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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION AND FORCED DEGRADATION STUDIES OF NAPROXEN AND ESOMEPRAZOLE MAGNESIUM IN DELAYED RELEASE DOSAGE FORM

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ABSTRACT

A simple stability indicating reverse phase high performance liquid chromatography method was developed and validated for simultaneous estimation of naproxen and esomeprazole magnesium in combined delayed release dosage form 500/20 mg, 375/20 mg, using a single unit of tablet. Chromatographic separation was achieved with Agilent's high performance liquid chromatography and Xterra RP-18 column, with the mobile phase-1 of perchlorate buffer (pH 8.7): acetonitrile methanol (700:200:100, v/v/v) and mobile phase-2 of per chlorate buffer (pH 8.7): acetonitrile (700:300, v/v) by gradient elution technique. The flow rate was maintained at 1.5 ml/min and the detection wavelength was 305 nm. Naproxen and esomeprazole were eluted at 3.3 min and 6.1 min respectively using the developed method. Analytical method validation was performed according to International Conference on Harmonization Q2 (R1) guidelines. The method was linear in the range of 60-1500 µg/ml for naproxen and 2-60 µg/ml for esomeprazole, with r^2 value of 0.9996 and 0.9997 respectively. The sample recoveries observed were 100.38-101.39% and 99.67-99.94% respectively for naproxen and esomeprazole magnesium, which confirm the non-interference of formulation additives in the estimation. The forced degradation studies were carried out and the stressed samples were analyzed using the developed method. The purity angle of the peak was observed lesser than the threshold angle, which confirms the non-interference from degradants in quantitating naproxen and esomeprazole in bulk and marketed formulation.

Keywords: Naproxen, esomeprazole magnesium, RP-HPLC, content uniformity, forced degradation, method validation

INTRODUCTION

Product quality is defined in terms of specifications, critical quality standards and attributes. A critical quality attribute is a physical, chemical, biological property or characteristic that would be within appropriate limit, range or distribution to ensure the desired product quality[1]. Some important critical quality attributes to ensure the quality of drug product are assay, dissolution, uniformity of dosage units and related substances. Most of the research works were carried out in method development for assay and related substances. Developing a method for content uniformity of dosage units for a combination product is a challenging process, since the drug concentrations of two drugs would be varying very high. The term "uniformity of dosage unit" is defined as the degree of uniformity in the amount of the drug substance among dosage units. The test for content uniformity of preparations presented in dosage units is based on the assay of the individual content of drug substance(s) in a number of dosage units to determine whether the individual content is within the limits set. It ensures that a consistent dose of the active pharmaceutical ingredient is maintained between batches so that the patient receives the correct dose[2,3]. A stability indicating method is an analytical procedure used to quantitate the decrease in the amount of the active pharmaceutical ingredient in drug product due to degradation. Forced degradation is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of the drug substance. Stress testing is carried out to demonstrate specificity of the developed method to measure the changes in concentration of drug substance when little information is available about potential degradation products[4].

The chemical name for Naproxen (NPX: figure-1a) is (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid. NPX has a molecular weight of 230.26 and a molecular formula of $C_{14}H_{14}O_3$; white to off-white, practically odour less, crystalline powder. It is soluble in chloroform, in dehydrated alcohol and in alcohol, sparingly soluble in ether and practically insoluble in water. The octanol/water partition coefficient of NPX at pH 7.4 is 1.6 to 1.8.[5,6]

The chemical name of Esomeprazole magnesium trihydrate (EPM: Figure-1b) is bis (5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2- pyridinyl) methyl] sulfinyl] -1*H*-benzimidazole-1-yl) magnesium trihydrate. EPM has the molecular weight of 767.2 and molecular formula of $(C_{17}H_{18}N_3O_3S)_2$ Mg.3 H_2O. The magnesium salt is a white to slightly coloured crystalline powder. It contains 3 moles of water of solvation and is slightly soluble in water. The stability of EPM is a function of pH, it rapidly degrades in acidic media, but it has acceptable stability under alkaline conditions. At pH 6.8 (buffer), the half-life of the magnesium salt is about 19 hours at 25°C and about 8 hours at 37°C.[7,8]

A combination of NPX and EPM, is used for adult and adolescent patients 12 years of age and older weighing at least 38 kg, requiring NPX for symptomatic relief of arthritis. The NPX

component is indicated for relief of signs and symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis in adults, juvenile idiopathic arthritis in adolescent patients. The EPM component is indicated to decrease the risk of developing NPX associated gastric ulcers[9].

