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Research Article

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 easy, exact, accurate, and long-lasting reverse phase RP-HPLC technique has been developed and validated for the simultaneous quantification of Pregabalin and Etorcoxib in bulk and formulation. This procedure employs a simple isocratic mobile phase of acetonitrile, a 150 x 4.6 mm, 2.7 m ascentis C18 column, with a flow rate of 0.8 ml/min of 0.01N Potassium dihydrogen phosphate (50:50). Average retention durations for pregabalin and etoricoxib were 2.313 and 2.840 minutes, respectively. Pregabalin and etoricoxib's percentage recovery were found to be 100.24% and 99.98%, respectively, showing that the approach is appropriate for routine analysis. With an R2 of 0.999 for all drugs, it was shown that Pregabalin and Etorcoxib are linear, demonstrating the approach's potential for producing results with high sensitivity. The% RSD should not exceed 2.0%, showing that the procedure is exact, according to the precision acceptance standards.

Keywords: Excision, Phytochemicals, Tetrapleura tetraptera, Re-epithelilization, Alloxa monohydrate

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 Ca^{2+} 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 recognized 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

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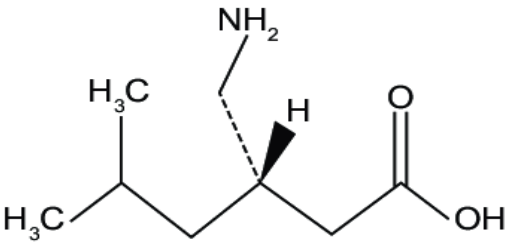
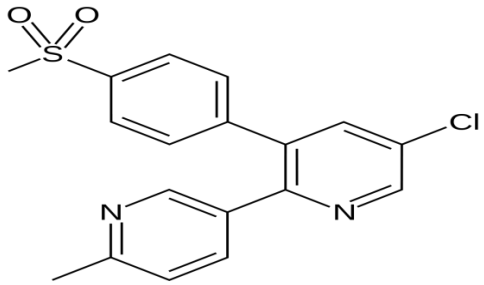
and inflammatory symptoms of different types of arthritis. It can be used to treat rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis-related joint and muscle pain and inflammation in individuals aged 16 and older. [8,9]. The cyclo-oxigenase enzyme (COX-2) isoform 2 is specifically inhibited 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.

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 utilised 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, an Ascentis (C18 150 mm x 4.6 mm, 2.7 m) column was employed. Acetonitrile is employed as mobile phase B, while 0.01N potassium di hydrogen phosphate is used as mobile phase A. (50:50 Ratio). The analysis was done in isocratic mode with an injection volume of 10 µL and a flow rate of 0.8 mL/min. The duration was five minutes. The measurements were made at 228 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: Weigh accurately about 37.5 mg of preghabalin and 30mg of etoricoxib working standard and transfer to a 50 ml volumetric flask. Add 10 ml of diluent and sonicate to dissolve. Dilute upto volume with diluent and mix. Transfer 1.0 ml of this solution into a 10 ml of volumetric flask and dilute upto volume with the diluent and mix. Filter the solution through 0.2µm nylon membrane filter

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 1.0 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

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 75 ppm and Etoricoxib 60 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	Pregabalin			Etoricoxib			
Inj	RT	NTP	TF	RT(Min)	NTP	TF	RS
1	2.307	5809	1.13	2.838	5458	1.12	4.1
2	2.312	5864	1.15	2.839	5472	1.10	3.9
3	2.312	5815	1.19	2.843	5482	1.09	4.0
4	2.312	5879	1.14	2.845	5476	1.10	3.9
5	2.317	5809	1.12	2.848	5415	1.11	4.1
6	2.330	5805	1.19	2.857	5465	1.11	4.0

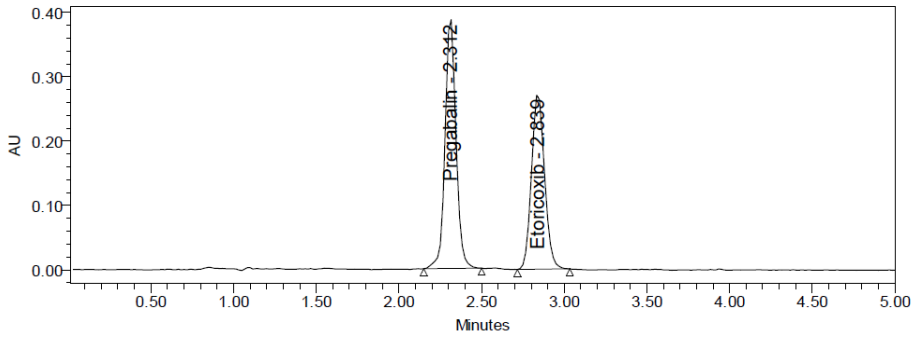
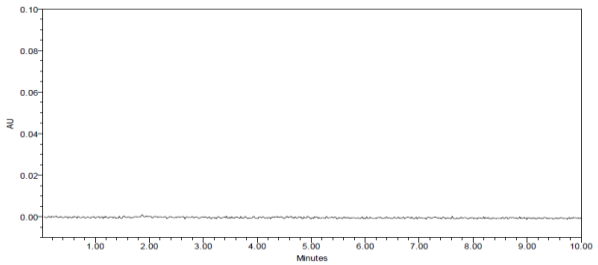


