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Research Article

SIMULTANEOUS ESTIMATION OF THE PREGABALIN AND ETORICOXIB IN TABLET DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Pregabalin and Etoricoxib in Tablet dosage form. Chromatogram was run through Agilent C18 150 x 4.6 mm, 5m. Mobile phase containing Buffer 0.01N KH2PO4: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 0.8 ml/min. Buffer used in this method was 0.01N KH2PO4 buffer. PH adjusted to 5.4 with dil. Orthophosphoric acid solution. Temperature was maintained at 30°C. Optimized wavelength selected was 240.0 nm. A simple, Accurate, precise method was developed for the simultaneous estimation of the Pregabalin and Etoricoxib in Tablet dosage form. Retention time of Pregabalin and Etoricoxib were found to be at 2.302 min and 3.219 min %RSD of the Pregabalin and Etoricoxib were and found to be 0.5 and 0.6 respectively. %Recovery was obtained as 100.27% and 100.22% for Pregabalin and Etoricoxib respectively. LOD, LOQ values obtained from regression equations of Pregabalin and Etoricoxib were 0.27, 0.83 and 0.24, 0.72 respectively. Regression equation of Pregabalin is y = 23826x + 8530.6 y = 27243x + 3474.3 of Etoricoxib. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Pregabalin, Etoricoxib, RP-HPLC

INTRODUCTION

An anticonvulsant, pregabalin is useful for treating fibromyalgia and neuropathic pain and, in conjunction with other anticonvulsants, for partial onset seizures. The inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and its structural analogue, pregabalin, are very similar1,2. Pregabalin combines antiepileptic and analgesic properties via its interaction with the alpha-II-delta subunit; it is a voltage-damaged Ca2+canal antagonist 4,5,6. The exact mechanism of action of pregabalin is still a mystery, although previous research with structurally similar medications has shown that its antiseizure and antinociceptive actions in animal models7 are

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due to its presynaptic binding to voltage-gated calcium channels. The molecular formula of pregabalin is (3S) 5-methyl-3-(aminomethyl) hexanoic acid 7. The chemical formula for pregabalin is (3S)-3-(aminomethyl)-5-methyl hexanoic acid 7.6. The brand name under which it is offered is Lyrica.

For moderate pain after dental surgery or inflammatory signs of various forms of arthritis, the short-term treatment option is etoricoxib, a COX-2 inhibitor. In adults (16 and up), it may alleviate inflammation and discomfort in the joints and muscles caused by autoimmune diseases such as rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Etoricoxib blocks the production of prostaglandins (PGs) from arachidonic acid by inhibiting the cyclo-oxigenase enzyme (COX-2) isoform 2. In its chemical composition, etoricoxib contains 5-chloro-3-(4-methanesulfonylphenyl)-6'-methyl-2,3'-bipyridine10. The phrase "fixed-dose drug" refers to products that include mixed doses of two or more drugs. The treatment of neuropathic chronic pain involves the combination of pregabalin (75 mg) and etoricoxib (60 mg).

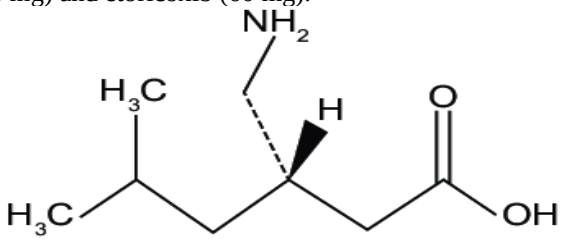

Figure 1: Structure of Pregabalin

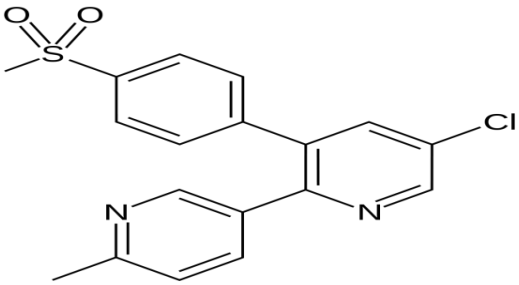

Figure 2: Structure of Etoricoxib

According to a literature review, there are some techniques for the simultaneous estimate of these medicines as well as others for assessment of the drugs alone or in combination with other drugs. Utilizing UV-Spectrophotometry RP-HPLC . There is no established technique for the stability-indicating simultaneous measurement of Pregabalin and Etoricoxib by RP-HPLC in pharmaceutical dosage form, according to a survey of the literature. The primary goal of this work is to provide an efficient, quick, and accurate RP-HPLC approach for estimating of Pregabalin and Etoricoxib in medicinal dose and tablet form. According to ICH recommendations, a proven approach was also used to estimate the amounts of Pregabalin and Etoricoxib. 11-22

