DEVELOPMENT AND VALIDATION OF METHOD FOR DETERMINATION OF ESOMEPRAZOLE AND NAPROXEN FROM COBINATION PRODUCT BY HPLC

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ABSTRACT
A simple selective and rapid reversed phase high performance liquid chromatographic (RPHPLC) method for the analysis Esomeprazole and Naproxen has been developed and validated. The separation was achieved from HPLC Column (Cogent, C18 250mm x 4.6 mm, 5 μm) with a mobile phase consisting (Buffer: Acetonitrile: Methanol = 50:40:10, add 0.1% v/v Triethylamine in above mixture and finally adjust with glacial acetic acid to a pH 7.0) flow rate 1.0 ml/min with UV detection at 303 nm. The method was specific and it was observed that no interference with diluents. Proposed method is accurate with (99.87%-100.08%) recovery for Naproxen and (99.44%-99.87%) recovery for Esomeprazole. The proposed method was accurate, and precise for the quantification of Esomeprazole and Naproxen in the tablet. The proposed method can also be used for routine analysis in quality control. The method was validated for the parameters like selectivity, sensitivity, precision, intermediate precision, accuracy, linearity, recovery & stability. This RP -HPLC method is suitable for determining the concentration of Esomeprazole and Naproxen in tablet and it can applied for routine analysis for determination of the Esomeprazole and Naproxen from dosage form.
Key Words: ICH, Gastroesophegeal,Laryngopharyngeal, NSAID, Proton pump inhibitor.

INTRODUCTION

The Naproxen is a Non-steroidal anti-inflammatory drug (NSAID) having chemical name (+)-(*S*)-6-Methoxy-α-methyl-2-naphthaleneacetic acid. The empirical formula is $C_{14}H_{14}O_3$ and its molecular weight 230.26 [1]. Naproxen is white to off white practically odorless crystalline powder. Soluble in chloroform, in dehydrated alcohol, sparingly soluble in ether, practically insoluble in water [2].

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Naproxen is used to inhibit prostaglandin synthesis as a result it acts as analgesic, anti-inflammatory and anti-pyretic properties [3]. The Esomeprazole is a proton pump inhibitor having chemical name 1 H-Benzimidazole, 5-methoxy -2 [(s)-[4-methoxy -3,5-dimethyl -2-pyridenyl)methyl] sulfinyl], magnesium salt trihydrate. The empirical formula is $C_{34}H_{36}MgN_6O_6S_2.3H_2O$ and its molecular weight is 767.17.[4] Esomeprazole is white to slightly colored powder, soluble in methanol, slightly soluble in water, practically insoluble in heptanes [5]. Esomeprazole is used to treat dyspepsia, peptic ulcer disease, gastroesophageal reflux disease, laryngopharyngeal reflux, and Zollinger–Ellison syndrome and NSAID associated ulcer. Validation is a fast growing and evolving subject. It is a requirement that has always made sense from both a

regulatory and quality perspective. [6], [7]. The analytical process are generally validated following the general non-mandatory guidelines as this process varies so widely and there is no universal approach from regulatory bodies such as US FDA and EC.[8],[9]. The most common reason for validation is to guarantee as per as possible that all process and machinery in the pharmaceutical manufacturing process are being used in a way which will ensure safety, integrity, quality and strength of the product for use by general public.[10], [11]. Densitometric determination of Esomeprazole with Domperidone was also established [12]. Spectroscopic estimation of Esomeprazole magnesium in solid dosage form with some other NSAID'S [13-16] Physico-chemical characterization, UV Spectrophotometric method development and validation studies for Esomeprazole Magnesium Trihydrate was reported in Literature [17-18]. A UPLC stability indicating method for determination of impurities in Esomeprazole Magnesium gastro resistant tablets was also reported in the literature [19].

