

Simultaneous Estimation of Pregabalin and Etoricoxib using Novel HPLC Method: An Application in Quantitative Analysis of Pharmaceutical Dosage Forms

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ABSTRACT

Introduction: A new optimised method was developed for simultaneous estimation of new FDA approved drugs using RP-HPLC method that would emphasize the use of these drugs in various industries for quality measurements. **Objectives:** The objective of the present study to develop and validate a new simple, novel, precise, rapid, reliable, accurate and reproducible reverse-phase high-performance liquid chromatography (RP-HPLC) technique for simultaneous estimation of Pregabalin (PREG) and Etoricoxib (ETOR) in bulk and formulation. **Materials and Methods:** This separation was done by using a mixture of methanol: acetonitrile: phosphate buffer (pH 5) at the ratio of (40:20:20) as mobile phase, with a hypersil ODS, C₁₈ (250 mm × 4.6 mm, i.d.2.5 μm). The flow rate was 1.0 ml min⁻¹ and effluents were monitored at 215 nm by using UV-visible detector with injection volume was 20 μl. **Results:** They were found to be linear over a range of 12.5–37.5 μg ml⁻¹ and 150–450 μg ml⁻¹ for PREG and ETOR respectively. The retention time of PREG and ETOR was 3.523 min and 4.702 min, respectively. The mean percentage recoveries of PREG and ETOR were found to be 100.27% and 99.91%, respectively. The LOD was found 1.89 and 2.26 μg ml⁻¹ and the LOQ were found 5.73 and 6.84 μg ml⁻¹, for PREG and ETOR, respectively. **Conclusion:** This novel validated economical methods could be applicable for analysis of PREG and ETOR in pharmaceutical industries.

Key words: Pregabalin, Etoricoxib, Method Validation, Simultaneous, ICH.

INTRODUCTION

Pregabalin (PREG) which is an anti-convulsant and antiepileptic drug, is used for neuropathic pain and as an addition therapy for partial seizures in adults. IUPAC name of Pregabalin is (3S)-3-(Aminomethyl)-5-methylhexanoic acid which shown in Figure 1.^{1,2} Its binding to the alpha2-delta subunit (voltage-gated calcium channels) may be involved for the anti-nociceptive and antiseizure effects of Pregabalin in animals. Etoricoxib (ETOR) is 5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine represented with the help of Figure 2. It is a selective cyclooxygenase 2 inhibitor with a non-steroidal

and anti-inflammatory properties used in the treatment of rheumatoid arthritis, osteoarthritis and acute gouty arthritis with low GI toxicity and is without effects on platelet function.^{3,5} On detailed literature survey, it was found that ETOR in dosage form are spectrophotometry, RP-HPLC and similarly for the estimation of PREG in dosage form are spectrophotometry, HPTLC, RP-HPLC.⁶⁻¹² Previously, some analytical methods for determining PREG individually or in combination with nortriptyline, tapentadol, amitriptyline, mecobalamin, and alpha lipoic acid were established. Since no

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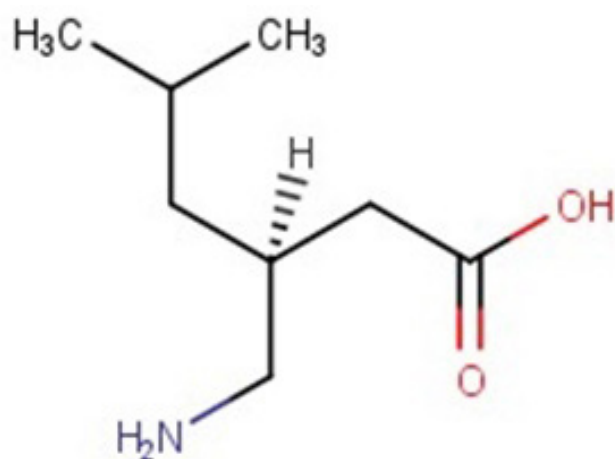


