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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Pregabalin and Etoricoxib in Pharmaceutical Dosage Form

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ABSTRACT

For the simultaneous estimation of Pregabalin and Etoricoxib in pharmaceutical dosage form, a straightforward, accurate, and precise method has been developed. The chromatogram was run through Supelco C18 150 x 4.6 mm, 2.7µ. Mobile phase containing Buffer 0.01N Methanol: KH_2(0.1%Acetic acid is added to adjust pH5.4) taken in the ratio 70:30 was pumped through column at a flow rate of 1ml/min. The temperature was maintained at 30°C. The optimized wavelength selected was 240nm. The retention time of Pregabalin and Etoricoxib was found to be 2.204min and 3.058 min. %RSD of the Pregabalin and Etoricoxib was found to be 0.6 and 0.5 respectively. %Recovery was obtained as 100.04% and 99.83% for Pregabalin and Etoricoxib respectively. LOD and LOQ values obtained from regression equations of Pregabalin and Etoricoxib were 0.11, 0.32, and 0.09, 0.32 respectively. The regression equation of Pregabalin is y = 15346x + 1398.4, and y = 12031x + 2587.4Of Etoricoxib. The method was designed to be simple and affordable due to shorter retention times and shorter run times, and it may be used for standard Quality Control Tests in Industries.

INTRODUCTION:

When compared to etoricoxib alone, FDC of pregabalin and etoricoxib significantly improved functional status and reduced pain in patients with CLBP. As it addresses both the neuropathic and nociceptive components of pain, pregabalin prolonged release-etoricoxib FDC may be one of the treatment options for CLBP, providing early and long-lasting pain relief as well as an improvement in quality of life [1]. Products with two or more active ingredients combined in a single dosage form are referred to as FDCs. Pregabalin IP (75 mg) and Etoricoxib (60 mg), a recently approved FDC, are suggested for treating neuropathic chronic back pain [2]. Using chromatographic methods like HPLC, GC, and HPTLC, among others, drug combinations are frequently simultaneously estimated. Although these methods are accurate and repeatable, the cost of analysis is very high due to the expensive techniques, materials, and skills needed. Therefore, it is crucial to develop a simpler and more cost-effective method for the simultaneous estimation of medications for regular formulation analysis. When estimating the medication combination simultaneously is required, spectrophotometric analysis meets this need with efficacy on par with chromatographic methods [3,4]. For their combination, no effective and trustworthy analytical method has been documented. To learn more about the various analytical instrumental methods currently being used for the individual quantitation of PGB, ETC in different matrices, as per Ich Q2R1 guidelines, the validation parameters are conducted as per criteria acceptance limits the tests are carried out [5]. The description outlines of drugs performing in technique are described. Pregabalin has structural similarities with the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) [6].is an anticonvulsant used in conjunction with other anticonvulsants to treat partial onset seizures, fibromyalgia, and neuropathic pain conditions. The cyclooxygenase enzyme's isoform 2 is specifically inhibited by etoricoxib (COX-2). It selects COX-2 inhibition over COX-1 by a factor of about 106. As a result, fewer prostaglandins (PGs) are produced from arachidonic acid [7]. pregabalin's presynaptic binding to voltage-gated calcium channels is essential for the antiseizure and antinociceptive effects Pregabalin controls the release of several excitatory neurotransmitters, such as glutamate, substance-P, norepinephrine, and calcitonin gene-related peptide, by a binding presynaptic ally to the alpha2-delta subunit of voltage-gated calcium channels in the central nervous system[9], Pregabalin along with other combination is the onset of action is better in action showing towards seizures [7]. There are some other RP-HPLC methodologies have been published. [10,11,12,13,14,15]

