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## **“Reverse Phase HPLC Method for Estimation of Acelofenac and Pregabaline in Combined Dossage Form”.**

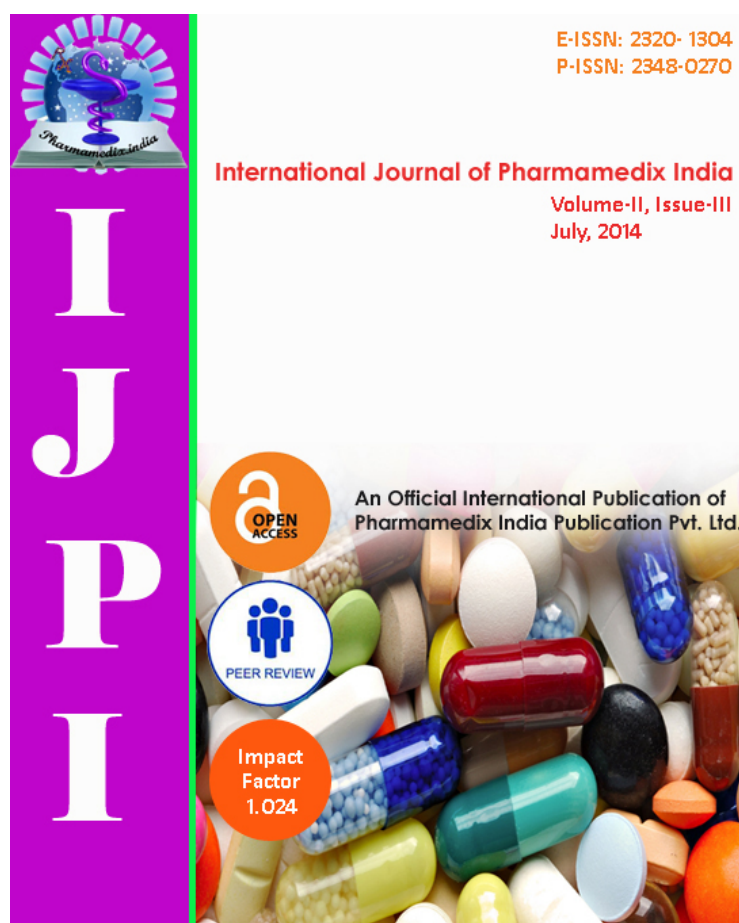
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### Abstract:

A simple, precise, accurate and rapid reverse phase high performance liquid chromatographic isocratic method has been developed for the estimation of Aceclofenac (ACF), and Pregabalin in tablet dosage form. The mixed standard solution containing of Pregabalin and Aceclofenac were chromatographed with mobile phase of different ratio of Buffer and acetonitrile, respectively at different pH values. (0.02MKH<sub>2</sub>PO<sub>4</sub>)-ACN-TEA 65-35-0.1 pH4.5 with H<sub>3</sub>PO<sub>4</sub>), RP-18 (E-Merck, 5  $\mu$ m, 4.0 x 250 mm) column was selected over the other columns. This RP-18 (E-Merck, 5  $\mu$ m, 4.0 x 250 mm) column has an embedded polar groups and which are more stable at lower pH and high Carbon loads, which provide high peak purity and more retention to polar drugs and facilitates the separation of impurity peaks within a very short run time.

- Injection volume – 10  $\mu$ l
- Column temperature – 25<sup>o</sup> C
- Sample temperature – 25<sup>o</sup> C
- Flow rate- 1.5ml/min
- $\lambda_{\text{max}}$ - 205nm

**Keyword:** Aceclofenac, Pregabalin RP-HPLC

### Introduction

ACF medication is a nonsteroidal anti-inflammatory drug, prescribed for fever, pain, ankylosing spondylitis and arthritis. It blocks the action of a substance in the body (cyclo-oxygenase), which may cause pain and inflammation.<sup>1</sup>

Pregabalin is an anticonvulsant drug used for neuropathic pain and as an adjunct therapy for partial seizures with or without secondary generalization in adults.<sup>2</sup> Pregabalin binds with high affinity to the

alpha2-delta site (an auxiliary subunit of voltage-gated calcium channels) in central nervous system tissues. Although the mechanism of action of pregabalin is unknown, results with genetically modified mice and with compounds structurally related to pregabalin (such as gabapentin) suggest that binding to the alpha2-delta subunit may be involved in pregabalin's antinociceptive and antiseizure effects in animal models. In vitro, pregabalin reduces the calcium-dependent release of several

neurotransmitters, possibly by modulation of calcium channel function. Studies also suggest that the descending noradrenergic and serotonergic pathways originating from the brainstem may be involved with the mechanism of pregabalin. Interestingly, although pregabalin is a structural derivative of inhibitory neurotransmitter gamma-aminobutyric acid (GABA), it does not bind directly to GABA or benzodiazepine receptors. The sodium channels, opiate receptors, and cyclooxygenase enzymes are not involved with the mechanism of pregabalin. It is also inactive at serotonin and dopamine receptors and does not inhibit dopamine, serotonin, or noradrenaline reuptake.<sup>3</sup>

According to the literature survey carried out, pregabalin is not official in pharmacopoeia, but

methylcobalamine is official in USP, EP and BP. A simple sensitive, selective, simultaneous determination has already been developed for determination of Pregabalin, Gabapantin and Vigabatrin in human serum, and also for methylcobalamine a routine estimation in liver, plasma, milk, intestinal fluid and faeces has been reported. But there was no analytical method reported for estimation of both drugs in the combination. Hence a RP-

HPLC<sup>4,5</sup> method was developed and validated as per ICH guidelines.

Figure 1 shows chemical structure of analytical compounds.

