DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PREGABALIN AND ETORICOXIBE

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ABSTRACT

Background: Accurate and reliable analytical methods are essential for ensuring the quality of pharmaceutical formulations, particularly for the simultaneous estimation of multiple active ingredients. Pregabalin and Etoricoxib are widely used therapeutic agents, necessitating a precise, efficient, and cost-effective method for their quantification in tablet formulations.

Aim: To develop and validate a simple, accurate, and economical RP-HPLC method for the simultaneous determination of Pregabalin and Etoricoxib in pharmaceutical tablets, ensuring suitability for routine quality control.

Methodology: The RP-HPLC method was developed using a Kromasil C18 column (250 × 4.6 mm, 5µ), with a phosphate buffer-acetonitrile (60:40) mobile phase, 1 mL/min flow rate, and detection at 226 nm. The retention times for Etoricoxib and Pregabalin were 2.213 and 2.702 minutes, respectively. The method was validated for precision, accuracy, and sensitivity. Results: The method exhibited excellent precision (%RSD: 0.8% for Pregabalin, 0.4% for Etoricoxib) and high accuracy (99.71% and 99.77%, respectively). Sensitivity analysis showed LOD of 0.09 µg/mL (Pregabalin) and 0.04 µg/mL (Etoricoxib), with strong linearity ($R^2 > 0.999$).

Conclusion: The validated RP-HPLC method is precise, accurate, robust, and cost-effective for the simultaneous estimation of Pregabalin and Etoricoxib in tablets. Its optimized chromatographic conditions and reduced retention time make it ideal for routine pharmaceutical quality control.

KEYWORDS: RP-HPLC, Pregabalin, Etoricoxib, Simultaneous Estimation.

INTRODUCTION

The quality of pharmaceutical drugs is a fundamental aspect of ensuring their safety and therapeutic efficacy. The pharmaceutical industry relies on rigorous quality control processes to guarantee that medications meet the required standards before they reach consumers. Analytical techniques play a crucial role in assessing the purity, potency, and stability of drug formulations, ensuring their compliance with regulatory guidelines. Among the various analytical methods available, chromatographic techniques have emerged as indispensable tools for drug analysis due to their high precision, accuracy, and sensitivity.

Pregabalin and Etoricoxib are widely used pharmaceutical compounds with distinct therapeutic roles. Pregabalin is commonly prescribed for neuropathic pain, epilepsy, and generalized anxiety disorders, whereas Etoricoxib is a selective COX-2 inhibitor used to manage inflammation and pain associated with arthritis and other musculoskeletal conditions. Given their combined presence in certain pharmaceutical formulations, it is essential to establish a reliable analytical method for their simultaneous quantification [1].

High-Performance Liquid Chromatography (HPLC) has become the preferred technique for the analysis of pharmaceutical compounds due to its ability to separate complex mixtures with high specificity. Reverse-phase HPLC (RP-HPLC) is particularly favored because of its efficiency, reproducibility, and suitability for routine quality control applications. However, the development of an optimized method for the simultaneous estimation of Pregabalin and Etoricoxib presents challenges, including achieving adequate separation, minimizing retention time, and ensuring method robustness [2].

In pharmaceutical analysis, method validation is a critical step to ensure that an analytical technique meets regulatory standards for accuracy, precision, linearity, sensitivity, and robustness. The International Council for Harmonisation (ICH) guidelines provide a framework for validating analytical methods, ensuring consistency and reliability in drug testing. Developing a stability-indicating RP-HPLC method is particularly important for detecting any degradation products that may arise due to environmental factors such as temperature, humidity, and light exposure. A well-validated method not only enhances quality control processes but also supports regulatory compliance and patient safety. Given the growing demand for combination drug formulations, a simultaneous estimation method for Pregabalin and Etoricoxib will significantly streamline analytical workflows, reducing time and costs associated with separate drug assessments while maintaining high accuracy and efficiency in pharmaceutical quality assurance [3].

This study focuses on developing and validating a simple, cost-effective, and accurate RP-HPLC method for the simultaneous estimation of Pregabalin and Etoricoxib in tablet formulations. By optimizing chromatographic conditions, this method aims to provide pharmaceutical industries with a reliable tool for routine quality control, ensuring the safety and efficacy of these drugs in therapeutic applications.

MATERIALS AND METHODS

Materials

The study utilized pure active pharmaceutical ingredients (APIs) of Pregabalin and Etoricoxib, along with their combination tablet formulation (PBREN - ET). The solvents and reagents used for analysis included distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen orthophosphate buffer, and ortho-phosphoric acid. All chemicals and solvents were procured from Rankem to ensure high purity and reliability.[4]

Instrumentation

The analytical procedures were carried out using a WATERS HPLC 2695 SYSTEM, which is equipped with quaternary pumps, a Photo Diode Array detector, and an auto sampler, all integrated with Empower 2 Software for data analysis. A UV-VIS spectrophotometer (PG Instruments T60), featuring a 2 mm to 10 mm bandwidth and matched quartz cells, was employed for absorbance measurements. Additional equipment used included a Denver Electronic Balance for precise weighing, a BVK Enterprises pH meter, and an ultrasonicator from BVK Enterprises for sample preparation.[5]

Methodology

Diluent Preparation

A diluent mixture of phosphate buffer and distilled water (50:50) was selected based on the solubility characteristics of Pregabalin and Etoricoxib to ensure optimal dissolution and stability.

Preparation of Standard Stock Solutions

Accurately weighed 37.5 mg of Pregabalin and 30 mg of Etoricoxib were transferred into separate 50 mL volumetric flasks. Approximately three-fourths of the diluent was added, followed by sonication for 10 minutes to ensure complete dissolution. The final volume was adjusted with diluent, yielding 750 µg/mL of Pregabalin and 600 µg/mL of Etoricoxib, labeled as standard stock solutions 1 and 2.

Preparation of Standard Working Solutions (100% Solution)

To prepare the working solution, 1 mL from each standard stock solution was pipetted into a 10 mL volumetric flask and diluted with the diluent, resulting in final concentrations of 75 µg/mL for Pregabalin and 60 µg/mL for Etoricoxib.

Preparation of Sample Stock Solutions

The powdered tablet sample, equivalent to the drug combination dosage, was accurately weighed and transferred into a 100 mL volumetric flask. Fifty milliliters of diluent was added, and the mixture was sonicated for 25 minutes to achieve complete dissolution. The volume was then adjusted with diluent and filtered using Milli-Q filters, yielding a final concentration of 750 µg/mL of Pregabalin and 600 µg/mL of Etoricoxib.

Preparation of Sample Working Solutions (100% Solution)

A 1 mL aliquot of the filtered sample stock solution was transferred into a 10 mL volumetric flask and diluted with the diluent to obtain working concentrations of 75 µg/mL for Pregabalin and 60 µg/mL for Etoricoxib.

Preparation of Buffer

A 0.1% ortho-phosphoric acid (OPA) buffer was prepared by diluting 1 mL of concentrated OPA with 1000 mL of water.[7-9]

Method Validation

System Suitability Parameters

The system suitability was evaluated by injecting standard solutions of Pregabalin (75 ppm) and Etoricoxib (60 ppm) six times. Key parameters assessed included peak tailing, resolution, and USP plate count. The method was deemed suitable if the %RSD of the peak areas did not exceed 2%.[10]

Specificity

The specificity of the method was confirmed by ensuring no interfering peaks were present in blank and placebo samples at the retention times of Pregabalin and Etoricoxib.