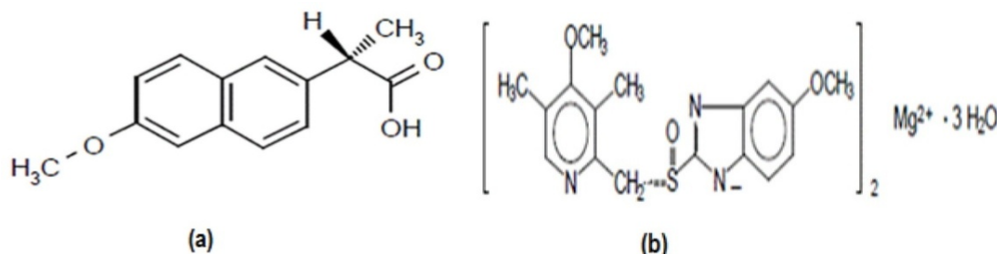


Figure 1: Chemical structure (a) Naproxen and (b) Esomeprazole magnesium trihydrate

The literature data revealed that there are simultaneous estimation methods developed for NPX and EPM in combined dosage form for assay and related substances using high performance liquid chromatography[10-14] and using ultraviolet spectroscopy[15-19]. No methods were established for content uniformity test. The sample size for content uniformity was a limiting factor, whereas for evaluation of assay and related substances, the sample size shall be optimized to achieve the required final concentration of drug substance for analysis. The reported liquid chromatographic methods for simultaneous estimation of NPX and EPM have used isocratic system, where the chromatography run time was with 5-10 minutes, in which impurities were not quantified. EPM is highly sensitive to at acidic pH, heat, humidity and oxidation; the impurities are getting eluted up to 20 minutes. The degradants need to be separated from main analyte to make the method stability indicating. So the aim of present study is to develop and validate a simple, accurate and precise stability indicating reverse phase HPLC method for simultaneous estimation of NPX and EPM from single delayed release tablet and by extending the run time to confirm the non-interference of excipients and degradants.

MATERIAL AND METHODS

Chemicals and reagents

Working standards, impurities for NPX and EPM were obtained from Dr. Reddy's Laboratories. The finished dosage form VIVOMO was procured from local pharmacy. Excipients were obtained from Signet Chemical Corporation. N-Butyl amine, acetonitrile, sodium perchlorate, perchloric acid, methanol of suitable HPLC and AR grade were purchased from E. Merck Co., Mumbai.

Instrumentation

The analysis was carried out using Agilent 1200 RP-HPLC system with binary pump and photo diode array detector, with an auto sampler and column heater. Data were collected and processed using Empower software. Photostability chamber, over head stirrer, sonicator, hot plate and pH meters were used for performing the analysis. Polyvinyl difluoride filters (0.45 micron) used for sample filtration were purchased from Rankem, India.

Preparation of mobile phase

The buffer solution was prepared by dissolving 0.71g of sodium perchlorate in 1000 ml of milli-Q water and 5 ml of N-Butyl amine. The pH was adjusted to 8.7 using 0.01 mol/L perchloric acid in anhydrous acetic acid. The resulting solution was filtered through 0.45 μ membrane filter. Mobile phase-1 was prepared by mixing the buffer, acetonitrile and methanol in the ratio (700:200:100, v/v/v) and degassed in a sonicator for about 10 minutes. Mobile phase-2 was prepared by mixing the buffer and acetonitrile in the ratio (300:700, v/v) and degassed in a sonicator for about 10 minutes.

Diluents

Three diluents were used in the analysis. Diluent-1: It was prepared by mixing 800 ml of methanol, 196 ml of milli-Q water and 4 ml of triethylamine, and degassed in a sonicator for about 10 minutes. Diluent-2: 0.25N Sodium hydroxide. Diluent-3: Mobile phase.

Preparation of standard solution

Preparation of EPM standard stock solution: About 60 mg of EPM was weighed accurately and transferred into a 250 ml volumetric flask, 150 ml of diluent-1 was added and sonicated for 10 minutes to dissolve the material completely and volume was made up with diluent-2 and mixed for 10 minutes. Preparation of NPX standard stock solution: About 126 mg of NPX was weighed accurately and transferred into a 25 ml volumetric flask, 15 ml of diluent-1 was added and sonicated for 10 minutes to dissolve the material completely and volume was made up with diluent-1 and mixed for 10 minutes. Standard preparation: The standard solution was prepared by transferring 5 ml standard stock solution of EPM and 5 ml standard stock solution of NPX into a 50 ml volumetric flask. The volume was made up with diluent-3 and mixed well.

Preparation of test solution

One tablet is weighed and transferred to 100 ml volumetric flask. 60 ml of diluent-1 was added and sonicated for 30 minutes with intermediate shaking, by maintaining the temperature at 10°C to 15°C during sonication. 30 ml of diluent-2 was added to the above solution and sonicated for 45 minutes with intermediate shaking to ensure the tablet is completely disintegrated, volume was made up with diluent-2 and mixed well. The solution was centrifuged at 4000 rpm for 10 minutes. 5 ml of clear centrifugate was transferred into a 50 ml volumetric flask; the volume was made up with diluent-3 and mixed well.

