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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PREGABALIN IN PHARMACEUTCAL DOASAGE FORM BY USING UV-VISIBLE SPECTROSCOPY

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Abstract: A simple, specific, accurate and precise UV spectrophotometric method was developed and validated for the estimation of Pregabalin in pharmaceutical dosage form. The stock solution was prepared by weighing 1000mg of standard Pregabalin in 1000ml of 0.2M Hcl in volumetric flask. The final stock solution was made to produce 1g/µl with water. Further dilution was prepared as per procedure and were scanned at 225nm. The linearity was found in the concentration range of 1µg/ml. the correlation coefficient was 0.9907. The regression equation was found to be Y= 0.155X+0.1773. Recovery of Pregabalin was found to be in the range of 98-102%. The method was validated for limit of detection limit of quantification for estimation of Pregabalin was found to be µg/ml and µg/ml respectively. The Proposed method can be successfully applied for the quantitative determination of Pregabalin in pharmaceutical dosage form.

Keywords: UV spectrophotometry, Regression, Recovery, Precision, Linearity.

I. INTRODUCTION

Pharmaceutical Analysis [1, 2]is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative chemical analysis of the substances present in bulk and pharmaceutical preparations.

UV-Visible Spectroscopy:

Spectroscopy is the branch of science dealing with the study of electromagnetic radiation with matter i.e., the measurement of electromagnetic radiation emitted or absorbed by analyte. Spectroscopy is one of the most powerful tools available for study of atomic and molecular structure. Molecular Spectroscopy is based upon UV, Visible and infrared radiation is widely used for the identification and measurement of inorganic, organic and biochemical species. Molecular UV or Visible absorption Spectroscopy is employed primarily for Quantitative analysis and is probably more widely used in chemical laboratories throughout the world than any other single method.

The scope of absorption Spectroscopy is further extended by use of color reactions often with concomitant increase in Sensitivity and / or Selectivity. Such reactions are used to modify the spectrum of an absorbing molecule, so that it could be detected in visible region, well separated from other interfering components. Chemical modification is also useful for transforming non-absorbing molecule to a stable derivative possessing significant absorption spectral behavior. Selectivity can further be enhanced by number of chemical or instrumental techniques such as difference Spectroscopy, derivative Spectroscopy, derivative-difference Spectroscopy and dual wavelength Spectroscopy; however, such methods should be validated.

The prescribed unit used in UV-Visible Spectroscopy is wavelength expressed as nanometers (nm). The position of maximum Absorbance of a peak is designated as λ_{max}. The wavelength in these regions is divided into two ranges i.e. 200-400 nm (UV region) and about 400-800 nm (Visible region).

II. Plan of work

Method development and validation of Pregabalin in tablet dosage form by UV Visible Spectrophotometer methods.

Survey of literature review on selected drugs and the mode of analysis.

Analytical method development

1. Selection of Wave length
2. Selection of Solvent

Analytical method validation for the estimation of compound.

The developed method is validated according to ICH guidelines for various parameters specified in ICHQ2B.

III. MATERIALS AND METHODS

The following materials used were either AR/LR grade or the best possible Pharma grade available as supplied by the manufacturer or supplier without further purification or investigation.

table no 3.1. materials

S.NO	MATERIALS	SOURCE
1	Pregabalin	Swefn Pharmaceuticals private Ltd, Ahmedabad
2	Methanol	Merck Mumbai Ltd, Mumbai.
3	Ethanol	Merck Mumbai Ltd, Mumbai.
4	Sulphuric acid	Merck specialities Pvt Ltd, Mumbai.
5	Glacial acetic acid	Merck Mumbai Ltd, Mumbai.
6	Hydrochloric acid	Nice Chemicals Pvt Ltd, Kerala
7	Water	Nishanth, Kurnool.
8	Nitric acid	Merck specialist Pvt Ltd, Mumbai.
9	Chloroform	Merck specialist Pvt Ltd, Mumbai.

3.1 UV SPECTROSCOPY

An Analytical Technologies Limited 2080N Spectrophotometer was used with 1 cm matched quartz cells. The data processing was performed using Analytical Technologies software.