Table 2: Specificity data

Sample name	retention time(Mins)	Response
Pregabaline	2.312	325924
Etoricoxib	2.839	174427



Blank Chromatogram

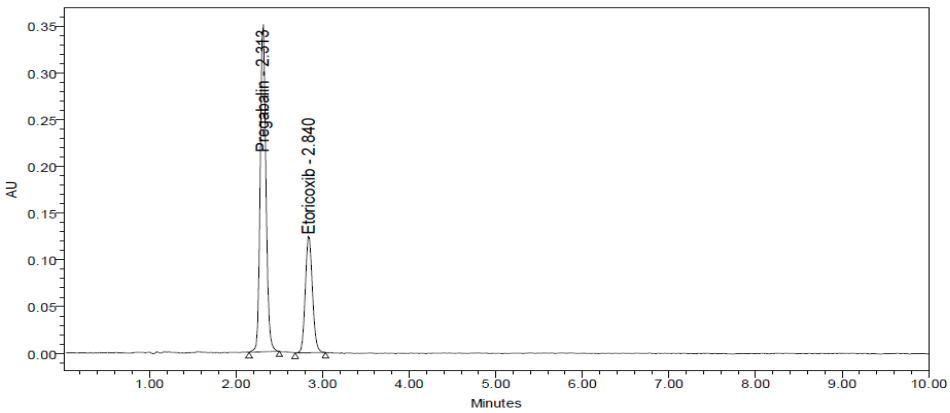


Figure 4: Specificity Chromatograms of pregabalin & etoricoxib

Table 3: pregabalin and etoricoxib Linearity

Pregabalin		Etoricoxib	
CON (PPM)	**Response**	**CON (PPM)**	**Response**
18.75	82432	15	43651
37.5	162937	30	87546
56.25	240535	45	136437
75	328061	60	173658
93.75	403539	75	216631
112.5	485291	90	264874