Chromatographic system

HPLC analysis was performed on Agilent HPLC system with photo diode array detector. Chromatographic separation of EPM and NPX were carried on Xterra RP18 column (150 × 4.6 mm, 5 μm particle size). Gradient condition with the mobile phase-1 and mobile phase-2 are programmed and 1.5 ml/min flow rate was used for analysis, with a run time of 20 minutes. The detection

wavelength selected for the estimation of the two drugs was at 305 nm. HPLC column was maintained at a temperature of 40°C. The chromatographic elution mode was in gradient mode, which was presented in Table 1.

Table 1: Gradient programme for chromatographic system

Time (Minute)	Mobile phase 1 (%)	Mobile phase 2 (%)
0	100	0
4	100	0
8	0	100
12	0	100
16	100	0
20	100	0

System Suitability

System suitability was performed by injecting 10 µl of standard solution into the chromatographic system and the area was measured for EPM and NPX peaks. The tailing factor, number of theoretical plates and % RSD for peak areas are presented in Table 2.

Table 2: System suitability for Esomeprazole magnesium and Naproxen

Parameters	Drug	Mean ± SD	% RSD
Retention time (R_t)	NPX	3.35 ± 0.02	0.5
	EPM	6.27 ± 0.13	2.15
Peak area	NPX	649252 ± 801	0.12
	EPM	16467 ± 90	0.54
Tailing factor(T)	NPX	1.3 ± 0.02	1.37
	EPM	1.0 ± 0.05	5.00
Theoretical plates (N)	NPX	5297 ± 21	0.40
	EPM	7762 ± 61	0.79

Peaks identity was confirmed by retention time comparison. Diluent-3 was used as blank for the analysis, samples were injected and the chromatograms are recorded for the response of analyte peak. The % content of both the drugs was calculated using the formula presented below:

$$\text{Quantity of NPX present in portion of tablets as \% of labelled amount} = \frac{A_T \times WS \times 5 \times 100 \times 50 \times P \times 100}{A_S \times 25 \times 50 \times 1 \times 5 \times 100 \times L}$$

A_T = Peak area of NPX for Test preparation
A_S = Peak area of NPX for Standard preparation
WS = Weight of NPX working standard / reference standard taken, in mg
P = Potency of NPX standard calculated as NPX
L = Labelled amount of NPX in mg, per tablet

$$\text{Quantity of EPM present in portion of tablets as \% of labelled amount} = \frac{A_T \times WS \times 5 \times 100 \times 50 \times P \times 100}{A_S \times 250 \times 50 \times 1 \times 100 \times L}$$

A_T = Peak area of EPM for Test preparation
A_S = Peak area of EPM for Standard preparation
WS = Weight of EPM working standard / reference standard taken, in mg.
P = Potency of EPM standard calculated as EPM
L = Labelled amount of EPM in mg, per tablet

Calculation for the Acceptance value

Acceptance value (AV) = [M-X] +ks
k = Acceptability constant, for 10units, the acceptability constant is 2.4
s = Sample standard deviation
X = mean of the individual contents (expressed as % of label claim)

M = is based on the X value.
If 98.5% ≤ X ≤ 101.5%, then M = X. if X > 101.5%, then X = 101. 5%. if X< 98.5, then M = 98.5%.

If the AV value is less than 15.0, 10 units value for content uniformity is adequate. The Vivomo tablets were analysed for content uniformity and the data has been presented in Table 3.

Table 3: Content uniformity results of Naproxen and Esomeprazole magnesium delayed release tablets 375/20 mg and 500/20 mg

S. No.	Content uniformity			
	500 mg/20 mg (Lot.No:WH0198)		375 mg/20 mg (Lot.No:WH3750)	
	NPX	EPM	NPX	EPM
1	98.8	100	98.5	100.3
2	96.8	98.7	97.8	99.8
3	98.8	99.8	97.4	99.3
4	97.2	99	97.6	99.5
5	98.9	98.8	96.6	98.1
6	95.2	96.8	97.6	99.5
7	99	99.9	96.2	97.6
8	95	96.5	97.5	99.3
9	98.7	100.9	97.2	99.9
10	98.2	100.4	97.4	100.1
Average(X)	**97.7**	**99.1**	**97.4**	**99.3**
s	**1.54**	**1.46**	**0.63**	**0.86**
% RSD	**1.58**	**1.47**	**0.65**	**0.86**
k	**2.4**	**2.4**	**2.4**	**2.4**
M	**98.5**	**99.1**	**98.5**	**99.3**
AV	**4.54**	**3.52**	**2.63**	**2.02**

Analytical method validation

HPLC method was validated to ensure consistent, reliable and accurate results are obtained to determine the level soft drugs in all the samples. The validation parameters linearity, accuracy, precision, limit of detection, limit of quantitation and specificity were evaluated.[20,21]

Linearity

The linearity of an analytical method was carried out to check the ability of the method to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different concentrations of standard solutions were prepared by diluting aliquots of standard stock solution (5040 µg/ml of NPX and 233 µg/ml for EPM) into 10 ml volumetric flasks to obtain concentrations in the range of 60-1500 µg/ml for NPX and 2-60 µg/ml for EPM and then injected each concentration into the chromatographic system and the chromatograms were recorded.