Figure 1: Structure of Pregabalin

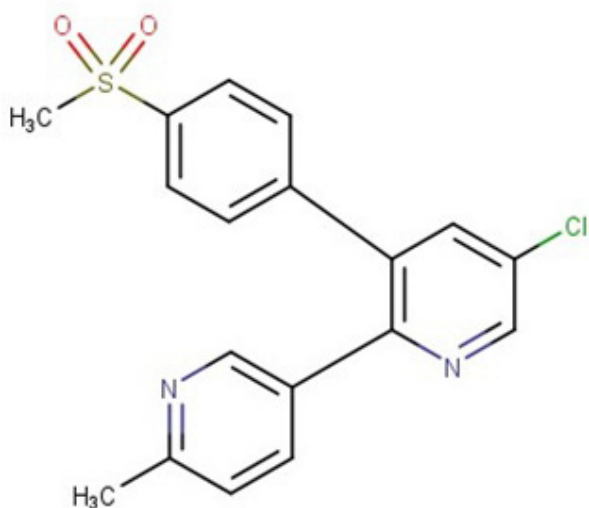


Figure 2: Structure of Etoricoxib

HPLC methods for simultaneous estimation of PREG and ETOR in a mixture of drugs have been published, the current study made an effective attempt to estimate both drugs using a simple, optimised, isocratic RP-HPLC method.¹³⁻¹⁷ The proposed method was developed and validated as per ICH guidelines ICH Q2 A and ICH Q2 B.¹⁸⁻²⁰

MATERIALS AND METHODS

Instrumental and analytical conditions

For the simultaneous estimation of drugs with an isocratic HPLC (Shimadzu®) having two LC-20AD pumps, with adaptable wavelength programmable SPD-M20A photo diode array detector, CBM-20A system controller and LC solution software of Shimadzu® with

the hypersil ODS-C₁₈ (250 mm × 4.6 mm, i.d.2.5 μm) columns and wavelength 215 nm was used for detection. The run time of the sample was found to be 15 min of 20 μl injection volume. The flow rate of an isocratic mobile phase was found to be 1 ml ml⁻¹ which was consisted of methanol: acetonitrile: phosphate buffer (pH 5) at the ratio of (40:20:20) as membrane filter of 0.45 μm porosity was used to filter the mobile phase was and same was degassed for further processing.

Reagents and chemicals

PRE and ETOR bulk drug was obtained from AKUMS drugs pharmaceutical Ltd, Haridwar, Utrkhand, India as a gift sample. ETOSHINE® NP brand of Sun Pharmaceutical Industries Ltd was obtained from local pharmacy containing Pregabalin (75mg) and Etoricoxib (60mg). Methanol, acetonitrile served by Merck (India) Limited. All other chemicals included in the study were of AR grade. A Millipore and Milli-Q system was utilized to obtain filtration and ultrapure water.

Preparation of Mobile Phase

The mobile phase used in this study was composed of methanol: acetonitrile: phosphate buffer (pH 5) at the ratio of 40:20:20 v/v, with isocratic elution. Filtration was done by 0.45-micron nylon membrane (Millipore) filter and an ultrasonic bath was used to degassed before further use to circumvent conflicts and clogging of the column due to small particles. Standard and sample preparations were achieved with methanol and acetonitrile as diluent. The mobile phase was screened based on system suitability.

Preparation of Standard Solution

The primary standard stock solution was prepared by dissolving 75 mg PRE and 60 mg ETOR in 100 ml volumetric flask in 75 ml in diluent and sonicated for 15 min with the help of sonicator for complete dissolve the drugs and after sonication volume make up 100 ml with the same solvent. Take the volume of primary standard stock solution (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml) and make up to 10 ml with diluent. The final concentration of PRE (10 to 105 μg ml⁻¹) and ETOR (6 to 84 μg ml⁻¹) was used for the study purpose. The final solution was filtered through a 0.45 μ nylon Millipore membrane filter.

