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Development and Validation of UV Spectrophotometric Method Of Pregabalin In Bulk And Pharmaceutical Formulation.

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Abstract: A simple and sensitive UV spectrophotometric method was developed and validated for the determination of pregabalin in bulk & pharmaceutical formulations. The method was linear in the range of 6–14 µg/ml with a correlation coefficient of 0.9986. There is no generally accepted method for the determination of pregabalin. The absorbance was measured at 210 nm. The method was validated with respect to accuracy, precision, Stability, limit of detection and limit of quantitation. This method is found to be simple, specific, precise, accurate, reproducible and low cost UV Spectrophotometric method.
Keywords: Pregabalin; validation; bulk drug, pharmaceutical formulations; UV spectrophotometric method.

Background:[1]

Pregabalin (PRG), (S)-3-(aminomethyl)-5-methylhexanoic acid (Figure 1), is an antiepileptic and structurally related to the inhibitory neurotransmitter aminobutyric acid (GABA) It was recently approved for adjunctive treatment of partial seizures in adults in United States and Europe and for the treatment of neuropathic pain from post therapeutic neuralgia and diabetic neuropathy. Currently, there is no official analytical procedure for pregabalin in any pharmacopeia. Several reports are there in literature for PRG determination based on chromatographic methods, i.e., gas chromatography-mass spectrophotometry (GC-MS), LC-MS MS, HPLC coupled with varying detection techniques like tandem mass spectrometry, fluorometry and enantiospecific analysis. These methods may involve procedural variations including pre- and post- column derivatization. Recently, capillary electrophoresis and nuclear magnetic resonance technique was reported for PRG involving complexation with cyclodextrins. All these are complex trace analysis techniques most of which have been employed for PRG determination in biological fluid samples. However, routine analysis of the drug in bulk powder and pharmaceutical preparations in research laboratories and pharmaceutical industry requires a relatively uncomplicated and a more cost effective method like UV/visible spectrophotometry or

spectrofluorometry. Pregabalin, as such, has a poor UV/ visible absorbance profile and very few reported methods have relied on generation of a chromophoric product by reaction of the drug with some suitable reagent. Considering the limited literature reports available in this area, we found it very pertinent to investigate and develop a novel spectrophotometric method for determination of pregabalin in bulk powder and pharmaceutical preparations.

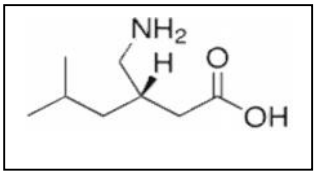

Figure -1:-Structure of pregabalin

Introduction:-[2]

Pregabalin (PGB), (S) - 3 - amino methyl hexanoic acid, is a structural analogues of γ - amino butyric acid (GABA) as shown in (Figure1). It is a white crystalline solid. It is soluble in water and in both basic and acidic aqueous solutions. It is a new anticonvulsant 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. It is both structurally and pharmacologically related to the anticonvulsant and analgesic medication gabapentin and both compounds were originally synthesized with the hope of modulating brain GABA receptors and GABA synthetic enzymes. These compounds are inactive at GABAA and GABAB recap-tors. The mechanism of action of pregabalin has been characterized only partially and in particular, the cellular and molecular details of its action to reduce neurotransmitter release are incompletely known. The primary high - affinity binding site for pregabalin in forebrain tissues is the α2 – δ type 1 auxiliary subunit of voltage - gated calcium channels and this interaction seems to the required for the pharmacological actions of the medications. The identification of the α2 – δ binding sites has lead to the speculation that pregabalin act pharmacologically specifically in neurons by modulating the action of synaptic calcium channels. This hypothesis is supported by several findings that pregabalin reduce calcium influx into synaptosomes prepared from human brain and it subtly reduce calcium dependent overflow of neurotransmitters from several different neuronal tissues and reduce synaptic responses. PGB is thought to be useful for treating any other conditions, pain, physiological conditions associated with psychomotor stimulants, inflamemation, gastrointestinal damage, alcoholism, insomnia, and various psychiatric disorders, including mania and bipolar disorder. There is no official method developed for the analysis of pregabalin till now and therapeutic importance of the drug has engendered development of assays for the quantification of PGB. A through literature search has revealed that only a few analytical methods are available for determination of pregabalin in bulk drugs and pharmaceutical formulations. Liquid chromatography - mass spectrophotometry (LC - MS), LC with fluorescence detection were used to determine pregabalin in human plasma and serum. All

