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Research Article

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Rapid simultaneous determination of naproxen and esomeprozole magnesium in combined tablets by validated ultra performance liquid chromatographic method

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ABSTRACT

A stability- indicating ultra Performance liquid chromatography (UPLC) method has been developed and validated for the simultaneous determination of Naproxen and Esomeprozole Magnesium in pharmaceutical preparations. An Agilent Zorbax SB Phenyl column (50X4.6mm i.d., 1.8µm particle size) was used. The mobile phase consisted of a mixture of 10 mm Ammonium Bicarbonate (adjusted to pH 7.0 with Phosphoric acid) and acetonitrile in the ratio 50:50 Ultraviolet (UV) detection was performed at 215 nm. Total run time was 5 min; these two drugs were eluted at the retention times of 0.766 and 1.484 min for Naproxen and Esomeprozole Magnesium respectively. The method was validated in terms of linearity, range, specificity, accuracy, and precision, limit of detection (LOD) and limit of quantitation (LOQ). The linearity for both the drugs was found in the range of 18.7-150 µg $mL^{-1}$of Nap and 1-8 µg mL^{-1}. The % recoveries of Naproxen were found to be 98.2-100.2% and Esomeprozole Magnesium were found to be 99.8-101.6% . The utility of the procedure is verified by its application to marketed formulations that were subjected to accelerated degradation studies. The method distinctly separated the drug and degradation products even in actual samples. The products formed in marketed tablet dosage forms are similar to those formed during stress studies.

Key words: Method development, Validation, Simultaneous, Naproxen sodium and Esomeprozole Magnesium Stability-indicating.

INTRODUCTION

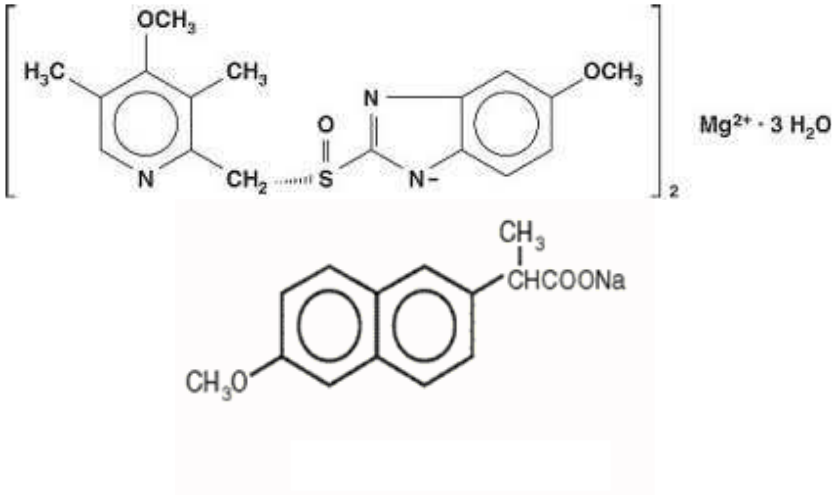
Naproxen is a member of arylacetic acid group of nonsteroidal anti-inflammatory drugs (NSAIDS). Chemically it is (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid, sodium salt. The empirical formula is $C_{14}H_{13}NaO_3$, representing a molecular weight of 252.23.

Esomeprazole Magnesium is a proton pump inhibitor. Chemically it is bis(5-methoxy-2- [(S)- [(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl] - lH-benzimidazole-1 -yl) magnesium trihydrate. Esomeprazole is the S-isomer of omeprazole. The empirical formula is $(C_{17}H_{18}N_3O_3S)_2$ Mg x 3 H_2O, representing a molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis.

A tablet formulation containing 375mg of Naproxen and 20mg of Esomeprozole has recently approved for treatment of migraine. The combination product was proved to have superior efficacy compared to individual components for the acute treatment of migraine. Naproxen sodium helps relive the headaches, while the anti-inflammatory effect decreases the neurogenic inflammation in the trigeminal ganglion, thus preventing the development of central sensitization.

