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A Novel Method For Spectrophotometric Method Development And Validation For The Estimation Pregabalin In Pure Form And Tablet Formulation

Prabhat Kumar Jain*, C. K. Tyagi
Sri Satya Sai University of Technology and Medical Science Sehore M.P. India
Corresponding Author
Prabhat Kumar Jain
Prabhatjain16@gmail.com

Abstract
A simple, accurate, cheap and quick spectrophotometric method was developed for the estimation of pregabalin in pharmaceutical pure and dosage forms. The method was based on the methyl orange reaction with drug pregabalin. This reaction produces a purple colour. The absorption of the pregabalin-methyl orange complex at 506nm had a linear relationship with the pregabalin concentration ranging from 10-50 μg/ml. The %RSD was less than 2%, showing high degree of precision of the proposed method. The methods were satisfactory applied for the determination of drugs in both bulk and pharmaceutical dosage forms. The colorimetric pregabalin assay reported herein is of great practical value because it is reproducible, sensitive, simple and extremely inexpensive.
Keywords: Pregabalin, Methyl orange, Spectrophotometry, Pharmaceutical dosage forms

Introduction

Pregabalin (PGB) is a new active substance known chemically as (S)–3–amino methyl–5–methyl hexanoic acid and is structurally related to the naturally occurring amino acids L – leucine and gamaa aminobutyric acid (GABA) Figure 1. It is a white to off – white crystalline, non – hygroscopic and water soluble (freely soluble below pH–3.7) powder. It contains one chiral centre, but is synthesized as the single enantiomer S. PGB exists as a single anhydrous and not solvated crystal form. Pregabalin undergoes minimal metabolism in human with unchanged parent representing the majority (≥90 %) of drug – derived material [1]. This contrasts with gabapentin, which is absorbed via a capacity limited L – amino acid transport system from the proximal small bowel into the blood stream [2-3].

The therapeutic importance of Pregabalin was behind the development of numerous methods for its determination. The methods adapted to the analysis of PGB include high – performance liquid chromatography (HPLC) [4], liquid chromatography – mass spectrophotometry (LC–MS) [5-6] and spectrofluorimetry [7]. In addition, these methods require long and tedious pretreatment of the samples and laborious clean up procedures prior to analysis. An official monograph of PGB does not exist in any pharmacopoeia and determination of PGB in bulk and pharmaceutical formulations has not been yet described. Since pregabalin poorly absorbs ultraviolet and visible light, an indirect spectrophotometric method is necessary for its assay. Methyl orange colorimetric reaction is commonly used as a general method for the qualitative identification of several drugs containing amino groups [8]. In the present work a new spectrophotometric procedure for the quantitative analysis of pregabalin using methyl orange as derivatizing agent was developed and validated.

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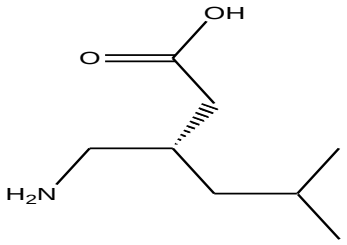

Fig. 1 Chemical structure of Pregabalin

Materials and Methods

Chemicals and Reagents

Pregabalin was received as gift sample from Scan Research Laboratories, Bhopal (M.P.). All solvents and reagents were of analytical grade. All the solutions were protected for light and were analyzed on the day of preparations. Triple distilled water was generated in house. All these were used of AR grade. Distilled water was obtained by Mili Q apparatus by Millipore (Milliford, USA) for whole experimental work.

Instrument

In UV-spectrophotometric method, Labindia model- 3000 + series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

Reagents and solutions

Methyl Orange: 2% w/v aqueous solution.

Preparation of calibration curve

Pregabalin samples were prepared in the 0.1 N HCl at concentration of l000µg/ml. Assay samples (5ml) were mixed with freshly prepared Methyl orange dye (1ml) and 3 ml of chloroform and shake and stand for 10 min. Pipette out the chloroform layer and UV-visible spectra over wavelength range of 400-800 nm were measured using UV visible spectrophotometer. Prepared suitable dilution to make different concentration of standard with concentration range of 10-50µg/ml and analyzed for drug content by UV spectrophotometer at a λ_{max} of 506 nm.

Assay of tablet formulation

Take 20tablets and determine the average weight, amount equivalent to 100 mg of drug was transferred to 100ml standard flask. The solution was dissolved in 0.1 N HCl and made up to volume with of 0.1 N HCl. Assay samples (5ml) were mixed with Methyl orange dye (1ml) and 3 ml of chloroform and shake and stand for 10 min. Pipette out the chloroform layer and UV-visible spectra over wavelength range of 400-800 nm were measured using UV visible spectrophotometer and analyzed for drug content by UV spectrophotometer at a λ_{max} of 506 nm using of chloroform as blank.

