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

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ABSTRACT

An HPLC method was developed to simultaneously quantify Pregabalin and Etoricoxib in tablet dosage forms. A Kromasil column was used, with a mobile phase of 0.1% Perchloric Acid and Acetonitrile in a 40:60 ratio. The flow rate and temperature were set at 1 mL/min and 30°C, respectively, with detection at 230 nm. The retention times for Pregabalin and Etoricoxib were 2.463 and 2.815 minutes. Regression equations showed high linearity for both drugs (Etoricoxib: y = 19095x + 21003; Pregabalin: y = 19173x + 28445). %RSD values were 0.3% for Pregabalin and 0.4% for Etoricoxib, indicating good accuracy. Recoveries confirmed the method's accuracy at 99.14% for Pregabalin and 99.95% for Etoricoxib. The limits of detection (LOD) for Pregabalin were 0.32, and the quantification limit (LOQ) was 0.97; for Etoricoxib, the LOD was 0.30, and the LOQ was 0.91. This method is effective for routine quality control analysis of both drugs in the pharmaceutical industry, offering shorter retention and run times.

Keywords: Etoricoxib (ETIB), Pregabalin (PREN), RP-HPLC.

I. INTRODUCTION

Pregabalin and Etoricoxib are often used in combination for their complementary effects in managing pain. Pregabalin is effective for neuropathic pain, while Etoricoxib is a non-steroidal anti-inflammatory drug (NSAID) that helps alleviate inflammatory pain. Together, they can provide broader pain relief. Using both medications may allow for lower doses of each, potentially minimizing side effects associated with higher doses of either drug. The combination may improve overall pain control in conditions where both neuropathic and inflammatory components are present, such as in certain types of arthritis or after surgery. Pregabalin is a medication that belongs to the class of anticonvulsants or antiepileptics, which is primarily used to manage certain types of nerve pain, epilepsy, and anxiety disorders. It is chemically similar to the neurotransmitter (GABA), although it does not act directly on GABA receptors. Pregabalin binds to the alpha-2-delta subunit of voltage-gated calcium channels in the central nervous system, which reduces the release of excitatory neurotransmitters such as glutamate, norepinephrine, and substance P. This modulation helps lower nerve cell hyperexcitability, reducing symptoms of neuropathic pain, seizures, and anxiety. The chemical name of pregabalin is **(S)-3-(aminomethyl)-5-methylhexanoic acid**.

Etoricoxib is a selective cyclooxygenase-2 (COX-2) inhibitor used primarily for its anti-inflammatory and analgesic properties. COX-2, an enzyme responsible for the synthesis of prostaglandins that cause pain and inflammation. Nonsteroidal anti-inflammatory drug (NSAID) that selectively inhibits the enzyme COX-2 (cyclooxygenase-2). Which are designed to reduce pain and inflammation with less gastrointestinal risk than traditional NSAIDs.The chemical name of etoricoxib **: 5-chloro-3-(4-methanesulfonylphenyl)-2-(6-methylpyridin-3yl)pyridine.** Using pregabalin and etoricoxib together can be beneficial in managing chronic pain, particularly when pain has both neuropathic and inflammatory components.

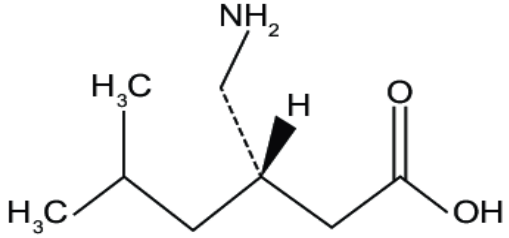

Figure 1: Structure of Pregabalin

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Description:

Molecular Weight: 159.2261

Molecular Formula: $C_8H_{17}NO_2$

IUPAC Name: (3S)-3-(aminomethyl)-5-methylhexanoic acid

Physical State: Solid

Solubility: 11.3 mg/mL

Storage: Store at -20° C

Melting Point: 176 - 178ºC

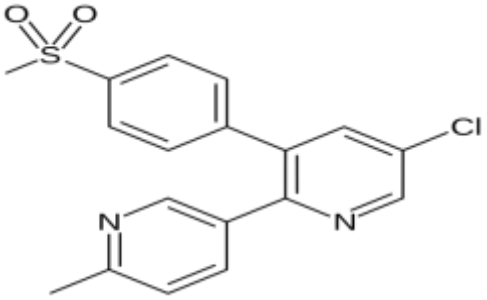

Figure 2: Structure of Etoricoxib

Description:

Molecular Weight: 358.842

Molecular Formula: $C_{18}H_{15}ClN_2O_2S$

IUPAC Name: 5-chloro-3-(4-methanesulfonylphenyl)-2-(6-methylpyridin-3yl)pyridine

Protein binding: 97% binding-albumin

Physical State: Solid

Storage: Store at -20° C

Melting Point: >172° C (dec.)

pKa value: .94

According to the literature survey, various techniques have been developed for the simultaneous estimation of these drugs, whether used alone or in combination with others. Using methods such as UV-Spectrophotometry and RP-HPLC. But this method is particularly effective and suitable for routine quality control analysis in the pharmaceutical industry, providing shorter retention and run times.