MATERIALS AND REAGENTS

Pregabalin and Etoricoxib pure drugs were received from Spectrum Pharma research solutions, Hyderabad. The combination tablet Pregabalin and Etoricoxib (Emaxgalin tab) was purchased from a local pharmacy store. Rankem in India provided all of the chemicals and buffers utilised in this method like Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen Ortho phosphate buffer, Ortho-phosphoric acid, Distilled water.

Instrumentation and Chromatographic Conditions

For the development and validation method, an automated sample injector was employed with a WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector. For the separation, a Discovery 150 (C18 250 mm x 4.6 mm, 5µm) column was employed. Acetonitrile is employed as mobile phase B, while 0.1% ortho phosphoric acid is used as mobile phase A. (35:65 Ratio). The analysis was done in isocratic mode with an injection volume of 10 mL and a flow rate of 1 mL/min. The duration was six minutes. The measurements were made at 254 nm.

PREPARATION OF SOLUTIONS

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of buffer:

0.1% OPA Buffer: 1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water. 0.01N NA2HPO4 Buffer: Accurately weighed 1.42gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 4.0 with dil. Orthophosphoric acid solution.

Preparation of Standard stock solutions: Accurately weighed 37.5 mg of Pregabalin, 30 mg of Etoricoxib was and transferred to 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (750µg/ml of Pregabalin and 600µg/ml of Etoricoxib)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (75µg/ml Pregabalin of and 60µg/ml of Etoricoxib)

Preparation of Sample stock solutions: Accurately weighed equivalent weight of the combination powder sample transfer into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by milli-Q filters. (750µg/ml of Pregabalin and 600µg/ml of Etoricoxib)

Preparation of Sample working solutions (100% solution): 1 ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (75µg/ml of Pregabalin and 60µg/ml of Etoricoxib)

METHOD VALIDATION

To prove that the technique is suggested for routine analysis, the HPLC method's validation was done for the simultaneous estimation of Pregabalin and Etoricoxib drug material in accordance with the ICH criteria.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: stock solutions of Pregabalin and Etoricoxib is taken in to 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Accuracy: Preparation of Standard stock solutions: Accurately weighed 37.5 mg of Pregabalin, 30 mg of Etoricoxib was and transferred to 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (750µg/ml of Pregabalin and 600µg/ml of Etoricoxib)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Pregabalin and Etoricoxib, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Pregabalin and Etoricoxib, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System suitability parameters: The The system suitability parameters were determined by preparing standard solutions of Pregabalin (75ppm) and Etoricoxib (60ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Degradation studies:

Oxidation: To 1 ml of stock solution of Pregabalin and Etoricoxib, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 75µg/ml&60µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock s solution Pregabalin and Etoricoxib, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c .The resultant solution was diluted to obtain 75µg/ml&60µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution Pregabalin and Etoricoxib, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 75µg/ml&60µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 1hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 75µg/ml & 60µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 750µg/ml&60µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 75µg/ml&60µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hr at a temperature of 60º. For HPLC study, the resultant solution was diluted to 75µg/ml & 60µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSIONS:

Table 1. System suitability table

S.No	Pregabalin			Etoricoxib			
Injection	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	RS
1	2.302	4202	1.36	3.215	6768	1.23	5.9
2	2.303	3799	1.36	3.216	5861	1.28	5.8
3	2.303	3954	1.35	3.217	5678	1.27	5.6
4	2.305	4030	1.34	3.219	6594	1.23	5.7
5	2.305	4132	1.32	3.221	5699	1.26	5.8
6	2.310	4440	1.31	3.226	5546	1.25	5.8

Table 2. Specificity data

Sample name	Retention time (Mins)	Area
Pregabalin	2.302	1835493
Etoricoxib	3.219	1715539

