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Development and Validation of HPLC Method for Estimation of Pregabalin in Bulk & Capsule Dosage Form

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Abstract

A simple, rapid, specific, precise and accurate HPLC method has been developed for the estimation of Pregabalin in bulk drugs and in capsule dosage forms. The mobile phase consisted of 80: 10: 10 (v/v/v) of Disodium Hydrogen Phosphate Buffer: Acetonitrile: Methanol. The flow rate is 1 ml/min. Chromatographic determination of Pregabalin was performed on Inertsil ODS -3V, C18 (250 X 4.6 mm Id, 5µm) column. The wavelength of detection is 210 nm. The injection volume is 20µL. The retention time of Pregabalin is 4.7 minutes. The developed method was validated in terms of specificity, accuracy, precision, linearity, solution stability, ruggedness, robustness and system suitability. The influence of Acid, Alkaline, Oxidative Stress, Photolytic stress, Thermal stress, and Humidity stress conditions on pregabalin was studied. Results indicated that Pregabalin is stable under the experimental conditions. The proposed method has been successfully used for the routine analysis of pregabalin in capsule dosage forms.

Keywords: HPLC; Pregabalin; Estimation; Bulk drug; Capsule dosage form

Introduction

Pregabalin [1] chemically known as (S)-2(amino methyl)-5-methyl hexanoicacid (Figure 1), is a white crystalline solid, which is soluble in water and in both basic and acidic aqueous solution sand used as anticonvulsant, analgesic medication and neurotransmitter. [2,3] Pregabalin is the 3 isobutyl substituted analogue of δ amino butyric acid (GABA) but is inactive at GABA receptors. [4] This drug produces its actions by binding to the alpha 2 delta (α2δ) subunit of the voltage gated calcium channels. It is well absorbed after oral administration and largely excreted by renal excretion [5] (Figure 1).

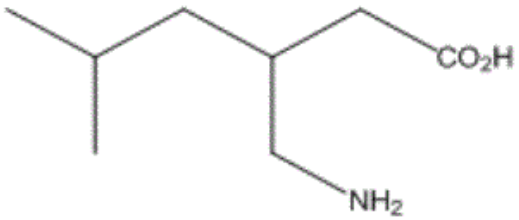

Figure 1: Structure of Pregabalin.

It was designed as a more potent successor to gabapentin. Pregabalin and gabapentin bind with high affinity to α2δ protein, an auxiliary subunit of Q type voltage sensitive calcium channels in the peripheral and central nervous system. [6] Binding to α2δ protein, results in calcium influx reduction at nerve terminals which leads to reduction of neurotransmitters such as glutamate and noradrenaline and abnormal neuronal excitability. [7,8] Pregabalin is thought to be useful for treating any other conditions, pain, physiological conditions associated with psychomotor stimulants, inflammation, gastrointestinal damage, alcoholism, insomnia, and various psychiatric disorders, including mania and bipolar disorder [9].

Pregabalin approved for a number of indications in the US and Europe that include adjunctive therapy of partial seizures in adults, pain from diabetic neuropathy or post-herpetic neuralgia in adults, and the treatment of anxiety disorders. [10] It received U.S.FDA approval for use in treating neuropathy pain and post herpetic neuralgia in 2004, appeared on the U.S market in fall 2005. [11] Recent studies have shown that pregabalin is effective at treating chronic pain in disorders such as fibromyalgia [12] and spinal card injury. [13] It is consider having a low potential for abuse, and a limited dependence liability if missed, and is thus classified as a schedule V drug in the U.S [14].

Although various bio analytical methods for estimation of pregabalin in human serum [15] and spectrophotometric method for estimation of pregabalin in dosage form [16,17] have been reported in the literature. Recently a new validated HPLC method [18] was developed for determination of pregabalin in bulk drug and capsule dosage forms. All of these methods are very expensive because these methods require long and tedious pre-treatment of the samples, laborious clean up procedures and derivatization for the analysis of pregabalin. It requires simple new HPLC method for analysis of pregabalin in bulk and the determination of pregabalin in capsules. So the attempt was made to develop a simple, efficient and selective method for the analysis of pregabalin in bulk and the determination of pregabalin in capsules. The UV detection, which is readily available in most analytical and pharmaceutical laboratories, was used for HPLC instrumentation. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Materials and Methods

Reagents and Chemicals

Disodium hydrogen phosphate (AR Grade), Phosphoric acid (AR Grade), Methanol (HPLC Grade), Acetonitrile (HPLC Grade), Sodium hydroxide (AR Grade), Hydrochloric acid (AR Grade), Hydrogen peroxide (AR Grade), Waters (Mili Q), Filter paper (Whatmann-42), Filter paper (Nylon 0.45µ), Pregabalin working Standard, Pregabalin Capsules, Pregabalin placebo.