Precision

The precision of the method was assessed by injecting six replicates of test solutions containing 75 ppm of Pregabalin and 60 ppm of Etoricoxib. The %RSD values were required to be below 2% to demonstrate reproducibility.[11-12]

Linearity

Linearity was evaluated by preparing standard solutions at six concentration levels ranging from 25% to 150% of the target concentration. A calibration curve was constructed, and regression equations were derived to confirm a strong correlation (R^2 close to 1.000).

Accuracy (Recovery Studies)

Recovery studies were conducted at 50%, 100%, and 150% spiking levels by adding known amounts of standard solutions to pre-analyzed samples. The acceptance criterion was set between 98% and 102% recovery.[13-15]

Robustness

To assess the robustness of the method, deliberate variations were introduced in flow rate (±0.1 mL/min), mobile phase composition, and column temperature (±5°C). The method was considered robust if system suitability parameters remained within acceptable limits and %RSD was below 2%.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined based on the standard deviation of the response and slope of the calibration curve. The LOD values were 0.09 µg/mL for Pregabalin and 0.04 µg/mL for Etoricoxib, while the LOQ values were 0.27 µg/mL and 0.11 µg/mL, respectively.[16]

Forced Degradation Studies

To evaluate the stability of the method, forced degradation studies were performed under different stress conditions:

- Oxidation: Exposure to 20% hydrogen peroxide (H_2O_2) at 60°C for 30 minutes.
- Acid Hydrolysis: Treatment with 2N hydrochloric acid at 60°C for 30 minutes.
- Alkaline Hydrolysis: Treatment with 2N sodium hydroxide at 60°C for 30 minutes.
- Thermal Degradation: Heating at 105°C for 1 hour.
- Photostability: Exposure to UV light for 24 hours.
- Neutral Hydrolysis: Refluxing the drug in water at 60°C for 1 hour.[17]

RESULTS

Method Development and Optimization

The development of an efficient RP-HPLC method for the simultaneous estimation of Pregabalin and Etoricoxib involved the optimization of chromatographic conditions. Various mobile phase compositions, buffer systems, and column parameters were tested to achieve optimal separation with minimal retention time. After several trials, the optimized method employed a Kromasil C8 column (4.6 × 250 mm, 5µm) with a mobile phase of 0.01N KH_2PO_4 and acetonitrile (60:40) at a flow rate of 1 mL/min. The column temperature was maintained at 30°C, and detection was performed at 226 nm. The retention times for

Pregabalin and Etoricoxib were found to be 2.702 minutes and 2.213 minutes, respectively, ensuring efficient resolution with acceptable peak symmetry and reproducibility.

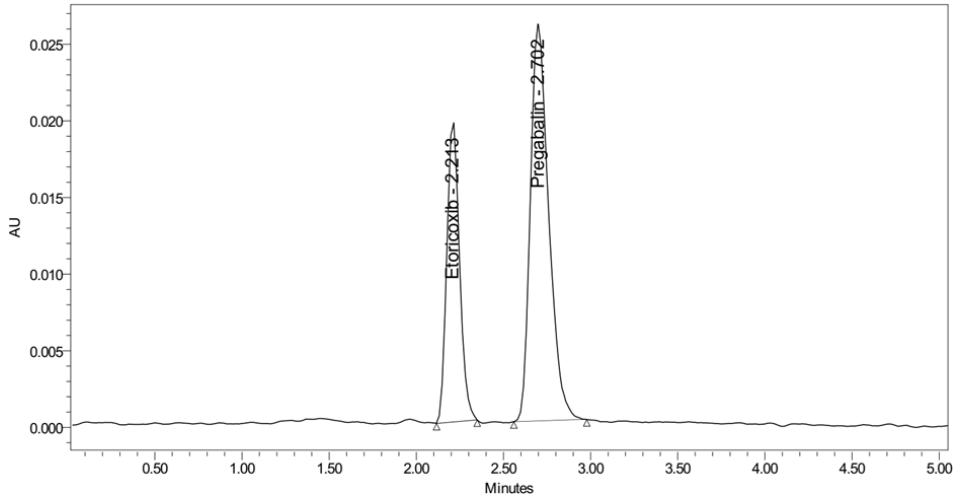

Figure 1. Chromatogram of Etoricoxib and pregabalin

System Suitability and Precision

The system suitability was assessed by injecting six replicates of the standard solution, and key parameters such as retention time, peak area, resolution, theoretical plate count, and tailing factor were evaluated. The results confirmed that all parameters were within the acceptable limits as per ICH guidelines. The precision of the method was determined by repeatability and intermediate precision studies, where the %RSD for Pregabalin and Etoricoxib was found to be 0.8% and 0.4%, respectively, indicating high reproducibility.

Table 1. System suitability parameters for Pregabalin and Etoricoxibe

S No	Etoricoxibe			Pregabalin			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.219	3879	1.19	2.724	2793	1.47	2.8
2	2.235	3893	1.19	2.747	2676	1.51	2.8
3	2.236	3910	1.20	2.750	2641	1.48	2.8
4	2.236	3955	1.20	2.754	2698	1.47	2.8
5	2.237	3960	1.20	2.754	2694	1.52	2.8
6	2.237	4040	1.20	2.754	2680	1.49	2.8

Table 2. Repeatability precision table of Pregabalin and Etoricoxibe

S. No	Area of Pregabalin	Area of Etoricoxibe
1.	605910	309936
2.	615675	309644
3.	608453	312096
4.	616863	308693
5.	612289	310021
6.	617434	310943
Mean	612771	310222
S.D	4751.3	1168.4
%RSD	0.8	0.4

Figure .2 Repeatability- precision

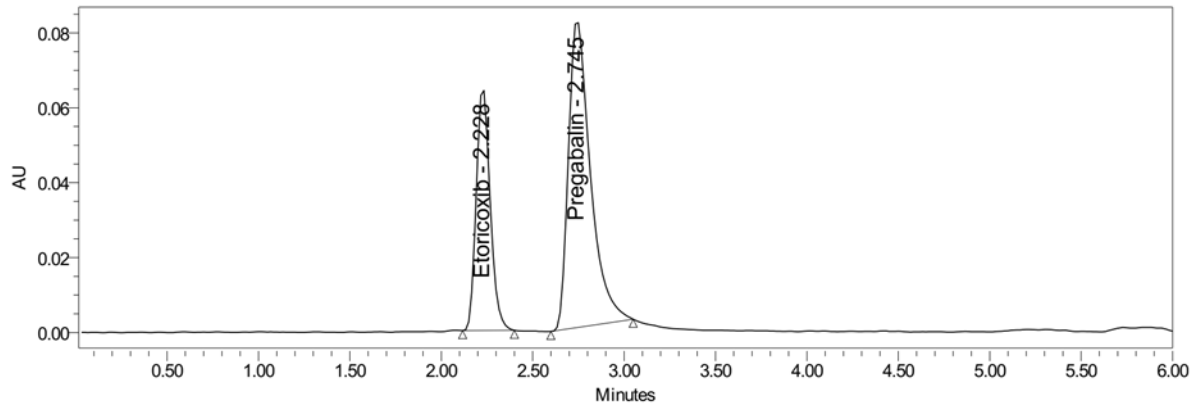

Table 3. Intermediate precision table of Pregabalin and Etoricoxibe

S. No	Area of Pregabalin	Area of Etoricoxibe
1.	606096	313896
2.	608147	308039
3.	601682	308352
4.	606306	307471
5.	601244	312102
6.	609067	309165
Mean	605424	309838
S.D	3267.7	2572.7
%RSD	0.5	0.8

