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Stability Indicating Development and Validation for Simultaneous Estimation of Pregabalin and Epalrestat RP-HPLC method

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Pregabalin and Epalrestat in Tablet dosage form. Chromatogram was run through ODS (250mm 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrile and methanol in the ratio of 60:40 was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength for Pregabalin and Epalrestat was 240nm. Retention time of Pregabalin and Epalrestat were found to be 2.354 min and 3.031 min. %RSD of the Pregabalin and Epalrestat were and found to be 0.4 and 0.4 respectively. %Recovery obtained was 99.69% and 99.76% for Pregabalin and Epalrestat. LOD, LOQ values were obtained from regression equations of Pregabalin and Epalrestat were 0.54, 1.63ppm and 0.58ppm, 1.75ppm respectively. Regression equation of Pregabalin y = 9554x + 19918, and of Epalrestat is y = 13505x +60097. Retention times are decreased and run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in industries.

Keywords: Pregabalin, Epalrestat, RP-HPLC.

1. INTRODUCTION

The Pregabalin was approved in Europe in the year July 2004, for the treatment of peripheral neuropathic pain and partial suizers in patients with epilepsy. Approval by the US FDA for the management of neuropathic pain associated with diabetic peripheral neuropathy and post-herpetic neuralgia followed in December 2004.

Epalrestat is a novel aldose reductase inhibitor which has been proven to have beneficial effects in diabetic neuropathy in many controlled clinical trials. It inhibits sorbitol production and prevents further complications. This agent has no influence on blood glucose concentrations.

2. MATERIALS AND METHODS

2.1 Apparatus and Chromatographic Parameters

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Pregabalin and Epalrestat.

2.2 Drug Samples

Pregabalin: It is an anticonvulsant drug used for neuropathic pain, partial seizures. It was designed as a more potent successor to gabapentin. It is marketed by Pfizer under the trade name Lyrica. It is classified as a schedule V drug in the U.S.

Epalrestat: It is a carboxylic acid derivative and a non-competitive and reversible used to treat most common long-term complications in patients. It is the only Aldose Reductase Inhibitors (ARI) commercially available. It is easily absorbed into the neural tissue and inhibits the euzyne with minimum side effects.

2.3 Reagent and solutions

Epalrestat and Pregabalin pure drugs (API), combination Epalrestat & pregabalin tablets (SYNJARDY), Distilled water, Acetonitrile, phosphate

buufer, ortho-phosphoric acid, all of the above chemicals and solvents are from Rankem.

2.4 PREPARATION OF THE PREGABALIN & EPALRESTAT STANDARD & SAMPLE SOLUTION:

2.4.1 Standard Solution Preparation

Accurately weighed 15mg of Epalrestat, 7.5mg of Pregabalin and transferred to 10ml volumetric flask. 3/4th of diluents was added and solicited for 10 minutes. Flasks were made up with diluents and labeled as standard stock solutions 1 and 2 (1500ug/ml EPAL and 750 ug/ml PREGA). Then 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask is made up with diluent. (150ug/ml of EPAL and 75ug/ml of PREGA).

2.4.2 Sample Solution Preparation

5 tablets were weighed and average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (1500 ug/ml of EPAL & 750 ug/ml of PREGA). Then 1 ml of filtered above solutions was transferred to 10ml volumetric flask and made up with diluent. (150ug/ml of EPAL and 75ug/ml of PREGA).

3. RESULTS AND DISCUSSION

3.1 METHOD DEVELOPMENT

Five trials were performed for the method development and both were eluted with good peak shape, good tailing factor. The tailing factor was found to be the fifth peak with RT of:

Pregabalin	=	2.354 ± 0.3 min
Epalrestat	=	3.031± 0.3 Min

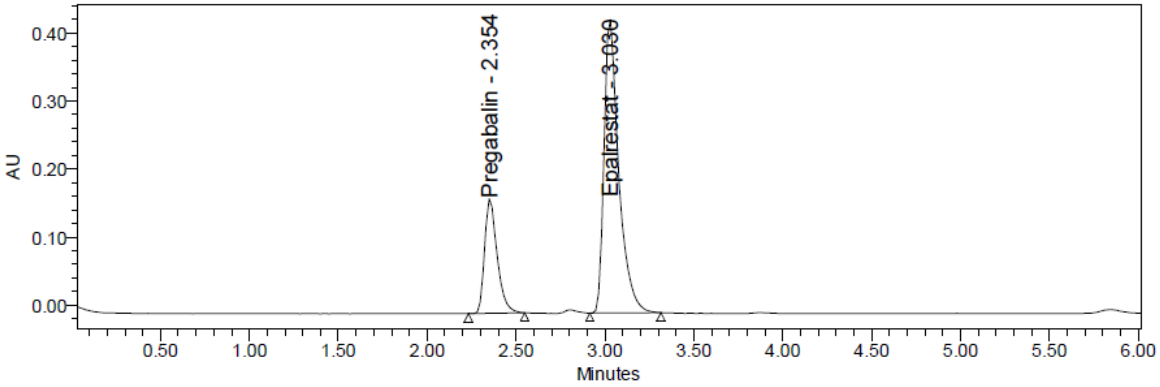


Figure 1. Chromatogram Peak of Pregabalin & Epalrestat

3.2 METHOD VALIDATION

3.2.1 Precision:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

3.2.2 Acceptance Criteria

The % RSD for the area of standard inactions results for pregabalin and Epalrestat were found to be 0.4% and 0.4% respectively.

Table 1: Precision results for Pregabalin and Epalrestat

S. No.	Pregabalin	Epalrestat
1	730819	2055480
2	738930	2049135
3	732302	2063977
4	737870	2054349
5	736914	2061002
6	733680	2073843
Mean	735086	2059631
Std. Dev.	3280.3	8695.3
%RSD	0.4	0.4

3.2.3 Accuracy:

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner Amount added for Pregabalin and Epalrestat and the values.

3.2.4 Acceptance Criteria

The % Recovery for each level should be between 99.0% to 100.0%.

Table 2: Recovery data

Sample	% Concentration at	Amount added (µg/ml)	Recovery (%)	% RSD
Pregabalin	50%	37.5	99.40	0.93
	100%	75	99.98	1.5
	150%	112.5	99.68	0.35
Epalrestat	50%	75	99.06	0.85
	100%	150	100.43	0.80
	150%	225	99.79	0.36

3.3 Recovery Studies

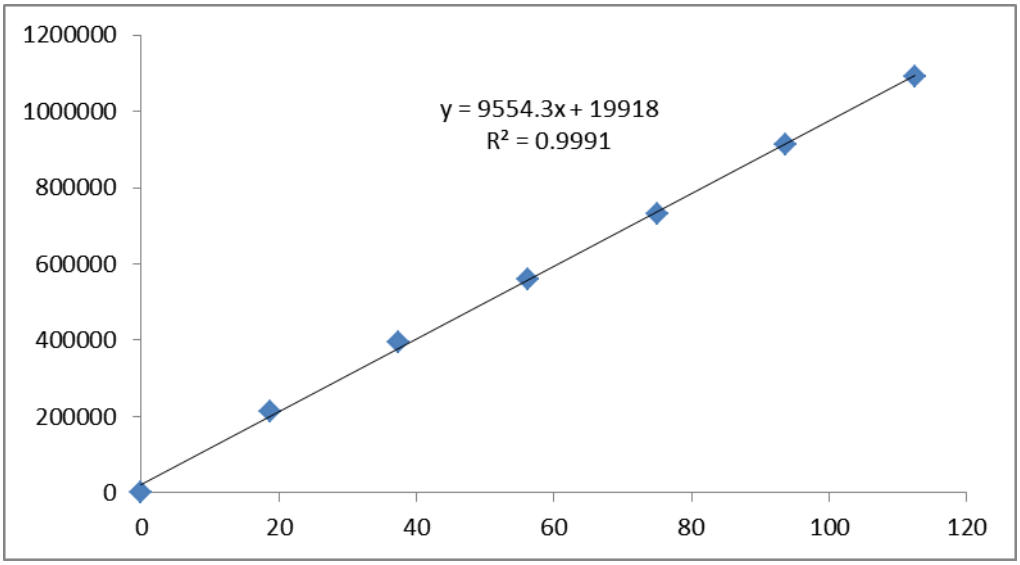
To determine the accuracy and precision of the proposed method recovery studies were carried out. A fixed amount of sample was taken and standard drug was added at 50%, 100% and 150% levels. The results were analyzed and the results were within the limits. The % recovery and % Relative standard deviation value for Pregabalin found to be 99.40, 99.98, 99.68 and 0.93, 1.5, 0.35 respectively and for Epalrestat 99.06, 100.43, 99.79 and 0.85, 0.80, 0.36 respectively.