Only few methods are available for determination of Naproxen and Esomeprazole in the product. So present work was undertaken with the aim to develop and validate a rapid and consistent reversed phase high performance liquid chromatographic method for determination of Naproxen and Esomeprazole according to ICH guideline. [20].

MATERIALS AND METHODS [21-22]
Reagents and chemicals
HPLC grade Acetonitrile, HPLC grade Methanol & Triethylamine, Glacial acetic acid, Disodium Hydrogen Phosphate, Potassium Dihydrogen Phosphate and Sodium Hydroxide, from Merck Germany. Naproxen WS, from Dr. Reddy's Laboratories Ltd, Mexico. Esomeprazole Magnesium WS from Cameo Healthcare, India and Millipore water.

Instruments
Analytical balance Sartorious (model: TE214S). HPLC Dionex Ultimate 3000, HPLC Shimadzu Prominence, Hanna pH meter, Column (Cogent, C18, 250mm x 4.6mm, 5μ).

Method Development [23-28]
Preparation of mobile phase
A mixture of Buffer, Acetonitrile and methanol in the ratio of 50:40:10 was added with 0.1% v/v Triethylamine and finally adjusted with glacial acetic acid to a pH of 7.0.

Preparation of Buffer
Solution was prepared by adding dibasic sodium phosphate (1.42 mg/ml) and monobasic potassium phosphate (1.36 mg/ml) in HPLC grade water.

Preparation of Diluents
A mixture of 0.1M Sodium Hydroxide and Methanol was prepared in the ratio of 50: 50.

Chromatographic Condition
Cogent C_{18}, 250 mm x 4.6 mm, 5 μ, Injection volume 20μl, wavelength 303 nm, temperature 30°C± 2°C, Flow rate 1.0 ml/min.

Standard preparation

Naproxen stock solution

At first, about 38.0 mg of Naproxen WS was transferred into 25 ml volumetric flask then added about 20 ml of diluting solvent and sonicated sufficiently to completely dissolve the content. The solution was kept for few minutes to cool the content at room temperature and made volume up to the mark with diluting solvent.

Esomeprazole Stock solution
About 22.3 mg of Esomeprazole Magnesium WS was transferred into 100 ml volumetric flask then added about 70 ml of diluting solvent and sonicated sufficiently to completely dissolve the content. The solution was kept for few minutes to cool the content at room temperature and made volume up to the mark with diluting solvent.

Final standard solution
Transferred 5 ml stock solution from Naproxen and 2 ml stock solution from Esomeprazole magnesium into 50 ml volumetric flask and made the volume up to the mark with mobile phase.

Sample preparation
An average weight of randomly selected 20 tablets was determined and crushed to fine powder with mortar and pestle. Accurate weight of powder that contains about 500 mg Naproxen and 20 mg Esomeprazole was transferred into 200 ml volumetric flask. Then about 150 ml of diluting solvent was added and Sonicated about 60 minutes with intermittent shaking. The sample was kept for few minutes to cool at room temperature and made volume up to the mark with diluents. The solution was filtered through Whatman filter paper No 42 and collected the filtrate discarding first few ml. 3 ml of filtrate was diluted to 50 ml with mobile phase. Then the solution was filtered through 0.45 μ disk filter and collected the filtrate.

System suitability solution
The final standard solution was used as system suitability solution. 20 μl of five replicate injections of standard solution were injected. The chromatogram was recorded and the system suitability parameters of the injections were checked for % RSD of area within 2.0 %. The resolution and

the plate count should not be less than 1.5 and 2000 respectively. However, the tailing factor should not be more than 2.0.

Method Validation [23-28]

System suitability

The system was deemed suitable if the following acceptance criteria were satisfied. The relative standard deviation (% RSD) of the peak area responses for Naproxen and Esomeprazole from five replicate injections of standard solution was not more than 2.0%. The resolution and the plate count should not be less than 1.5 and 2000 respectively. However, the tailing factor should not be more than 2.0.

Specificity

For specificity study identification, placebo interference and RT ratio of sample and standard were observed.