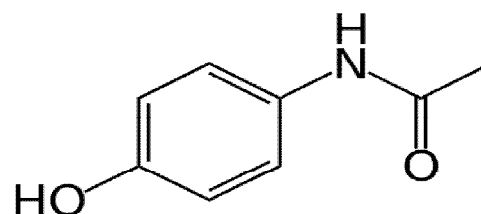


Figure 1 (a) Paracetamol

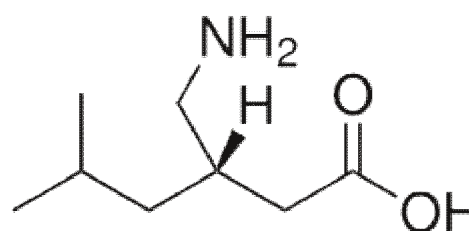


Figure 1 (b) Pregabalin

#### Figure 1 Chemical structure of studied drug

Literature survey reveals that there are some HPLC methods available and some methods are with few other ingredients in combination with ACF. The reported HPLC methods so far in the literature were considered to be uneconomical, time consuming and have poor symmetry. After thoroughly evaluating the existing methods, the authors have attempted to develop an alternative simple, rapid, precise, accurate and economical RP-HPLC method for the estimation of ACF and Pregabalin in bulk drug samples and in pharmaceutical dosage forms. Figure 1: shows the chemical structures for analytical compounds.

## **MATERIAL & METHODS**

### **Chromatographic System**

#### **Instrumentation**

The HPLC system (Agilent 1100 series), consisted of a HPLC pump Agilent 1100 series G1311A Quat. Pump, Serial DE43635137, Detector Agilent 1100 series G1315B DAD, Serial DE54627481, Injector Agilent 1100 series G1329A ALS, Serial DE43616697, Control module Agilent interface 35900E, Software Chemstation.

#### **Drugs and chemicals used**

Pharmaceutical pure samples of ACF and Pregabalin were obtained as gift from IIM Jammu, Commercial tablets of ACF and Pregabalin were purchased from local drug market containing Pregabalin 150 mg tablet ACF 100 mg.

#### **Development and optimization of stability indicating RP-HPLC method**

##### **Selection of Chromatographic Conditions**

Proper selection of the HPLC method depends upon the nature of the sample (ionic or ionizable or neutral molecule), its molecular weight and solubility. HPLC was selected for the initial separation because of its simplicity and suitability. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied, and the chromatographic parameters such as capacity factor,

asymmetric factor, and resolution and column efficiency were calculated. The condition was chosen that given the best resolution, symmetry and capacity factor was selected for estimation.

##### **Selection of Ratio of Mobile Phase**

The mixed standard solution containing of Pregabalin and Aceclofenac were chromatographed with mobile phase of different ratio of Buffer and acetonitrile, respectively at different pH values.

##### **Selection of Proper Column**

For HPLC method, various columns are available but our main aim is to resolve the the drugs in the presence of degradation products and other impurities. So the RP-18 (E-Merck, 5  $\mu$ m, 4.0 x 250 mm) column was selected over the other columns. This RP-18 (E-Merck, 5  $\mu$ m, 4.0 x 250 mm) column has an embedded polar groups and which are more stable at lower pH and high Carbon loads, which provide high peak purity and more retention to polar drugs and facilitates the separation of impurity peaks within a very short run time.

##### **Preparation of solutions and Reagents**

##### **Standard stock solution of Aceclophenac:**

Weigh and transfer 50 mg of Aceclophenac working standard into a 100 ml volumetric flask, add 5 ml Acetonitrile and sonicate to

dissolve. Make up the volume upto 100ml with diluent and mix.

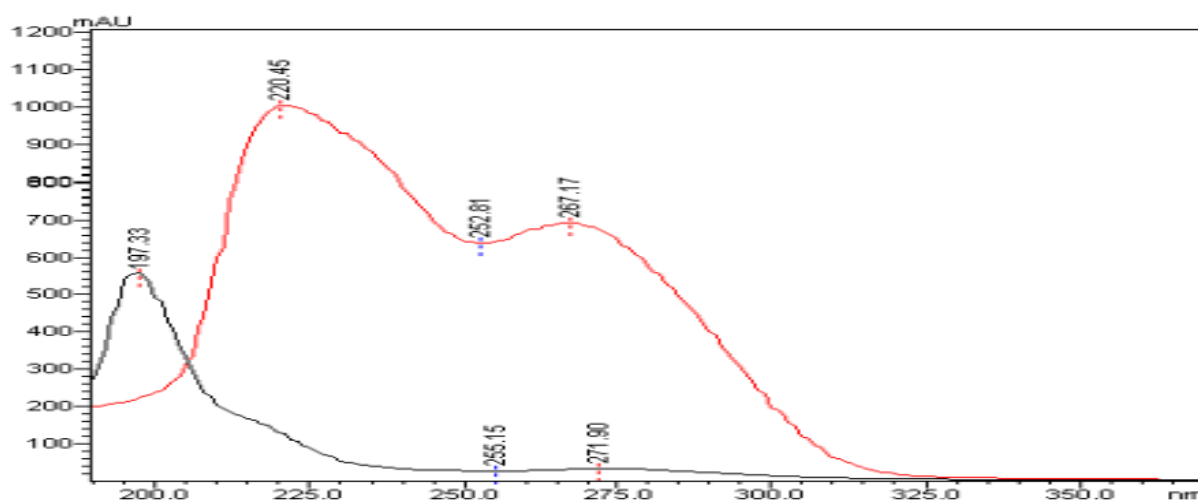
#### **Standard stock solution of Pregabalin:**

Weigh and transfer 37.5 mg of Pregabalin working standard into a 100ml Volumetric flask, add 5ml Acetonitrile and sonicate to dissolve. Make up the volume upto 100ml with diluent and mix.

