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# DEVELOPMENT AND VALIDATION OF NEW STABILITY INDICATING ANALYTICAL METHOD FOR THE SIMULTANEOUS DETERMINATION OF PREGABALIN AND ETORICOXIB IN PHARMACEUTICAL FORMULATION BY USING RP-HPLC

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**ABSTRACT**

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Pregabalin and Etoricoxib in pharmaceutical dosage form. Chromatographic separation of Pregabalin and Etoricoxib was achieved on Waters Alliancee2695by using X-Terra RP-18(150 mm X4.6mm, 3.5µm) column and the mobile phase containing acetonitrile and 0.1% triethyl amine in the ratio of 50:50% v/v. The flow rate was 1.0 mL/min; detection was carried out by absorption at 214nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Pregabalin and Etoricoxib were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements was less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, precise, accurate and robust method for quantitative analysis of Pregabalin and Etoricoxib study of its stability.

**Keywords:** Pregabalin, Etoricoxib, HPLC, Validation.

## INTRODUCTION

Pregabalin (Fig. 1) is an anticonvulsant drug used to treat neuropathic pain conditions and fibromyalgia and for the treatment of partial onset seizures in combination with other anticonvulsants[1]. Chemically it is (S)-3-(aminomethyl)-5-methylhexanoic acid. Pregabalin subtly reduces the synaptic release of several neurotransmitters, apparently by binding to alpha2-delta subunits, and possibly accounting for its actions *in vivo* to reduce neuronal excitability and seizures[2].

Etoricoxib (Fig. 2) is used to relieve moderate post-surgical dental pain as a short-term treatment and inflammatory and painful symptoms of various forms of arthritis[3]. Chemically it is 5-chloro-2-(6-methylpyridin-3-yl)-3[4(trideuterio methyl sulfonyl) phenyl] pyridine. Etoricoxib is a selective COX-2 inhibitor and selectively inhibits isoform 2 of cyclo-oxigenase enzyme (COX-2), preventing production of prostaglandins (PGs) from arachidonic acid[4].

Literature survey reveals that few HPLC methods have been reported for estimation of these drugs alone as well as combination with other drugs in pharmaceutical dosage forms. The present work is aimed to develop and validate a new stability indicating RP-HPLC method for simultaneous estimation of Pregabalin and Etoricoxib in pharmaceutical dosage form in accordance with ICH guidelines[5,6].

## MATERIALS AND METHODS

### MATERIALS

Pregabalin and Etoricoxib pure drugs (API) were procured from Shree Icon Pharmaceutical Laboratories, Vijayawada, India. The marketed formulation of Pregabalin and Etoricoxib tablets (EBOV PG contains 75 mg of Pregabalin and 60mg of Etoricoxib) were purchased from local market. Acetonitrile, triethyl amine and HPLC grade water were obtained from Rankem Chemicals Ltd., Mumbai, India.

### Instrumentation

The analysis of drugs was carried out on Waters Alliance e2695 separation module HPLC system with PDA Detector at 214nm on X-Terra RP-18 (150 mm X 4.6 mm, 3.5 µm). The instrument is equipped with auto injector with 10 µL sample loop. A 10 µL hamilton syringe was used for injecting the samples. Data was analyzed by using Empower 2 software. A double-beam Schimadzu UV-1800 UV-Visible spectrophotometer was used for measuring absorbance for Pregabalin and Etoricoxib solutions. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Sartorius balance was used for weighing the materials.

### Mobile phase

A mobile phase consisting of mixture of acetonitrile and 0.1% triethyl amine in the ratio of 50:50% v/v was prepared.

### Diluent

Mobile phase used as diluent.

### Preparation of standard stock solution

Accurately weigh and transfer 75 mg of Pregabalin and 60 mg of Etoricoxib working standards into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it completely and make volume upto the mark with the same solvent to get stock solution. Further pipette 5 mL of the above stock solutions into a 50 mL volumetric flask and dilute upto the mark with diluent (75µg/mL of Pregabalin and 60 µg/mL of Etoricoxib).

