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Regular Article

Development of a Method and its Validation for Estimation of Pregabaline in Pharmaceutical and Bulk Formulation

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Received 15 September 2015; Revised 1 December 2015; Accepted 1 December 2015; Published 25 December 2015

Editor: Chih-Yuan Tseng

Abstract

Simple HPLC isocratic reversed phase method was validated after its development for the analysis of Pregabalin in pharmaceutical and bulk formulations. A C18 5 µm BDS hypersil column (250 mm × 4.6 mm) using phosphate buffer solution (pH 6.9) and acetonitrile in the ratio of 94:6 as a mobile phase was used for the completion of separation. The compound eluted isocratically having flow rate of 1.0 ml /min. The wavelength of 210 nm was set in UV detector for the detection of pregabalin. The linearity of the method was maintained within the range of 0.60 - 0.89 µg/ml. The method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation. For the evaluation of the impact of minor changes and for the establishment of appropriate system suitability parameters robustness test also done. According to ICH guidelines, the method was validated in terms of specificity, linearity, accuracy, precision and robustness. The proposed HPLC method was found specific, accurate as well as precise for the estimation of pregabalin from its pharmaceutical dosages form an also found suitable for routine analysis and quality control of pharmaceutical preparation containing pregabalin active pharmaceutical ingredient.

Keywords: Pregabalin, Isocratic system, Validation, High Performance Liquid Chromatography (HPLC), Pharmaceutical formulations.

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1. Introduction

Pregabalin is an antiepileptic drug that is both structurally and pharmacologically related to gabapentin. It is newly approved drug which having therapeutic action against neuropathic pain from post therapeutic neuralgia, diabetic neuropathy and partial seizures in adults [1-3]. Literature survey has revealed that only few analytical methods are available for estimation of pregabalin in pure drug and pharmaceutical formulations such as HPLC with fluorescence detector [4], LC-MS-MS from human plasma [5], Tandem mass spectrometry [6], Enantiospecific analysis [7], Spectrophotometric and spectroflourimetric methods [8], UV- Vis Spectrophotometric technique [9].

All the reported methods are quite complicated and cannot be utilized for routine analysis of pregabalin. Among these methods UV-Vis spectrophotometric technique is most simple for routine analysis, but it cannot be utilized for estimation of pregabalin. Because, pregabaline is less UV sensitive due to lack of strong chromophoric group, which is the basic condition to be UV-VIS sensitive [8]. As MS based systems are expensive, complex and unavailable in many laboratories and at the same time requires quantification of matrix effect as a must, HPLC/MS-MS methods are less preferable to HPLC-UV methods in many occasions [10]. Hence, the aim of this investigation was to develop and validate a simple HPLC-UV method for the rapid quantification of pregabalin in bulk and pharmaceutical formulation, and its application to assess the effectiveness for its intended purpose.

2. Materials and Methods

2.1 Chemicals/reagents used in the study

All the chemicals and reagents used in this study was analytical grade. The drug used are Pregabalin (Kopran ltd, India) & other chemicals used are Lactose Monohydrate, Maize Starch, Talc (Dr. Paul, India), Acetonitrile, Phosphoric Acid (Merck, India), Potassium dihydrogen phosphate (Qualigens fine chemicals, Mumbai, India) & Purified water (HPLC grade).

2.2 Instruments used in this study

Instruments used in this study are listed in Table 1.

Table 1. Instruments used in the study

Name of the Instruments	Origin
HPLC	Dionex, Ultimate 3000, Germany
HPLC column C18 5 µm ODS hypersil column (250 mm × 4.6 mm)	Thermo Scientific, UK
Mobile phase filtration unit	Pall Life Sciences, Mumbai, India
Analytical balance	Thermo Scientific, UK
pH meter	Thermo Scientific, UK

2.3 Chromatographic conditions

The HPLC system (Dionex, Ultimate 3000, Germany) equipped with binary pump, a degasser, and an autosampler having thermostat column oven was used. The analysis was performed using BDS C_{18} column (4.6 mm x 25 cm) at ambient temperature. The volume of injection was 20 µL having flow rate of 1.0 ml per minute and run time was 9.0 minutes with an isocratic system.