So far, several liquid chromatography procedures have been described for the determination of Naproxen and Esomeprozole. But, these procedures were developed to estimate both Naproxen and Esomeprozole individually and in combination with other drugs from formulation, plasma, urine, intestinal perfusion sample and bulk drugs. For simultaneous determination of Naproxen and Esomeprozole in formulation, there are two spectrometric methods {} and HPTLC method was reported. Where as no single liquid chromatographic method has been reported for simultaneous estimation from combined tablets. Hence, it is necessary to develop a rapid, accurate and validated LC method for the simultaneous determination of naproxen and Esomeprozole from combined dosage form.

Fig. 1: Chemical structures of Esomeprozole Megnesium and Naproxen sodium



EXPERIMENTAL SECTION

Instrumentation and Chromatographic Conditions

The Waters UPLC Acquity system we used consists of a binary solvent manager, a sample manager and a UV detector. Zorbax SB Phenyl, 50 mm x 4.6 mm i.d with 1.8 µm particles was used as stationary phase. 10mm ammonium Bicarbonate (pH 7.0 with ortho Phosphoric acid) as solvent A and acetonitrile was as solvent B used for mobile phase. The mobile phase prepared in the ratio (50:50) prior to use, the mobile phase was mixed thoroughly and degassed. The simple Isocratic Mobile phase pumped at 0.6 mL min^{-1}. The eluants were monitored at 215 nm. The injection volume for samples and standards were 2 µL. Acetonitrile and water in the ratio, 50:50; *v/v,* respectively was used as diluent.

Reagents

Standards were supplied by D.C.O. Hyderabad, India. HPLC grade acetonitrile, analytical grade ortho phosphoric acids were purchased from Merck (Mumbai, India). Water was prepared by Millipore MilliQ Plus water purification system. Commercial pharmaceutical preparation of Vimovo combined tablets were purchased from the market. The declared content of tablets was Naproxen375 mg and 20 mg Esomeprazole per tablet.

Preparation of Solutions

Standard Solutions

A standard solution containing 75 µg mL^{-1} of NS and 4 µg mL^{-1} of ES were prepared by dissolving appropriate amount of NS and ES in diluent. All the solutions were covered with aluminium foil to prevent photolytic reaction until the time of analysis.

Sample Preparation
Ten tablets, each containing 375 mg of NS and 20 mg of ES were dissolved in 1000 mL diluents to get 3750 µg mL^{-1} of SS and 200 µg mL^{-1} of ES. 1mL of above solution was diluted to 50 mL to get 75 µg mL^{-1} of NS and 4 µg mL^{-1} of ES. The solution was filtered through 0.45 µm Millipore PVDF filter. Then 2 µL of these solutions were injected in the column and chromatogram was. The retention times of NS and ES were found to be 0.7 min and 1.4 min, respectively.

System Suitability Solution Criteria
The system suitability was assessed by five replicate analyses of the drugs at concentrations of 75 µg mL^{-1} of NS and 4 µg mL^{-1} of ES. The acceptance criteria was not more than 2.0% for the RSD for the peak areas and not more than 2.0 for tailing factor for the peaks of the both the drugs.

Method Validation
Method validation was performed as per ICH guidance [5-6] for simultaneous determination of NS and ES in the formulations. The following validation characteristics were addressed: linearity, detection limit, quantification limit, precision, accuracy and specificity.

System Suitability Criteria
The system suitability test solution was injected and the chromatographic parameters like relative standard deviation for replicate injections of both NS and ES and the tailing factor for NS and ES peaks were evaluated for proving the system suitability.

Specificity – Forced Degradation Studies
Forced degradation studies were performed on NS and ES combined tablets to prove the stability indicating property of the method. The stress conditions employed for degradation study of NS and ES include acid hydrolysis (1 N HCl), base hydrolysis (1N NaOH), water hydrolysis and oxidation (3% H_2O_2). For light studies, the monitoring period was 10 days whereas for heat, acid, base and water hydrolysis it was 48 h. Oxidation was carried out for 24 h. Peak purity of the principal peak in the chromatogram of stressed samples of NS and ES tablets was checked using photo diode array detector.