### Preparation of sample stock solution

Accurately weighed and transfer 228 mg of Pregabalin and Etoricoxib sample into a 100 mL clean dry volumetric flask, add diluent and sonicate it upto 30 minutes to dissolve and centrifuge for 30 minutes to dissolve it completely and make volume upto the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter to get stock solution. Further pipette 5 mL of the above stock solutions into a 50 mL volumetric flask and dilute upto the mark with diluents (75 µg/mL of Pregabalin and 60 µg/mL of Etoricoxib).

### Procedure

The column was maintained at a temperature of $25^0$C. The run time was set at 6 minutes. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solutions. Inject 10 µL of the standard and sample solutions six times into the chromatographic system at a flow rate of 1.0 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed.

## METHOD VALIDATION

### Linearity

Several aliquots of standard solutions of Pregabalin and Etoricoxib were taken in six different 10 mL volumetric flasks and diluted upto the mark with diluent such that the final concentrations were in the range of 18.75-112.50 µg/mL for Pregabalinand 15-90 µg/mL for Etoricoxib. The above solutions were injected into the HPLC system keeping the injection volume constant. The drugs were eluted with UV detector at 214 nm, peak areas was recorded for all the peaks. The linearity curves were constructed by plotting concentration of the drugs against peak areas. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of drug in tablet dosage form.

### Precision

Precision for Pregabalin and Etoricoxib was determined in terms of system precision, repeatability and intermediate precision. Every sample was injected six times. The measurements for peak areas were expressed in terms of % RSD.

### Accuracy

The accuracy of the method was assessed by recovery studies of Pregabalin and Etoricoxib at three concentration levels 50%, 100% and 150%. Fixed amount of pre-analyzed sample was spiked with known amount of Pregabalin and Etoricoxib. Each level was repeated three times. The % recovery of Pregabalin and Etoricoxibwere calculated.

**System suitability**
The system suitability parameters like retention time, theoretical plate count, tailing factor and resolution were evaluated by six replicate analysis of Pregabalin and Etoricoxib and compared with standard values. The acceptance criteria for theoretical plates number (N) at least 3000 per each peak, tailing factors not more than 2.0 and % RSD of peak areas not more than 2% for Pregabalin and Etoricoxib.

**Limit of detection and limit of quantitation**
The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions of Pregabalin and Etoricoxib using the developed HPLC method. LOD and LOQ were estimated from signal-to-noise ratio.

**Robustness**
The robustness of the method was determined by making small deliberatechanges in method like variation of flow rate, mobile phase ratio and temperature.

**Assay**
Standard preparations are made from the bulk drug and sample preparations are made from formulation. Both standard and sample solutions were injected in six homogeneous samples. 10 µL of sample solution was injected into the chromatographic system and measure the peak areas of Pregabalin and Etoricoxib and calculate the % assay by using the formula. The results were compared with the label claim of Pregabalin and Etoricoxib in tablet dosage form.

**DEGRADATION STUDIES**
**Acid degradation**
Pipette 5 mL of above stock solution into a 50 mL volumetric flask and 3 mL of 1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1N NaOH and make upto 50 mL with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

**Alkali degradation**
Pipette 5 mL of above stock solution into a 50 mL volumetric flask and 3 mL of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1N HCl and make upto 50 mL with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

**Peroxide degradation**
Pipette 5 mL above stock solution into a 50 mL volumetric flask and 1 mL of 3% v/v of hydrogen peroxide added in 50 mL of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

**Thermal degradation**
Pregabalin and Etoricoxib samples were taken in petridish and kept in hot air oven at $110^0$C for 24 hours. Then the sample was taken and diluted with diluent and injected into HPLC and analyzed.

