mumbai)

Marketed formulation Vimovo contains 20mg of Esomeprazole magnesium trihydrate& 375mg of Naproxen.

Vimovo approved by US-FDA recently in 2011 & it is not available in India so I procured it from US market (Astrazeneca ltd.)

HPLC conditions

A SUPELCO 516 C_{18} –DB (250× 4.6 mm,particle size 5µ) column was used as the stationary phase. A mixture of 0.01M phosphate buffer pH 7.5, ACN and methanol(40:50:10v/v, with addition of 0.01% triethyl amine)was used as a mobile phase. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 0.8 ml/min. The eluents were monitored at 303nm.The injection volumes of samples and standard were 20µl.

Standard solutions

A stock solution containing 1000µg/ml of ESO and NAP were prepared separately by dissolving ESO and NAP in methanol & 0.02M phosphate buffer(2:8). standard stocks solutions of NAP(1000 µg/ml) and ESO (1000 µg/ml) transferred 5 ml aliquots to 50 ml volumetric flasks and making up the volume with methanol & 0.02M phosphate buffer(2:8)& that will become 100 µg/ml. From this, 1.5, 3.0, 4.5, 6.0, 7.5 and 9 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol & 0.02M phosphate buffer(2:8). This gives 15 to 90 µg/ml of NAP. and for ESO from 10 µg/ml solution 0.8, 1.6, 2.4, 3.2, 4 and 4.8 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol & 0.02M phosphate buffer(2:8). This gives 0.8 to 4.8 µg/ml of ESO. All the solutions were covered with aluminum foil to prevent photolytic degradation until the time of analysis.

Assay in formulations

Twenty tablets, (Vimovo, US-Astrazeneca) each containing 20mg of ESO, 375mg of NAP were weighed and finely powdered. A quantity of powder equivalent to 20mg of ESO and 375mg of NAP were weighed and transferred in to 100ml 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. One ml of this solution was further diluted to 100ml to get a concentration of 2 µg/ml of ESO and 37.50µg/ml of NAP. From this, 4ml were then diluted to 10ml to get the 1.6 &30 µg/ml of ESO and NAP respectively. 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 Esomeprazole and Naproxen present in each tablet were calculated by comparing the peak area of the standard solution and sample. The results are tabulated in Table 1.

Table 1: Table for Assay

S. No.	TabletSample	Label claim (mg/tablet)	*Amount Present (mg/tablet)	*Percentage (Label claim)(%w/w)
1	Esomeprazole magnesium trihydrate	20	20.11±0.02	101.23±0.05
2	Naproxen	375	374.76±0.04	99.65±0.18

*Average of six determinations, mean ± Standard Deviation

Optimized HPLC conditions

A SUPELCO 516 C_{18} –DB (250× 4.6 mm,particle size 5µ) column maintained at ambient temperature was used as stationary phase. An isocratic mobile phase constituting 0.01M phosphate bufferpH 7.5, Acetonitrile and Methanol in ratio 40:50:10 (%v/v/v), at a flow rate of 0.8 ml/min was used.Inthat 0.01% Triethyl amine was added for decreasing the tailing of the peak. The mobile phase was filtered using 0.45 µm filter paper and degassed for 10 min by sonication. Samples of 20µl were injected into the HPLC system with the runtime of 10 min.Retention time of the drugs obtained under these conditions were 4.6 and 3.2 min for ESO and NAP respectively. For the quantitative analytical purposes the wavelength was set at 303nm.The typical chromatogram of the mixture was shown in Figure 3.

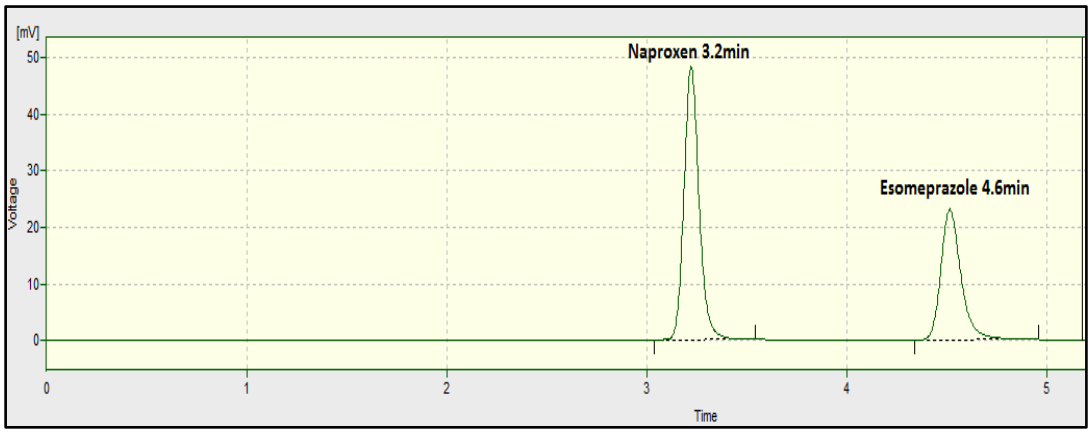

Fig. 3: chromatogram of the mixture(ESO & NAP)

Method Validation

The chromatographic conditions were validated by evaluating linearity, recovery, method and system precision,accuracy, system suitability, solution stability, limit of detection(LOD), Limit of Quantification (LOQ), robustness, ruggedness studies in accordance with ICH guideline Q2(R1).