**Working standard solution:** Transfer 5ml of Aceclofenac standard stock solution and 5ml of Pregabalin standard stock solution in 50 ml volumetric flask. Make up the volume with diluent and mix.

#### **Selection of Detection Wavelength**

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study, standard solution of Aceclofenac and Pregabalin were scanned over the range of 200–400 nm wavelengths. The both drugs showed absorbance at 205 nm. So the 205 nm wavelength was selected for simultaneous estimation of Pregabalin and Aceclofenac dosage forms. This wavelength was also selected for simultaneous estimation of Pregabalin and Aceclofenac in their dosage forms.



#### **Analytical Method Development For The Simultaneous Estimation Of Pregabalin and Aceclofenac By HPLC And Its Validation.**

To ensure transferability of the method and to ensure the method will operate successfully in a QC site, the method

variation should be known and monitored. The method variation is an estimate of the variation that will be experienced in routine use of the method. Chromatographic Separation can be demonstrated by a sufficient separation of the substances

present. For the assay, appropriate separation means an adequate resolution between the peak of interest and other peaks (e.g., impurities, placebo or matrix components). So, Several trials for the development purpose were taken for the simultaneous estimation of Pregabalin and Aceclofenac. Finally, a better separation condition characterized by better resolution, decorous peak shape, short runtime, minimal peak tailing and best reproducibility of the results were identified.

#### Trial :

**Mobile phase-** (0.02MKH<sub>2</sub>PO<sub>4</sub>)-ACN-TEA 65-35-0.1 pH4.5 with H<sub>3</sub>PO<sub>4</sub>

#### Conditions:

- Column – RP-18 (E-Merck, 5 µm, 4.0 x 250 mm) column
- Injection volume – 10 µl.
- Column temperature – 25<sup>0</sup> C
- Sample temperature – 25<sup>0</sup> C
- Flow rate- 1.5ml/min
- λ<sub>max</sub>- 205nm

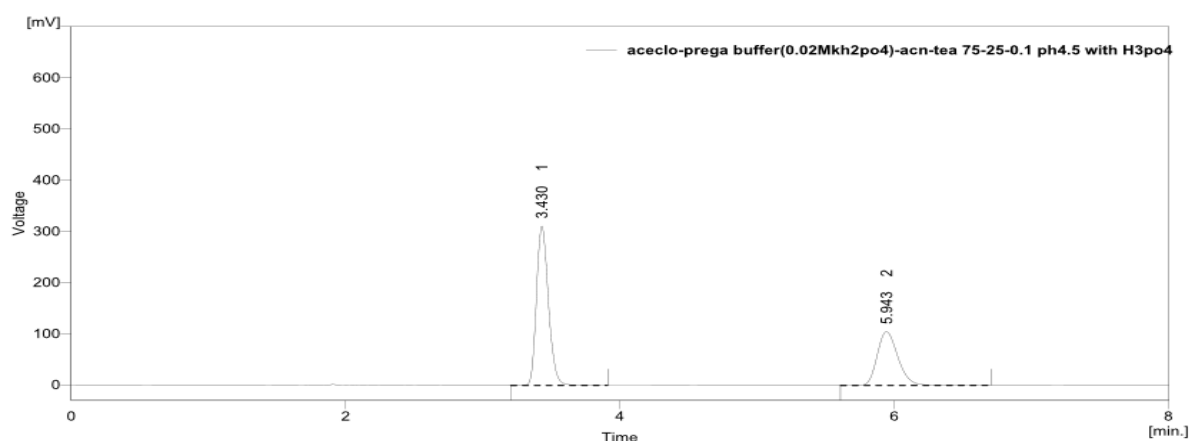


**Figure: Trial Calibration data for standard Aceclo.**

| Sr. No.                 | % Concentration with respect to test concentration | Concentration ppm(µg/ml) | Peak Area mean* |
|-------------------------|----------------------------------------------------|--------------------------|-----------------|
| 1                       | 50                                                 | 25                       | 771.708         |
| 2                       | 75                                                 | 37.5                     | 1131.884        |
| 3                       | 100                                                | 50                       | 1483.366        |
| 4                       | 125                                                | 62.5                     | 1860.093        |
| 5                       | 150                                                | 75                       | 2233.068        |
| Correlation Coefficient |                                                    | 0.9998                   |                 |
| Regression Equation     |                                                    | $y = 29.207x + 35.652$   |                 |

**Table 6.4: Calibration data for standard Pregabalin**

| Sr. No.                 | % Concentration with respect to test concentration | Concentration ppm( $\mu\text{g/ml}$ ) | Peak Area mean* |
|-------------------------|----------------------------------------------------|---------------------------------------|-----------------|
| 1                       | 50                                                 | 18.75                                 | 340.379         |
| 2                       | 75                                                 | 28.135                                | 506.842         |
| 3                       | 100                                                | 37.5                                  | 664.343         |
| 4                       | 125                                                | 46.875                                | 833.159         |
| 5                       | 150                                                | 56.25                                 | 1000.218        |
| Correlation Coefficient |                                                    | 0.9999                                |                 |
| Regression Equation     |                                                    | $y = 17.557x + 10.59$                 |                 |

