STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PREGABALIN AND ETORICOXIB IN BULK AND TABLET DOSAGE FORM BY UPLC

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Abstract

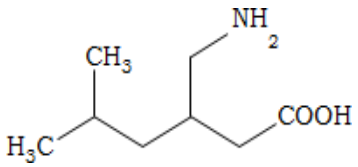
A simple, accurate and precise reverse phase ultra-performance liquid chromatography method was developed and validated for simultaneous estimation of Pregabalin and Etoricoxib in formulations as well as in pure form. The developed method was shown acceptable separation of pregabalin and Etoricoxib. The retention times for Pregabalin and Etoricoxib were 1.134 min. & 1.535 min. respectively. The limits of detection and quantification were obtained from regression equations and observed to be 0.85ppm and 0.54ppm for Pregabalin, 2.58 and 1.62ppm for Etoricoxib, respectively. Degradation studies of the tablet dosage form under stress conditions of acid, base, oxidation , temperature, water, UV light indicated that the method was particular with no interferences from any of the potential impurities that could form over the shelf life. The new developed RP-UPLC method which was sensitive, accurate stability indicating and fast, for active pharmaceutical ingredients and dosage forms.

Key words: *Pregabalin, Etoricoxib, Simultaneous, Reverse Phase - UPLC.*

1.Introduction

Pregabalin (Figure 1) chemically known as (S) - 3- (aminomethyl) - 5- methylhexanoic acid (1) which is an analogue of gabapentine is very potent drug (2,3). It is used as an anticonvulsant, analgesic medication and neurotransmitter. It is permitted in the US and Europe countries for adjunctive therapy of partial seizures in adults, and also has been permitted for the treatment of pain from diabetic neuropathy or post- herpetic neuralgia in adults (4). It binds with high specificity and affinity to voltage-gated calcium channel alpha (2)-delta proteins (5,6). Recent studies have shown that pregabalin is effective at treating chronic pain in disorders such as fibromyalgia (7) and spinal cord injury (8). It is freely soluble water, both in acid and basic aqueous solution. It is well absorbed after oral administration and largely excreted by renal excretion (9)

Figure1.Chemical structure of Pregabalin



Etoricoxib (Figure 2) is a COX-2 selective inhibitor (approx. 106.0 times more selective for COX-2 inhibition over COX-1), chemically known as 5-chloro-6'-methyl-3-[4-(methyl sulfonyl) phenyl]- 2,3'- bipyridine; $C_{18}H_{15}ClN_2O_2S$ (10) , It is used in the treatment of rheumatoid arthritis (11) , ankylosing spondylitis, chronic low back pain, acute pain and gout (12-14), psoriatic arthritis, osteoarthritis (15). It inhibits the synthesis of prostaglandins by inhibiting cyclooxygenase -2 enzyme (16-18). It is an off-white crystalline powder, freely soluble in alkaline aqueous solution and relatively insoluble in water (19).

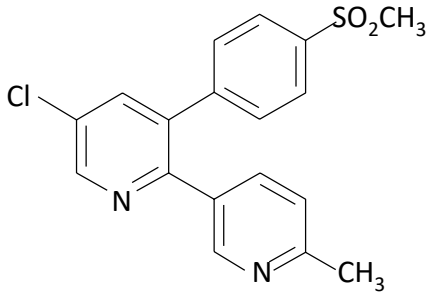


Figure 2. Chemical Structure of Etoricoxib

A literature review revealed that there are many HPLC based methods which have been developed for Pregabalin alone and with other combinational drugs also reported (20-24). Few spectrometry (25) , RP-HPLC (27) and HPTLC (28) methods reported for determination of Etoricoxib alone in bulk and dosage form and also in combined dosage form , impurities determination (29) Few RP-

UPLC methods reported for simultaneous estimation of Etoricoxib with other combined drugs (30) as well as Pregabalin with other combined drugs (31). However, there is no published data on the use of the reverse phase ultra performance liquid chromatography (UPLC) method for simultaneous estimation for both Pregabalin and Etoricoxib.

The objective of this study is to develop and validate a simple, sensitive, accurate, stability-indicating, and rapid RP-UPLC method for the simultaneous estimation of Pregabalin and Etoricoxib in tablet formulation as well as for active pharmaceutical ingredients, and also carry out degradation studies of tablet dosage form under forced stress conditions of oxidation, acid, base, temperature, water, and UV light to indicate that the method is specific with no interference from any of the potential impurities that could form so that it can be applied for routine analysis in testing laboratories.

2.Materials and methods

Materials

Chemicals and solvents

Pregabalin (purity: 99.30%) and Etoricoxib (purity: 99.7%) drugs [Active Pharmaceutical Ingredient (API)] and Pregabalin and Etoricoxib tablets (Emaxgalin) were obtained from Sun pharmaceutical industries as gift samples. Potassium dihydrogen ortho phosphate, acetonitrile, methanol, orthophosphoric acid, and sodium dihydrogen orthophosphate were of analytical reagent grade and procured from Rankem. UPLC purified water was obtained through Milli-Q water generation system. For sample solution filtrations 0.45µ polyvinylidene fluoride (PVDF) filters of Millipore were used

Instrumentation and equipment

Waters Acquity UPLC system with an Auto Injector and Tunable UV Detector was used; the software used is Empower 2, ultrasonicator (Labman),digital pH meter (Digisun electronics); electronic balance (Denver); and UV-VIS spectrophotometer integrated with UV WIN 6 software (PG instruments T 60); vacuum pump (Crompton); hot air oven (Survewell instruments) were also used.

Preparation of solutions

Buffer - 0.1% phosphate buffer (pH 2.5)

1 ml of orthophosphoric acid was taken in a 1000ml volumetric flask, about 100ml of milli - Q water was added and the final volume was made up to 1000ml with milli-Q water.

Mobile phase

Mobile phase: Mixture of 0.1% phosphate buffer solution and acetonitrile in the ratio of 60:40 (v/v).

Diluents

Depends on the drug solubility, water and acetonitrile with in the proportion of 50:50 (v/v) was chosen as diluents

Preparation of Standard stock solution

Accurately weighed and transferred 37.5mg of Pregabalin and 30mg of Etoricoxib working standards into a 50 ml clean dry volumetric flasks, added 25mlof diluent, sonicated for 10 minutes and made up to the final volume with diluents. (750µg/ml Pregabalin & 600µg/ml Etoricoxib).

