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DEVELOPMENT AND VALIDATION OF RP-HPLC FOR SIMULTANEOUS ESTIMATION OF PREGABALIN AND ETORICOXIB IN PHARMACEUTICAL TABLET DOSAGE FORM

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Keywords:

Pregabalin, Etoricoxib, RP-HPLC, ICH guidelines

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ABSTRACT: A simple, precise, rapid, linear and economical RP-HPLC method was developed and validated for simultaneous estimation of Pregabalin and Etoricoxib in pharmaceutical tablet dosage form. The determination was performed on Phenomenex C18 (250 mm × 4.6 mm, ID, 5µ) column at 30 ºC temperature using a mobile phase Acetonitrile: Water (55:45 %v/v) at a flow rate of 0.8 ml/min was employed. The RP-HPLC detection wavelength was 200 nm and 235 nm for Pregabalin and Etoricoxib, respectively. This method obeys Beers law in employed concentration range 2.4-12 µg/ml and 0.8-4 µg/ml for Pregabalin and Etoricoxib, respectively. The correlation coefficient of Pregabalin and Etoricoxib was found to be 0.9993 and 0.9997, respectively. The retention time was 2.680 and 7.383 minutes. The percentage RSD for accuracy and precision was found to be less than 2%. The method was validated as per ICH guidelines for its selectivity, specificity, system suitability, linearity, range, precision, accuracy, LOD, LOQ, robustness, assay. The method was successfully employed for routine quality control analysis of Pregabalin and Etoricoxib in pharmaceutical formulation.

INTRODUCTION: Pregabalin is 3-isobutyl derivative of gamma-aminobutyric acid (GABA) – an inhibitory neurotransmitter. It may be used in the treatment of neuropathic pain, postherpetic neuralgia, and fibromyalgia, among other conditions. It has anticonvulsant, anti-epileptic, anxiolytic and analgesic activity [1, 2]. Etoricoxib is a synthetic, nonsteroidal anti-inflammatory drug (NSAID) with antipyretic, analgesic and potential antineoplasmic properties.

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Etoricoxib is a member of the class of bipyridines that is [2, 3] 'bipyridine, which is substituted at 3, 5, and 6' positions by 4-(methylsulphonyl) phenyl, chlorine, and methyl groups, respectively. Etoricoxib is a new COX-2 selective inhibitor [3, 4].

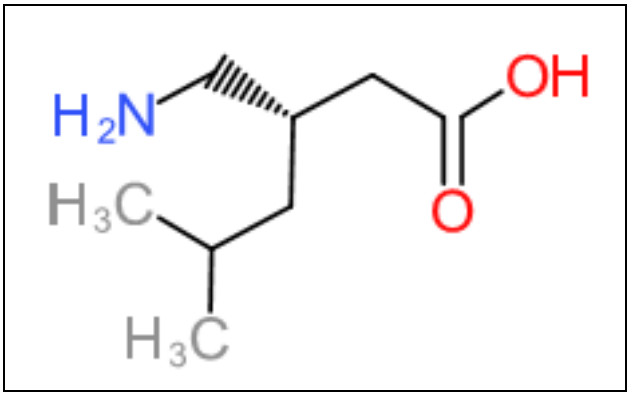

FIG. 1: PREGABALIN

The literature review explains that there are various methods available for the determination of Pregabalin and Etoricoxib individually or in combination with other drugs. There is no method that has been reported for the estimation of Pregabalin and Etoricoxib simultaneously [5-12].

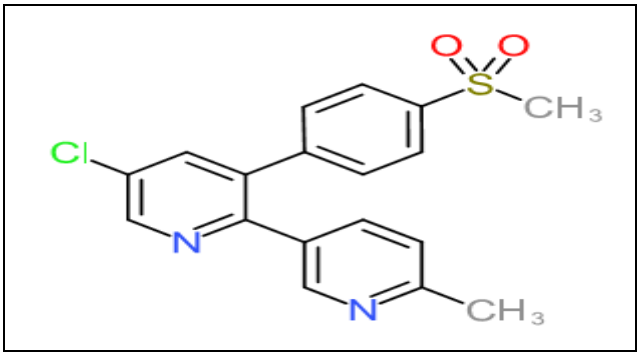

FIG. 2: ETORICOXIB

MATERIALS AND METHODS: Standard of Pregabalin and Etoricoxib were purchased from yarrow chem products Mumbai. Water, acetonitrile was obtained from merk Ltd. India.