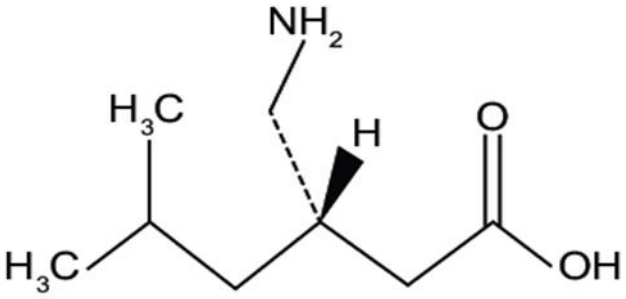

Figure-1.1 Chemical structure of Pregabalin

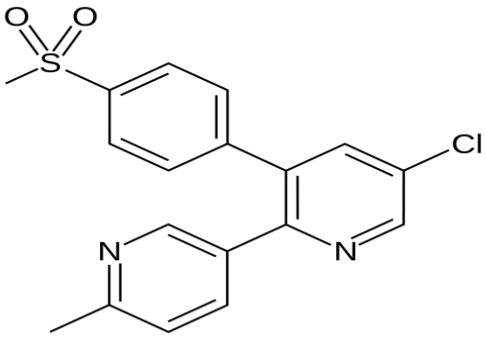

Fig 1.2: Chemical Structure of Etoricoxib

MATERIALS AND METHODS

Chemicals and reagents

Pregabalin and Etoricoxib tablets (EMAXGALIN), and some of the other ingredients such as Ortho-phosphoric acid, distilled water, acetonitrile, and phosphate buffer, The chemicals, and solvents mentioned above are all from Rankem.

Instrumentation:

It is the most adaptable, secure, dependable, and quick chromatographic method for determining the quality of drug ingredients[16], WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector, and Autosampler integrated with Empower 2 Software, Supelco C18 150 x 4.6 mm, 2.7m., electronic balance, sonicator, hot air oven, digital pH meter, and UV-Visible chamber.

Preparation of Standard stock solution: 30 mg of Etoricoxib and 37.5 mg of Pregabalin were separately weighed and transferred to 50 ml volumetric flasks. 3/4 th of the diluents were added before being sonicated for 10 minutes. Flasks labeled "Standard stock solution 1 and 2" were filled with diluents. (600 g/ml of etoricoxib and 750 g/ml of pregabalin).

Preparation of Standard working 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 solution: Accurately weighed equivalent weight of the combination powder sample transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the 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 solution:1 ml of filtered sample stock solution was transferred to a 10ml volumetric flask and made up with diluent. (75µg/ml of Pregabalin and 60µg/ml of Etoricoxib)

Chromatographic conditions:

Flow rate:	1ml/min
Column:	Supelco 150(4.8 x 150mm,2.7µm)
Wavelength:	228nm
Column temperature:	30°C
Injection volume:	10.0µL
Run time:	6minutes

Observation: At 2.204 and 3.058 minutes, respectively, pregabalin and etoricoxib were successfully eluted. This method was optimized and will be validated because the plate count and tailing factor were very satisfactory.

Method Validation: The method was validated following ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are among the validation parameters.

RESULTS AND DISCUSSION

System suitability parameters: Plate count should be greater than 2000, tailing factor should be lower than 2, and resolution should be greater than 2. Every system-appropriate

parameter was successful and within tolerances. The % RSD for the area of six standard injection results should not be more than 2%.

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.

Linearity: Six linear concentrations of Pregabalin (18.75-112.5µg/ml) and Etoricoxib (15-90µg/ml) were injected in a duplicate manner. Average areas were mentioned above and the linearity equations obtained for was y = 15346x + 1398.4of Pregabalin. and Etoricoxib of was y = 12031x + 2587.4, Correlation coefficient obtained was 0.999 for the two drugs.

Precision:

Repeatability: Six working sample solutions of the same concentrations were made after performing multiple samplings from a sample stock solution. After giving each injection from a working sample solution, the obtained areas were noted in the previous table. For two drugs, average area, standard deviation, and percent RSD were calculated; the results were 0.6% and 0.5% for Pregabalin and Etoricoxib, respectively. The system precision was exceeded using this method because the precision limit was less than "2."