Preparation of standard working solutions

Sample stock solution (1ml) was transferred to 10ml volumetric flask and made up with diluent. (75µg/ml Pregabalin & 60µg/ml Etoricoxib).

Preparation of sample stock solutions

Accurately weighed equivalent weight of the combination powder sample was transferred into a 100ml volumetric flask, 50ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters (750µg/ml Pregabalin & 600µg/ml Etoricoxib)

Preparation of sample working solution

In 10ml volumetric flask, 1ml of filtered sample stock solution was transferred and made up with diluent. (75µg/ml Pregabalin & 60µg/ml Etoricoxib).

Chromatographic conditions

The Waters Acquity UPLC chromatographic system with HSS C18 column of dimension 100 × 2.1 mm, 1.8 µm, was used. The mobile phase was a mixture of 0.1% orthophosphoric acid and acetonitrile in the ratio of 60:40 (v/v). The flow rate was 0.3 ml/minute, with 1 µl injection volume, column oven temperature of 30°C, and detection at 215 nm using Acquity Tunable UV detector. Data collation, instrument operation, processing were carried out with the help of Waters Empower 2 software.

Method development

Mobile phase was pumped for around 30 minutes to saturate the column and correct the base line. Various mobile phase ratios, buffers, and other parameters were changed to create the

method.

Validation parameters

Assay of pregabalin and etoricoxib

The assay of the marketed formulation of Pregabalin (75mg) and Etoricoxib (60 mg) tablets batch was carried out in line with the RP-UPLC assay method. Six sample sets were prepared and assayed

Linearity and range

To demonstrate the linearity and range of the RP-UPLC assay method, six standard solutions with concentrations of about 18.75-112.5µg/ml of Pregabalin and 15–90 µg/ml of Etoricoxib were prepared corresponding to 25%, 50%, 75%, 100%, 125%, and 150%. The prepared solutions were injected in to the chromatographic system and plotted graphs between concentration and peak area to find the correlation coefficient.

Accuracy

To demonstrate the accuracy of the Pregabalin and Etoricoxib RP-UPLC assay method, sample solutions of concentration 75ppm Pregabalin and 60ppm Etoricoxib were prepared by taking one tablet equivalent to the powdered Pregabalin and Etoricoxib tablet, followed by sonication with the diluent and filtration. Known quantities of Pregabalin and Etoricoxib standard stock solution were spiked on to the sample solution at levels of 50%, 100%, and 150%. Each of the sample solution spiked with a known concentration of the standard (50%, 100%, and 150% each of Pregabalin and Etoricoxib was injected in triplicate).

Precision

The method precision of the RP-UPLC assay method as part of the repeatability was checked by injecting six replicates of Pregabalin and Etoricoxib solutions of concentration 75ppm of Pregabalin and 60ppm of Etoricoxib. Intermediate precision was established by injecting six sample solutions of concentration 75ppm of Pregabalin and 60ppm of Etoricoxib into the chromatograph after 24 hours of preparation. The %amount of Pregabalin and Etoricoxib was calculated, and relative standard deviation (RSD) was determined.

LOQ and LOD

The LOD was determined using regression analysis and calculated using the following equation:

$$LOD = 3.3\sigma / S$$

The LOQ was calculated with the following formula:

$$LOQ = 10\sigma / S$$

Where σ = standard deviation of calibration curve S = slope of the calibration curve.

System suitability

Pregabalin and Etoricoxib working standard (75ppm Pregabalin and 60ppm of Etoricoxib) solution was prepared and injected six replicates into the UPLC system. From standard chromatograms various parameters of system suitability like resolution, retention time, plate count, variation and relative standard deviations were evaluated.

Specificity and selectivity

Specificity was demonstrated by injection of blanks, pharmaceutical placebo, and potential degradation impurities from degradation studies performed in degradation studies using the ICH guideline RP-UPLC method. The absence of adjuvant interference during the application of the planned approach to the study of pharmaceutical formulations demonstrated its selectivity.

Robustness

The robustness of the pregabalin and etoricoxib RP - UPLC assay is intended for methods that can occur in a lab environment by using both sides of chromatographic variables such as flow (+0.3 mL / min), composition, etc. Demonstrated by major changes. Mobile phase (+ 2%) and temperature (+ 5 ° C). Under each of these conditions, sample pretreatment was introduced twice into optimized chromatographic conditions with the above modifications.

Forced degradation studies

Oxidation

To 1 ml of stock solution of Pregabalin and Etoricoxib, 1 ml of 20% hydrogen peroxide was added separately. At 60°C solutions were kept for thirty minutes . To obtain (75ppm and 60ppm) solution, the resultant solution was diluted and 10 µl were injected into the system and the chromatograms were recorded to assess the sample stability for UPLC study.

Acid degradation studies

To 1 ml of stock solution of Pregabalin and Etoricoxib, 1 ml of 2 N hydrochloric acid was added and refluxed for thirty minutes at 60°C. The resultant solution was diluted to obtain (75ppm and 60ppm) solution and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Alkali degradation studies

To 1 ml of stock solution of Pregabalin and Etoricoxib, 1 ml of 2 N sodium hydroxide was added and refluxed for thirty minutes at 60°C. The resultant solution was diluted to get (75ppm and 60ppm) solutions and 10 µl was injected into the system and the chromatograms were recorded to estimate the stability of the sample.

Dry heat degradation studies

To study dry heat degradation, standard drug solution was placed in an oven at 105°C for 6 hours. For UPLC study, the resultant solution was diluted to obtain (75ppm and 60ppm) solutions and 10 µl was injected into the system and the chromatograms were recorded to evaluate the stability of the sample.

Photo stability studies

The photochemical stability of the drug was also studied by exposing the (75ppm and 60ppm) solution to UV light by keeping the beaker in a UV chamber for 7 days or 200-Watthours/min in a photo stability chamber. For the study of UPLC , the resultant solution was diluted to get (75ppm and 60ppm) solution and 10 µl solution was injected into the system and the chromatograms were recorded to estimate the stability of the sample.

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hours at a temperature of 60°C. For UPLC study, the resultant solution was diluted to obtain (75ppm and 60ppm) solutions and 10 µl was injected into the system and the chromatograms were recorded to estimate sample stability.