Precision

The system precision was carried out to ensure that the analytical system is working properly by injecting standard solution preparation containing NPX 504 µg/ml and EPM 23.3 µg/ml six times into the HPLC system as per the test procedure. The retention time and peak areas for both the drugs in all the sample solutions were measured and % RSD was calculated. In method precision, a homogenous sample containing of NPX and EPM of a single tablet was analysed six times and % RSD was calculated.

Accuracy

Accuracy was performed by calculating percentage recovery by standard addition method. Known concentration of (403.2, 504 and 604.8 µg/ml of NPX and 18.64, 23.3 and 27.96 µg/ml of EPM) were added to the pre-analyzed sample solution having NPX (504 µg/ml) and EPM (23.3 µg/ml). Peak area of each solution at 305 nm was taken in triplicates and recovery was calculated using regression equation.

Limit of Detection (LOD)

Limit of detection is the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyte can be detected is determined from the linearity curve.

The detection limit (DL) may be expressed as: $DL = 3.3\ \sigma/S$
Where, σ = the standard deviation of the response
S = the slope of the calibration curve

Limit of Quantification (LOQ)

Limit of quantification is the lowest concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantitation can be obtained from linearity curve.

The detection limit (DL) may be expressed as: $DL = 10\ \sigma/S$
Where, σ = the standard deviation of the response
S = the slope of the calibration curve

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the chromatograms obtained from the drug standards with that of obtained from the tablet preparations. The retention times of the drug standards and the drug from sample preparations were same, so the method was specific without interference from excipients in the tablets

i. Placebo Interference: A study was conducted to establish the interference of placebo was conducted. Assay was performed on Placebo (Placebo contains without NPX and EPM and with other excipients in equivalent to about the weight of placebo present in portion of test preparation as per test method.

ii. Impurity interference: A study was conducted on the impurity interference, by spiking the standard preparation with all impurities in the concentration of 0.3% of test preparation and injected into the HPLC system, by following the test method.

Forced degradation studies (stress testing)

In order to develop stability indicating method for estimation of NPX and EPM, stress studies were carried out to develop and validate the stability indicating property of the proposed method. The chromatograms of the stressed samples were evaluated for peak purity of NPX and EPM peak using Empower networking software

Acid degradation studies

1.0 ml of stock solution of NPX and 1.0 ml of stock solution of EPM was taken in a 10 ml volumetric flask. To the above solution 1.0 ml of 2N hydrochloric acid solution was added and refluxed for 15 minutes at 25 ± 2°C and then neutralized the solution with 1.0 ml of 2N sodium hydroxide solution. The resultant solution was suitably diluted with diluent-3 to obtain a concentration of 504 µg/ml and 23.3 µg/ml solutions of NPX and EPM respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Base degradation studies

1.0 ml of stock solution of NPX and 1.0 ml of stock solution of EPM was taken in a 10 ml volumetric flask. To the above solution 1.0 ml of 2N sodium hydroxide solution was added and refluxed for 4 hours at 60 ± 2°C and then neutralized the solution with 1.0 ml of 2N hydrochloric acid solution. The resultant solution was suitably diluted with diluent-3 to obtain a concentration of 504 µg/ml and 23.3 µg/ml solutions of NPX and EPM respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Oxidation studies

1.0 ml of stock solution of NPX and 1.0 ml of stock solution of EPM was taken in a 10 ml volumetric flask. To the above solution 2.0 ml of 5% hydrogen peroxide solution was added exposed for 1 hour at 25 ± 2°C and then the resultant solution was suitably diluted with diluent-3 to obtain a concentration of 504 µg/ml and 23.3 µg/ml solutions of NPX and EPM respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Photolytic degradation studies

1.0 ml of stock solution of NPX and 1.0 ml of stock solution of EPM was added in a 10 ml volumetric flask. Four samples were prepared, out of which two were wrapped with aluminium foil for control and loaded into photo stability chamber. Two samples were wrapped with aluminium foil and remaining two was kept un-wrapped; and exposed to ultra violet light and visible light as per ICH guidelines. The exposed solution was suitably diluted with diluent-3 to obtain a concentration of 504 µg/ml and 23.3 µg/ml solutions of NPX and EPM respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.[22]

Thermal degradation studies

1.0 ml of stock solution of NPX and 1.0 ml of stock solution of EPM were added in a 10 ml volumetric flask, exposed for 2 hours at 105 ± 2°C using hot air oven and then the resultant solution was suitably diluted with diluent-3 to obtain a concentration of 504 µg/ml and 23.3 µg/ml solutions of NPX and EPM respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

