The RP-HPLC method for simultaneous estimation of esomeprazole and naproxen in binary combination

Abstract

Objective: A simple, precise, reliable, rapid, sensitive and validated RP-HPLC method has been developed to determine esomeprazole magnesium trihydrate (ESO) and naproxen (NAP) in synthetic mixture form. **Materials and Methods:** Chromatographic separation achieved isocratically on Phenomenex, Luna C18 column (5 µm, 150mm x 4.60mm) and acetonitrile: phosphate buffer (pH 7.0) in the ratio of 50:50 (v/v) as the mobile phase, at a flow rate of 0.5 ml/min. Detection was carried out at 300 nm. The retention times for NAP and ESO was found to be 2.67 ±0.014 and 5.65 ±0.09 min respectively. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. **Results:** The method was linear in the concentration range of 50-250 µg/ml for NAP and 2-10 µg/ml for ESO with correlation coefficient of 0.999 and 0.998 respectively. The mean recoveries obtained for NAP and ESO were 100.01% and 97.76 % respectively and RSD was less than 2. The correlation coefficients for all components are close to 1. **Conclusions:** Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of NAP and ESO.

Key words: Esomeprazole magnesium trihydrate, naproxen, RP-HPLC, validation

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INTRODUCTION

Esomeprazole magnesium trihydrate[1] (ESO), bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl]-1-H-benzimidazole-1-yl)magnesium trihydrate [Figure 1a], is a compound that inhibits gastric acid secretion. ESO is cost-effective in the treatment of gastric oesophageal reflux diseases. ESO is the S-isomer of omeprazole, the first single optical isomer proton pump inhibitor, generally provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to omeprazole.[2] Several methods have been employed for the estimation of ESO alone and combination with other drugs such as UV and RP-HPLC methods.[3-11] Naproxen (NAP) is chemically, (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid [Figure 1b] 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, NAP is capable of producing disturbances in the gastrointestinal tract. Several chromatographic methods have been reported for determination of NAP in raw material,[12] tablets,[13-15] plasma,[16-21] urine,[22-26] plasma and urine,[27] serum,[28] intestinal perfusion samples,[29] and pharmaceutical preparations.[30] There are no pharmacokinetic drug interactions between NAP and ESO. The NAP/ESO tablet is bioequivalent to EC naproxen, and as expected, the bioavailability of non-EC esomeprazole from the NAP/ESO tablet is lower than the EC esomeprazole formulation.[31] According to the information collected from the literature, there is no reported method for simultaneous determination of ESO and NAP as The US Food and Drug Administration (FDA) has recently approved a fixed-dose tablet combination of delayed-release enteric-coated NAP and immediate-release ESO magnesium (*Vimovo*: AstraZeneca and Pozen, Inc). The tablet is available

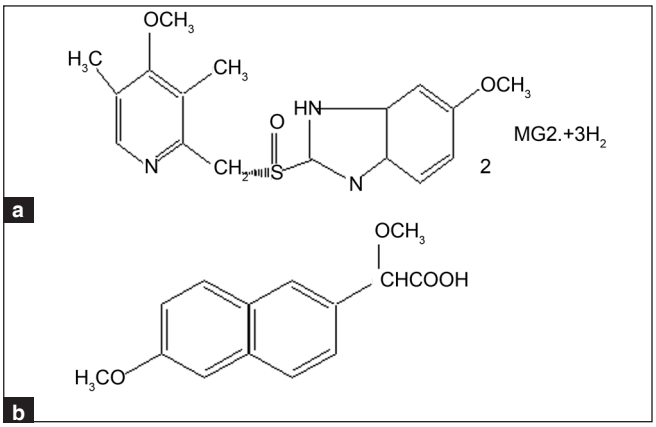

Figure 1: Chemical structures of (a) esomeprazole magnesium trihydrate and (b) naproxen

in the US market not in India. In the present work, we are therefore focused on to achieve the optimum chromatographic conditions for the simultaneous determination of ESO and NAP in a synthetic mixture. The developed method could be applied to quality control of the tablet dosage form whenever it available in Indian market. We are using the same excipients which are used by the manufacturer in tablet formulation. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines,[32] which is mandatory also.