3.Results and Discussion

Optimization of chromatographic conditions

The UPLC method sensitivity based on the choice of detection wavelength. An ideal wavelength is which gives good response for all impurities and analyte peak to be detected. Wavelength was set at 215 nm by considering the UV maxima of Pregabalin and UV minima of Etoricoxib. 0.01 N potassium dihydrogen phosphate and 0.1% orthophosphoric acid (pH 2.5) buffer and acetonitrile combination was estimated with UPLC columns such as HSS, Hibar, and BEH. Ethylene Bridged Hybrid (BEH) column was estimated with 0.1% orthophosphoric acid (pH 2.5) and acetonitrile

mix. The resolutions were not satisfactory when Ethylene Bridged Hybrid (BEH) was assessed with 0.1% orthophosphoric acid (pH 2.5) and acetonitrile mix. 0.1% orthophosphoric acid (pH 2.5)/0.01 N potassium hydrogen phosphate buffer and acetonitrile were checked with High Strength Silica (HSS) column. The resolution was promising but peak splitting was observed for Pregabalin peak. By increasing the ratio of the buffer to solvent mix it was further optimized . With combination of 0.1% orthophosphoric acid (pH 2.5) and acetonitrile at 60:40 (v/v), worked well with fast elution, good separation, and peak shapes.With Waters Acquity HSS C18 column of dimensions 100 × 2.1 mm, 1.8 µm, with a flow rate of 0.3 ml/minute, column oven temperature of 30°C, and mobile phase consisting of 0.1% orthophosphoric acid (pH 2.5) and acetonitrile in the ratio of 60:40(v/v), the final optimized condition was achieved shown in figure 3. Both Pregabalin and Etoricoxib peaks eluted within 3 minutes, thus making it a quick method and hence the possibility to run multiple samples in a short time.

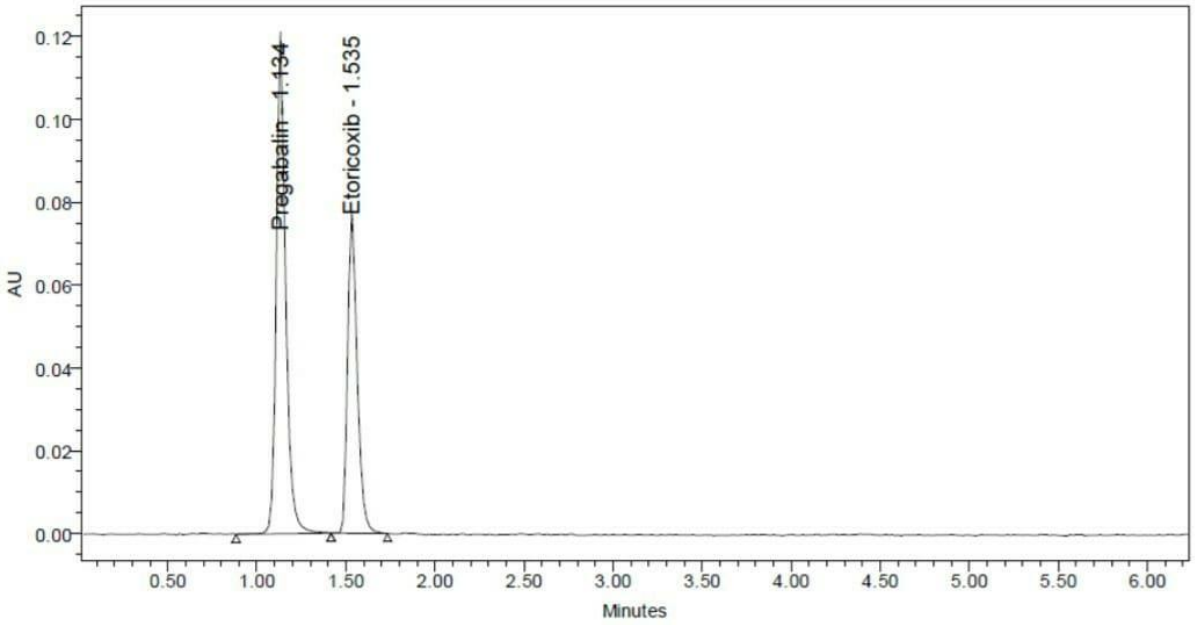


Figure 3. Chromatogram of optimization

Assay of Marketed Formulation

The assay of the marketed formulation of Pregabalin (75mg) and Etoricoxib (60 mg) tablets batch was carried out in line with the RP-UPLC assay method. Six sample sets were prepared and assayed. The assay results were observed to be 100% with RSD of 0.7% for Pregabalin and 99.54% with RSD of 0.78% for Etoricoxib shown in table 1.

Table 1. Results of marketed formulation analysis.

Compound name	Brand name	Label claim	% Content

Pregabalin	Emaxgalin	75mg	100
Etoricoxib	Emaxgalin	60mg	99.54

Linearity and Range

The linearity and range of the RP-UPLC test method was demonstrated by plotting the concentration and peak area. The linear equations obtained with pregabalin and etoricoxib: y = 8133x + 3133 and the linear equations obtained with etoricoxib: y = 6766x + 4154 The correlation coefficients obtained were greater than 0.999 for both agents. This shows that the method is linear in the range of 25% to 150%. The details of the level of linearity are shown in table 2-4. The linearity plot of pregabalin and Etoricoxib were shown in figure 4-5

Table 2. Linearity of Pregabalin

S.No	% Linearity Level	Conc. in ppm(Pregablin)	Avg peak area
1	25	18.75	158858
2	50	37.5	312199
3	75	56.25	463528
4	100	75	612314
5	125	93.75	760522
6	150	112.5	916475

Table 3. Linearity of Etoricoxib

S.No	% Linearity Level	Conc. in ppm(Etoricoxib)	Avg peak area
1	25	15	102108
2	50	30	212100
3	75	45	314188
4	100	60	413818
5	125	75	514294
6	150	90	609830

Table 4. Optical characteristics of pregabalin and etoricoxib

Parameters	pregabalin	etoricoxib
Linearity (ppm)	18.75-112.5 ppm	15-90 ppm
Regression equation	y = 8133x+3113	y = 6766x + 4154
Slope	8133	6766
Intercept	3113	4154
Correlation coefficient (R^2)	0.999	0.999

