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STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION OF PREGABALIN IN PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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Abstract:

A simple, precise, accurate, reproducible Stability indicating RP-HPLC method was developed for the determination of Pregabalin in pharmaceutical dosage forms. After optimization chromatographic separation was achieved by Isocratic mode with a mixture of dipotassium hydrogen phosphate: methanol in the ratio of 60:40% v/v as the mobile phase with AGILENT ZORBAX SB- ODS C18 (250 × 4.6 mm I.D) 5 µm column as stationary phase at flow rate of 1 mL/min and detection wavelength of 247 nm. The retention time for Pregabalin found to be 2.00 min respectively. The linearity of this method was found in the concentration range of 50 µg/mL to 150 µg/mL for pregabalin. The correlation coefficient R^2 value is found to be 0.99 for Pregabalin. The LOD and LOQ for pregabalin were found to be 0.53 µg/mL and 1.61 µg/mL respectively. Percentage recovery for pregabalin were found to be 100% which indicates that the proposed method is highly accurate. The drug was subjected to acid hydrolysis, base hydrolysis, peroxide, heat and sunlight degradation and the method was found to be specific and stability indicating as no interfering peaks of degradants and excipients were observed.

Keywords: *Pregabalin, RP-HPLC, Dosage form, Stability indicating.*

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INTRODUCTION:

Pregabalin [(3S)-3-(amino methyl)-5-methyl hexanoic acid is a drug used to treat partial epilepsy, generalized anxiety disorder, neuralgia and fibromyalgia. It is a more potent successor of Gabapentin.

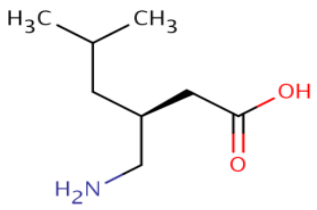

Fig 1: Structure of Pregabalin.

Literature review revealed that various analytical methods were available for the evaluation of Pregabalin in pharmaceutical dosage forms, but there were very few stability indicating methods available so far. This forms the basis for development of new stability indicating RP-HPLC method for the estimation of Pregabalin in pharmaceutical dosage forms.

The proposed method was validated with respect to stability indication besides specificity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) according to ICH guidelines to show it could be used for determination of Pregabalin in pharmaceutical formulations.

MATERIALS AND METHODOLOGY:

Chemicals and Reagents:

Pregabalin standard drug, di potassium hydrogen phosphate(laboratory reagent), HPLC grade methanol, HPLC grade water. All other chemicals of analytical grade were procured from local laboratories. All dilutions were performed in standard class-A, volumetric glassware.

Instrument:

Theanalysis of drug was carried out on WATERS HPLC system using PDA detector. Separation was achieved under optimized conditions on Agilent ZorbaxSb-Ods C_{18} (250×4.6mm I.D) 5µm.The out put of signal was monitored and integrated using Empower-2 software.

Preparation of Buffer Solution (0.1N K_2HPO_4):

8.709g of di potassium hydrogen phosphate was accurately weighed and dissolved in 400 mL HPLC grade water. Mixed well and sonicated to remove dissolved gases. Finally the volume was made with water and pH was adjusted to 4.8 using dil.OPA.

Preparation of Mobile Phase:

To 40 mL of buffer solution, 60 mL of methanol was added. Mixed well and degassed in an ultrasonic water bath for 10 minutes. Filtered through 0.45µ membrane filter.

Preparation of Diluent:

Water is used as a diluent.

Preparation of Standard Solution:

Accurately weighed amount of 150mg of Pregabalin working standard was transferred into 100 mL volumetric flask, to which 50 mL of methanol was added and sonicated to dissolve the material completely, then the volume was made upto 100 mL using water. 1mL of this solution was diluted to 25 mL using water and mixed well.

Preparation of Sample Solution:

10 capsules were weighed and the average weight of capsules was recorded. An accurately weighed amount of 338 mg of fine powder equivalent to 150 mg of Pregabalin was transferred to 100 mL of volumetric flask to which 70 mL of diluent was added and sonicated for 30 minutes. Finally the volume was made upto the mark with water. From the above solution 1 mL was taken in 10 mL of volumetric flask and was made upto the mark.The solution was filtered through a 0.45µ membrane filter.

Chromatographic Conditions:

Agilent ZorbaxSb-Ods C_{18} (250×4.6mm I.D) 5µm column was used for analysis at 25ºC column temperature. The mobile phase was pumped through the column at a flow rate of 1 mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 247 nm for the detection and chromatographic run time was 5 minutes.