2.4 Method development

The impact of different chromatographic variables such as flow rate, mobile phase, pH and solvent ratio were studied for the optimization of chromatographic conditions. Various solvent systems were tried for the development of a suitable method based on HPLC for estimation of pregabalin in pharmaceutical and bulk drug formulations. Mobile phase tried for this purpose were acetonitrile: buffer (30:70 V/V), acetonitrile: buffer (50:50 V/V) and acetonitrile: buffer (96:4 V/V). The condition, acetonitrile: buffer (96:4 V/V) was selected that showed the better resolution and symmetry. Same solvent system was used for the extraction of the drug from the formulation containing excipients which was used for quantification.

2.5 Calibration curve

Different concentrations (0.60mg/ml, 0.67mg/ml, 0.75mg/ml, 0.82mg/ml, 0.89 mg/ml) from the stock solution were made for the preparation of calibration curve. The filtration of mobile phase was done by using 0.45 µm membrane filter and delivered at 1.0 mL/min for column standardization, and during the process baseline was continuously monitored. The prepared

dilutions were injected serially and areas under the peaks were calculated for each dilution.

2.6 Method validation

2.6.1 Linearity

The concentrations of pregabalin within 0.60-0.89 mg/mL were prepared and areas under peak were obtained. The graph was plotted between concentration and area under peak for linearity.

2.6.2 Precision

Precision was considered at two levels i.e. intermediate precision and repeatability. Intraday variations as well as inter-day variation were carried out to determine repeatability of sample application and intermediate precision for the determination of pregabalin at 0.75mg/ml concentration.

2.6.3 Accuracy as recovery

Assessment of accuracy of this method was done by the addition of known amount of drug solution to a drug solution of known concentration and subjecting the samples to the proposed HPLC method. The recovery studies were done in triplicate manner where accuracy was expressed with respect to recovery.

By the addition of known amount of drug concentration corresponding to three concentration levels of 80, 100 and 120% (i.e. 60, 75 and 90 mg) of target analyte concentration along with the excipients in triplicate. The accuracy was expressed as the percentage of analyte recovered by the assay method. From results it confirms that the method is highly accurate.

2.6.4 Specificity

The specificity and selectivity of the proposed method was evaluated by estimating the amount of pregabalin in the presence of common excipients such as Corn starch, lactose and talc. By assessing the resolution between the peaks corresponding to various substances, which is a demonstration of the ability to separate excipients along with other substances from Pregabalin.

2.6.5 Robustness

For the evaluation of influence of small but deliberate variations in the chromatographic conditions robustness was carried out for the estimation of Pregabalin. Robustness of the method was estimated by changing the flow rate (0.7 & 1.5 mL/min), inject volume (50 µL), mobile phase ratio (±10%), pH (±10%), and temperature (±10%)

2.6.6 Assay of commercial dosage form

Accurately weighed quantity of Gaba-P 75, gifted sample from Renata Limited, Bangladesh equivalent to about 75 mg of pregabalin was transferred in to a 100ml volumetric flask. About 50ml of mobile phase was added, in which the sonication of solution was done for 15min with continuous shaking at 30ºC. Volume was made up with mobile phase. The solution was filtered through a 0.22mm syringe filter paper by discarding first few ml of the filtrate. A solution of final concentration of 0.75 mg/mL of pregabalin was prepared. Standard solution in the concentration range of about 0.75 mg/mL was also prepared.

3. Results and Discussion

3.1 Method development

3.1.1 Selection of the mobile phase

The optimization of chromatographic conditions, especially mobile phase composition was done by several trials to get symmetric peak shapes and good resolution for the analytes and an optimum run time. A composition of phosphate buffer solution and acetonitrile (ACN) was found theoretically suitable for the analysis as per the previous bioanalytical research works conducted on Pregabalin [11]. While trying to achieve symmetric peaks for this drug, the major challenge was to attain separation of the peak from excipients mixture. Isocratic flow of the mobile phase at different compositions failed to provide the intended resolution. For instance, when buffer and acetonitrile was used at a ratio of 94:6, pregabalin gave symmetric peak at around 9.0 minute.

