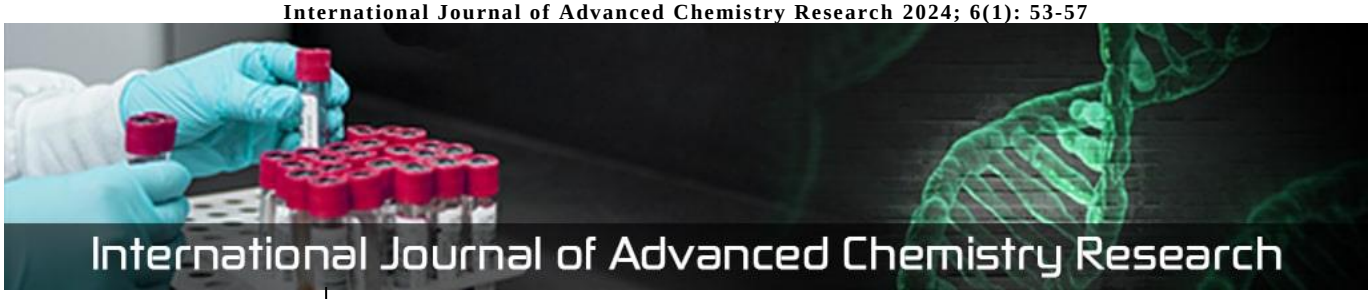

ISSN Print: 2664-6781
ISSN Online: 2664-679X
Impact Factor: RJIF 5.32
IJACR 2024; 6(1): 53-57
www.chemistryjournals.net
Received: 20-12-2023
Accepted: 25-01-2024

Kiran Rapolu
CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India

Dr. Raja Reddy Aleti
CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India

Dr. Rama Rao Tadikonda
CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India

Dr. Pravalika Patlolla
CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India

Dr. Narender Boggula
CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India

Corresponding Author:
Dr. Narender Boggula
Associate Professor, Department of Pharmaceutical Chemistry, CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India

Method development and validation of pregabalin in bulk and tablet dosage forms by UV spectroscopy

Kiran Rapolu, Dr. Raja Reddy Aleti, Dr. Rama Rao Tadikonda, Dr. Pravalika Patlolla and Dr. Narender Boggula

DOI: https://doi.org/10.33545/26646781.2024.v6.i1a.168

Abstract
A simple and sensitive UV-spectrophotometric method was developed and validated for the determination of pregabalin in bulk and pharmaceutical formulations. The estimation carried out by using 1.2 pH HCl buffer as solvent. The absorbance was measured at 210 nm. The method was linear in the range of 2-10 µg/ml with correlation coefficient value 0.997. The recovery was found to be 98.12-101.17%. The relative standard deviation was found to be less than 2. The method was validated with respect to accuracy, precision, assay, ruggedness, robustness, limit of detection and limit of quantitation. This is found to be simple, specific, precise, accurate, reproducible and low-cost UV-spectrophotometric method. The method can be useful for the day-to-day routine analysis in the quality control departments of bulk and pharmaceutical formulations industries.

Keywords: Pregabalin, validation, pharmaceutical formulations, UV-spectrophotometry

Introduction
Pregabalin (PGB) is chemically as (S)-3-amino methyl-5-methyl hexanoic acid and is structurally related to the naturally occurring amino acids L-leucine and gamma aminobutyric acid (GABA). It is a white to off-white crystalline, non-hygroscopic and water soluble (Freely soluble below pH-3.7) powder. It contains one chiral center, but is synthesized as the single enantiomer S. PGB exists as a single anhydrous and not solvated crystal form. PGB is an anti-convulsant and analgesic medication that was recently approved for adjunctive treatment of partial seizures in adults in both the United States and Europe and for the treatment of neuropathic pain from postherpetic neuralgia and diabetic neuropathy [1-3].