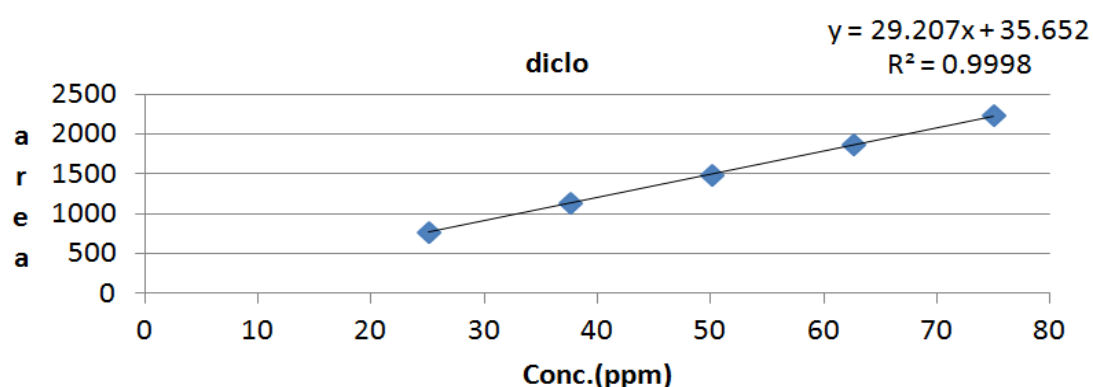


**Figure 6.12: Calibration Curve of Aceclofenac**

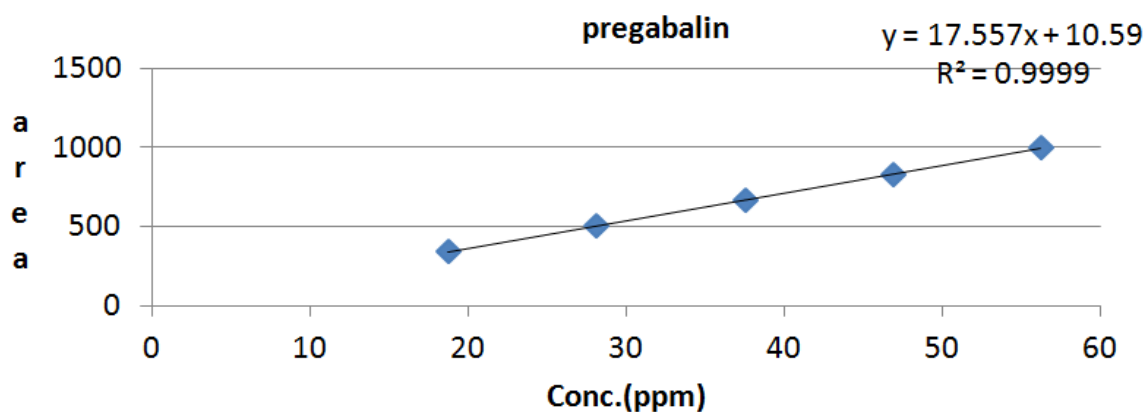


**Figure 5.13: Calibration Curve of Pregabalin**

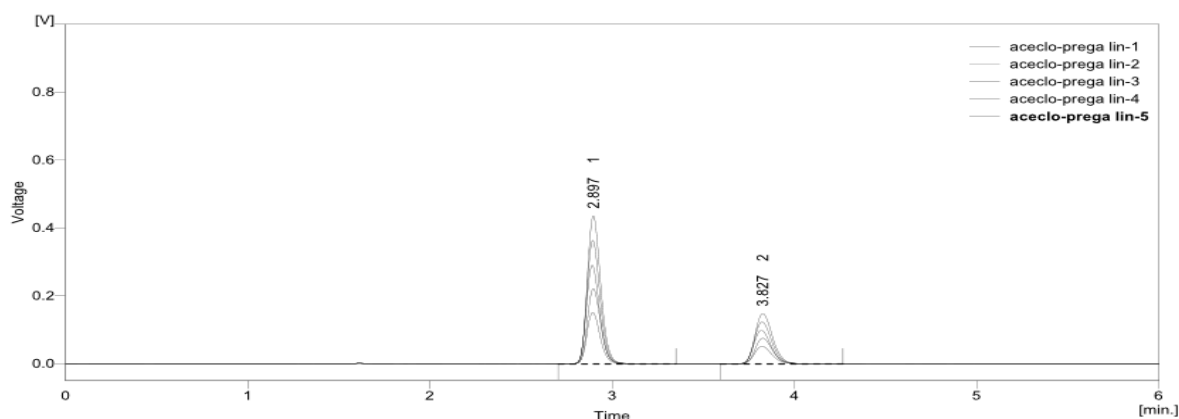


**Figure 5.14: Overlay Chromatogram for Calibration curve of Pregabalin and Aceclofenac**

### Conclusion:

The results obtained lies well within the acceptance criteria. The method can therefore be termed as linear.

### ASSAY OF ACECLOFENAC AND PREGABALIN:

#### Procedure:

#### Inject blank preparation

Inject standard preparation five times, record the chromatograms and check for compliance for system suitability test, make any necessary changes in to meet the system suitability requirements Standard solution (five injections) and Sample solution (in duplicate) of Aceclofenac. and Pregabalin was injected into the chromatographic system and the chromatograms were recorded.

#### Calculation:

The Assay of Aceclofenac and Pregabalin calculated as per the formula given below:

$$\text{Assay (ACLO) (mg/Tab)} = \frac{A_{\text{Sam}_1}}{A_{\text{Std}_1} \text{DT}} \times \frac{DS_1}{100} \times \frac{P_1}{W_1}$$

$$\text{Assay (mg/Tab)} \\ \% \text{Assay (\% of label claim as ACLO.)} = \frac{\text{Assay (mg/Tab)}}{C_1} \times 100$$

$$\text{Assay (Pregabalin) (mg/Tab)} = \frac{A_{\text{Sam}_2}}{A_{\text{Std}_2}} \times \frac{DS_2}{DT} \times \frac{P_2}{100} \times W_1$$

$$\text{Assay (XYZ) (mg/Tab)} \\ \% \text{Assay (\% of label claim as Pregabalin)} = \frac{\text{Assay (XYZ) (mg/Tab)}}{C_2} \times 100$$