II. MATERIALS AND METHOD

Chemicals & Reagents

Pregabalin and etoricoxib ,the respective pure drugs were acuquired from Specturm pharma research solutions. The Pregabalin (75mg), Etoricoxib(60mg), combination tablet (**PBREN - ET**), was purchased from Indian martin hyderabad. The chemical and buffers utilized in this analysis were obtained from Rankem, an indian supplier.

Instrumentation

Electronics Balance-Denver, p^H meter -BVK enterprises, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.,UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Pregabalin and Etoricoxib solutions.

Objective

The main objective of this study is to develop a highly reliable, accurate, sensitive, specific, consistent, and efficient analytical method for the simultaneous quantification of Pregabalin and Etoricoxib in both their pure forms and tablet formulations.

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Chromatographic conditions:

Mobile phase	:	40% Perchloric acid (0.1%) : 60% Acetonitrile
Flow rate	:	1ml/min
Column	:	Kromasil C8 (4.6 x 250mm, 5µm)
Detector wave length	:	230nm
Column temperature	:	30°C
Injection volume	:	10µL
Run time	:	**6** min
Diluent	:	Water and Acetonitrile in the ratio 50:50
Results	:	Pregabalin and Etocoxib had eluted at 2.436 and 2.815 min and it had a good plate count and resolution, tf. So, further validation is continued.

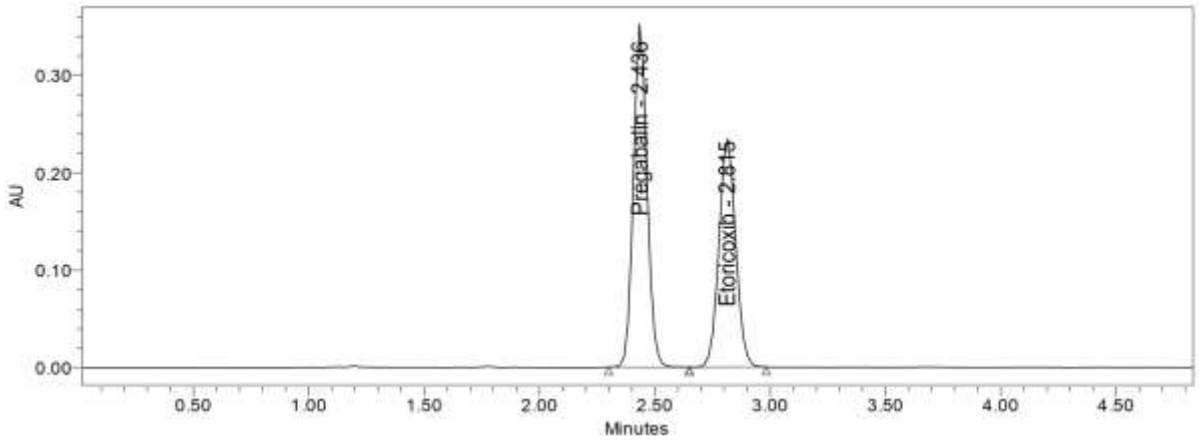

Figure 3: Optimized Chromatogram

Preparation of Buffer:

0.1% Perchloric Acid Buffer:1ml of Conc Perchloric acid was diluted to 1000ml with water.

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

Preparation of Standard solution:

Accurately weighed 37.5 mg of Pregabalin, 30 mg of Etoricoxib and transferred to 50 ml volumetric flasks separately. 3/4 th of diluents was adde and sonicated for 10 minutes. Volume was 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, 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

The HPLC method was validated to simultaneously determine the drug substances Pregabalin and Etoricoxib in accordance with ICH guidelines. This validation demonstrates that the method is suitable for routine analysis.