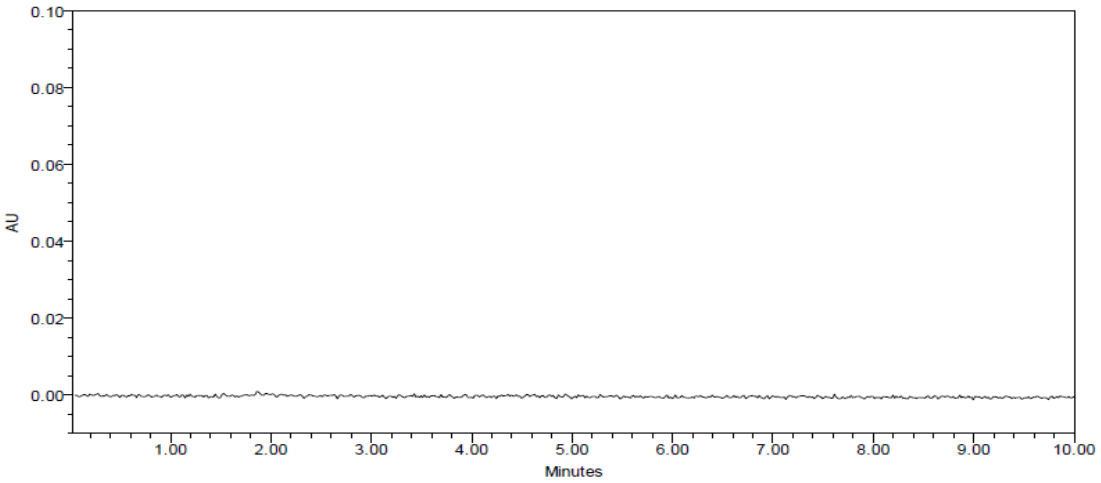

Figure 3. Blank Chromatogram

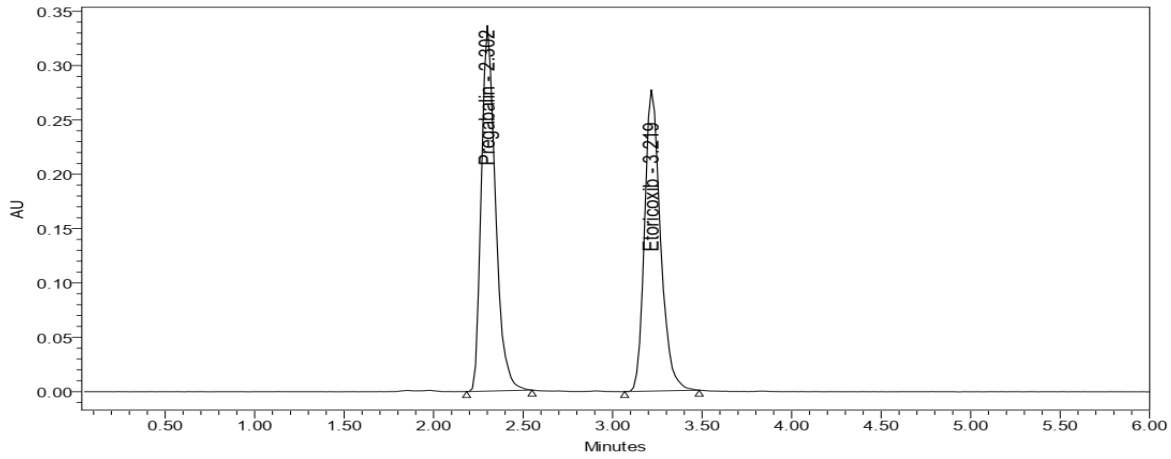

Figure 4. Specificity Chromatograms of Pregabalin and Etoricoxib

Linearity

Table 3. Linearity table for Pregabalin and Etoricoxib:

Pregabalin		Etoricoxib	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	**0**	0
18.75	454188	**15**	406195
37.5	906381	**30**	822280
56.25	1355442	**45**	1243513
75	1795800	**60**	1655349
93.75	2260367	**75**	2014073
112.5	2668905	**90**	2464376

