A NEW VALIDATED METHOD FOR THE ESTIMATION OF PREGABALIN AND ETORICOXIB AN USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND OF ITS DEGRADATION

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ABSTRACT:

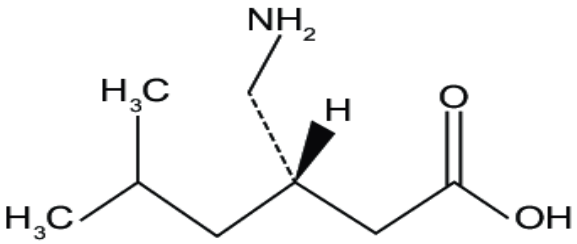
A simple, Accurate, precise method was developed for the simultaneous estimation of the Etoricoxib and Pregabalin in Tablet dosage form. Chromatogram was run through BDS C18 150 x 4.6 mm, 5µm. Mobile phase containing 0.1%Orthophosphoric acid: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 240 nm. Retention time of Etoricoxib and Pregabalin were found to be 2.219min and 2.783.%RSD of the Etoricoxib and Pregabalin were and found to be 0.3 and 0.4 respectively. %Recovery was obtained as 99.80% and 99.78% for Etoricoxib and Pregabalin respectively. LOD, LOQ values obtained from regression equations of Etoricoxib and Pregabalin were 0.07, 0.09, and 0.21, 0.28 respectively. Regression equation of Etoricoxib is y = 26616x + 4951.1, and y = 37650x + 4959.1 of Pregabalin. 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: Method development, Pregabalin. Etoricoxib, Method Validation

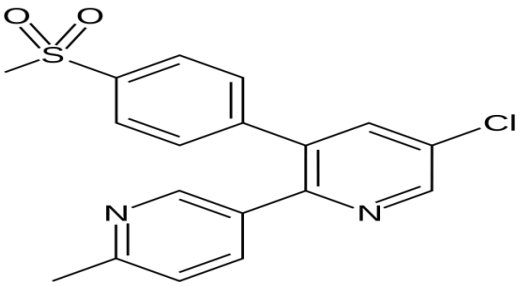
INTRODUCTION:

Pregabalin belongs to an anticonvulsant class,it helps to treat neuropathic pain and fibromyalgia, and used in partial onset seizures in combination with other anticonvulsants.Pregabalin is structurally similar to gamma-aminobutyric acid (GABA) - an inhibitory neurotransmitter[1,2].Pregabalin is a voltage-damaged Ca2+canal antagonist that interact with alpha-II-delta subunit to serve as both an antiepileptic as well as analgesic agent [4,5,6].Studies with structurally comparable medicines show that pregabalin's presynaptic binding to voltage-gated calcium channels is essential for the antiseizure and antinociceptive effects seen in animal models7, even if the mechanism of action has not yet been fully explained. Pregabalin is recognised structurally as (3S) 5-methyl-3-(aminomethyl)hexanoic acid[7]. Structurally Pregabalin is known as (3S)-3-(aminomethyl)-5-methylhexanoic acid[7]. It is sold under the brand name of Lyrica.

Etoricoxib, a COX-2 inhibitor, is used as a short-term therapy for mild post-surgical dental pain as well as the painful and inflammatory symptoms of different types of arthritis. It can be used to treat rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis-related joint and muscle pain and inflammation in individuals aged 16 and older. [8,9].The cyclo-oxigenase enzyme (COX-2) isoform 2 is specifically inhibited by etoricoxib, inhibiting the formation of prostaglandins (PGs) from arachidonic acid10. Etoricoxib's chemical makeup includes 5-chloro-3- (4-methanesulfonylphenyl) -6'-methyl-2,3'-bipyridine[10]. The term "fixed-dose drug " mention to goods that have two or more drug medications together in a single dose formulation[11]. In combination pregabalin (75mg) and Etoricoxib (60mg) both are used for the treatment of neuropathic persistent pain.



Structure of Pregabalin



Structure of Etoricoxib

Figure-1: Structures of Etoricoxib and Pregabalin.

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 [12-16] RP-HPLC [17-22]. 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 pregabalin and etoricoxib in medicinal dose and bulk form. According to ICH recommendations, a proven approach was also used to estimate the amounts of pregabalin and etoricoxib.

MATERIALS AND REAGENTS

Pregabalin and Etoricoxib pure drugs purchased from BMR chemicals, Hyderabad. The combination tablet Pregabalin and Etoricoxib (**Ebov PG**) was purchased from a local pharmacy store. Merch in India provided all of the chemicals and buffers utilized in this Method.

