Abstract
A simple, accurate, precise and highly selective reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for Pregabalin and Tapentadol. Chromatographic separation was achieved isocratically using Waters Alliance 2695 separation module, X Bridge C18 (100 x 4.6 mm, 5µ) at ambient temperature. Chromatographic conditions of 1ml/min flow rate and both drugs are identified with UV visible PDA detector at 210nm. Mobile phase employed was Phosphate buffer of pH 6.85 and acetonitrile in the ratio of 55:45 which resulted better resolution and sensitivity. Developed method was validated in terms of linearity range (187.5-1125 µg/ml for Pregabalin and 175-750 µg/ml for Tapentadol), precession (correlation coefficient is less than 0.999), robustness, accuracy (recovery of Pregabalin and tapentadol were 100.77% and 99.9% respectively). The validation of proposed method was verified by recovery studies and can be applicable in routine pharmaceutical analysis.

Keywords: Pregabalin, Tapentadol, RP-HPLC method.

Cite this article as:
INTRODUCTION
Pregabalin is an anticonvulsant drug used for neuropathic pain and as an adjunct therapy for partial seizures with or without secondary generalization in adults [1]. Chemically Pregabalin is (S) – 3 -(aminomethyl) -5-methylhexanoic acid. pregabalin binding to the alpha2-delta subunit may be involved in Pregabalin’s anti-nociceptive and antiseizure effects in animals[2]. Tapentadol is a centrally acting analgesic with a dual mode of action as an agonist of the μ-opioid receptor and as a norepinephrine reuptake inhibitor. Structurally Tapentadol is 3-[(2R, 3R)-1-(dimethylamino)-2-methylpentan-3-yl] phenol. It is needed to develop a method without any draw back because no methods are reported for Pregabalin and Tapentadol.

MATERIALS AND METHOD
Chromatographic separation was carried by using WATERS Aliance 2695 model with empower2 software, Weighing Balance model no ER200A, Sonicator with SE60US and pH Meter AD102U model was used. Pregabalin and Tapentadol standards are obtained as gift from AUROBINDO labs, Hyderabad, the tablet dosage forms as lyrica 75 (Pregabalin) and TAPAL 50 (Tapentadol). The entire chemicals and reagents user were HPLC grade or analytical reagent grade purchased from Qualigens, Merck (CHEMICALS), Mumbai, India.

EXPERIMENT
Chromatographic conditions:
Mobile phase : Phosphate buffer of pH 6.85 : Acetonitrile(55:45)
Flow rate : 1.0 ml/min
Column : X Bridge C18(100 x 4.6 mm, 5m).
Detector wavelength : 210 nm
Column temp : 30oC
Injection volume : 10 µl
Run time : 10 min

Assay procedure
SAMPLE PREPARATION:
5 tablets of pregabalin and Tapentadol were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 ml volumetric flask, 80ml of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluents, , to get final concentrations 750 ppm for Pregabalin and 500ppm for Tapentadol . The resulting solutions was injected for quantitative analysis the amount of Pregabalin and Tapentadol was calculated by using the calibration the results are reported in the table.

STANDARD PREPARATION:
Accurately Weighed and transferred 75mg of Pregabalin and 50mg of Tapentadol working Standards into a 10 ml clean dry volumetric flasks, add 7ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Amount found(ppm)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>TAP</td>
</tr>
<tr>
<td>1</td>
<td>753.13</td>
<td>496.68</td>
</tr>
<tr>
<td>2</td>
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<td>496.60</td>
</tr>
<tr>
<td>6</td>
<td>752.36</td>
<td>496.60</td>
</tr>
</tbody>
</table>

Table 1: Assay results

Figure 1: Standard chromatogram for pregabalin and tapentadol

Construction of calibration curve:
Standard stock solution of Pregabalin and Tapentadol are prepared individually to get concentration of 7500ppm of and 5000ppm respectively. From the standard stock solutions different dilutions were prepared, injected and their peak area were measured and calibration curves were constructed by using the physical mixture containing Pregabalin and Tapentadol in the ratio of 1:1

Validation and method development
The proposed analytical method was validated was validated with respect to parameters which are specificity, linearity, precision, accuracy, robustness and ruggedness are executed as per ICH guidelines. The results obtained with respect to the individual parameters are within the acceptance criteria and are stated earlier are validated as per ICH guidelines. The results obtained are narrated in the table ahead (Table 1-4)

Parameters
System suitability
Solution containing both Pregabalin and Tapntadol was injected and system suitability parameters were determined. The results are given in the table.
Limit of detection and limit of quantification:
The proposed method was estimated for the terms of limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were calculated by using signal to noise ratio (s/n) method. The LOD was found to be 3.1 µg/ml and 3.2 µg/ml for Pregabalin and Tapentadol respectively. The LOQ was found to be 9.2 µg/ml and 9.1 µg/ml for Pregabalin and Tapentadol respectively.

Robustness:
Robustness was established by analyzing system suitability parameters of sample at 25°C and 30°C at and flow rates of 0.8 ml/min and 1.2 ml/min and the %RSD of peak areas were calculated. The results were within the limit.

DISCUSSION
In RP-HPLC method development preliminary study on column selection was revealed that C₁₈ column gave a better resolution than C₈ column. Mobile phase and flow rate selection based on the peak parameter (height, area, tailing, theoretical plate and resolution) and run time. The best separation is achieved by using phosphate buffer pH 6.85 and acetonitrile in the ratio of 55:45 as mobile phase. The both drugs shown maximum absorption at 210 nm in UV-Spectra hence this wavelength was considered under optimized chromatographic conditions. At this the peaks are well separated and there is no interfering peaks are from placebo, thus the method has specified. The retention time obtained for Pregabalin and Tapentadol were 2.411 and 2.411 respectively. The capacity factor, tailing factor, theoretical plate count and resolution are within the acceptance criteria. By calculating the mean recovery we can confirm that the method was accurate. The mean recovery for Pregabalin and Tapentadol was found to be 102.0% and 101.87% respectively. As per the ICH guidelines the results were within the limit.
Small changes in the experimental parameters like flow rate and temperature does not affect the chromatographic separation.

**CONCLUSION**

The method developed for simultaneous estimation of Pregabalin and Tapentadol was a simple, precise and accurate. 10 min require for the development, which enabled the rapid determination process of bulk and pharmaceutical dosage forms. Hence the proposed method is suitable for routine analysis of dosage forms containing Pregabalin and Tapentadol.

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