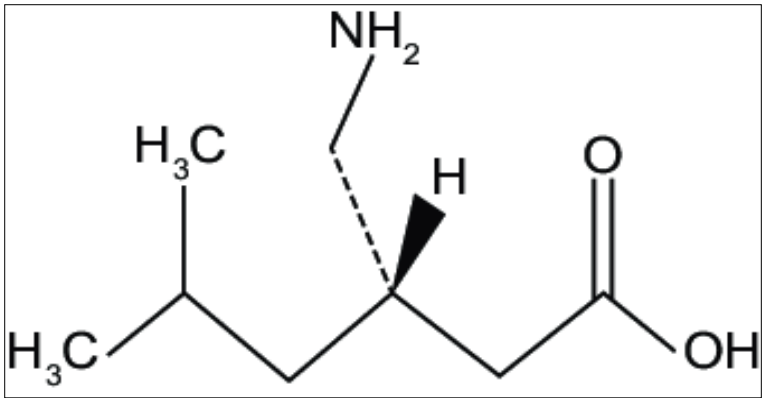

Fig 1: Molecular structure of pregabalin

The mechanism of action is still unclear, pregabalin decreases central neuronal excitability by binding to an auxiliary subunit (α-δ protein) of a voltage-gated calcium channel on neurons in the central nervous system.

PGB reduces the release of several neurotransmitters include glutamate, norepinephrine, and calcitonin gene related peptide from certain brain tissues and also reduce calcium influx in synaptosomes [4, 5].

Pregabalin undergoes minimal metabolism in human with unchanged parent representing the majority (≥90%) of drug-derived material. 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. 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), liquid chromatography-mass spectrophotometry (LC-MS) and spectrofluorimetry. 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. Therefore, it is very imperative to develop a simple and suitable analytical method for the determination of PGB in bulk and pharmaceutical formulations [6-9].

UV-Visible spectrophotometry is the technique of choice in research laboratories, hospitals and pharmaceutical industries due to its low cost and inherent simplicity. This paper reports a simple, sensitive and accurate spectrophotometric method for the determination of PGB. The method is based on the direct measurement of native absorbance of the drug at 210 nm against the reagent blank. The proposed method was extended to the determination of PGB in bulk, pharmaceutical formulations.

Materials and Methods

Instruments used

UV-Visible spectrophotometer with UV Win software. Weighing balances and matching quartz cells with a 1 cm cell path length were utilized along with the mentioned equipment, which had automatic wavelength accuracy of 0.1 nm.

Chemicals and reagents

Pharmaceutical grade pregabalin (API) was procured as gift sample from Hetero Drugs Ltd., Hyderabad, Telangana, India. The marketed pharmaceutical dosage form of pregabalin sitagliptin tablets (preganerve100 mg) was purchased from local Pharmacy, Hyderabad, Telangana, India. All chemicals and reagents were of analytical grade. Solvent selection: A number of trails were done to find out the ideal solvent for dissolving the drug. The solvents such as double distilled water, methanol and HCl buffer were tried based on the solubility of the drug.

Selection of detection wavelength

Appropriate volume 1 ml of standard stock solution of pregabalin was transferred into a 10 ml volumetric flask, diluted to a mark with HCl buffer to give concentration of 10 µg/ml. The resulting solution was scanned in the UV range (200-400 nm).

Preparation of stock solution

A precisely weighed, 10 mg of pregabalin was transferred to 10 ml volumetric flask (Clean and dry). Then few ml of HCl buffer was added and dissolved the drug by vigorous shaking. The volume was then made up to the mark with HCl buffer to obtain the stock solution of 1000 µg/ml.

Preparation of working standard solution

From stock solution 1 ml was pipetted out further diluted to 10 ml with HCl buffer to get the solution having the concentration of 100µg/ml.

Preparation of calibration curve

From the working standard solution, pipetted out 0.2ml, 0.4ml, and 0.6 ml,0.8ml,1ml and diluted to 10 ml using HCl buffer to produce, 2,4,6,8,10µg/ml, solutions, respectively. The absorbance of the solutions at the λ_{max} of 210 nm using HCl buffer as blank was measured. The calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis. The curve shows linearity in the concentration range of 2 to 10 µg/ml. The correlation co-efficient (r^2) was found to be 0.9998.

Assay of pharmaceutical formulation

20 Tablets of pregabalin marketed formulations were weighed and powdered. A quantity of tablet powder equivalent to 50mg of pregabalin was transferred to 100 ml volumetric flask and volume was made up to the mark with HCl buffer. The absorbance of the resulting solution was measured at 210 nm and the amount of pregabalin was computed from its calibration plot.