**Hydrolytic degradation**
Pipette 5 mL above stock solution into a 50 mL volumetric flask and 1 mL of water added in 10 mL of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

**RESULTS AND DISCUSSION**
The HPLC procedure was optimized with a view to develop a simple, specific, accurate and precise method for simultaneous estimation of Pregabalin and Etoricoxib in tablet dosage form using X-Terra RP-18 (150 mm X 4.6 mm, 3.5 µm)column in isocratic mode with mobile phase composition of acetonitrile and 0.1% triethyl amine in the ratio of 50:50% v/v resulted in peak with maximum separation, good shape and resolution. A flow rate of 1.0 mL/min gave an optimum signal-to-noise ratio with reasonable separation time. Total run time was 6 minutes. The drug components were measured with UV detector at 214 nm. The results of optimized chromatographic conditions were shown in Table 1.
Linearity was obtained in the range of 18.75-112.50 µg/mL for Pregabalin and 15-90 µg/mL for Etoricoxib. The correlation coefficient ($r^2$) was found to be 0.999 for both Pregabalin and Etoricoxib respectively. The regression equation of the linearity plot of concentration of Pregabalinover its peak area was found to be y=46612.08x+21509.21, where x is the concentration of Pregabalin(µg/mL) and y is the corresponding peak area. The regression equation of the linearity plot of concentration of Etoricoxib over its peak area was found to be y=22860.89x+4433.07, where x is the concentration of Etoricoxib(µg/mL) and y is the

corresponding peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. The linearity results were shown in Table 2 and the calibration curves were shown in Fig. 3 and Fig. 4.

The % RSD for system precision, repeatability and intermediate precision for Pregabalin were found to be 0.34%, 1.06% and 0.66% respectively (limit % RSD<2.0%). The % RSD for system precision, repeatability and intermediate precision for Etoricoxib were found to be 0.32%, 1.06% and 1.09% respectively (limit % RSD<2.0%) and hence the method is precise. The precision data of Pregabalin and Etoricoxib were furnished in Table 3, 4 and 5.

The mean % recovery of the drugs Pregabalin and Etoricoxib were found to be 98.80% and 100.94% respectively and the high percentage of recovery of Pregabalin and Etoricoxib indicates that the proposed method is highly accurate. The results of accuracy studies of Pregabalin and Etoricoxib were shown in Table 6 and Table 7.

The retention times for the drugs Pregabalin and Etoricoxib was 2.932 minutes and 3.884 minutes respectively. The number of theoretical plates calculated for Pregabalin and Etoricoxib was 2487 and 5321 respectively. The tailing factor for Pregabalin and Etoricoxib was 1.24 and 0.91 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantitation (LOQ) for Pregabalin were found to be 2.25 µg/mL and 7.5 µg/mL; 1.8 µg/mL and 6.0 µg/mL for Etoricoxib respectively, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 8.

The robustness studies indicated that no considerable effect on the determination of the drugs. Therefore the test method is robust for the quantification of the drugs. In all deliberately varied conditions, the % RSD for replicate injections of Pregabalin and Etoricoxib were found to be within the acceptable limits.

Validated method was applied for the simultaneous estimation of Pregabalin and Etoricoxib in commercial tablet dosage forms. The % Assay of Pregabalin and Etoricoxib were found to be 99.10% and 100.11% respectively. The results for the drugs assay showed good agreement with label claims. No interfering peaks were found in the chromatogram of the tablet formulation within the run time indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the drugs Pregabalin and Etoricoxib by the proposed HPLC method. The assay results are shown in Table 9.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showed no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and were found to be within limits. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks. The typical chromatogram of Pregabalin and Etoricoxib standard were shown in Fig. 5. All the degradation products formed during forced degradation studies were well separated from the analyte peaks demonstrating that the developed method was specific and stability indicating**.** The results of the degradation studies are presented in Table 10.

**CONCLUSION**

The developed RP-HPLC method for the estimation of selected drugs Pregabalin and Etoricoxib is simple, specific, accurate, precise and robust. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested non-interference of formulation recipients in the estimation and can be used in laboratories for the routine analysis of selected drugs. The present work concluded that stability indicating assay method by RP-HPLC has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Pregabalin and Etoricoxib.

