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Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Epalrestat and Pregabalin in Bulk and Tablet Dosage Form

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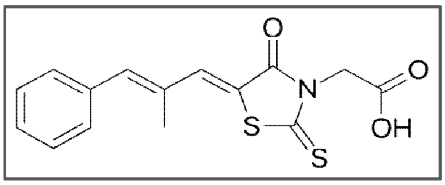
ABSTRACT

The purpose of the investigation was to develop a new RP-HPLC method for simultaneous estimation of Epalrestat at and Pregabalin in pharmaceutical dosage forms. Chromatography was carried out on Kromasil 250 C-18 column (4.6 x 250mm, 5µ particle size) with a isocratic mobile phase composed of ortho phosphoric acid buffer, Acetonitrile, 47:53v v^{-1}) at a flow rate of 1 mL min^{-1}. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 210 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. The retention times for Epalrestat and Pregabalin and were 2.575 min and 3.406 min respectively. The percentage recoveries of Epalrestat and Pregabalin were 99.29 % and 100.34 % respectively. The relative standard deviation for assay of tablets was found to be less than 2 %. The method was fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and pharmaceutical industries.

Graphical Abstract



Chemical Structure of Epalrestat

Keywords: Epalrestat, Pregabalin, High performance liquid chromatography, Acetonitrile, Ortho-phosphoric acid, Terbutaline.

INTRODUCTION

Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can, over time, develop nerve damage throughout the body. A total of fifty patients having diabetic neuropathic pain are recruited based on the inclusion and exclusion criteria. There is a higher prevalence of DM with 4.3% in india [1] and and 1-2% in the west [2]. Asian Indians are response more prone towards insulin resistance and cardiovascular mortality [3]. 19.1% of south indian peoples were type II diabetic patients with peripheral neuropathi [4]. Diabetic autonomic neuropathy accounts concluded that 25% to 50% patients over a period of 5-10 years were leading to death due to silent myocardial infarction [5, 6].

At first visit, patients are randomly assigned to one of the two treatment groups either Epalrestat or Pregabalin. Statistical analysis using student unpaired t-test. The scales used are Dallas pain questionnaire scale, Pain drawing scale, Lower extremity function scale, Biothesiometry score and plasma glucose post prandial. In the Dallas pain questionnaire scale, Pain drawing scale, Lower extremity function right and left toe scale in visit II and visit III are is $P>0.05$ significant value. In the Biothesiometry score right and left toe scale the 'p' value is is 0.0445 and it is <0.05, it has found that the reduction of PGPP between two treatment groups during visit II and III is statistically differs.

The study concludes that there was rapid reduction of pain Pregabalin therapy when compared to Epalrestat therapy. The above information indicates that the efficacy observed for diabetic peripheral neuropathic pain relief, was more with Pregaballin therapy at a dose of 150 mg daily.

MATERIALS AND METHODS

Chemicals and Reagents: The reference samples of Epalrestat and Pregabalin were provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade acetonitrile, HPLC grade methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial tablets (Pre Aldonil: dose Eplarestat 150 mg, Pregabalin 75 mg) were purchased from the local pharmacy.

Instrument and chromatographic conditions: RP-HPLC waters 2695 separation module equipped with 2996 Photodiode Array Detector was employed in this method**.** The Empower 2 software was used for LC peak integration along with data acquisition and data processing. The column used for separation of analytes is Hypersil Kromasil 250 C18, (250 x 4.6 mm, 5µ). Mobile phase consisting of ortho phosphoric acid Buffer: Acetonitrile in the ration of 47:53 % v/v at a flow rate of 1.0 ml min^{-1}. It was filtered through 0.45µm nylon filter and sonicated for 5 min in ultrasonic bath. Samples were analysed at 210 nm at an injection volume of 10 µL.

Preparation of ortho phosphoric acid: 1 mL of ortho phosphoric acid was taken in a 1000 mL of Volumetric flask add about 900 mL of milli-Q water added and degas to sonicate and finally make up the volume with water.