RESULT AND DISCUSSION

Method Development

The initial screening for diluents was performed based on the chemical nature of molecule. Since, EPM is sensitive to acidic condition; diluents selected are of basic nature with higher pH values. Chromatographic parameters were preliminary optimized to develop a stability indicating method for NPX and EPM with a short analysis time (20 min). To separate the degradants from main analytes, gradient technique was developed to elute the impurities, thus capturing all the possible degradants of both the components. Retention time of analyte increases with increased column length. Hence, a shorter column (150 × 4.6 mm, 5 µm particle size) was selected to have a shortest possible runtime without compromising on the resolution. In order to identify a suitable organic modifier, various organic solvents like acetonitrile and methanol were tested. A solvent combination of methanol and acetonitrile produced better selectivity with low column back pressures. N-Butyl amine, as a buffer gave sharp peaks for both the components compared to other buffers. Diluents selected for the preparation standard and sample solutions were based on the extraction and stability of both the drugs. EPM is highly sensitive to acidic environment. To prevent the degradation of NPX which also is weakly acidic and EPM, alkaline chemicals like triethyl amine and sodium hydroxide solutions were used in the diluents. Different gradient programs were tried to separate all the impurities from main analyte with high resolution.

The system suitability was performed by injecting 10 µl combined standard preparation into the chromatographic system for five times, the chromatograms were recorded and responses were measured for the NPX and EPM peaks. The system suitability parameters summary is presented in Table 2 and the typical chromatogram for standard is presented in Figure 2a. The % RSD for peak area of five replicate injections was observed with 0.12% for NPX and 0.54% for EPM. The marketed product vivo mo tablets 375/20 mg and 500/2 mg was analyzed using the developed method, and results were presented in Table 3. The sample chromatograms were presented in Figure 2b. The acceptance value is meeting the acceptance criteria of below 15.

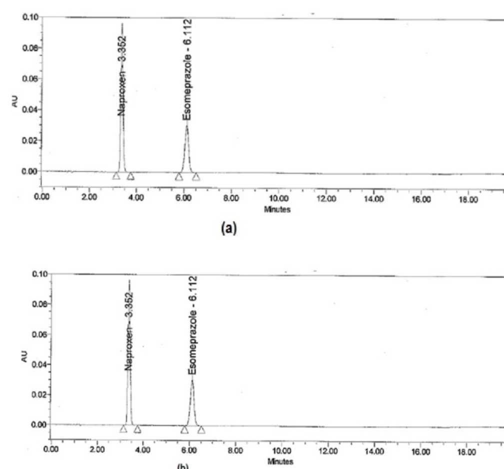


Figure 2: A typical chromatogram of (a) standard for Naproxen and Esomeprazole, (b) sample for Naproxen and Esomeprazole magnesium Delayed Release Tablets

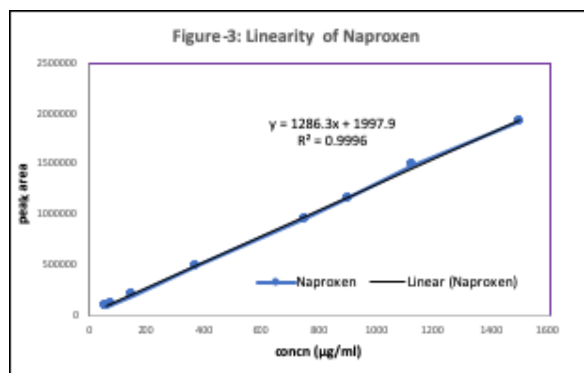
A series of solutions of NPX and EPM standards were prepared in the concentration range (NPX 60 µg/ml to 1500 µg/ml and EPM 2 µg/ml to 60 µg/ml) and analyzed as per test method. The results are presented in Table 4 and the validation parameters established are presented in Table 5.

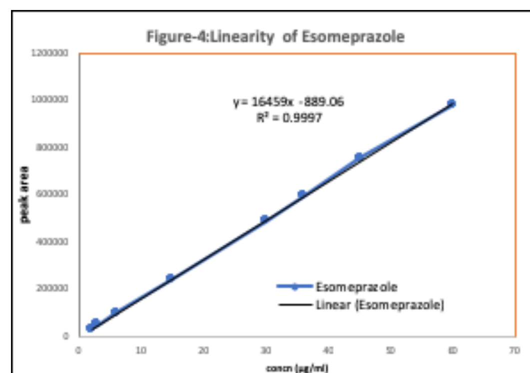
Table 4: Linearity Study

	Naproxen		Esomeprazole magnesium	
S. No.	**Concentration (µg/ml)**	**Peak Area**	**Concentration (µg/ml)**	**Peak Area**
1	60	80326	2	32659
2	75	99664	3	49128
3	150	196390	6	99037
4	375	479920	15	242239
5	750	953292	30	487525
6	900	1157766	36	590874
7	1125	1478272	45	753923
8	1500	1918340	60	979961