Instruments and Columns

HPLC with UV Detector/PDA detectors, Weighing Balance, Centrifuge, Humidity chamber, Photo-stability chamber, Drying oven, pH meter, HPLC columns, Glass apparatus, Sonicator.

Chromatographic Conditions

The proposed method was performed using a liquid chromatography of model Shimadzu Lab solution. The chromatographic separation was achieved on an Inertsil ODS -3V C18 column (250 X 4.6 mm Id, 5µm). The mobile phase consisted of 80: 10: 10 (v/v/v) of Buffer: Acetonitrile: Methanol. The flow rate was 1.0 ml /min and the detection wavelength was 210 nm. The injection volume is 20µL and the retention time of Pregabalin is 4.7 minutes.

Experimental

Preparation of Solutions

Preparation of buffer for mobile phase: Weigh and dissolve 5.68 g of disodium hydrogen phosphate in 950 ml of water, adjust the pH of the solution to 6.5 with phosphoric acid and dilute to 1000 ml with water. Filter the solution through Nylon filter paper of 0.45µ filter.

Preparation of the mobile phase: Prepare a mixture of Buffer: Acetonitrile: Methanol in the ratio 80: 10: 10 sonicate and degas.

Preparation of Standard solution: Weigh and transfer 30 mg of Pregabalin standard into a 100 ml volumetric flask. Add 50 ml of methanol sonicate to dissolve. Dilute up to the mark with mobile phase and mix.

Preparation of sample solution: Weigh and transfer powder (triturate of not less than 20 capsules) equivalent to 150 mg of Pregabalin in a 100 ml volumetric flask, add 70 ml of the methanol, sonicate for 45 min, cool and dilute to volume with methanol. Centrifuge / filter through suitable filter discarding first few ml of filtrate. Dilute 5 ml of the filtrate to 25 ml with mobile phase.

Analysis of Pregabalin from Capsule Dosage form: Inject 20 µl of the blank (mobile phase) once (Figure 2) and standard preparation (Figure 3) in replicate. Inject 20 µl of sample preparation (Figure 4) in duplicate into the HPLC, record the chromatograms, and measure the responses of the peak due to Pregabalin. Calculate the percent content of mg/capsule (Figure 2, 3, 4 & 5).

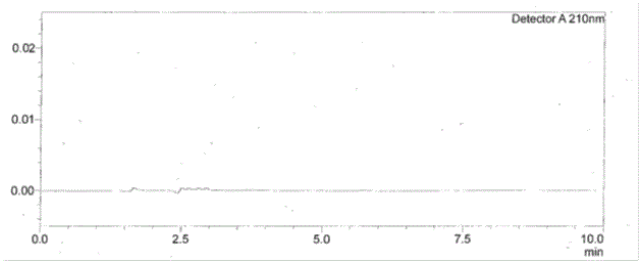

Figure 2: Chromatogram of Blank (Mobile Phase).

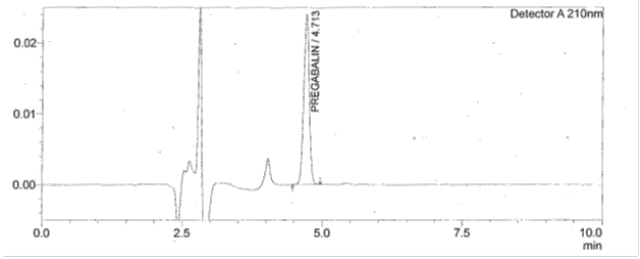

Figure 3: Chromatogram of Standard Preparation of Pregabalin.

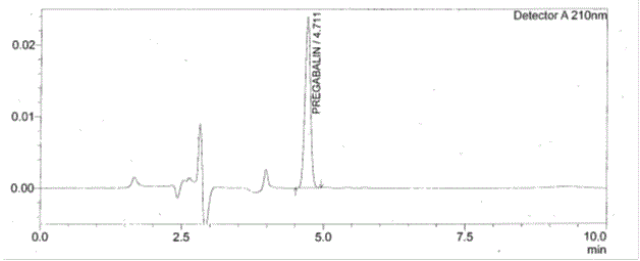

Figure 4: Chromatogram of Sample Preparation of Pregabalin.

