International Journal of Medicine and Pharmaceutical Research

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Research Article **Open Access**

Development, Validation & Degradation Studies of RP-HPLC Method for the Determination of Pregabalin in Oral Suspension Powder

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A B S T R A C T

A simple, rapid, selective, precise and accurate reverse phase HPLC method has been developed for the estimation of Pregabalin in oral dosage forms. The mobile phase consisted of 50:50 (v/v) of Acetonitrile & Buffer. The flow rate is 1 ml/min. Chromatographic determination of Pregabalin was performed on Phenomenex C18 column (150 X 4.6 mm Id, ODS 5µm). The wavelength of detection is 262 nm. The injection volume is 10µL. The retention time of Pregabalin is 2.501 ± 0.09 minutes. The developed method was validated in terms of accuracy, precision, linearity, and limit of detection, limit of quantitation, solution stability, ruggedness, and robustness. The influence of Acid, Alkaline, Oxidative Stress, Photolytic stress conditions on Pregabalin was studied. Results indicated that pregabalin is stable under the experimental conditions with a high baseline noise observed in alkaline medium. The proposed method has been successfully used for the routine analysis of pregabalin in oral suspension powder.

Keywords: Pregabalin, GABA, Anticonvulsant, HPLC

A R T I C L E I N F O

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Article History: Received 18 July 2015, **Accepted** 21 August 2015, **Available Online** 10 October 2015

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Manuscript ID: IJMPR2671

PAPER-QR CODE

Citation: Eswara Rao Bammidi, et al. Development, Validation & Degradation Studies of RP-HPLC Method for the Determination of Pregabalin in Oral Suspension Powder. *Int. J. Med. Pharm, Res.*, 2015, 3(5): 1151-1156.

1. Introduction

Pregabalin (PGB), (*S*)-3-(aminomethyl)-5-methylhexanoic acid, is a structural analogue of γ - amino butyric acid (GABA) as shown in **(Figure 1)**. It is a white crystalline solid. It is soluble in water and in both basic and acidic aqueous solutions. It is a new anticonvulsant and analgesic medication that was recently approved as an add on therapy for partial seizures in adults and for the treatment of neuropathic pain from post-therapeutic neuralgia and diabetic neuropathy[1,2]. It was designed as a more potent successor to gabapentin.

Pregabalin, an antiepileptic drug similar to gabapentin produces its actions by binding to the alpha2-delta (α2δ) subunit of the voltage-gated calcium channels[3]. It reduces calcium influx into synaptosomes prepared from human brain[4,5] and it subtly reduce calcium dependent overflow of neurotransmitters like glutamate and substance P from several different neuronal tissues and reduce synaptic responses.

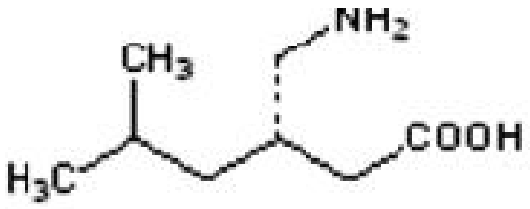


Figure 1: Structure of Pregabalin

PGB 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. Pregabalin is not official in any pharmacopoeia. A thorough literature search has revealed that only a few analytical methods are available for determination of Pregabalin in bulk drugs and pharmaceutical formulations[6]. Determination of Pregabalin without pre-column derivatisation using RP-HPLC has not been reported thus far. Therefore, in the present investigation an attempt has been made to determine pregabalin in oral dosage forms using RP-HPLC. This method was validated as per ICH guidelines.

2. Materials and Methods

Reagents and chemicals:

Methanol-HPLC grade, Acetonitrile-HPLC grade,Water-HPLC grade, Sodium dihydrogen phosphate, Disodium hydrogen phosphate , Potassium dihydrogen phosphate, Disodium EDTA, Mercuric chloride, Distilled water, Toluene, n-Butanol, Ethyl acetate, Triethylamine, Sodium hydroxide, Hydrochloric acid, Hydrogen peroxide, Nitric acid, Chromic acid, Glacial acetic acid, Sucrose, Pregabalin.