System suitability:

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,

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resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

System suitability chromatogram was shown in figure 4 and values are mentioned in the table 1

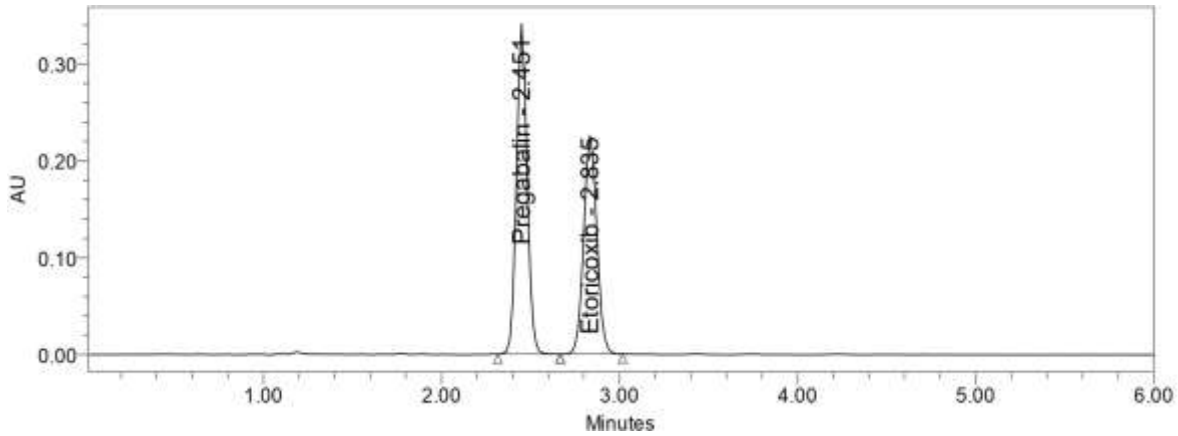


Figure 4: System suitability chromatogram

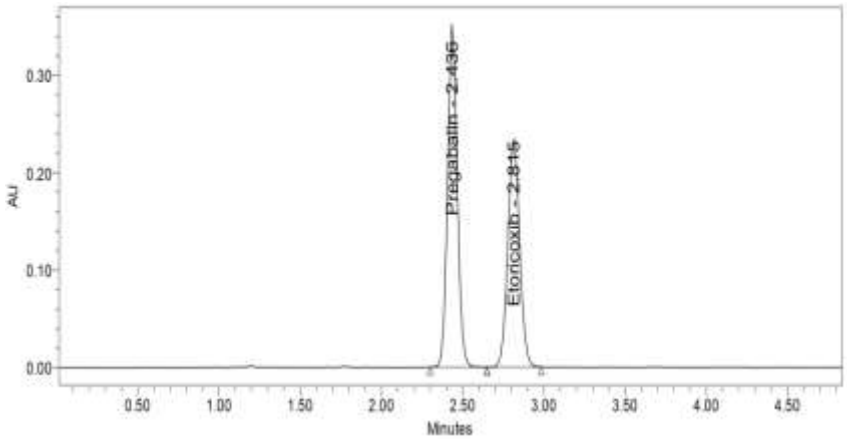
Table 1: System suitability parameters for Pregabalin and Etoricoxib

S no	Pregabalin			Etoricoxib			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.451	7886	1.07	2.835	6736	1.04	3.0
2	2.454	7484	1.06	2.839	7071	1.04	3.0
3	2.462	7390	1.03	2.847	6948	1.03	3.0
4	2.463	7472	1.04	2.848	6933	1.04	3.0
5	2.465	7849	1.05	2.849	6702	1.04	3.0
6	2.467	7834	1.07	2.853	6941	1.05	3.0

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. Figure 5 displays a representative chromatogram, while Table 2 presents the experimental data.

Table 2: Specificity data

Sample name	Retention time(mins)	Area
Pregabalin	1.173	315860
Etoricoxib	0.578	145499



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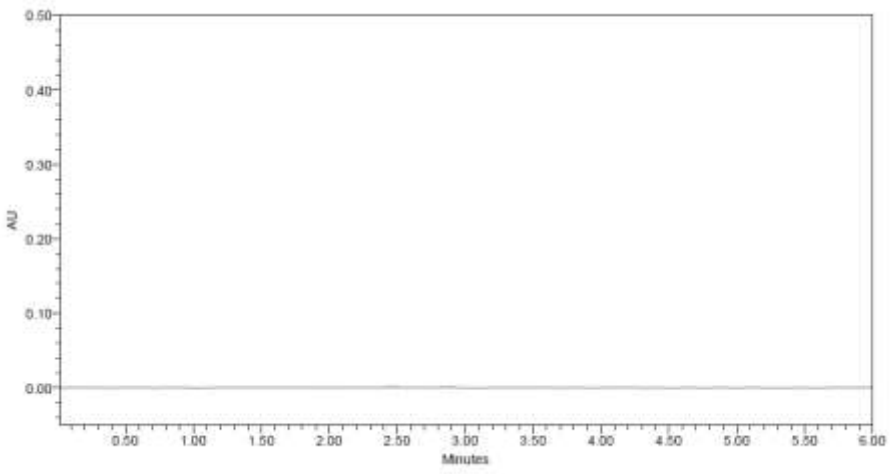

Figure 5: Specificity Chromatogram of pregabalin and Etoricoxib.