Intermediate Precision: Each injection from each working sample solution was given on the following day of the sample preparation, and the obtained areas were noted in the above table. Multiple sampling from a sample stock solution was carried out, and six working sample solutions of the same concentrations were prepared. For two drugs, average area, standard deviation, and percent RSD were calculated; the results were 1.0% and 0.3% for Pregabalin and Etoricoxib, respectively. The system precision was exceeded using this method because the precision limit was less than "2."

Accuracy: Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy and %Recovery was obtained as 100.04% and 99.83% for Pregabalin and Etoricoxib respectively.

Robustness: Flow minus (0.9 ml/min), Flow plus (1.1 ml/min), mobile phase minus (65B:35A), mobile phase plus (75B:25A), temperature minus (27°C), and temperature plus (33°C) robustness conditions were maintained, and samples were injected in duplicate. The

parameters for system suitability were not significantly impacted, and all of the parameters were met. The limit was reached for % RSD.

Assay: (EMAXGALIN), bearing the label claim Pregabalin 75mg, Etoricoxib 60mg. An assay was performed with the above formulation. The average % Assay for Pregabalin and Etoricoxib obtained was 100.42% and 100.06% respectively.

Degradation Studies: Degradation studies were performed with the stock standard solution and the degraded samples were analyzed using the proposed method. The assay % of the injected samples was calculated and all the samples passed the limits of degradation. The results were shown in table 7.

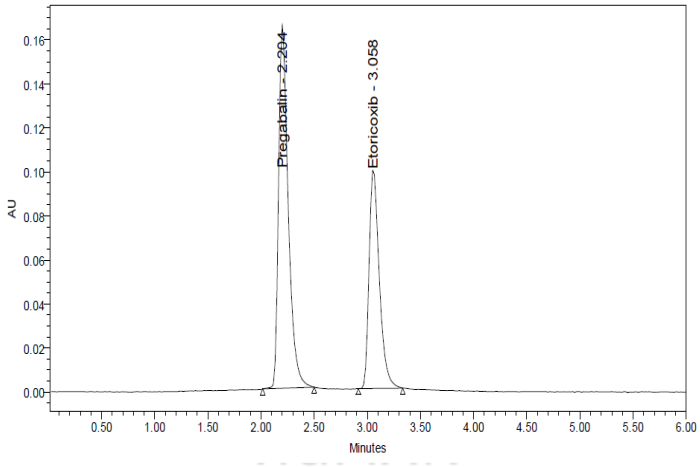

Fig No.2: Optimised Chromatogram

Table No.1: System suitability parameters

S no	Pregabalin			Etoricoxib			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.195	2779	1.46	3.058	4783	1.40	4.9
2	2.198	2764	1.45	3.219	4946	1.38	5.7
3	2.200	2792	1.46	3.234	4982	1.38	5.7
4	2.203	2778	1.42	3.245	4848	1.36	5.7
5	2.204	2857	1.46	3.275	4915	1.39	5.9
6	2.206	2703	1.46	3.285	4958	1.36	6.1