Preparation of Solutions

Eplarestat stock preparation (1500µg ml^{-1}): Accurately weighed and transferred 15 mg of Epalrestatin to 10 mL of clean dry volumetric flask, add 7mL of diluent (water : ACN50:50), then sonicated for 10 min and make up the volume with diluent.

Pregabalin stock preparation (750µg mL^{-1}): Accurately weighed 7.5mg of Pregaballin and transferred into 10 mL of clean dry volumetric flask, add 7mL of diluent (water : ACN 50:50), then sonicated for 10 min and make up the final volume with diluent.

Standard Preparations

Eplarestat Standard Preparation (150µg mL^{-1}): From the above Epalrestat stock solution 1ml was pipette out into 10 mL of clean dry volumetric flask and make up the final volume with diluent as shown in figure 1.

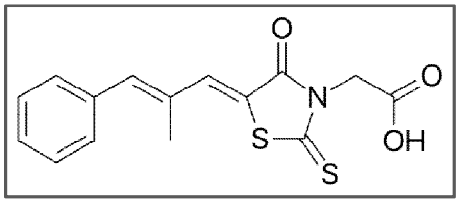


Figure 1. Chemical structure of Epalrestat

Pregabalin standard Preparation (75µg mL^{-1}): From the above Pregaballin stock solution 1ml was pipette out into a 10ml clean dry volumetric flask and make up the final volume with diluent as shown in figure 2.

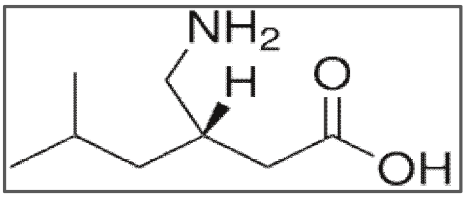


Figure 2. Chemical structure of Pregabalin

Method validation: The validation of the method was carried out as per ICH Guidelines. The parameters assessed were specificity, linearity, precision, accuracy, stability, LOD and LOQ.

Specificity: Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products and related substances.

Accuracy: The accuracy was determined by calculating % recoveries of Epalrestat and Pregabalin. It was carried out by adding known amounts of each analyte corresponding to three concentration levels (50, 100, and 150%) of the labelled claim to the excipients. At each level, six determinations were performed and the accuracy results were expressed as percent analyte recovered by the proposed method.

Precision: Precision of an analytical method is usually expressed as the standard deviation. The repeatability studies were carried out by estimating response of Epalrestat and Pregabalin six times. The intra-day and inter-day precision studies (intermediate precision) were carried out by estimating the corresponding responses three times on the same day and on three different days for three different concentrations and the results are reported in terms of relative standard deviation.

Linearity: The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. The linear regression data for the calibration plot is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance.

Robustness: Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no affect on the peak tailing, peak area and theoretical plates and finally the method was found to be robust.

Limit of Detection and Limit of Quantitation: The LOD can be defined as the smallest level of analyte that gives a measurable response and LOQ was determined as the lowest amount of analyte that was reproducibly quantified. These two parameters were calculated using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations,

$$LOD=3.3 \times SD/slope \text{ and } LOQ=10 \times SD/slope,$$

Where, s = standard deviation, S= slope of the calibration curve.

Assay of Epalrestat and Pregabalin in Tablet: Assay of marketed product was carried out by using the developed method. Sample solutions were prepared and injected into RP-HPLC system. The sample solution was scanned at 210 nm. The % drug estimated was found to be 99.29 for Epalrestat and 100.34 for Pregaballin in figure 3. The chromatogram showed two single peaks of Epalrestat and Pregabalin was observed with retention times of 2.573 and 3.397 min.