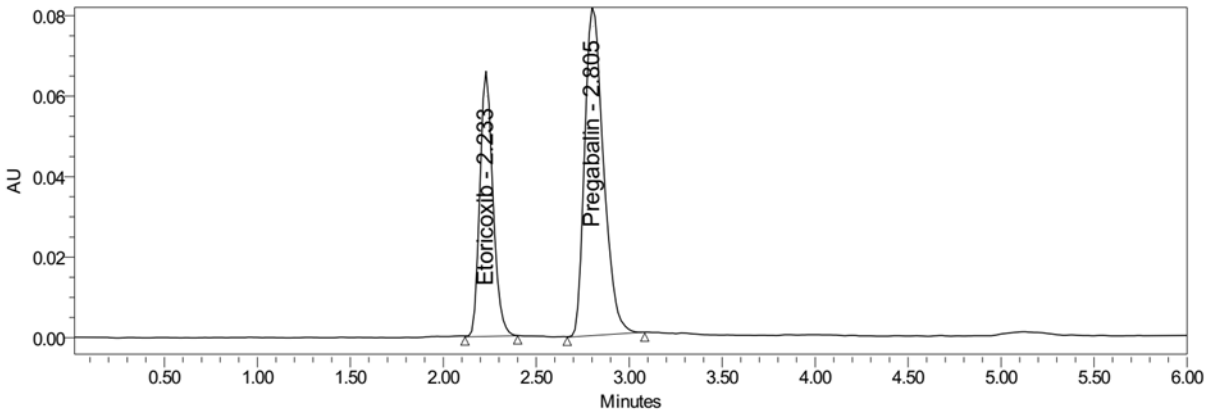

Figure 3. Inter day precision chromatogram

Linearity and Sensitivity

The linearity of the method was established over a concentration range of 18.75–112.5 µg/mL for Pregabalin and 15–90 µg/mL for Etoricoxib, with a correlation coefficient (R^2) of 0.999 for both drugs. The regression equations obtained were y = 8100x + 936.21 for Pregabalin and y = 5195.1x + 967.86 for Etoricoxib, demonstrating excellent linearity.

Table 4. Linearity table for Pregabalin and Etoricoxibe

Pregabalin		**Etoricoxibe**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
18.75	149897	15	78599
37.5	306378	30	154042
56.25	455342	45	238693
75	614644	60	314192
93.75	764958	75	394129
112.5	904699	90	463577

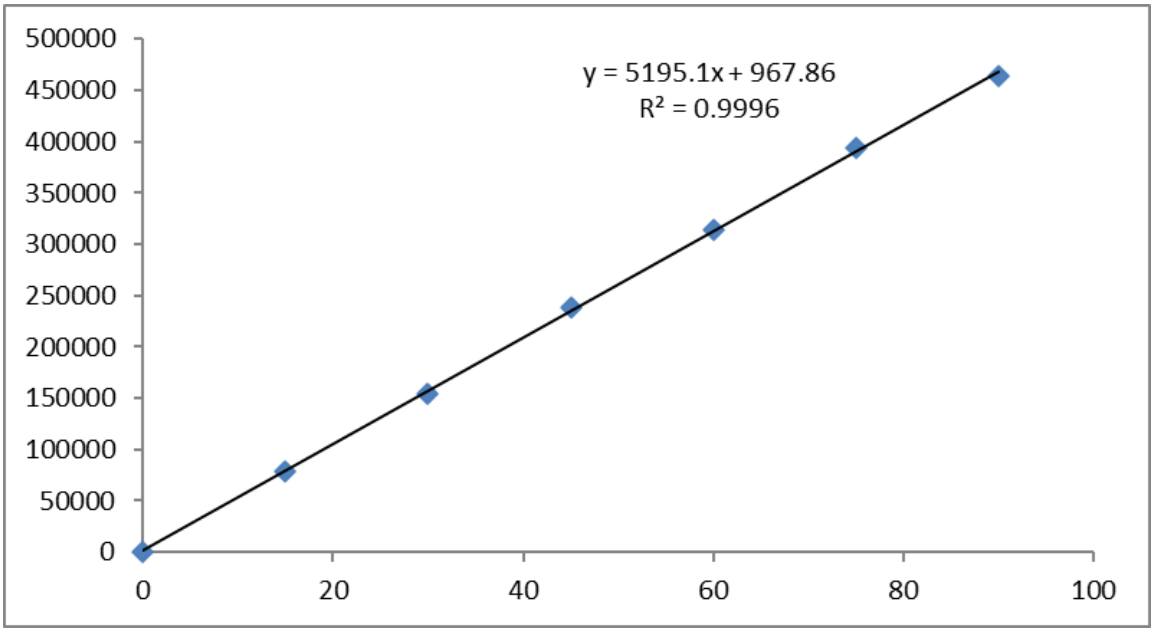

Figure 4. Calibration curve of Pregabalin

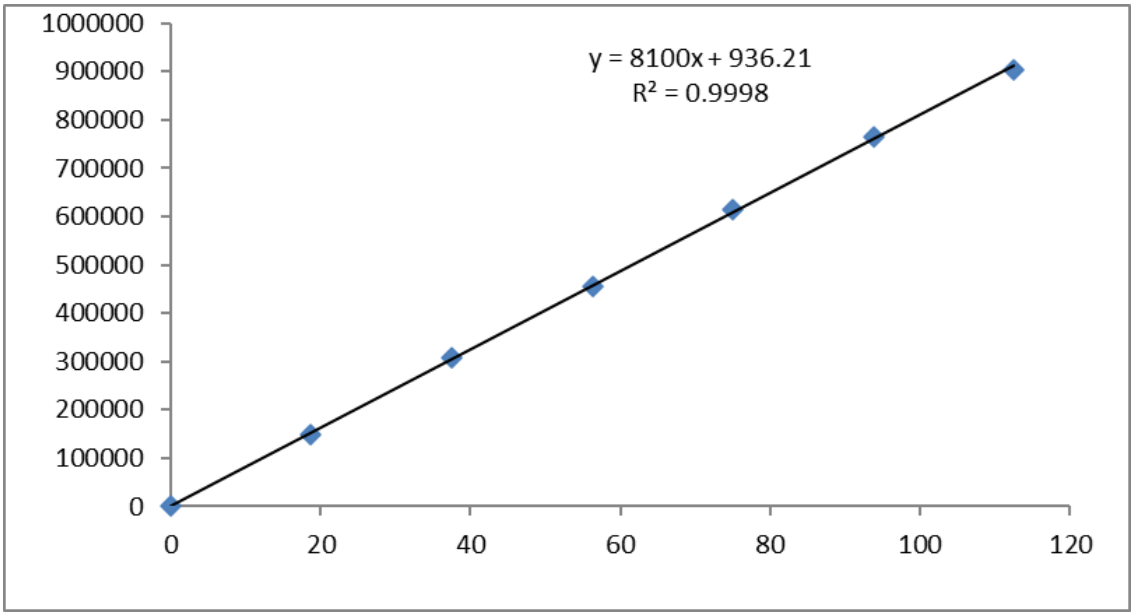

Figure 5. Calibration curve of Etoricoxibe

The sensitivity of the method was evaluated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD values were determined to be 0.09 µg/mL for Pregabalin and 0.04 µg/mL for Etoricoxib, while the LOQ values were 0.27 µg/mL and 0.11 µg/mL, respectively.