RESULTS AND DISCUSSION:

Method development

Spectroscopic analysis of compound showed that Pregabalin has maximum UV absorbance at 247 nm.Therefore, the chromatographic detection was performed at 247nm using photo diode array detector.

Chromatographic conditions were optimized using mobile phase of Di potassium hydrogen phosphate and methanol (40:60), column temperature of 25ºC, injection volume of 10µL on column Agilent ZorbaxSb-Ods C_{18} (250×4.6mm,5µ) with a flow rate of 1 mL/min.

A typical chromatogram for estimation of Pregabalin obtained by using the above mentioned mobile phase from 10µL of the assay preparation is illustrated in **Fig.2.**

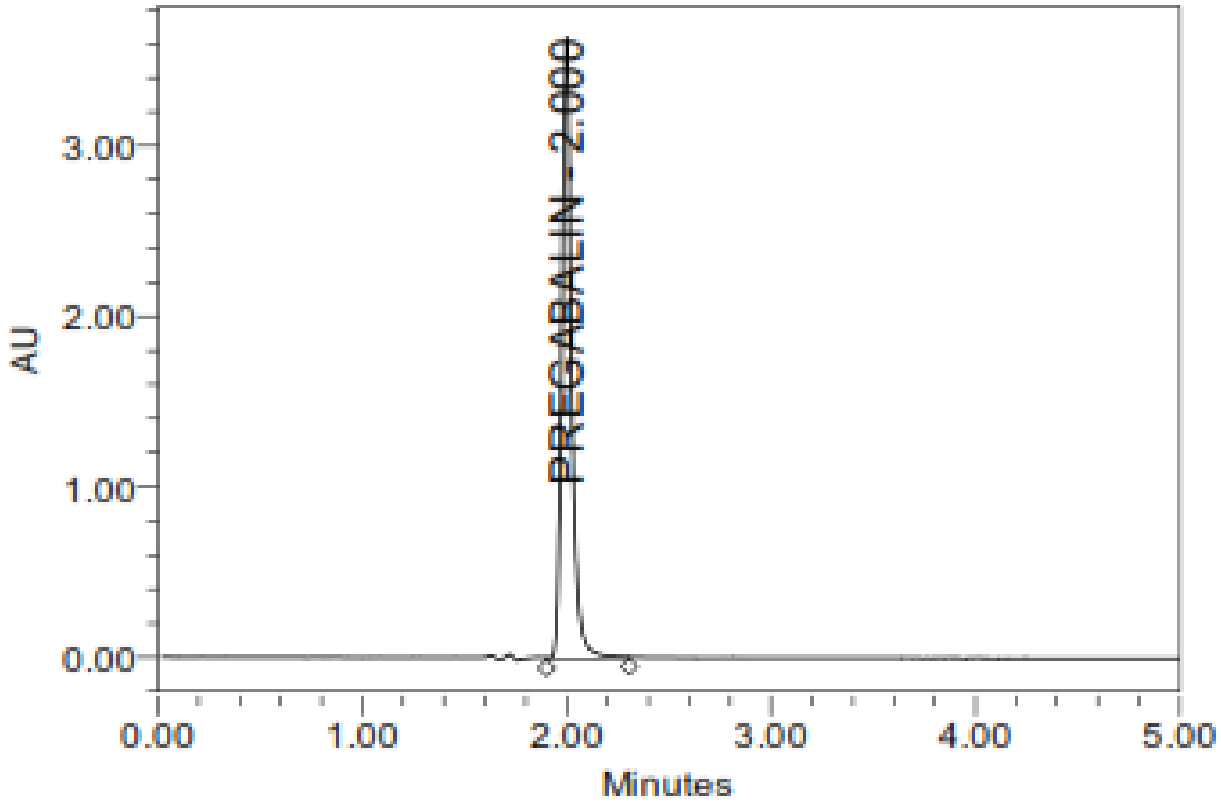

Fig 2: A typical HPLC Chromatogram Showing the Peak Of Pregabalin

Method Validation

The developed RP-HPLC methodwas extensively validated for assay of Pregabalin using the following parameters.

System suitability:

Table 1: System Suitability Parameters of Pregabalin by Proposed Method

S.NO	RetentionTime	USP plate count	USP tailing
Injection 1	1.992	6724	1.30
Injection 2	1.985	6596	1.32
Injection 3	1.989	6754	1.33
Injection 4	1.970	6973	1.35
Injection 5	1.985	6762	1.33

Specificity:

Blank and Placebo Interference:

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **(Fig.3)**showed no peaks at the retention time of Pregabalin peak. This indicates that the diluent solution used in sample preparation donot interfere in estimation of Pregabalin in Pregabalin capsules. Similarly chromatogram of placebo solution **(Fig.4)**showed no peaks at the retention time of Pregabalin peak. This indicates that the placebo used in sample preparation donot interfere in estimation of Pregabalin in Pregabalin capsules.