Apparatus:

Volumetric flask (5, 10, 25, 50, 100, 250 ml)**,** Graduated Pipette (1, 2, 5, 10 ml)**,** Measuring cylinder (10, 100 ml)**,** Thermometer (300^0C)**,** Funnel (25, 50, 100 ml)**,** Petridish**,** Conical flask (25, 100, 250 ml)**,** Glass Beaker (100, 250, 500 ml)**,** Plastic beaker (500 ml)**,** Centrifuge tube (5, 10 ml). 0.45 µ HPLC Filter, Polypropylene micro centrifuge tune, Centrifuge (SEMI), Whatmann Filter paper No 41.

Chromatographic conditions:

The Chromatographic system consisted of a Shimadzu Class LC-2010CHT, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, PDA SPD-M20ADiod array UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software. The mobile phase consisted of 50:50 % (v/v) of Acetonitrile and buffer. The flow rate is 1ml/min. Chromatographic determination of Pregabalin was performed on Phenomenex C18 column (250 X 4.6 mm id, ODS 5µm). The wavelength of detection is 262 nm. The injection volume is 10µL.

Preparation of Solutions:

Preparation of the mobile phase:

HPLC grade solvents were used in separate bottle of gradient pumps as mobile phase. Acetonitrile and buffer in the ratio of 50:50 v/v were adjusted by gradient pump operated by LC solution software. Mixed solvents were degassed by the instrument and used as mobile phase.

Standard Preparation:

(Pregabalin 1000µg/ml) accurately Weighed 1mg of Impurity-A of pregabalin and transferred to 10ml volumetric flask labeled Impurity stock and diluted to 10ml with diluents. 10mg Pregabalin drug was weighed and taken into a 10 ml clean dry volumetric flask labeled working solution, add 5ml of diluents, and sonicated for 30 minutes. 0.5ml from the impurity stock solution was pipette out and transferred to flask labeled working solution and make up to the final volume with diluents.

Assay:

Accurate quantity of oral suspension powder equivalent to the powder of 10 mg of Pregabalinwas weighed and transferred to 100 ml volumetric flask, dissolved in methanol (60 ml) and sonicated for 30 minutes. The solution was filtered through Whatmann filter paper No. 41 and residue was washed with methanol. To ensure, the solution was filtered again through 0.45 µ filter paper. The solution was diluted up to the mark with methanol. Accurately measured 10 ml of standard sample stock solution was transferred to 100 ml volumetric flask, diluted up to the mark with methanol to get final working concentration of10 µg/ml. The assay content was evaluated using the regression equation of linear calibration curve.

Method validation

Linearity and range:

The calibration curve was plotted over the concentration range of 25% to 125% of pregabalin. Accurately measured standard working solutions of pregabalin (25% to 125%) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with mobile phase[7]. The solutions were filled in the vials of auto sampler rack. Aliquots (20 µl) of each solution were injected from auto sampler under the operating chromatographic conditions described above. The linearity was evaluated by linear regression analysis, which was calculated by least square method. It is depicted in (**Figure 1**).

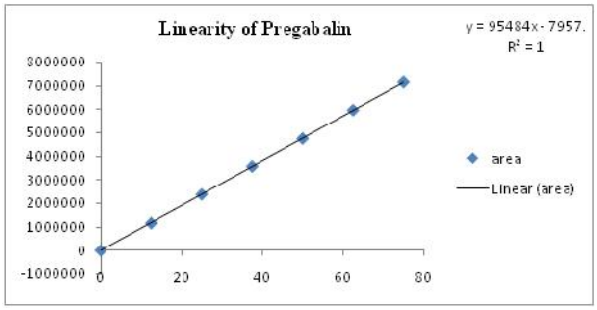
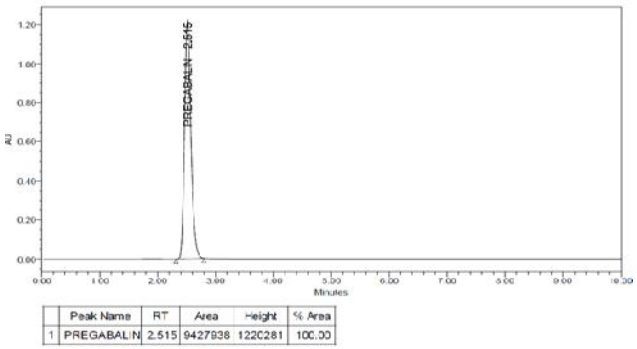