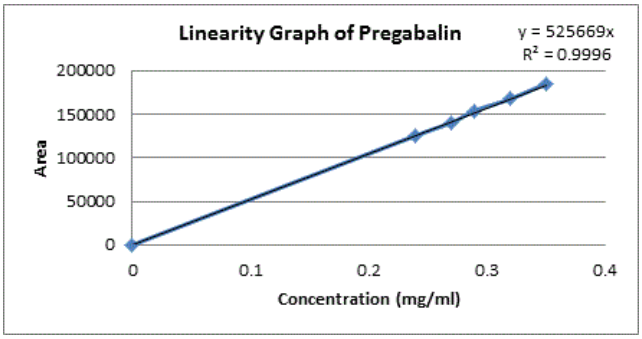

Figure 5: Linearity Graph of Pregabalin.

Method Validation

A full method validation was performed according to guidelines set by the ICHGuidelines. The validation of this procedure was performed in order to evaluate the method in terms of Specificity, accuracy, precision, linearity, solution stability, ruggedness, robustness and system suitability.

Specificity: Inject blank, placebo, Standard and Sample preparation. Check any interference from diluents and placebo at the Pregabalin peak.

Forced degradation: Stress the sample at the following conditions and evaluate the purity index of Pregabalin peak. Sample stress by Hydrogen Peroxide, Thermal, Humidity, Hydrochloric Acid, Sodium Hydroxide and Photo-Stability. The results are shown in the Table 1.

S.No	Mode of degradation	Condition	% w/w Assay	Peak Threshold	Purity Index	Peak Purity
1	As such sample	As is sample	100.2	0.999	0.999	Pass
2	Acid treated	0.1N HCl	92.8	0.999	0.999	Pass
3	Base treated	0.1N NaOH	96.3	0.999	0.999	Pass
4	Peroxide treated	3% v/v H_2O_2	99.5	0.999	0.999	Pass
5	Thermal treated	At 60°C	98.2	0.999	0.999	Pass
6	Humidity treated	40°C/ 75%RH	97.3	0.999	0.999	Pass
7	Photo-stability	Open exposure	99.9	0.999	0.999	Pass
8	Photo-stability	Primary pack	100.1	0.999	0.999	Pass
9	Photo-stability	Secondary pack	100.6	0.999	0.999	Pass

Table 1: Specificity (forced degradation) results.

Linearity: Linearity shall be performed in the range from 80% to 120% of working concentration of specification limit and Y-intercept shall be noted. Linearity of pregabalin was shown in Table 2 & Figure 5.

% Level	Concentration(mg/ml)	Area
80	0.24	125512
90	0.27	140176
100	0.29	154148
110	0.32	167567
120	0.35	184962
Correlation Coefficient	0.999	
Y-Intercept	525669	

Table 2: Linearity.

Accuracy (Recovery): Prepare the sample solution in triplicate by spiking placebo with known standard at about 80%, 100% and 120 % of Pregabalin to its specification limit. % recoveries were calculated and the results are shown in the Table 3.

Sample ID	Amount added-mg	Amount found-mg	%Recovery	Average	%RSD
Rec.80%-1	120.19	118.95	99		
Rec.80%-2	120.1	119.28	99.3	99.2	0.15
Rec.80%-3	121.01	120.01	99.2		
Rec.100%-1	149.44	148.04	99.1		
Rec.100%-2	149.5	148.17	99.1	99	0.23
Rec.100%-3	148.61	146.74	98.7		
Rec.120%-1	180.95	180.66	99.8		
Rec.120%-2	180.84	180.11	99.6	99.8	0.25
Rec.120%-3	180.55	180.8	100.1		
Overall Average				99.3	
Overall SD				0.44	
Overall %RSD				0.44	

Table 3: Accuracy (Recovery).

System Precision: Prepare the standard solution as per the method and inject in replicates as per the method. Calculate the tailing factor and theoretical plate from the first standard chromatograph (Table 4).

Sample ID	Area Response
Injection -1	164140
Injection -2	163998
Injection -3	163977
Injection -4	163847
Injection -5	163717
Average	163936
SD	160
%RSD	0.1

Table 4: System Precision.

Method precision: Prepare 6 samples of single batch and analyze the same as per the test method and calculate the % RSD of % Assay (Table 5).

Sample ID	% Pregabalin
Sample -1	99.1
Sample -2	98.9
Sample -3	98.7

Sample -4	98.9
Sample -5	98.8
Sample -6	98.8
Average	98.9
SD	0.14
%RSD	0.14

Table 5: Method Precision.