of these methods are very expensive because these methods require long and tedious pretreatment of the samples, laborious clean up procedures (including extraction with solvent) and derivatization for the analysis of PGB. There is no UV method without derivatization for the analysis of PGB. So there is need for the development of a UV method for the analysis of PGB. Hence, an attempt has been made to develop a simple, efficient and selective method for the analysis of PGB in bulk & pharmaceutical formulations. The method requires no derivatization steps. The method was used successfully to evaluate PGB.

Material And Method:

Pregabalin pure drug was obtained from Alkem Laboratories Ltd, Mumbai, as a gift sample & Pregabalin tablet was obtained from local pharmacy. Instrument used were Shimadzu UV/Visible 1601 Spectrophotometer and Shimadzu analytical balance. All other chemicals used were of analytical grade.

Determination of appropriate UV wavelength: A suitable wavelength was required for the determination of Pregabalin. The appropriate wavelength for the determination of PGB was determined by wavelength scanning over the range 190–450 nm with a Shimadzu UV/Visible 1601 Spectrophotometer.

Standard PGB Solution:

A stock solution of PGB (50 µg/ml) was prepared by dissolving 5 mg PGB in 100 ml volumetric flasks with double distilled water. The stock solution (50 µg/ml) was used to prepare the working solutions by suitable dilutions with distilled water. The solutions were stable at least 10 days in room temperature.

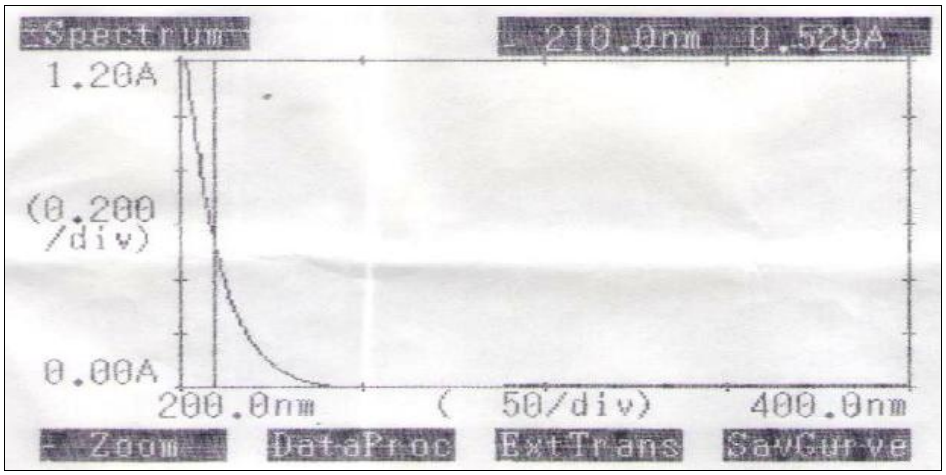

Figure-2: UV/Visible scan of pregabalin without derivatization λmax 210 nm).

Procedure for the determination of PGB:

Aliquots of stock solution (50 µg/ml) were transferred into a set of 50 ml volumetric flasks and volumes were completed to the mark with distilled water to produce solutions in the concentration range 6-14 µg/ml. Absorbance was measured at 210 nm against the reagent blank. Calibration graphs were constructed by plotting absorbance against the final concentration of PGB.

Table No: - 1 Absorbance obtained from respective concentrations..