Instrumentation: Schimadzu HPLC system used for method development and validation. The separation was achieved on Phenomenex C18 (250 mm × 4.6mm, ID, 5µ) column. Digital Ultrasonic Cleaner (LMUC 2), Electronic Balance (Afcoset FX-300), Vacuum Filtration Unit, PH Meter (M PH System -361), Hot Air Oven (IS: 3119), pipettes, volumetric flask used during the study.

Chromatographic Conditions:

Mobile Phase: Acetonitrile: Water 55:45 v/v

Diluent: Mobile phase was used as diluent

Column: Phenomenex C18

Flow Rate: 0.8 ml/minute

Detection of Wavelength: 200 nm for Pregabalin and 235 nm for Etoricoxib

Injection Volume: 20 µl

Column Temperature: 30 ºC

Run Time: 10 min

Selection of Mobile Phase: Standard Solution of Pregabalin and Etoricoxib was injected into the HPLC system and run -in a different solvent system. Different mobile phase like acetonitrile and water in the varying proportion of mobile phase components, varying condition of pH were tried in order to obtain desired system suitability parameter for the Pregabalin and Etoricoxib. Acetonitrile and water in a ratio of 55:45% v/v were chosen as the mobile phase, which has good resolution & acceptable peak parameters.

Preparation of Mobile Phase: Mobile phase was prepared by mixing acetonitrile and water in the ratio of 55:45%v/v. It was then filtered through 0.45 µm membrane filter paper using vacuum filtration assembly and then sonicate on an ultrasonic water bath for 20 min.

Preparation of Standard Stock Solution of Pregabalin: Standard stock solution of pregabalin prepared by transferring 2 mg drug in 5 ml volumetric flask.

Added mobile phase in sufficient amount and shake vigorously. Make up the final volume up to 5 ml with mobile phase that is acetonitrile: water 55:45%v/v. Sonicate on an ultrasonic water bath for 20 min.

Preparation of Standard Stock Solution of Etoricoxib: Standard stock solution of Etoricoxib prepared by transferring 2 mg of drug in a 5ml volumetric flask. Added mobile phase in sufficient amount and shake vigorously. Make up the final volume upto 5 ml with mobile phase that is acetonitrile: water 55:45%v/v. Sonicate on an ultrasonic water bath for 20 min.

Preparation Of sample Solution: One tablet weighed and powdered. An accurately weighed powder equivalent to 2 mg of pregabalin and transferred into 5 ml volumetric flask. Dissolved in mobile phase that is acetonitrile: water 55:45%v/v and sonicate on ultrasonic water bath for 20 minutes. Filter through syringe filter (0.22 µm).

Selection of Wavelength: From standard stock solutions of pregabalin and etoricoxib, further dilutions were done using mobile phase and scanned over the range 190-400 nm. The spectrum was obtained for both drugs. It was observed that the drug showing maximum absorbance at 200 nm for pregabalin and 235 nm for etoricoxib by UV spectrophotometer.

System Suitability: The system suitability studies are concerned as per ICH guidelines.

The parameter like capacity factor, tailing factor, asymmetry factor & number of theoretical plate, and resolution were calculated.

Validation of Analytical Methods: A method was validated as per ICH Q2 (R1) guidelines.

Specificity:

a) Pregabalin and Etoricoxib Identification: Solutions of standard and sample were prepared and injected into the HPLC system. The chromatograms were recorded.

b) Blank Interference: A study to establish the interference of blank was conducted. Diluent injected into HPLC system and chromatogram recorded.