Linearity

The linearity was carried out by observing the correlation coefficient (r) of standard solution.

System Precision

System precision was carried out by performing six replicate injections of standard at 100% of the test concentration and calculating the % RSD of the measured area.

Method precision

To demonstrate method precision, six replicate of sample against standard at 100% of test concentration was carried out and the precision of method was calculated by computing % RSD of six measurements.

Intermediate precision (Ruggedness)

Intermediate precision or ruggedness study of an analytical method is the degree of reproducibility of the test results obtain by the analysis of the same samples under a variety of normal test conditions. Test sample of Naproxen and Esomeprazole representing single batch was analyzed by two different analysts in two different equipments at different days. The ruggedness of the test method was calculated by measuring % RSD of six results and % RSD of results of two analysts.

Accuracy

Study was carried out over a range of 80% - 120% (3 replicate each) of the test concentration. The % recovery and RSD of % recovery for each concentration was also measured.

Range

Data generated in linearity, precision and accuracy were considered for establishing the range of the analytical method.

Robustness

Robustness of the method was investigated by changing flow rate (±0.1%), column temperature (±5⁰C) and ratio of components of mobile phase.

Stability study

The solution stability experiments were carried out under room temperature at intervals of 0h, 6h, 12h, 18 h, 24 h, 30h, and, 48 h.

RESULTS AND DISCUSSION

System suitability

System suitability is an integral part of analytical procedures. The result obtained from the System suitability is shown in table 01&02. In optimized chromatographic conditions, Relative Standard Deviation (% RSD) of area and retention time of Naproxen and Esomeprazole were 0.07%, 0.116% and 0.049% and 0.034%, respectively. The average tailing factor for Naproxen and Esomeprazole were 1.036 and 0.92, respectively at average resolution 8.078. The obtained results satisfy the USP requirements.

Specificity

Specificity of an analytical method is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.[29] From the specificity study it was observed that the chromatogram for naproxen and Esomeprazole sample with reference standard showed positive response and Blank (Placebo) had no response, So the method was specific.

System precision

System precision was carried out by performing six replicate injections at 100% of the test concentration and calculating the %RSD, Tailing factor, resolution and Theoretical plate count. From the data (Table 01 & 02), it is observed that Relative Standard Deviation (% RSD) of area and retention time of Naproxen and Esomeprazole were 0.07%, 0.12% and 0.049%, 0.034%, respectively. The average tailing factor for Naproxen and Esomeprazole were 1.036 and 0.92, respectively at average resolution 8.078, whereas, theoretical plate count for Naproxen and Esomeprazole were 5407 and 6008 respectively. The results correspond the USP requirements.

Method precision

The result shows that the % RSD of six samples was found to be 0.78% and 0.074% for Naproxen and Esomeprazole (table 03) .

Intermediate precision or Ruggedness

Assay result by two different analysts at different days have been found quite close to each other and % RSD of two analysts (12 samples) is 1.244% for

Naproxen and 0.28% for Esomeprazole which was within acceptance criteria. So the method can be considered to be rugged enough (Table-03)

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The result shows that average % recovery at different accuracy levels are 99.98% and 99.70% for Naproxen and Esomeprazole respectively (Table-04). Correlation coefficient for Naproxen and Esomeprazole is 1.0000 (Figure 03 & 04).

Linearity

The linearity of an analytical method is its ability to elicit test results directly proportional to the concentration of the analyte in samples within given range. [30]. Linearity of the method was evaluated from the correlation coefficient of calibration curves that were constructed from mean peak area of Naproxen and Esomeprazole at different concentrations level of 80%, 90%, 100%, 110% and 120% (Table 05). Correlation coefficient for Naproxen and Esomeprazole were 0.9998 and 0.9997, respectively (Figure 03 & 04).