Method Validation

The developed method was validated as per ICH guidelines [8] with respect to linearity, precision, selectivity, recovery, accuracy and stability.

Linearity and construction of calibration curve

Solutions containing 10-50µg/ml of pregabalin were prepared from standard solution to determine the linearity range. The detection was carried out at 506nm. Spectrums were recorded and absorbance was recorded for all the concentrations. A calibration plot of concentration over the absorbance was

constructed and was shown in Fig 2. The optical characteristics such as Beer's law limits, regression equation and correlation coefficient, mean absorbance value and statistical data of the calibration curve were calculated and results are presented in Table 1 & 2.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of clindamycin to pre analysed tablet solutions. The resulting solutions were then reanalyzed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

Results and Discussion

The proposed spectrophotometric methods are indirect and based on the determination of the pregabalin in marketed formulation using methyl orange as reacting dye. Calibration curves have correlation coefficients (r) 0.999 indicating good linearity over a concentration range of 10-50 µg/ml. The regression characteristics were reported in Table 2. The accuracy of the methods was determined by investigating the recovery of drugs at concentration levels covering the specified range (five replicates of each concentration) Table 3. The %RSD was less than 2%, showing high degree of precision of the proposed method Table 4. The results of the method lie within the prescribed limit, showing that method is free from interference from excipients.

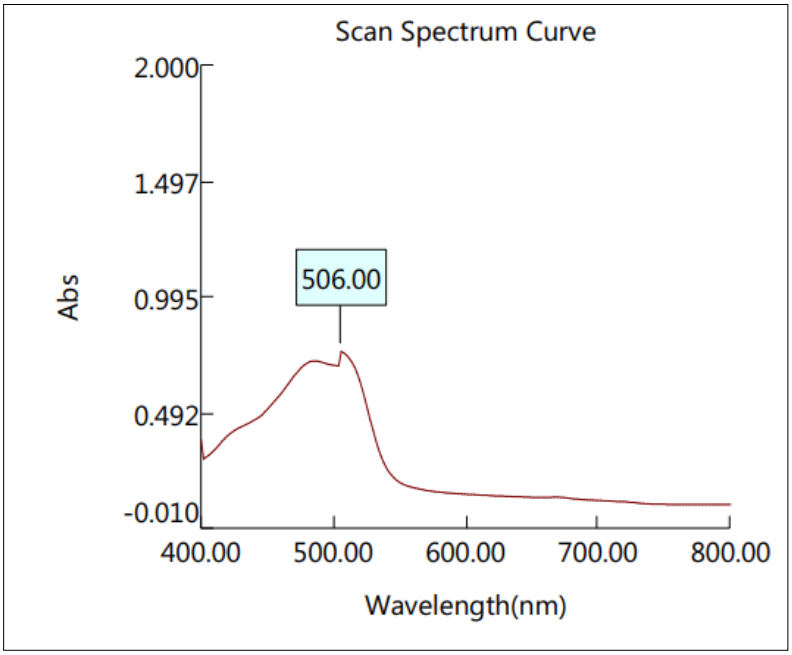

Fig. 2 Calibration curve of pregabalin at 506nm

Table 1 Readings for Linearity of pregabalin

S. No.	Concentration	Absorbance*	S.D.
1	0	0	0
2	10	0.125	0.001
3	20	0.235	0.002
4	30	0.345	0.001
5	40	0.458	0.003
6	50	0.565	0.002

Table 2 Optical characteristics of the proposed method

Parameters	Results
Wavelength	506nm
Beer's law limit (μg/mL)	10-50
Regression equation (Y=mx+c)	Y=0.011X+0.007
Slope (m)	0.011
Intercept (c)	0.007
Correlation Coefficient (r)	0.999

Table 3 Results of recovery studies on marketed formulations

Recovery level %	% Recovery (Mean±SD)*
80	99.12±0.142
100	99.45±0.135
120	99.05±0.137

*Average of five determination

Table 4 Results of Precision (% R.S.D.)

Parameter	(Mean±SD)*	% RSD
Repeatability	99.12±0.025	0.074
Day to Day	98.15±0.021	0.057
Analyst to Analyst	99.45±0.032	0.083
Reproducibility	99.45±0.014	0.014

*Average of five determination

Conclusion

The results of present study demonstrated the developed colorimetric assay can be successfully applied for routine analysis of pregabalin in bulk and pharmaceutical dosages form. The method was validated according to ICH guideline. This method is simple, selective, cost effective and less time consuming can be successfully applied to pharmaceutical formulations and pure drug sample.

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