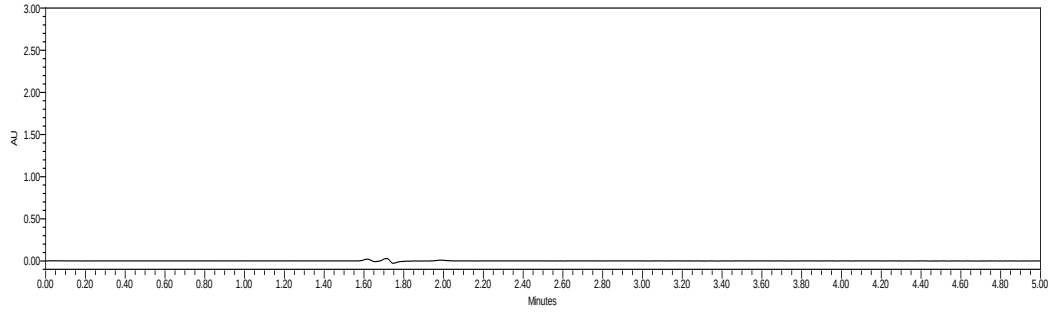

Fig.3. Typical Chromatogram Showing No Interference of Diluent for Pregabalin

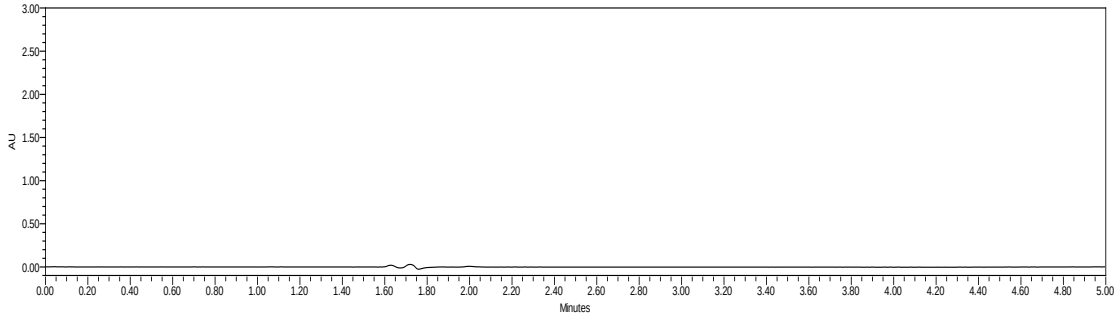

Fig.4. Typical Chromatogram Showing No Interference of Placebo for Pregabalin

The HPLC chromatograms recorded for the placebo showed almost no peaks at the retention time of Pregabalin. The peak for Pregabalin is clearly is separated from other excipients of the formulations. As there is no blank interference observed at the retention time of Pregabalin, the HPLC method presented in this study is specific for Pregabalin.

Accuracy:

The solutions were injected thrice each separately at a concentration ranging from 50-150%. The individual recovery and mean recovery values were calculated from the amount added and amount founded reports were shown in table 4..

Table 2: Specificity Parameters for Pregabalin Standard

Pregabalin standard	Inj-1	Inj-2	Inj-3	Avg	%RSD
RT	2.000	1.989	1.989	1.992	0.260
Area	4347057	4336950	4345660	4343222	0.102

Table 3: Specificity Parameters for Pregabalin Sample

Pregabalin sample	Inj-1	Inj-2	Inj-3	Avg	%RSD
RT	1.989	1.987	1.985	1.987	0.082
Area	4451085	4398605	4305630	4385107	0.370

Table 4:. Accuracy data of Pregabalin

S.NO	Accuracy level	injection	Sample area	RT
1	50%	1	2226819	1.992
		2	2228924	1.992
		3	2228047	1.990
2	100%	1	4454088	1.996
		2	4453008	1.991
		3	4458568	2.003
3	150%	1	6689683	1.999
		2	6688162	2.006
		3	6684305	2.002

Table 5: Recovery Data of Pregabalin

S.NO	Accuracy Level	Injection	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	169.00	29.700	29.69	100	100
		2	169.00	29.700	29.72	100	
		3	169.00	29.700	29.71	100	
2	100%	1	338.00	59.400	59.38	100	100
		2	338.00	59.400	59.37	100	
		3	338.00	59.400	59.44	100	
3	150%	1	507.00	89.100	89.19	100	100
		2	507.00	89.100	89.17	100	
		3	507.00	89.100	89.12	100	

Precision:

The solution was injected for six times and measured the area for all six injections in HPLC. Mean and percentage RSD were calculated.