Method development and validation [10-14]

These current validation characteristics describe the validation parameters stated by the International Conference on Harmonization [ICH] guidelines.

Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. A linearity correlation coefficient above 0.998 is acceptable for most methods, especially for major components in assay methods. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision was determined by intra-day and inter-day study. The repeatability of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for the sample solution. The percent relative standard deviation (% RSD) was calculated.

Accuracy (Recovery studies)

The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different levels (80%, 100% and 120%) by standard addition method and the samples were analyzed in triplicate by the proposed

method. Known amount of standard sitagliptin at 80%, 100% and 120% of predetermined sample was added to a pre-quantified tablet sample.

Ruggedness

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. The absorbance was measured and assay was calculated for six times.

LOD and LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$

Where, σ = Standard deviation of the response, and
S = Slope of the calibration curve.

Results and Discussion

Determination of absorbance maxima

From the above working standard solution, 1 ml was pipetted out into a 10 ml volumetric flask and the volume was made up to the mark with HCl buffer to prepare a concentration of 10 µg/ml. The sample was then scanned in UV/Vis-spectrophotometer in the range 200-400nm using HCl buffer as blank and the wavelength corresponding to maximum absorbance was found to be 210 nm. It was observed that the drug showed maximum absorbance at 210 nm which was selected as the wavelength for detection. The absorption maxima curve was shown in Figure 2.

