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"Analytical Method Development and Validation of Pregabalin by RP-HPLC Method"

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KEYWORDS

Pregabalin, RP-HPLC, Validation.

ABSTRACT:

A simple, precise, reproducible Reverse Phase High-Performance Liquid Chromatography method was developed and validated for Pregabalin in the capsule dosage form. Chromatographic separation was achieved by Kromasil C18, (250 mm x 4.6 ID, Particle size- 5 microns) column and Acetonitrile: 0.1% TFAA (15:85 %V/V) as mobile phase, at a flow rate of 1 ml/min (milliliter per minute) Using UV detection at 210nm. The method has been validated for linearity, accuracy, precision, LOD, and LOQ. The linearity of Pregabalin was found to be in the range of 50.0 - 750.0 µg/mL. (R2=0.99992) respectively. The accuracy of the present method was evaluated at 50%, 100%, and 150%. Recovery was found to be in a range from 99.06-99.60. Values of LOD (11.572 µg/ml for PRE) and LOQ (35.068 µg/ml for PRE) indicated good sensitivity of the method. Pregabalin was estimated precisely and accurately using the proposed analytical method, which was validated under ICH requirements.

INTRODUCTION:

Epilepsy affects about 1% of people worldwide, which makes it a serious health issue. Epilepsy becomes the most prevalent serious brain illness and has a severe impact on quality of life if seizures are not well controlled. Seizures, which are caused by incorrect signaling among clusters of brain cells, are the defining feature of this illness. [1] Pregabalin is a structural analog of γ-amino butyric acid (GABA), also known as (S)-3-amino methyl hexanoic acid. The Food and Drug Administration (FDA) authorized this medication for the treatment of neuropathic pain, fibromyalgia, and diseases of the central nervous system (CNS). [2-3] Pregabalin is a calcium channel antagonist that blocks the VGCC, which lowers Ca2+ inflow and reduces glutamate and sensory neuropeptide release (substance P and CGRP) at the synapse. Pregabalin produces a decrease in the synaptic availability of glutamate by increasing the activity of excitatory amino acid transporters (EAATs). Reduced glutamate levels also prevented NMDA from being activated, which in turn reduced neuronal activity. Furthermore, pregabalin also helps the suppression of neuronal excitation by activating the KATP channels. In a variety of neuropathic pain situations, pregabalin eventually offers substantial pain relief through all of these pathways [4-6]

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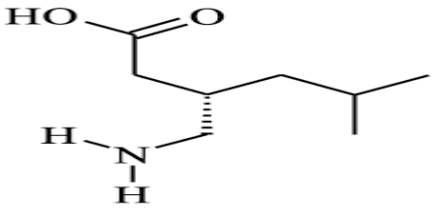

Figure.1: Structure of Pregabalin

MATERIALS AND METHODS:

1. Pregabalin (MSN Laboratories, Hyderabad, AP, India).

CHEMICAL AND REAGENT USED:

Table No.1. Chemical and reagent used

Sr.No	Chemicals	Make
1.	Acetonitrile	Qualigens (Thermo fisher scientific)
2.	Methanol	Qualigens (Thermo fisher scientific)
3.	Ortho-phosphoric acid	Qualigens (Thermo fisher scientific)
4.	Ammonium acetate	Qualigens (Thermo fisher scientific)
5.	Trifluoroacetic acid	Qualigens (Thermo fisher scientific)
6.	HPLC water	Moreshwar Ent.

INSTRUMENT AND EQUIPMENT USED

Table No.2. Instrument and equipment used

Sr.No	INSTRUMENT	Make
1.	Balance	Aczet
2.	pH-Meter	Labman
3.	UV-spectrophotometer	jasco
4.	Vortexer	Remi
5.	Centrifuge	Remi
6.	HPLC	Agilent
7.	Ultrasonicator	Bio Technics India

SOFTWARE

Table No.3. Equipment Software

Sr. No	INSTRUMENT	Software
1.	HPLC	Openlab Ezchrom workstation
2.	UV-spectrophotometer	Spectra manager

Optimization of mobile phase

Method development for Pregabalin was started with combinations of various solvents like acetonitrile, Methanol, and ortho-phosphoric acid. Ammonium acetate Trifluoroacetic acid, HPLC water.
Different mobile phase compositions were attempted to obtain a sharp peak and good resolution. Comprising varying proportions of TFAA, acetonitrile, water, and methanol. Acetonitrile and 0.1% TFAA (15:85) ratio were selected.

Preparation of mobile phase:

AR grade solvents acetonitrile: 0.1% TFAA (15:85 %V/V) were used as mobile phase.

