Research Article

Modification and Validation of HPLC Analytical Method for the Estimation of Pregabalin in Capsule Dosage Form

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ABSTRACT

The major goal of this study was to modify and validate a HPLC analytical method to quantify the pregabalin in its solid dosage form. The separation and quantification were done on waters 3.9 x 300 mm column with 10 µm internal diameter. Mobile phase consisted of acetonitrile: phosphate buffer (KH_2PO_4) in the ratio of 50:950. Flow rate was 1.5 ml/min whereas the detection was done at 210 nm. The retention time for pregabalin was 6.5 min with average USP tangent of 5730.195± 0.376. There was a good correlation between concentration of pregabalin and their dilutions with r^2 =0.9993. The capacity of method for percent recoveries at three levels over a range of 70-130% were 97.68 - 102.48% with % CV value of 0.73 - 1.95 %. The % CV of intra and inter-day variation was between 0.46 -0.79 and 1.37-0.072 for the concentration of 50, 100 and 150 µg/ml respectively. The results indicated the repeatability, reproducibility and robustness of the method with limit of detection 0.25 µg/ml and 1.0 µg/ml as limit of quantification. The stability study of 160 µg/ml of pregabalin solution (mobile phase) was done to assess the possible decomposition. It was concluded that the proposed method is suitable for routine analysis of pregabalin in its dosage forms. It could be beneficial for the estimation of stability of pregabalin in in-vitro pharmacokinetic studies.

Keywords: Optimization and validation of analytical method, ICH parameters for validation, Pregabalin, stability study.

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INTRODUCTION

The analytical method development is a process of demonstrating that the method developed is proper for its intended use. An accurate and reproducible analytical method proves that the obtained data is true and validated. In this regard, HPLC plays an important role in the separation and quantitative identification of components as raw in single or in a mixture. Amount of drug and their metabolites in the unit of pharmaceutical dosage forms are the main and crucial quality characteristics that help to check and confirm the quality, safety, and efficacy of drug substances and drug products.

Pregabalin is a structural analogue to gamma-aminobutyric acid (GABA), just as with gabapentin (GBP)[1]. Initially it was used for the treatment of epilepsy, but latter studies described their usefulness in the treatment of neuropathic pain (NeP)[2-4]. It is chemically described as 5-methyl-hexanoic acid. It is white to off-white crystalline solid, freely soluble in water and in basic and acidic aqueous solution[5]. Due to highly soluble and highly permeable compound; the oral absorption is almost 90% and is not dependent of dose and rate of administration. On the other hand, Pregabalin is classified as a schedule V drug in the U.S under Administrative Controlled Substances Code Number (ACSCN)[6] # 2782. It has narrow therapeutic Index and is highly potent drug. It may cause serious side effects/adverse effects if the percentage content of drug per unit is not as per label claim.

The main efforts of the current study were to modify and validate HPLC analytical method for the determination of pregabalin in their dosage forms, depending on the availability and feasibility of the facilities. Pregabalin is errant in nature, so the reliable scrutiny of active component in its dosage forms is essential to control the content uniformity and to establish the quality and safety of products. To achieve this goal, HPLC is the most used technique. The basic concept of this method was adapted from Indian Pharmacopoeia[7] 2014.

MATERIALS AND METHODS

Materials

Pregabalin was obtained as gift sample from Dr Reddy Laboratories, Hyderabad, India. Potassium hydroxide, hydrochloric acid (Merck), Potassium dihydrogen phosphate (Merck), Sodium Hydroxide (Sigma Aldrich), and distill water was freshly prepared by distillation

method. PGB capsules (150 mg) were randomly purchased from local market of UAE

Instruments

The projected study was completed by using HPLC system comprises of a LC-20AD liquid chromatography equipped with SPD-20A UV-VIS detector. Chromatographic separations were performed on waters column, C18 (3.9 × 300 mm; 10 µm packing) which was attached with loop 20 µL and attached with HPLC-Dell system. Electronic balance (Mettler Toledo, England), pH meter (Hanna).

Preparation of Mobile phase

A mixture of Acetonitrile and KH_2PO_4 buffer (pH=6.9) was prepared in the ratio of 50:950. Mobile phase was filtered through filtration unit (Millipore, 0.45 µm pore size) and degassed before use.