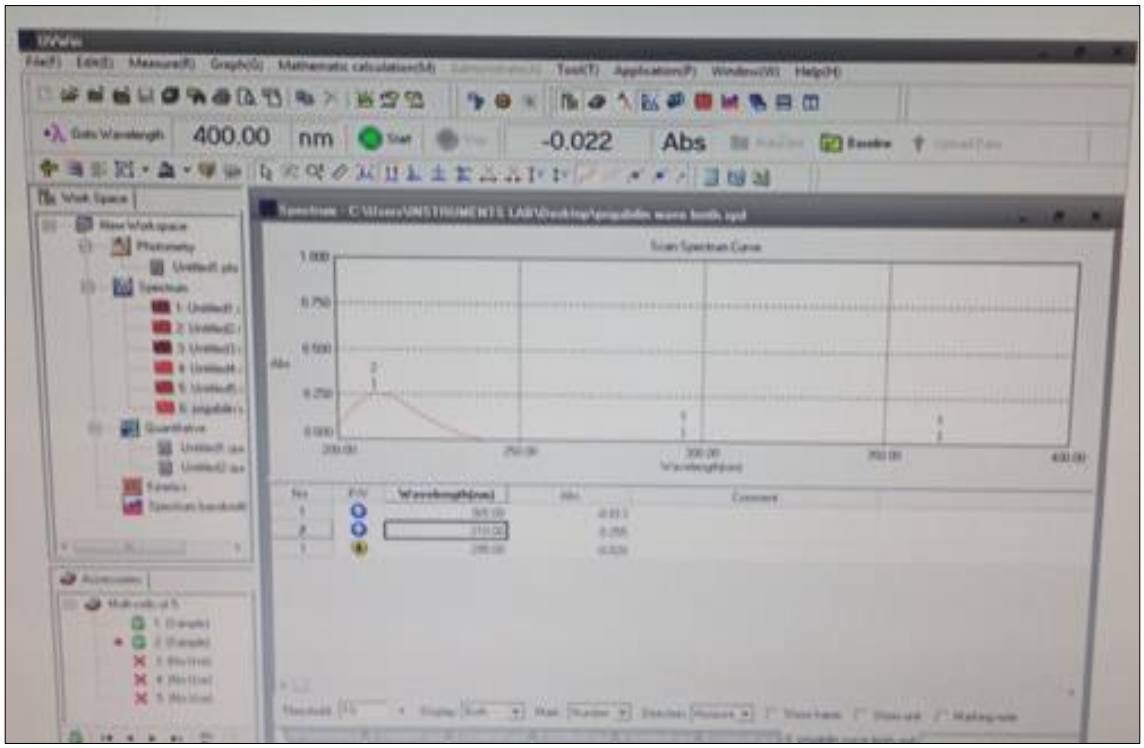

Fig 2: Absorbance maxima of pregabalin

Table 1: Linearity of pregabalin

Concentration (µg/ml)	Absorbance
2	0.154
4	0.321
6	0.441
8	0.614
10	0.768

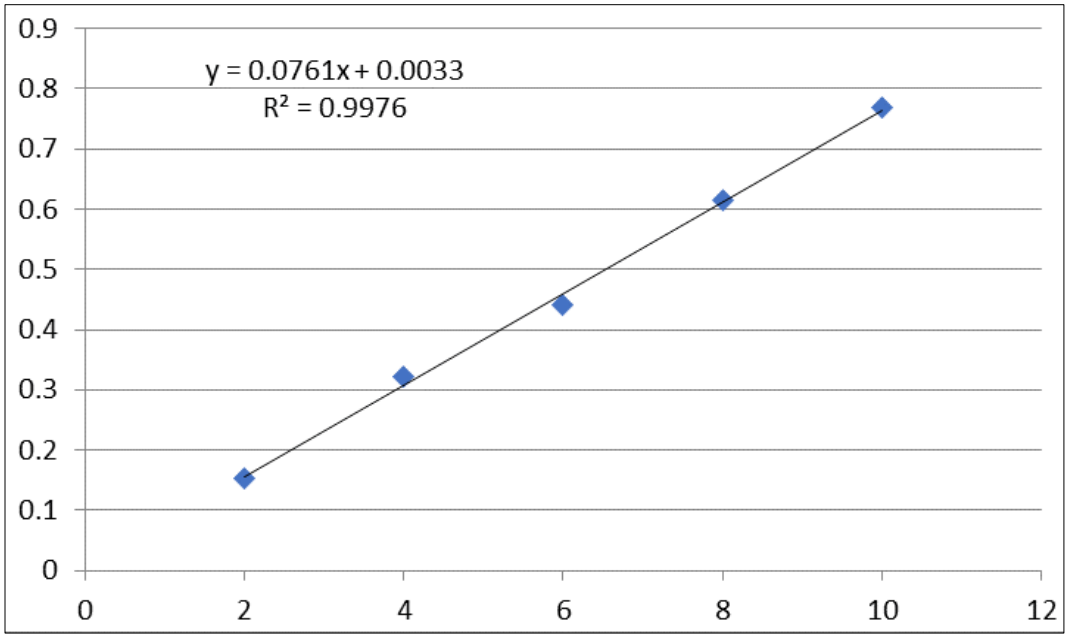

Fig 3: Linearity graph of pregabalin

Table 2: Precision

S. No.	Concentration (µg/ml)	Absorbance (intraday)	Absorbance (interday)
1	10	0.771	0.781
2	10	0.774	0.783
3	10	0.772	0.782
4	10	0.769	0.780
5	10	0.772	0.789
6	10	0.775	0.783
Mean		0.773	0.782
Std. Dev.		0.002828	0.001414
% RSD		0.362	0.180

Table 3: Results for accuracy

S. No.	Level of adding	Amount added (µg/ml)	Amount recovered (µg/ml)	Average
1	80	4.8	4.77	98.12
2	100	6	5.9	99.15
3	120	7.2	7.21	101

Table 4: Results for robustness

S. No.	Wavelength	Absorbance
1.	205	0.781
2.	210	0.777
3.	215	0.792

Table 5: Results for ruggedness study

S. No.	Analyst	% RSD
1	Analyst-1	0.919
2	Analyst-2	0.901

Table 6: Assay of tablets

Drug	Labelled amount	Mean	SD	% Assay	% RSD
Pregabalin	75mg	100.115	1.40	99.12	0.0139

Table 7: LOD and LOQ of pregabalin

LOD	0.616 (µg/ml)
LOQ	1.872 (µg/ml)

New spectrophotometric methods were developed for the determination of pregabalin in bulk and pharmaceutical dosage form. The absorption spectra were recorded in the wavelength region of 200-400 nm by UV. The proposed method obeyed Beer's law in the concentration range of 2-10 µg/ml with good correlation coefficient of r^2=0.997. Calibration data was represented in Table 1. Beer's law range was confirmed by the linearity of the calibration curve of pregabalin was show Figure 3. Precision of the method was reported in terms of relative standard deviation and it should be evaluated by using a minimum of 3 determinations over which shows % RSD less than 2 indicates that the method was precise and the results are presented in Table 2.