Where,

|                   |                                                                                                                                |
|-------------------|--------------------------------------------------------------------------------------------------------------------------------|
| ASam <sub>1</sub> | Average area counts of ACLO peak observed in the chromatogram of sample solution.                                              |
| AStd <sub>1</sub> | Average area counts of ACLO peak observed in the chromatogram of standard solution as obtained under system suitability.       |
| ASam <sub>2</sub> | Average area counts of Pregabalin peak observed in the chromatogram of sample solution.                                        |
| AStd <sub>2</sub> | Average area counts of Pregabalin peak observed in the chromatogram of standard solution as obtained under system suitability. |
| DS <sub>1</sub>   | Dilution factor for ACLO standard preparation, in mg/ml.                                                                       |
| DS <sub>2</sub>   | Dilution factor for Pregabalin standard preparation, in mg/ml.                                                                 |
| DT                | Dilution factor for sample preparation, in mg/ml.                                                                              |
| P <sub>1</sub>    | Percent potency of ACLO reference/working standard, on as is basis.                                                            |
| P <sub>2</sub>    | Percent potency of Pregabalin reference/working standard, on as is                                                             |

|                |                                            |
|----------------|--------------------------------------------|
|                | basis.                                     |
| C <sub>1</sub> | Label claim, in mg, ACLO per Tablet.       |
| C <sub>2</sub> | Label claim, in mg, Pregabalin per Tablet. |
| W <sub>1</sub> | Average weight in mg per tablet.           |

**Table 6.4 %Assay of Calculation**

| Parameters  | Aceclofenac  | Pregabalin   |
|-------------|--------------|--------------|
| Sample Area | 1459.405     | 657.074      |
|             | 1441.521     | 652.444      |
|             | 1480.418     | 668.752      |
| Mean Area   | 1460.468     | 659.423      |
| %Assay      | 97.17765546% | 97.97422114% |

## VALIDATION OF THE PROPOSED METHOD

Validation of the developed analytical procedure is the process by which it is established by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications. Validation was performed in accordance with the International Conference on Harmonization (ICH) <sup>10-12</sup>. Validation parameters checked were precision, robustness, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

### Precision

The precision of an analytical method is the closeness of agreement (degree of scatter) between series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed condition.

### Method Precision

#### A) Intraday Precision

Combined standard solutions containing mixture of 50 ppm of Aceclofenac and 37.5 ppm of Pregabalin were analyzed 6 times on the same day. The % R.S.D for the Pregabalin and Aceclofenac was calculated.

**Table 6.5: Intraday Precision For Aceclo. And Pre.**

| ACECLOFENAC (Concentration: 50 Ppm) |          | PREGABALIN (Concentration: 37.5 Ppm) |          |
|-------------------------------------|----------|--------------------------------------|----------|
| Determination                       | Area     | Determination                        | Area     |
| 1                                   | 1472.847 | 1                                    | 659.697  |
| 2                                   | 1436.143 | 2                                    | 643.269  |
| 3                                   | 1453.74  | 3                                    | 651.14   |
| 4                                   | 1490.55  | 4                                    | 667.644  |
| 5                                   | 1457.247 | 5                                    | 655.106  |
| 6                                   | 1489.017 | 6                                    | 664.286  |
| Average                             | 1466.591 | Average                              | 656.857  |
| Standard Deviation                  | 21.42725 | Standard Deviation                   | 8.944259 |
| % R.S.D.                            | 1.461025 | % R.S.D.                             | 1.361675 |

### Chromatogram for Intraday Study

Combined standard solutions containing mixture of 50 ppm of Aceclofenac and 37.5 ppm of Pregabalin were analyzed 6 times on the different different day. The % R.S.D for the Pregabalin and Aceclofenac was calculated.

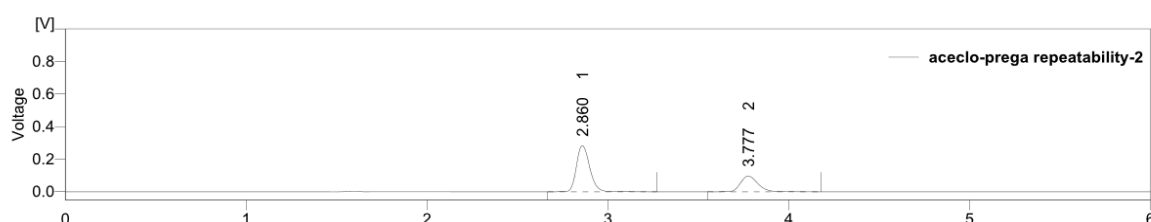
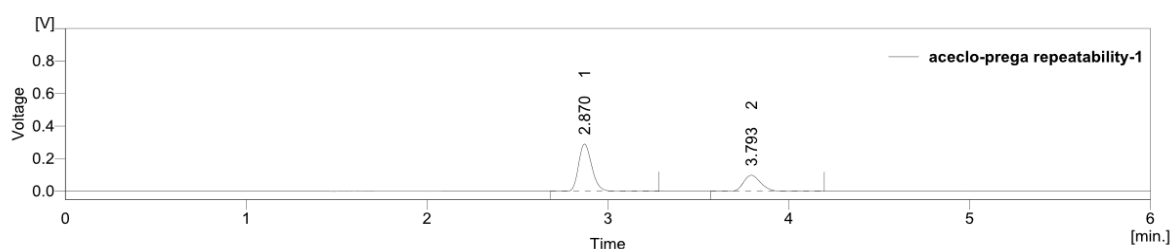