3.4 Linearity and Calibration Curve

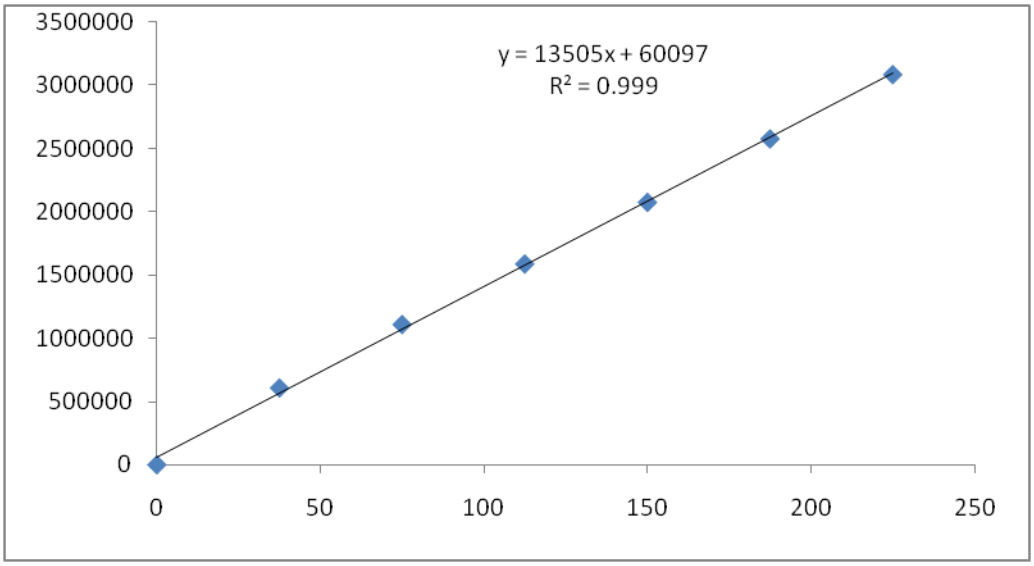
Seven linear concentrations of Pregabalin (18.75 – 112.5 ppm) and Epalrestat (37.5ppm to 225pm) are prepared and injected Regression equation of the Pregabalin and Epalrestat are found to be 1 y=9554x = 19918 and y= 13505x 60097 and the regression coefficient was 0.999.

Table 3: Different concentration values of Pregabalin and Epalrestat

S. No	Concentration Pregabalin (µg/ml)	Response	Concentration Epalrestat (µg/ml)	Response
1	0	0	0	0
2	18.75	211974	37.5	608042
3	37.5	393475	75	1110567
4	56.25	558183	112.5	1589541
5	75	732785	150	2077684
6	93.75	914411	187.5	2580420
7	112.5	1090611	225	3089338



Calibration curve of Pregabalin



Calibration curve of Epalrestat

3.5 Limit of Detection and Limit of Quantification

Limit of Detection (LOD) is the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio. The LOD will not only depend on the procedure of analysis but also on the type of instrument. In chromatography, detection limit is the injected amount that results in a peak with a height at least twice or thrice as high as baseline noise level. The LOD for Pregabalin and Epalrestat was found to be 0.54 and 0.58 respectively.

Limit of Quantification (LOQ) is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio. In chromatography, limit of quantification is the injected amount that results in a peak with a height, ten times as high as base line noise level. The LOQ for Pregabalin and Epalrestat was found to be 1.63 and 1.75 respectively.

3.6 Robustness

Robustness is determined by making deliberate changes in the chromatographic conditions like change in flow rate, mobile phase composition and temperature and evaluated for the impact on the method. It was observed from the chromatograms that the results were within the limits. This indicates that the method developed is robust.

Table 4: Acceptance criteria values of Pregabalin and Epalrestat

S. No.	Parameter	Acceptance Criteria	
		Pregabalin	Epalrestat
1	Accuracy	99.0% – 100.0%	99.0% - 101.0%
2	Precision (%RSD)	0.4%	0.4%
3	% Recovery	99.69	99.76
4	LOD (ug/ml)	0.54	0.58
5	LOQ (ug/ml)	1.63	1.75

4. CONCLUSION

The proposed study describes new and simple RP-HPLC method for the estimation of Pregabalin and Epalrestat. The method validated was found to be simple, accurate and precise. Therefore the proposed study method can be used for quantification of Pregabalin and Epalrestat in bulk and pharmaceutical dosage form.

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6. REFERENCES

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