3.2 UV METHOD DEVELOPMENT

The parameters for the development were as follows:

1) Selection of solvent

2) Selection of wavelength

3.3 VALIDATION OF PROPOSED METHOD

The parameters for the validation were as follows:

1) Linearity

2) Accuracy

3) Precision

4) Robustness

5) Ruggedness

6) Limit of detection

7) Limit of quantitation

3.4 Selection of solvent:

In order to select suitable solvent for determination of Pregabalin, various solvents like 1N Sulphuric acid, ethanol, 0.1N NaOH, DMSO [dimethyl sulfoxide], water, Acetate buffer, 2M hydrochloric acid, isopropyl, chloroform and methanol were tried for the solubility studies and it was found that Pregabalin was soluble in 2M HCL, so in present investigation 2M HCL was selected as a solvent.

3.5 Selection of wavelength:

10µg/ ml of Pregabalin solution was scanned in the range of 200-400 nm. The maximum absorbance was found at 225 nm.

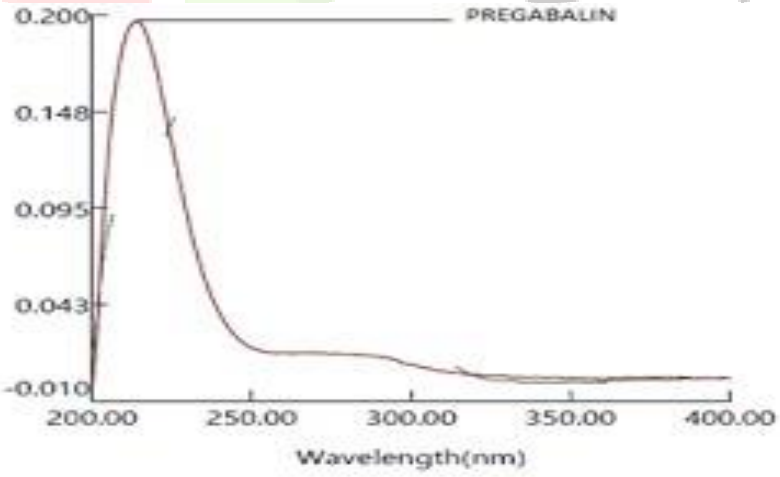

fig.3.5 uv spectrum of pregabalin

3.6 Preparation of Solvent (2M HCL)

Take 16.6 ml of concentrated HCL and transfer it into the 1000 ml volumetric flask and make up the volume up to 1000 ml with the water.

3.7 Preparation of stock solution

A stock solution of Pregabalin was prepared by accurately weighing 100 mg of drug, &transfer it in to 100 ml volumetric flask and dissolve it with 2M HCL and make up the volume to 100 ml with solvent 2M HCL (1000 µg/ml) name it as solution-1.

3.8 Preparation of working standard solution

An accurately measured quantity of 10 ml of standard stock solution-1 was transferred to 100 ml volumetric flask and diluted up to 100 ml with 2M HCL to give a working standard solution having strength of (100 µg/ml) and consider it as solution-

An accurately measured quantity of 10ml of solution-2 was transferred to 100 ml volumetric flask and diluted up to 100 ml with 2M HCL to give a working standard solution having strength of (10µg/ml) and consider it as working solution-3.

3.9 VALIDATION OF THE METHOD

Once the UV method development was over, the method was validated in terms of parameters like linearity, accuracy, precision, ruggedness and robustness.

3.10 Linearity

To evaluate the linearity, serial dilution of analyte was prepared from the working solution was diluted with solvent to get a series of concentration ranging from 2, 4, 6, 8, 10 and 12µg/ml.

The prepared solutions were filtered through Whatman filter paper (No.41). Calibration curve was constructed by plotting the absorbance on Y-axis against the concentration on X-axis. The results which are given in **Table 4.2** were within acceptable limits.

2µg/ml: 1 ml of working standard solution-3 was taken in 10 ml of volumetric flask diluted up to the 10ml with solvent 2M HCL.

4µg/ml: 1 ml of working standard solution-3 was taken in 10 ml of volumetric flask diluted up to 10ml with solvent 2M HCL.

6µg/ml: 1 ml of working standard solution-3 was taken in 10 ml of volumetric flask diluted up to 10ml with solvent 2M HCL.

8µg/ml: 1 ml of working standard solution-3 was taken in 10 ml of volumetric flask diluted up to 10ml with solvent 2M HCL.

10µg/ml: 10ml of working standard solution was taken in 10 ml of volumetric flask.