MATERIALS AND METHODS

Instrumentation

Liquid chromatographic Shimadzu (LC-20AT) system was manufactured by Shimadzu Science park drive Pasteur, Singapur Science Park, Gapore 118227, comprising of a manual injector, double reciprocating plunger pump LC-20ATVp for constant flow and constant pressure delivery and Photodiode array detector SPD-M20A connected to software LC solution, (Version-1.23SP1) for controlling the instrumentation as well as processing the data generated, was purchased from SpincoBiotech Pvt. Ltd., No. 3 Sector-II Shanti Nikatan Colony, Gautam Nagar, Bhopal 462023. Weighing was done on a Digital Micro Balance (CX-265) manufactured by Citizen Scale (I) Pvt. Ltd. and pH of buffer was maintained by using a Systronics pH meter.

Chemicals and reagents

Analytically pure sample of ESO was a generous gift from Glenmark Pharma Ltd., Baddi, and NAP was an obtained from Aurbindo Pharma Ltd., Hyderabad. Potassium dihydrogen phosphate, disodium hydrogen phosphate, and acetonitrile (HPLC Grade) were purchased from E. Merck Ltd. Worli, Mumbai, India. The 0.45 μm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd., Chandigadh, India. All excipients used were of pharmaceutical grade. Triple distilled water was generated in house.

Chromatographic conditions

The isocratic mobile phase consisted of acetonitrile–phosphate buffer (pH 7.0) in the ratio of (50:50v/v), flowing through the column at a constant flow rate of 0.5 ml/min. A Phenomenex, Luna C_{18} column (5 μm, 150 × 4.60 mm^2) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of the method for two drugs, 300 nm was selected as the detection wavelength for UV-PDA detector. The HPLC system was operated at a room temperature of 25°C.

Standard preparation

Standard stock solution

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 100 ml of diluent which was a mixture of acetonitrile and phosphate buffer in the ratio of 50:50 (pH 7.0) to get a concentration of 1000 μg/ml.

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 50 to 250, 2 to 10 μg/ml for NAP and ESO, respectively.

Sample preparation

A synthetic mixture was prepared by taking powdered equivalent to 500 mg NAP and 20 mg ESO, and the other tablet excipent such as carnauba wax, colloidal silicon dioxide, croscarmellose sodium, iron oxide yellow, glyceryl monostearate, hypromellose, iron oxide black, magnesium stearate, methylparaben, polysorbate 80, polydextrose, polyethylene glycol, povidone, propylene glycol, propylparaben, titanium dioxide, and triethyl citrate, which are very close to the composition of tablet formulation in 100 ml diluents and then sonicated for 15 min and filtered through Whatman paper no. 41. Then different concentrations of solution were prepared by a serial dilution technique as per standard and each dilution was analyzed.

RESULTS AND DISCUSSION

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water, and

buffer solutions in various proportions and at different pH values. A mobile phase consisting of acetonitrile/phosphate buffer (50:50, v/v, pH 7.0) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 min were studied. A flow rate of 0.5 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using a reversed-phase C18 column, the retention times for NAP and ESO were observed to be 2.67 ± 0.014 and 5.65 ± 0.09 min, respectively. Total time of analysis was less than 6 min. The maximum absorption of NAP and ESO together as detected at 300 nm, and this wavelength was chosen for the analysis [Figure 2].

System suitability

System suitability parameters such as number of theoretical plates, HETP, and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for ESO and NAP were 2948 and 1614, respectively.

Table 1: System suitability parameters

Parameter	Esomeprazole	Naproxen
Retention time*	5.65 ± 0.09	2.64 ± 0.014
Number of theoretical plate*	2948.54 ± 25.05	1614.9 ± 9.50
Tailing factor*	0.85 ± 0.01	1.05 ± 0.008
HETP*	0.05 ± 0.00	0.0928 ± 0.0005
Calibration range (µg/ml)	2–10	50–250

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 2–10 µg/ml for ESO and 50–250 µg/ml for NAP. The linearity was represented by a linear regression equation as follows:

Y (NAP)= 6066.07conc. + 17036.93 (r^2 = 0.999),

Y (ESO)= 34935.04conc. + 2042.686 (r^2 = 0.998)