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 BDS C18 150 x 4.6 mm, 5µm. column was employed. Acetonitrile is employed as mobile phase B, while 0.1%Orthophosphoric acid is used as mobile phase A. (60:40 Ratios). The analysis was done in isocratic mode with an injection volume of 10 µL and a flow rate of 1 mL/min. The duration was five minutes. The measurements were made at 240 nm.

PREPARATION OF SOLUTIONS

Preparation of 0.01N potassiumdihydrogen phosphate Buffer: In a 1000 ml volumetric flask, accurately weigh 1.36 g of potassium di hydrogen phosphate. Add 900 ml of milli-Q water, degas, sonicate, and fill the remaining volume with water.

Preparation of Standard solution: Accurately weighed 15 mg of Pregabalin, 12 mg of Etoricoxib and transferred to individual 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. (300µg/ml of Pregabalin and 240µg/ml of Etoricoxib), 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.

Preparation of Sample solution: weigh 720.0mg of tablet powder (Equivalent to 75 mg of Pregabalin and 60mg of Etoricoxib) into a 100 ml volumetric flask. Add about 10 ml of diluent and shake for 20 minutes by mechanical means or manually and further sonicate for 30 minutes. Dilute up to mark with diluent. Transfer 0.4 ml of this solution into another 10 ml volumetric flask and make up the volume with diluent. Filter the solution through 0.2 µm nylon membrane filter (750µg/ml of Pregabalin and 600µg/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.

System suitability: By injecting a reference solution containing Pregabalin 30 ppm and Etoricoxib 24 ppm, the system appropriateness was carried out for each validation parameter. Figure 2's system suitability chromatogram and Table 1's results illustrate the chromatogram.

Specificity (Selectivity): Checking of the interference in the optimized method. At these medications' retention durations using this approach, we didn't detect any conflicting peaks in the blank or placebo. Thus, it was claimed that this procedure was particular. Figure 3 displays a representative chromatogram, and Table 2 contains experimental data.

Table 1:System suitability results

S no	Etoricoxib			Pregabalin			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.220	6650	1.08	2.784	13479	1.20	5.4
2	2.220	6939	1.11	2.785	13522	1.19	5.5
3	2.221	6644	1.07	2.787	13391	1.19	5.4
4	2.223	6465	0.99	2.793	13528	1.11	5.5
5	2.224	6551	1.04	2.793	13418	1.11	5.5
6	2.224	6784	1.07	2.794	13699	1.11	5.6

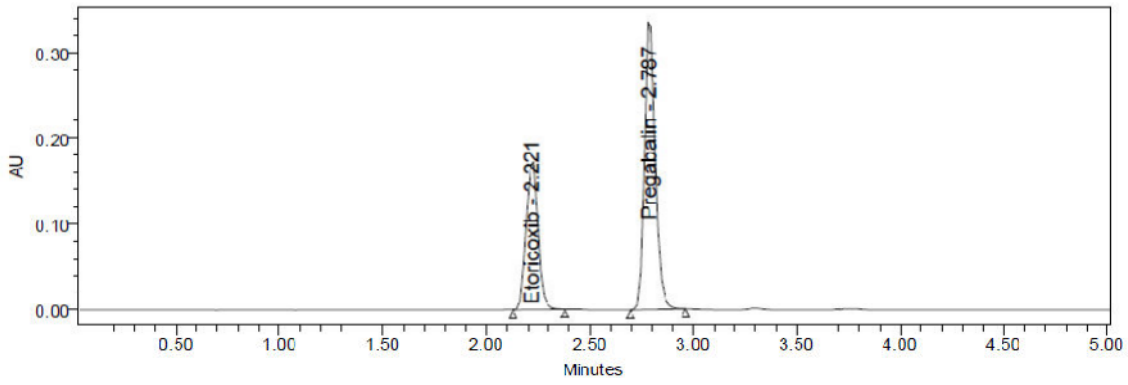
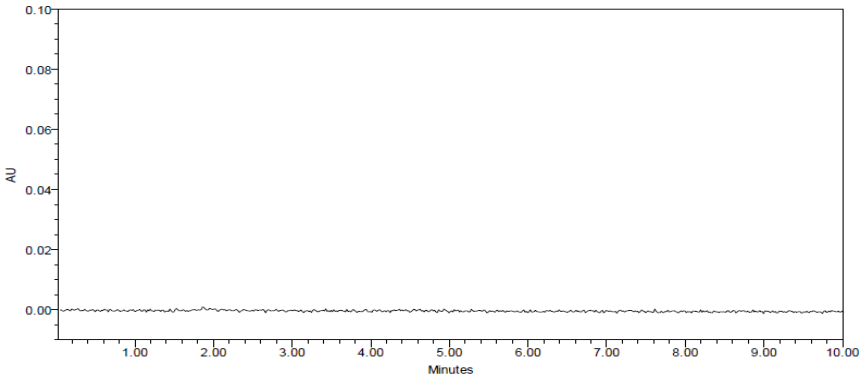


Figure 3: System suitability Chromatogram of Pregabalin and Etoricoxib.