**Fig. 6.14 And 6.15: Chromatogram Of Intraday Study(1-2)**

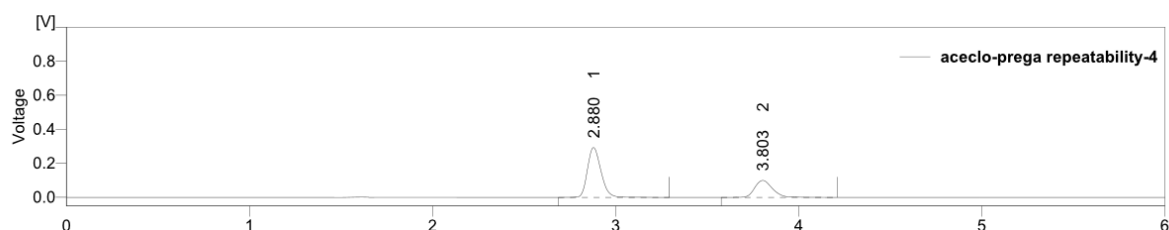
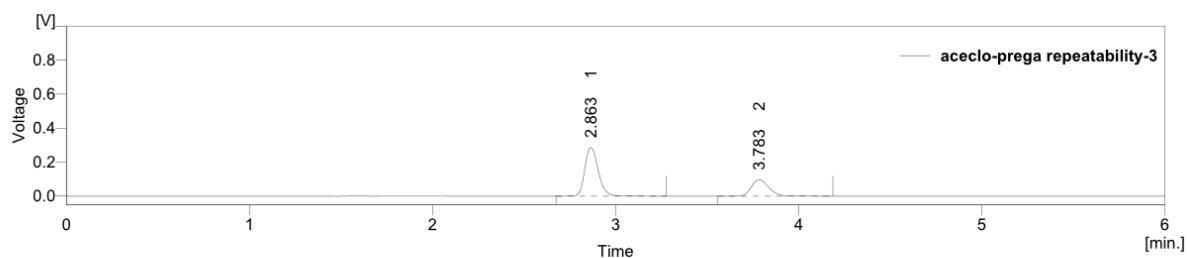


**Fig. 6.15,6.16: Chromatogram Of Intraday Study(3-4)**

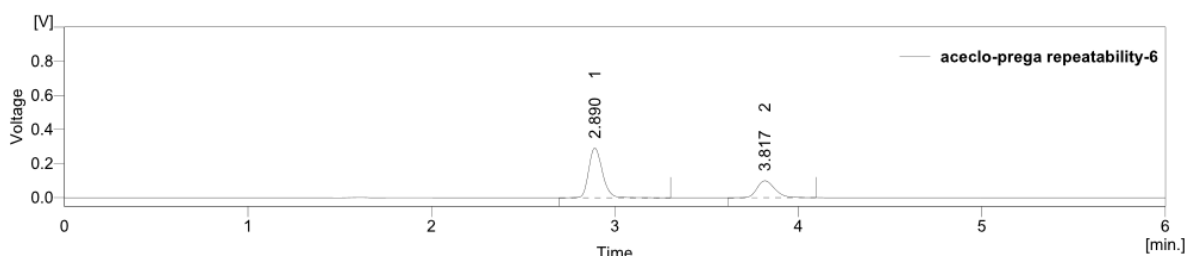
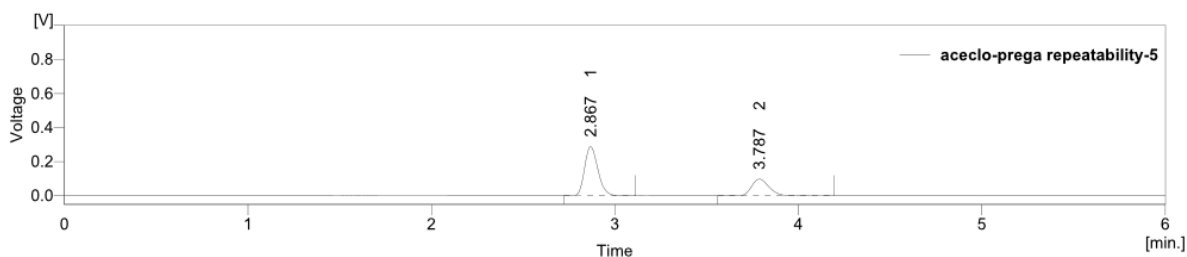


**Fig. 6.17.6.18: Chromatogram Of Intraday Study(5-6)**

#### Interday Precision:

**Acceptance criteria:** %RSD of interday precision should not more than 2.0%.

**Table 6.6: Interday precision**

| Aceclo, (Concentration: 50 Ppm) |          | Pregabalin (Concentration: 37.5 Ppm) |         |
|---------------------------------|----------|--------------------------------------|---------|
| Determination                   | Area     | Determination                        | Area    |
| 1                               | 1547.318 | 1                                    | 693.06  |
| 2                               | 1533.445 | 2                                    | 673.199 |

|                    |          |                    |          |
|--------------------|----------|--------------------|----------|
| 3                  | 1531.78  | 3                  | 686.067  |
| 4                  | 1572.166 | 4                  | 704.172  |
| 5                  | 1551.948 | 5                  | 695.128  |
| 6                  | 1528.863 | 6                  | 684.755  |
| Average            | 1544.253 | Average            | 689.3968 |
| Standard Deviation | 16.47412 | Standard Deviation | 10.57899 |
| % R.S.D.           | 1.066802 | % R.S.D.           | 1.534528 |

