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Method Development and Validation of Pregabalin in Bulk and Tablet Dosage forms by UV Spectroscopy

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Abstract

For determining Pregabalin in bulk and pharmaceutical formulations, a straightforward and accurate UV spectrophotometric method was created and verified. Distilled water was used as the solvent in the estimation. It was determined that the absorbance was 210 nm. A correlation coefficient of 0.997 indicated that the method's range was linear, between 2 and 10 µg/ml. 98.12–101.17% was the recovery rate determined. There was less than a 2-fold relative standard deviation found. Assay, robustness, ruggedness, quantitation limit, accuracy, and precision were all evaluated in the validation process. It has been discovered that this UV spectrophotometric approach is straightforward, exact, accurate, repeatable, and reasonably priced. In the bulk and pharmaceutical formulations sectors, the method can be helpful for routine daily analysis carried out by quality control departments.

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

Introduction

Pregabalin (PGB) shares structural similarities with the naturally occurring amino acids L-leucine and gamma aminobutyric acid (GABA). Its chemical formula is (S)-3-amino methyl-5-methyl hexanoic acid. It is a crystalline powder that is white to off-white in color, non-hygroscopic, and water soluble (freely soluble below pH 3.7). Although it is manufactured as the single enantiomer S, it only has one chiral center. PGB is only found in a single, non-solvated, anhydrous crystal form. PGB is an anti-convulsant and analgesic drug that was recently approved in the US and Europe for the treatment of neuropathic pain from diabetic neuropathy and postherpetic neuralgia, as well as for the adjunctive treatment of partial seizures in adults. [1-2]

Pregabalin reduces central neuronal excitability via binding to the auxiliary subunit (α-δ protein) of a voltage-gated calcium channel on central nervous system neurons, albeit the exact mechanism of action is yet unknown.

PGB decreases synaptosomal calcium influx as well as the release of many neurotransmitters from specific brain areas, such as glutamate, norepinephrine, and calcitonin gene related peptide. [3-4]

Pregabalin is metabolized minimally in humans, with the majority (≥90%) of drug-derived material remaining unaltered from the parent compound. In contrast, gabapentin enters the bloodstream from the proximal small bowel through a capacity-limited L-amino acid transport pathway. Numerous techniques for determining pregabalin's level were developed because of its medicinal relevance. High-performance liquid chromatography (HPLC), liquid chromatography-mass spectrophotometry (LC-MS), and spectrofluorimetry are among the techniques modified for PGB analysis. These methods also necessitate time-consuming sample pretreatment and strenuous cleanup steps before analysis. There is no formal PGB monograph in any pharmacopoeia, and there is currently no description available for determining PGB in pharmaceutical formulations or bulk. Consequently, it is crucial to

Because of its inherent simplicity and low cost, UV-visible spectrophotometry is the method of choice in research laboratories, hospitals, and the pharmaceutical industry. This study presents a straightforward, accurate, and sensitive spectrophotometric technique for determining PGB. The technique is based on measuring the drug's natural absorbance at 210 nm directly against the reagent blank. The suggested technique has been expanded to include PGB measurement in pharmaceutical formulations in bulk.[5]

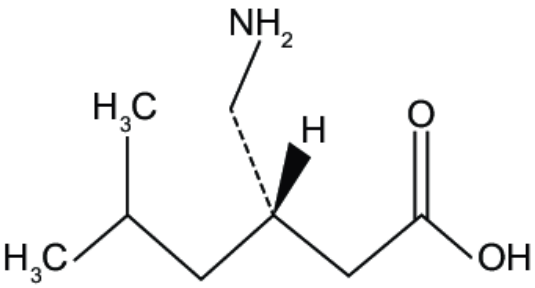

Fig.1 Molecular structure of pregabalin

Materials and Methods

Instruments used

UV-Visible spectrophotometer with UV Win application installed. The aforementioned apparatus, which had an automated wavelength accuracy of 0.1 nm, was used in conjunction with matched quartz cells and weighing balances with a 1 cm cell path length.