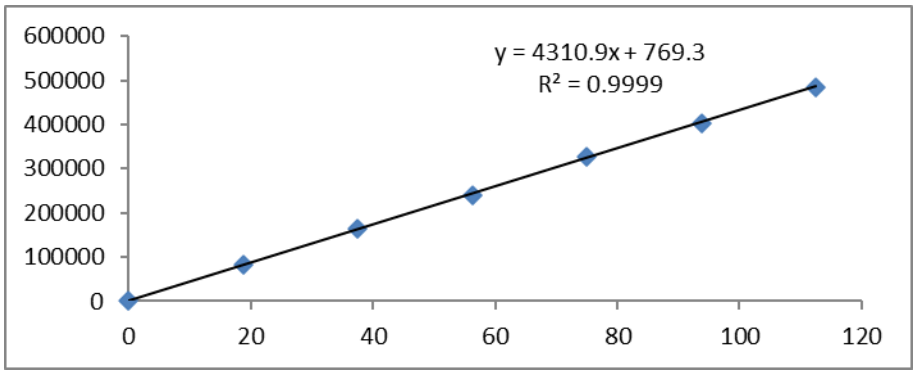


Figure 5: pregabalin calibration Curve

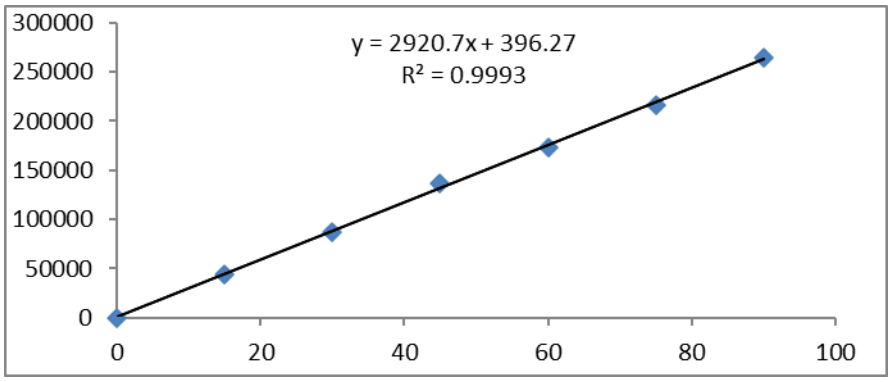


Figure 6: etoricoxib calibration Curve

Table 4: Recovery data

%level	**%recovery**			
	pregabaline		**etoricoxib**	
	amt found	%rec	amt found	%rec
50% level	37.74	100.65	29.74	99.12
	37.67	100.45	29.85	99.50
	37.58	100.22	30.14	100.48
100%level	74.97	99.96	60.66	101.54
	75.32	100.43	59.64	99.40
	75.27	100.36	60.58	100.97
150%level	112.62	100.11	89.30	99.22
	112.41	99.92	90.08	100.09
	112.55	100.40	89.94	99.94
mean%		**100.24**		**99.98**

System Precision: With regard to the working strength of Pregabalin and Etorcoxib, 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	323520	177722
2	328707	173003
3	326713	176399
4	328951	173190
5	323793	172393
6	323862	173855
Avg	325924	174427
Std dev	2533.9	2133.9
%RSD	0.8	1.2

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 Etorcoxib 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	325202	173539
2	322129	172628
3	326454	176778
4	323506	173781
5	325592	172006
6	325015	174806
Avg	324650	173923
Std dev	1564.9	1700.0
%RSD	0.5	1.0

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(-)	1.1	0.1
flow(+)	0.8	0.6
temp(-)	0.7	0.9
temp(+)	0.4	1.0
mobile phase(-)	0.5	0.5
mobile phase (+)	0.5	0.5

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

	pregabalin			etoricoxib		
Degradation condition	**% degraded**	**Purity angle**	**Purity threshold**	**% degraded**	**Purity angle**	**Purity threshold**
Acid	7.06	0.319	0.532	8.04	0.430	0.557
Base	4.88	0.311	0.543	5.81	0.502	0.578
Oxidation	9.29	0.844	1.536	7.58	0.443	0.756
Thermal	2.59	0.226	0.446	4.56	0.248	0.462
Photolytic	1.30	0.457	0.54	2.83	0.296	0.538
Hydrolytic	1.11	0.285	0.517	0.41	0.272	0.496

