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Research Article

Development and Validation of a Stability Indicating RP-HPLC-UV Method for the Simultaneous Determination of Epalrestat and Pregabalin in Combined Pharmaceutical Formulation

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ABSTRACT

An accurate, rapid, selective and specific stability indicating RP-HPLC-UV method was developed for the simultaneous determination of Epalrestat and Pregabalin in combined pharmaceutical formulation. Chromatographic separation was achieved on Discovery ODS C18 column (250 × 4.6 mm, 5 µm) with UV detection at 241 nm. The mobile phase consisting of 0.1% Ortho Phosphoric Acid (OPA) and acetonitrile in a ratio of 50:50, v/v and at a flow rate of 1.0 mL/min. The method was linear over the concentration range for Epalrestat 37.5-225 µg/mL and for Pregabalin 18.75-112.5 µg/mL. The retention times for Epalrestat and Pregabalin were found to be 2.166 min and 3.020 min respectively. The mean percentage recoveries of Epalrestat and Pregabalin were found to be 100.32% and 100.29% respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Epalrestat and Pregabalin in combined tablet dosage form.

INTRODUCTION

Epalrestat (Figure 1), chemically 2-[(5Z)-5-[(E)-2-methyl-3-phenylprop-2-enylidene]-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl]acetic acid is an aldose reductase inhibitor used for the treatment of diabetic neuropathy [1,2]. It reduces the accumulation of intracellular sorbitol which is believed to be the cause of diabetic neuropathy [3].

Pregabalin (Figure 2), chemically (3S)-3-(aminomethyl)-5-methylhexanoic acid is an anticonvulsant drug used for neuropathic pain, as an adjunct therapy for partial seizures and in generalized anxiety disorder [4,5]. It binds to the alpha2-delta subunit of the voltage-gated calcium channel in the central nervous system [6].

In literature review there are a few analytical methods were reported for estimation of Epalrestat and Pregabalin alone or in combination with other drugs in pharmaceutical dosage forms. But only few methods are available for the simultaneous estimation of Eparlestat and Pregabalin by using RP-HPLC [7-12]. The main objective of the present work describes a simple, rapid, precise and accurate reversed phase stability indicating HPLC method for the simultaneous determination of Epalrestat and Pregabalin in combined pharmaceutical dosage forms as per ICH guidelines [13,14].

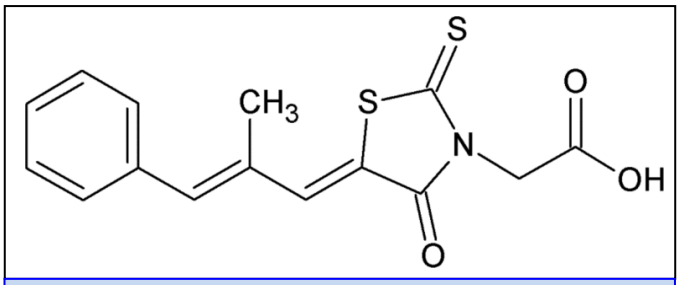

Figure 1: Structure of Eparlestat.

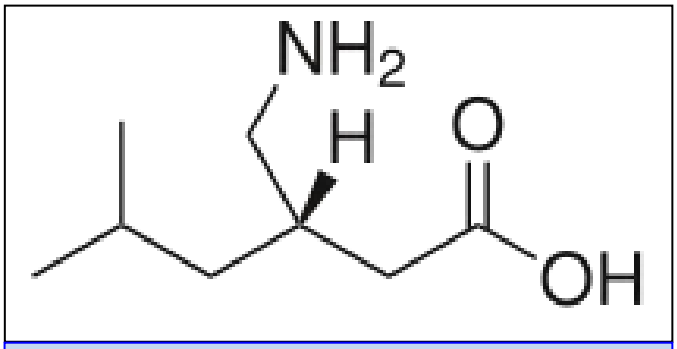

Figure 2: Structure of Pregabalin.

MATERIALS AND METHODS

Materials

Epalrestat and Pregabalin pure drugs (API) were obtained from Spectrum Pharma Research solutions, Hyderabad, India. Combination of Epalrestat and Pregabalin tablets were procured from local pharmacy store. HPLC grade Acetonitrile, ortho phosphoric acid and water were obtained from Rankem chemicals Ltd., Mumbai, India.