Figure 1: Linear Calibration Curve of Pregabalin

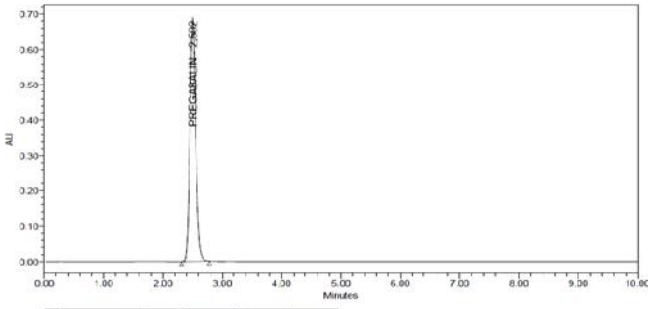
Accuracy (% Recovery): Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. A known amount of pregabalin (50, 100, and 150 %) of standard powder was added to the pre analysed solution of formulation. This solution was analyzed as previously described[8]. The assay was repeated over 3 consecutive days to obtain intermediate precision data. The resultant % CV for this study was found to be < 2.0 % with a corresponding percentage recovery value is shown as follows in Fig 2.



	Peak Name	RT	Area	Height	% Area
1	PREGABALIN	2.515	9427938	1220281	100.00

Figure 2: Chromatogram of Pregabalin showing 100%accuracy

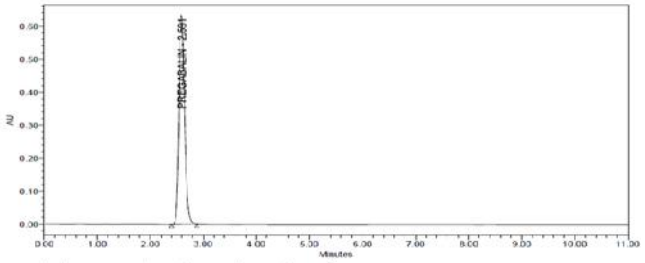
Precision: Precision was evaluated in terms of intraday and interday precision. The intraday precision was investigated using three different concentrations of sample solutions prepared as discussed above, from stock solution. The intraday and interday precision of the proposed method was determined by analysing the corresponding concentration 3 times on the same day and on different days over a concentration of pregabalin (0.6, 0.8, and 1.0 µg/ml). The results were reported in terms of % coefficient of variance (% CV).



	Peak Name	RT	Area	Height	% Area
1	PREGABALIN	2.502	4693625	638093	100.00

Figure 3: Precision of Pregabalin

Robustness: Method robustness was performed by applying small changes in the ratio of mobile phase, injection volume, and column temperature and flow rate[9]. Robustness of the method was done at three different concentration levels. The results were expressed in terms of % CV.



	Peak Name	RT	Area	Height	% Area
1	PREGABALIN	2.591	4637346	637694	100.00

Degradation Studies:

For Stress Degradation Analysis, 1 mL aliquots (induplicate) of samples containing MQC level concentration are treated separately with 100 µL of 0.1N HCl (Acid stress), 0.1N NaOH (Alkaline stress), 5% v/v Hydrogen Peroxide (OxidativeStress), for 24 Hrs. Samples for Photolytic stress are placed in a transparent glass vial & placed in a UV chamber for 24 Hrs. Samples are then injected for analysis. The results of analysis are then compared with similarly prepared fresh samples (**figure 2**).