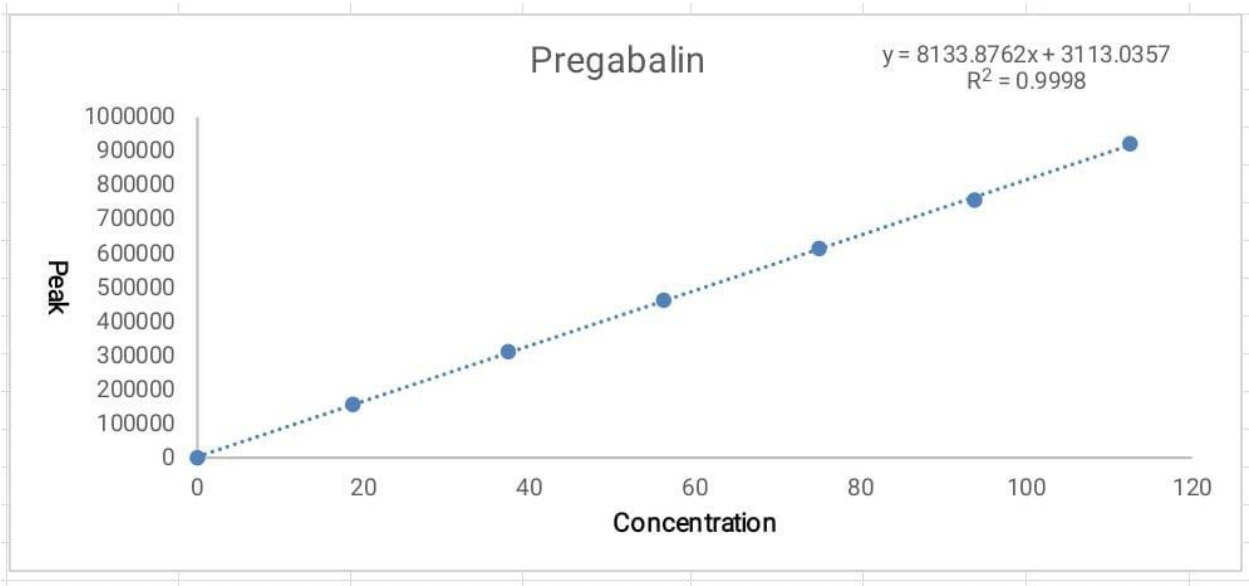


Figure 4. Linearity of Pregabalin

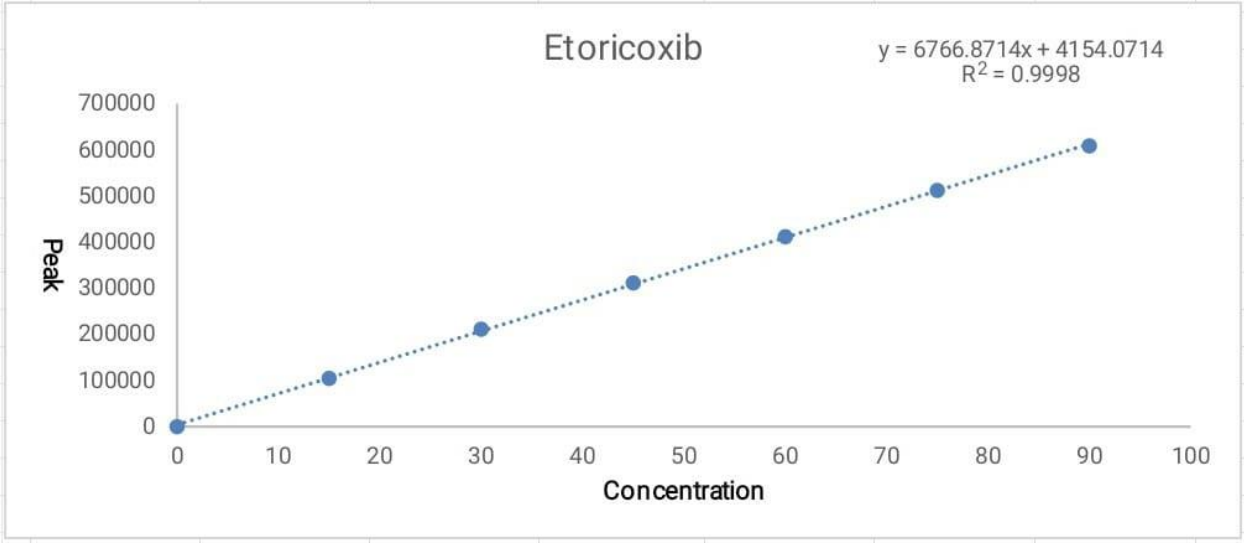


Figure 5.Linearity of Etoricoxib

Accuracy

Accuracy of the Pregabalin and Etoricoxib RP-UPLC assay method was demonstrated with mean recovery observed to be 100% for Pregabalin and 99.87% for Etoricoxib. The detailed percent recovery for Pregabalin and Etoricoxib was presented in table 5-6

Table 5. Recovery studies of Pregabalin

Accuracy- Pregabalin				
% Level	Amount Spiked(ppm)	Amount Recovered (ppm)	% Recovery	Mean % Recovery
50	37.5	37.5	100	100
	37.5	37.9	101.1	
	37.5	37.3	99.5	
100	75	74.9	99.8	
	75	74.3	99.1	
	75	73.7	98.3	
150	112.5	113.7	101.1	
	112.5	113.0	100.5	
	112.5	113.2	100.6	

Table 6. Recovery studies of Etoricoxib

Accuracy- Etoricoxib				
% Level	Amount Spiked(ppm)	Amount Recovered (ppm)	% Recovery	Mean % Recovery
50	30	29.61	98.71	99.87
	30	29.92	99.74	
	30	29.82	99.39	
100	60	59.67	99.41	
	60	60.18	100.30	
	60	59.44	99.06	
150	90	90.62	100.69	
	90	89.62	99.58	
	90	91.28	101.42	

Precision

The method precision of the RP-UPLC assay method as part of repeatability was checked and the percentage relative standard deviations for Pregabalin and Etoricoxib were observed to be 0.7% and 0.8%, respectively. As part of intermediate precision, the percentage amount of Pregabalin and Etoricoxib was calculated, and RSD was found to be 0.7% and 1.1%, respectively. Details of the precision method and intermediate precision are summarized in table 7-8