3.1.2 Detector parameters selection

Different wavelengths were tried again to achieve the best possible resolution. After conducting trials at different detector settings, 200 nm was found to be the optimum wavelength for detection of the target compound. For instance, UV detection at a wavelength of 210 nm (Figure 1) was tried to achieve a better resolution and but did not show any improvement over 200 nm.

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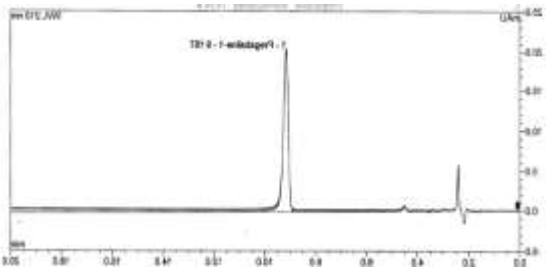

Figure 1. Detector response at 210 nm wavelength.

3.2 Method Validation

3.2.1 System suitability

Repeatedly inject the standard preparation for six times and record the response. Parameters for System Suitability mentioned in Table 2, is within the limit which indicates that assay method is validated considering system suitability.

S I. no.	Concentration (%)	Sample weight (mg)	Concentration (ppm)	Peak area/Absorbance
1	80%	60.77	601.9	3.942
2	90%	67.82	671.7	4.449
3	100%	75.73	750.0	4.916
4	110%	82.82	820.2	5.501
5	120%	90.44	895.7	5.973

Table 2. System Suitability parameter

3.2.2 Selectivity and specificity

The selectivity and specificity of the proposed method was scrutinized by estimating the amount of pregabalin in the presence of common excipients such as Talc, Maize Starch, Lactose monohydrate etc. By assessing the resolution between the peaks corresponding to various substances, which is a demonstration of the ability to separate excipients along with other substances from Pregabalin (Figure 2). Specificity of an assay ensures that the signal measured comes from the desire compound, and no interference occurs from Placebo and Mobile phase.

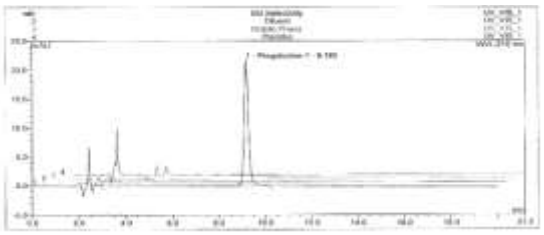

Figure 2. Chromatogram of selectivity.

3.2.3 Linearity

The linearity range was obtained as 80%, 90%, 100%, 110% and 120% of pregabalin solutions. For calibration curve of Pregabalin following equation of linear regression is required:

$$y = 348.97x + 4872.8$$

The equation was based on the data set mentioned in Table 3.

Table 3. Peak areas and different concentrations

Parameter	Specification	Results
Tailing Factor for the analyze peak	Not more than 2	1.5
Theoretical Plate for the analyze peak	Not less than 2000	3141
Area	RSD value not more than 2%	0.100%
Retention Time	RSD value not more than 2%	0.100%

of Pregabaline

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Sample no.	Different percentage of sample	Sample amount taken(mg)	A	B	B/AX 100 (% Recovered)	Mean value %	%RSD of mean value
1	80%	190.46	60.34	59.54	98.65	98.31	0.31
2.		190.46	60.34	59.21	98.12		
3.		190.46	60.34	59.24	98.18		
1	100%	191.83	75.76	74.55	98.40	98.52	0.29
2.		191.83	75.76	74.89	98.85		
3.		191.83	75.76	74.48	98.31		
1.	120%	192.34	90.83	89.19	98.19	98.47	0.25
2.		192.34	90.83	89.52	98.56		
3.		192.34	90.83	89.61	98.66		