Instrumentation

The analysis of drugs was carried out on a Waters HPLC 2965 system on Discovery C18 column (250 × 4.6 mm, 5 µm). The instrument is equipped with a 2695 pump with inbuilt degasser, 2996 PDA detector and auto injector with 20 µL sample loop. A 20 µL Hamilton syringe was used for injecting the samples. Data was analysed by using Waters Empower 2 software. A double-beam PG Instruments T60 UV-Visible spectrophotometer with special bandwidth of 2 mm and 10 mm and matched quartz was be used for measuring absorbance for Epalrestat and Pregabalin solutions. Degassing of the mobile phase was done by using an ultrasonic bath sonicator.

Mobile phase

A mobile phase consisting of mixture of 0.1% OPA and acetonitrile in the ratio of 50:50, v/v was prepared.

Diluent

Based up on the solubility of the drugs, diluent was selected, acetonitrile and water taken in the ratio of 50:50, v/v.

Preparation of standard stock solution

Accurately weighed and transferred 15 mg and 7.5 mg of Epalrestat and Pregabalin working standards into 10 mL clean dry volumetric flask. Add 3/4th volume of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above each stock solution, 1 mL was pipetted out in to a 10 mL volumetric flask and then make up to the final volume with diluent. (150 µg/mL Epalrestat and 75 µg/mL Pregabalin)

Preparation of standard working solution

20 tablets were weighed and calculate the average weight of each tablet then the tablet powder weight equivalent to 150 mg of Epalrestat and 7.5 mg of Pregabalin was transferred into a 10 mL volumetric flask, 70 mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered 0.25µm membrane filter. From the filtered solution 1 mL was pippeted out into a 10 mL volumetric flask and made upto 10 mL with diluent.

RESULTS

Method development

Various trails were performed by using different mobile phases and based on peak parameters the chromatographic conditions (Table 1) were optimized and optimize chromatogram was shown in (Figure 3).

Table 1: Optimized chromatographic conditions.

Mobile phase	0.1% OPA:acetonitrile, 50:50 (v/v)
Flow rate	1 mL/min
Column	Discovery C18 (250 x 4.6 mm, 5 µm)
Detector wave length	241 nm
Column temperature	25°C
Injection volume	10 µL
Run time	8 min
Diluent	Water:acetonitrile, 50:50 (v/v)
Retention time	Epalrestat: 2.166 min; Pregabalin: 3.020 min
Theoretical plates	Epalrestat: 6158; Pregabalin:8839
Tailing factor	Epalrestat: 1.3; Pregabalin: 1.3
Resolution	Epalrestat: 0; Pregabalin: 6.8

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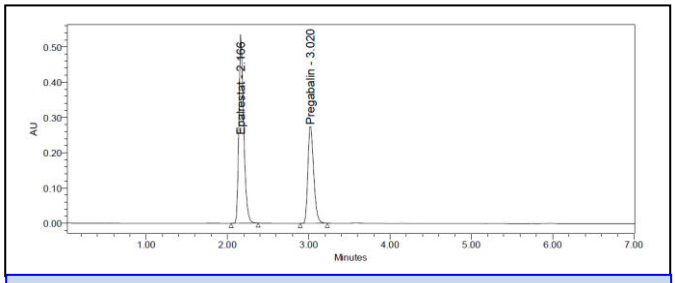

Figure 3: Optimized Chromatogram of Epalrestat and

Method validation

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Epalrestat (150 µg/mL) and Pregabalin (75 µg/mL) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The system suitability parameters like tailing factor was observed below 2, high theoretical plate counts were observed which satisfies limits of ICH guidelines.

Specificity: Specificity is the parameter used to check the interference in the optimized method. We should not find interfering peaks in blank, placebo, standard and sample at retention times of these drugs in this method. So this method was said to be specific.

Table 2: Linearity results for Eparlestat and Pregabalin.

S. No.	Concentration Epalrestat (µg/mL)	Response	Concentration Pregabalin (µg/mL)	Response
1	0	0	0	0
2	37.5	447896	18.75	276074
3	75	864179	37.5	541134
4	112.5	1291524	56.25	807510
5	150	1711870	75	1069021
6	187.5	2112093	93.75	1337480
7	225	2557000	112.5	1586803
Slope		11283	Slope	14116
Intercept		14178	Intercept	8527
Correlation Coefficient		0.999	Correlation Coefficient	0.999

Linearity: Linearity solutions are prepared such that 0.25 mL, 0.5 mL, 0.75 mL, 1 mL, 1.25 mL, 1.5 mL from the stock solutions Epalrestat and Pregabalin are taken in to 6 different volumetric flasks and diluted to 10 mL with diluents to get 37.5 µg/mL, 75 µg/mL, 112.5 µg/mL, 150 µg/mL, 187.5 µg/mL, 225 µg/mL of Epalrestat and 18.75 µg/mL, 37.5 µg/mL, 56.25 µg/mL, 75 µg/mL, 93.75 µg/mL, 112.5 µg/mL of Pregabalin. Six linear concentrations of Epalrestat (37.5-225 µg/mL) and Pregabalin (18.75-112.5 µg/mL) are prepared and injected. The results were furnished in (Table 2) and calibration curves were shown in (Figure 4,5).