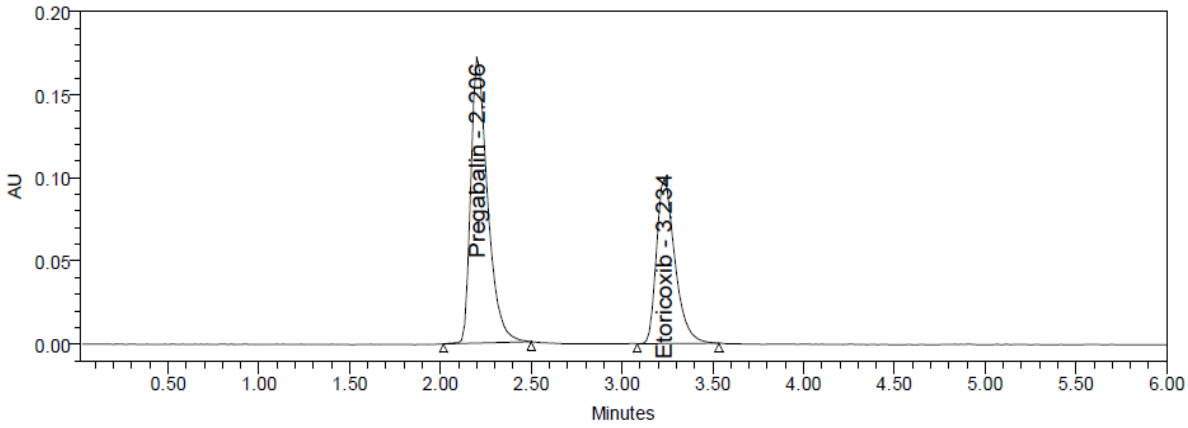

Figure No.3: Standard solution chromatogram

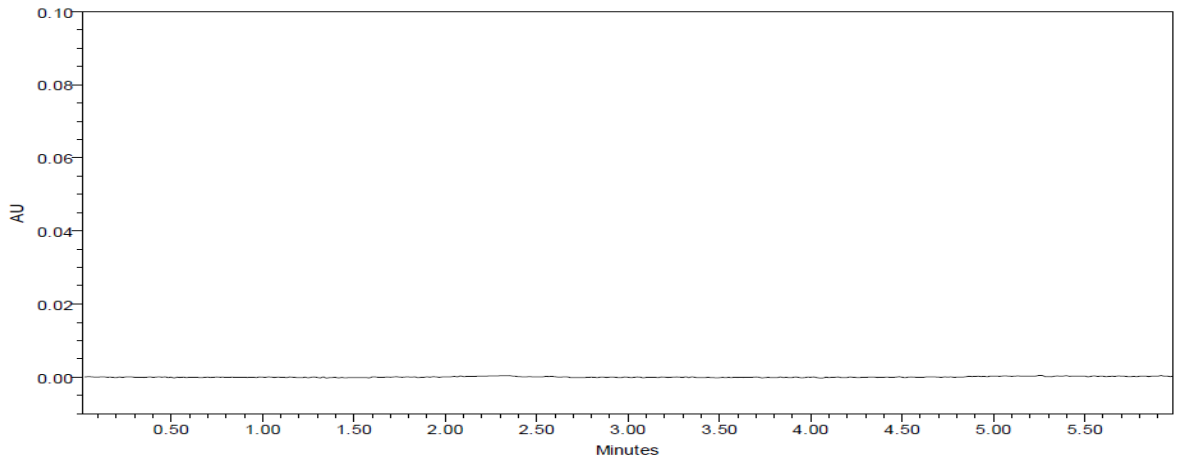

Figure No.4: Blank chromatogram

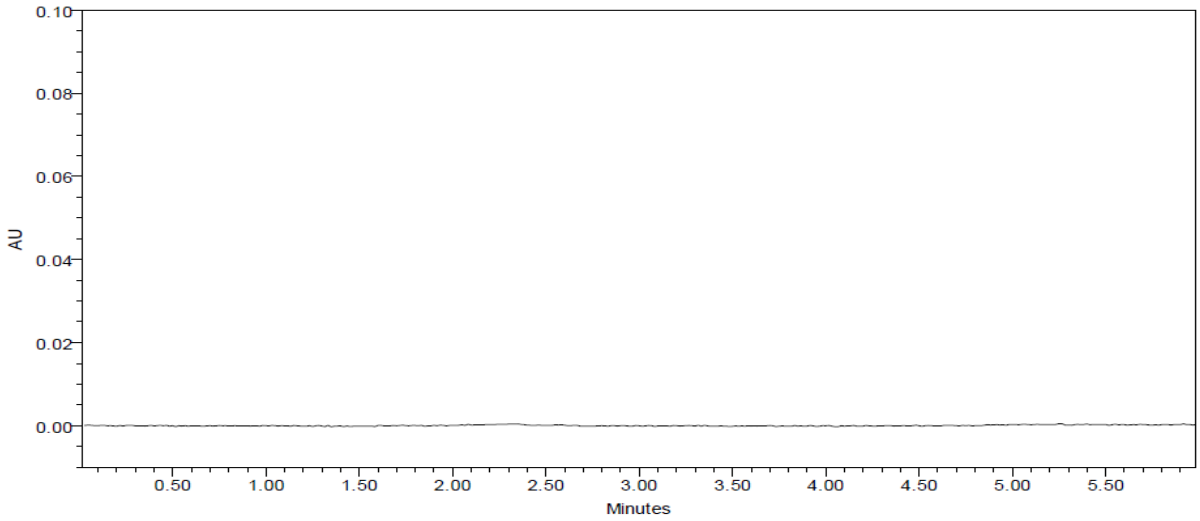

Figure No.5: Placebo chromatogram

Table No.2: Linearity table for Pregabalin and Etoricoxib,

Pregabalin		Etoricoxib	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
18.75	287165	15	182961
37.5	577586	30	364594
56.25	863339	45	541665
75	1161499	60	732351
93.75	1441015	75	907852
112.5	1721684	90	1078524