Preparation of Sample Solution

20 tablets of Etoshine NP were accurately weighed and pulverized. The powder equivalent to 75 mg PRE was weighed and then transferred into a clean and dried 100 ml volumetric flask. The tablet powder was dissolved into the mobile phase (methanol: acetonitrile:

phosphate buffer (pH 5) at the ratio of 40:20:20 v/v) using sonication and then the volume was made up to 100 ml with same. Then it was filtered using 0.45 μ filter and diluted to get 7.5 and 6.0 μ g ml⁻¹ of PRE and ETOR respectively. The 20 μ l of working sample was injected through hypersil ODS-C₁₈ (250 mm \times 4.6 mm, i.d.2.5 μ m) column with the flow rate of 1 ml min⁻¹ and the eluents were measured at 215 nm.

RESULTS AND DISCUSSION

Method Development

The main objective of this study to develop and validate a simple, precise, reliable, rapid, more accurate, as well as least time-consuming High-Performance Liquid Chromatography (HPLC) method for simultaneous PRE and ETOR in API as well as in various formulations comply with ICH guideline. The method was optimized and validated by changing different parameter like mobile phase composition, pH, elution technique, flow rate and appropriate columns to achieve sharp peak, maximum theoretical plates, less tailing factor and short analysis time. A typical chromatogram found by the same condition mentioned above illustrated in Figure 3. The system suitability test is of importance in the validation of analytical technique as well as confirmation of resolution amongst numerous peaks of the interest. All perilous parameters (theoretical plates, retention time and tailing factor) in the study met complete acceptance every time. % RSD for peak area of six replicate was found to be 0.099 and 0.237 for PRE and ETOR respectively. % RSD for retention time was found to be 1.23 and 1.14 for PRE and ETOR. Tailing factor was under 2 and theoretical plate more than 2000 show that the method was met under condition during the validation.

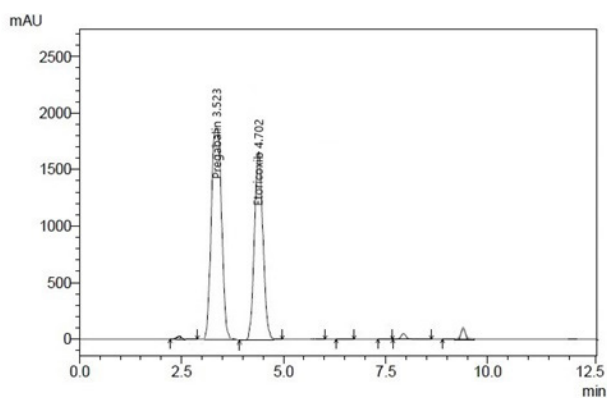


Figure 3: Chromatogram representing the separation of PRE and ETOR System suitability

Method Validation

Specificity

Good resolution and no interference from blank and excipients were observed that this method is specific for simultaneous analyzing of PRE and ETOR. The explanatory chromatogram did not show any other peak confirming to the specificity of this method.

Stress (forced decomposition) studies on formulation

In a 100 mL volumetric flask, a quantity of the powder equivalent to 75 mg of PRE and 60 mg of ETOR was transferred and added 40 mL of water, acid, base, and H₂O₂ solutions individually. The formulation was subjected to physico-chemical conditions in order to degrade 10% of the compound. The stressed samples were neutralised and diluted with mobile phase, then filtered through 0.45 filtered, sonicated for 10 minutes, and sufficiently diluted with mobile phase to have a final concentration of 7.5 μ g ml⁻¹ PRE and 6 μ g ml⁻¹ ETOR. Since the drugs peak was well differentiated even in the presence of degradation products, this stability-indicating approach for the determination of PRE and ETOR in tablet dosage form is unique. Overall, the data showed that the excipients and degradation products had no effect on the drug peak, suggesting that the process had the best selectivity. Based on the peak area shown in Figure 4, quantification was achieved using UV detection at 215 nm. The main goal of developing the chromatographic method was to isolate the degradation products obtained from stress studies from the drug peak.

Accuracy

Then recovery was estimated at 50 %, 100 % and 150 % of the selected concentrations. Three samples were prepared for each recovery level and recovery values for PRE and ETOR were found to be 99.53-101.70 % and 99.70 to 101.29 %, respectively as described in Table 1 and Figure 5.