Preparation of Standard Stock Solution of Pregabalin

Standard Stock solution preparation of 1000ppm of individual drug.
Accurately weighed 50mg of pure drug dissolved in 50ml of solvent (solvent was used as your mobile phase only); this gives 1000ppm solution. The stock solution was further diluted to a sub-stock of 100 µg/ml. The 10 µg/ml solution was prepared by diluting 1 ml of sub-stock solution to 10 ml with the mobile phase.

Selection of analytical wavelength for measurement

After chromatographic development bands were scanned over the range of 200-400 nm. From the overlain spectra it was observed that the drug showed considerable absorbance at 210 nm. So, 210 nm was selected as the wavelength for measurement

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Fig. No. 2: Typical chromatogram of Blank

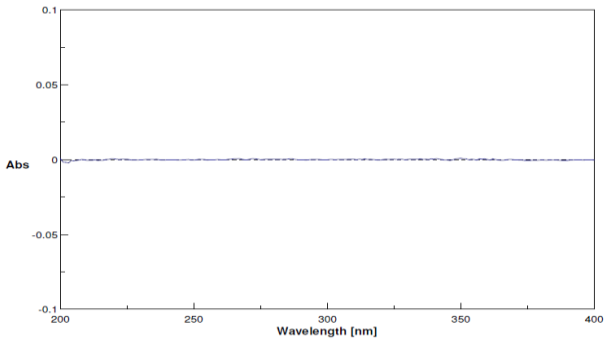
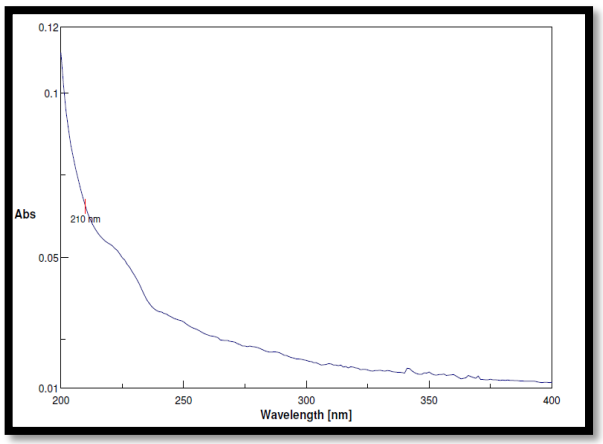


Fig. No. 3 UV spectrum of Pregabalin



Preparation of sample solution:

Computed the Maxgalin 50 mg capsule's average weight. The powder equivalent to 100 mg of pregabalin was weighed and then transferred to a 100 mL volumetric flask that had been cleaned and dried. Added 70 mm of water and sonicated for 15 minutes while shaking occasionally. Allow the solution to reach room temperature after 15 minutes, then add water to bring the volume up to the appropriate level
Using the mobile phase, further dilute 5 ml of the filtered stock solution to 10 ml. (500 ppm of Pregabalin),

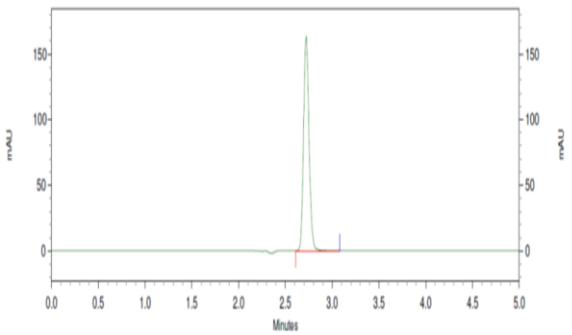
Optimization of the HPLC method

Table No.4. Optimized of the HPLC method

Parameter	**Description**
Mode	Isocratic
Column Name	Kromasil C18, 250 mm X 4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	210 nm
Column Oven temp	35 ºC
Mobile Phase	Acetonitrile: 0.1% TFAA (15:85 %V/V)
Flow Rate	1.0 ml/min
Run time	05 Minutes

Fig. No. 4: Standard chromatogram of Pregabalin

Chromatogram:



Method Validation

System suitability test

Table No. 5 Results for System Suitability Test of Pregabalin

Sr No.	**Standard solution**	**Area**	**Asymmetry**	**Theoretical plates**
1	Standard_1	5638240	1.17	5646
2	Standard_2	5652583	1.17	5636
3	Standard_3	5622140	1.17	5640
4	Standard_4	5635469	1.16	5653
5	Standard_5	5660317	1.17	5662
Mean		**5641750**	**1.17**	**5647**

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STD Dev	**14986.749**	
% RSD	**0.27**	

SPECIFICITY: Specificity is the ability to access unequivocally the analyte in the presence of components that may be expected to be present.