Linearity: Linearity was tested for both drugs *i.e.* pregabalin and etoricoxib, for the concentration 2.4 -12 µg/ml of pregabalin and 0.8-4.0 µg/ml of etoricoxib. The response factors were plotted against corresponding concentration of pregabalin and etoricoxib to obtain the calibration curve.

Accuracy: To checked the accuracy of the method, recovery studies were carried out by adding the standard drug to at three different level 80%, 100%, 120% and amount recovered and % RSD was calculated for both the drug.

Precision: Precision of the developed method was demonstrated by intra and inter-day variation to absorbance for validation.

Intraday Precision: A concentration that is 7.2 µg/ml of pregabalin and 4.8 µg/ml of Etoricoxib injected into HPLC system, six replicate was taking to obtain chromatogram. Peak was recorded. From which SD, % RSD, and mean were calculated.

Inter-day Precision: The procedure was carried out for 3 days with freshly prepared solution from stock solution a concentration that is 7.2 µg/ml of Pregabalin and 4.8 µg/ml of Etoricoxib in six replicate at 24 h interval. Peak was recorded. From which SD, %RSD and mean was calculated.

Robustness: Standard stock solution of Pregabalin (400 µg/ml) and Etoricoxib (400 µg/ml). Pipetting 0.12 ml of standard stock solution of Pregabalin in to 5 ml volumetric flask to acquire 9.6 µg/ml of pregabalin and 0.04 ml of standard stock solution of Etoricoxib into 5 ml volumetric flask to acquire 3.2 µg/ml of Etoricoxib, respectively. Then robustness was performed by a small, deliberate change in flow rate and wavelength and the calculating the % RSD of Pregabalin and Etoricoxib, respectively.

Limit of Detection: The linearity study was carried out six times. The detection limit of Pregabalin and Etoricoxib was calculated by using average slope and standard deviation of intercept.

Limit of Quantitation: The linearity study was carried out six times. The quantification limit of Pregabalin and Etoricoxib was calculated by using average slope and standard deviation of intercept.

Assay: Analysis was carried out as mentioned under procedure was repeated three times. A sample solution of 9.6 µg/ml was injected into the HPLC system, and the area was recorded. Concentration and percentage purity were determined from the linearity equation.

RESULTS AND DISCUSSION:

Analytical Method Development: Several trials were made to get good peak resolution, acceptable plate count & tailing factor. Method was optimized for the simultaneous estimation of pregabalin and etoricoxib from pharmaceutical tablet dosage form.

Optimized Method:

Chromatographic Conditions:

Mobile Phase: Acetonitrile: Water 55:45 v/v

Diluent: Mobile phase was used as diluent

Column: Phenomenex C18

Flow Rate: 0.8 ml/minute

Detection of Wavelength: 200 nm for Pregabalin and 235 nm for Etoricoxib

Injection Volume: 20µl

Column Temperature: 30 ºC

Run Time: 10 min

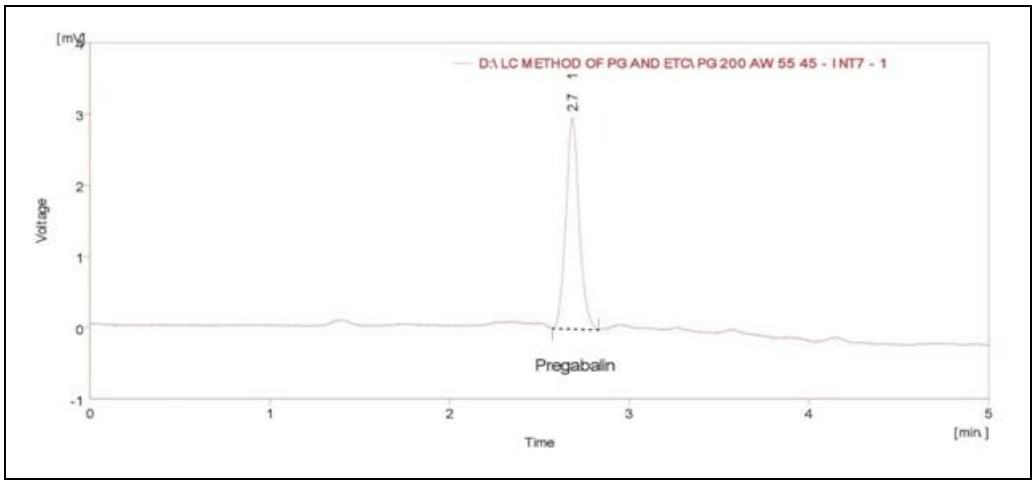

FIG. 3: STANDARD CHROMATOGRAM OF PREGABALIN

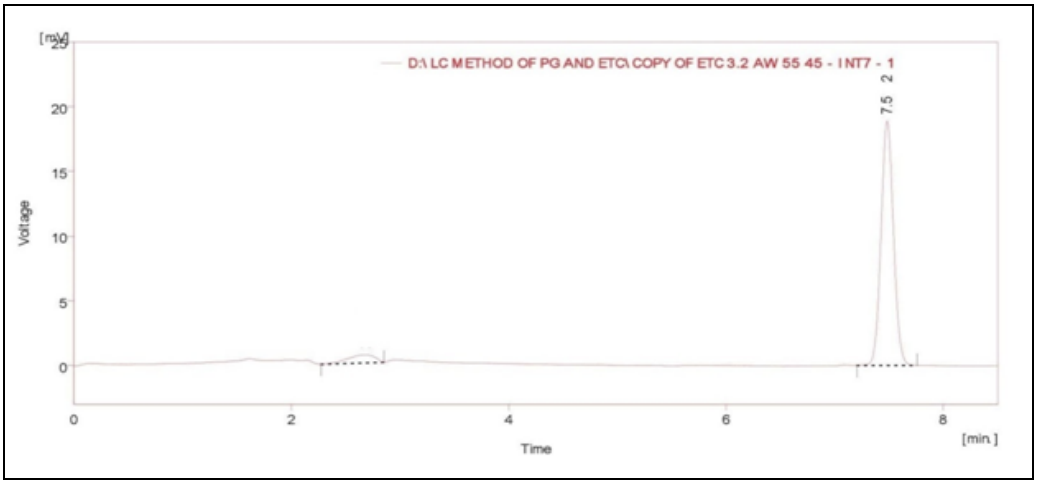

FIG. 4: STANDARD CHROMATOGRAM OF ETORICOXIB

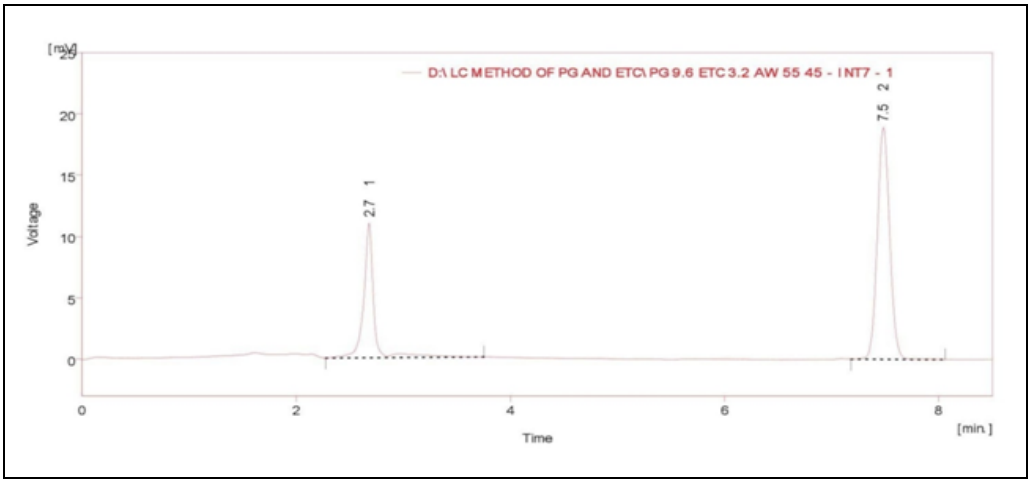

FIG. 5: STANDARD CHROMATOGRAM OF OPTIMISED METHOD

The retention time for Pregabalin and Etoricoxib was found to be 2.680 and 7.483, respectively. The resolution between two analytes is good. No peak asymmetry was observed. No other impurity interference was seen. All the results were found to be within acceptance criteria. Hence the method was considered to be optimized.