Recovery studies were carried out for the developed method by addition of known amount of standard drug solution of Pregabalin to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery (Table 3) was in the range of 98.12-101.17%. The results were reported to be within the limits. In fact, there was no difference in mean assay results of the method obtained from two instruments of different manufacturers.

Conclusion

For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influences of the variables were determined. The results are within the specified limits which states that this method is robust. The developed method was applied to the analysis of tablet formulations of pregabalin found to be within the proposed limits. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method. The developed method is validated according ICH guidelines, method is fast, accurate, precise and robust. Present method can be therefore used for routine analysis of pregabalin. The result shows the developed method of pregabalin is yet another suitable method for assay which can help in the analysis of pregabalin in different formulations.

Acknowledgement

We express our indebtedness and sense of gratitude the management of CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India for providing the necessary equipment for research, facilities and support.

Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

References

1. Hamandi K, Sander JW. Pregabalin: A new antiepileptic drug for refractory epilepsy. Seizure. 2006; 15(2):73-78.
2. Jampala B, Bandi R, Naidu NVS. Analytical RP-HPLC method for development and validation of pregabalin in bulk and the determination of pregabalin in capsule dosage form. International Journal of Innovative Research in Science Engineering and Technology. 2014; 3(4):11094-11098.
3. Pawar PY, Zanje LS, Tambe SS, Nandgaonkar AU, Funde PV, Vyavhare AA. Simultaneous estimation of pregabalin and paracetamol by UV spectroscopic method in bulk and tablet formulation. World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(4):743-750.
4. Bhatt KK, Patelia EM, Mori A. Simultaneous estimation of pregabalin and methylcobalamine in pharmaceutical formulation by RP-HPLC method. Journal of Analytical and Bioanalytical Techniques. 2013; 4(1):1-4.
5. Karavadi TM, Challa BR. Bioanalytical method development and validation of pregabalin in rat plasma by solid phase extraction with HPLC-MS/MS: application to a pharmacokinetic study. Journal of Liquid Chromatography and Related Technologies. 2014; 37:130-144.
6. Sowjanya P. RP HPLC method development of pregabalin in bulk, dosage form and validation parameters. Journal of Hospital and Clinical Pharmacy. 2016; 2(4):61-75.
7. Akula G, Sapavatu SN, Jadi RK, Battineni JK, Boggula N. Analytical method development and validation for the estimation of tramadol in bulk and its formulations by UV-spectroscopy. Journal of Advanced Scientific Research. 2021; 12(2):77-83.
8. Ashu M, Parmar S, Nagarajan K, Singh V. Development and Validation of Rapid HPLC Method for Determination of Pregabalin in Bulk and Capsule Dosage Forms. Der Pharma Chemica. 2011; 3(1):482-489.
9. Sriveni T, Vanamala N, Vemula SR, Aeruva R, Porika S, Akiful Haque M, *et al*. Development and Validation of Dolutegravir in Bulk and Formulation: An Anti-Retroviral Drug Using UV-Spectroscopy. International Journal of Pharmaceutical Quality Assurance. 2021; 12(1):57-60.
10. David B, Millington C. Analysis of Pregabalin at Therapeutic Concentrations in Human Plasma/Serum by Reversed-Phase HPLC. Ther Drug Monit. 2005; 27(4):451-456.
11. Gujral RS. A novel method for the determination of pregabalin in bulk pharmaceutical formulations and human urine samples. Afr J Pharm Pharmacol. 2009; 3(6):327-334.
12. Kasawar GB, Farooqui MN. Development and validation of HPLC method for the determination of pregabalin in capsules. Indian J Pharm Sci. 2010; 72(4):517-519.
13. Lode M, Tadikonda RR, Rapolu K, Boggula N. Development and Validation by UV Spectrophotometric Method for the Determination of Sitagliptin in Bulk and its Formulation. International Journal of Development Research. 2024; 14(1):64716-64719.
14. Shaalan RAA. Spectrofluorimetric and Spectrophotometric Determination of Pregabalin in Capsules and Urine Samples. Int. J Biomed Sci. 2010; 6(3):260-267.