Table 2: Specificity data

Sample name	retention time(Mins)	Response
Pregabaline	2.785	1142675
Etoricoxib	2.221	643968



Blank Chromatogram

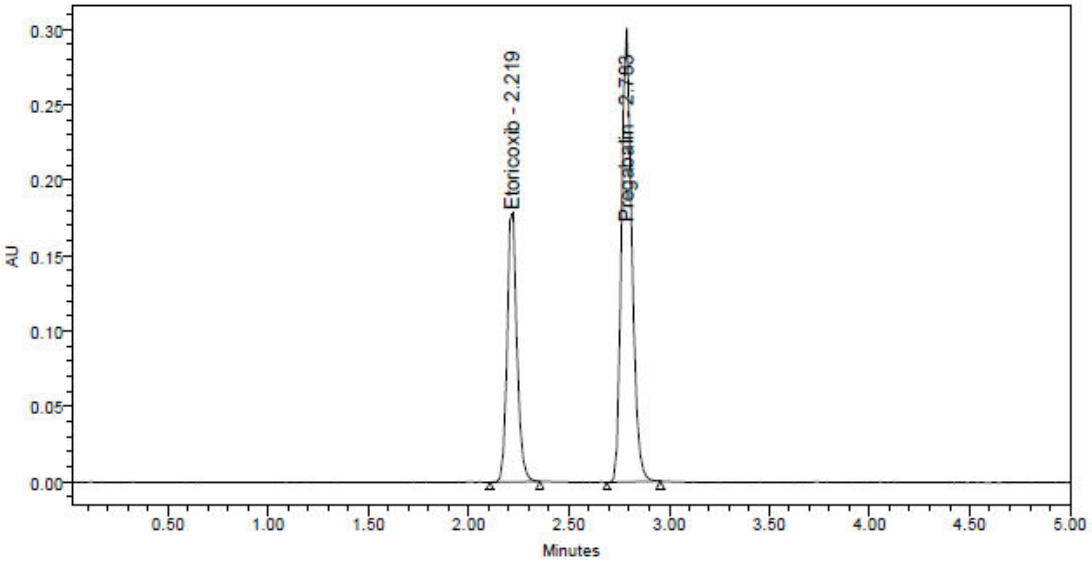


Figure 4: Specificity Chromatograms of pregabalin&etoricoxib.

Table 3: Pregabalin and Etoricoxib Linearity

Etoricoxib		Pregabalin	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
6	167363	7.5	283267
12	326755	15	580378
18	484942	22.5	853314
24	643968	30	1142675
30	806689	37.5	1404034
36	958527	45	1700863