Linearity:

Table 3: Of Etoricoxib and Pregabalin linearity

% Level	ETIB		PREN	
0%	Conc	area	Conc	area
25%	15	316652	18.75	412468
50%	30	603270	37.5	754396
75%	45	911156	56.25	1124511
100%	60	1147452	75	1444535
125%	75	1441207	93.75	1836984
150%	90	1742198	112.5	2175518

*Concentration is measured in (µg/mL)

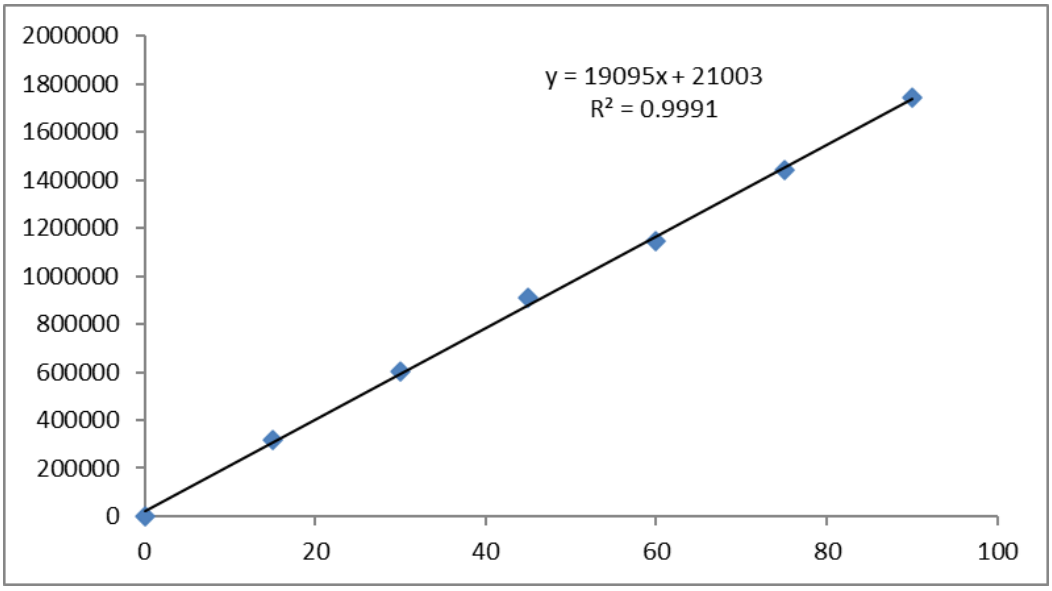

Figure 6: Calibration data of Etooricoxib

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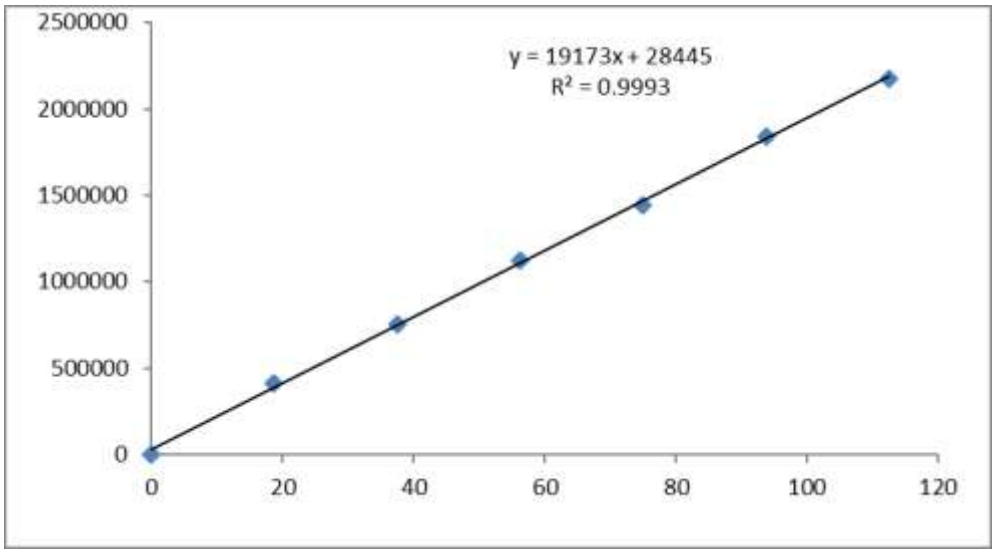

