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Original Article

SIMULTANEOUS ESTIMATION OF RELATED COMPOUNDS IN ESOMEPRAZOLE AND NAPROXEN TABLETS BY USING ION PAIR REVERSE PHASE HPLC

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ABSTRACT

Objective: To develop and validate a novel gradient reverse phase HPLC method for quantitative estimation of Naproxen and Esomeprazole impurities in pharmaceutical dosage form.

Methods: Chromatographic separation was achieved on X-Bridge C18,150x4.6 mm, 3.5 µm column. Detection wavelength was set at 302 nm. The mobile phase A consists of Buffer and Acetonitrile in the ratio of 90:10, where Buffer was prepared by dissolving di ammonium hydrogen phosphate (2.64 gm per Liter) and 1-hexane sulphonic acid sodium salt (1.0 gm per Liter), pH adjusted to 6.5±0.05 with orthophosphoric acid. A mixture of acetonitrile and 1-propanol in the ratio of 90:10 was used as mobile phase B. Flow rate was set to 0.7 mL/minute in gradient elution mode, with a retention time for Naproxen and Esomeprazole 29 and 46 minute respectively.

Results: The calibration curve was linear over the concentration range of 4.621 µg/mL – 99.026 µg/mL for Naproxen and 0.254 µg/mL–3.806 µg/mL for Esomeprazole (r= 0.999). The proposed method was found to be (considered)accurate and precise and linear within the desired range. The limit of quantitation (LOQ) was calculated. The purity angle was found less than purity threshold for forced degradation peaks, which shows there was no interference from the common excipient, known impurities and degradents indicating separation, accuracy and reliability of the method. The method was validated as per ICH guidelines and found to be specific, accurate, linear, precise and stability indicating.

Conclusion: A Novel, simple, selective and rapid reversed phase high performance liquid chromatographic (HPLC) method was developed and validated for the estimation of Naproxen and Esomeprazole impurities in pharmaceutical dosage form. Hence, the method can be used for routine analysis in various pharmaceutical industries.

Keywords: Naproxen, Esomeprazole magnesium, Impurities, Simultaneous estimation and Stability indicating.

INTRODUCTION

Esomeprazole magnesium is a proton pump inhibitor [1]. Esomeprazole is the S-isomer of omeprazole, the first single optical isomer proton pump inhibitor. It is used in treatment of gastro esophageal reflux disease [GERD]. Esomeprazole Mg has a chemical name as bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole-1-yl) magnesium trihydrate. [1,2]

Naproxen is a non steroidal anti inflammatory drug [NSAID], commonly used for reduction of moderate to severe pain, fever, inflammation and stiffness. Like other NSAIDs Naproxen is capable of producing disturbances in the gastrointestinal tract. [3] Naproxen has a chemical name as (S)-6-methoxy-α-methyl-2-naphthalene acetic acid. Combination of both Naproxen and Esomeprazole is used for the treatment indicated for the relief sign and symptoms of osteoarthritis, rheumatoid arthritis and decrease the risk of developing gastric ulcers during continuous treatment.

A literature survey reveals that several methods have been reported for determination of assay of Naproxen and Esomeprazole, individually and in combination. Few methods have been reported for the estimation of impurities of each drug individually. [4 - 12] However there are no methods found to determine the impurities of both drugs in a single method. Hence an attempt was made to develop and validate a single method for the estimation of impurities in Naproxen and Esomeprazole tablets.

For this study Citizen Impurity A, Omeprazole sulphone impurity, Ufiprazole impurity [fig. 1] of Esomeprazole mg dihydrate and Naproxen impurity A, Naproxen impurity B, Naproxen impurity C [fig. 2] of Naproxen has been considered.