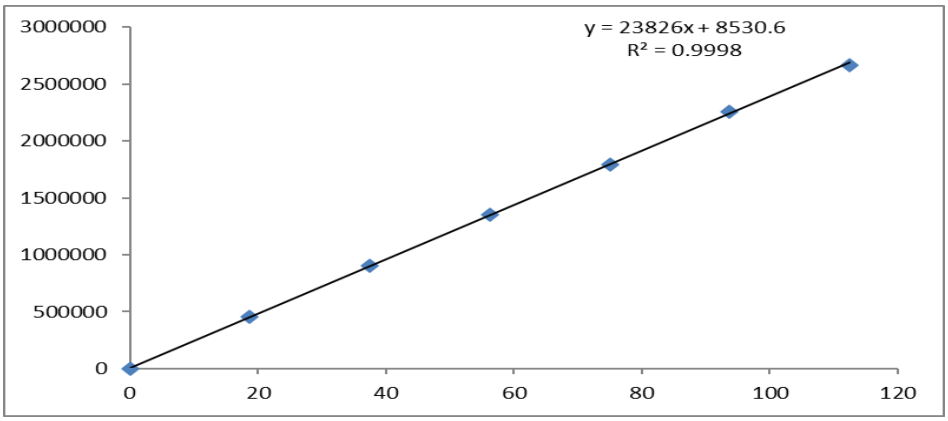

Figure 5. Calibration curve of Pregabalin

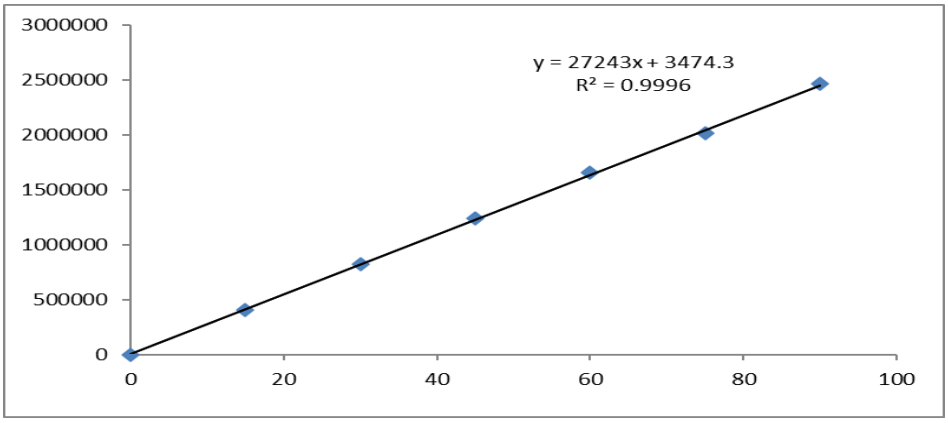

Figure 6. Calibration curve of Etoricoxib

Accuracy:

Table 4. Accuracy table of Pregabalin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	37.5	37.745841	100.66	100.27%
	37.5	37.829363	100.88	
	37.5	37.382918	99.69	
100%	75	74.555502	99.41	
	75	74.439537	99.25	
	75	75.744833	100.99	
150%	112.5	113.75251	101.11	
	112.5	113.58224	100.96	
	112.5	111.94625	99.51	

Table 5. Accuracy table of Etoricoxib

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	30	29.967	99.89	100.22%
	30	29.881	99.60	
	30	29.692	98.97	
100%	60	59.721	99.54	
	60	59.976	99.96	
	60	60.220	100.37	
150%	90	88.711	98.57	
	90	90.199	100.22	
	90	90.457	100.51	

System Precision: With regard to the working strength of Pregabalin and Etoricoxib, six duplicate injections of the standard solution at 100% of the prescribed limit were analysed to determine the system accuracy. In Table 5, the results of the peak area are compiled.

Table 6. System precision

S. No	Area of Lamivudine	Area of Tenofovir
1.	1811555	1651404
2.	1801637	1656887
3.	1819971	1655306
4.	1824286	1652494
5.	1808668	1668344
6.	1806319	1659628
Mean	**1812073**	**1657344**
S.D	**8548.9**	**6154.4**
%RSD	**0.5**	**0.4**

The % RSD for the peak areas of Pregabalin and Etoricoxib obtained from six replicate injections of standard solution was within the limit of (<2%).

Method precision: Analyzing a sample of Pregabalin and Etoricoxib allowed researchers to gauge the method's accuracy (Six individual sample preparations). Table 6 provides a summary of the data.