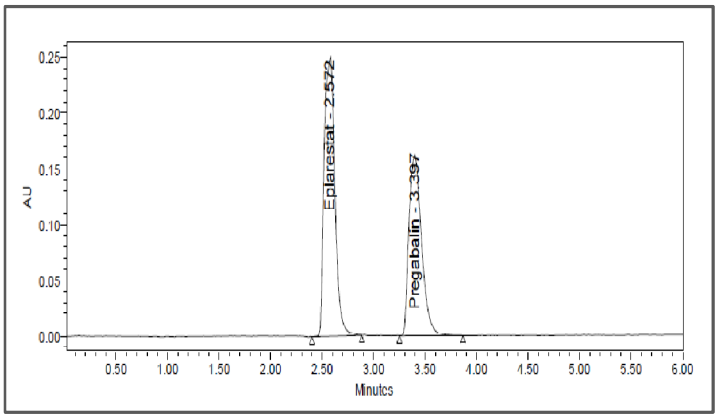


Figure 3. A Typical Chromatogram of Epalrestat and Pregabalin in tablet dosage form

Forced Degradation Studies: Stress studies had performed according to the ICH guidelines, under conditions of hydrolysis (acidic and alkaline), photolysis, oxidation, and thermal studies.

Oxidation: To 1 mL of stock solution of Epalrestat and Pregabalin, 1 mL of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 150 µg mL^{-1} and 75µg mL^{-1} solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 mL of stock solution Epalrestat and Pregabalin, 1 mL of 2N Hydrochloric acid was added and refluxed for 30 min at 60°C .The resultant solution was diluted to obtain 150µg/ml & 75µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 mL of stock solution Epalrestat and Pregabalin, 1 mL of 2N sodium hydroxide was added and refluxed for 30 min at 60°C. The resultant solution was diluted to obtain 150 µg mL^{-1} and 75 µg mL^{-1} solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 150 µg mL^{-1} and 75 µg mL^{-1} solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability Studies: The photochemical stability of the drug was also studied by exposing the 1500µg mL^{-1} and 750 µg ml^{-1} solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt h m^{2-1} in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 150µg mL and 75µg mL^{-1} solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

Optimized Chromatographic conditions: To establish and validate an efficient method for analysis of these drugs in pharmaceutical formulations, preliminary tests were performed. Different chromatographic conditions were employed for the analysis of the Epalrestat and Pregabalin in both bulk and pharmaceutical dosage form. Finally the analysis was performed by using ortho phosphoric acid Buffer: Acetonitrile in the ration of 47:53 % v v^{-1} at a flow rate 1.0 mL min^{-1}. Samples were analysed at 210 nm at an injection volume of 10 µL and separation was carried by using Kromasil 250C18, (250 x 4.6 mm, 5µ) column. The proposed method was optimized to give a sharp peak with minimum tailing for Epalrestat and Pregabalin (Fig. 4). The optimized conditions were given in table 1.

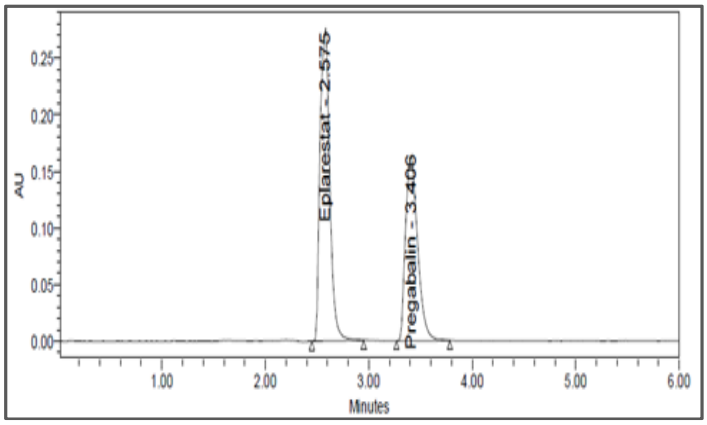


Figure 4. Standard Chromatogram of and Pregabalin

Table 1. Optimized Chromatographic conditions

Parameter	Conditions
Mobile phase	Ortho phosphoric acid Buffer Acetonitrile 47:53% V V^{-1} pH adjusted to 4.8
Column	Kromasil 250 C18, 250x4.6mm, 5µ
Wavelength	210 nm
Flow rate	1.0 mL min^{-1}
Injection volume	10 µL
Run time	6 min
Diluent	Water :Acetonitrile (50:50)

Forced degradation studies were performed to establish the stability indicating property and specificity of the proposed method. Degradation studies were carried out under conditions of hydrolysis, dry heat, oxidation, UV light and photolysis and the drug substances were degraded in all conditions.