Chromatogram and System Suitability Parameter of Pregabalin and Etoricoxib: The column was equilibrated with the mobile phase. Working standard solution was injected into the HPLC system. All the system suitability parameters are within range and satisfactory as per ICH guidelines.

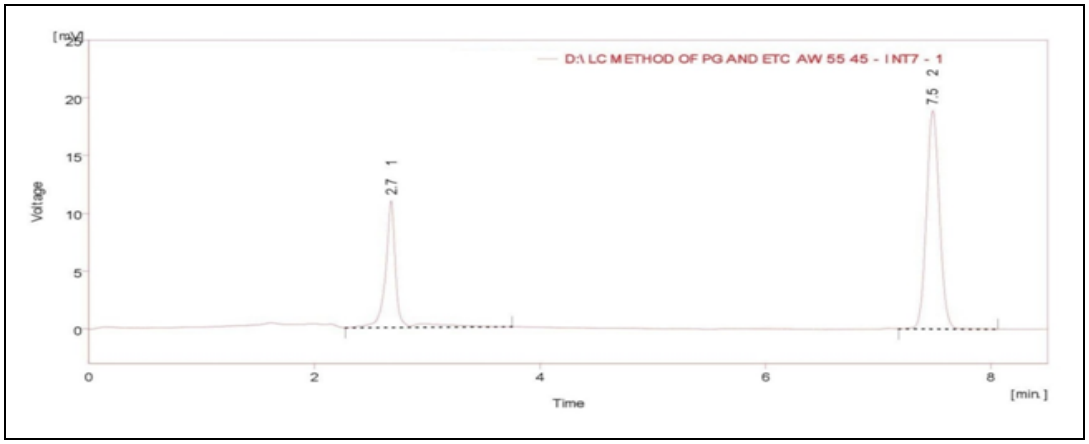

FIG. 6: SYSTEM SUITABILITY CHROMATOGRAM OF PREGABALIN AND ETORICOXIB

TABLE 1: SYSTEM SUITABILITY STUDIES OF PREGABALIN AND ETORICOXIB

Property	Pregabalin	Etoricoxib
Retention Time (t_R)	2.680(≥1)	7.383(≥1)
Theoretical Plate (N)	6316(≥2000)	19453(≥2000)
Tailing Factor	1.250(≥2)	1.074(≥2)

Method Validation:

1. Specificity: No interference at the retention time, which shows that the method was specific. The chromatograms for specificity studies (standard) are represented as **Fig. 4-6**.

2. Linearity: Five linear concentrations of Pregabalin (2.4-12 µg/ml) and Etoricoxib (0.8-4.0 µg/ml) were prepared. The calibration curve was plotted using concentration against peak area. The procedure was repeated for six times.

Regression equation of Pregabalin and Etoricoxib was found to be, y = 2.0293x + 0.8062 and y = 59.689x + 1.085. Correlation coefficient for the Pregabalin and Etoricoxib was 0.9993 and 0.9997, respectively.

TABLE 2: CALIBRATION DATA OF PREGABALIN AND ETORICOXIB

S. no.	Conc. of Pregabalin (µg/ml)	Peak Area	Conc. of Etoricoxib (µg/ml)	Peak Area
1	2.4	5.786	0.8	48.528
2	4.8	10.260	1.6	96.826
3	7.2	14.321	2.4	146.584
4	9.6	20.276	3.2	190.112
5	12	25.275	4.0	240.640