Chemicals and reagents

Pregabalin of pharmaceutical grade (API) was received as a gift from the supplier. Pregabalin sitagliptin tablets (preganerve 100 mg), the marketed pharmaceutical dosage form, was acquired from a small pharmacy in Hyderabad, Telangana, India. Each and every chemical and reagent were analytical grade. Choosing a solvent: Several trials were conducted to determine the best solvent for the drug's dissolution. Various solvents, including methanol, double distilled water, and HCl buffer, were tested in relation to the drug's solubility.

Selection of detection wavelength

The right amount of volume A 10 ml volumetric flask was filled with 1 ml of pregabalin standard stock solution, which was then diluted to the appropriate level with distilled water to yield a concentration of 10 µg/ml. A UV (200–400 nm) scan was performed on the resultant solution.

Preparation of stock solution

Ten milligrams of pregabalin, carefully weighed, were added to a ten-milliliter volumetric flask (dry and clean). After that, a small amount of distilled water was added, and the medication was thoroughly shaken to dissolve it. After that, the volume was adjusted with distilled water to get the 1000 µg/ml stock solution.

Preparation of working standard solution

To obtain a solution with a concentration of 100µg/ml, 1 ml of the stock solution was pipetted out and then further diluted to 10 ml using distilled water.

Preparation of calibration curve

Using distilled water, create 2,4,6,8, and 10µg/ml solutions by pipetting out 0.2, 0.4, and 0.6 milliliters, 0.8 milliliters, and 1 milliliter from the working standard solution. Using distilled water as a blank, the absorbance of the solutions was measured at the λmax of 210 nm. Plotting the calibration curve involved placing the absorbance on the Y-axis and the concentration on the X-axis. The curve exhibits linearity between 2 and 10 µg/ml of concentration. 0.9998 was determined to be the correlation coefficient (r^2).

Assay of pharmaceutical formulation

Weighed and powdered were 20 pregabalin tablets from marketed formulations. A 100-milliliter volumetric flask was filled to capacity with distilled water after an amount of tablet powder equal to 50 milligrams of pregabalin was added. Pregabalin concentration was calculated from the calibration plot of the resultant solution's absorbance, which was measured at 210 nm.

Method development and validation

The International Conference on Harmonization [ICH] guidelines validation parameters are described by these contemporary validation features.

Linearity

When test findings are obtained that are directly proportional to the analyte concentration in the sample, the analytical technique is said to be linear. Measurements at different analyte concentrations can be taken individually to evaluate linearity. Most techniques, particularly those involving key components in assay procedures, may tolerate a linearity correlation coefficient higher than 0.998. The difference between the highest and lowest analyte concentration in the sample is the analytical procedure's range.[6]

Precision

The degree of agreement between several measurements made from repeated samplings of the

same homogeneous material under specified circumstances is expressed as the precision of an analytical method. The investigation conducted intra- and inter-day yielded results on precision. [16-17] Three test runs on the same day were used to assess the method's repeatability, and three assay runs for the sample solution over three days were used to assess the assay's intermediate precision. It was calculated to get the percent relative standard deviation, or % RSD.[7]

Accuracy (Recovery studies)

The degree to which the value that is acknowledged as either a conventional true value or an approved true value agrees with the analytical procedure's accuracy is expressed. The standard addition method was used to conduct accuracy studies at three distinct levels (80%, 100%, and 120%), and the suggested approach was used to examine the samples in triplicate. A tablet sample was pre-quantified and then given known amounts of standard sitagliptin at 80%, 100%, and 120% of the predefined sample.[8]

Ruggedness

The reproducibility of outcomes under real-world use settings is referred to as method ruggedness. This covers various laboratories, instruments, columns, sources of chemicals, solvents, and reagents, among other things. When a method is devised, its roughness may not be known at first, but as it is used again, it becomes clear.[9]