Table 5: Validation parameters established by Linearity and Precision

Parameters	NPX	EPM
Linearity (µg/ml)	60-1500	2 -60
correlation co-efficient (r^2)	0.9996	0.9997
Regression equation	y = 1286.3x + 1997.9	y = 16459x - 889.06
Method precision (% RSD) (375/20 mg)	0.65	0.86
Method precision (% RSD) (500/20 mg)	1.58	1.47
System precision (%RSD)	0.39	1.21
LOD (µg/ml)	2.49	0.095
LOQ (µg/ml)	7.55	0.29





Linearity was established by plotting a graph between concentrations versus peak area and determined the correlation coefficient, which was presented in Figure 3 for NPX and Figure 4 for EPM. The detector response was found to be linear with a correlation coefficient of 0.9996 and 0.9997 respectively for NPX and EPM.

The LOD was evaluated by determining the minimum levels of concentration for NPX and EPM that could be detected using developed analytical method. The LOQ was studied by estimating the minimum concentration that could be quantified with acceptable accuracy and precision. The LOD values for NPX and EPM were determined to be 2.49 µg/ml and 0.095 µg/ml, and the LOQ values were 7.55 µg/ml and 0.29 µg/ml respectively.

The precision of test method was evaluated by repeatability studies, by evaluating ten test samples of NPX and EPM delayed release tablets 500 mg/20 mg and 375 mg/20 mg of each strength. The % relative standard deviation of NPX and EPM is presented in Table 5, which was observed within the acceptance criteria limit of not more than 15% according to the ICH guideline.

The recovery experiments were performed by adding a known quantity of pure standard drug into the solution of the tablet powder. The sample was spiked with standard at levels 80%, 100% and 120% of test concentration were evaluated for content uniformity in triplicate, which was observed with the % RSD less than 2%, and the results are presented in Table 6.

Table 6: Accuracy (Results of recovery study)

Level of Recovery (%)	concentration actual (µg/ml)		concentration added (µg/ml)		Mean % recovery ± SD		% RSD	
	NPX	**EPM**	**NPX**	**EPM**	**NPX**	**EPM**	**NPX**	**EPM**
80	504	23.3	403.2	18.64	100.18 ± 0.35	100.93 ± 1.43	0.35	1.41
100	504	23.3	504	23.3	99.81± 0.56	100.86 ± 1.72	0.56	1.70
120	504	23.3	604.8	27.96	99.82 ± 0.64	101.26 ± 1.45	0.64	1.43

Placebo interference: Chromatograms of placebo showed no peaks at the retention times of NPX and EPM peaks. This indicates that the excipients used in the formulation do not interfere in estimation of NPX and EPM in Tablets. The placebo chromatogram is shown in Figure 5a.

Impurity interference: The chromatogram recorded by spiking the standard preparation with all impurities in the concentration of 0.3% of test preparation was found that all the impurities are separated from the main analyte NPX and EPM. Chromatogram of impurity interference is shown in Figure 5b.

Figure 5: A Typical chromatogram of (a) placebo for interference and (b) Impurity interference

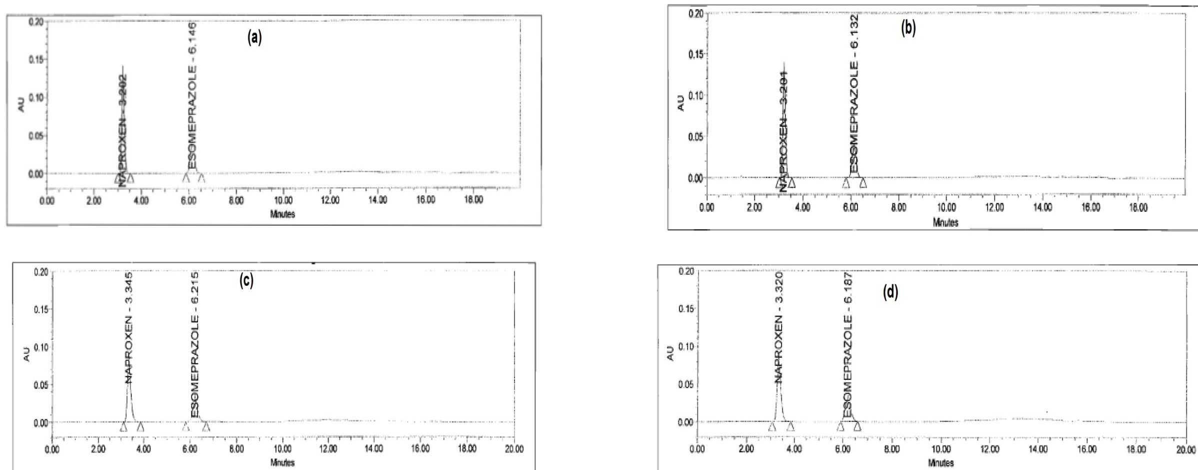
Forced degradation studies (stress testing)

From the forced degradation sample chromatograms all degradants peaks were resolved from NPX and EPM peak in the chromatograms of all samples. For all forced degradation samples, the purity angle found to be less than threshold angle, which indicates that there is no interference from degradants in quantitating the NPX and EPM in tablets. The percentage drug content after forced degradation, purity threshold and purity angle performed for all the stressed samples and unstressed samples were presented in Table 7.