Table 5. Sensitivity table of Pregabalin and Etoricoxibe

Molecule	LOD	LOQ
Pregabalin	0.09	0.27
Etoricoxibe	0.04	0.11

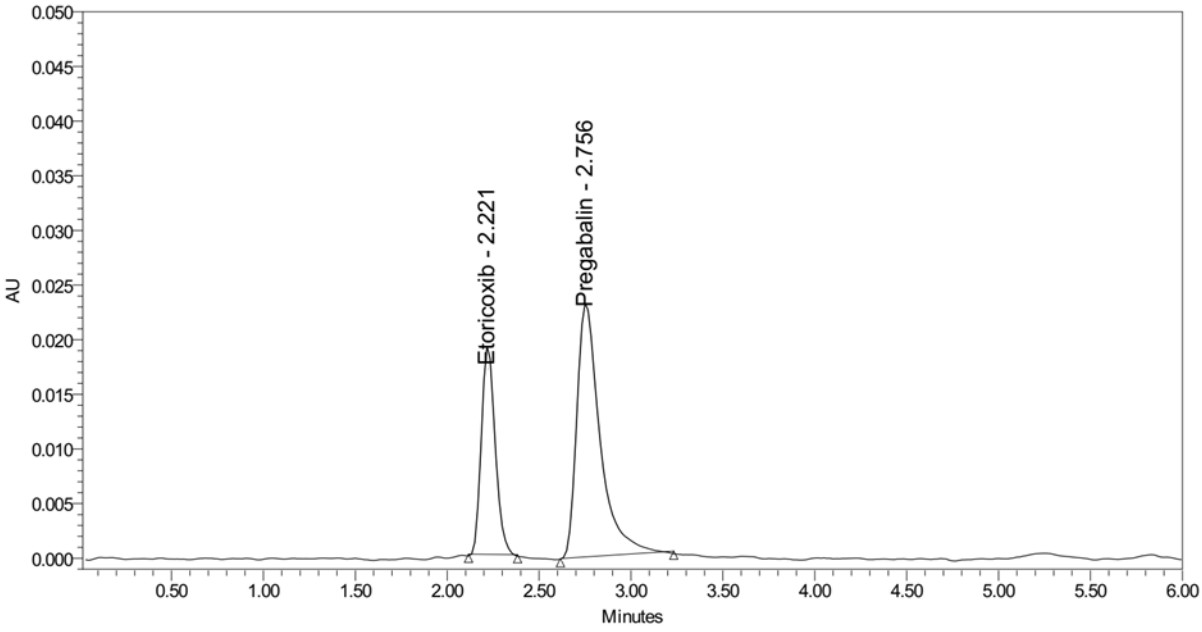

Figure 6. LOQ Chromatogram of Standard

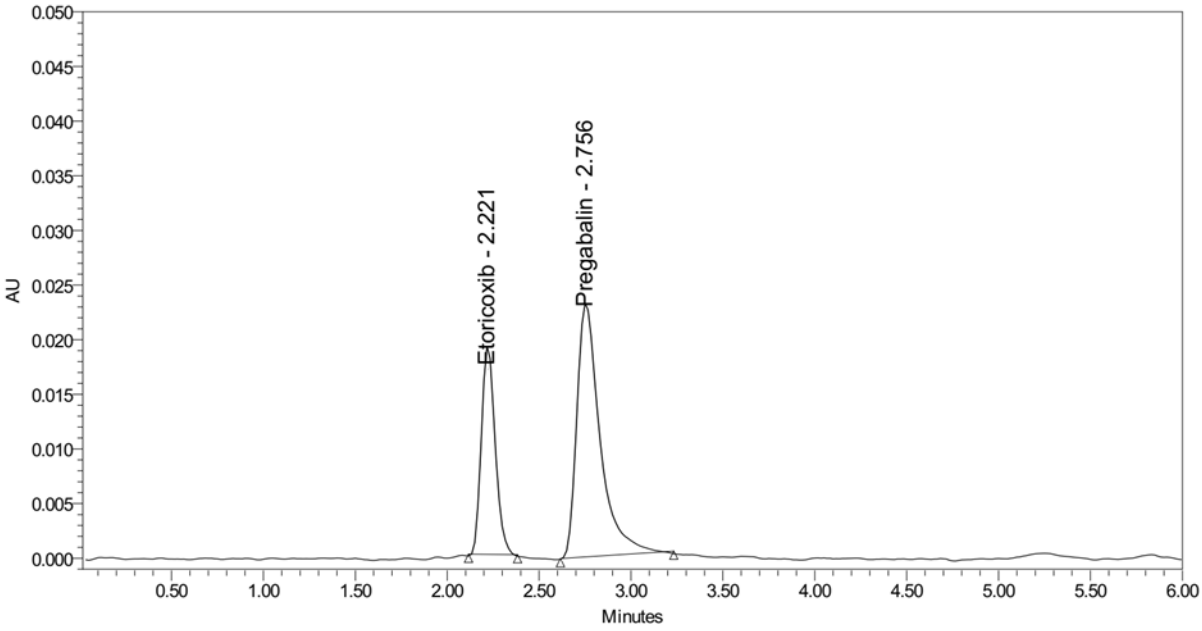

Figure 7. LOQ Chromatogram of Standard

Accuracy and Recovery Studies

Accuracy was assessed through recovery studies at 50%, 100%, and 150% levels, using the standard addition method. The percentage recovery values were found to be 99.71% for Pregabalin and 99.77% for Etoricoxib, which were within the acceptable range of 98–102%, confirming the method's reliability in estimating drug content without interference.

Table 6. Accuracy table of Pregabalin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	37.5	37.234	99.29	99.71%
	37.5	37.669	100.45	
	37.5	37.436	99.83	
100%	75	74.719	99.62	
	75	74.382	99.18	
	75	75.363	100.48	
150%	112.5	112.205	99.74	
	112.5	111.716	99.30	
	112.5	111.909	99.48	

Table 7. Accuracy table of Etoricoxibe

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	30	29.812	99.37	99.77%
	30	29.736	99.12	
	30	30.049	100.16	
100%	60	59.850	99.75	
	60	59.940	99.90	
	60	59.952	99.92	
150%	90	90.242	100.27	
	90	89.638	99.60	
	90	89.834	99.82	