Acid and base hydrolysis was performed by exposing the drug substances with 2N HCl and 2N NaOH at 60°C for 30min and it was showed degradation of Epalrestat and Pregabalin with degraded products peak at retention time 2.572 min and 3.397 min respectively. Degradation studies under oxidative conditions were performed by heating the drug sample with 20% H_2O_2 at 60°C and

degraded product peaks were observed. Both Epalrestat and Pregabalin are sensitive to acid and alkali and there was no degradation occurs under UV light and thermal conditions. The results of forced degradation studies were given in table 2.

Table 2. Results of Forced Degradation Studies

S. No.	Injection	Epalrestat		Pregabalin	
		% Assay	% Degradation	% Assay	% Degradation
1.	Acid Degradation	98.0	2.0	96.0	40.
2.	Base Degradation	97.8	2.2	96.09	3.91
3.	Peroxide	98.4	1.6	98.13	1.87
4.	Thermal Degradation	99.0	1.0	99.31	0.69
5.	UV Degradation	98.4	1.6	99.41	0.59
6.	Neutral Degradation	98.9	1.1	99.80	0.2

Precision was evaluated by a known concentration of Epalrestat and Pregabalin was injected six times and corresponding peaks were recorded and % RSD was calculated and found within the limits. The low % RSD value was indicated that the method was precise and reproducible and the results were shown in the table (Table 3).

Table 3. Precision method of proposed RP-HPLC method

Drug	Mean Area	%RSD
Epalrestat	1774612	0.9
Pregabalin	131803	0.

Accuracy of the method was proved by performing recovery studies on the commercial formulation at 50, 100 and 150% level. % Recoveries of Epalrestat and Pregabalin ranges from 99.29% and 100.34%in simultaneous equation method and the results were shown in table 4. Linearity was established by analyzing different concentrations of Epalrestat and Pregabalin respectively.

The calibration curve was plotted with the area obtained versus concentration of both Epalrestat and Pregabalin (Fig. 5 and 6).

