



Short communication



Received on: 25/08/2014

Accepted on: 30/09/2014

Published on: 15/10/2014

Gumpula Swapna

Srikakatiya Institute of Pharmaceutical Sciences, Vaddepaly, Warangal 506370 A.P. INDIA

Email:

purastulaswapna.may30@gmail.com



QR Code for Mobile users

Conflict of Interest: None Declared !

DOI: [10.15272/ajbps.v4i36.573](https://doi.org/10.15272/ajbps.v4i36.573)

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

Merugu Manasa^{1,2}, Gumpula Swapna¹, Sarigomula Prakash¹

¹ Srikakatiya Institute of Pharmaceutical Sciences, Vaddepaly, Warangal 506370 A.P. India.

² Unity College of Pharmacy, Bhuvanagiri, 508116, A.P. INDIA

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 alliaance 2695 separation module, X Bridge C₁₈ (100 x 4.6 mm, 5 μ) 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 μ g/ml for Pregabalin and 175-750 μ g/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:

Merugu Manasa, Gumpula Swapna and Sarigomula Prakash . RP-HPLC Method for Simultaneous Estimation of Pregabalin and Tapentadol in Bulk and Pharmaceutical Dosage Form. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (36); 2014, 63-66.

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 Alliance 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 μ l
 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.

Replicate	Amount found(ppm)		% Recovery	
	PRE	TAP	PRE	TAP
1	753.13	496.68	100.41%	99.33%
2	752.61	497.51	100.31%	99.50%
3	752.86	496.55	100.38%	99.31%
4	752.56	496.60	100.34%	99.32%
5	752.87	496.60	100.38%	99.32%
6	752.36	496.60	100.31%	99.32%

Table 1: Assay results

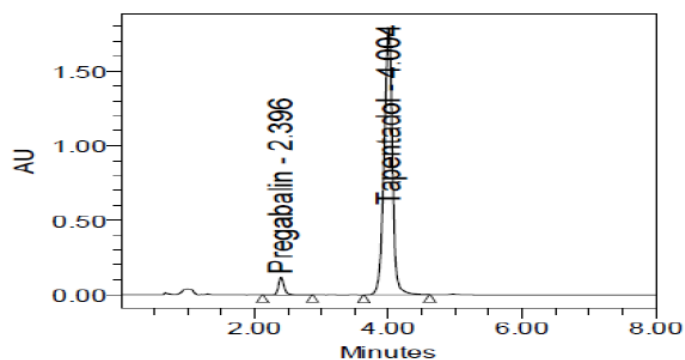


Figure 1: Standard chromatogram for pregabalin and tapentadol

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.

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

Table 2: System suitability parameters**Linearity:**

Linearity was evaluated from calibration curve data and linear response was observed between 187.5 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

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

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

Level	Concentration (µg/ml) Pregabalin	Concentration (µg/ml) Tapentadol	Response Pregabalin	Response Tapentadol
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	1125	750	442839	9260399

Table 3: Linearity results**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.

Drug	Amount added		Amount found		% Recovery	
	PRE	TAP	PRE	TAP	PRE	TAP
50%	375	250	378.46	248.94	100.9%	99.5%
	375	250	377.70	247.34	100.92%	98.9%
	375	250	378.47	248.14	100.92%	99.25%
	750	500	752.36	496.60	100.31%	99.3%
100	750	500	750.96	496.30	100.12%	99.2%
	750	500	751.57	497.01	100.21%	99.3%
	1125	750	1130.20	756.41	100.46%	100.85%
150%	1125	750	1128.91	756.44	100.34%	100.85%
	1125	750	1130.13	756.44	100.45%	100.85%

Table 4: Recovery study**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.

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
Standard deviation	2498.221	13856.92
% RSD	0.8	0.2

Table 5: Intraday precision result**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 of sample at 25°C and 30°C and at 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 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.

REFERENCES

1. Baveja S.K. et al., journal of chromatography A, Pg. no 337-344 (1987)
2. Salo J.P, J.Pharm. Biomed. Anal. 14, Pg.no 1261-1266 (1996)
3. Loyd .R Snyder, et al. John wiley & Sons publishers, INC, New York, 2nd edition, Pg. no 686-706.
4. www.science direct.com 18/9/09
5. 4th version Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005).
6. Salo J.P, J.Pharm. Biomed. Anal. 14, published in 1996, Pg .no 1261-1266
7. Loyd .R Snyder, et al., Practical HPLC Method Development , John wiley & Sons publishers , INC, New York, 2nd edition, Pg. no 686-706.
8. www.science direct.com 18/9/09
9. D.Helmeste et al., J.Chromatogr. Published in 1997, Pg. no 195-201.
10. Internal ICH of technical requirements for the registration of pharmaceuticals for human use, validation of analytical parameters; methodology adopted in 1996, Geneva.
11. ClinicalTrials.gov.htm
12. International Journal of Natural Product Science 2012: Spl Issue 1:115.
13. Kasawar GB, Farooqui MN. Development and validation of HPLC method for the determination of pregabalin in capsules. Indian J Pharm Sci 2010;72:517-9
14. Journal of Applied Chemical Research, 13, 85-89 (2010)
15. Der Pharma Chemica, 2011, 3(1) : 482-489 (<http://derpharmachemica.com/archive.html>)
16. scientificpca.org/paper/2012/09/30/201209302309140A.doc
17. International Journal of Chemical and Pharmaceutical Sciences 2012, June., Vol. 3(2)
18. Narmadha p et al., Int.j.Res.Pharm.Sic.,4(1),25-29
19. Journal of Chemical and Pharmaceutical sciences ISSN: 0974-2115
20. Journal of Global Trends in Pharmaceutical Sciences Vol.3, Issue 3, pp -755-762, July-September 2012
21. Vol-3, Issue-4, Suppl-1, Nov 2012 ISSN: 0976-7908 Gandhi et al
22. www.ijpbs.com (or) www.ijpbsonline.com ISSN: 0975-7619 Research Article www.ditonline.info
23. IJPBS |Volume 2| Issue 3 |JULY-SEPT |2012|01-09
24. International Journal of ChemTech Research CODEN(USA): IJCRGG ISSN : 0974-4290 Vol.5, No.1, pp 34-41, Jan-Mar 2013