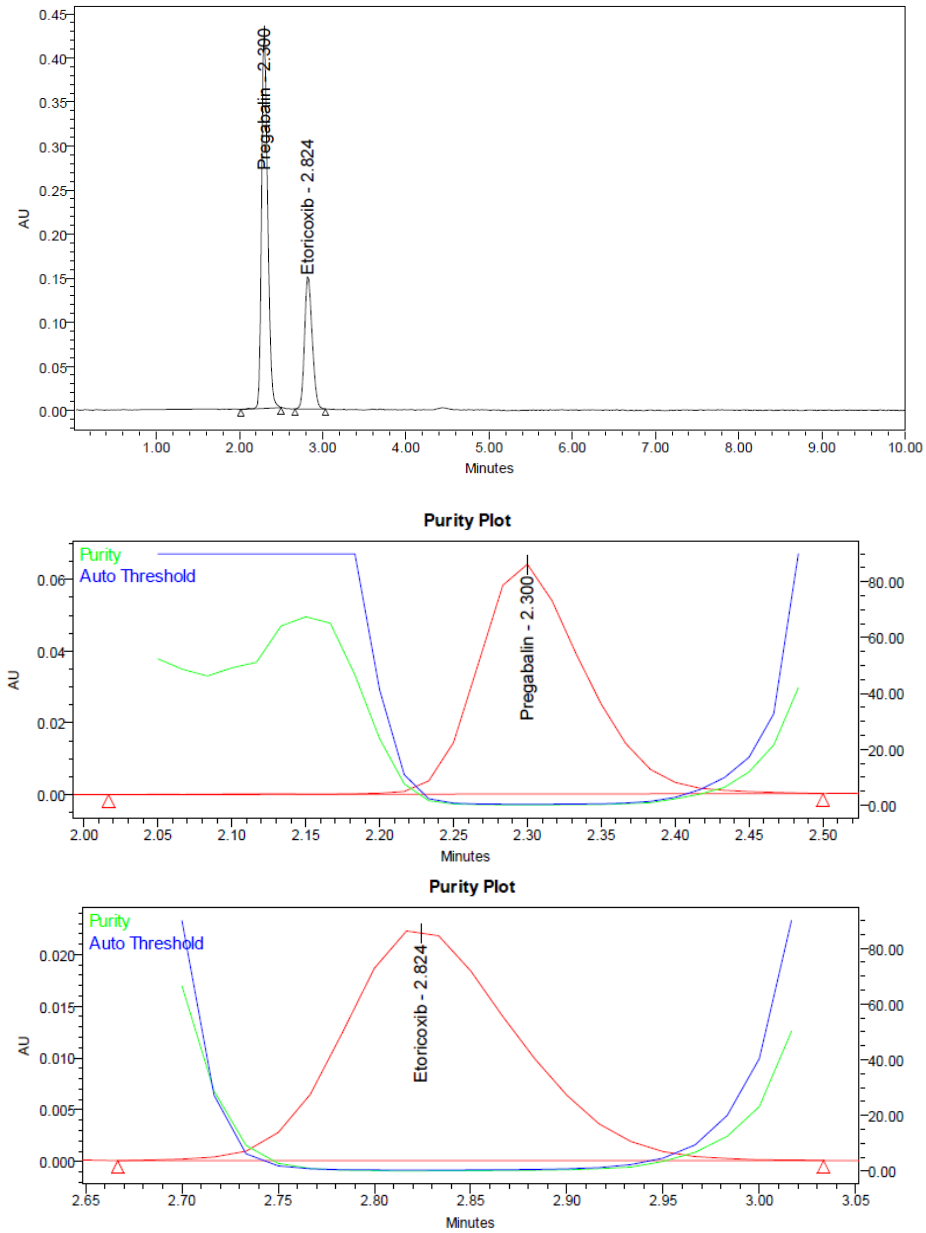


Fig no 7. Acidic stability peaks and purity polts