Preparation of Diluent

Weighed 1.2g of monobasic potassium phosphate and dissolved in 900ml of distilled water. The pH was adjusted to 6.9 ± 0.05 with 5N KOH and volume was made up to 1L with distilled water[7]

Preparation of Standard solutions

50 mg of Pregabalin (Ref) powder was weighed accurately and transferred carefully in to 50 ml volumetric flask. It was diluted with diluents and shaken for 5 min. After shaking, 4 ml of solution was pipetted out and was diluted to 25 ml to get the final standard solution of 160 µg/ml. It was filtered through 0.45µm filter paper.

Specification of HPLC

Mobile Phase was a mixture of Acetonitrile: KH_2PO_4 (50:950). The separation was carried out at wavelength of 210 nm with flow rate of 1.5 ml/min. System suitability parameters were as per USP[8] (2018) i.e included, Theoretical plate numbers > 2000; Tailing factor < 2.0 and Resolution > 2.0 (indicates method variation and column aging) whereas RSD of replicate injections must be < 2.0% gives the idea about the system performance.

Construction of Calibration curve

The calibration curve was constructed by using pregabalin (Ref.) powder. Series of different concentrations (250-50 µg/ml) were made in diluent and absorbance of solutions was measured by HPLC at λ = 210 nm.

Optimization and Validation of HPLC method

The study was conducted to optimize and validate HPLC analytical method to determine pregabalin. The experiment was carried-out according to the specifications of United State Pharmacopeias[8] (USP 41–NF 36, 2018) and validated as per International Conference on Harmonization[9] (ICH-1996). The method was validated for the parameters as system suitability, specificity, range and linearity, limit of detection, limit of quantification, accuracy, precision, ruggedness and robustness.

RESULTS AND DISCUSSION

To generate a reliable and accurate quantitative data, it is important to evaluate and validate an analytical method to ensure that they produce valid results, appropriate for their intended purpose. Steps involved in experimental procedure must be simple and easy. Depending on the extent of the adjustment, it is essential to understand the nature of compound that help to gather the basic information regarding the sample pre-treatment, ideas about the separation techniques, wavelength for detection and analytical conditions to ensure that they work properly in its local environment.

Pregabalin is freely soluble in water and high polar compound. Beside this, it has narrow therapeutic Index, that means it is highly potent in its function. It may cause major side effects or adverse effects if the amount of drug in their dosage form is not estimated properly. Pregabalin is an antiepileptic drug, functions by binding to the alpha2-delta (α2δ) subunit of the voltage-gated calcium channels[10]. These actions might fluctuate during the transportation of drug from site of administration to site of action due to change of temperature and pH of GIT.

A rapid and accurate method of analysis to get a satisfactory result from pharmaceutical products is the most important part of any research and developments. Adjustment of the capacity factors like analysis time and detection sensitivity, for good chromatographic separation are the challenging point in the optimization of method. In the present study, the focusing point of pregabalin is its high solubility, polarity and UV absorption.

A Reverse phase HPLC isocratic method was developed. The basic component of mobile phase is monobasic potassium phosphate and the idea regarding the selection of this salt was adapted from Indian Pharmacopeia[7], keeping in mind the system suitability parameters like resolution factor (Rf) between peaks, tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness.

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. This test is based on feasibility of equipment, analytical procedure and samples used to analyze[11]. System suitability test was done by injecting six replicate injections of standard pregabalin solution of 1mg/ml.

The percentage coefficient of variation (% CV) of the retention times and the peak areas of pregabalin were 0.052% and 0.753% respectively. Whereas the Mean theoretical plates count, based on USP tangent calculations[5] for pregabalin peak was 5730.195 (Table 1). The higher plate counts define the efficiency of column, whereas the tailing factor 1.2, indicates almost the symmetrical pictures of peak.

Table 1: Summary of System suitability results for Pregabalin

Injection Number	Retention Time of Pregabalin (min)	Peak Area of Pregabalin	USP Tailing Factor	USP Tangent
1	6.583	433339	1.273	5760.62
2	6.584	430312	1.269	5741.01
3	6.583	428920	1.264	5713.46
4	6.577	428878	1.263	5717.41
5	6.580	435892	1.266	5704.76
6	6.587	435767	1.261	5743.91
Mean	6.582	432184.7	1.266	5730.195
% CV	0.052	0.753	0.346	0.376

The calibration curve was linear over the range of 250-50 µg/ml and regression analysis (R^2) was calculated by examining the samples of five different concentrations that was found to be 0.9993. The value of R^2 indicated that the proposed analytical procedure has ability to give test results (quantitative data) with acceptable level of accuracy and precision (Figure 1).