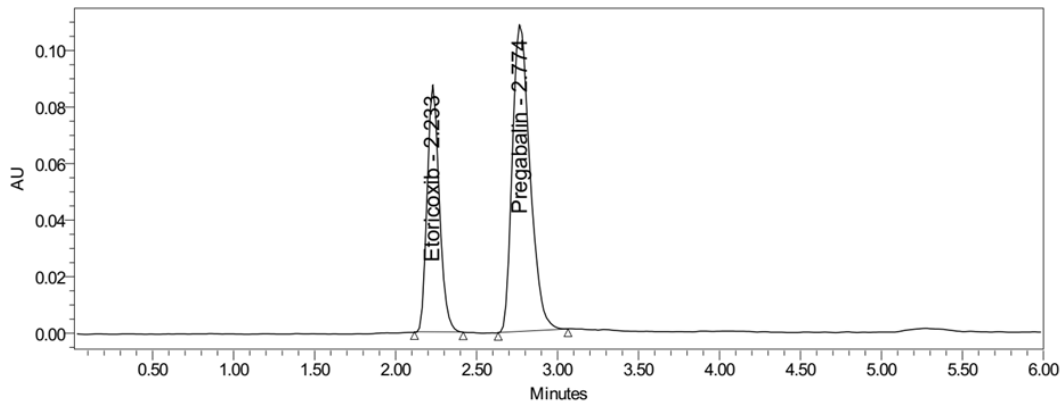

Figure 8 . Accuracy 50% Chromatogram of Pregabalin and Etoricoxibe

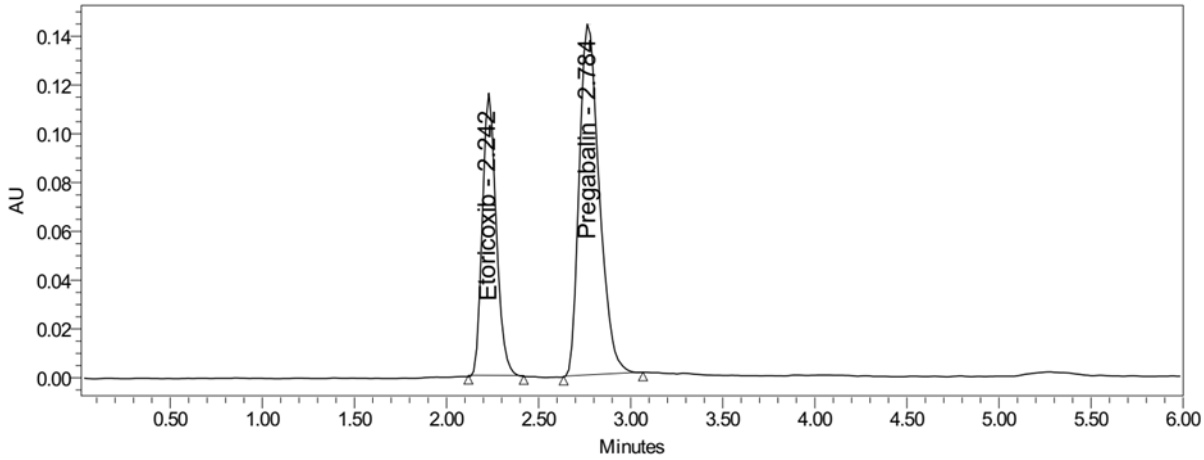

Figure 9 . Accuracy 100% Chromatogram of Pregabalin and Etoricoxibe

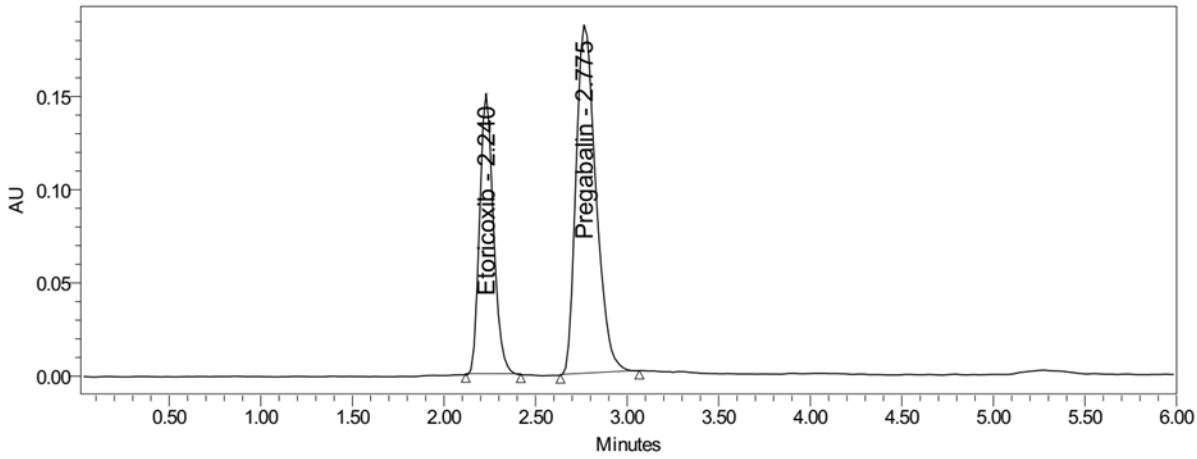

Figure 10 . Accuracy 150% Chromatogram of Pregabalin and Etoricoxibe

Robustness and Forced Degradation Studies

To determine the robustness of the method, small deliberate changes were made to the flow rate (±0.1 mL/min), column temperature (±5°C), and mobile phase composition. : Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), 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. The results showed minimal variation in system suitability parameters, with %RSD values remaining below 2%, indicating that the method is robust and can withstand minor modifications without affecting accuracy.

Table 8. Robustness data for Pregabalin and Etoricoxibe.

S.No	Condition	%RSD of Pregabalin	%RSD of Etoricoxibe
1	Flow rate (-) 0.9ml/min	0.5	1.3
2	Flow rate (+) 1.1ml/min	0.2	0.6
3	Mobile phase (-) 65B:35A	1.0	0.8
4	Mobile phase (+) 55B:45A	0.5	0.9
5	Temperature (-) 25°C	0.4	0.1
6	Temperature (+) 35°C	0.7	0.9

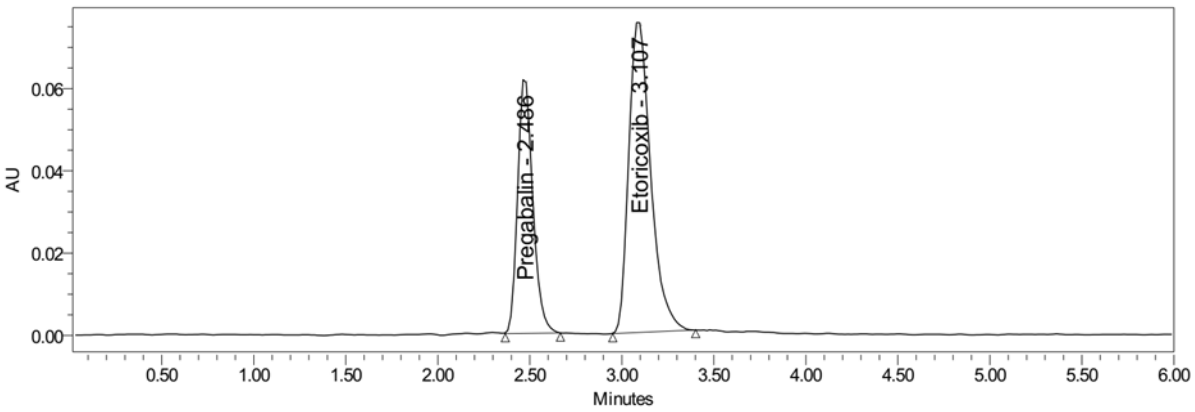

Figure 11. Robustness Chromatogram of Pregabalin and Etoricoxibe.

Forced degradation studies were conducted under acidic, basic, oxidative, thermal, photolytic, and neutral conditions to assess the stability-indicating nature of the method. The degradation percentages varied depending on the stress conditions, with the highest degradation observed in oxidative (6.00%) and acidic (5.52%) conditions. The method successfully separated the degradation products from the main analytes, confirming its stability-indicating capability.