Figure 7: Calibration data of Pregabalin

Table 4: Accuracy (%Recovery data)

	ETIB	PREN
Concentration range (µg/ml)	15-90	18.75-112.5
Regression Equation	y = 19095x + 21003	y = 19173x + 28445
Co-relation	0.999	0.999

Accuracy of ETIB and PREN

Table 4.1:

	PREGABALIN			ETORICOXIB		
%Level	Amount taken	recovered	% of recovery	Amount taken	recovered	% of recovery
50%	37.5	37.71538	100.57	30	29.95653	99.86
	37.5	36.82585	98.20	30	30.1072	100.36
	37.5	37.34815	99.60	30	29.82414	99.41
100%	75	74.48052	99.31	60	59.73595	99.56
	75	73.67559	98.23	60	60.17795	100.30
	75	74.89381	99.86	60	60.3149	100.52
150%	112.5	110.2571	98.01	90	88.83655	98.71
	112.5	111.0417	98.70	90	90.3447	100.38
	112.5	112.2992	99.82	90	90.41864	100.47
Mean % recovery	99.14			99.95		

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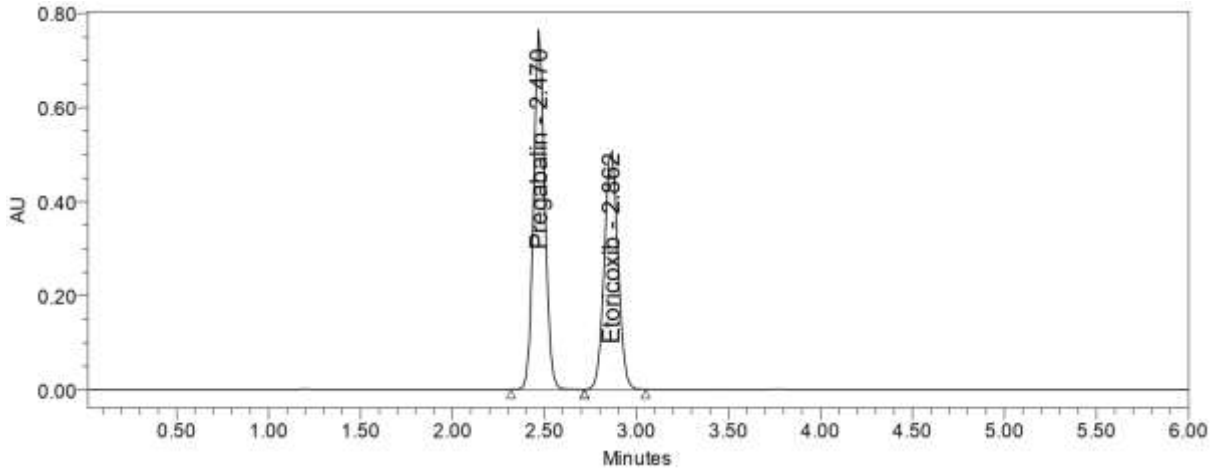