Table 7: Assay and Peak purity results of Forced degradation studies

Stress Condition	Naproxen			Esomeprazole magnesium		
	% Assay	Purity Angle	Purity threshold	% Assay	Purity Angle	Purity threshold
Unstressed	100.9	0.36	0.529	99.5	0.091	3.203
Refluxed with 2N HCl solution for about 15 min at 25 ± 2°C	99.7	0.331	0.522	98.8	0.09	3.291
Refluxed with 2 N NaOH solution for about 4 hours at 60 ± 2°C	94.8	0.368	0.55	97.6	0.091	2.921
Exposed to 5% Hydrogen peroxide (H_2O_2) for about 1 hour at 25 ± 2°C	92.8	0.106	0.375	90.2	0.03	0.22
Exposed to visible light for about 1.2 Million Lux hours	99.3	0.101	0.355	98.5	0.031	0.218
Exposed to UV light for about 200 watt Hours/square meter	99.8	0.089	0.315	98.2	0.03	0.215
Dry heating done at 105 ± 2°C for about 2 hours	98.5	0.103	0.316	96.9	0.028	0.215

The typical chromatogram of stressed ample and purity plots derived through Empower software were presented in Figure 6, 7 and Figure 8.



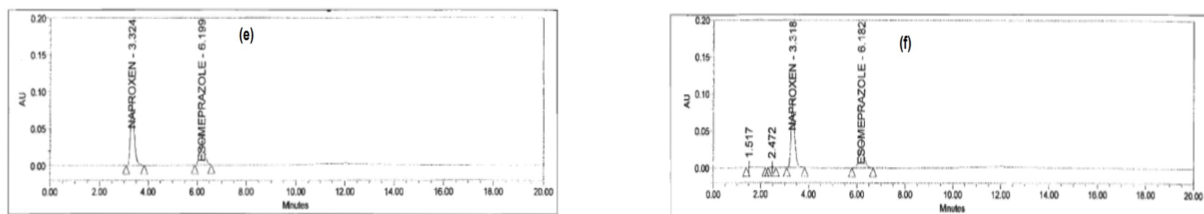


Figure 6: Chromatogram of Naproxen and Esomeprazole Magnesium sample (a) Acid stressed, (b) Base stressed, (c) Oxidation stressed, (d) Visible light stressed, (e) UV light stressed, and (f) Heat stressed samples