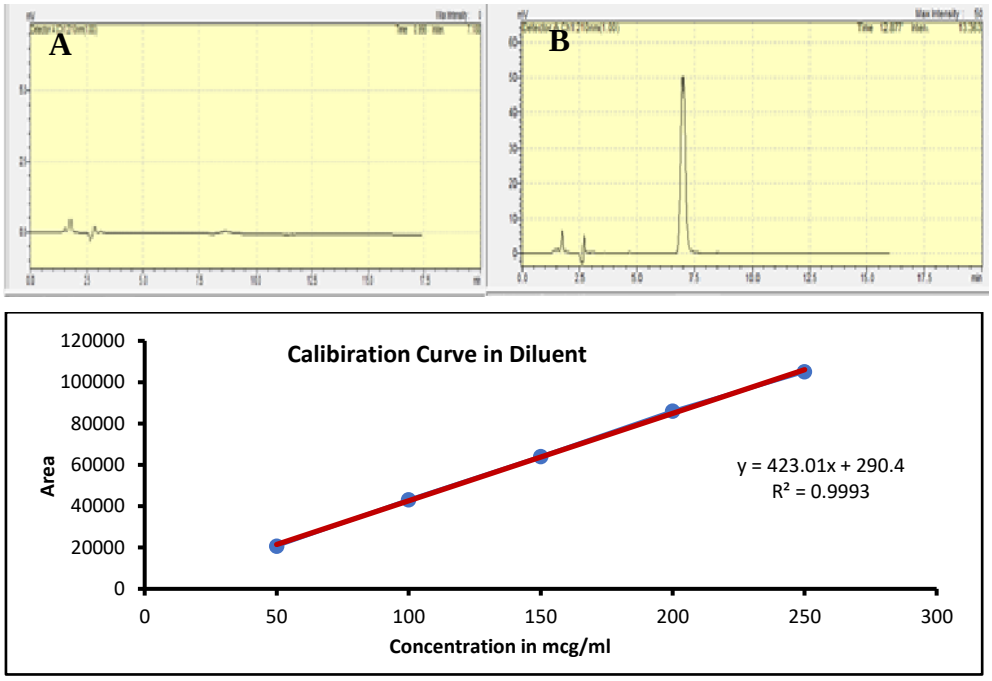


Figure 1: Calibration curve shows linearity over the concentration range 250-50 µg/ml

The ability of method for percent recoveries (accuracy) was calculated by samples solution prepared in triplicate at three levels over a range of 70-130% and performed as per procedure. The recovery ranges were from 97.68 to 102.48% with % CV value of 0.73 to 1.95 % (Table 2).

Table 2: Accuracy data for the assessment of pregabalin

Level	Sample Number	Sample Amount (mg)	Pregabalin		% Recovery	% Mean Recovery	% CV
			Theoretical mg	Measured mg			
70%	**1**	183.26	105.73	103.28	97.68	98.81	1.33
	2	183.75	106.01	106.29	100.26		
	3	183.68	105.97	104.37	98.49		
100%	**1**	255.6	147.46	151.11	102.48	100.31	1.95
	2	262.5	151.44	151.092	99.77		
	3	261.3	150.75	148.74	98.67		
130%	**1**	343.98	198.45	197.32	99.43	100.023	0.73
	2	338.52	195.3	196.94	100.84		
	3	339.17	195.68	195.28	99.8		
Mean						99.71	1.34

% CV = coefficient of variation (help to precise the estimation)

These results demonstrate the closeness of recovery values from their theoretical values and expressed that the intended method is fast and accurate for the quantification of pregabalin.

Precision of the method was demonstrated at two different levels such as repeatability and intermediate precision[12]. Repeatability was carried out by injecting six (6) consecutive samples of 1000µg/ml of the test concentration (system suitability), that help to understand the operating conditions of equipment over a short period of time, as the result is tabulated in Table 1. It is also expressed as inter and intra-day assay.