Linearity of Response
Linearity solutions were prepared from stock solution at five concentration levels from 18.75 µg mL^{-1} to 150 µg mL^{-1} for NS and from 1 µg mL^{-1} to 8µg mL^{-1} for ES. The slope, Y-intercept and correlation coefficient were calculated.

Precision
Repeatability (intra-day*)*
The precision of the assay method was evaluated by carrying out six independent assays of NS and ES (3.75 mg mL^{-1} of NS and 0.2 mg mL^{-1} of ES) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated.

Intermediate Precision (inter-day)
Different analyst from the same laboratory evaluated the intermediate precision of the method. This was performed by assaying the six samples of NS and ES tablets against qualified reference standard**.** The percentage of RSD of six assay values was calculated.

Accuracy (Recovery study)
The accuracy of the method was evaluated in triplicate at six concentration levels, i.e. 50%, 100% and 150% of target test concentration (3.75 mg mL^{-1} of NS and 0.2 mg mL^{-1} of ES) in combined tablets. The percentages of recoveries were calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
The LOD and LOQ for SS and NS were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration.

Robustness

To determine the robustness of the method the experimental conditions were deliberately changed and the resolution of NS and ES, tailing factor and % RSD for five replicate injections was evaluated. The mobile phase flow rate was 0.6 mL min^{-1}; to study the effect of flow rate on resolution it was changed to 0.5 and 0.7 mL min^{-1}. The effect of pH was studied at pH 7.5 and 6.5 (instead of pH 7.0). The effect of column temperature was studied at 35 and 45 °C (instead of 40 °C). In all these experiments the mobile phase components were not changed.

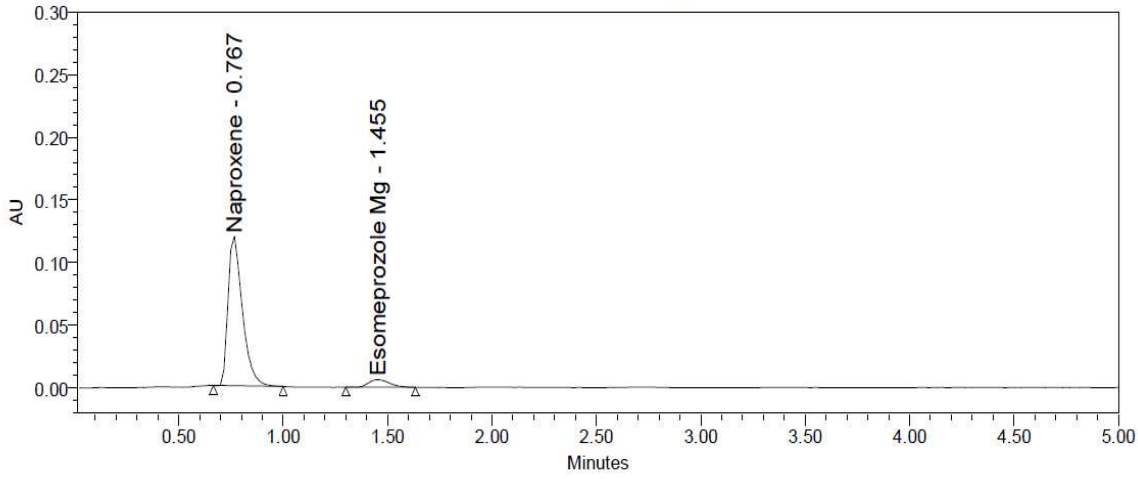
Solution Stability and Mobile Phase Stability

The stability of NS and ES in solution was determined by leaving test solutions of the sample and reference standard in tightly capped volumetric flasks at room temperature for 48 h during which they were assayed at 24 h intervals. Stability in the mobile phase was determined by analysis of freshly prepared sample solutions at 24 h intervals for 48 h and comparing the results with those obtained from freshly prepared reference standard solutions. The mobile phase was prepared at the beginning of the study period and not changed during the experiment. The RSD (%) of the results was calculated for both the mobile phase and solution-stability experiments.