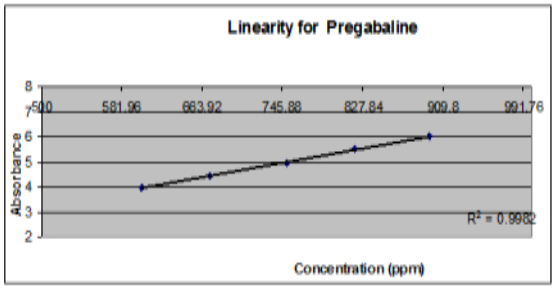

Figure 3. Calibration curve for Pregabaline.

A plot of Concentration vs. Absorbance shown as straight line has not forced to intercepted Zero, R^2 value = 0.998. So the method of analysis for Pregabalin in sample complies with linearity test.

3.2.4 Accuracy

Analyte percentage was taken as the expression of accuracy. High accuracy of the method reflected by the data that were obtained from the results. The average recovery of Pregabaline from excipients mixture is 98.43%. The method showed good efficiency in terms of recovery. The efficiency of the bioanalytical method was based on the data set mentioned in Table 4.

Table 4. Accuracy of Pregabalin at three concentrations (A= Theoretical Concentration; B= Concentration recovered)

The three replicates of three different concentration of Pregabalin from sample matrix analysis show that the percent recovery remains within the range of 98-102%. This indicates that the following method of Gaba-P 75 mg Capsule is validated considering accuracy.

3.2.5 Precision- Repeatability

By carrying out six independent assays of test samples against qualified working standard method repeatability was examined. The percentage relative standard deviation of assay values for repeatability (n=6) is 0.038%. The repeatability of the bioanalytical method was based on the data set mentioned in Table 5.

Table 5. Precision-Repeatability of Pregabalin at 100% concentration (A= Theoretical Concentration; B= Concentration recovered)

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Sample no.	Different percentage of sample	Sample amount taken (mg)	A (mg)	B (Recovered) (mg)	B/AX100 (% Recovered)	Mean value %	Relative Standard Deviation
1	100 %	115.99	75.01	74.4732	99.28	98.93	0.038
2.		115.99	75.01	74.1370	98.83		
3.		115.99	75.01	74.1175	98.10		
4.		115.99	75.01	74.3117	99.06		
5.		115.99	75.01	74.3258	99.08		
6.		115.99	75.01	74.4520	99.25		

Results of Mean Assay 98.93 %, Relative Standard Deviation 0.032 %, which indicate that assay method for Pregabalin in Gaba-P 50 mg Capsule is validated considering precision.

Precision- Reproducibility (Intermediate precision):

By the analysis of samples intermediate precision was done by different analyst employing different instrument and column on different day. By carrying out six independent assays of test samples Reproducibility of the method was examined against qualified working standard. The percentage relative standard deviation of assay values for reproducibility (n=6) is 1.67%. The reproducibility of the bioanalytical method was based on the data set mentioned in Table 6.

Table 6. Precision-Reproducibility of Pregabalin at 100% concentration (A= Theoretical Concentration; B= Concentration recovered)

Sample no.	Different percentage of sample	Sample amount taken (mg)	A (mg)	B (Recovered) (mg)	B/AX100 (% Recovered)	Mean value %	Relative Standard Deviation
1	100 %	190.91	75.02	75.1556	100.18	99.51	0.47
2.		190.91	75.02	74.8218	99.73		
3.		190.91	75.02	74.1434	98.83		
4.		190.91	75.02	74.3778	99.14		
5.		190.91	75.02	74.7422	99.62		
6.		190.91	75.02	74.6715	99.53		

Results of Mean Assay 99.51 %, Relative Standard Deviation 0.047%, which indicate that assay method for Pregabalin in Gaba-P 50 mg Capsule is validated considering precision.

