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Development and Validation of Stability indicating HPLC method for simultaneous estimation of Metformin and Ertugliflozin

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ertugliflozin and Metformin in Tablet dosage form. Chromatogram was run through phenomenex c18 150 x 4.6 mm, 5m. Mobile phase containing Buffer: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA (2.2ph) buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 220.0nm Retention time of Ertugliflozin and Metformin were found to be 2.365 min and 2.868 min. %RSD of the Ertugliflozin and Metformin were and found to be 0.6 and 0.6 respectively. %Recovery was obtained as 100.04% and 99.66% for Ertugliflozin and Metformin respectively. LOD, LOQ values obtained from regression equations of Ertugliflozin and Metformin were 0.34, 1.03 and 0.01, 0.03 respectively. Regression equation of Ertugliflozin is y= 94666x + 1023.and y = 66010x + 12210 of Metformin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Metformin, Ertugliflozin, Mobile phase, Retention time, RP-HPLC

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1. Introduction

Pregabalin is structurally similar to gamma-aminobutyric acid (GABA) - an inhibitory neurotransmitter. It may be used to manage neuropathic pain, postherpetic neuralgia, and fibromyalgia among other conditions.20 Although as per the FDA Label the mechanism of action has not been definitively defined, there is evidence that pregabalin exerts its effects by binding to the α2δ subunit of voltage-dependent calcium channels.2022 Pregabalin is marketed by Pfizer under the trade name Lyrica and Lyrica Cr (extended release).It may have dependence liability if

misused but the risk appears to be highest in patients with current or past substance use disorders.

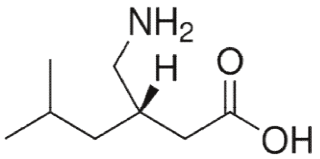

Fig 1: Chemical structure of Pregabalin

Literature review reveals that there is no analytical method reported for the analysis of Pregabalin by estimation by UV–Visible Spectrophotometer. Spectrophotometer and Spectroscopy are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new Spectrophotometer method development for the estimation of Pregabalin in pharmaceutical dosage form.

2. Methodology

Materials:

Pregabalin was a gift sample from Dr. Reddys Lab, Hyderabad. All chemicals (distilled water, methanol) and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

Apparatus:

A Labindia UV–visible spectrophotometer (UV-T60-India) was used for all absorbance measurements with matched quartz cells.

Method Development

Preparation of standard stock solution:

Accurately weighed 10 mg of Pregabalin was transferred to a 100 ml volumetric flask, dissolved in 20 ml distilled water by shaking manually for 10 min. The volume was adjusted with the same up to the mark to give the final strength, i.e. 100 µg/ml.

Selection of wavelength for analysis of Pregabalin:

Appropriate volume 0.5 ml of standard stock solution of Pregabalin was transferred into a 10 ml volumetric flask, diluted to a mark with distilled water to give concentration of 5 µg/ml(and also 10, 15µg/ml). The resulting solution was scanned in the UV range (200–400 nm). In spectrum Pregabalin showed absorbance maximum at 288 nm

Validation of the method

The method was validated in terms of linearity, accuracy, precision, and ruggedness. The method was statically validated according to ICH guidelines $Q_2(R_1)$.

Linearity:

Different aliquots of Pregabalin in the range 0.5–3 ml were transferred into series of 10 ml volumetric flasks, and the volume was made up to the mark with distilled water to get concentrations 5, 10, 15, 20, 25, and 30 µg/ml, respectively. The solutions were scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 288 nm. The calibration plot was constructed as concentration vs. absorbance

Accuracy:

To the pre analyzed sample solutions, a known amount of standard stock solution was added at different levels, i.e. 50%, 100%, and 150%. The solutions were reanalyzed by the proposed method.

Precision: Precision of the method was studied as intraday and interday variations. Intraday precision was determined by analyzing the 10, 15 and 20 µg/ml of Pregabalin solutions for three times in the same day. Interday precision was determined by analyzing the 10, 15, and 20 µg/ml of Pregabalin solutions daily for 3 days over the period of week.