Selection of wavelength

From the UV-visible Spectrophotometric results, a detection wavelength of 305nm for Esomeprazole magnesium trihydrateand 272nm for Naproxen was selected. Because at this wavelength they shows maximum absorbance and then 303nm was selected as common wavelength for simultaneous estimation of both the drugs,

as these are eluting in the same mobile phase with good absorbance. The maximum absorbance with good peak intensity, good peak shape and height was observed at 303nm (Fig. 4).

System suitability

The column efficiency, resolution and peak symmetry were calculated for the standard solutions. (Table.2). The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall within ± 2% standard deviation range during performance of the method. Here asymmetric factor for peaks of ESO and NAP was less than 2% and resolution was satisfactory. The peaks obtained for ESO and NAP were sharp and have clear base line separation.

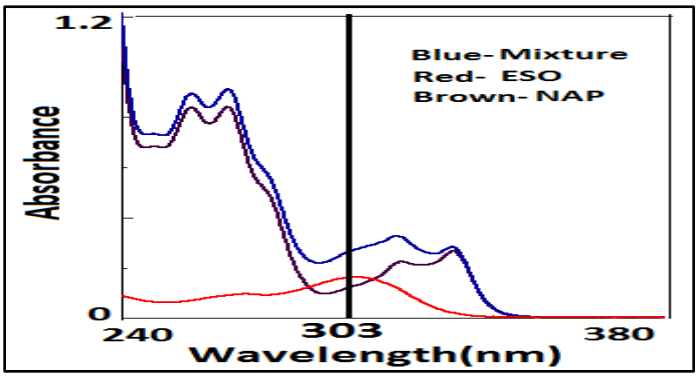

Fig. 4: Selection of wavelength

Table 2: system suitability

S. No.	Parameters	Esomeprazole	Naproxen	Acceptance criteria
1	Retention time	4.6	3.2	-
2	RSD of replicate injections	0.165	0.368	Not more than 2%
3	Asymmetric factor	1.47	1.46	Not more than 2
4	Theoretical plates per meter	1,96,908	1,63,722	More than 10000
5	Resolution factor	8.02		More than 2

Solution stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results indicated that both the solutions, retention time and peak area of ESO and NAP did not show much variation (%RSD less than 2.0). There was no significant degradation with in the indicated period. Hence, it was concluded that both the solutions were stable for 24 hr at room temperature.

Specificity of the method

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 60 µg/ml of NAP and 4µg/ml of ESO were injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both ESO and NAP from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

Linearity study

The peak areas of ESO and NAP were linear with respect to the concentrations over the range of 0.8-4.8µg/ml and 15-90µg/ml respectively. The slope and intercept value for calibration curvey=33.4286x+1.0667(R^2= 0.9999) for ESO and y= 2.4467x+0.5000(R^2= 0.9997) for NAP. Overlay chromatogram of ESO & NAP is shown in figure 5and Calibration plot for ESO & NAP are shown in figure 6.

The results showed that excellent correlation exists between peak area and concentration of the drugs within the concentration range indicated previously.

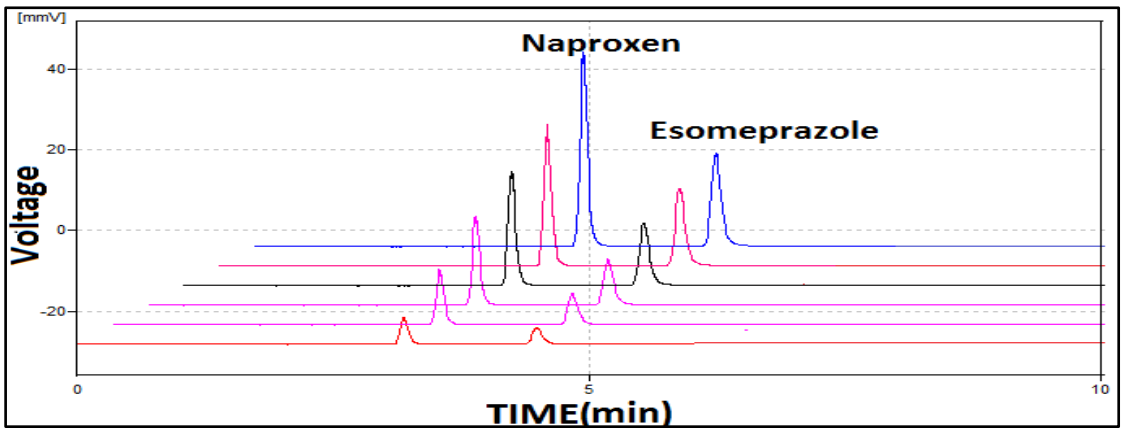

Fig. 5: overlay chromatogram(ESO 0.8 -4.8µg/ml & NAP 15-90µg/ml)