Table 9. Degradation Data of Pregabalin and Etoricoxibe

Type of degradation	Pregabalin			Etoricoxib		
	Area	%recovered	% Degraded	Area	%Recovered	% degraded
Acid	583136	94.48	5.52	295471	94.48	5.52

Base	584353	94.68	5.32	296384	94.68	5.32
Peroxide	580177	94.00	6.00	293765	94.00	6.00
Thermal	595049	96.41	3.59	306510	96.41	3.59
Uv	603764	97.82	2.18	308980	97.82	2.18
Water	611714	99.11	0.89	311254	99.11	0.89

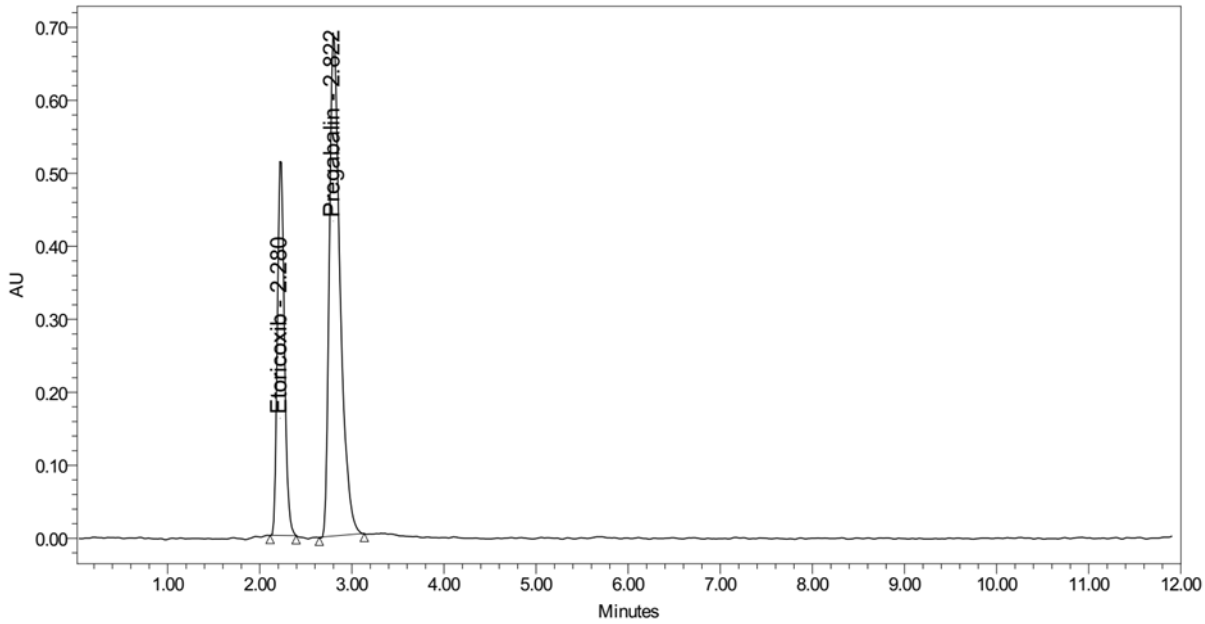

Figure 12. Acid chromatogram of Pregabalin and Etoricoxibe

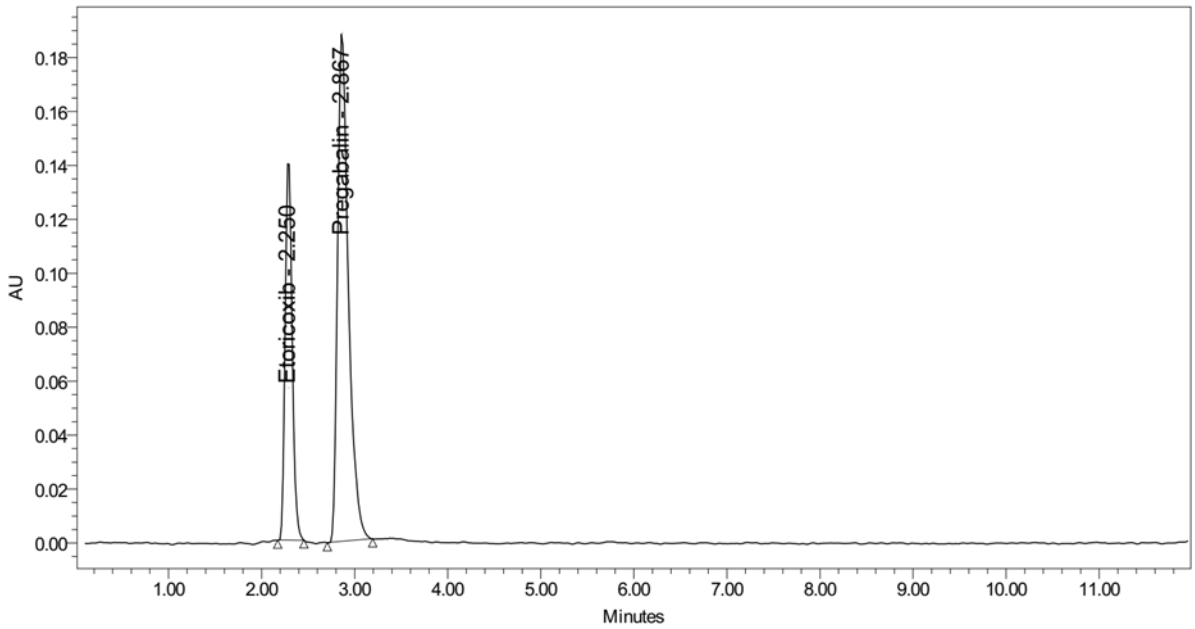

Figure 13. Base chromatogram of Pregabalin and Etoricoxibe

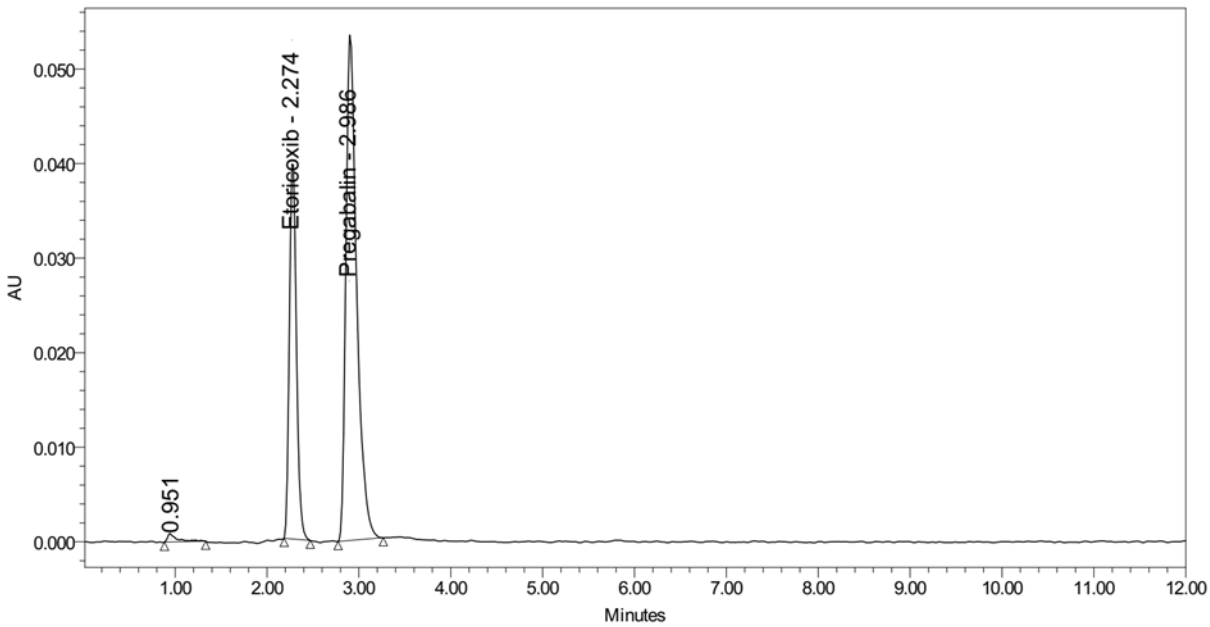

Figure 14. Peroxide chromatogram of Pregabalin and Etoricoxibe