Method Development and optimization of stability indicating assay method

The method was optimized to separate major degradation products formed under varies stress conditions from NS and ES. The main target of the chromatographic method is to get the separation for closely eluting degradation products, mainly for the degradation product which is eluting very closely to the NS. The degradation samples were run using different stationary phases like C18, C8 and Mobile phases containing buffers like phosphate and acetate with different pH (2-7) and using organic modifiers like acetonitrile and methanol in the mobile phase. But the separation was satisfactory in the adopted chromatographic conditions only. It indicated that the gradient elution with 10mm Ammonium bicarbonate in water and acetonitrile ratio 50:50; v/v, mobile phase was successful in separating drugs and all degradation products.

Fig.2: A typical chromatograms obtained from Naproxen sodium and Esomeprazole tablets and from stressed samples



RESULTS AND DISCUSSION

Method Validation

Validation of an analytical procedure is the process by which it is established, by laboratories studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications [28].

System Suitability

The system suitability test solution was injected and the chromatographic parameters like relative standard deviation for replicate injections of I and DC and the tailing factor for both NS and ES peaks are evaluated**.** The relative standard deviation for replicate injections of both NS and ES was 0.5% and 0.3% respectively. The tailing factor for both NS and ES peaks was 1.2% and 1.3%, respectively. This indicates the suitability of the system.

Table .I System Suitability Parameters

Parameter	NS	ES
Retention time	0.7min	1.4min
% RSD(n=6)	0.5	0.3
Asymmetric factor	1.18	1.2
Theoretical plates	4232	3642
Resolution factor	_	4.5

Specificity – Forced Degradation Studies

Degradation was not observed in NS and ES stressed samples that were subjected to light, heat, water and oxidation. However, the degradation was observed under base hydrolysis and acid hydrolysis. The peak purity test results derived from PDA (Photo Diode Array detector) confirmed that the SS and NS peaks were pure and homogeneous in all the analyzed stress conditions. This method indicates that the method is specific and stability indicating.

Linearity of Response

Calibration curve obtained by least square regression analysis between average peak area and the concentration showed (Table 1a and Table 1b) linear relationship with a regression coefficient of 0.999. The best fit linear equation obtained was Y =14165x+52851 for NS and Y=6166x+2836 for ES. Analysis of residuals indicated that the residuals were normally distributed around the mean with uniform variance across all concentrations suggesting the homoscedastic nature of data. Selected linear model with univariant regression showed minimum % bias indicating goodness of fit which was further supported by the low standard error of estimate and mean sum of residual squares.

Table 1a: Linearity results of Naproxene (peak Area Vs Concentration)

Concentration in µg/ml	Mean area Response achieved
18.75	301963
37.5	585381
75.0	1149259
112.5	1631895
150.0	2173125