The repeatability of the method was checked by injecting three different concentrations at different time intervals within a day or in different days. The tabulated results of all the samples (Table 3 & 4) were calculated for % CV and were found to be 0.46–0.79 and 0.072–1.37 respectively, which was well within the acceptance limit of ≤ 2%.

Table 3: Intra-day variation in the analysis of Pregabalin

Conc. (µg/ml)	9.00 am	12.0 am	3.00 am	Mean	±SD	% CV
50	21052	20674	20720	20815.33	206.25	0.99
	20976	21104	20857	20979	123.53	0.59
Mean	**21014**	**20889**	**20788.5**	**20897.2**	**112.97**	**0.54**
100	42337	42467	43038	42614	372.90	0.88
	42747	42472	43066	42761.7	297.27	0.70
Mean	**42542**	**42469.5**	**43052**	**42687.83**	**335.09**	**0.79**
150	63576	63148	63980	63568	416.06	0.65
	63416	63882	64048	63782	327.65	0.51
Mean	**63496**	**63515**	**64014**	**63675**	**293.74**	**0.46**

±SD = Standard deviation (Measures the amount of variability)

Table 4: Inter-day variation in the analysis of Pregabalin

Concentration (µg/ml)	1st day	2nd day	3rd day
50	21974	21008	20875
	21552	211467	20997
Mean	21763	21077	20936
% CV	**1.37**	**0.46**	**0.41**
100	42337	43116	42718
	42747	43072	43096
Mean	42542	43094	42907
% CV	**0.68**	**0.072**	**0.62**
150	63980	63917	63535
	64048	63429	63614
Mean	64014	63673	63574.5
% CV	**0.075**	**0.54**	**0.088**

Intermediate precision of the method, as per ICH guidelines[9] also known as reproducibility / inter-laboratory trial, was carried out by different analyst in different laboratory (Department of Pharmaceutics, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan), using different HPLC column and system (Table 5). The shorter retention time of method illustrating lower solvent consumption, made it easy to implement daily in routine QC analysis. It is also leading that the procedure is environmentally friendly and allows the analysis of large number of samples in a short period of time which is supporting the need of study.

The result of samples by using different HPLC system, was calculated for % CV, was found between 0.28-4.96%. It indicates that the method adequately meets the acceptance criteria for inter-batch precision which is appropriately less than 15%. The %CV of LLOQ concentration is 4.96%, which is also within the acceptance limit of ≤ 20%[13] (Table 5).

Table 5: Intermediate Precision Data of Pregabalin

Nominal True Concentration (µg/mL)	Measured Value (µg/mL)	% Result	Statistical Analysis		
			Mean	±SD	%CV
10	10.702	107	102.1	5.06	4.96
	9.69	96.9			
	10.24	102.4			
50	49.04	98.08	98.1	0.27	0.28
	49.19	98.38			
	48.92	97.84			
100	98.69	98.69	99.59	0.78	0.78
	100.01	100.01			
	100.07	100.07			
150	151.30	100.87	99.89	0.87	0.87
	149.39	99.59			
	148.82	99.21			

% CV = Coefficient of variation (help to precise the estimation); ±SD = Standard deviation (Measures the amount of variability)

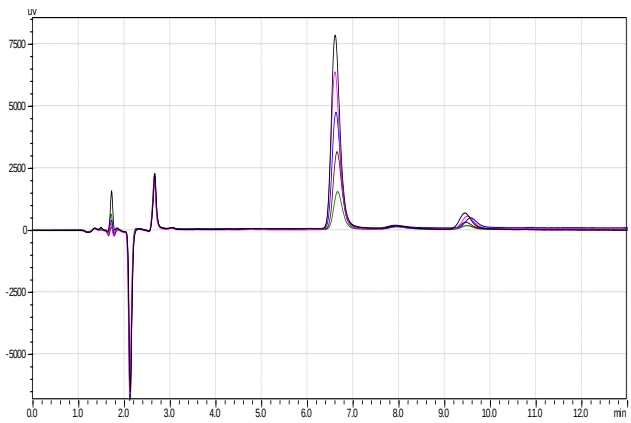
For more verification and validation Robustness study was also carried out on a sample concentration of 160 µg/ml by making slight changes in flow rate, concentration of acetonitrile and change in pH of mobile phase (Table 6). The results demonstrated that there were no significant variations due to the changes in flow rate from 1.0 ml/min. to 1.5 ml/min, with % CV 0.26- 0.078, due to change in pH from 6.7 to 6.9, with coefficient variation of 0.773-0.74 % and the variation in the concentration of acetonitrile from 3.5 to 5.0 % indicated a recovery of 99.77 and 99.8% with % CV 0.071 and 1.634 respectively.