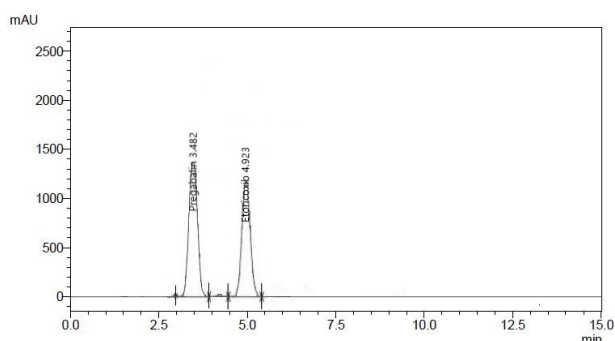
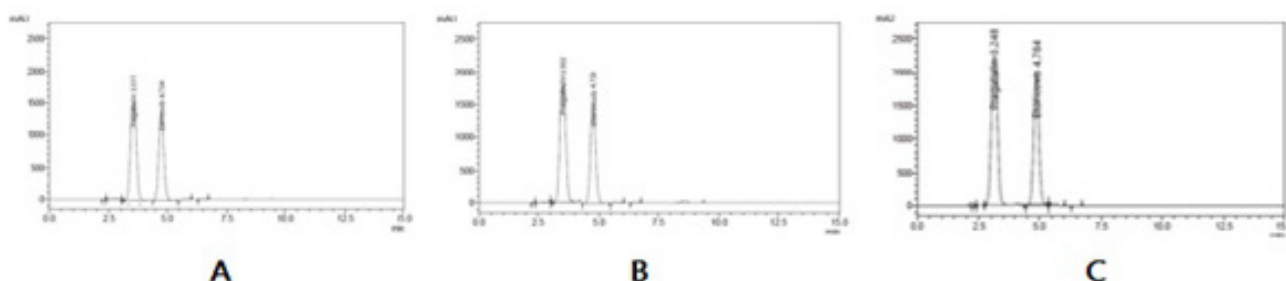


Figure 4: Chromatogram of acid degradation

Table 1: Accuracy study of PRE and ETOR.

Name of the drug	Spiked amount	Amount of drug (Tablet) μg	Amount of drug (Standard) μg	Total Drug (μg)	Total Found (μg) Mean \pm SD	% RSD	% Recovery
PRE	50%	15	5	20	20.34 \pm 0.320	1.58	101.70
	100%	15	10	25	25.16 \pm 0.336	1.34	100.64
	150%	15	15	30	29.86 \pm 0.391	1.31	99.54
ETOR	50%	12	5	17	17.22 \pm 0.164	0.95	101.30
	100%	12	10	22	22.22 \pm 0.370	1.67	101.00
	150%	12	15	27	26.92 \pm 0.443	1.65	99.70

**Figure 5: Accuracy study of PRE and ETOR at 50% (A), 100% (B), and 150% (C). Precision****Table 2: System and Method precision study for PRE and ETOR.**

Injection Number	System Precision		Method Precision	
	Peak areas		% Assay	
	PRE	ETOR	PRE	ETOR
1	2245768	1945874	101.23	102.34
2	2194854	1894873	99.34	99.27
3	2189463	1934574	99.45	99.45
4	2249463	1946286	100.45	100.35
5	2276623	1967975	99.57	99.58
6	2224523	1956783	101.45	101.45
Mean	2230116	1941061	100.25	100.41
SD (\pm)	33792.20	25288.00	0.93	1.25
RSD (%)	1.52	1.30	0.93	1.24
Acceptance criteria	% RSD should not be more than 2			

For the precision study, overall % RSD of system, method precision was found to less than 2 which indicate that the precision achieved successfully in the limit. The results are given in Table 2.

Intermediate precision (Ruggedness)

Interday and interday fluctuations were achieved using six replicate injections of standard and sample solutions, those were prepared and analyzed by different analysts on three different days over one week. Ruggedness was also expressed in terms of percentage RSD and statistical analysis revealed no significant difference between obtained results retaining different analyst. Interday and interday precision % RSD was found to be under significant value and showed in Table 3.