Figure 8: Accuracy-50%

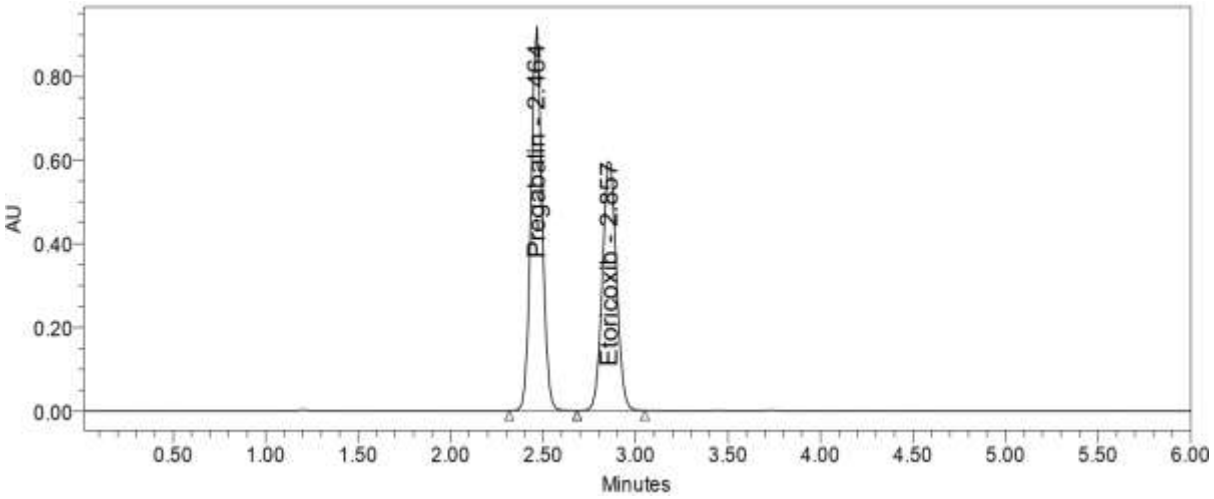

Figure 9: Accuracy-100%

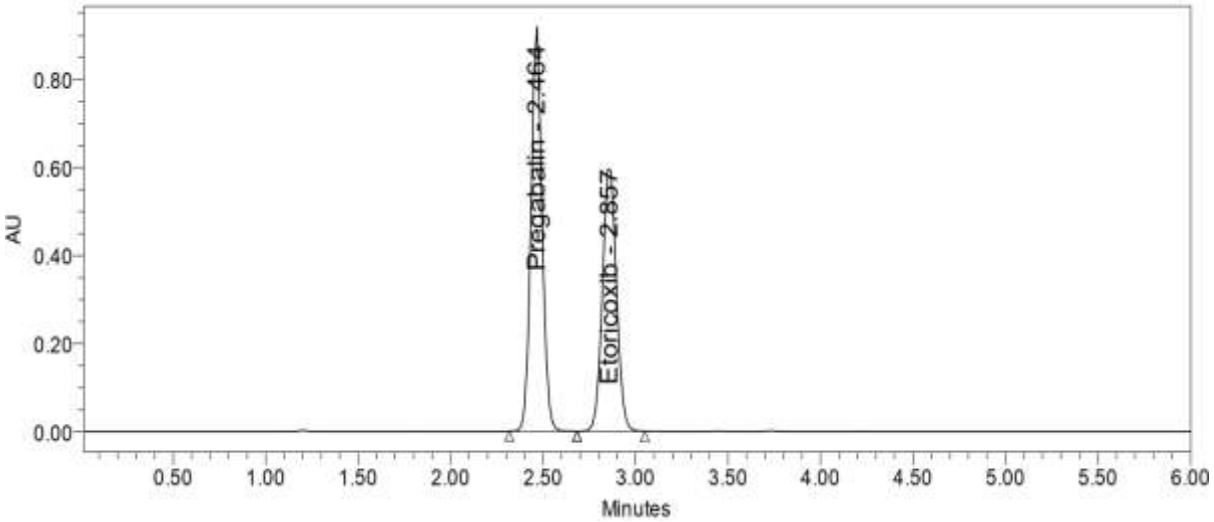

Figure 10: Accuracy-150%

Precision:

System Precision: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned below. Average area, standard deviation and % RSD were calculated for two drugs.% RSD obtained as 0.8%and 0.7% respectively for Pregabalin and Etoricoxibe .As the limit of Precision was less than "2" the system precision was passed in this method.

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Table 5: System precision table of Pregabalin and Etoricoxib

S. No	Area of Pregabalin	Area of Etoricoxib
1.	1413496	1152714
2.	1437153	1169733
3.	1433951	1168798
4.	1438747	1174698
5.	1439434	1167483
6.	1449748	1176809
Mean	1435422	1168373
S.D	11985.8	8475.8
%RSD	0.8	0.7

Repeatability:

Table 5.1: Repeatability table of Pregabalin and Etoricoxib

S. No	Area of Pregabalin	Area of Etoricoxib
1.	1425113	1159607
2.	1421554	1159788
3.	1413374	1164131
4.	1418841	1154310
5.	1422607	1155840
6.	1423966	1166628
Mean	1420909	1160051
S.D	4274.8	4708.7
%RSD	0.3	0.4

The precision of the method was determined by analyzing a sample of Pregabalin and Etoricoxib.. (Six individual sample preparations). Data obtained is summarized in Table 5.1. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.3% and 0.4% respectively for Pregabalin and Etoricoxib. As the limit of Precision was less than "2" the system precision was passed in this method.

Sensitivity:

Limit of Detection and Limit of quantitation were Listed in below tab**le.**

Table 6: Sensitivity table of Pregabalin and Etoricoxib

Molecule	LOD	LOQ
Pregabalin	0.32	0.97
Etoricoxib	0.30	0.91

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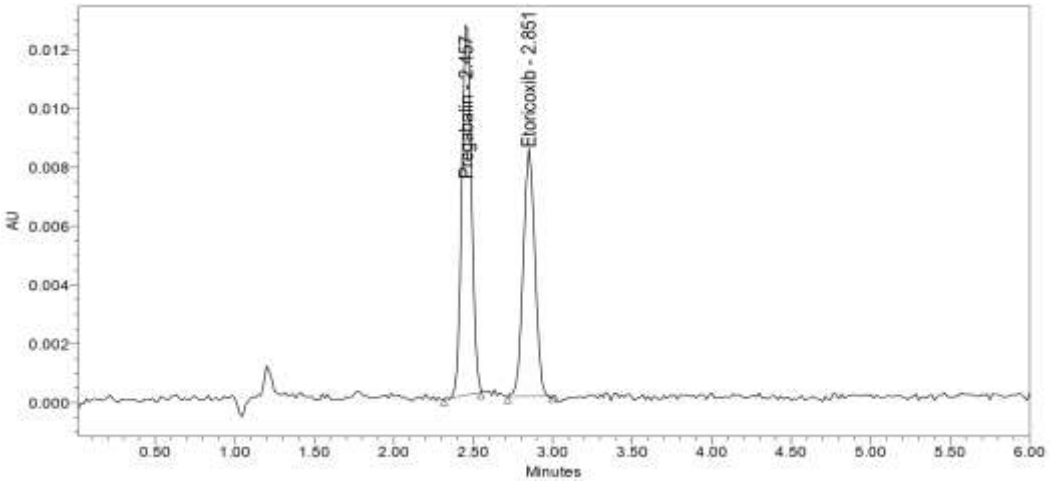

Figure 11: LOD Chromatogram of Standard

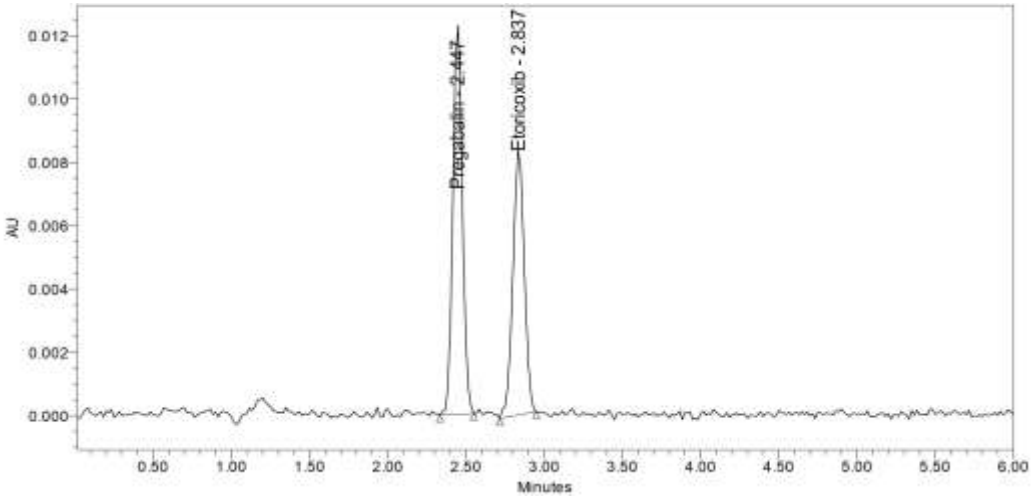

Figure 12: LOQ Chromatogram of Standard

Robustness:

Table 7: Robustness data for Pregabalin and Etoricoxib.

S.no	Condition	%RSD of Pregabalin	%RSD of Etoricoxib
1	Flow rate (-) 0.9ml/min	0.2	0.3
2	Flow rate (+) 1.1ml/min	0.1	0.2
3	Mobile phase (-) 35B:65A	0.2	0.1
4	Mobile phase (+) 45B:55A	0.6	0.3
5	Temperature (-) 25°C	0.4	0.5
6	Temperature (+) 35°C	0.1	0.1

System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Degradation Studies:

Table 8: Forced degradation conditions for Pregabalin and Etoricoxib

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins

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Base	2N NAOH	60^{0}c	30 mins
peroxide	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

Table 9: Degradation Data of Pregabalin and Etoricoxibe

Types of Degradation	**Pregabalin**			**Etoricoxib**		
	Area	%Recovered	%Degraded	Area	%Recovered	%Degraded
Acid	1383708	95.94	4.06	1112522	95.12	4.88
Base	1386850	96.16	3.84	1131824	96.77	3.23
Peroxide	1412559	97.95	2.05	1147842	98.14	1.86
Thermal	1428018	99.02	0.98	1164428	99.57	0.43
Uv	1433743	99.41	0.59	1167910	99.86	0.14
Water	1435080	99.51	0.49	1169302	99.98	0.02

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.

Assay of Tablets:

The labelled EBOV- PG Tablet states the contents of Pregabalin 75mg and Etoricoxib 60mg. assay of Pregabalin and Etoricoxibe were 99.06% and 99.19% respectively.