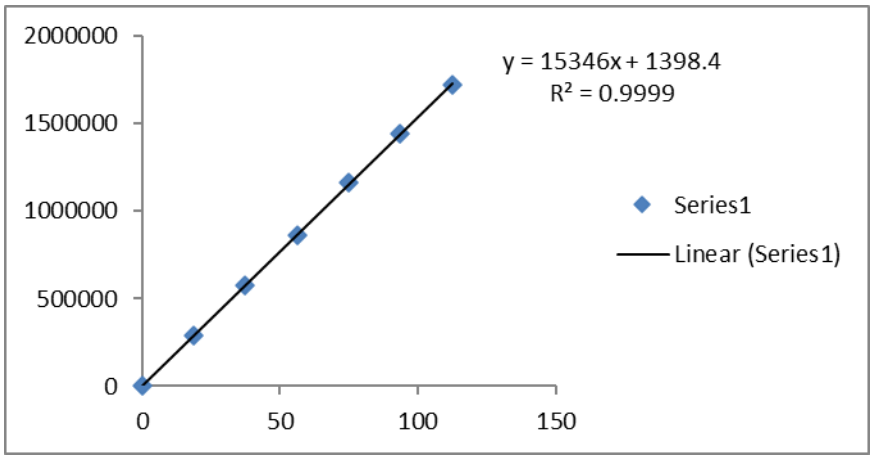

Figure No 6: Calibration curve of Pregabalin

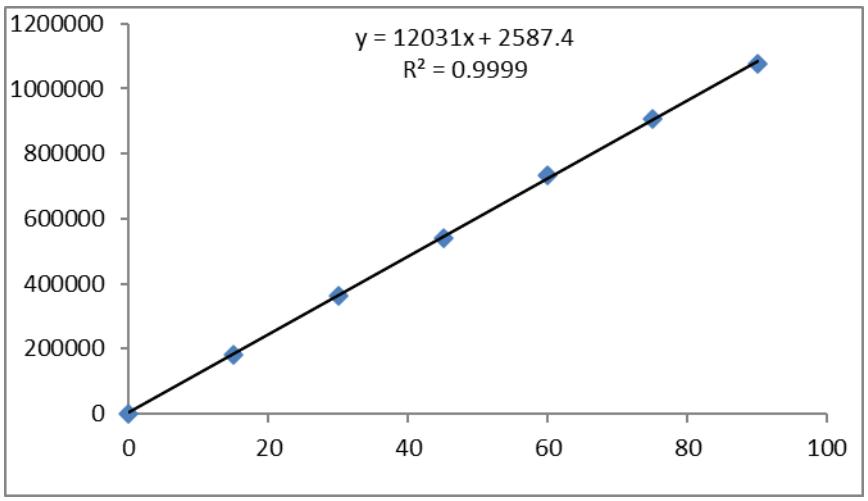

Figure No.7 Calibration curve of Etoricoxib

Table No.3: Repeatability table of Pregabalin and Etoricoxib

S. No	Area of Pregabalin	Area of Etoricoxib
1.	1160051	721105
2.	1161750	720449
3.	1144589	725733
4.	1154450	729782
5.	1161426	724355
6.	1155314	728353
Mean	1156263	724963
S.D	6505.1	3766.6
%RSD	0.6	0.5

Table No.4: Intermediate precision table of Pregabalin and Etoricoxib

S. No	Area of Pregabalin	Area of Etoricoxib
1.	1145314	721277
2.	1151965	724474
3.	1164472	722566
4.	1130066	721720
5.	1153897	721190
6.	1150840	725733
Mean	1149426	722827
S.D	11371.3	1872.6
%RSD	1.0	0.3

Table No.5 Accuracy table of Pregabalin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	37.5	37.76	100.70	100.04%
	37.5	37.66	100.44	
	37.5	37.24	99.31	
100%	75	75.69	100.91	
	75	74.89	99.86	
	75	75.07	100.09	
150%	112.5	111.91	99.47	
	112.5	111.65	99.25	
	112.5	112.92	100.37	

Table no 5.1 Accuracy table of Etoricoxib

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	30	30.14	100.46	99.83%
	30	29.78	99.27	
	30	29.89	99.64	
100%	60	59.94	99.90	
	60	59.64	99.40	
	60	60.41	100.69	
150%	90	89.38	99.31	
	90	89.13	99.03	
	90	90.70	100.78	

Table No.6: Robustness data for Pregabalin and Etoricoxib.

S.no	Condition	%RSD of Pregabalin	%RSD of Etoricoxib
1	Flow rate (-) 0.9ml/min	0.7	0.3
2	Flow rate (+) 1.1ml/min	0.9	0.3