Linearity and range

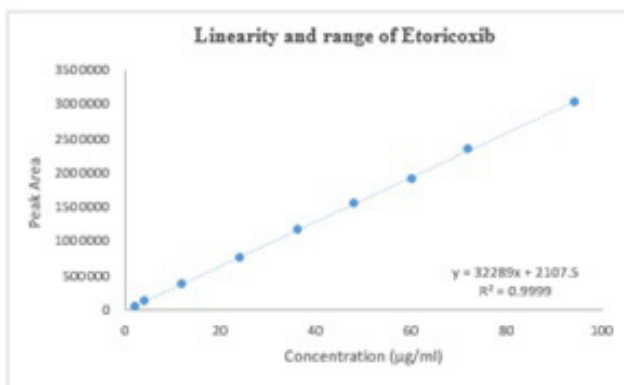
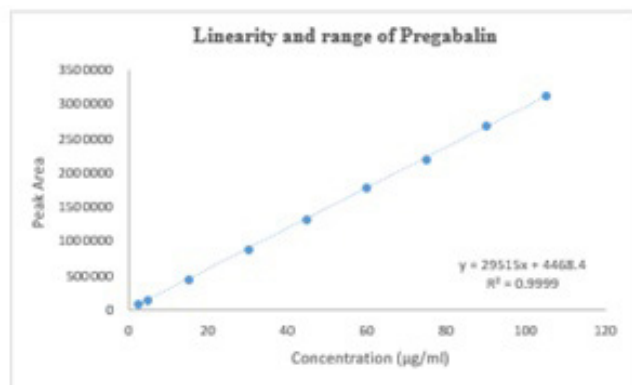
The method for linearity was evaluated from the calibration curve data and the linear value was observed between range from 2.5- 105 $\mu\text{g ml}^{-1}$ and 2.0- 94 $\mu\text{g ml}^{-1}$ for PRE and ETOR respectively. During simultaneous determination, the calibration curves were linear and the correlation coefficients (r^2) were found to be 0.999 for both drugs which are shown in Figure 6.

Robustness

The robustness of the analytical procedure was established and developed by changing of flow rate, pH, wavelength and mobile phase composition. The robustness data of both drugs have been incorporated in Table 4.

Table 3: Intraday and Interday precision study for PRE and ETOR.

Concentration	Intraday Precision		Interday Precision (Day 1,2,3)	
	Area of PRE	Area of ETOR	Area of PRE	Area of ETOR
PRE 60 ($\mu\text{g ml}^{-1}$) and ETOR 48 ($\mu\text{g ml}^{-1}$)	1758302	1566838	1754632	1581836
	1748457	1576385	1734653	1573330
	1747658	1554392	1742382	1562364
Mean	1751472.3	1565872	1743889	1572510
SD	5928.14	11028.30	10074.39	9761.86
%RSD	0.34	0.70	0.58	0.62
PRE 75($\mu\text{g ml}^{-1}$) and ETOR 60 ($\mu\text{g ml}^{-1}$)	2173254	1956233	2175284	2041483
	2153728	1945732	2166345	1983747
	2099463	1944599	2184735	1987743
Mean	2142148.30	1948855	2175455	2004324.33
SD	38234.07	6414.89	9196.19	32242.32
%RSD	1.78	0.33	0.42	1.61
PRE 90 ($\mu\text{g ml}^{-1}$) and ETOR 72 ($\mu\text{g ml}^{-1}$)	2637685	2347568	2597393	2297473
	2695736	2394738	2603726	2347422
	2637646	2374432	2589302	2337374
Mean	2657022.30	2372246	2596807	2327423
SD	33527.02	23660.86	7229.83	26419.55
%RSD	1.26	1.00	0.28	1.14

**Figure 6: Linearity and range of PRE and ETOR****Detection and quantitation limits**

The limit of detection and limit of quantitation were found to be 1.89, 5.73 $\mu\text{g ml}^{-1}$ for PRE and 2.26, 6.84 $\mu\text{g ml}^{-1}$ for ETOR, respectively.