Robustness

By deliberately changing the chromatographic conditions robustness of the method was done. Mobile phase flow rate was changed from 1.0 to 0.7 and 1.5 ml/min. The mobile phase organic strength was fluctuated by ±10% while buffer pH was fluctuated by±0.2 units. The solution of standard and three different sample preparations were injected in each varied conditions and checked the assay. Percentage of relative standard deviation for the assay values (n=3) for pregabalin were found within the acceptance range of 2%, for all deliberated varied conditions. The tailing factor of the pregabalin peak was found < 1.5 indicating the method robustness.

Representative different chromatograms obtained by changing chromatographic conditions are follows:

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By Changing Column

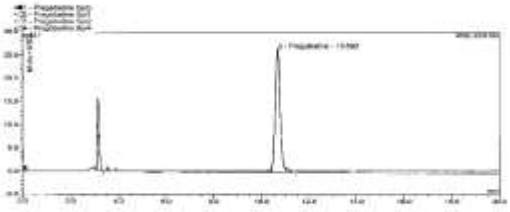

Figure 4. 100% solution of Pregabalin (by changing column).

By Changing flow rate 1ml/min to 0.75ml/min

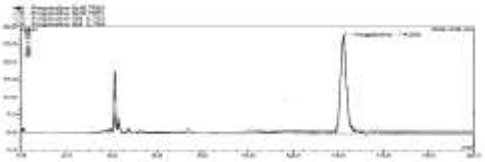

Figure 5. 100% solution of Pregabalin (by changing flow rate from1ml/min to 0.75ml/min).

By Changing flow rate 1ml/min to 1.5ml/min

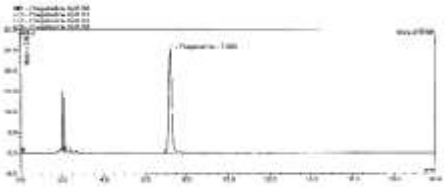

Figure 6. 100% solution of Pregabalin (by changing flow rate from 1ml/min to 1.5ml/min).

By Changing Inject Volume 20µl to 50µl

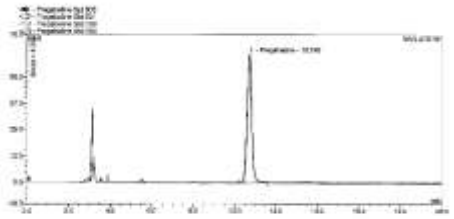

Figure 7. 100% solution of Pregabalin (by changing Inject Volume 20µl to 50µl).

By Changing pH 6.9 to 6.7 and 7.1

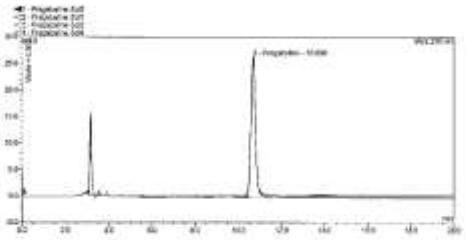

Figure 8. 100% solution of Pregabalin (by changing pH from 6.9 to 6.7).

Robustness of the method was performed by changing column, flow rate, inject volume and pH. The standard solution and three different sample preparations were injected and checked the assay. The recovery was excellent.

4. Conclusion

In conclusion, the proposed HPLC method is a simple, accurate and reproducible method for routine in vitro tests of PGB in bulk and pharmaceutical formulations. Although several HPLC methods are now available for determination of PGB with UV detection. The major advantages of this method include short retention time, without derivatization with other reagent, stability of the solution, no need for prior separation or purification before analysis, and the applicability of a common HPLC system (isocratic system, UV detector). The short chromatographic time makes this method suitable for the processing of multiple

samples in a limited amount of time. In addition, the method has wider linear dynamic range with good accuracy and precision. The method shows no interference from common excipients. The statistical parameter and recovery data reveals good accuracy and precision of the proposed method.

Conflict of Interests

The authors declare no conflict of interests regarding the publication of this article.

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