Range

 The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of the analyte within the extremes of the specified range of the analytical procedure. Based on the linearity, precision and accuracy results, the Range of the method was determined as 80% to 120% of the target concentration.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage.[31] The robustness of this method was determined by analyzing the same batch of sample by deliberately changing the method parameters like machine, P^H of mobile phase and ratio of mobile phase. From the results presented in table 06 it is cleared that the system suitability criteria meet with the acceptance limit. Hence the method is robust.

Stability study

The result of the stability study is presented in Table .From the data it is observed that the test sample solution is stable up to 48 h at ambient condition.

Table-01: Data for System Precision (Naproxen)

Standard Concentration (µg/ml)	No. of Measurement	Retention Time(mins)	Peak Area	Theoretical Plate	Tailing Factor	Resolution
	01	3.540	931803	5518	1.03	8.14
	02	3.537	929857	5475	1.04	8.14
	03	3.537	930783	5443	1.05	8.13
	04	3.537	930402	5363	1.04	8.04
	05	3.540	930423	5342	1.02	8.03
	06	3.537	930735	5301	1.04	7.99
Average	Average	3.538	930667	5407	1.036	8.078
Relative standard deviation	Relative	0.049%	0.070%	1.5661%	0.920%	0.804%

Table-02: Data for System Precision (Esomeprazole Magnesium)

Standard Concentration (µg/ml)	No. of Measurement	Retention Time	Peak Area	Theoretical Plate	Tailing Factor	Resolution
	01	5.447	616657	6098	0.93	8.14
	02	5.443	614857	6103	0.92	8.14
	03	5.447	614909	6074	0.90	8.13

04	5.443	614972	5957	0.91	8.04
05	5.447	614859	5940	0.91	8.03
06	5.443	615555	5874	0.91	7.99
Average	5.445	615301	6008	0.920	8.078
Relative standard deviation	0.034%	0.116%	1.608%	0.681%	0.804%

Table-03: Data for Intermediate Precision

Sample No.	% of label claim			
	Day-1		Day-2	
	Naproxen	Esomeprazole	Naproxen	Esomeprazole
1	99.35	99.70	100.12	100.25
2	99.42	99.75	99.83	99.65
3	99.45	99.55	100.25	99.50
4	99.26	99.75	100.53	99.20
5	99.42	99.70	100.06	99.25
6	99.47	99.70	99.67	99.35
% of RSD	0.078%	0.074%	0.304%	0.399%
% of RSD of 12 samples	Naproxen		0.415%	
	Esomeprazole		0.280%	

Table-04: Data for Accuracy

Concentration Level	Sample No.	Amount added in (µg/ml)		Amount Recovered in (µg/ml)		% Recovery	
		Naproxen	Esomiprazole	Naproxen	Esomeprazole	Naproxen	Esomeprazole
80%	1	120.09	17.86	120.80	17.81	100.59	99.72
	2	120.11	17.85	120.56	17.75	100.38	99.44
	3	120.09	17.88	120.69	17.78	100.49	99.44
100%	1	150.12	22.30	149.22	22.27	99.40	99.87
	2	150.12	22.32	149.02	22.24	99.27	99.64
	3	150.18	22.31	149.03	22.28	99.23	99.87
120%	1	180.18	26.78	177.98	26.70	98.78	99.70
	2	180.18	26.78	178.44	26.74	99.03	99.85
	3	180.18	26.78	178.70	26.72	99.18	99.78

Table-05: Data for Linearity

% Concentration		Concentration (µg/ml)	Peak Area	Correlation co-efficient	
				Naproxen	Esomeprazole
80	Naproxen	121.6	753720		
	Esomeprazole	6.4	495424	0.9998	0.9997
90	Naproxen	137.2	845731		
	Esomeprazole	7.2	550784		

100	Naproxen	152.4	936386		
	Esomeprazole	8.0	620801		
110	Naproxen	167.2	1017969		
	Esomeprazole	8.8	671827		
120	Naproxen	182.8	1129734		
	Esomeprazole	9.6	735618		