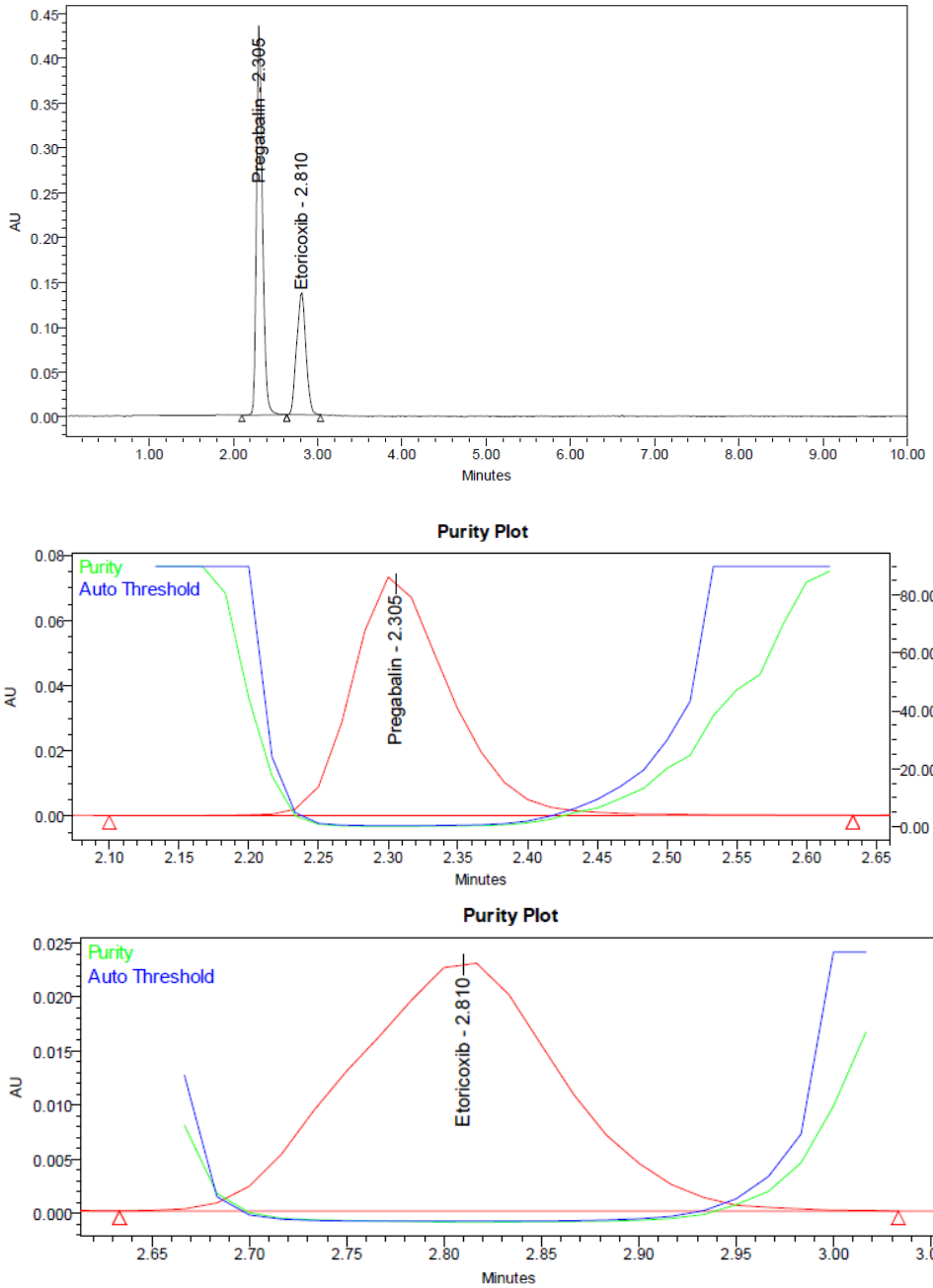
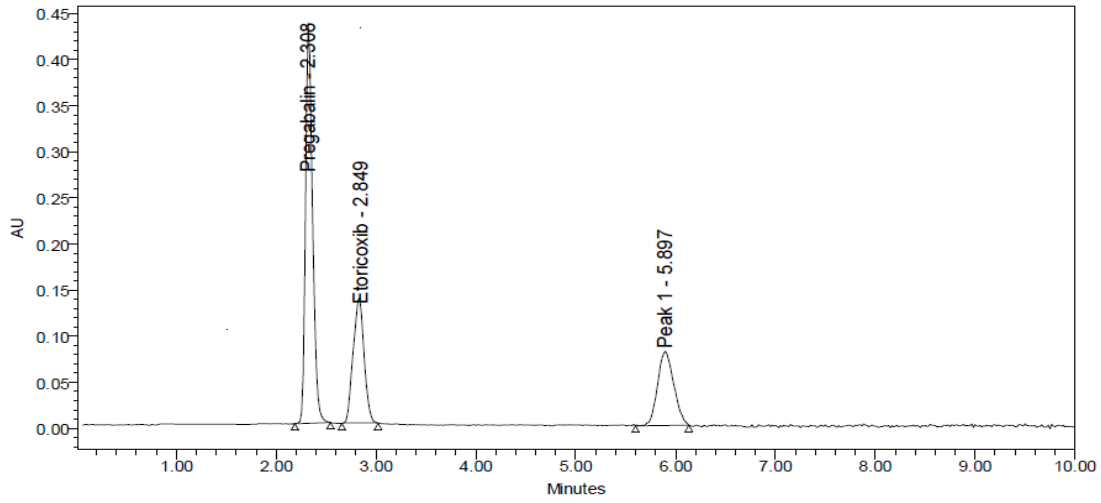