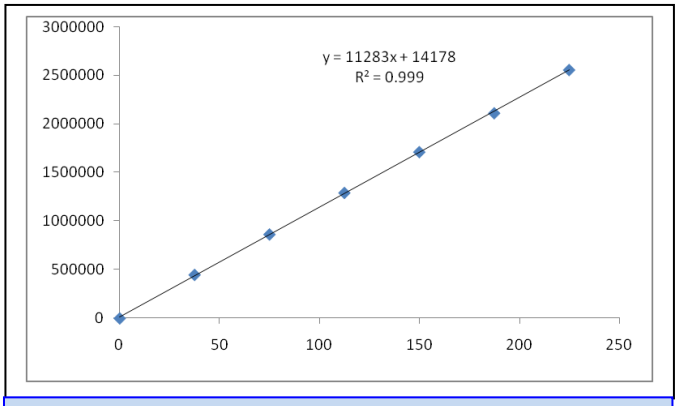

Figure 4: Calibration curve of Epalrestat.

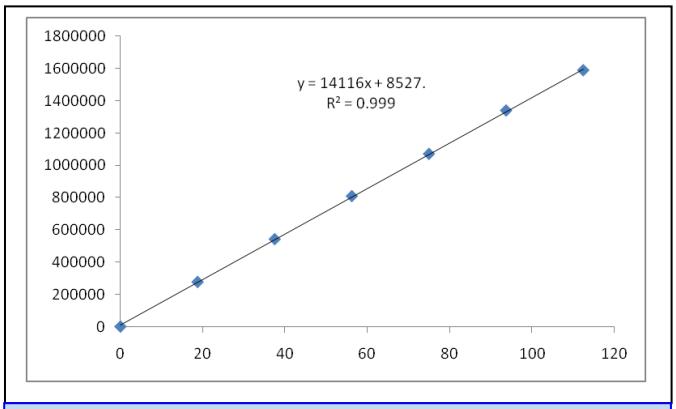

Figure 5: Calibration curve of Pregabalin.

Precision: Precision of method was studied by performing intra-day and inter-day precision. Intra-day precision (Table 3) and inter-day precision (Table 4) was studied by injecting the 6 replicates of standard solution in a single day and six days. Calculate the %RSD and it should not be more than 2.0.

Accuracy: The accuracy of the method was established by calculating percentage recovery of Epalrestat and Pregabalin by the method of addition. Known amount of Epalrestat and Pregabalin at 50%, 100% and 150% was added to a pre quantified sample solution. The recovery studies (Table 6,7) were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery at each level was not less than 99% and not more than 101%.

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Table 3: Intra-day precision results for Epalrestat and Pregabalin.

S. No.	Epalrestat	Pregabalin
1	2161531	1362804
2	2164164	1362804
3	2169594	1367678
4	2175663	1369414
5	2140324	1351145
6	2143278	1351145
Mean	2159092	1360832
Std. Dev.	14275.0	7949.5
%RSD	0.7	0.6

Table 4: Inter-day precision results for Epalrestat and Pregabalin.

S. No.	Epalrestat	Pregabalin
1	2165754	1354153
2	2171754	1354888
3	2166871	1361432
4	2166887	1353897
5	2174256	1357783
6	2171607	1352185
Mean	1785910	1355723
Std. Dev.	3461.5	3342.2
%RSD	0.2	0.2

Sensitivity: Limit of detection was calculated by standard deviation method. LOD for Epalrestat and Pregabalin were found to be 0.10 and 0.03 µg/mL respectively. Limit of Quantification was calculated by standard deviation method. LOQ for Epalrestat and Pregabalin were found to be 0.29 and 0.11 µg/mL respectively.