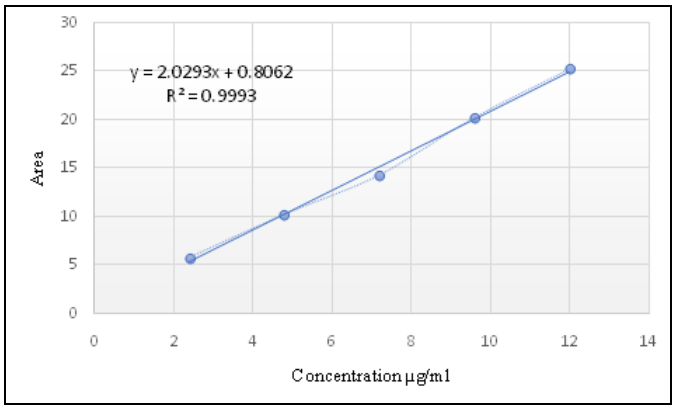

FIG. 7: CALIBRATION CURVE OF PREGABALIN (CONC. V/S PEAK AREA)

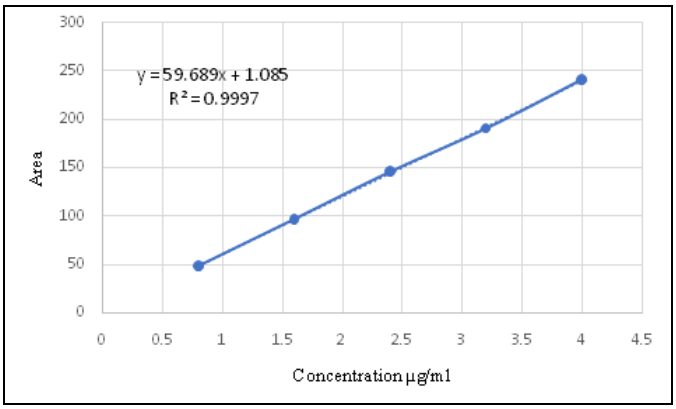

FIG. 8: CALIBRATION CURVE OF ETORICOXIB (CONC. V/S AREA)

3. Range: The concentration range of Pregabalin and Etoricoxib was found to be,

Pregabalin = 2.4-12µg/ml

Etoricoxib = 0.8-4 µg/ml

4. Precision:

Intraday Precision: Intraday precision was performed and % RSD for Pregabalin and Etoricoxib were found to be 0.30 and 0.05 respectively.

TABLE 3: INTRADAY PRECISION OF PREGABALIN AND ETORICOXIB

S. no.	Pregabalin (7.2 µg/ml)	Etoricoxib (4.8 µg/ml)
1	15.218	282.369
2	15.219	282.478
3	15.217	282.587
4	15.199	282.280
5	15.101	282.235
6	15.209	282.439
Mean	15.209	282.421
SD	0.0461	0.130
%RSD	0.30	0.05

Inter-day Precision: Inter-day precision was performed and % RSD for Pregabalin and Etoricoxib were found to be 0.53 and 0.13 respectively.

TABLE 4: INTER-DAY PRECISION OF PREGABALIN AND ETORICOXIB

S. no.	Pregabalin			Etoricoxib		
	Day I	Day II	Day III	Day I	Day II	Day III
1	15.315	15.484	15.326	282.352	282.389	282.567
2	15.218	15.326	15.489	282.551	282.450	282.489
3	15.321	15..475	15.212	282.410	282.557	282.399
4	15.363	15.411	15.342	282.489	282.453	282.490
5	15.431	15.489	25.380	282.387	282.489	282.489
6	15.361	15.310	15.281	282.376	282.370	282.989
Mean	15.334	15.416	15.338	282.427	282.45	282.60
SD	0.070	0.081	0.093	0.0765	0.067	0.217
% RSD	0.46	0.53	0.61	0.03	0.02	0.08

5. Accuracy: Three different level 80%, 100%, 120% concentration prepared and injected into HPLC system in triplicate to obtain chromatogram. Amount recovered and % recovery was displayed in table.