Table 7. Method precision of pregabalin and etoricoxib

Method Precision		
S.No	Area of Pregabalin	Area of Etoricoxib
1	610643	410037
2	603296	405801
3	609062	401019
4	607953	408195
5	610192	408650
6	615775	407495
Avg	609487	406866
Std.dev	4055.5	3185.0
%RSD	0.7	0.8

Table 8. Intermediate precision of pregabalin and etoricoxib

Intermediate precision		
S.No	Area of Pregabalin	Area of Etoricoxib
1	606789	406971
2	615156	411309
3	602309	403255
4	610824	399390
5	607363	403206
6	609506	408993
Avg	608658	405521
Std.dev	4815.4	4375.9
%RSD	0.7	1.1

LOQ and LOD

LOD and LOQ were obtained by using regression analysis. The LOD values were observed to be at 0.85 and 0.54ppm for Pregabalin and Etoricoxib, respectively. Similarly, the LOQ values were observed to be 2.58 and 1.62 ppm for Pregabalin and Etoricoxib, respectively.

System suitability

The system suitability parameters were evaluated from six replicate injections of pregabalin and etoricoxib. Plate count was greater than two thousand. The retention time, tailing factor, less than 2. and resolution should be > 3. All of the system's appropriate parameters were accepted, and they were all within acceptable bounds. System suitability details were presented in table I0.

Table 9. System suitability parameter of pregabalin and etoricoxib

S.No	Pregabalin			Etoricoxib			
Inj	RT(Min)	USP Plate Count	Tailing	RT(Min)	USP Plate Count	Tailing	Resolution
1	1.133	2197	1.23	1.502	3821	1.29	3.8
2	1.135	2195	1.23	1.519	3771	1.30	3.8
3	1.136	2248	1.21	1.519	3795	1.29	3.9
4	1.136	2248	1.21	1.519	3811	1.29	3.8
5	1.137	2210	1.21	1.521	3845	1.32	3.8
6	1.137	2242	1.22	1.531	3853	1.30	3.9

Robustness

Samples were injected under conditions of flow (-) (0.3ml/min), flow (+) (0.6ml/min), mobile phase (-) (58B:42A), mobile phase (+) (62B:38A), temperature (–) (25°C), and temperature (+) (35°C). The robustness of an analytical process is a measure of its capacity to persist uninfluenced by modest but purposeful changes in method parameters, and it serves as a marker of its dependability in routine use. The tailing factor of pregabalin and etoricoxib was < 2.0 in all of the changed chromatographic settings. In all of the robustness scenarios, there was a very minimal variance in the resolution and tailing factor values, demonstrating the method's robustness. The results were tabulated in table 11.

Table 10:Robustness of Pregabalin and Etoricoxib

Parameter	% RSD of Pregabalin	% RSD of Etoricoxib
Flow(0.6ml/min)	0.3	0.4
Flow(0.03ml/min)	0.6	0.7
Mobile phase((62:38)	0.4	0.5
Mobile Phase(58:42)	0.5	1.2
Temperature (35°C)	0.4	0.3
Temperature (25°C)	0.8	0.6

Specificity and selectivity

Specificity for the RP-UPLC method was demonstrated by no interference from the blank, formulation placebo.There were no interferences in the chromatograms of blank and placebo samples. As a result, the procedure was specific and selective. Figure 6-7 shown in chromatograms of blank and working placebo solution respectively.