Stability in analytical solution: Prepare one sample as per test method and inject in duplicate into HPLC at initial and at different time intervals up to 24hrs. Determine the % Assay at different time interval samples (Table 6).

Time interval	% Pregabalin
Initial	99.1
6hrs	99
10hrs	98.1
24hrs	98.6
Correlation (6hrs~ Initial)	1
Correlation (10hrs~ Initial)	0.99
Correlation (24hrs~ Initial)	0.99

Table 6: Solution stability.

Ruggedness: Perform precision of test method by different analyst by using different column, different system (HPLC) on different day. Evaluate the system suitability criteria. Calculate the % RSD of % Assay of six samples; calculate the overall % RSD of % Assay of twelve samples between two analysts (Table 7).

S.No	Analyst-1	Analyst-2
Sample -1	99.1	98.5
Sample -2	98.9	100.2
Sample -3	98.7	99.2
Sample -4	98.9	98.6
Sample -5	98.8	99
Sample -6	98.8	100.6
Average	98.9	99.4
SD	0.14	0.86
%RSD	0.14	0.87
Overall Avg	99.1	
Overall SD	0.64	
Overall %RSD	0.65	

Table 7: Ruggedness.

Robustness: Prepare the three samples and analyze under the conditions by changing the flow rate by ± 0.1ml, wavelength ± 2nm, mobile phase pH ± 0.2 units and solvent composition ± 2%. Evaluate the system suitability criteria. Calculate the % RSD of % Assay of three samples; calculate the overall % RSD of % Assay of nine samples between normal and variable condition (Table 8).

ID	Normal condition	Low flow (0.9ml/min)	High flow (1.1ml/min)	Low λmax 208nm	High λmax 212nm	At Low pH 6.3	At High pH 6.7	Low com (82:9:9)	High com (78:11:11)
Sample -1	99.1	99	100.5	98.6	98.8	99	98.2	100.3	99.4
Sample -2	98.9	99.5	101	99.7	99.2	98.6	98.1	99.1	99
Sample -3	98.7	98.9	100.5	98.5	100.9	98.9	98.4	99.7	99.5
Sample -4	98.9	-	-	-	-	-	-	-	-
Sample -5	98.8	-	-	-	-	-	-	-	-
Sample -6	98.8	-	-	-	-	-	-	-	-
Average	98.9	99.1	100.7	98.9	99.6	98.8	98.2	99.7	99.3
SD	0.14	0.32	0.29	0.67	1.12	0.21	0.15	0.6	0.26
%RSD	0.14	0.32	0.29	0.67	1.12	0.21	0.16	0.6	0.27
Overall Avg	-	99	99.5	98.9	99.1	98.9	98.7	99.1	99
Overall SD	-	0.24	0.92	0.35	0.69	0.15	0.34	0.52	0.28
Overall%RSD	-	0.24	0.92	0.36	0.69	0.15	0.35	0.53	0.28

Table 8: Robustness.

Results and Discussion

The HPLC method was developed and validated of pregabalin and determination of pregabalin in capsule dosage form. It was validated for Specificity, accuracy, precision, linearity, solution stability, ruggedness, robustness and system suitability. Detail results of each parameter as shown in Table 9.

S.No	Validation Parameters	Acceptance Criteria	Results/Conclusion
1	Specificity (Selectivity)	No peak shall be eluting at the retention time of Pregabalin peak in blank and placebo.	No peaks eluted at the retention of Pregabalin peak in blank and placebo.
2	Forced degradation	No peak shall be eluting at the retention time of Pregabalin peak upon forced degradation. Purity index of Pregabalin peak shall pass.	No peaks eluted at the retention time of Pregabalin peak upon forced degradation. Purity index of Pregabalin peak passes.
3	Linearity and range	Correlation coefficient 'r' should not be less than 0.99.	Correlation coefficient 'r' was within the specified limit.
4	Accuracy (Recovery)	% Recovery in each level of concentration and mean recovery shall be between 98.0 -102.0.	% Recovery in each level and mean recovery was within the specified limits.
5	System Precision	Tailing factor shall be not more than 2.0. Theoretical plate shall be not less than 1500. % RSD of Pregabalin peak area shall be not more than 2.0.	Tailing factor is 1.04 Theoretical plate is 9704 %RSD is 0.10
6	Method Precision	% RSD of % Assay shall be not more than 2.0 of six samples.	% RSD is 0.14
7	Stability in analytical solution	Sample solution will be considered stable from the time of preparation, up to the correlation of % Assay at different time interval sample against initial % Assay lies between 0.98 -1.02.	Solution is stable up to 24 hrs from the time of preparation.
8	Ruggedness	System suitability criteria shall pass. a) % RSD of % Assay shall be not more than 2.0 of six samples. b) Overall % RSD of % Assay shall be not more than 2.0 of twelve samples between two analysts.	A system suitability criterion passes. % RSD is 0.87 Overall %RSD is 0.65
9	Robustness	System suitability criteria shall pass at each variable condition. a) % RSD of % Assay shall be not more than 2.0 of three samples b) Overall % RSD of % Assay shall be not more than 2.0 of nine samples between normal and variable condition.	A system suitability criterion passes at each variable condition. % RSD of % Assay of three samples & Overall % RSD of % Assay of nine samples between normal and variable condition was within the specified limit.