A blank, standard solution was prepared and injected to check peak purity.

Table No. 7 Specificity stability

Description	Observation
Blank	No interference at R.T. of Pregabalin due to blank
Placebo	No interference at R.T. of Pregabalin due to placebo
Standard solution	Peak purity was 0.991
Test Solution	Peak purity was 0.986

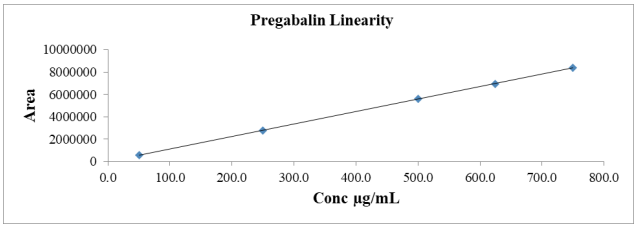
Linearity and Range

The linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range.

Table No.8 Linearity Data for Pregabalin

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	50.0	555605	555760	0.267
		554358		
		557317		
50%	250.0	2768024	2758301	0.305
		2753178		
		2753702		
100 %	500.0	5616048	5631111	0.301
		5649471		
		5627813		
125 %	625.0	6912006	6921538	0.206
		6937894		
		6914713		
150 %	750.0	8374072	8353277	0.225
		8348346		
		8337413		

Fig. No.9 Calibration curve of Pregabalin



Limit of detection and limit of quantification

The LOD and LOQ values were calculated using the formulas LOD = 3.3 σ/S and LOQ = 10 σ/S, respectively. In this method, σ represents the standard deviation of the responses, while S is the mean of the calibration curve slopes.

Table No.9 LOD and LOQ

Sr. No.	Drug	LOD	LOQ
1	Pregabalin	**11.572 µg/mL**	**35.068 µg/mL**

ACCURACY (RECOVERY):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyze samples to which known amounts of analyte have been added.

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Table No. 10 Result and statistical data of Accuracy of Pregabalin

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	2813485	249.35	250.50	99.54	**99.60**	**0.930**
	2784584	246.78	250.00	98.71		
	2847917	252.40	251.00	100.56		
100	5713045	506.32	500.50	101.16	**99.99**	**1.041**
	5594521	495.81	500.00	99.16		
	5628403	498.82	500.50	99.66		
150	8399456	744.40	751.00	99.12	**99.06**	**0.223**
	8368034	741.62	750.50	98.82		
	8404570	744.85	750.50	99.25		

PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. The precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on the Test sample.

Table No. 11 Result of Intra-day and Inter-Day Precision for Pregabalin

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	640.3	5498545	97.48
	Sample 2	640.6	5626103	99.69
	Sample 3	640.4	5660043	100.32
	Sample 4	640.1	5606710	99.43
	Sample 5	640.5	5507891	97.61
	Sample 6	640.2	5544384	98.30
	Mean			**98.81**
	STD DEV			**1.1754**
	% RSD			**1.190**
Intermediate precision (Inter-Day)	Sample 1	640.2	5619273	99.63
	Sample 2	640.7	5473794	96.98
	Sample 3	640.5	5567014	98.66
	Sample 4	640.3	5699145	101.03
	Sample 5	640.6	5529427	97.98
	Sample 6	640.2	5607340	99.42
	Mean			**98.95**
	STD DEV			**1.4082**
	% RSD			**1.423**
Repeatability Plus Inter-day	**Mean**			**98.878**
	STD DEV			**1.2390**
	% RSD			**1.253**

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ROBUSTNESS:

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

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Table No. 12 Result of Robustness Study of Pregabalin

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (213 NM)	2.69	4986302	1.18	5669
Wavelength by -3 NM (207 NM)	2.70	7195786	1.16	5592
Flow rate by +10% (1.1mL/min)	2.49	5143584	1.21	5456
Flow rate by -10% (0.9mL/min)	3.01	6287691	1.16	5712
Column oven temp by +2ºC (37 ºC)	2.74	5563614	1.20	5727
Column oven temp by -2ºC (33 ºC)	2.72	5547684	1.16	5681

CONCLUSION

The good point of this method is its simplicity, without any change in the analyte properties or increasing the phase of analysis the sample can be prepared in one step and can be used for routine QC analysis of pregabalin in its dosage form. The retention time is only 2.71 minutes, so a large number of samples can be run on the same day, economical, or cost-effective. The inter-day, intraday, and short-term stability study indicated that the validated method is good enough with the quality of reproducibility and repeatability. It was concluded that the modified method can be used confidently for the precise and accurate analysis of Pregabalin.

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