Table 7. Method precision

S. No	Area of Lamivudine	Area of Tenofovir
1.	1819633	1655923
2.	1815502	1677108
3.	1814392	1667684
4.	1799826	1650913
5.	1806051	1659586
6.	1799299	1654947
Mean	**1809117**	**1661027**
S.D	**8617.4**	**9698.5**
%RSD	**0.5**	**0.6**

Results shows, the % RSD of Repeatability study was within the range for Pregabalin and Etoricoxib is (<2%)

Table 8. Robustness

S.No.	Condition	%RSD of Pregabalin	%RSD of Etoricoxib
1	Flow rate (-) 0.9ml/min	0.5	0.3
2	Flow rate (+) 1.1ml/min	0.5	0.2
3	Mobile phase (-) 60B:40A	0.4	0.3
4	Mobile phase (+) 70B:30A	0.6	0.3
5	Temperature (-) 25°C	0.2	0.4
6	Temperature (+) 35°C	0.2	0.2

Table 9. Forced degradation for Pregabalin and Etoricoxib

Stress condition	Solvent	Temp (^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxidation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	-

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Table 10. Degradation results of Pregabalin and Etoricoxib

Type of degradation	Pregabalin		Etoricoxib	
	% RECOVERED	% DEGRADED	% RECOVERED	% DEGRADED
Acid	91.72	8.28	90.66	9.34
Base	93.90	6.10	92.18	7.82
Peroxide	93.12	6.88	94.51	5.49
Thermal	97.14	2.86	98.09	1.91
Uv	98.74	1.26	98.57	1.43
Water	98.74	1.26	99.65	0.35