Sr. no.	Conc. µg/ml	Absorbance
1	0	0.00
2	6	0.274
3	8	0.375
4	10	0.487
5	12	0.562
6	14	0.652

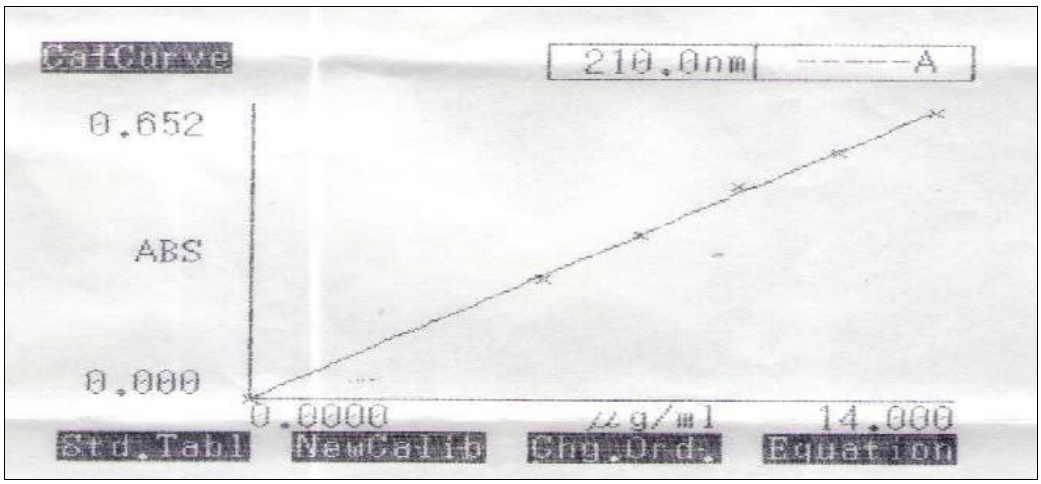

Figure-2 Standard Calibration curve of Pregabalin.

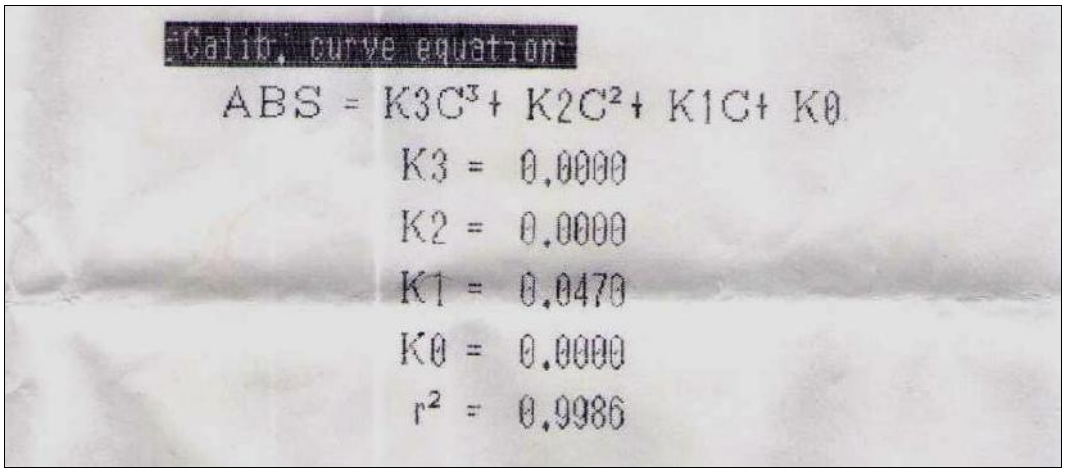

Figure-3 Equation for Standard Calibration curve.

Method validation: [3]

The method was validated for selectivity, linearity, precision, accuracy, recovery and stability according to the principles of the Food and Drug Administration (FDA) industry guidance. Validation of analytical procedures is a vital aspect not just for regulatory purposes, but also for their efficient and reliable long – term application. The ICH guidelines achieved a great deal in harmonizing the definitions of required validation parameters, their calculation and interpretation. It is the responsibility of the analyst to identify parameters which are relevant to the performance of given analytical procedure as well as to design proper validation protocols including acceptance criteria and to perform an appropriate evaluation. The International Conference on the Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use has harmonized the requirements in two guidelines. The first one summarizes and defines the validation characteristics needed for various types of test procedures, the second one extends the previous test to include the experimental data required and some statistical interpretation. These guidelines serve as a basis worldwide both for regulatory authorities and industry and bring the importance of a proper validation to the attention of all those involved in the process of submission.