Fig no 8. Basicstability peaks and purity plots



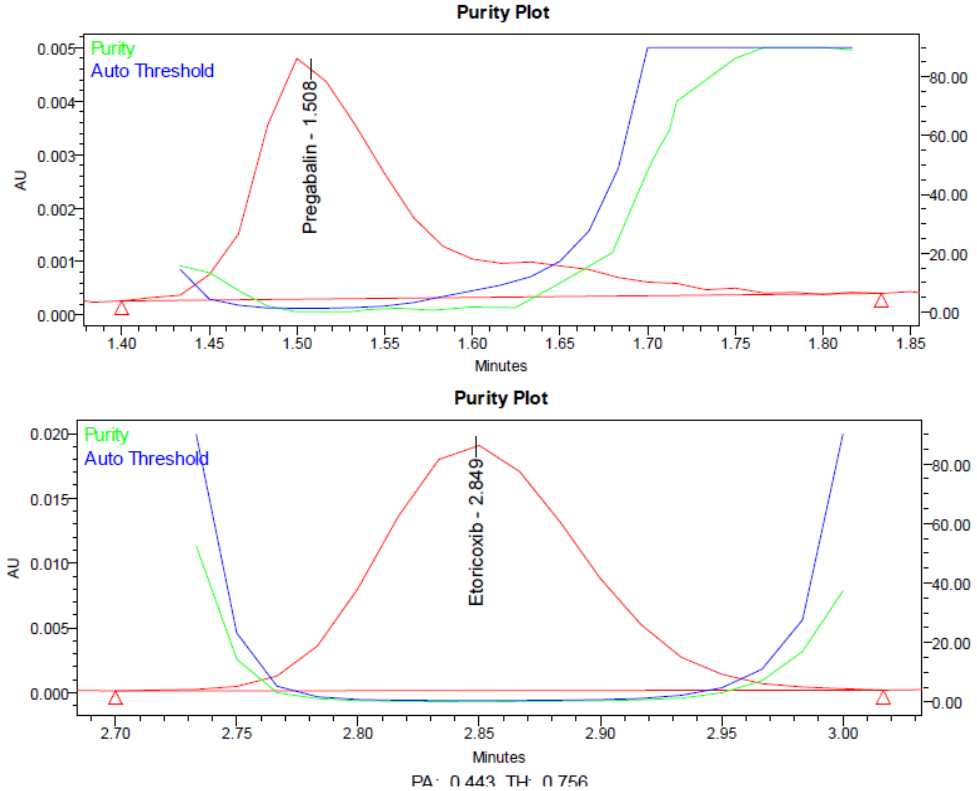
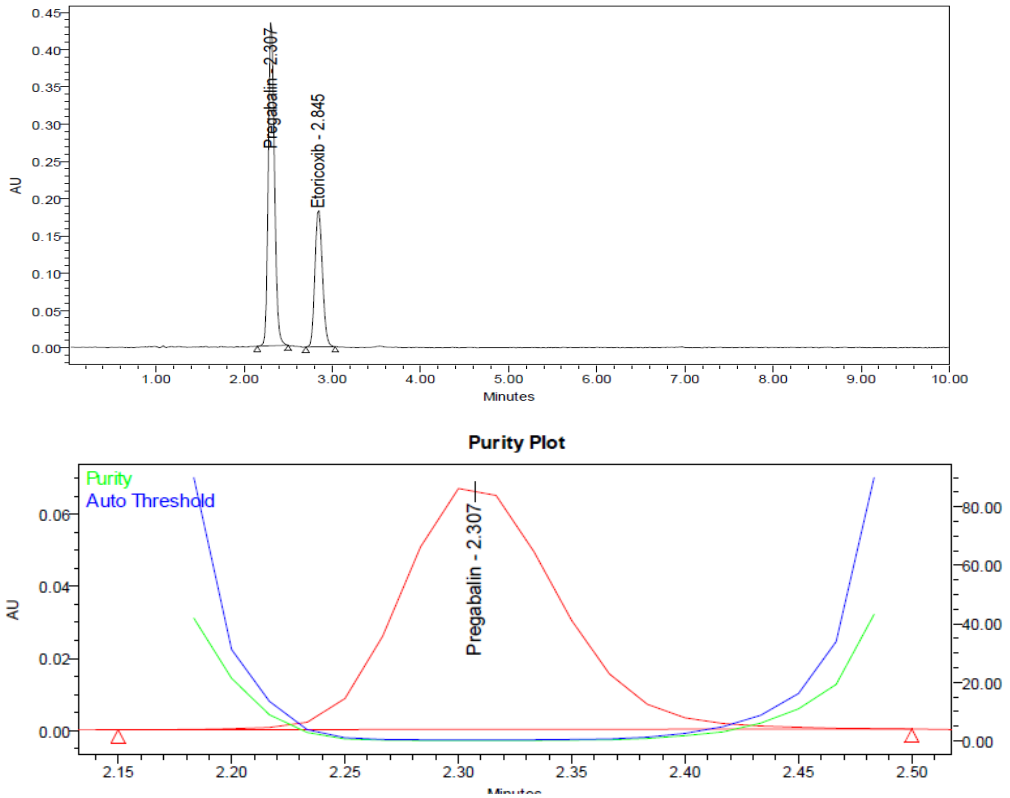