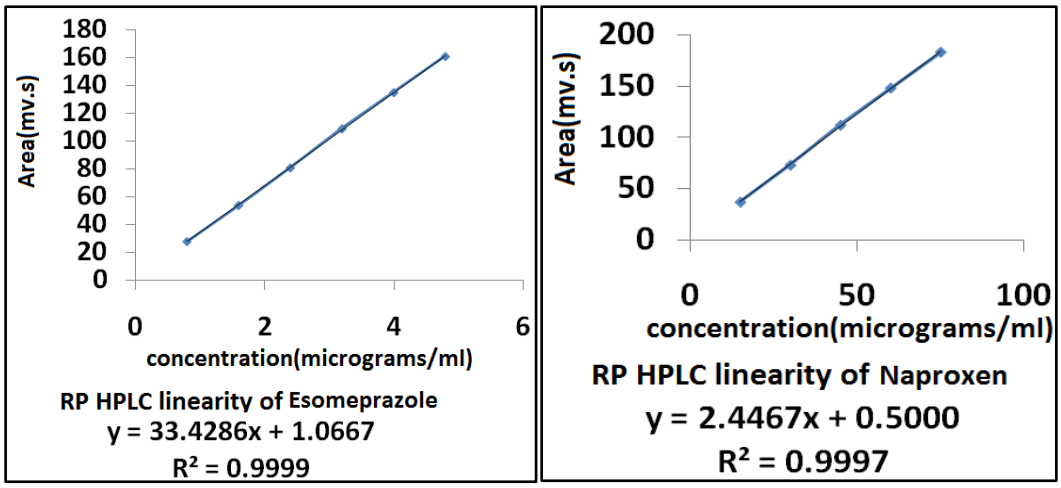

Fig. 6: Calibration plot of ESO & NAP at 303nm

Limit of detection and Limit of quantification

The linearity for ESO was performed from 0.8-4.8µg/ml and that for NAP from 15-90µg/ml. Linearity graph was plotted and the correlation coefficient (R^2) determined. The limit if detection (LOD) was calculated from the linearity curve using the formula

LOD= 3.3X {Residual Standard deviation/Slope}.

The LOD for ESO was confirmed to be 0.016µg/ml and for NAP it was confirmed to be 0.170µg/ml. The Limit of quantification (LOQ) was calculated from the linearity curve using the formula.

LOQ= 10X {Residual Standard deviation/Slope}

The LOQ for ESO was confirmed to be 0.048µg/ml and for NAP it was confirmed to be 0.51µg/ml. LOD &LOQ are shown in table 3.

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. It was confirmed by studying the recovery at three different concentrations, 80%, 100%, and 120 % of those expected by spiking a previously analyzed test solution with additional drug standard solutions, the analysis being done in replicate. The %RSD in all cases were within the acceptable limit (≤ 2%)

Interday and Intraday precision were evaluated by analyzing three concentration, three times and % RSD values obtained were calculated to determine any intraday and interdayvariation.The %RSD in all cases were within the acceptable limit (≤ 2%)

Table 3: Summary of validation parameter

S. No.	Parameters		ESO	NAP
1	Analytical wavelength (nm)		303	303
2	Beer's range (µg/ml)		0.8 to 4.8	15 to 90
3	Equation		y = 33.4286x+1.0667	y =2.4467x+0.5000
4	Correlation coefficient		0.9999	0.9997
5	Intraday precision (%RSD)		0.98	1.2
6	Interday precision (%RSD)		1.03	1.34
7	LOD (µg/ml)		0.016	0.170
8	LOQ (µg/ml)		0.048	0.51
9	Retention time (min.)		4.6	3.2
10	% Recovery	80% standard addition †	99.83	100.2
		100% standard addition †	101.25	101.5
		120% standard addition †	100.95	99.87

† Mean value of five determinations

Robustness

The Robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP- HPLC method developed is robust.

CONCLUSION

The developed method was validated in terms of accuracy, repeatability, and precision. A good linear relationship was observed for ESO and NAP in the concentration ranges of 0.8-4.8µg/mL and 15-90µg/mL respectively. The correlation coefficient for ESO was found to be 0.9999 and that for NAP was 0.9997. The precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of ESO and NAP estimated in the tablet dosage form were free from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise, and accurate, and could be conveniently adopted for the routine quality control analysis of ESO and NAP simultaneously, from its pharmaceutical formulations and bulk drug.

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