Table 6: Method Precision Data of Pregabalin (Repeatability)

S.NO	RT	Area	%Assay
injection1	1.989	4451085	99
injection2	1.995	4452131	99
injection3	1.991	4456244	99
injection4	1.993	4459358	99
injection5	1.993	4450742	99
injection6	1.998	4454852	99
Mean			99
Std. Dev.			0.08
% RSD			0.08

Intermediate Precision (analyst to analyst variation)

Performed on different day by using different make column of same dimensions by different analyst.

Table 7: Intermediate Precision Data of Pregabalin

Sample No.	% Assay for Pregabalin	
	Analyst-1	Analyst-2
1	99.2	98.9
2	99.2	99.2
3	98.9	99.1
4	99	99
5	98.8	98.4
6	99.1	98.6
Average	99.03	98.86
% RSD	0.15	0.28

Linearity:

A standard curve was obtained in the concentration range of 50-150µg/mL. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient of standard curve were calculated and given in **Fig.5** and **Table.8** to determine the linearity of the method.

Table.8 Linearity Data of Pregabalin

S.No	Conc(µg/ml)	RT	Area
1.	50	1.982	2227692
2.	75	2.006	3347889
3.	100	1.995	4453515
4.	125	1.T996	5578059
5.	150	1.995	6684752
Slope			44583
Correlation coefficient (r^2)			0.99

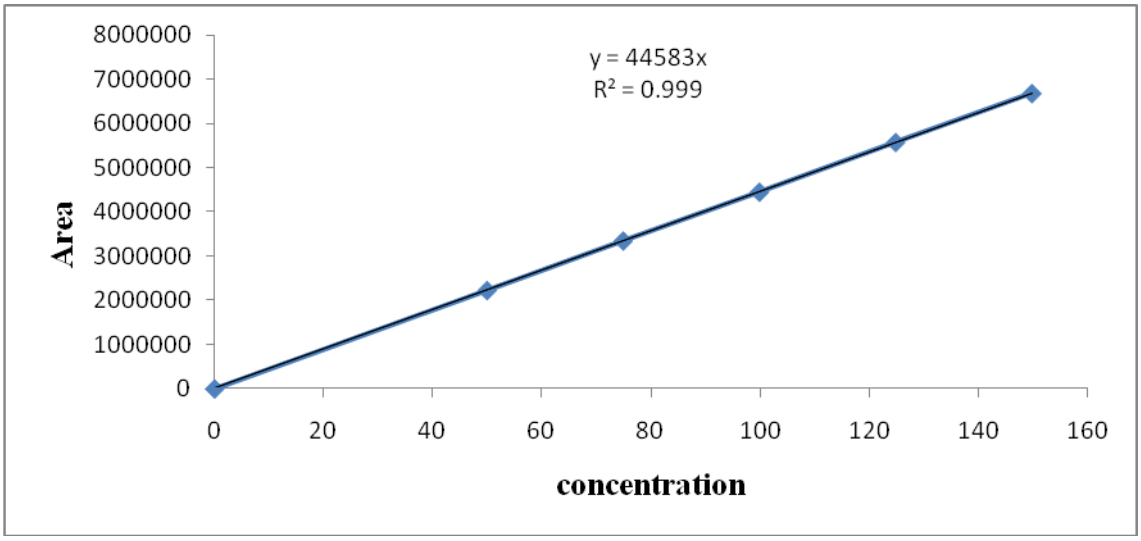

Fig 5: Linearity Curve for Pregabalin

Robustness:

Effect of Variation In Flow Rate:

The system suitability solution was prepared as per the test method and injected into the HPLC system with ±0.2 mL of the method flow. The system suitability values were evaluated as required by the test method for both the flow rates. Actual flow rate was 1 mL/min and it was changed to 0.8 mL/min and 1.2 mL/min and injected into HPLC and system suitability was checked.

Effect of Variation in Temperature:

The system suitability solution was prepared as per the test method and injected into the HPLC with ±5°c of the method temperature. The system suitability values were evaluated as required by the test method for both the temperatures. The results were reported in **Table. 9.**

Table 9: Robustness Data of Pregabalin

Parameter	RT	Theoretical plates	USP tailing
Decreased flow rate	2.478	7508	1.47
Increased flow rate	1.658	6313	1.28
Decreased temperature	2.466	7162	1.42
Increased temperature	1.663	6266	1.29

LOD and LOQ

Limit of detection was found to be 0.53µg/mL and Limit of quantitation was found to be 1.61µg/mL.