Table 9: Results and Discussion

Conclusion

A simple, rapid, specific, precise and accurate HPLC method has been developed for the estimation of Pregabalin in bulk drugs and its capsule dosage forms. The test method is validated for Specificity (Selectivity), Linearity (Range), Precision (System, Method), Accuracy (Recovery), Ruggedness, Stability of analytical solution, Filter suitability and Robustness, found to be within the specified limit. The HPLC method for the determination Assay in Pregabalin capsules is accepted as valid since it complies with the requirements for Specificity, system suitability, Accuracy (recovery), Linearity and range, method precision, Ruggedness (intermediate precision), Robustness, Stability of analytical solution and Filter suitability. The influence of Acid, Alkaline, Oxidative Stress, Photolytic stress, Thermal stress, and Humidity stress conditions on Pregabalin was studied. Results indicated that Pregabalin is stable under the experimental conditions. The proposed method has been successfully used for the routine analysis for the determination of Assay in Pregabalin capsules.

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References

1. Bhatacharya K, Sen P, Ray A (1994) Pharmacology and Toxicology 2:192.

2. Donald JA, Burgers (2003) Medicinal Chemistry and Drug discovery (6th edn) Wiley, New Jersey: 312-313.
3. Warner G, Figgitt DP (2005) Pregabalin as Adjunctive Treatment of Partial Seizures. CNS Drugs 19: 265-272.
4. Dworkin RH, Kirkpatrick P (2005) Nat Rev Drug Discov.
5. (2010) Indian Pharmacopeia (6th edn), Ghaziabad.
6. Taylor CP, Angelotti T, Fauman E (2007) Prevalence of acute repetitive seizures (ARS) in the United Kingdom. Epilepsy Res 73: 137.
7. Fink K, Meder W, Dooley DJ, Gothert M (2000) Inhibition of neuronal Ca2+ influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices. Br J Pharmacol 130: 900.
8. Van Hooft JA, Dougherty JJ, Endeman D, Nichols RA, Wadman WJ (2002) Eur J Pharmacol 449: 221.
9. Agarwal S, Bose A, Bhaumik U, Ghosh D (2008) Determination of Pregabalin in Human Plasma using LC/MS/ MS, Chromatographia 67: 237-243.
10. Taylor CP, Angelotti T, Fauman E (2007) Pharmacology and mechanism of action of pregabalin: the calcium channel alpha2-delta (alpha2-delta) subunit as a target for antiepileptic drug discovery. Epilepsy Res 73: 137-150.
11. Dworkin RH, Kirkpatrick P (2005) Improving R&D productivity, Nat Rev Drug Discov 4: 455-456.
12. Crofford LJ, Rowbotham MC, Mease PJ (2005) Pregabalin for the treatment of fibromyalgia syndrome: Results of a randomized, double-blind, placebo-controlled trial. Arthritis Rheum 52: 1264-1273.
13. Siddall PJ, Cousins MJ, Otte A, Griesing T et al (2006) Neurology 67: 1792.
14. Anonymous (2005) Schedules of controlled substances: Placement of pregabalin in to Schedule V Final rule. Fed Regist 70: 43633-43635.
15. Beery D, Millington C (2005) Analysis of Pregabalin at Therapeutic Concentrations in Human Plasma/Serum by Reversed-Phase HPLC. Ther drug moni 27: 451-456.
16. Gujral RS, Haque SKM, Shanker P (2009) Int J of Biomed Res 5: 1-6.
17. Onal A, Sagiril A (2009) Spectrochimica Acta Part A: molecular and biomedical spectroscopy 72: 68-71.
18. Ashu M, Parmar S, Nagarajan K, Singh V (2011) Development and validation of rapid HPLC method for determination of Pregabalin in bulk drug and capsule dosage forms. Der Pharma Chemica 3: 482-489.