Table-06: Data for Robustness

SL. No.	Changing Parameters	Assay results (%)	
		Naproxen	Esomeprazole
01.	HPLC: Dionex-Ultimate 3000 Column: Cogent HPLC Column – Serial No. M11-ST04-433	99.26	99.75
02.	HPLC: Simadzu Prominance Column: Cogent HPLC Column – Serial No. M12-ST06-003	101.33	99.20
03.	Mobile Phase (Actual) Buffer : Acetonitrile : Methanol = 50:40:10	100.92	100.25
04.	Mobile Phase: Buffer : Acetonitrile : Methanol = 40:50:10	99.50	99.75
05.	Mobile Phase: Buffer : Acetonitrile : Methanol = 60:30:10	101.97	100.40
06.	pH of Mobile phase 6.8	99.93	100.70
07.	pH of Mobile phase 7.2	100.29	100.25

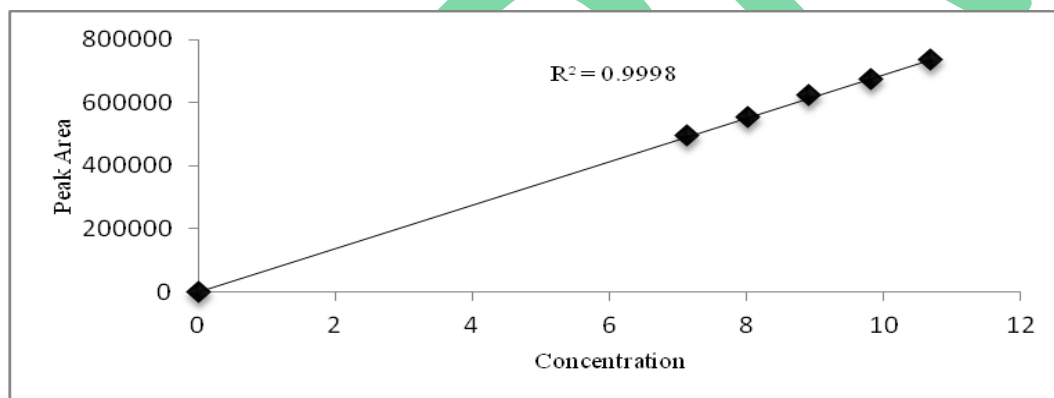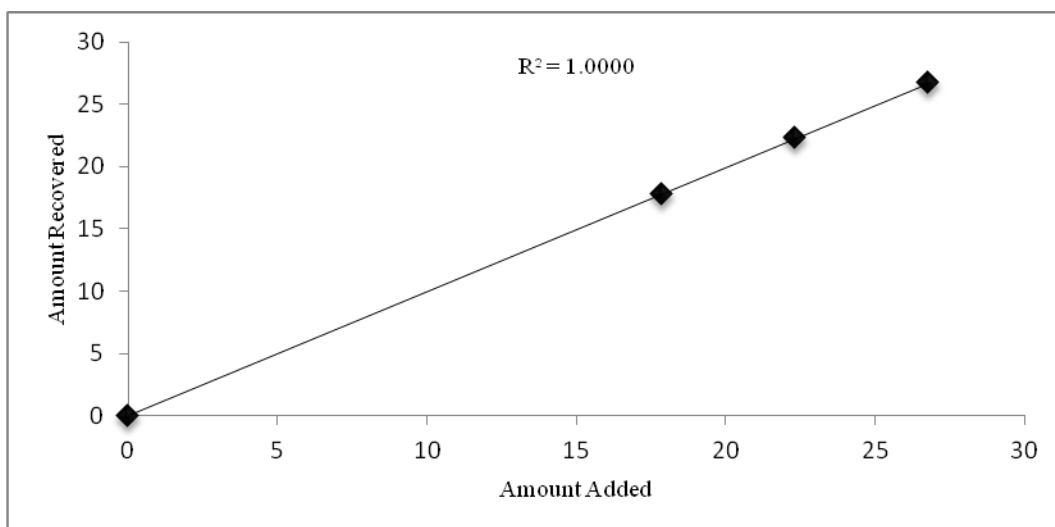$R^2 = 1.0000$