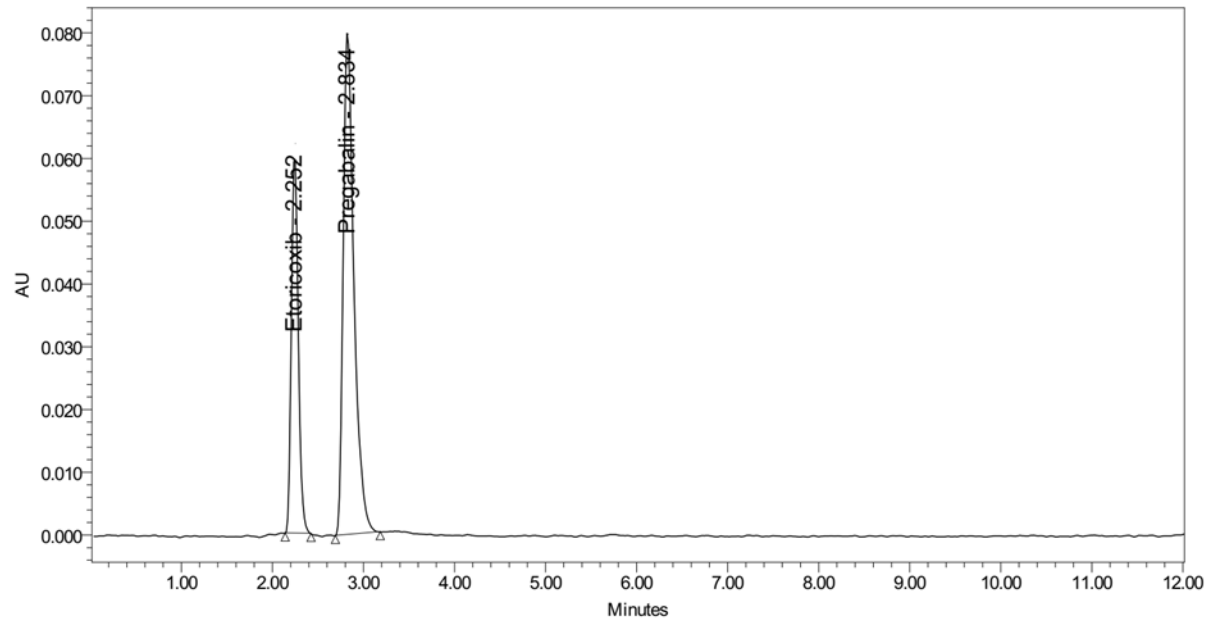

Figure 15. Thermal chromatogram of Pregabalin and Etoricoxibe

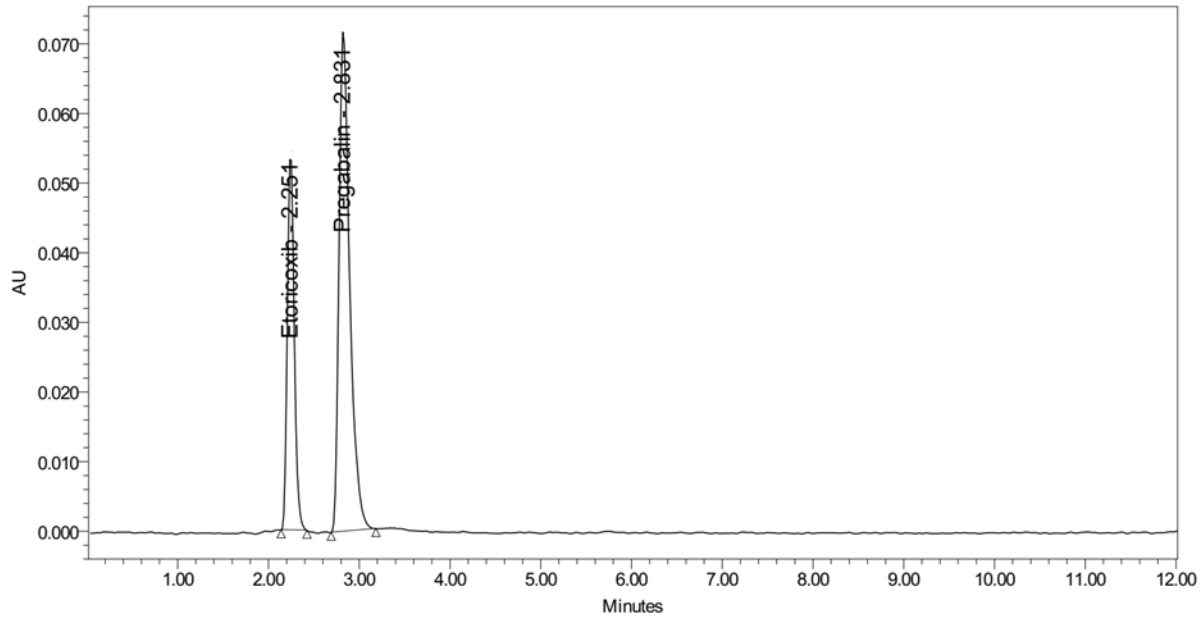

Figure 16. UV chromatogram of Pregabalin and Etoricoxibe

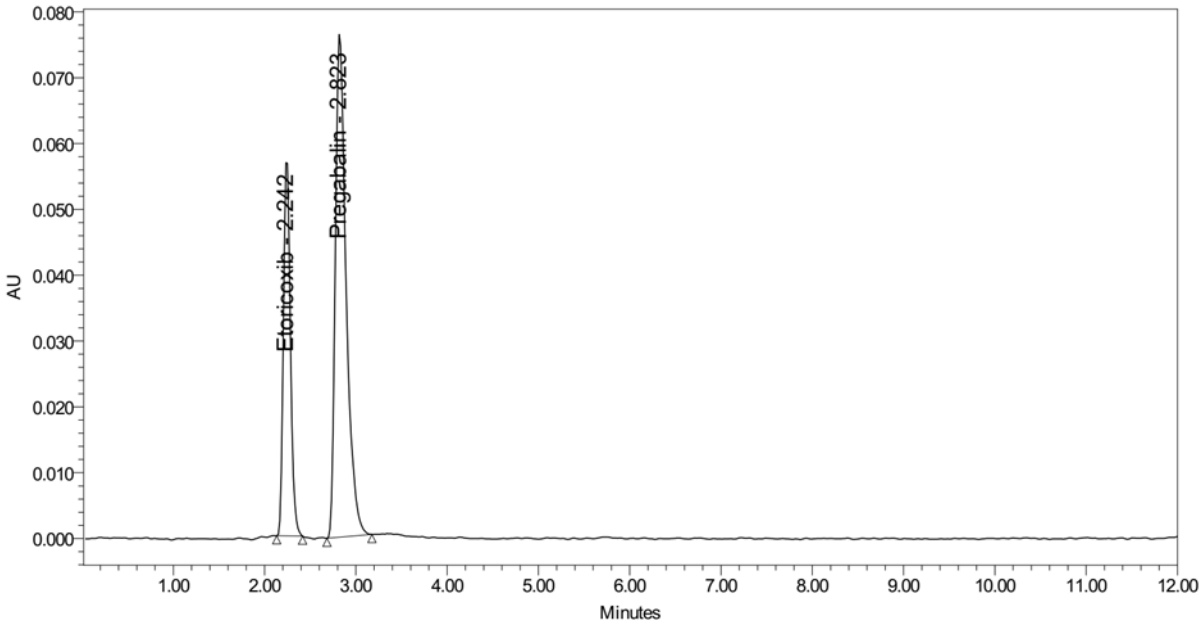

Figure 17. Water chromatogram of Pregabalin and Etoricoxibe

Assay of Marketed Formulation

The validated RP-HPLC method was applied to the estimation of Pregabalin and Etoricoxib in a commercial tablet formulation (PBREN-ET, La Renon Healthcare Pvt Ltd). The percentage assay values were found to be 99.55% for Pregabalin and 99.19% for Etoricoxib, confirming the method's suitability for routine quality control analysis in pharmaceutical industries.