Fig no 9. Oxidation stability peaks & purity plots



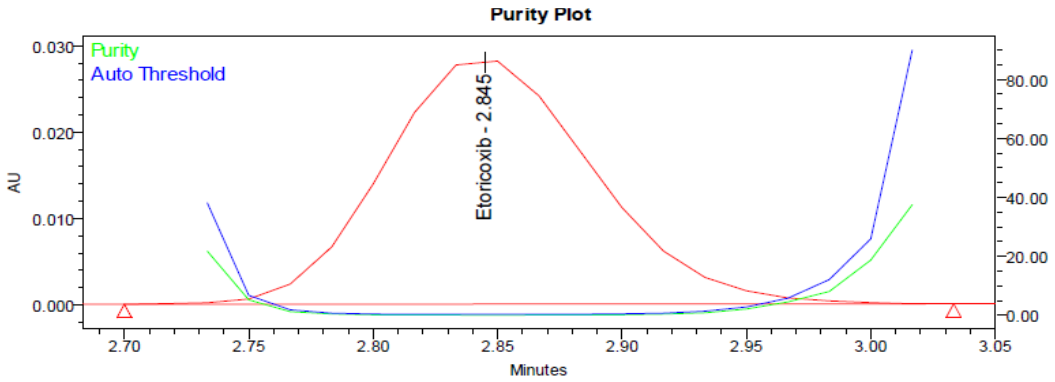


Fig no 10. Heat stability peaks & purity plots

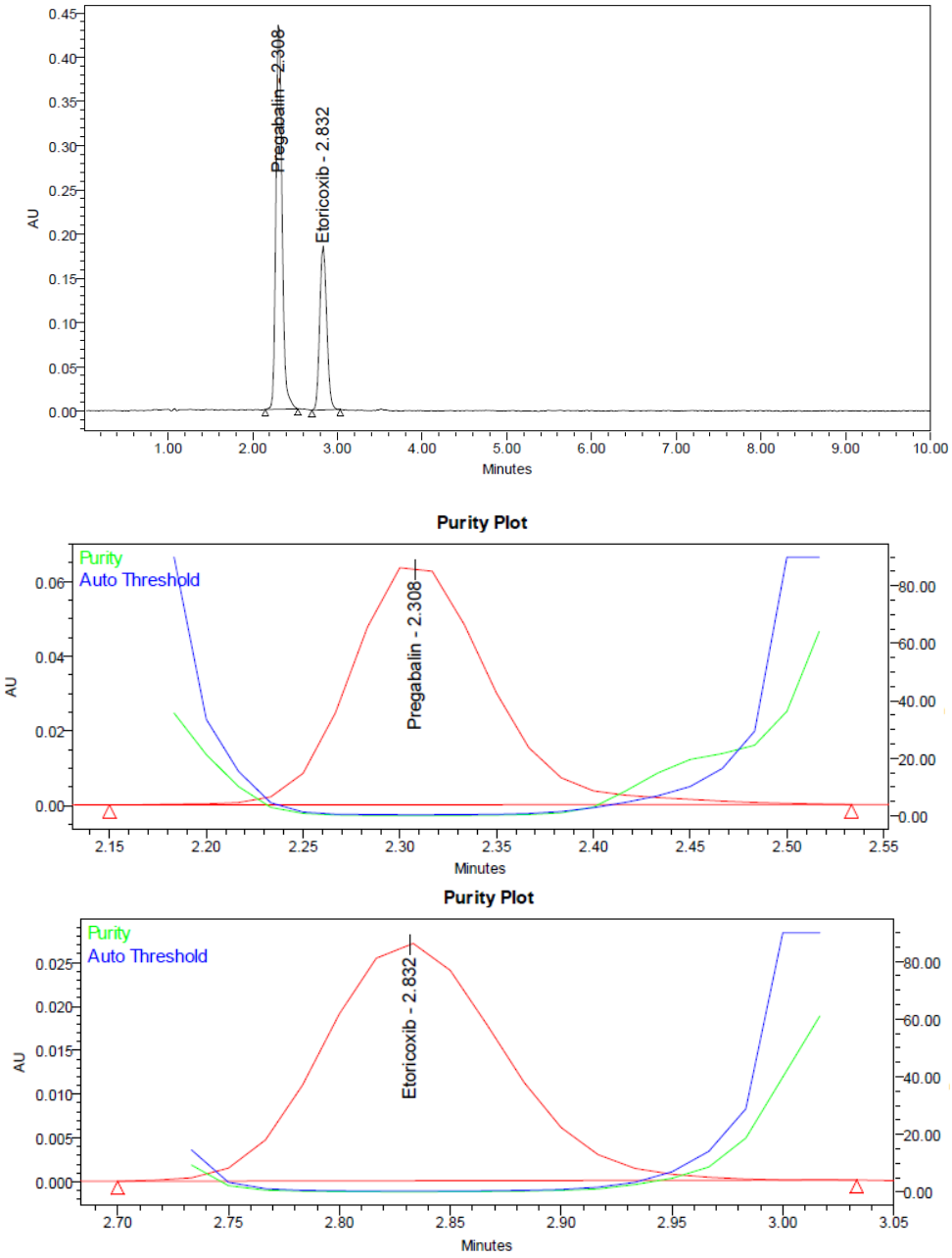


Fig no 11. Sun lightstability peaks & purity polts

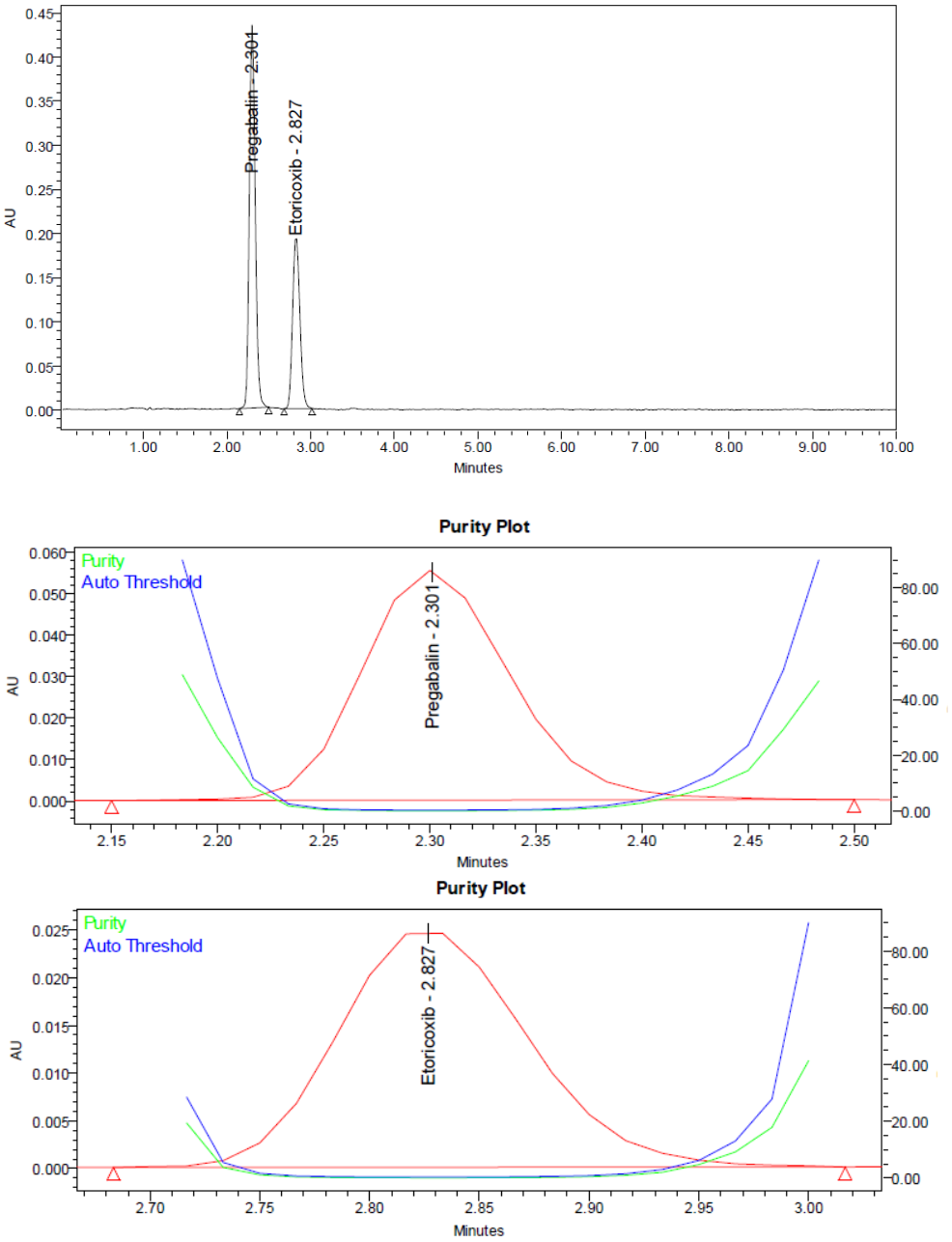


Fig no 12. Hydrolytic stability peaks & purity plots

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.