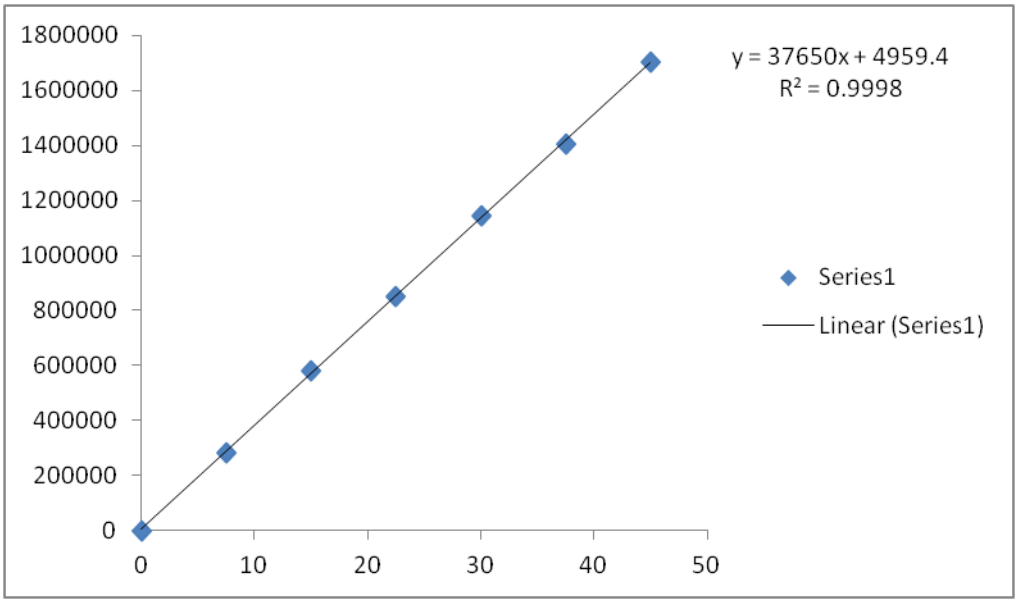


Figure 5: Pregabalin calibration Curve

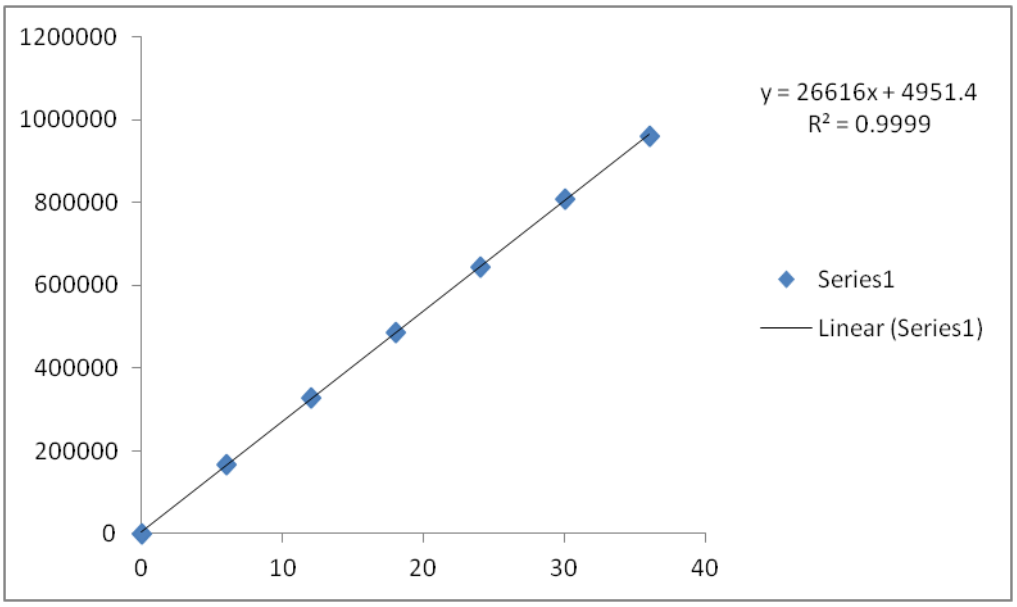


Figure 6: Etoricoxib calibration Curve

Table 4: Recovery data

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12	11.92	99.32	99.80%
	12	12.00	100.03	
	12	12.00	99.98	
100%	24	24.11	100.44	
	24	23.91	99.61	
	24	24.00	100.01	
150%	36	35.83	99.52	
	36	35.84	99.56	
	36	35.91	99.75	

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	15	14.83	98.87	99.78%
	15	15.12	100.81	
	15	15.06	100.37	
100%	30	29.93	99.75	
	30	29.90	99.68	
	30	29.95	99.82	
150%	45	44.80	99.55	
	45	44.78	99.50	
	45	44.82	99.60	

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 6, the results of the peak area are compiled.

Table 5: System precision data

Inj	**Pregabalin**	**Etoricoxib**
1	1228135	669950
2	1227803	670156
3	1229668	670117
4	1221670	676337
5	1226402	669699
6	1226556	670142
Avg	1226706	671067
Std dev	2738.4	2587.7
%RSD	0.2	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 7 provides a summary of the data.

Table 6: Method precision data

Injection	Pregabalin	Etoricoxib
1	668610	1219900
2	672192	1226720
3	670118	1218428
4	670535	1216699
5	674226	1217616
6	671722	1228069
Avg	671234	1221239
Std dev	1935.3	4901.5
%RSD	0.3	0.4

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

Table 7: Robustness results

Chromatographic condition	Pregabalin (%RSD)	Etoricoxib (%RSD)
Flow rate (-) 0.9ml/min	0.2	0.2
Flow rate (+) 1.1ml/min	0.2	0.3
Mobile phase (-) 65W:35A	1.1	1.2
Mobile phase (+) 55W:45A	1.1	1.2
Temperature (-) 25°C	0.8	0.7
Temperature (+) 35°C	1.0	1.2

Table 8:stability conditions for Pregabalin & Etoricoxib.