Degradation Studies

Acid degradation studies: To 1mL of stock solutions of Epalrestat & Pregabalin, 1mL of 2N Hydrochloric acid was added and refluxed for 30 min at 600C. The resultant solution was diluted to obtain 150 µg/mL & 75 µg/mL solution and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Table 5: Accuracy results of Epalrestat and Pregabalin.

Sample	Concentration (µg/mL)	%Recovery	Average
Epalrestat	75	100.04	100.04
	75	100.02	
	75	100.05	
	150	100.11	100.10
	150	100.08	
	150	100.13	
	225	100.85	100.85
	225	100.89	
	225	100.82	
Pregabalin	37.5	100.72	100.74
	37.5	100.74	
	37.5	100.76	
	75	100.57	100.57
	75	100.59	
	75	100.55	
	112.5	99.65	99.62
	112.5	99.61	
	112.5	99.62	

Alkali degradation studies: To 1 mL of stock solution Epalrestat & Pregabalin, 1 mL of 2N sodium hydroxide was added and refluxed for 30 min at 600C. The resultant solution was diluted to obtain 150 µg/mL & 75 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies: To 1 mL of stock solution of Epalrestat & Pregabalin, 1 mL of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 600C. For HPLC study, the resultant solution was diluted to obtain 150 µg/mL & 75 µg/mL solution 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 hrs to study dry heat degradation. For HPLC study, the resultant solution was diluted to 150 µg/mL & 75 µg/mL solution and 10 µL were injected

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Table 6: Degradation data of Epalrestat and Pregabalin.

Type of degradation	Epalrestat			Pregabalin		
	Area	%Recovered	%Degraded	Area	%Recovered	%Degraded
Acid	2082493	95.47	4.53	1320313	96.17	3.83
Alkali	2099544	96.25	3.75	1331583	96.99	3.01
Oxidative	2152960	98.70	1.30	1356655	98.82	1.18
Dry heat	2159544	99.00	1.00	1361626	99.18	0.82
UV	2169635	99.47	0.53	1367295	99.59	0.41
Neutral	2174170	99.68	0.32	1368924	99.71	0.29

Table 7: A summary table.

Validation Parameters	Results	
	Eparlestat	Pregabalin
Detection wavelength (nm)	241	241
Linearity(µg/mL)	37.5-225	18.75-112.5
Regression Equation	Y=11283x+14178	Y=14116x+8527
Correlation coefficent(R^2)	0.999	0.999
Retention time(min.)	2.166	3.020
Accuracy (%recovery)	100.32	100.29
Intra-day Precision (% RSD)	0.7	0.6
Inter-day Precision (% RSD)	0.2	0.2
Limit of Detection(µg/mL)	0.10	0.03
Limit of Quantitation (µg/mL)	0.29	0.11

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 1500 µg/mL & 750 µg/mL solution to UV Light by keeping the beaker in UV Chamber for 1 day or 200 Watt hours/m2 in photo stability chamber.. For HPLC study, the resultant solution was diluted to obtain 150 µg/mL & 75 µg/mL solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1 hr at a temperature of 600C. For HPLC study, the resultant solution was diluted to 150 µg/mL & 75 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Degradation studies results were computed in (Table 6).

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DISCUSSION

A stability indicating RP-HPLC method was developed and validated for the simultaneous determination of Epalrestat and Pregabalin by using mobile phase consisting of 0.1% orthophosphoric acid and acetonitrile in the ratio of 50:50, v/v. The retention times for Epalrestat and Pregabalin were found to be 2.166 min and 3.020 min respectively. The method was validated as per ICH guidelines. Linearity range was found to be 37.5-225 µg/mL for Epalrestat and 18.75-112.5 µg/mL for Pregabalin. The %RSD values for intra-day precision values of Epalrestat and Pregabalin were 0.7 and 0.6. The %RSD values for inter-day precision values of Epalrestat and Pregabalin were 0.2 and 0.2. The proposed method is precise. The mean percentage recoveries of Epalrestat and Pregabalin were found to be 100.32% and 100.29% respectively and the method is found to be accurate. Degradation studies are also carried out in acid, alkali, oxidative, dry heat, UV light and neutral stressed conditions. The results revealed that both the drugs are stable in described conditions. Thus it is evident that the described method can be adopted for routine estimation of Epalrestat and Pregabalin in combined pharmaceutical dosage form. The results were summarized in (Table 7).

CONCLUSION

The present method was proposed for the simultaneous determination of the Epalrestat and Pregabalin by using RP-HPLC in tablet dosage form is found to be simple, accurate, rapid and precise. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be applied in regular quality control tests in industries.

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