**Conclusion:** Percentage R.S.D. of interday was found to be NMT 2% for both the drugs

### Chromatogram for Interday Study

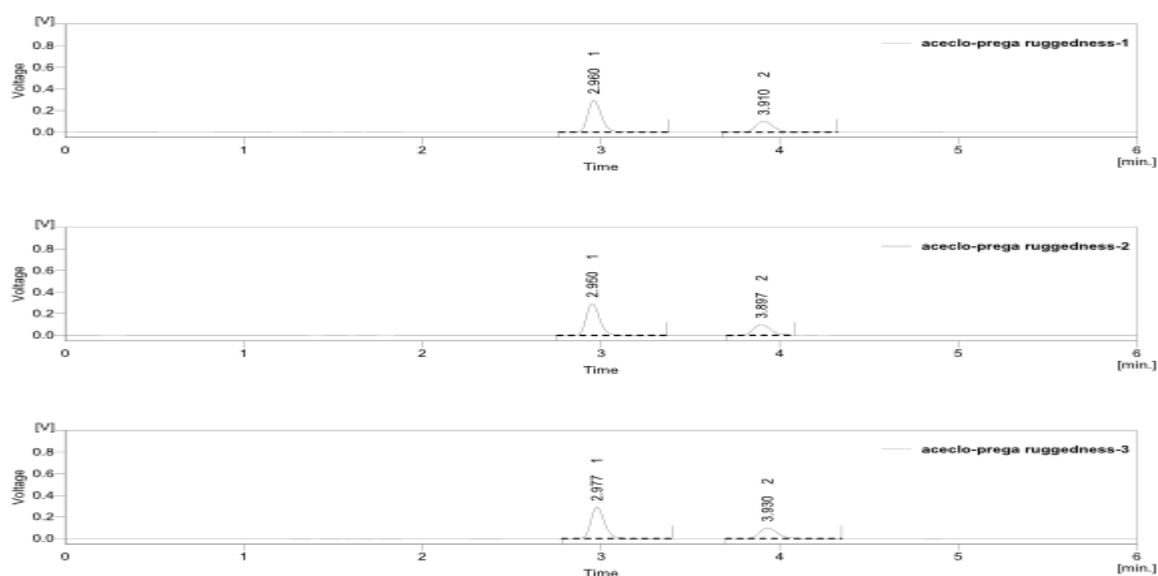


**Fig. 6.19: Chromatogram of InterdayStudy(1-3)**

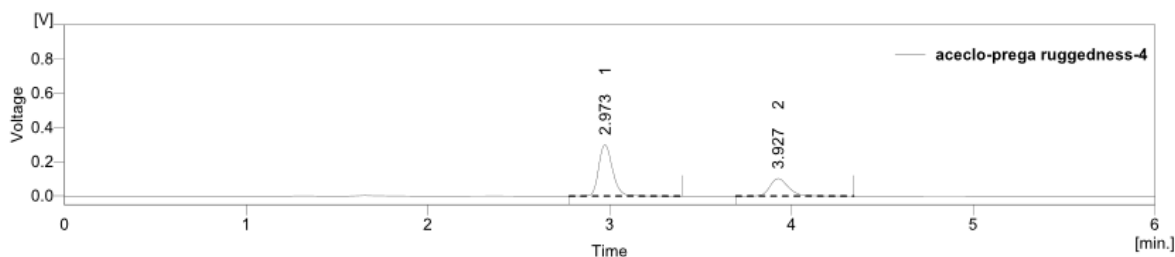


**Fig. 6.20: Chromatogram of InterdayStudy(4)**

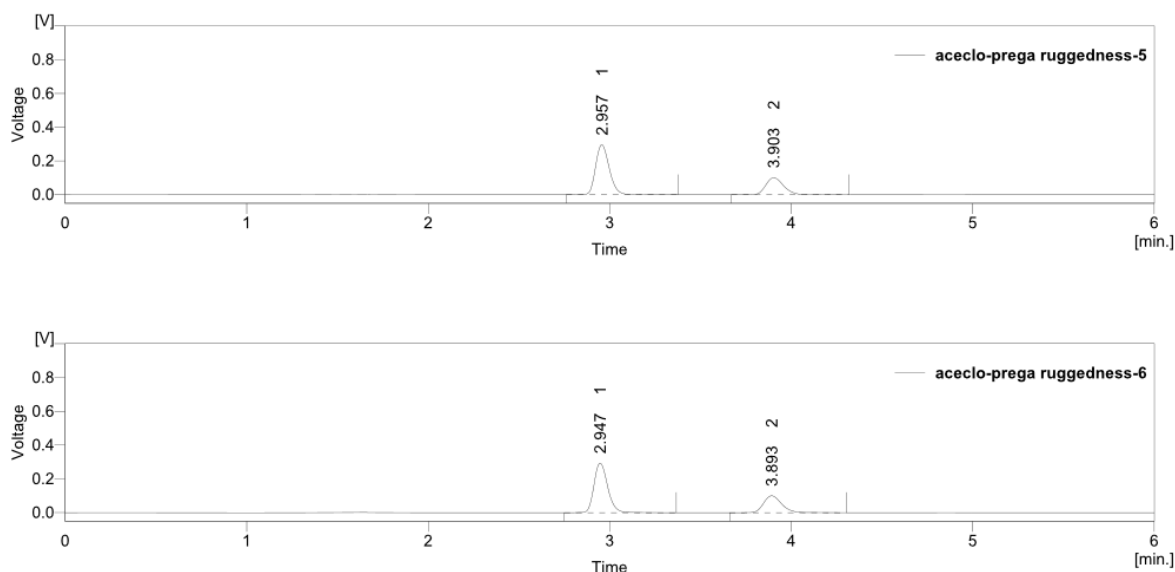


**Fig. 6.21: Chromatogram Of InterdayStudy(5-6)**

**Conclusion:** The results obtained lies well within the acceptance criteria. The method can therefore be termed as precise

#### LOD and LOQ

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity. It may be calculated as

SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

**Table 6.7: Result of LOD and LOQ**

| PARAMETER                  | Aceclo.     | Pregabalin  |
|----------------------------|-------------|-------------|
| S.D. of Intercept          | 9.012413896 | 3.278839241 |
| Slope of Calibration Curve | 146.7       | 45.33       |
| LOD (ppm)                  | 0.202733237 | 0.238697761 |
| LOQ (ppm)                  | 0.614343142 | 0.723326548 |

#### 6.5.5 Robustness

**Procedure:** Change following parameters, one by one and observe their effect on system suitability test and assay.

- Change mobile phase composition by  $\pm 2$  ml of organic solvent.
- Change flow rate  $\pm 0.2$  ml/min.