Table 2: Results of recovery studies with static evaluation

Conc. of drug in preanalyzed samples (µg/ml)		Std. drug sol. added (µg/ml)		Recovered amount* (µg/ml)		% Recovered	
ESO	NAP	ESO	NAP	ESO	NAP	ESO	NAP
4	100	4	80	7.84	180.01	98.08	100.05
4	100	5	100	8.68	200.05	96.51	100.02
4	100	6	120	9.87	219.91	98.70	99.96
					Mean	97.76	100.01
					S.D	0.025	0.477
					%R.S.D	0.29	0.245

*Mean of nine determinations (three replicates at three concentration level)

Table 3: Results of precision and robustness

Validation parameter	Percentage mean ± S.D*(*n* = 5)		Percentage RSD*	
	ESO	NAP	ESO	NAP
Repeatability	97.69 ± 0.68	100.37 ± 0.15	0.69	0.15
Intermediate precision				
Day-to-day	98.60 ± 1.10	99.60 ± 0.14	1.11	1.4
Analyst to analyst	100.62 ± 0.98	99.72 ± 0.27	0.99	0.27
Robustness	100.22 ± 1.30	100.33 ± 0.61	1.29	0.6

*Mean of 15 determinations (three replicates at five concentration level)

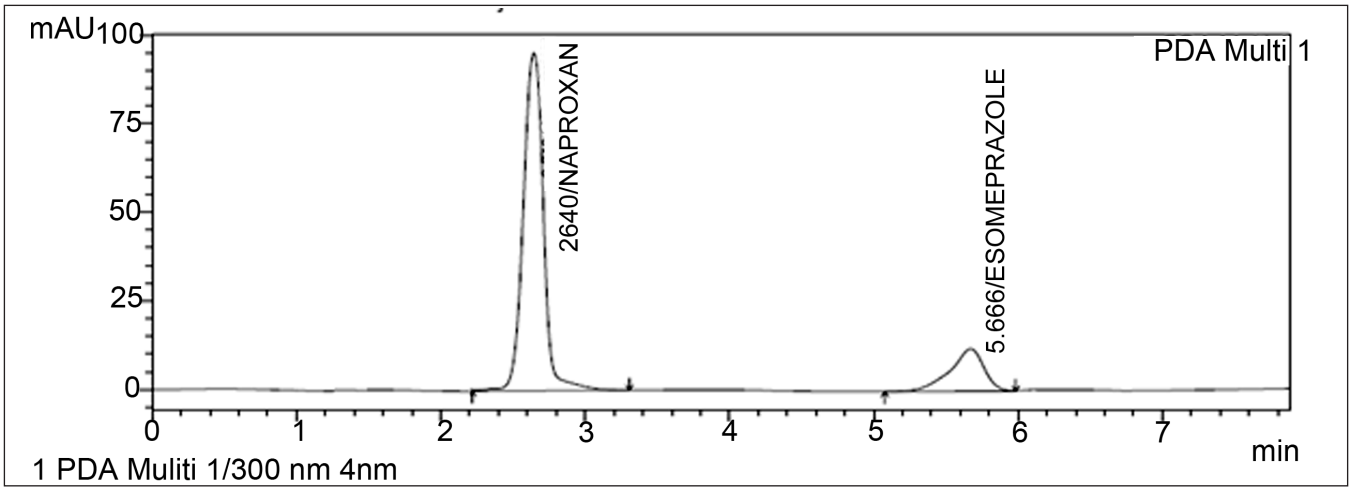

Figure 2: Chromatograms of NAP (200 µg/ml) and ESO (8 µg/ml) reference substances