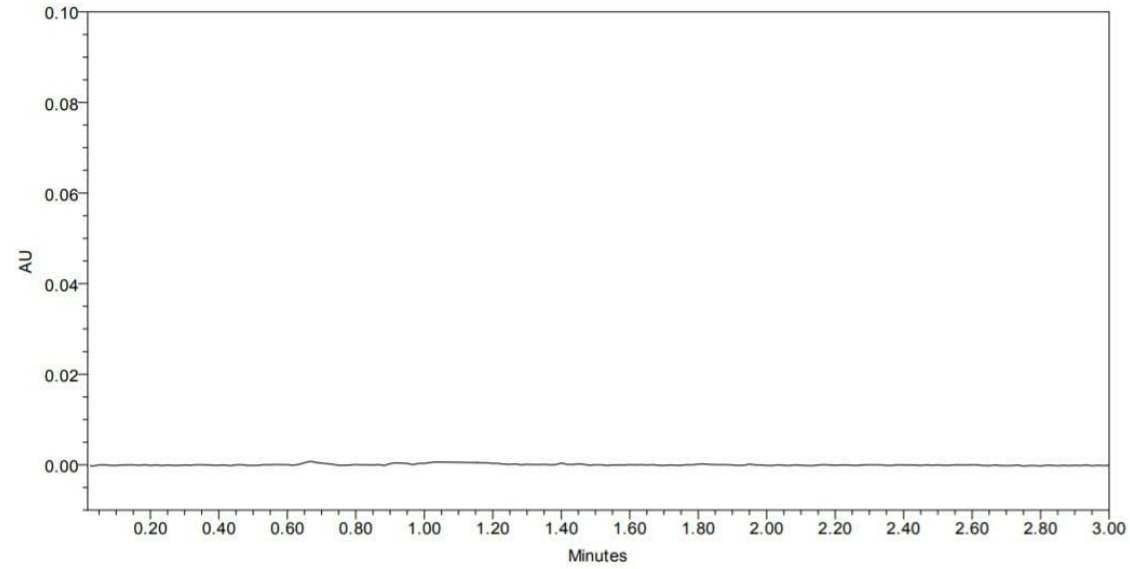


Figure 6. Chromatogram of blank

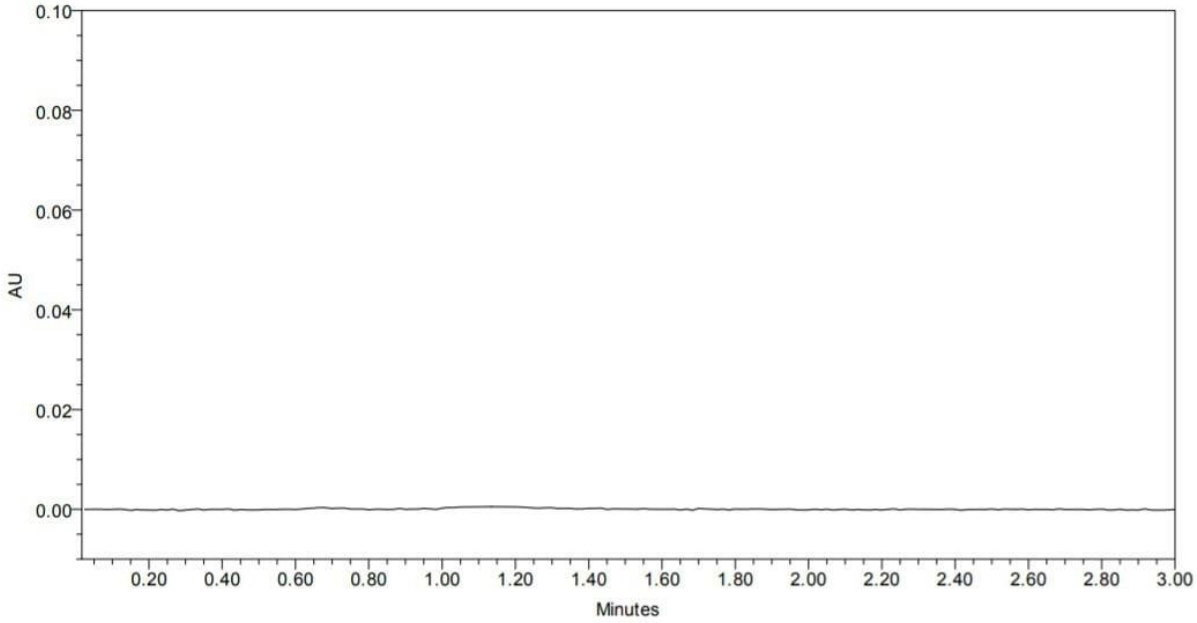


Figure 7. Chromatogram of placebo

Forced degradation studies

The method stability-indicating capability was demonstrated by degradation studies in accordance with the ICH guidelines. Pregabalin and Etoricoxib was observed in exposure to acidic conditions to an extent of 5.11% and 5.45%, respectively. Least degradation was observed in conditions of exposure to water to the extent of about 0.75 and 0.71% in both Pregabalin and Etoricoxib . Under each condition of degradation, there were no interferences at the retention time of Pregabalin and Etoricoxib . The details of degradation under various stress conditions are detailed in table 11 for Pregabalin and Etoricoxib respectively.

Table 11 . Degradation data of Pregabalin and Etoricoxib

Degradation data of Pregabalin			Degradation data of Etoricoxib		
S.NO	Degradation Condition	% Drug undegraded	% Drug degraded	% Drug undegraded	% Drug degraded
1	Acid	94.89	5.11	94.55	5.45
2	Alkali	95.26	4.74	95.34	4.66
3	Oxidation	95.69	4.31	95.82	4.18
4	Thermal	97.47	2.53	97.54	2.46
5	UV	98.77	1.23	98.35	1.65
6	Water	99.25	0.75	99.26	0.71