**Change mobile phase composition:** Inject std. working solution (50ppm of Aceclofenac and 37.5ppm of Pregabalin) three times by change in the mobile phase composition by  $\pm 2.0$  ml of organic solvent of developed method.

**Change Flow rate:** Inject std. working solution (50ppm of Aceclofenac and 37.5ppm of Pregabalin) three times by change in the flow rate by  $\pm 0.2$  ml/min of developed method. Calculate the %RSD of mean area for change in method parameter. And results showed in table 6.8

**Table 6.8.: Result of Robustness**

| Condition                | Aceclofenac |                    |            | Pregabalin |                    |          |
|--------------------------|-------------|--------------------|------------|------------|--------------------|----------|
|                          | Area        | Standard Deviation | % RSD      | Area       | Standard Deviation | % RSD    |
| Increasing pH +0.2       | 1585.28     | 0.69832758         | 1.32551524 | 709.992    | 0.512986           | 1.296869 |
|                          | 1550.47     |                    |            | 694.398    |                    |          |
|                          | 1626.81     |                    |            | 728.573    |                    |          |
| Decreasing pH -0.2       | 1633.2      | 0.64498723         | 1.19389608 | 731.431    | 0.481505           | 1.187117 |
|                          | 1610.39     |                    |            | 721.203    |                    |          |
|                          | 1595.84     |                    |            | 714.665    |                    |          |
| Decreasing Solvent 0.2%  | 1610.16     | 0.83063169         | 1.53930931 | 721.177    | 0.617538439        | 1.536101 |
|                          | 1587.67     |                    |            | 711.082    |                    |          |
|                          | 1636.14     |                    |            | 732.741    |                    |          |
| Increasing Solvent 0.2%  | 1471.69     | 0.54548211         | 1.10576096 | 659.216    | 0.407452           | 1.099075 |
|                          | 1493.78     |                    |            | 669.144    |                    |          |
|                          | 1462.86     |                    |            | 655.265    |                    |          |
| Decreasing Flow rate 0.2 | 1578.27     | 0.5574061          | 1.05232205 | 706.896    | 0.415133           | 1.043596 |
|                          | 1600.28     |                    |            | 716.736    |                    |          |
|                          | 1568.5      |                    |            | 702.509    |                    |          |
| Increasing Flow rate 0.2 | 1477.49     | 0.13874202         | 0.28189276 | 661.756    | 0.13369            | 0.361751 |
|                          | 1470.48     |                    |            | 658.602    |                    |          |
|                          | 1470.48     |                    |            | 657.17     |                    |          |

### **\*Average of three determination**

The data indicate that there is no significant difference between the results obtained under conditions and varied method parameters. Therefore method is robust.

### **Conclusion**

A novel RP- HPLC method has been developed for the simultaneous estimation of Aceclofenac and Pregabalin in marketed formulations. The method gave good resolution for both the drugs with a short analysis time of 10 minutes. The developed method was validated. It was found to be simple, precise and accurate. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of Aceclofenac and pregabalin in combined dosage form. It can be also used in the quality control in bulk manufacturing.

### **Acknowledgement**

The Authors are highly thankful to Ahamed Noor Mansoori for providing the necessary help for literature search .

### **Reference**

1. Ravisankar p., devadasuch., devalarao g, nageswararao m. (2013), development and validation of rp-hplc method for simultaneous determination of paracetamol,

aceclofenac sodium and chlorzoxazone in combined dosage form, world journal of pharmacy and pharmaceutical sciences, 3(1), 667-681

2. <http://en.wikipedia.org/wiki/Pregabalin>

3. <http://www.drugbank.ca/drugs/DB00230>

4. A. H. Becket, J. B. Stanlek, (1997), Practical Pharmaceutical Chemistry, 4th edition, CBS, publishers.

5. L.R Snyder, J.J Kirkland,(1997), Practical HPLC method development, Wiley inter science publications, New York

6. Momin M Y, Yeole P G, Puranik M P, Wadher S J. (2006), Reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form. Indian J Pharm Sci, 68(3), 387-389

7. Patel T., Bhadani S., Chaudary A., Vaghela K.(2014) , Simultaneous Determination of Aceclofenac and Pregabalin in Combined Dosage form by using RP-HPLC Method, American Journal of Pharmatech Research 4 (3), 518-525.

8. N. Kannapan, S. P. Nayak, T. Venkatachalam, V. Prabhakaran (2010), Analytical RP-HPLC Method for Development and Validation of Pregabalin and Methylcobalamine in Combined Capsule

Formulation, Journal of Applied Chemical Research, 13, 85-89.

9. Seerapu S, Srinivasan B P., (2010), Development and validation of RP-HPLC method for the estimation of ivabradine hydrochloride in tablets., Indian J Pharm Sci; 72 (5), 667-71.

10. ICH draft Guidelines on Validation of Analytical Procedures: Definitions and Terminology, Federal register, Vol.60, IFPMA, Switzerland, 1995, PP.11260

11. International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use, Validation of analytical procedures: text and methodology Q2 (R1) , November 2005. International Conference on Harmonization, Q2B: Validation of Analytical Procedures: Methodology and Availability, Federal Register, 1997, 62 (96), 27463–27467.