3	Mobile phase (-) 65B:35A	0.8	0.4
4	Mobile phase (+) 75B:25A	0.4	0.4
5	Temperature (-) 27°C	0.6	0.3
6	Temperature (+) 33°C	0.4	0.4

Table No.7: Degradation Data

Type of degradation	Pregabalin			Etoricoxib		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	1124612	97.67	2.33	709721	97.95	2.05
Base	1136640	98.71	1.29	711767	98.23	1.77
Peroxide	1124194	97.63	2.37	707776	97.68	2.32
Thermal	1072993	93.19	6.81	675910	93.29	6.71
Uv	1139371	98.95	1.05	714035	98.55	1.45
Water	1142143	99.19	0.81	721285	99.55	0.45

CONCLUSION:

To estimate Pregabalin and Etorcoxib simultaneously in bulk and pharmaceutical dosage form, a straightforward, accurate, and precise method was developed. Both Pregabalin and Etorcoxib had retention times of 2.204 and 3.058 minutes, respectively. The %RSD for

Pregabalin and Etorcoxib were determined to be 0.6 and 0.5, respectively. % The percentages of recovery for Pregabalin and Etorcoxib were 100.04% and 99.83%, respectively. Pregabalin and etoricoxib's LOD and LOQ values were 0.11, 0.32, and 0.09, 0.32, respectively, from regression equations. Etoricoxib's regression equation is y = 12031x + 2587.4 and Pregabalin's is y = 15346x + 1398.4. Retention times decreased, resulting in a decrease in run time. As a result, the method developed was straightforward and cost-effective, and it could be used for routine Quality Control Tests in Industries.

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