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RP-HPLC Method for Simultaneous Estimation of Pregabalin and Tapentadol in Bulk and Pharmaceutical Dosage form

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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 allaiance 2695 separation module, X Bridge C_{18} (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 6.8 and acetonitrile in the ratio of 70:30 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.

Key words: *Pregabalin, Tapentadol, RP-HPLC method.*

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.

Pregabalin and Tapentadol are existed in individual and combination can be estimated by various methods reported in the literature such as HPLC detection [3-12].

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 (Tapentdol). 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 C_{18} (100 x 4.6 mm, 5µ).
Detector wavelength : 210 nm
Column temp : 30°C
Injection volume : 10 µl
Run time : 10 min

Assay Procedure:

Sample Preparation:

5 tablets of pregabalin and Tapentadol were weighed and caluculate 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 were 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 Pregablin 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.

RESULTS

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

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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 2**.

Table No. 1: Assay Results

Replicate	Amount found (ppm)		% Recovery	
	PRE	TAP	PRE	TAP
1	753.11	496.66	100.41%	99.33%
2	752.63	497.53	100.31%	99.50%
3	752.85	496.53	100.38%	99.31%
4	752.57	496.62	100.34%	99.32%
5	752.88	496.61	100.38%	99.32%
6	752.35	496.59	100.31%	99.32%

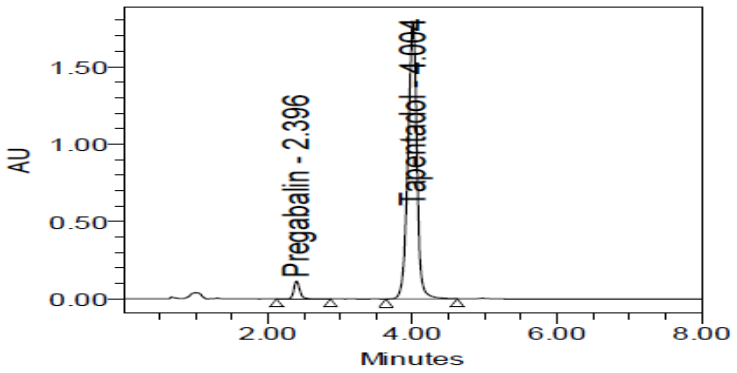

Fig. 1: Standard Chromatogram for Pregabalin and Tapentadol

Table No. 2: System Suitability Parameters

Parameter	PRE	TAP
Retention time	2.411	4.005
Tailing factor	1.22	0.94
Theoretical plate count	4501	5358

Linearity:

Linearity was evaluated from calibration curve data and linear response was observed between 187.5 5 µg/ml to 1125 µg/ml of Pregabalin and 125 µg/ml - 750 µg/ml of Tapentadol. The regression equations were constructed for both drugs are given below

$Y_{pre} = 392.3X + 543.1$ ($r^2 = 0.999$)

$Y_{tap} = 12419X - 10524$ ($r^2 = 0.999$)

***Accuracy*:**

Accuracy was confirmed by doing recovery studies at three different concentration levels 50%, 100% and 150% each in triplicate. The results are reported in **Table 4**.

***Precision*:**

The sample solutions are prepared by using the tablets of Pregabalin and Tapentadol, injected six times in the same day to determine the intraday precision. The results are shown in the **Table 5**.

Table No. 3: Linearity Results

Level	Conc.^{n} (ug/ml) PRE	Conc.^{n} (ug/ml) TAP	Response PRE	Response TAP
I	187.5	125	75724	1388724
II	375	250	148967	3000304
III	562.5	375	218973	4440446
IV	750	500	292485	6083860
V	937.5	625	369492	7689934
VI	112	150	442839	9260399

Table No. 4: Recovery Studies

Drug	Amount added		Amount found		% Recovery	
	PRE	TAP	PRE	TAP	PRE	TAP
50 %	375	250	378.46	248.9408	100.9%	99.5%
	375	250	377.70	247.34	100.92%	98.9%
	375	250	378.47	248.14	100.92%	99.25%
100 %	750	500	752.36	496.60	100.31%	99.3%
	750	500	750.96	496.30	100.12%	99.2%
	750	500	751.57	497.01	100.21%	99.3%
150 %	1125	750	1130.20	756.41	100.46%	100.85%
	1125	750	1128.91	756.44	100.34%	100.85%
	1125	750	1130.13	756.44	100.45%	100.85%

Table No. 5: Intra-day Precision Results

Injection	Area of Pregabalin	Area of Tapentadol
Injection-1	296551	6148603
Injection-2	299707	6153129
Injection-3	295025	6154783
Injection-4	293010	6127838
Injection-5	298788	6122394
Injection-6	297910	6148809
Average	296831.8	6142593
SD	2498.221	13856.92
% RSD	**0.8**	**0.2**

***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 3.1µg/ml and 3.2µg/ml for Pregabalin and Tapentadol respectively. The LOQ was found to be 9.2µg/ml and 9.1µg/ml for Pregabalin and Tapentadol respectively.

Robustness:

Robustness was established by analyzing system suitability of sample at 25°C and 30°C and at flow rates of 0.8ml/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 C_{18} column gave a better resolution than C_8 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 70:30 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 4.005 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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