Assay of tablets

Currently, in the market, only one manufacturer brand name ETOSHINE[®] NP (Sun Pharmaceutical Industries

Ltd) available and its percentage assay was found to be 101.34 % and 102.65 % for PRE and ETOR, respectively.

CONCLUSION

A new RP-HPLC method was developed and validated for simultaneous estimation of PRE and ETOR in bulk and tablet dosage form. The proposed novel, fast, accurate and precise method was successfully applied in

Table 4: Robustness of PRE and ETOR.

Condition		PRE			ETOR		
		% RSD	Tailing Factor	% Recovery	% RSD	Tailing Factor	% Recovery
Change in Flow rate	Normal Flow rate (1.0 ml per minute)	0.78	1.09	100.18	0.87	1.11	100.67
	Change in flow rate (0.8 ml per minute)	0.89	1.11	98.57	0.91	1.14	100.89
	Change in flow rate (1.2 ml per minute)	0.78	1.02	99.45	0.78	1.32	100.56
Change in pH	Normal Condition (5.0)	0.23	1.10	99.78	0.23	1.04	99.45
	Oven temperature (4.5)	0.45	1.23	100.98	0.47	1.21	100.12
	Oven temperature (5.5)	0.46	1.31	100.45	1.02	1.09	100.18
Change in Wave Length	Normal: Wave Length 215nm	0.34	1.08	99.46	0.67	1.00	100.67
	Wave Length 210nm	0.56	1.10	101.78	0.78	1.15	100.45
	Wave Length 220nm	0.67	1.31	98.99	0.23	1.35	100.18
Change composition of mobile phase	Normal Condition (Methanol: ACN: Buffer) (50:20:30)	0.18	1.02	99.18	0.45	1.19	101.11
	(Methanol: ACN: Buffer) (40:30:30)	0.81	1.03	99.87	1.01	1.11	100.23
	(Methanol: ACN: Buffer) (60:10:30)	0.64	1.31	99.46	0.34	1.26	99.89

pharmaceutical industries for the routine quantitative analysis of new fixed-dose combinations approved by DCGI in December 2019.

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CONFLICT OF INTEREST

The authors have no conflict of interests.

ABBREVIATIONS

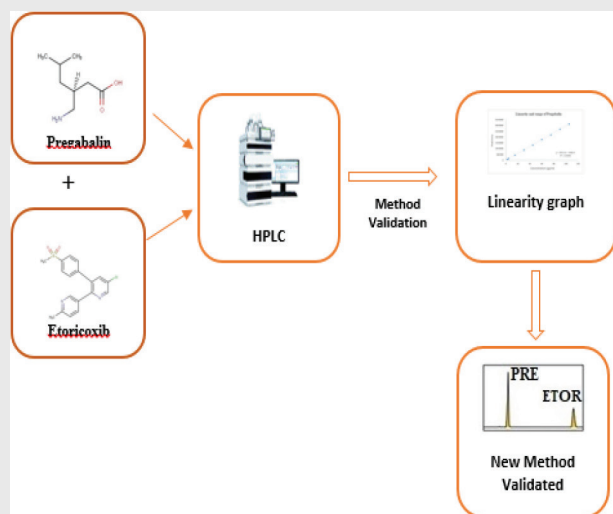
PRE: Pregabalin; **ETOR:** Etoricoxib; **ICH:** International Council for Harmonisation; **µl:** Microliter; **cm:** Centimeter; **HPLC:** High-Pressure Liquid Chromatography; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **nm:** Nanometer; **SD:** Standard Deviation.

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PICTORIAL ABSTRACT



SUMMARY

For quantification of Pregabalin and Etoricoxib with HPLC methods was made and it is applicable for the routine analysis in pharmaceutical industries.

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Dr. Amit Chaudhary, Dean, School of Pharmacy, Abhilashi University, Mandi, Himachal Pradesh, India. He had 18 Years experience in research and teaching field. He has published more than 45 national and international research article in reputed journals. He has a member of various professional bodies including APTI, IPGA etc



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