Assay of Etoricoxi band Pregabalin.

Table 10: Assay Data of Pregabalin and Etoricoxib

Name of the Drug	**Label claim dose**	**%Assay**
Pregabalin	75mg	99.06
Etoricoxib	60mg	99.19

Table 11:

	PREN			**ETIB**		
S.no	Std	Sample	% of Assay	Std	Sample	% of Assay
1	1413496	1425113	**98.88**	1152714	1159607	**99.15**
2	1437153	1431554	**99.33**	1169733	1159788	**99.17**
3	1433951	1433374	**99.46**	1168798	1164131	**99.54**
4	1438747	1428841	**99.14**	1174698	1154310	**98.70**
5	1439434	1422607	**98.71**	1167483	1155840	**98.83**
6	1449748	1423966	**98.81**	1176809	1166628	**99.75**
Avg	1435422	1427576	**99.06**	1168373	1160051	**99.19**
Stdev	11985.8	4355.0	**0.30**	8475.8	4708.7	**0.40**
%RSD	0.8	0.3	**0.31**	0.7	0.4	**0.41**

Procedure for Assay:

5 tablets were weighed, tablet which weight is equivalent to lable claim was taken (PRE-75mg, ETR-60mg) and transferred into 100ml VF, 50 ml diluent was added and sonicated for 25 min further volume was made up with

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diluent and filterd using 0.45µm nylon. 1 ml of the above solution was transferred to 10ml volumetric flask and made up with diluents to obtain the concentration of (75µg/ml of Pregabalin and 60µg/ml of Etoricoxib).

The relative standard deviation (RSD) for the area of the six standard injections should not exceed 2%.

Assay was calculated by:

Table 12:

% Assay =	**AT** / **AS**	**x**	**WS** / **DS**	**x**	**DT** / **WT**	**x**	**P** / **100**	**x**	**Av wt** / **LC**	**x**	**100**

Where,

- **AS:** Average peak area due to standard preparation
- **AT:** Peak area due to assay preparation
- **WS:** Standard Weight of PREGABALIN / ETORICOXIB in mg
- **WT:** Weight of sample in assay preparation
- **DT:** Dilution of assay preparation
- **DS:** Dilution of standard preparation
- **P:** Purity of PREGABALIN / ETORICOXIB
- **AV:** Average weight of tablets in mg
- **LC:** Labelled claim of PREGABALIN / ETORICOXIB

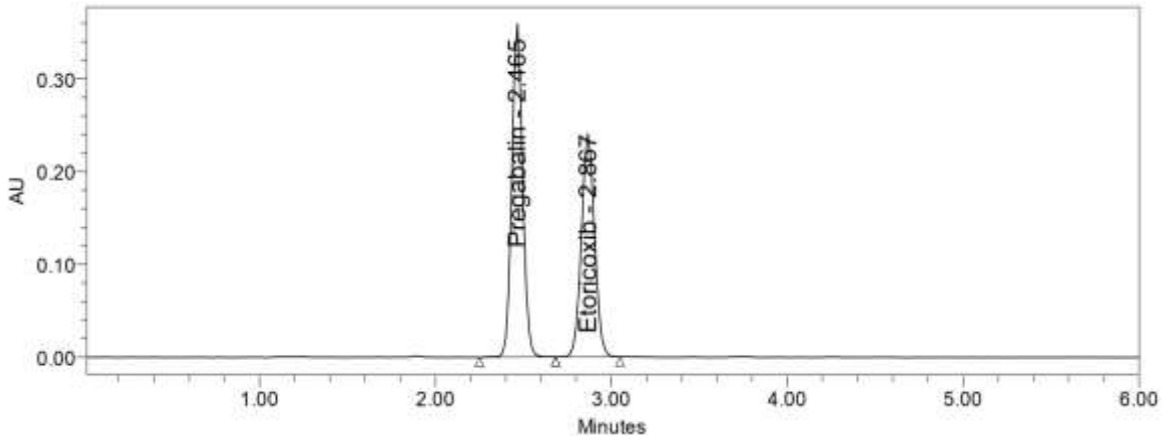

Figure 13: Assay Chromatogram

III. CONCLUSION

The method's remarkable precision, accuracy, linearity, and specificity provide reliable and precise measurement of medications in both bulk and dosage forms. The two compounds may be successfully separated and resolved using the optimal chromatographic conditions, which include the choice of the mobile phase and detection wavelength, without interference from excipients or degradation products. This method may be used in quality control labs to analyze pregabalin and etoricoxib on a regular basis, ensuring product quality, stability, and compliance with regulations. Furthermore, the method meets ICH standards, ensuring that it is suitable for routine analysis.

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