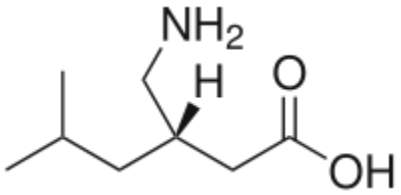


**Fig. 1: Chemical structure of Pregabalin**

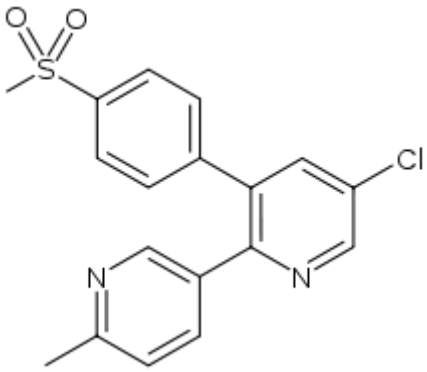


**Fig. 2: Chemical structure of Etoricoxib**

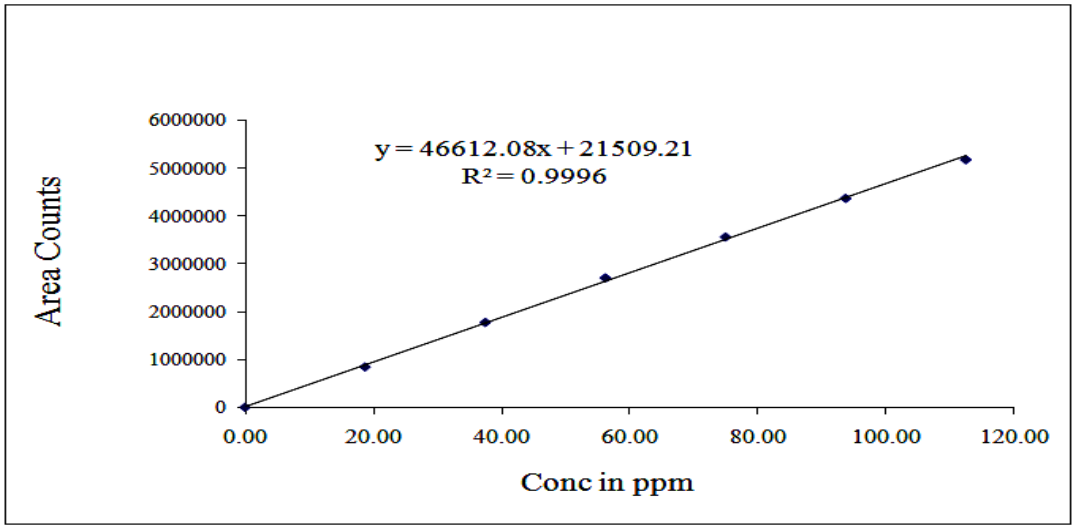


**Fig. 3: Calibration curve for Pregabalin**

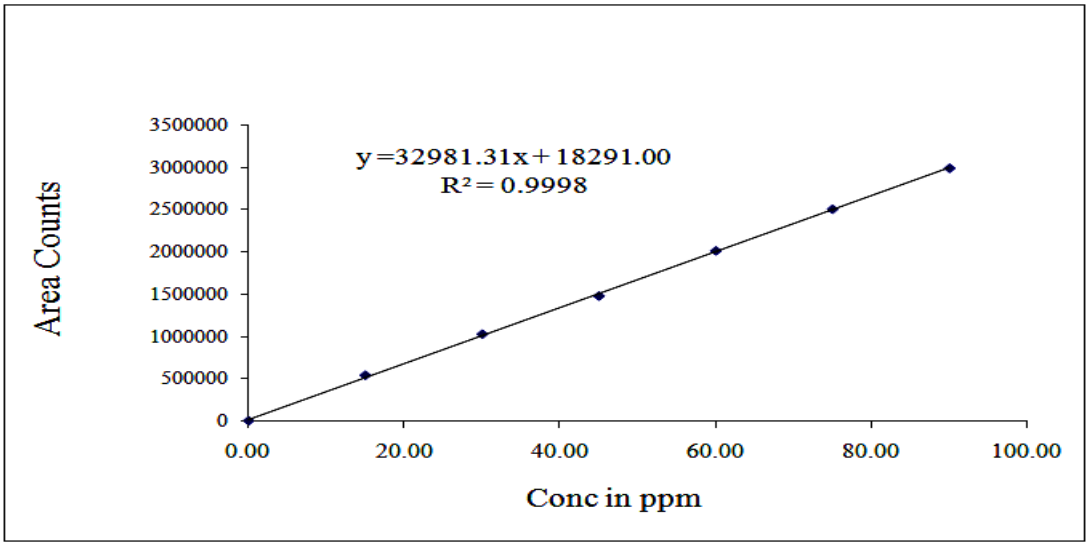


**Fig. 4: Calibration curve for Etoricoxib**

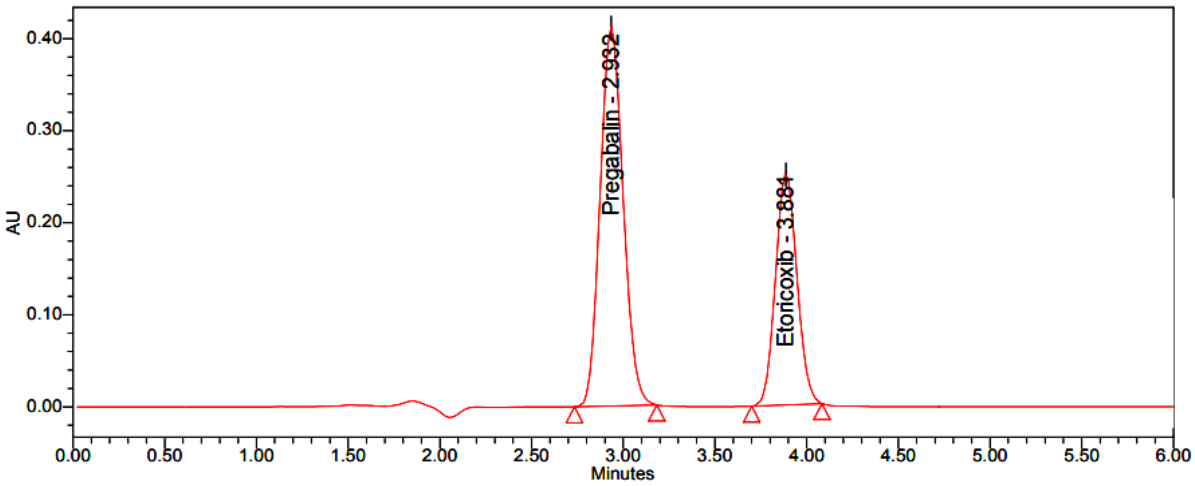


**Fig. 5: Chromatogram of Pregabalin and Etoricoxib**

**Table 1: Optimized chromatographic conditions**

| Parameter | Condition |
|---|---|
| Mobile phase | Acetonitrile:0.1% triethyl amine (50:50% v/v) |
| Diluent | Mobile phase |
| Column | X-Terra RP-18 (150 mm X 4.6 mm, 3.5 µm) |
| Column temperature | $25^0$C |
| Wave length | 214 nm |
| Injection volume | 10 µL |
| Flow rate | 1.0 mL/min. |
| Run time | 6 min. |

**Table 2: Linearity results of Pregabalin and Etoricoxib**

| S. No. | Concentration of Pregabalin (µg/mL) | Peak area | Concentration of Etoricoxib (µg/mL) | Peak area |
|---|---|---|---|---|
| 1 | 18.75 | 846844 | 15.00 | 536548 |
| 2 | 37.50 | 1781815 | 30.00 | 1021479 |
| 3 | 56.25 | 2713037 | 45.00 | 1472830 |
| 4 | 75.00 | 3576514 | 60.00 | 2006975 |
| 5 | 93.75 | 4387252 | 75.00 | 2498205 |
| 6 | 112.50 | 5198609 | 90.00 | 2981114 |