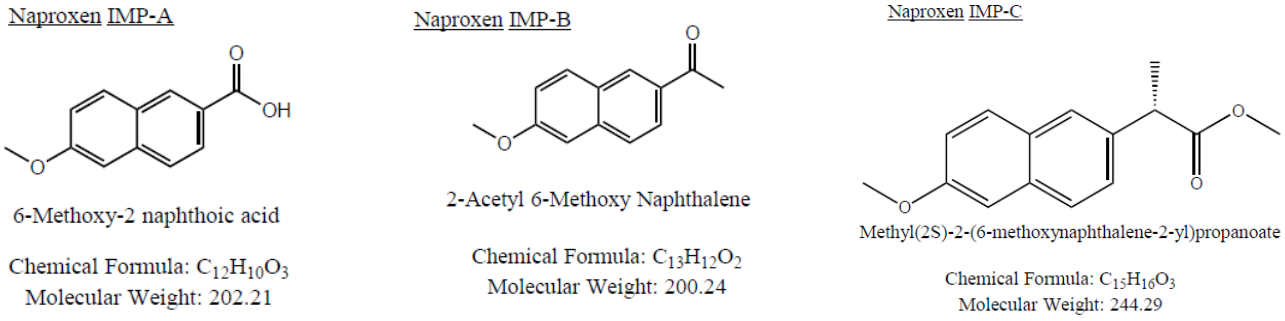

Fig. 1: Chemical structure of Naproxen impurities

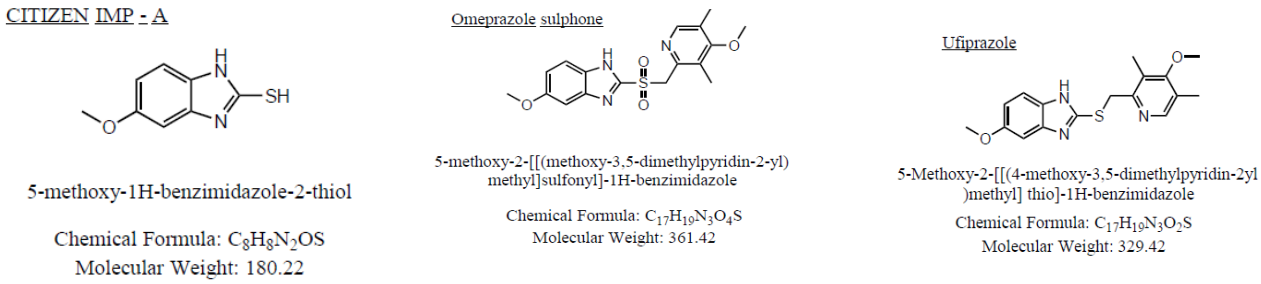

Fig. 2: Chemical structure of Esomeprazole impurities

MATERIALS AND METHODS

Materials, chemicals and reagents

Di-ammonium hydrogen phosphate, O-Phosphoric acid, Ammonia solution (25%) used were of analytical reagent grade. Ion pair 1-Hexane sulphonic acid sodium salt was of HPLC grade. Methanol, Acetonitrile and 1-Propoanol were also of HPLC grade. HPLC grade water was utilized throughout the experiment. Naproxen/ Esomeprazole magnesium tablets, analyte standards and all impurity standards were obtained from Hetero Labs Ltd (Hyderabad. India).

Equipment

High performance Liquid Chromatography system (from Waters) with auto sampler and with Photo Diode Array detector was used for the study. Data was gathered and processed by using Waters Empower software.

Chromatographic conditions

The analysis was carried out on XBridge, C18, 150 x 4.6 mm, 3.5µ particle size. The column Oven temperature was maintained at 30°C. The mobile phase A consists of Buffer and Acetonitrile in the ratio of 90:10 where Buffer is prepared by dissolving di ammonium hydrogen phosphate (2.64 gm per Liter) and 1-hexane sulphonic acid sodium salt (1.0 gm per Liter), pH adjusted to 6.5±0.05 with orthophosphoric acid. A mixture of acetonitrile and 1-propanol in the ratio of 90:10 was used as mobile phase B. Flow rate was set to 0.7 mL/minute in gradient elution mode. Gradient time program was set as T/%B: 0/0, 20/0, 25/10, 50/20, 55/25, 70/30, 80/60, 85/75, 90/75, 92/0 and 100/0.

The injection volume was 10 µL and the detection was performed at 302 nm using a photo diode array (PDA) detector. Various compositions of mobile phase A and mobile phase B with different ion-pairing agents and different gradient programs were tested for this study. The typical retention times of Naproxen and Esomeprazole are 29 minutes and 46 minutes respectively in the final optimized conditions. The criticality of this method is to eluting impurities of both the active ingredients with optimum separation and symmetric peak shapes with no interference due to placebo.

Sample preparation

Diluent preparation

A mixture of Water and Ammonia solution in the ratio of 500:10 v\v was used as diluent 1 to extraction and A mixture of Methanol and Ammonia solution in the ratio of 500:10 v\v was used as diluent 2 as make up diluent for better peak shapes.

Standard preparation

Esomeprazole magnesium Standard Stock Solution (ESM Stock) was prepared by weighing accurately 26mg of standard in 100 mL volumetric flask, added about 60 mL of diluent 2, sonicated to dissolve and diluted to volume with diluent 2. (0.26 mg/mL) Standard solution containing both Naproxen and Esomeprazole was prepared by weighing accurately 13mg of Naproxen standard in 200 mL volumetric flask, added about 120 mL of diluent 2, sonicated to dissolve, to this added 2.0 mL of ESM Stock, dissolved and diluted to volume with diluent 2 and mixed well.(2.6 µg/mL for Esomeprazole and 65 µg/mL for Naproxen)

A sensitivity standard solution of Esomeprazole was also prepared by diluting 2.0 mL of ESM Stock to 200 mL and further diluting 2.0 mL to 20 mL using diluent 2. (0.26 µg/mL)

Test preparation

Transferred 5 tablets (Hetero Labs Ltd Hyderabad, India) into a 200 mL volumetric flask, added about 30 mL diluent 1 and sonicated for not less than 30 minutes with intermediate shaking. Further added 30 mL of diluent 2 and continued for shaking for 15 minutes. Diluted to volume with diluent 2 and mixed well. Filtered the solution through 0.45 µm membrane filter by discarding the first few mL of the filtrate.