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 (minutes)
Base	2N NAOH	60^0c	30(minutes)
Oxdation	20% H_2O_2	60^0c	30(minutes)
Thermal		105^0c	6 hours

Photolytic		-	48 hr
Hydrolytic	Water	60^0c	1hr

Table 9: Degradation profile results

Type of degradation	**Etoricoxib**			**Pregabalin**		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	630323	93.55	6.45	1111728	90.26	9.74
Base	626932	93.05	6.95	1189591	96.59	3.41
Peroxide	636551	94.48	5.52	1170097	95.00	5.00
Thermal	648161	96.20	3.80	1174373	95.35	4.65
Uv	656351	97.42	2.58	1204401	97.79	2.21
Water	671806	99.71	0.29	1219645	99.03	0.97

According to the results, samples were degraded when they were subjected to an acid, base 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.

Table 11: Assay outcome for pregabalin&etoricoxib

Drug name	**Label claim dose**	**%Assay**	**Brand Name**
pregabalin	75mg	99.36%	Ebov PG
etoricoxib	60mg	99.62%	

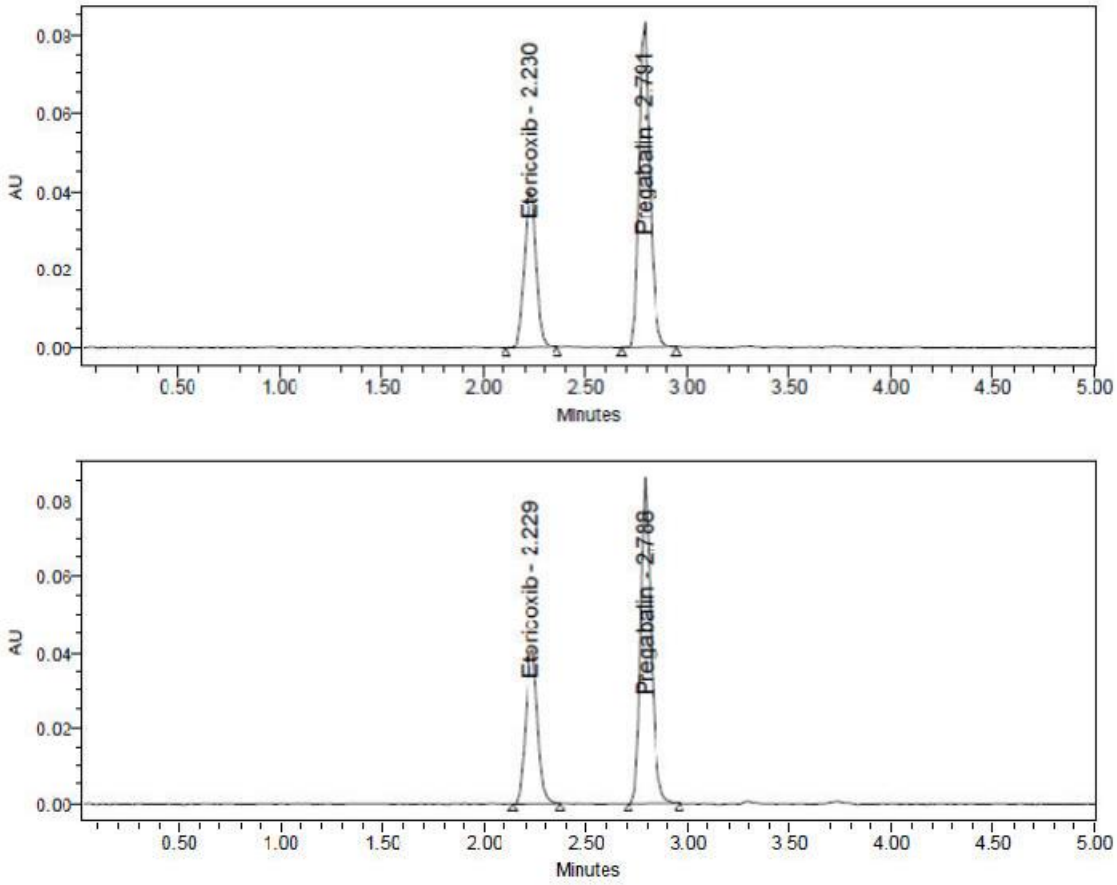


Fig No 13: Assay Chromatogram of Sample

CONCLUSION

This study demonstrates the use of a straightforward and well-established stability-indicating RP-HPLC method to simultaneous identifies Pregabalin and Etoricoxib in pharmaceutical dosage form. The strategy was precise, simple, linear, efficient, and long-lasting. The method can tell active pharmaceutical ingredients apart from degradation byproducts produced during forced degradation testing. The recommended method can be used in the quality-control department for routine quantitative Pregabalin and Etoricoxib analysis.

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