**Table 3: System precision data of Pregabalin and Etoricoxib**

| S. No. | Peak area of Pregabalin | Peak area ofEtoricoxib |
|---|---|---|
| 1 | 3581428 | 2064821 |
| 2 | 3596572 | 2053837 |
| 3 | 3560654 | 2049679 |
| 4 | 3582898 | 2066520 |
| 5 | 3590947 | 2057862 |
| 6 | 3578854 | 2055233 |
| Mean | 3581892 | 2057992 |
| SD | 12320.75 | 6532.4 |
| % RSD | 0.34 | 0.32 |

**Table 4: Repeatability data of Pregabalin and Etoricoxib**

| S. No. | Peak area of Pregabalin | Peak area of Etoricoxib |
|---|---|---|
| 1 | 3521984 | 2095653 |
| 2 | 3595491 | 2048731 |
| 3 | 3560482 | 2071604 |
| 4 | 3513734 | 2088056 |
| 5 | 3605487 | 2039175 |
| 6 | 3577890 | 2063440 |
| Mean | 3562511 | 2067777 |
| SD | 37939.112 | 21920.717 |
| % RSD | 1.06 | 1.06 |

**Table 5: Intermediate precision data of Pregabalin and Etoricoxib**

| S. No. | Peak area of Pregabalin | Peak area of Etoricoxib |
|---|---|---|
| 1 | 3572415 | 2087861 |
| 2 | 3567881 | 2035836 |
| 3 | 3545612 | 2028510 |
| 4 | 3585206 | 2071649 |
| 5 | 3597823 | 2053468 |
| 6 | 3534679 | 2065423 |
| Mean | 3567269 | 2057125 |
| SD | 23720.339 | 22404.993 |
| % RSD | 0.66 | 1.09 |

**Table 6: Accuracy results of Pregabalin**

| % Concentration level | Conc. added (µg/mL) | Conc. found (µg/mL) | % Recovery | % Mean recovery |
|---|---|---|---|---|
| 50% | 37.5 | 36.74 | 97.97 | 98.80 |
| 100% | 75.0 | 74.78 | 99.70 | |
| 150% | 112.5 | 111.1 | 98.75 | |

**Table 7: Accuracy results of Etoricoxib**

| % Concentration level | Conc. added (µg/mL) | Conc. found (µg/mL) | % Recovery | % Mean recovery |
|---|---|---|---|---|
| 50% | 30 | 30.50 | 101.66 | 100.94 |
| 100% | 60 | 60.55 | 100.91 | |
| 150% | 90 | 90.23 | 100.25 | |

**Table 8: System suitability parameters of Pregabalin and Etoricoxib**

| S. No. | Parameters | Pregabalin | Etoricoxib |
|---|---|---|---|
| 1 | Linearity (µg/mL) | 18.75-112.50 | 15-90 |
| 2 | Correlation coefficient | 0.999 | 0.999 |
| 3 | Retention time (min.) | 2.932 | 3.881 |
| 4 | Resolution | -- | 4.48 |
| 5 | Theoretical plates (N) | 2487 | 5321 |
| 6 | Tailing factor | 1.24 | 0.91 |
| 7 | LOD (µg/mL) | 2.25 | 1.8 |
| 8 | LOQ (µg/mL) | 7.5 | 6.0 |

**Table 9: Assay results of Pregabalin and Etoricoxib**

| Formulation | | Label claim | Amount found | % Assay |
|---|---|---|---|---|
| EBOV PG | Pregabalin | 75 mg | 74.33 mg | 99.10% |
| | Etoricoxib | 60 mg | 60.07 mg | 100.11% |

**Table 10: Degradation results for Pregabalin and Etoricoxib**

| S. No. | Degradation condition | Pregabalin | | Etoricoxib | |
|---|---|---|---|---|---|
| | | Peak area | % Degradation | Peak area | % Degradation |
| 1 | Acid | 3085241 | 13.9 | 1801240 | 12.5 |
| 2 | Alkali | 3042147 | 15.1 | 1782027 | 13.4 |
| 3 | Peroxide | 2994625 | 16.4 | 1701129 | 17.4 |
| 4 | Thermal | 3551247 | 0.9 | 1830687 | 11.1 |
| 5 | Hydrolytic | 3557642 | 0.7 | 2042097 | 0.8 |

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