TABLE 5: ACCURACY DATA FOR PREGABALIN

Level %	Theoretical conc.(µg/ml)	Peak Area	Mean	SD	%RSD	Estimate conc.	% Recovery
80%	4.8	10.429 10.379 10.529	10.440	0.076	0.73	4.74	98.75
100%	6	12.689 12.723 12.896	12.769	0.11	0.87	5.89	98.16
120%	7.6	15.876 15.973 16.123	15.990	0.12	0.78	7.48	98.42

TABLE 6: ACCURACY DATA FOR ETORICOXIB

Level %	Theoretical conc.(µg/ml)	Peak Area	Mean	SD	% RSD	Estimate conc.	% Recovery
80%	4.8	285.123 285.896 286.478	285.832	0.67	0.24	4.77	99.37
100%	6	353.963 354.654 353.879	354.165	0.42	0.37	5.89	98.16
120%	7.6	446.728 447.321 448.897	447.648	1.12	0.25	7.48	98.42

6. Robustness: Small deliberate change in method like flow rate, wavelength is made but there was no recognized change in the result and are within range as per ICH guidelines.

TABLE 7: ROBUSTNESS DATA OF PREGABALIN AND ETORICOXIB

S. no.	Robustness Condition	Pregabalin %RSD	Etoricoxib %RSD
1	Flow rate minus (0.6 ml/min)	1.14	0.2
2	Flow rate plus (1 ml/min)	1.11	0.11
3	Wavelength minus (198 and 233 nm)	0.27	0.12
4	Wavelength plus (204 and 237 nm)	0.77	0.06

7. Limit of Detection: The limit of detection was calculated by using formula, LOD = 3.3× SD of intercept/ slope, was found to be 0.992, 0.0837 µg/ml for Pregabalin and Etoricoxib, respectively.

8. Limit of Quantification: The limit of quantification were calculated by using the formula, LOQ = 10×SD of intercept/slope, which was found to be 2.98, 0.253 µg/ml for Pregabalin and Etoricoxib, respectively.

9. Assay: The average % assay was calculated and found to be 99.94% and 100.44% for Pregabalin and Etoricoxib.

TABLE 8: % ASSAY DATA OF PREGABALIN AND ETORICOXIB

S. no.	Amount present (mg/tab)		Amount obtained (mg/tab)		% Assay	
	Pregabalin	Etoricoxib	Pregabalin	Etoricoxib	Pregabalin	Etoricoxib
1	75	60	74.95	60.31	99.94	100.52
2	75	60	75	60.31	100	100.52
3	75	60	75	60	100	100
4	75	60	74.99	60.31	99.99	100.52
5	75	60	75	60.34	100	100.57
6	75	60	74.84	60.31	99.79	100.52
		Mean			99.94	100.44
		SD			0.0897	0.217
		% RSD			0.09	0.22

CONCLUSION: A new method of analysis is developed for simultaneous estimation of Pregabalin and Etoricoxib drugs in pharmaceutical tablet dosage form by the RP-HPLC method.

The analytical procedure was validated as per ICH Q2B guidelines and shown to be simple, accurate, precise, and specific. The method was validated for specificity, linearity, range, precision, accuracy, robustness, LOD, LOQ, assay.

In the case of RP-HPLC, Pregabalin and Etoricoxib were eluted on Phenomenex C18 (250 mm × 4.6 mm, ID, 5 µ) column. The mobile phase consists of acetonitrile: water (55:45v/v) which filtered through membrane filter paper using vacuum filtration assembly and sonicate to degas and delivered at a flow rate 0.8 ml/min at 30 ºC.

The retention time of Pregabalin and Etoricoxib was 2.680 and 7.383 minutes. A calibration curve was linear. The percentage RSD (%RSD) for precision and accuracy of the method was found to be less than 2%. The low % RSD values for recovery indicate that the method was found to be accurate. Good agreement seen in the assay result of pharmaceutical formulation by the developed method. Hence, it can be concluded that the proposed method using HPLC can be regarded as simple, fast reproducible, and sensitive methods for simultaneous estimation of Pregabalin and Etoricoxib in the combined dosage form. Hence, this method can be used for the in-process evaluation in pharmaceutical manufacturing firms and routine quality control of these drugs in drug testing laboratories.

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CONFLICTS OF INTEREST: There is no conflict of interest.

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