Table 6: Results for Pregabalin Robustness Test

Parameter	Changes	% Target	% Recovery	%CV
Target Conditions		100.73	99.93	0.57
Flow rate	1.0 ml/min	100.0	99.63	0.26
	1.5 ml/min	100.2	100.31	0.078
Buffer pH	6.7	101.5	100.4	0.773
	6.9	100.7	99.66	0.74
Acetonitrile Variation	5 %	102.1	99.77	1.634
	3.5 %	99.9	99.8	0.071

% CV = Coefficient of variation (help to precise the estimation)

Limit of detection (LOD) and Limit of quantification (LOQ) were also done based on peak responses of standard solution in the range of 20-1.0 µg/ml, in which LOQ (1.0 µg/ml) and LOD 0.25 µg/ml respectively with R^2 = 0.9994 (Figure 2). The value of LOD and LOQ was supported by the study of Kasawar and Farooqui[14], 2010.



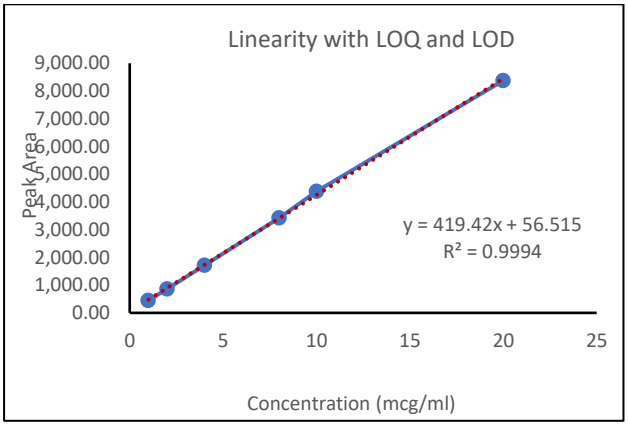


Figure 2: Estimation of LOD and LOQ of Pregabalin

Analysis of Pregabalin in their dosage form was also verified by the intended method. It is simple, easy and cost-effective method. After validation, it was applied for the assessment of active compound in its capsule dosage form. Retention time was between 6-7 minutes that indicated the effectiveness and suitability of method for routine quality control analysis of finish products

Stability

The standard solution of 160 µg/ml of pregabalin was kept in refrigerator for 7 days to estimate the possibility of decomposition of API in solution form. The results of analysis showed that the solution was relatively stable with % CV of 0.0086 - 0.234 (Table 7).

Table 7: Stability study of Pregabalin solution

Sample	Day 0	Day 1	Day 3	Day 7
Assay-1	73109	72325	73404	72568
Assay-2	73046	72125	73413	72328
MEAN	73077.5	72225	73408.5	72448
± SD	44.55	141.42	6.36	169.71
% CV	0.06	0.196	0.0086	0.234

% CV = Coefficient of variation (help to precise the estimation); ±SD = Standard deviation (Measures the amount of variability)

The study revealed that no significant degradation has occurred in pregabalin solution. The retention time and peak area of solution were almost unchanged, indicating that the application of Pregabalin sample (in mobile phase), can be used for the QC analysis without facing stability issue.

CONCLUSION

The good point of this method is its simplicity, without any change in the analyte properties or increasing the phase of analysis (derivatization), the sample can be prepared in one step and can be used for routine QC analysis of pregabalin in their dosage form. The retention time is only 6-7 minutes, so large number of samples can be run in the same day. The method is isocratic and the quantity of organic solvent used is very small (5ml/100), therefore the method is nonhazardous, economical or cost effective also. The inter day, intraday and short-term stability study indicated that the validated method is good enough with the quality of reproducibility and repeatability. It was concluded that the modified method can be used confidently for the precise and accurate analysis of Pregabalin

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