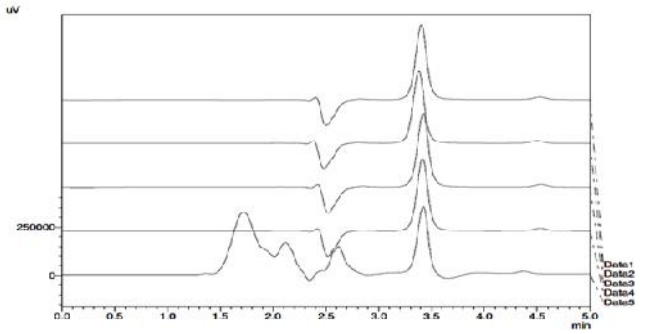


Figure 2: Overlay Chromatogram Showing the Influence of Various Stress Conditions on Pregabalin

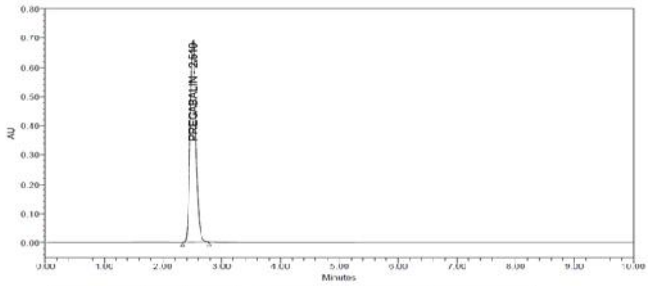
Data 1–Freshlyprepared Sample; Data 2 – Oxidative Stress; Data 3–Photolytic Stress; Data 4 – Acid Stress; Data 5– Alkaline Stress. Data 5 clearly indicates the spectral degradation of Pregabalin due to alkaline instability.

System suitability:

As system suitability test was an integral part of chromatographic methods development and were used to verify that the system is adequate for the analysis to be performed, the system suitability parameters for pregabalin were evaluated[10]. The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values, reported in Table with the acceptance criteria of the CDER guidance documents.

Solution stability: The stability of the drug is determined by placing the MQC samples for the short term stability by keeping at room temperature up to12 hours and then comparing the obtained peak area with that of the similarly

prepared fresh sample. Further, auto-sampler stability for up to24 hrs was studied and established.



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor
1	PREGABALIN	2.510	4783086	394665	100.00	3037.87	1.13

3. Results and Discussion

Method optimization: The HPLC procedure was optimized with a view to develop a stability indicating assay method. The aim of this study was to develop a gradient RP-HPLC assay method for the analysis of pregabalin in formulation. The published literature for the estimation of other azoles antifungal drug in combination and knowledge of the molecule suggest that reverse phase liquid chromatography (RPLC) is suitable for analysis of pregabalin[11]. In case of RP-HPLC various columns are available, but as the main aim of the method is to resolve drug at retention time from excipients found in formulation, C18 column (250 mm x 4.6 mm i.d., 5 µm particle size) was preferred over other columns. A Phenomenex C18 was preferred as it has high carbon loading with very closely packed material to give high performance over other C18 columns. Initial studies to optimise the mobile phase were involved with various mobile phases ratio containing acetonitrile and water in same proportion. In the study Pregabalin (1 µg/ml) showed a retention time greater than 6 minute. In this case, the optimized mobile phase was constituted by different (60:40 and 50:50 v/v) proportion of acetonitrile and buffer. It was observed that satisfactory resolution of pregabalin was obtained with less retention time from solvent peak for acetonitrile and buffer in the ratio of 50:50 v/v, while retention time was more than 4.5 for ratio of 60:40 v/v. Finally, acceptable resolution with reasonable peak shapes and peak purity were achieved by using acetonitrile and buffer in the ratio of 50:50 v/v with flow rate of 1 ml/min at 262nm. The method parameter was optimized to analyse the pregabalin in oral suspension powder.

Method validation: The results of validation studies on RP-HPLC method development for cinacalcet are involved. The developed method as described above was validated for various parameters like system suitability, specificity, linearity, precision, accuracy, LOQ and LOD.

Linearity and range: Linearity of the method was evaluated at six concentration levels by diluting the standard stock solution to give solutions in the concentration range from 25% to 125%. The results show that an excellent correlation existed between the peak area and concentration of analyte. The calibration curve was prepared by plotting the area under the response from the detector (AUC) line versus the concentration and analysed through linear regression. The response for the drug was linear ($r2 = 1$) in the concentration range between 25% to 125%. The linearity was observed in the expected concentration range, demonstrating its suitability for analysis seen in Table 1.