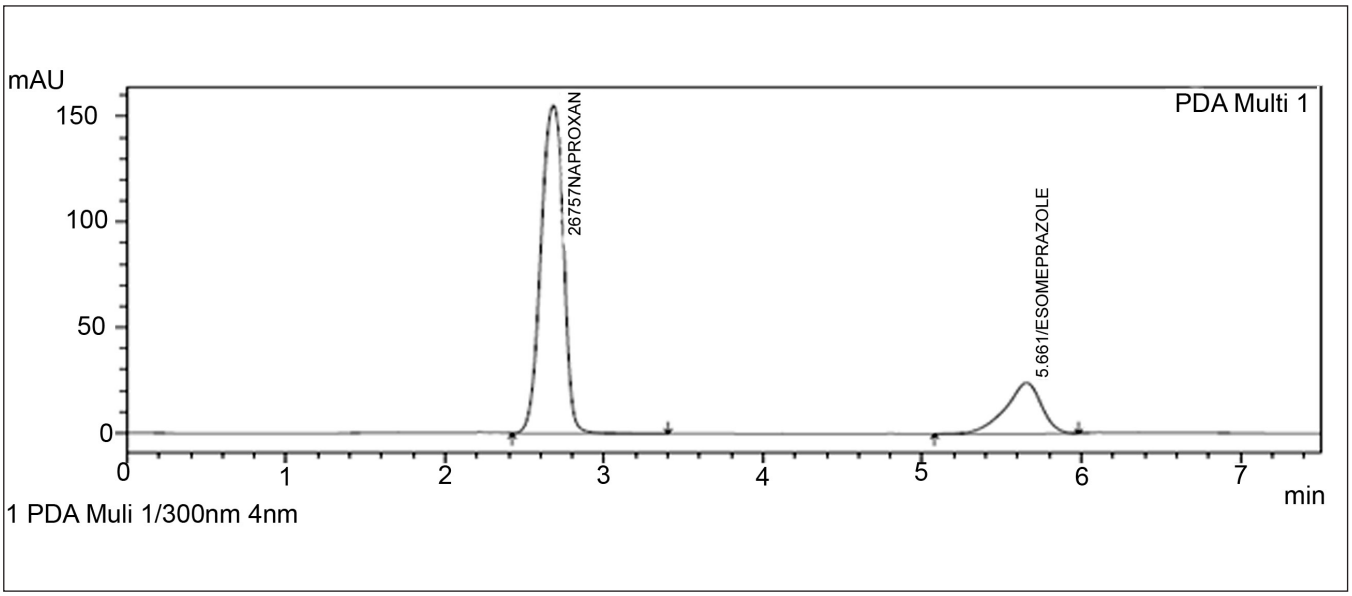

Figure 3: Chromatograms of NAP (150 µg/ml) and ESO (6 µg/ml) in a synthetic mixture

Table 4: Stability data of ESO and NAP

Hours	ESO, 4 µg/ml	NAP, 8 µg/ml
0	154762	647002
6	153618	649872
12	152871	641837

where Y isthe area under curve and r^2 is the correlation coefficient.

Accuracy

Method accuracy was performed by adding known amounts of NAP and ESO to the preanalysed synthetic mixture solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80%, 100%, and 120% of the nominal analytical concentration (4 µg/ml for ESO and 100 µg/ml for NAP). Each level was made in triplicate [Table 2]. The mean percentage recoveries obtained for NAP and ESO were 100.01% and 97.76%, respectively, and RSD was less than 2.

Repeatability

Five dilutions in three replicates were analyzed in the same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in Table 3.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variations, and results were found within acceptable limits (RSD < 2) as shown in Table 3.

Table 5: Statistical evaluation of synthetic mixture analysis

Parameter	Sample	
	ESO	NAP
Mean % estimated	100	98.77
Standard deviation (SD)	1.05	0.77
% Coefficient of variation	0.96	0.78
Standard error (SE)*	0.17	0.31

*Mean of nine determinations (three replicates at three concentration levels)

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method's capacity to remain unaffected. The change was made in the ratio of mobile phase, instead of acetonitrile:phosphate buffer (pH 7.0) (50:50 v/v), acetonitrile:phosphate buffer (pH 7.0) (55:45 v/v) was used as a mobile phase. Results of analysis were summarized in Table 3.

Stability of sample solution

The sample solution injected after 12 h do not show any appreciable change. Results are shown in Table 4.

Specificity and selectivity

Commonly used excipients were spiked in to a preweighed quantity of drugs. The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPLC method is illustrated in Figure 3. Where complete separation of NAP (naproxen) and ESO (esomeprazole) in presence of tablet excipients.

Synthetic mixture analysis

The concentration of ESO and NAP in the synthetic mixture was found to be 100% and 98.77%, respectively. The low values of % coefficient of variation indicate that the method is precise and accurate in Table 5.

CONCLUSION

A simple precise, reliable, rapid, sensitive, and accurate reverse phase HPLC method has been developed for the simultaneous determination of ESO and NAP. The developed method is suitable for the identification and quantification of binary combination of ESO and NAP. A high percentage of recovery and the run time of less than six minutes allow its application for the routine determination of ESO and NAP in the tablet dosage form.

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