Nowadays, the validation characteristics needed for the various test procedures and their general requirements are well understood. The essential question to be answered is on the suitability of the calibration mode to be used in the test procedure. It should be noted that in most cases only a

qualitative statement is needed. The stability of the working PGB sample solutions at room temperature was evaluated with the help of UV spectra.

The linearity of the proposed method was constructed for Pregabalin reference standard solution by plotting concentration of the compound versus the absorbance. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The accuracy and precision of the method was evaluated within the linear range. Five independent analysis were performed at each concentrations level within one day (intraday precision) as well as for five consecutive days (interday precision). The accuracy was ascertained by recovery studies using the standard addition method. The amount of PGB was determined from the regression equation.

Accuracy (Recovery Test):[4]

Accuracy of the method was studied by recovery experiments. Recovery experiments were performed by adding known amount to tablet. The recovery was performed at three levels, 80%, 100% and 120% of Pregabalin standard concentration. The recovery samples were prepared in afore mentioned procedure. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for pregabalin ranged from101.6632 ±1.0736 (Table no.1).

Linearity: [4]

The linearity of the response of the drug was verified at 6 to 14 µg/ml concentrations. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis (Table no.3). The equation of the calibration curve

for Pregabalin obtained y = 0.0470x + 0.000, the calibration curve was found to be linear in the aforementioned concentrations. The correlation coefficient (r2) of determination was 0.9986.

Precision: [4]

Precision of method (intra-day) was evaluated for Pregabalin. The reproducibility (inter-day precision) of the method was also evaluated on different days in the same laboratory. The relative standard deviation (RSD) and assay values obtained were 0.7123, 101.5975 and 0.7408, 101.8233 Respectively (Table no.2).

Limit of Detection and Limit of Quantitation: [5]

The LOD and LOQ were determined based on the standard deviation of the y-intercept and the slope of the calibration curves. LOD and LOQ for Pregabalin were found to be 2.457 mg/ml and 7.448 mg/ml respectively. (Table No.3)

Stability:

The stability of final measured sample solution was examined and responses were found to be stable for at least 10 days at room temperature. This allows the processing of large batches of samples and their comfortable measurements with convenience.

Table no. 1 Determination of Accuracy by percentage recovery method

Drug	Drug Amount (mg)	Level of Addition (%)	Amount added (mg)	Drug found (µg/ml)*	% Recovery	Average % Recovery
Pregabalin	1	80	0.8	16.2581	102.8732	101.6632
	1	100	1	20.6732	101.6342	
	1	120	1.2	24.6620	100.4824	

Table no. 2 Determination of Precision

Sample no.	Inter-day precision	Intra-day precision
1	101.56	101.7896
2	102.23	100.7971
3	102.67	102.2058
Mean±SD	101.8233±0.7543	101.5975±0.7237
%RSD	0.7408	0.7123

Table no: 3 Optical characteristics and validation data of pregabalin

Sr.No	Parameters	Values
1	λmax/ nm	210 nm
2	Beers law limits (µg/ml)	6-14
3	Molar absorptivity (1 /mol/cm)	3.693x10-4
4	Correlation coefficient (R)	0.9986
5	Sandell's sensitivity(µg cm-2)	0.378
6	Regression equation (y)	y = 0.0470x + 0.000
7	Slope, *b*	0.0470
8	Intercept, *c*	0.000
9	Limit of detection (mg/ml)	2.457
10	Limit of quantification(mg/ml)	7.448
11	Interday RSD	0.7408
12	Intraday RSD	0.7123

y = bx + c, where x is the concentration of drug in µg/ml; Average of six determinations

Conclusion:

The developed method was found to be simple, sensitive, accurate, precise, reproducible, and can be used for routine quality control analysis of Pregabalin in bulk and pharmaceutical formulation.

Acknowledgement

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