Experimental design

Method validation

The aim of method validation was to confirm that the present method was suitable for its intended purpose as described in ICH guidelines [15]. The described method has been widely validated in terms of specificity, precision, linearity, accuracy and Limit of Quantification. Specificity of the method was evaluated by injecting individual impurities and by subjecting the drug in to force stress conditions. The precision of the method was expressed in term of coefficient of variation for % of impurities. The accuracy was expressed in terms of percent recovery of the known amount of impurities added to the sample preparation. To perform the validation activity Naproxen impurity A, Naproxen impurity B and Naproxen impurity C of Naproxen, and Citizen impurity A, Omeprazole sulfone and Ufiprazole were selected.

RESULTS AND DISCUSSIONS

The proposed HPLC method, allows an accurate and precise quatitation of impurities of Naproxen and Esomeprazole in pharmaceutical dosage form. The obtained validation results were discussed as below in individual section.

System suitability

System suitability tests are part and parcel of a liquid chromatographic method. As integral part of chromatographic method system suitability parameters like USP Tailing, theoretical plates and Relative standard deviation (RSD) for replicate injections were evaluated and found to be satisfactory as per common chromatographic practices. Since it is related substances method, it is necessary to monitor the sensitivity of the method and it was done by injecting a sensitivity standard of Esomeprazole and determining S/N ratio. According to the results presented, the proposed method fulfills these requirements within the accepted limits. Results are shown in table No 1.

Specificity

Specificity is the ability to assess unequivocally the analytes in the presence of components which may be expected to be present. Typically these might include impurities, degradents, matrix (placebo), etc. Specificity was tested by injecting the impurity standards individually. Test spiked with all impurities (fig. 3), placebo preparation and forced degradation samples. Results of impurity interference study have been tabulated in table No 2.

Forced degradation studies were carried out to provide an indication of the stability indicating property and specificity of the

proposed method. Intentional degradation was attempted to stress conditions like acid hydrolysis (using 1 N HCl), base hydrolysis (using 1 N NaOH), and oxidative degradation (using 3.0% H2O2) to evaluate the ability of the proposed method to separate degradation products from active ingredients. To check and ensure the homogeneity (peak purity) of peaks in the stressed sample solutions, photo diode array detector was employed. In forced degradation it was observed that Esomeprazole is susceptible to degradation in acid and oxidative stress conditions, whereas Naproxen is found to be stable under all stress conditions. Peak purity in all the degradation conditions has been proven for both analytes. Results are listed in table No 3.

Table 1: Results of system suitability test

Name of Drug substances	Theoretical plates	USP Tailing factor	%RSD for replicate injections	S/N ratio(at 0.05%)
Esomeprazole	100466	1	0.6	106
Naproxen	79728	1.4	0.7	426

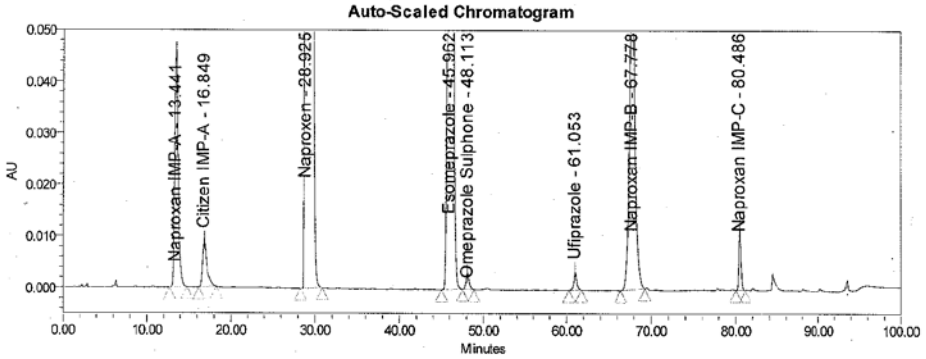

Fig. 3: Typical Chromatogram of Test spiked with impurities

Table 2: Results of Specificity - Impurity Interference

Impurity Name	RT (minutes)
Citizen Impurity A	17.453
Omeprazole sulphone	48.4
Ufiprazole	61.208
Naproxen Impurity A	13.011
Naproxen Impurity B	68.01
Naproxen Impurity C	80.59
Naproxen	29.124
Esomeprazole	46.182