Accuracy & Precision: Accuracy and precision calculated for the QC samples during the intra- and inter –day run are given in Table2 and Table 3. The intra-day (day-1) and inter-day accuracy ranged from 98.00 to 101.00 %. The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria.

System Suitability: The % RSD of the peak area and the retention time for both drug and internal standard are within the acceptable range (**Table 4**). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 3075±32 and the USP tailing factor was 1.13.

Robustness: Robustness is the measure of method capacity to remain unaffected by deliberate small changes in the chromatographic conditions. The experimental conditions were deliberately altered to evaluate the robustness of the method. The impact of flow-rate (1 ± 0.1ml/min), and effect of mobile-phase composition (± 5%) on chromatographic parameters such as retention time, theoretical plates, and tailing factor, were studied**.** There was no significant variation due to the variation of mobile phase composition or flow rate variation.

Stress Degradation: The stress studies involving acid, light (UV) and oxidation revealed that Pregabalin was stable under the stress conditions (**Figure 2**). However in alkaline conditions (0.1N NaOH), the baseline resulted in high noise without affecting the peak shape. For all stress conditions studied, the drug content was within 97 – 99 % indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

4. Conclusion

A validated RP-HPLC analytical method has been developed for the estimation of pregabalin in dosage form. The proposed method is simple, accurate, precise, specific, and has ability to separate drug from excipients if found in formulation. The method is suitable for routine analysis of pregabalin in oral suspension powder. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS. The prime importance was given to develop less time consuming and simple RP-HPLC-PDA method. The RP-HPLC method developed meets the system suitability criteria, peak integrity and resolution for the parent drug. Detection and quantification limits achieved, describe the method is very sensitive. High recoveries and acceptable % CV values confirm established RP-HPLC method is accurate and precise. The analytical results demonstrate the ability of the developed method to assay pregabalin in the presence of their excipients. Also the data for precision show that developed method was precise. Assay results found from the study show that the method is successfully applied for the analysis of pregabalin in formulation. Hence, the method is recommended for routine quality control analysis of pregabalin in formulation.

Table 1: Linearity and Range for Pregabalin

Concentration µg/ml	Peak Area	Statical Analysis
12.5	1172801	
25	2382564	Slope: 95484
37.5	3580737	
50	4751521	Intercept: 7957
62.5	5967584	
75	7153613	Correlation coefficient: 1

Table 2: Pregabalin Demonstrating Accuracy and Specificity of the Method

S. No	Sample ID	CONC µg/ml	Retention Time	Peak Area	Accuracy
1	BLANK	0	0	0	0
2	TS 1	12.5	2.512	7114884	98.27
3	TS 2	25	2.511	7112220	99.34
4	TS 3	37.5	2.510	7119812	101.11
5	TS 4	50	2.516	9454838	98.98
6	TS 5	62.5	2.515	9427938	100.42
7	TS 6	75	2.520	11761614	100.54

Table 3: Pregabalin Demonstrating Precision of The Method

S.No	Retention Time	Peak Area
1	2.51	4789005
2	2.51	4793556
3	2.508	4793528
4	2.508	4789555
5	2.507	4777266
6	2.507	4791100
Mean	2.5083	4789002
STD Dev	0.001366	6060.771
% RSD	0.05	0.13

Table 4: System Suitability Test for Pregabalin

S.No	Pregabalin			
	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
1	2.51	4783086	3037	1.13
2	2.512	4612220	3138	1.11
3	2.511	4607162	3091	1.12
4	2.516	4628589	3179	1.12
5	2.52	4737346	3071	1.14
6	2.515	4576016	3013	1.13
Mean	2.514	4657403	3088	1.125
STD Dev	0.0037	82712.18	62.18	0.0104
% CV	0.49	0.17	0.21	0.92

5. Abbreviations

PGB : Pregabalin
GABA: Gabapentin
LOD : Limit of Detection
LOQ : Limit of Quantification.
UV : Ultra violet
RSD : Relative Standard Deviation.
STD DEV: Standard deviation
CV : Coefficient of variance

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