Table 11: Assay outcome for pregabalin & toricoxib

Drug name	Label claim dose	%Assay	Brand Name
pregabalin	75mg	99.51	Ebov PG
etoricoxib	60mg	99.61	

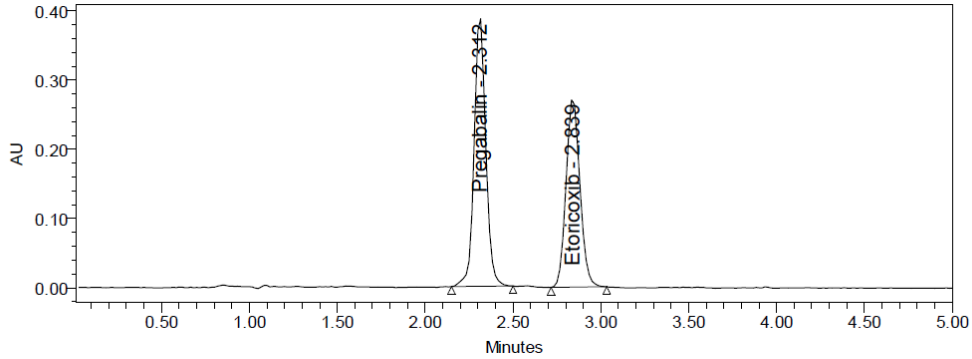


Fig No 13: Assay Chromatogram of Sample

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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