Robustness

The ICH defines robustness as "a measure of an analytical procedure's capacity to remain unaffected by small, but deliberate variations in method parameters." Developing techniques that accommodate anticipated fluctuations in the separation parameters is the most crucial component of resilience. [13-15] When determining a method's robustness, variables such detector wavelength variation are altered within a reasonable range, and the variables' quantitative influence is ascertained. The parameter is said to be inside the robustness range of the approach if its influence falls within a previously defined tolerance. Six times, the assay was computed and the absorbance was measured.[10]

LOD and LOQ

The smallest amount of analyte in a sample that can be identified but may not always be precisely measured is known as the detection limit of a particular analytical technique. The lowest amount of analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the quantitation limit of a particular analytical process. [18-20]

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = Standard deviation of the response, and

S = Slope of the calibration curve.

Results and Discussion

Determination of λmax

A 10 µg/ml concentration was prepared by pipetting 1 ml of the working standard solution mentioned above into a 10 ml volumetric flask and adding distilled water to get the volume up to the required level. After that, the sample was scanned in the 200–400 nm range using distilled as a blank in a UV/Vis spectrophotometer. It was discovered that 210 nm was the wavelength corresponding to maximal absorption. The medication was found to have maximal absorbance at 210 nm, thus that wavelength was chosen for detection.

With a strong correlation coefficient of r2=0.997, the suggested approach complied with Beer's law in the concentration range of 2–10 µg/ml. In Table 1, calibration data were displayed. The linearity of the pregabalin calibration curve (Figure 3) supported the range of Beer's law. The method's precision was expressed in terms of relative standard deviation, and it should be assessed using at least three determinations, with a percentage RSD of less than two indicating that the procedure was precise. The pre-analyzed tablet sample solution was subjected to three different concentration levels of a known amount of standard drug solution of pregabalin in order to conduct recovery experiments for the established method. The suggested techniques were used to analyze the final answers.

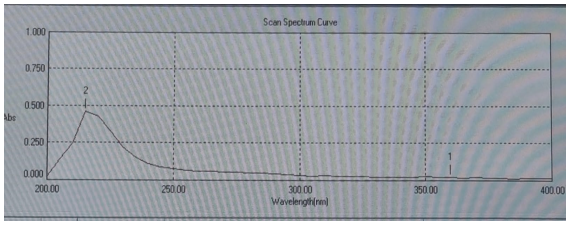

Fig.2 Absorbance maxima of pregabalin

CONCENTRATION(µg/ml)	ABSORBANCE
0	0
2	0.154
4	0.308
6	0.461
8	0.614
10	0.768

Table.1 Linearity of pregabalin

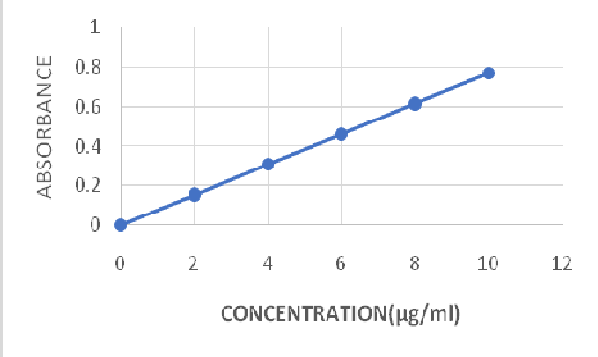

Fig.3 Linearity graph of pregabalin

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

Table.2 Results for ruggedness study

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 of precision

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 accuracy

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

Table.5 Results for robustness

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

Table.6 Assay of tablets

LOD	LOQ
0.616 (µg/ml)	1.872 (µg/ml)

Table.7 LOD and LOQ of pregabalin

Conclusion

A method's robustness can be assessed by varying certain parameters, like the detector wavelength, within a reasonable range and calculating the

quantitative effects of the changes. The fact that the outcomes fall within the given parameters indicates the method's robustness. The pregabalin tablet formulations that fell within the suggested bounds were analyzed using the described approach. The created method's excellent linearity, accuracy, and precision show the method's high caliber. The devised method is resilient, quick, accurate, and precise, and it has been validated in accordance with ICH requirements. Pregabalin can therefore be routinely analyzed using the current approach.

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