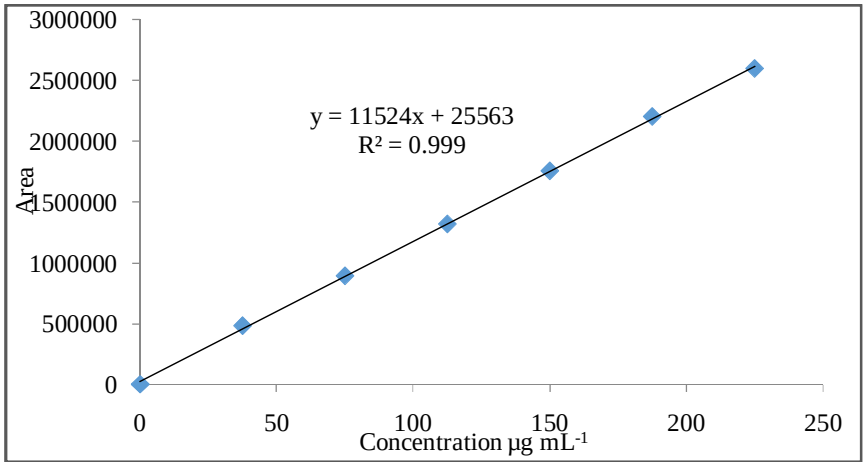


Figure 5. Linearity curve of Epalrestat

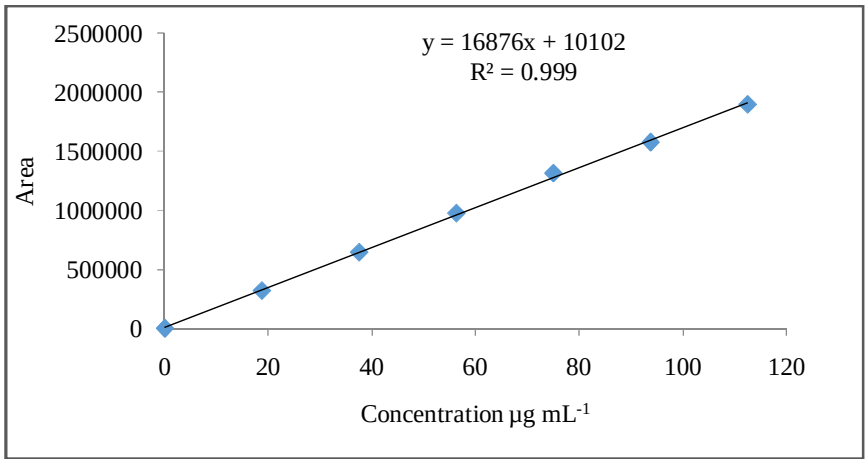


Figure 6. Linearity curve of Pregabalin

Table 4. % Recovery Data for Epalrestat and Pregabalin

Drug	Spiked Level %	% Recovery	% RSD
Epalrestat	75	100.15	0.69
	150	99.87	1.45
	225	99.80	0.59
Pregabalin	31.5	99.05	1.1
	75	100.10	0.80
	112.5	99.84	0.36

In the present study six concentrations were chosen ranging between 37.5-225 µg mL^{-1} of Epalrestat and 18.75-112.5 µg mL^{-1} of Pregabalin.

The regression equation and correlation coefficient for Epalrestat and Pregabalin was found to be y = 11524.x + 25563, R2=0.9996 and y = 16876.x + 10102, R2=0.9992 respectively and results were given in table 5.

Table 5. Results of Linearity

S. No.	Epalrestat		Pregabalin	
	Conc. (µg mL^{-1})	Peak Area	Conc. (µg mL^{-1})	Peak Area
1	37.5	483591	18.75	318611
2	75	891482	37.5	643861
3	112.5	1321019	56.25	973773
4	150	1756366	75	1311872
5	187.5	2203029	93.75	1573759
6	225	2598978	112.5	1893947

Robustness of the method is the ability of the method to remain unaffected by small deliberate changes in parameters like flow rate, mobile phase composition and column temperature. To study the effect of flow rate of the mobile phase it was changed to 0.1 units from 1.0 mL to 0.9 mL and 1.1 mL.

The effect of column temperature also checked by changing temperature to ± 5°C, this deliberate change in the above parameters has no significant effect on chromatographic behaviour of the samples and results were given in table 6.

Table 6. Robustness Data

Parameters	Changed Condition	Mean Peak Area		UPS plate count	
		Epalrestat	Pregabalin	Epalrestat	Pregabalin
Flow rate(mL min^{-1})	0.9 mL	1851653	1399910	3485	3506
	1.0 mL	1783756	1310964	3704	3952
	1.1 mL	1787560	1334975	3560	3824
Temperature (±5°C)	25°C	3531926	2678483	2664	2684
	30°C	1777871	1308442	3704	3924
	35°C	1775864	1342719	3357	3750
Mobile phase (±5%0	42:58 %v v^{-1}	1776588	1310776	3964	3456
	47:53 %v v^{-1}	1768821	1324218	3847	3924
	52:48 %v v^{-1}	1800500	1324939	3498	3923

LOD and LOQ of Epalrestat and Pregabalin were evaluated based on relative standard deviation of the response and slope of the calibration curve. The detection limits were found to be 1.49 µg mL^{-1} and 0.01µg mL^{-1} for Epalrestat and Pregabalin respectively. The quantitation limits were found to be 4.50 µg mL^{-1} and 0.05 µg mL^{-1} for Epalrestat and Pregabalin respectively. The results were given in the table 7.

Table 7. Results of LOD and LOQ

Drug	LOD (µg mL^{-1}	LoQ (µg mL^{-1}
Epalrestat	1.49	4.50
Pregabalin	0.01	0.05

APPLICATION

This method is useful sensitive and has the ability to separate the drug from degradation products and excipients found in the dosage form.

CONCLUSION

A new stability- indicating RP-HPLC method has been developed for estimation of Epalrestat and Pregabalin in bulk and pharmaceutical dosage form. The developed method was validated and it was found to be simple, sensitive, precise, robustness and it can be used for the routine analysis of Epalrestat and Pregabalin in both bulk and pharmaceutical dosage forms. The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study stability of Epalrestat and Pregabalin under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations.

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