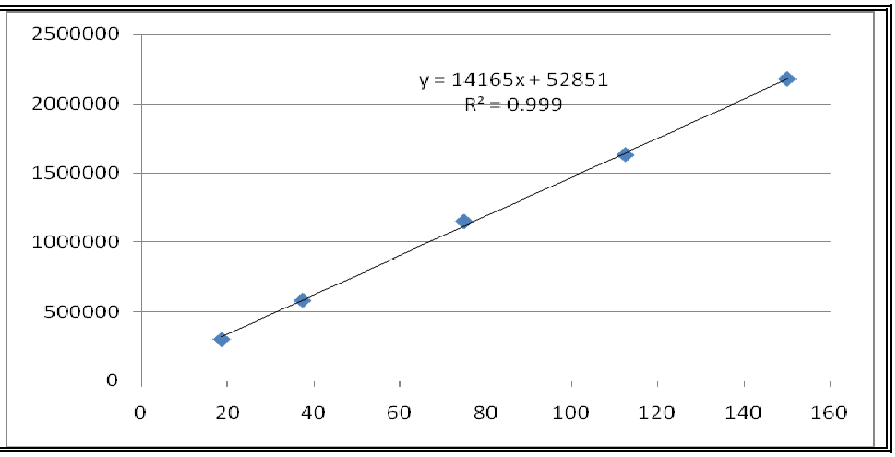
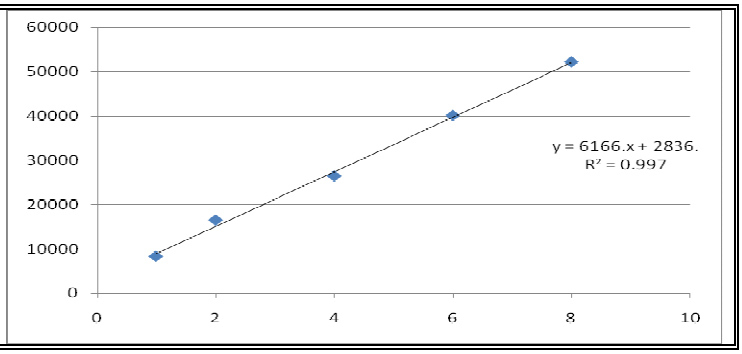


Table 1b: Linearity results of Esomeprazole (Peak area Vs concentration)

Concentration in µg/ml	Mean area Response achieved
1	8344
2	16521
4	26484
6	40124
8	52204



Precision

The precision of an analytical method gives information on the random error. It expresses of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The percentage RSD values for the precision study was 0.7%, 1.3% (inter-day precision) and 0.8%, 1.3% (intra-day precision) for NS and ES, respectively. This is confirming good precision of the method (Table 2).

Table 2: Precision results for Naproxen and Esomeprozole

S. No.	Parameter	Variation	% RSD for Assay	
			Naproxene	Esomeprazole
1	Repeatability (inter-day)	(a) Analyst-1 (b) Waters Acquity UPLC system with PDA detector. (c) Day-1	0.7	1.3
2	Intermediate precision (intra-day)	(a) Analyst-2 (b) Waters Acquity UPLC system with TUV detector. (c) Day-2	0.8	1.3

Accuracy-Recovery Test

The percentage recovery of NS was ranged from 98.2 to 100.2 and ES was ranged from 99.8 to 101.6. Excellent recoveries were made at each added concentration (Table 3).

Table 3. Accuracy data

S. No.	Concentration (%)	Mean recovery (%)	
		NS	ES
1	50	98.2	99.8
2	100	99.3	100.6
3	150	100.2	101.6

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection of NS and ES was 1.8 and 0.4 µg mL^{-1}, respectively for 2 µL injection volume. The limit of quantification of NS and ES was 5.4 and 1.2 µg mL^{-1}, respectively for 2 µL injection volume.

Robustness

When mobile phase flow rate, pH and column temperature were deliberately varied resolution between NS and ES was greater than 3.0, tailing factor and % RSD for five replicate injections of NS and ES was less than 1.5, illustrating the robustness of the method.

Stability in Solution and in the Mobile Phase

RSD (%) for assay of NS and ES during solution stability and mobile phase stability experiments was within 0.9%. No significant changes in the amounts of the two drugs were observed during solution stability and mobile phase experiments. The results from solution stability and mobile phase stability experiments confirmed that standard solutions and e mobile phase were stable for up to 48 h during assay determination

CONCLUSION

A simple specific stability indicating liquid chromatographic method is developed for the quantification of NS and ES simultaneously in combined dosage forms. This method is validated and it is found to be specific, precise, accurate, robust and linear for the detection and quantification of NS and ES.

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