Table 3: Results of Forced degradation Studies with Peak purity details

Stress Conditions	Naproxen		Esomeprazole		% degradation of Esomeprazole	% degradation of Naproxen
	PA	PT	PA	PT		
Acid Degradation	0.299	0.418	0.605	1.177	8.95	Nil
Alkali Degradation	0.174	0.357	0.794	1.237	Nil	Nil
Peroxide Degradation	0.152	0.353	0.578	1.190	19.24	Nil

PA = Purity Angle,PT= Purity Threshold, Note: Purity Angle should be less than Purity Threshold to meet Peak purity acceptance criteria

Linearity

The linearity of the method was checked in order to demonstrate the proportional relationship of response versus impurities concentration over the working range. It is usual practice to perform linearity experiments over a wide range of impurity concentration covering from about LOQ to a higher level. This gives confidence that the response and concentration are proportional and consequently ensures that calculations can be performed in the specified range.

The linearity of detector response to different concentrations of all impurities of Naproxen and Esomeprazole was studied by preparing a series of solutions. The data were subjected to statistical analysis using a linear-regression model. The results have indicated superior linearity. Results are given in table No 4.

Precision

Six sample solutions were prepared using the single sample lot of Naproxen and Esomeprazole tablets by spiking the known impurities and the precision of the method was tested. The % RSD calculated for all impurity results indicate that the proposed method has got an acceptable level of repeatability. Results are listed in table No 5.

Accuracy

Accuracy of the proposed method was established by recovery experiments. This study was employed by spiking of known amounts of impurities into samples of at 0.05%, 100% and 150% of targeted concentration, in triplicate and injected into the chromatography system.

The resulting mixtures were analyzed as described in the proposed method. Results obtained from recovery studies are given in table No 6.

Limit of quantification (LOQ)

The limit of quantification for all impurities of Naproxen and Esomeprazole were determined by the signal to noise ratio method.

As reporting threshold will be 0.05% for this product, LOQ was considered at that level for all impurities and it was found that, at 0.05% level signal to ratio was found to be more than 10 for all impurities. Hence 0.05% was considered as LOQ.

Table 4: Results of Linearity Studies (Response Vs Concentration)

Name of impurities	Correlation coefficient	Intercept	Slope
Citizen Impurity A	0.999	6259.8	107639.8
Omeprazole sulphone	0.999	963.9	31855.9
Ufiprazole	0.999	655.8	39989.3
Naproxen Impurity A	0.999	23242.1	21311.9
Naproxen Impurity B	0.999	58835.6	51049.2
Naproxen Impurity C	0.999	5738.6	2814.8

Table 5: Method Precision data

Impurity names	Mean from six samples (% impurity)	% RSD for six samples
Citizen Impurity A	0.616	0.86
Omeprazole sulphone	0.484	0.66
Ufiprazole	0.454	0.35
Naproxen Impurity A	0.489	0.35
Naproxen Impurity B	0.507	0.36
Naproxen Impurity C	0.544	0.33

Table 6: Results of Recovery Study at Different Levels

Name of impurity	0.05% level	100% level	150% level
Citizen Impurity A	97.9	98.3	101.2
Omeprazole sulphone	94.7	88.7	92.2
Ufiprazole	96.3	88.4	91.6
Naproxen Impurity A	91.2	94.6	96.2
Naproxen Impurity B	96.5	96.0	97.4
Naproxen Impurity C	90.4	97.4	98.3

Note: Number of samples analyzed at each level is in triplicate

Solution stability

Solution Stability studies were performed for Standard and test spiked solution for about 24 hours at 5°C. Results from this study were compared against the initial standard area and % impurity for the test solution. Data indicate that both the standard and test solution are stable at 5°C for about 24 hours with acceptance criteria of ± 0.05 for impurities and ±5 % for standards. Results obtained from solution stability studies are given in table No 7.

Table 7: Results of Solution Stability Studies

Name	Initial	After 24 hours	Difference from the initial
Standard for Esomeprazole	84348	86200	2.2%
Standard for Naproxen	185834	194770	4.8%
Citizen Impurity A	0.613	0.621	0.008
Omeprazole Sulphone	0.484	0.479	0.005
Ufiprazole	0.456	0.457	0.001
Naproxen Impurity A	0.486	0.49	0.004
Naproxen Impurity B	0.501	0.503	0.002
Naproxen Impurity C	0.543	0.559	0.016
Total impurities	3.083	3.109	0.026

CONCLUSION

The novel gradient RP-HPLC method developed for simultaneous quantitative analysis of impurities of Naproxen and Esomeprazole magnesium present in pharmaceutical dosage form is accurate, precise, linear and specific. Satisfactory results were obtained during method validation experiments. This method is appropriate for the routine analysis of production samples to check the quality of the product with respect to impurities.

CONFLICT OF INTEREST

Declared none

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