12µg/ml: 1.2ml of working standard solution-2 was taken in 10 ml of volumetric flask diluted up to 10ml with solvent.

Acceptance criteria: correlation coefficient should not be less than 0.99.

3.11 Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of Pregabalin (5µg/ml) were spiked with 80, 100, and 120 % pregabalin standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery of the pure drug. % RSD were calculated and reported in **Table 4.3.**

Procedure

The standard solution was analyzed for three times and then each level of sample solution was analyzed in replicate for three times and the absorbance of standard and sample solutions was compared.

3.12 Precision

The precision of the analytical method was studied by analysis of multiple sampling of homogenous sample. The precision is expressed as standard deviation or relative standard deviation.

The precision of the method was demonstrated by intra-day and inter-day variation studies.

3.12a Intra-day precision

In the intra-day studies, the standard solution (8µg/ml) was analysed for six times in different time interval within a day. % RSD was calculated presented in **Table 4.4.**

3.12b Inter-day precision

In the inter-day variation studies, the standard solution (8µg/ml) was analysed for six times in different days. % RSD was calculated presented in **Table 4.4a.**

3.13 Sensitivity

The Sensitivity of measurement of Pregabalin by use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of response and S is the average of the slope of the corresponding Calibration curve. The results for LOD & LOQ are reported in the **Table 4.5a & 4.5b.**

3.14 Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from analyst to analyst. The ruggedness of the method was determined by carrying out the experiment by different operators. The results of ruggedness testing are reported in the **Table 4.6.**

3.15 Robustness

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and response was evaluated. Variation of wavelength (223 and 227 nm) and temperature (45^{o}C&55^{o}C) had no significant effect on the absorbance of 8µg/ml solution, indicating that the method was robust. The results are shown in **Table 4.7& 4.7a.**

IV. RESULTS AND DISCUSSION

table 4.1: characteristic parameters of pregabalin for the proposed uv method

Parameters	UV
Calibration range (µg / ml)	2-12µg/ml
Wavelength(nm)	225
Regression equation (Y*)	0.0155x+0.1773
Intercept	0.1773
Slope (b)	0.0155
Correlation coefficient (r^2)	0.9907
LOD (µg / ml)	0.9282
LOQ (µg / ml)	2.8129

*Y = bx + a where x is the concentration of Pregabalin in µg / ml and Y is the absorbance at the respective λ_{max}.

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4.1 VALIDATION OF ANALYTICAL METHOD:

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters.

4.2 Linearity

Calibration graph was plotted using absorbance of standard drug v/s concentration of standard drug solutions. Linear regression data showed a good linear relationship over a concentration range 02-12µg/ml.

table 4.2: calibration curve for pregabalin

S. No.	Concentration(µg/ml)	Absorbance
1	2	0.1925
2	4	0.2059
3	6	0.2268
4	8	0.2426
5	10	0.2511
6	12	0.2710

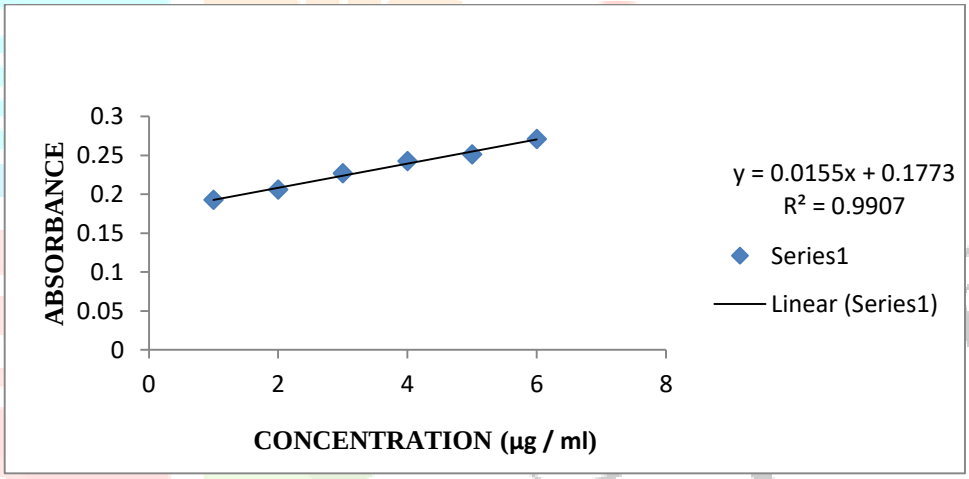

fig: 4.2. calibration curve of pregabalin

Observation:

1. The correlation coefficient for Pregabalin was found to be 0.9907respectively.
2. The Linearity range for Pregabalin was found to be 02-12µg/ml.