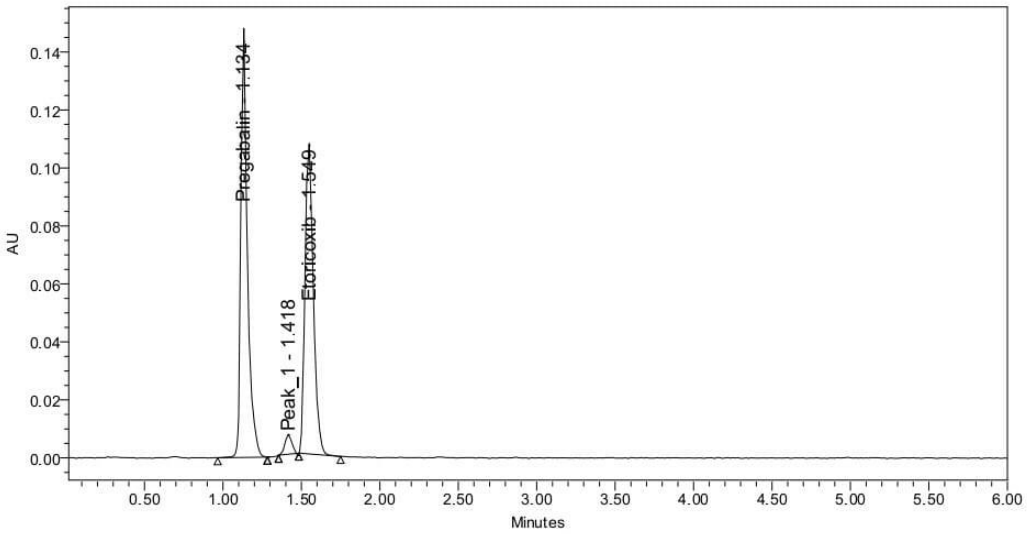


Figure. 8. Acid degradation of Pregabalin and Etoricoxib

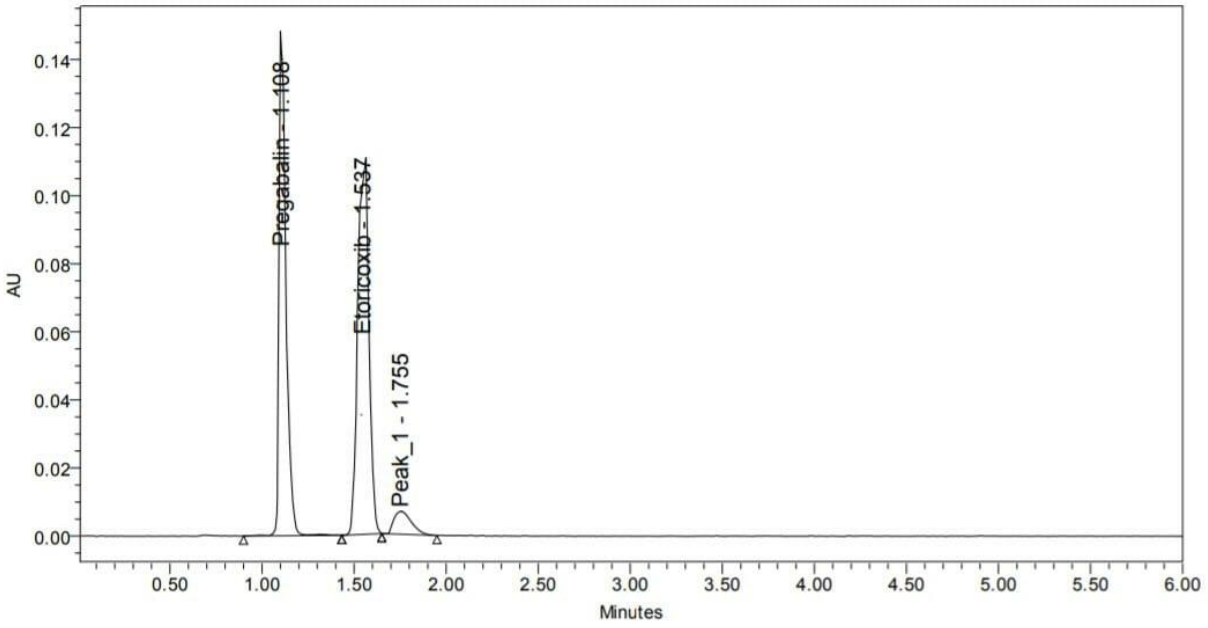


Figure 9. Chromatogram of base degradation

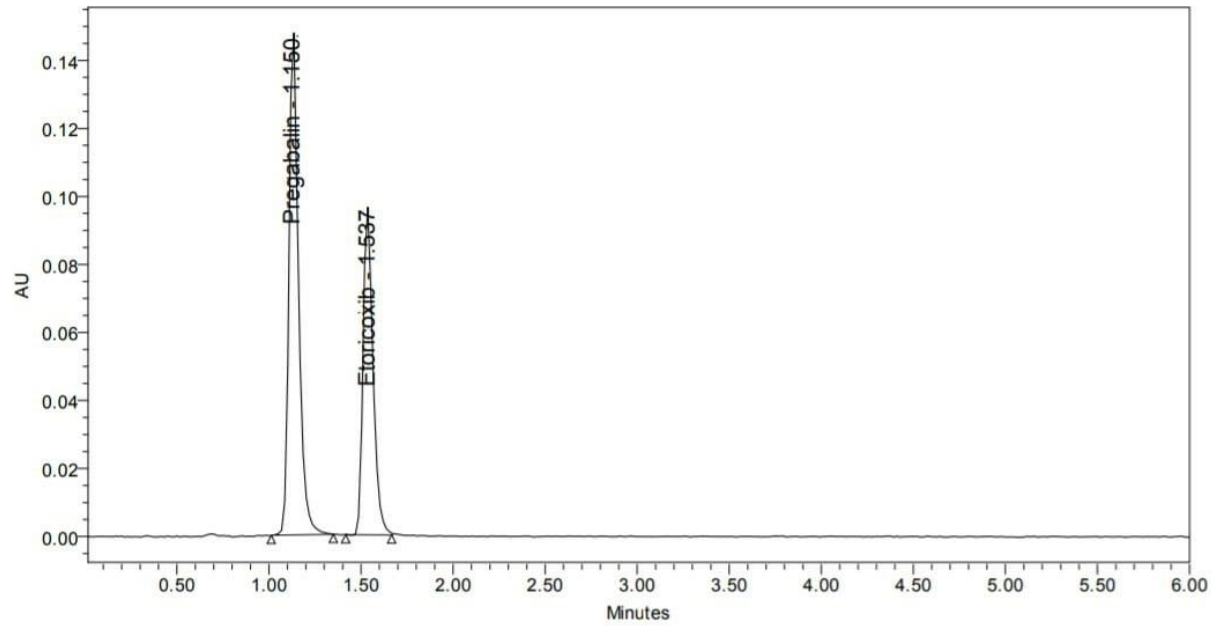


Figure 10. Chromatogram of peroxide degradation

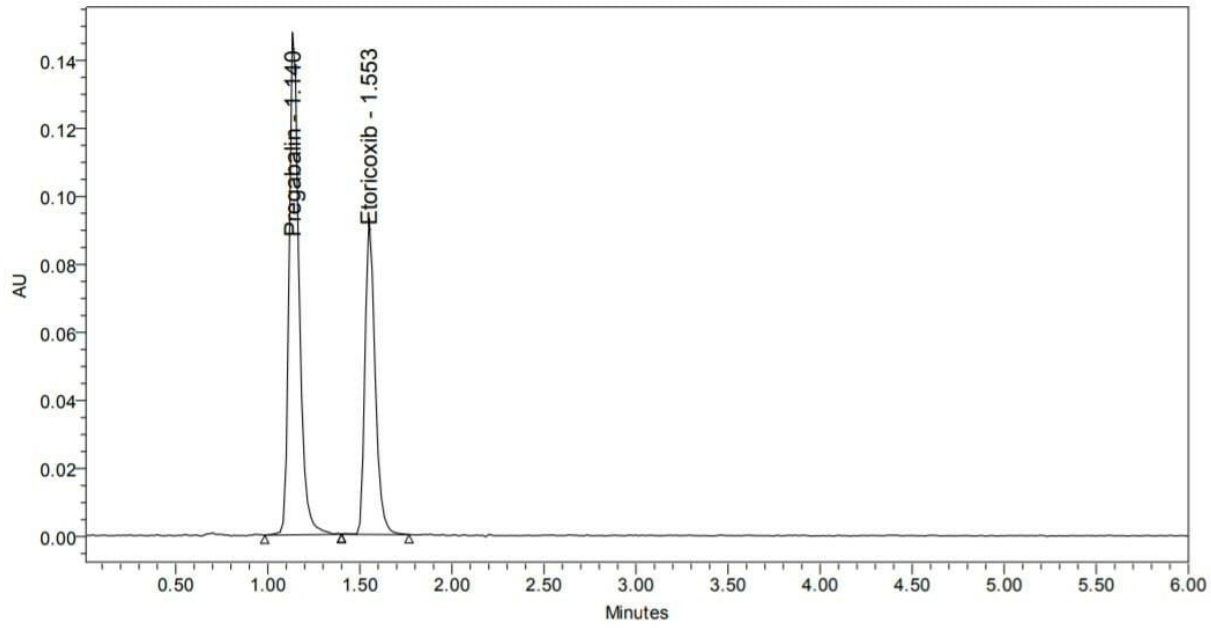


Figure 11. Chromatogram of Thermal degradation

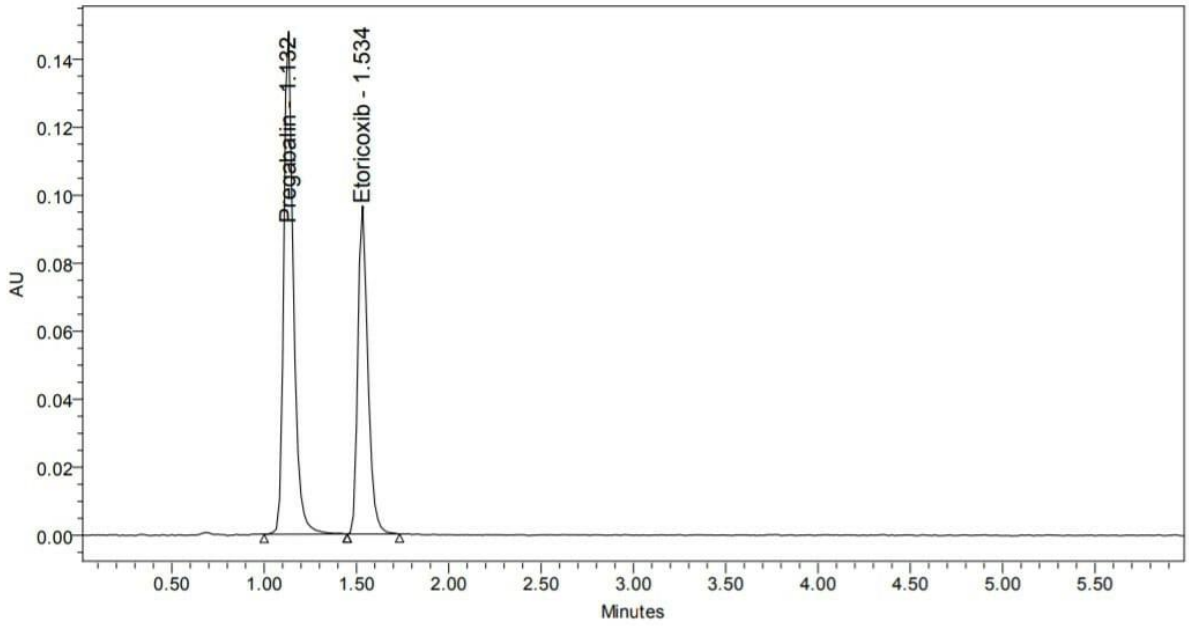


Figure 12. Chromatogram of UV degradation

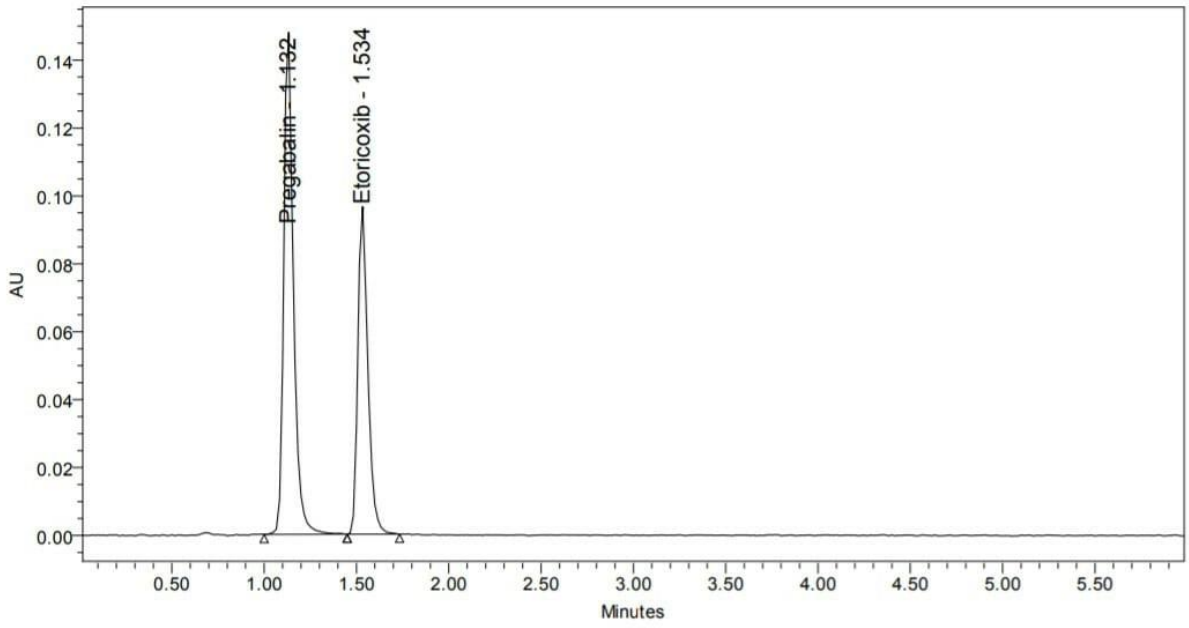


Figure 13. Chromatogram of neutral degradation

4.Conclusion

A sensitive, accurate, stability indicating, and quick RP-UPLC method was developed and validated for the simultaneous estimation of Pregabalin and Etoricoxib in bulk form and Pregabalin and Etoricoxib tablet formulation. The method demonstrated linearity in the range 25%–150% for both Pregabalin and Etoricoxib with an R^2 value greater than 0.999. Precision and accuracy were well within the 2% RSD. Both Pregabalin and Etoricoxib were well separated and eluted well within 3 minutes, thus making this estimation quick and at the same time avoiding solvent wastage. Degradation studies reiterate that there is no interference from any of the potential impurities arising out of exposure to conditions as specified in the ICH Q2 R1; Hence, the method can easily be applied to in process, routine, and stability monitoring in the laboratory. In addition, the low levels of LOD and LOQ extend the possibility of using the same method for monitoring of cleaning residues after equipment cleaning in production shop floor.

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Conflict of interest

The authors declare that there is no conflict of interest in the submission of this article.

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