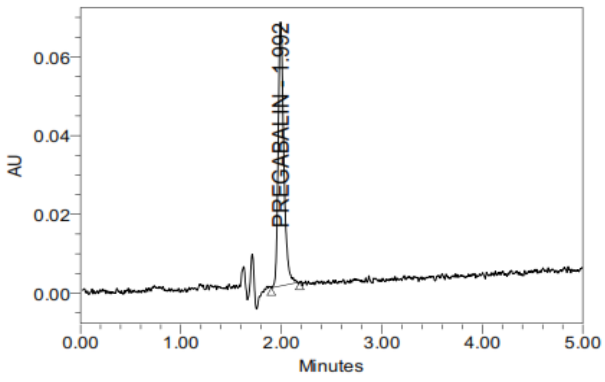

Fig 6: A typical Chromatogram for LOD of Pregabalin

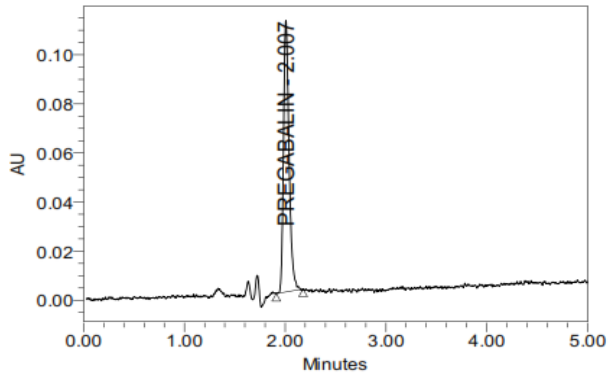

Fig 7: A typical chromatogram for LOQ of Pregabalin

Forced Degradation Studies:

Acid degradation: 150 mg equivalent weight of sample powder was transferred into a 50mL of volumetric flask and 10mL of 0.1N HCL was added and sonicated for 30minutes. To the above solution10ml of 0.1N NaOH was added and the volume was made upto the mark with water . 1 mL of the above solution was transferred into 25mL of volumetric flask anddiluted to volume with water.

Base degradation: 150 mg equivalent weight of sample powder was transferred into a 50mL of volumetric flask and 10mL of 0.1 N NaOH was added and sonicated for 30minutes. To the above solution 10mL of 0.1N HCl was added and the volume was made upto the mark with water. 1 mL of the above solution was transferred into 25 mL of volumetric flask and diluted to volume with water.

Peroxide degradation: 150 mg equivalent weight of sample powder was transferred into a 50mL of volumetric flask and 10ml of peroxide was added and sonicated for 30minutes. Thevolume was made upto the mark with water .From the above solution, 1mL of the solution was transferred into 25 mL of volumetric flask diluted to volume with water.

Heat degradation: The sample before weighing was exposed at 105◦C.Then 150 mg weight of sample powder was transferred into a 50mL volumetric flask and to that 10mL of peroxide was added and sonicated for 30minutes. The volume was made upto the mark with water .From the above solution, 1 mL was transferred into 25 mL of volumetric flask diluted to volume with water.

Sun light degradation: The sample before weighing was exposed in the light for 24hrs.Then 150 mg weight of sample powder was transferred into a 50mL volumetric flask and to that 10mL of peroxide was added and sonicated for 30minutes. The volume was made upto the mark with water .From the above solution, 1 mL was transferred into 25mL of volumetric flask diluted to volume with water. The results of forced degradation studies were reported in the **Table.10**

Table 10: Forced Degradation Data for Pregabalin

S.NO	RT	AREA	TAILING	PLATE COUNT	%ASSAY
ACID	2.013	3811416	1.15	5392	85
BASE	2.004	3821153	1.15	5260	85
PEROXIDE	2.018	3026144	1.15	5407	67
LIGHT	2.010	3438726	1.18	4915	76
HEAT	2.011	3131042	1.14	4916	70

CONCLUSION:

A simple, precise, accurate RP-HPLC method was optimized with a view to develop and validate stability indicating assay method for the determination of Pregabalin in pharmaceutical dosage forms. From the developed method it was found that the results of all validation parameters performed as per ICH guidelines were satisfactory. The method was acceptable for degradation studies of heat, sunlight, acid, base, peroxide which meet the acceptance criteria for forced degradation studies.

Moreover, the lower solvent consumption along with the shorter analytical run time of 5.0 minutes leads to an environmentally friendly chromatographic procedure that allows the analysis of large number of samples in a short period of time. Therefore, the proposed method can be used for routine analysis of drug without any interference from the excipients in laboratories and in the pharmaceutical industry.

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