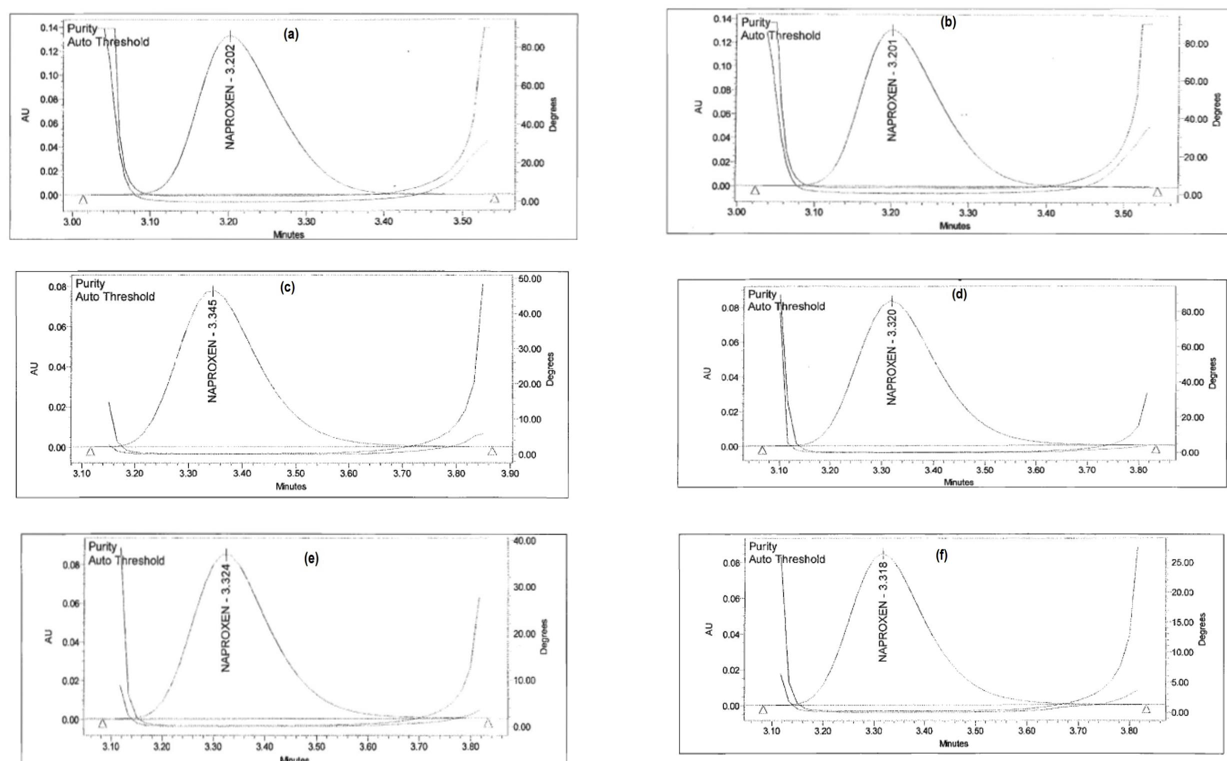
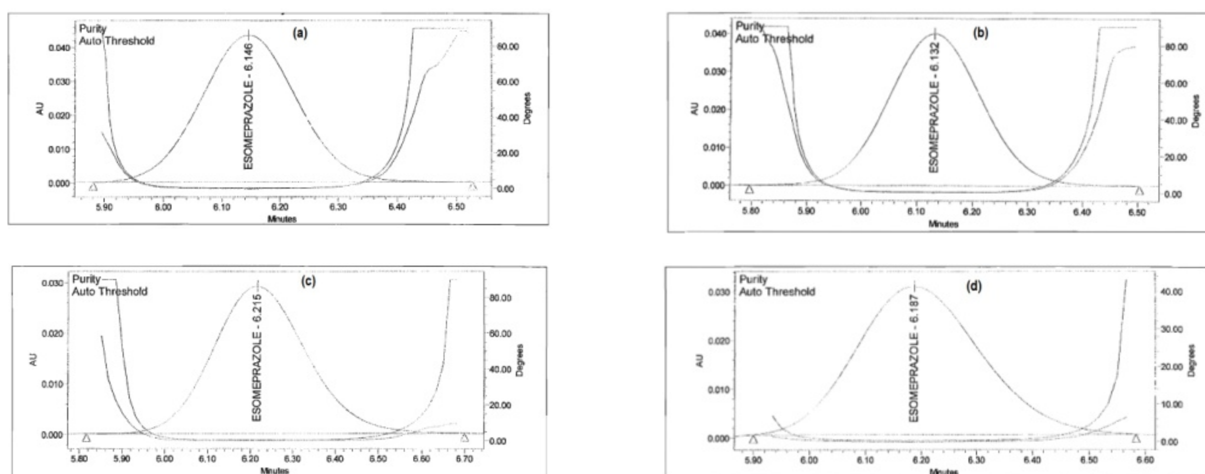


Figure 7: Purity plot of Naproxen in Naproxen and Esomeprazole Magnesium sample (a) Acid stressed, (b) Base stressed, (c) Oxidation stressed, (d) Visible light stressed, (e) UV light stressed, and (f) Heat stressed samples



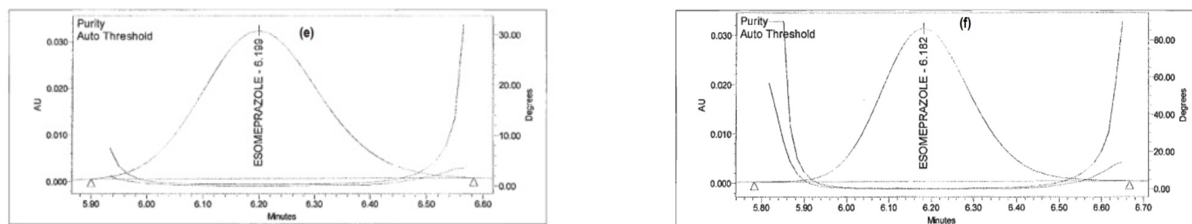


Figure 8: Purity plot of Esomeprazole in Naproxen and Esomeprazole Magnesium sample (a) Acid stressed, (b) Base stressed, (c) Oxidation stressed, (d) Visible light stressed, (e) UV light stressed, and (f) Heat stressed samples

CONCLUSION

A simple, accurate and precise stability indicating RP-HPLC method for simultaneous estimation of NPX and EPM for evaluation of content uniformity of tablet was developed. The developed method was capable of eluting degradation products. The drug peaks observed from chromatogram are not interfered by degradants and formulation additives. The developed method was validated in compliance with the ICH guidelines. Hence, this developed method can be conveniently adopted for routine quality control analysis of content uniformity of NPX and EPM delayed release tablets.

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