4.3 Accuracy

To study the accuracy of the method, recovery studies were carried out. The concentration of drug present in resulting solution was determined using developed procedure and percentage recovery and percentage RSD were calculated.

table 4.3: accuracy summary

Sample (%)	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered* (µg/ml)	%Recovery ± STDEV*	%RSD
80%	4	0.5	100.44	100.44±0.004365	1.14
100%	5	0.5	99.27	99.27±0.003716	1.50
120%	6	0.5	100.15	100.15±0.004952	1.68

*Average of three determinations

Acceptance criteria:

1. % recovery should be within the range of 98-102%
2. % RSD should be not more than 2%

4.4 PRECISION

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or relative standard deviation.

table 4.4: intraday precision results for pregabalin

S. No.	Concentration(µg/ml)	Absorbance
1	8	0.2442
2	8	0.2543
3	8	0.2518
4	8	0.2483
5	8	0.2330
6	8	0.2334
	STDEV	0.00436

	AVG	0.2441666
	%RSD	1.78

table 4.4.a: inter day precision results for pregabalin

S. No.	Concentration(µg/ml)	Absorbance
1	8	0.2495
2	8	0.2439
3	8	0.2439
4	8	0.2497
5	8	0.2445
6	8	0.2568
	STDEV	0.004768
	AVG	0.24805
	%RSD	1.98

Acceptance criteria:

% RSD of the six replicate injections should not more than 2.0%.

4.5 Sensitivity:

Limit of detection (LOD) and limit of quantitation (LOQ) were determined from standard deviation and slope method as per ICH guideline, for Pregabalin. LOD was found to be and LOQ was found to be 0.9282 and 2.8192.

table 4.5a: observation of limit of detection

S. No.	Slope	STDEV of precision	LOD
1	0.0155	0.00436	0.9282

table 4.5b: observation of limit of quantitation

S. No.	Slope	STDEV	LOQ
1	0.0155	0.00436	2.8129

4.6 RUGGEDNESS:

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

table 4.6: ruggedness studies of pregabalin by uv- visible spectroscopic method

S. No	Analyst-1		Analyst-2	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	8	0.2485	8	0.2450
2	8	0.2237	8	0.2440
3	8	0.2424	8	0.2379
4	8	0.2491	8	0.2474
5	8	0.2426	8	0.2424
6	8	0.2379	8	0.2381
	STDEV	0.004324	STDEV	0.00382187
	AVG	0.2407	AVG	0.24246667
	%RSD	1.79	%RSD	1.57

Acceptance criteria:

1) % RSD should not more than 2.0%.

4.7 Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Wavelength:

The solution was prepared and observed in replicate for six times with (±2) wavelength i.e. 223nm and 227nm respectively.

Temperature:

The solution was prepared and observed in replicate for six times with (±5) Temperature i.e. 45^0c & 55^0c respectively.

table 4.7: robustness summary for wavelength

S. No	Condition	Modification	Mean Absorbance ± STDEV	% RSD (for Absorbance
1	Wavelength (nm)	223	0.2189 ±0.003712	1.69
		227	0.21125±0.004027	1.90

*Average of six determinations

Acceptance Criteria:

% RSD should not more than 2.

table 4.7a: robustness summary for temperature

S. No	Condition	Modification	Mean Absorbance ± STDEV	% RSD (for Absorbance
1	Temperature (^{o}c)	45	0.2659 ±0.001353	0.5077
		55	0.28833±0.002909	1.0058

*Average of six determinations

Acceptance Criteria:

% RSD should not more than 2.

V. CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision.

There are few analytical methods appeared in the literature for the determination of the Pregabalin.

In view of the above, a simple and specific analytical method was planned to develop with sensitivity, accuracy, precision and economical.

In the present investigation of, UV method for the quantitative estimation of Pregabalin in pharmaceutical dosage form has been developed and validated.

The proposed UV method is more sensitive, accurate and precise and is suggested for routine analysis.

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