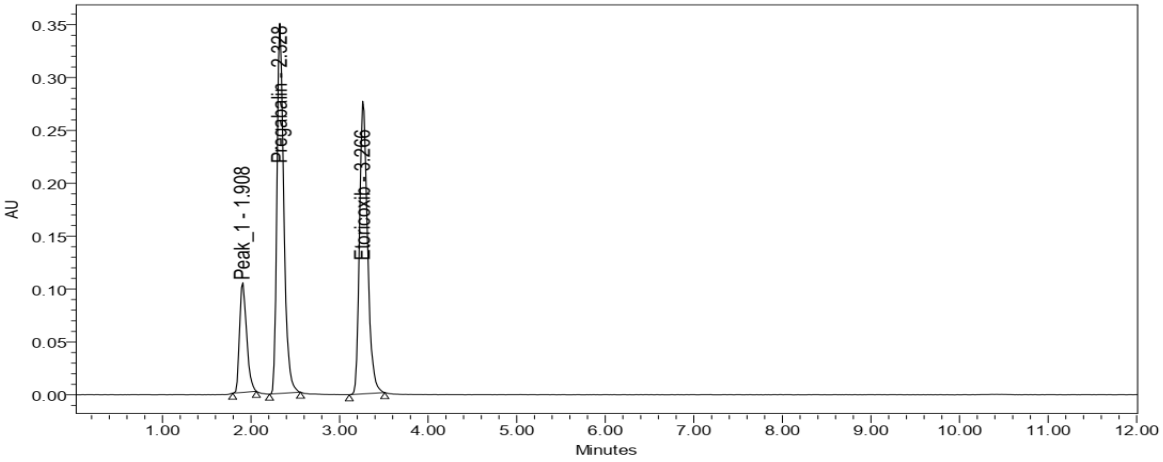

Figure 8. Acid chromatogram of Pregabalin and Etoricoxib

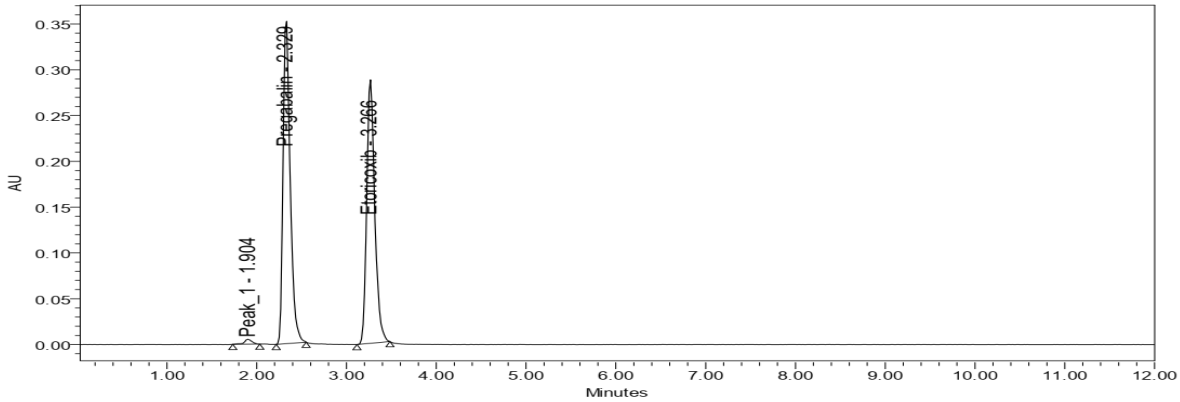

Figure 9. Base chromatogram of Pregabalin and Etoricoxib

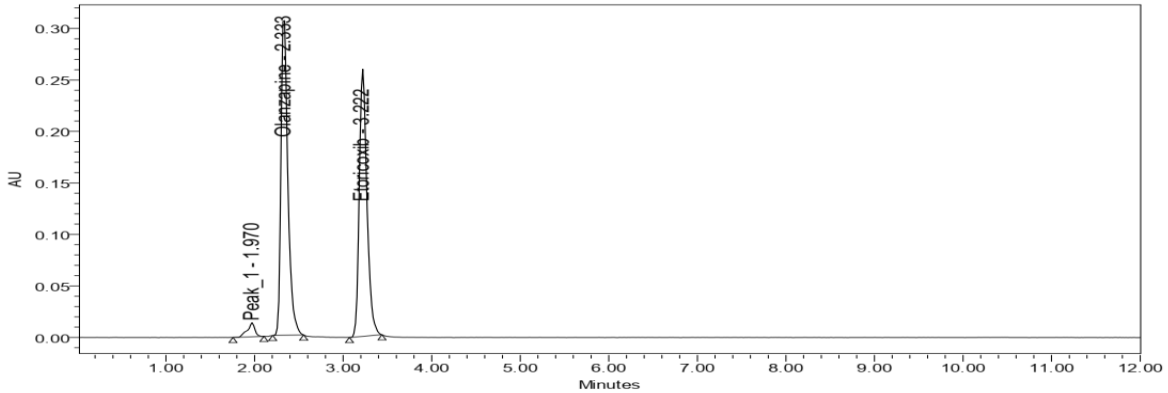

Figure 10. Peroxide chromatogram of Pregabalin and Etoricoxib

According to the results, samples were degraded when they were subjected to an acid, base, and oxidation interaction. Hydrolysis reaction, heat reaction, and light reaction all showed no deterioration. According to the stress research, none of the degradants co-eluted with the maxima of the active medication.

Assay: (Emaxgalin tab) bearing label claim, Pregabalin 75mg and Etoricoxib 60mg, assay was carried out by injecting sample into HPLC System.

Table 11. Assay Data of Pregabalin

S.no	Standard Area	Sample area	% Assay
1	1811555	1819633	100.32
2	1801637	1815502	100.09
3	1819971	1814392	100.03
4	1824286	1799826	99.22
5	1808668	1806051	99.57
6	1806319	1799299	99.20
Avg	**1812073**	**1809117**	**99.74**
Stdev	**8548.9**	**8617.4**	**0.48**
%RSD	**0.5**	**0.5**	**0.5**

Table 12. Assay data of Etoricoxib

S.no	Standard Area	Sample area	% Assay
1	1651404	1655923	99.71
2	1656887	1677108	100.99
3	1655306	1667684	100.42
4	1652494	1650913	99.41
5	1668344	1659586	99.94
6	1659628	1654947	99.66
Avg	**1657344**	**1661027**	**100.12**
Stdev	**6154.4**	**9698.5**	**0.6**
%RSD	**0.4**	**0.6**	**0.6**

Table 13. Assay outcome for Pregabalin and Etoricoxib

Drug Name	Label claim dose	%Assay	Brand Name
Pregabalin	75mg	99.74%	**Emaxgalin tab**
Etoricoxib	60mg	100.12%	

CONCLUSION

The proposed HPLC method was validated as per ICH guidelines and applied for the determination of Pregabalin and Etoricoxib in tablet dosage form. The method was found to be accurate, precise, robust and specific. Retention time of Pregabalin and Etoricoxib were found to be 2.250 min and 2.875 min. %RSD of the Pregabalin and Etoricoxib were and found to be 0.6 and 0.3 respectively. %Recovery was obtained as 99.92% and 99.62% for Pregabalin and Etoricoxib respectively. LOD, LOQ values obtained from regression equations of Pregabalin and Etoricoxib were 0.11, 0.32 and 0.27, 0.83 respectively. Regression equation of Lamivudine is y = 8638.7x + 10272, and y = 12913x + 5402 of Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

ACKNOWLEDGEMENT

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