Sensitivity: The sensitivity of measurements of Pregabalin by the use of the proposed method was estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD). The LOQ and LOD were calculated using equation LOD = 3 × N/B and LOQ = 10 × N/B, where 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability:

Repeatability was determined by analyzing 20 µg/ml concentration of Pregabalin solution for six times.

Ruggedness:

Ruggedness of the proposed method is determined for 20 µg/ml concentration of Pregabalin by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions.

3. Results and Discussion

Selection of wavelength for analysis of Pregabalin: During the development phase, the use of ethanol as the diluent resulted in preferable outcome in UV analysis. The pre-determined wavelength of maximum absorption (λmax) was 288 nm.

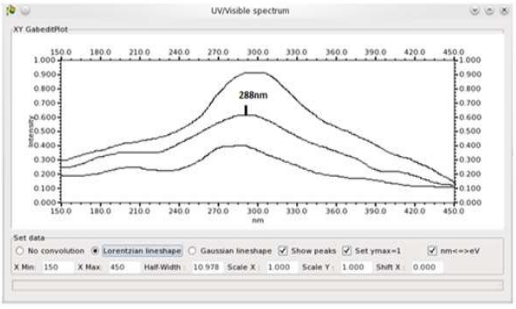

Fig 2: UV–Visible spectrum

Linearity: In this method pregabalin showed good linearity in the range of 5-30µg/ml. The correlation coefficient was found to be 0.9998. The linearity data are shown in table 2 and figure 3.

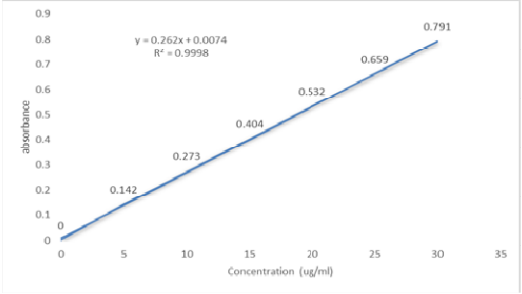

Fig 3: Calibration curve for Pregabalin

Accuracy:
The recovery studies with standard addition method at 50%, 100% and 150% levels of the test concentration showed good results with a percentage mean recovery was found be 99.65 %. The developed method was accurate. The results are shown in table 3.

Precision (Repeatability):
Both intra-day and inter-day precision was within the acceptable limit with% RSD less than 2%. So, the developed method was more precise and repeatable.

Sensitivity:
The linearity equation was found to be Y = 0.262X + 0.0074. The LOQ and LOD for Pregabalin were found to be 0.51 µg and 2.99 µg, respectively.

Ruggedness: The peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The result showed that the % RSD was less than 2%.

Table 1: Wave length selection

Stocks	Wavelentgth of stocks	Absorbance
5 µg/ml	288	0.356
10 µg/ml	288	0.594
15 µg/ml	288	0.912

Table 2: Results for Linearity

Concentration (ug/ml)	Absorbance(nm)
0	0
5	0.142
10	0.273
15	0.404
20	0.532
25	0.659
30	0.791

Table 3: Accuracy Results

%Concentration (at specification Level)N=3	Absorbance	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	0.4213	2.5	2.498	99.92	99.65
100%	0.6213	5.0	4.990	99.08	
150%	0.9199	10	9.995	99.95	

Table 4: Intra-day and inter-day precision determined for three different concentrations of Pregabalin (n=3)

Concentration (µg/mL)	Intra-day precision			Inter-day precision		
	Absorbance measured	RSD (%)	Average (%)	Absorbance measured	RSD (%)	Average (%)
10	0.4113	0.140	98.96	0.4110	0.240	98.96
15	0.6147	0.094	98.60	0.6153	0.094	98.70
20	0.9210	0.122	98.77	0.8213	0.070	98.81

Table 5: Results for Ruggedness

Analyst	Concentration (µg/mL)	Absorbance measured (Mean ± SD)	Amount Found (%)	RSD (%)
I	20	0.8116±0.0015	98.98	0.02
II	20	0.8214±0.0010	99.12	0.01

4. Conclusion
This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Pregabalin in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

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