$R^2 = 0.9998$
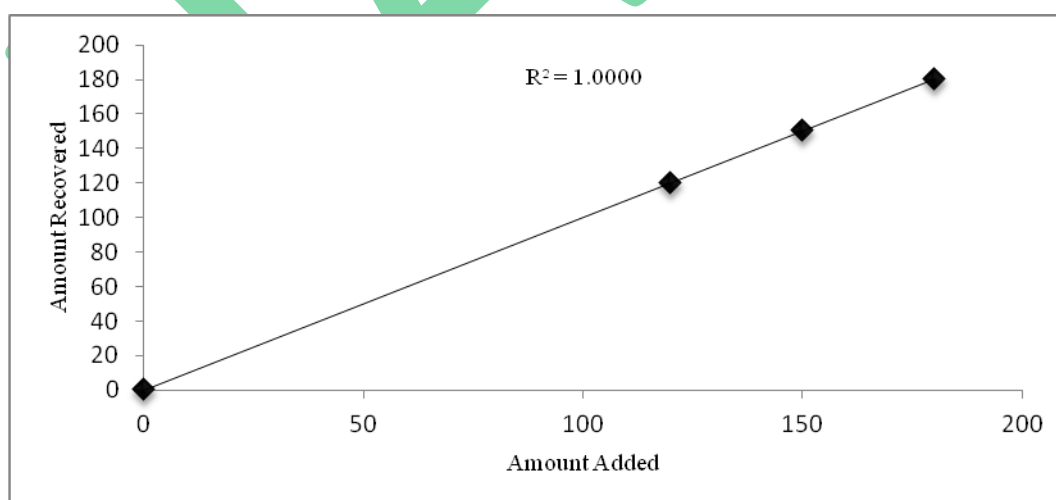
Figure-03: Graphical representation for Linearity of Naproxen
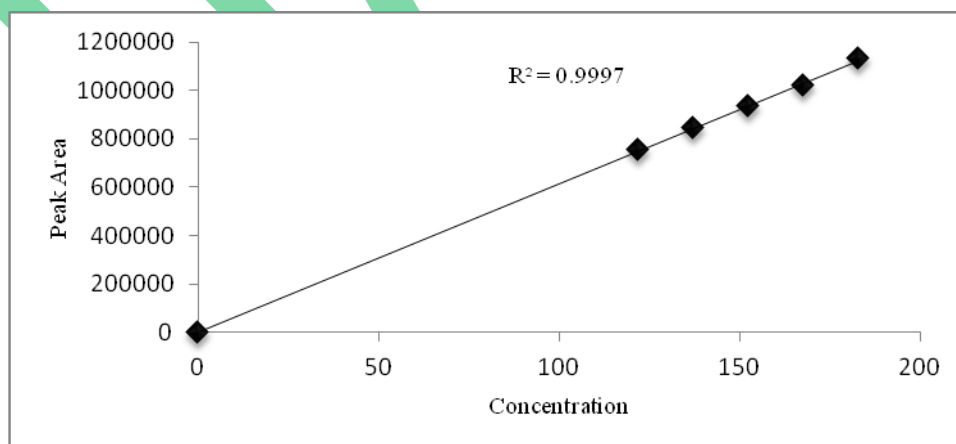

$R^2 = 0.9997$

Figure-04: Graphical representation for Linearity of Esomeprazole

CONCLUTION

The method adopted for estimation of Naproxen and Esomeprazole by HPLC is precise, linear, accurate, rugged and robust enough. The sample solution is found to be stable up to 48 h at ambient condition. Hence this method can be considered validated for its intended purpose to establish the quality of the drug substance during routine analysis with consistent and reproducible results.

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