Table 10. Assay Data of Pregabalin

S.no	Standard Area	Sample area	% Assay
1	617532	605910	98.43
2	606557	615675	100.02
3	617946	608453	98.84
4	606994	616863	100.21
5	614874	612289	99.47
6	614742	617434	100.30
Avg	613108	612771	99.55
Stdev	5080.6	4751.3	0.77
%RSD	0.8	0.8	0.78

Table 11. Assay Data of Etoricoxibe

S. no	Standard Area	Sample area	% Assay
1	317438	309936	99.10
2	312782	309644	99.00
3	313862	312096	99.79
4	307552	308693	98.70
5	314109	310021	99.13
6	308923	310943	99.42
Avg	312444	310222	99.19
Stdev	3637.8	1168.4	0.37
%RSD	1.2	0.4	0.38

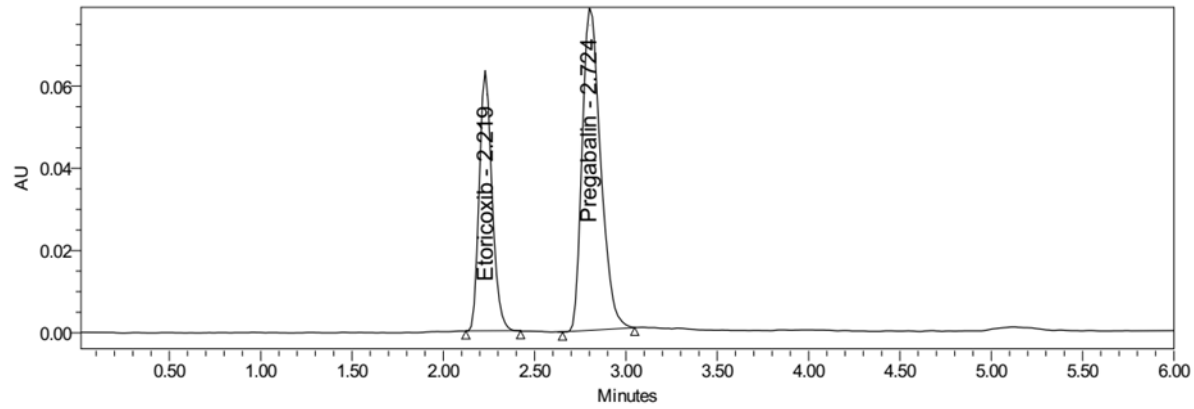

Figure 18. Chromatogram of working standard solution

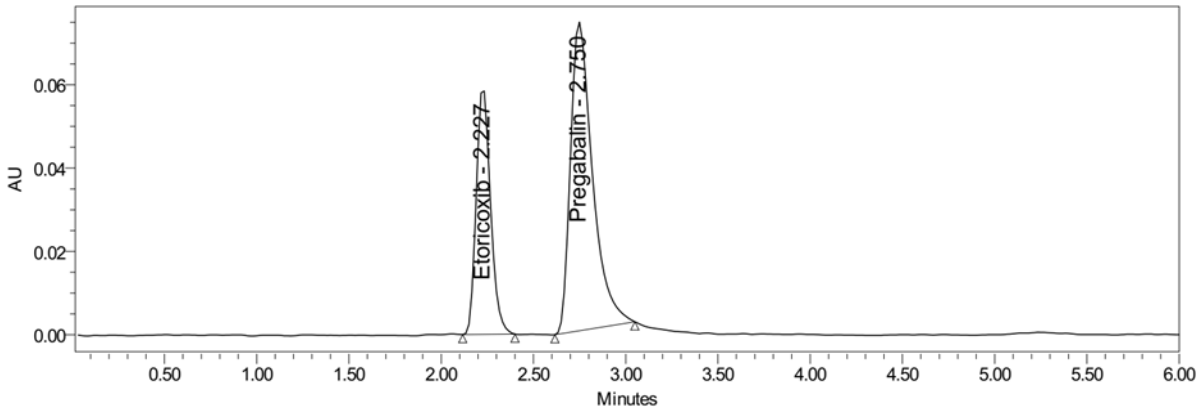

Figure 19. Chromatogram of working sample solution

DISCUSSION

The developed RP-HPLC method for the simultaneous estimation of Pregabalin and Etoricoxib demonstrated high efficiency, precision, and robustness, making it suitable for routine pharmaceutical quality control. The optimized chromatographic conditions, including the Kromasil C8 column, phosphate buffer-acetonitrile mobile phase (60:40), and a flow rate of 1 mL/min, resulted in well-resolved peaks with retention times of 2.702 minutes for Pregabalin and 2.213 minutes for Etoricoxib.

The method's precision was confirmed through repeatability and intermediate precision studies, with %RSD values of 0.8% for Pregabalin and 0.4% for Etoricoxib, well within acceptable limits. The calibration curves displayed excellent linearity across the tested concentration ranges, with correlation coefficients (R^2) above 0.999, confirming the method's reliability. Sensitivity assessments indicated LOD values of 0.09 µg/mL for Pregabalin and 0.04 µg/mL for Etoricoxib, while LOQ values were 0.27 µg/mL and 0.11 µg/mL, respectively.

Accuracy, evaluated through recovery studies at 50%, 100%, and 150% spiking levels, showed mean recovery rates of 99.71% for Pregabalin and 99.77% for Etoricoxib, ensuring the method's ability to quantify the drugs without interference. Robustness testing under

varied conditions, including changes in flow rate, temperature, and mobile phase composition, demonstrated minimal variation, with %RSD values remaining below 2%, affirming the method's stability.

Forced degradation studies under acidic, basic, oxidative, thermal, photolytic, and neutral conditions confirmed the stability-indicating capability of the method. The highest degradation was observed under oxidative (6.00%) and acidic (5.52%) conditions, yet the method successfully separated degradation products from the main analytes.

When applied to a marketed formulation (PBREN-ET), the validated method yielded assay results of 99.55% for Pregabalin and 99.19% for Etoricoxib, demonstrating its practical applicability in pharmaceutical analysis. The study concludes that the developed RP-HPLC method is a simple, accurate, precise, and cost-effective approach for simultaneous drug estimation, making it ideal for routine quality control in the pharmaceutical industry.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Pregabalin and Etoricoxibe in Tablet dosage form. Retention time of Etoricoxibe and Pregabalin were 2.213 min and 2.702 min. %RSD of the Pregabalin and Etoricoxibe were and found to be 0.8% and 0.4% respectively. %Recovery was obtained as 99.71% and 99.77% for Pregabalin and Etoricoxibe respectively. LOD, LOQ values obtained from regression equations of Pregabalin and Etoricoxibe were 0.09, 0.27 and 0.04, 011 respectively. Regression equation of Pregabalin is y = 8100x + 936.21, and y = 5195.1x + 967.86 of Etoricoxibe. 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.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest for this work.

ACKNOWLEDGEMENTS

The authors thank JKKN College of Pharmacy for their support.

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