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Assay Method for Simultaneous Estimation of Epalrestat and Pregabalin in Pure and its Dosage form by RP-HPLC

N.Veena[*1], Dr.T.Rama Mohan Reddy[2], Dr.K.Abbulu[3]

[*1]*Department of Pharmaceutical Analysis, CMR College of Pharmacy, Medchal, Hyderabad-501401*
[2]*Associate Professor, department of Pharmaceutical Chemistry, CMR College of Pharmacy, Medchal, Hyderabad-501401*
[3]*Principal, Department of Pharmaceutics, CMR College of Pharmacy, Hyderabad, 501401.*

***Corresponding Author: N.Veena**
Email: ramamohanreddy40@gmail.com

ABSTRACT

In the proposed method, a new RP-HPLC method has been developed for simultaneous estimation of Epalrestat and Pregabalin in pure and its dosage form. The present method was a sensitive, precise, and accurate RP-HPLC method for analysis of Epalrestat and Pregabalin. To optimize the mobile phase, various combinations of buffer and organic solvents were used on Std BDS C18 column (4.6 x 150mm,5µm). Then the mobile phase containing a mixture 0.1% OPA: Acetonitrile (52:48) was selected at a flow rate of 1ml/min at a detector wavelength of 240 nm foretention time, baseline stability and minimum noise. The retention times of Epalrestat and pregabalin were found to be 2.930 min and 2.179 min respectively. The limit of detection and quantification of Epalrestat and Pregabalin were found to be 0.20 and 0.62; 0.18 and 0.56 respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in formulations didn't interfere with the estimation of drugs by the proposed HPLC method.

Keywords: Epalrestat, Pregabalin, RP-HPLC.

INTRODUCTION: EPALRESTAT

Epalrestat [**1-8]** is a carboxylic acid derivative and a noncompetitive and reversible used for the treatment of which is one of the most common long-term complications in patients. Chemically, Epalrestat is 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl) butan-1-one. Chemically, Epalrestat is unusual in that it is a drug that contains a group. Aldose reductase is the key enzyme in the polyol pathway whose enhanced activity is the basis of diabetic neuropathy. Aldose reductase inhibitors (ARI) target this enzyme. Out of the many ARIs developed, ranirestat and fidarestat are in the trial stage. Others have been discarded due to unacceptable adverse effects or

weak efficacy. Epalrestat is the only ARI commercially available. It is easily absorbed into the neural tissue and inhibits the enzyme with minimum side effects. The chemical structure of Epalrestat was given in fig 1.

Pregabalin

Pregabalin [9-17] is an anticonvulsant drug used for neuropathic pain, as an adjunct therapy for partial seizures, and in generalized anxiety disorder. It was designed as a more potent successor to gabapentin. Chemically it is (3S)-3-(aminomethyl)-5-methylhexanoic acid. Pregabalin is marketed by Pfizer under the trade name Lyrica. It is considered to have a dependence liability if misused and is classified as a Schedule V drug in the U.S. The chemical structure of Pregabalin was given in fig 2.The review of literature revealed that several analytical methods have been reported for Epalrestat and Pregabalin **[**18-21] in Spectrophotometry, HPLC, HPTLC and LC/MS individually and in combination. To date, there have been no published reports about the stability indicating studies and simultaneous estimation of Epalrestat and Pregabalin by HPLC in bulk drug and in tablet dosage forms. This present study reports for the first time stability indicating simultaneous estimation of Epalrestat and Pregabalin by RP-HPLC in bulk drug and in tablet dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Epalrestat and Pregabalin were obtained as gift samples from Spectrum Pharma Research laboratory in Hyderabad and Tablets (Prealdonil, Zydus.) containing Epalrestat -150 mg and Pregabalin-75 mg were purchased from local market. Acetonitrile, Water were obtained from Merc, Mumbai and Potassium dihydrogen ortho phosphate, Triethylamine, Ortho phosphoric acid obtained from RANKEM, Mumbai. All solvents used in this work are HPLC grade.

Instrumentation

HPLC waters 2695 separation module equipped with Photo diode array detector was employed in this method. The Empower 2 software was used for peak integration along with data acquisition and data processing, UV-Visible spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Epalrestat and Pregabalin. Electronics Balance-Denver, p^h meter -BVK enterprises, India, Ultrasonicator-BVK enterprises

Preparation of solutions

Diluent: Based up on the solubility of the drugs, diluents was selected, Acetonitrile and Water taken the in the ratio of 50:50.

Preparation of Standard stock solutions

Accurately weighed 75 mg of Epalrestat, 37.5 mg of Pregabalin and transferred to individual 50ml volumetric flasks respectively, 30 ml of diluent was added and solicited for 10 minutes. Flasks were made up to volume with diluent and labeled as Standard stock solution 1and Standard stock solution 2.Pipetted out 1ml from each standard stock solution and was transferred into 10ml volumetric flask and made up to the volume with diluent.

Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluent was added and sonicated for 25 min, and made up the volume with diluent and filtered by HPLC filters. Transferred 1ml of filtered sample stock solution was into 10ml volumetric flask and made up the volume with diluent.

Preparation of buffer

% OPA Buffer: 1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water.

Chromatographic conditions

The column used for separation of analytes BDS C18 (4.6 x 150mm, 5µm). Mobile phase consisting of 0.1%OPA: Acetonitrile (52:48) at a flow rate of 1ml/min. Samples were analysed at a detector wavelength of 240nm at an injection volume of 10µL with a run time of 6 min. The proposed method of Epalrestat and Pregabalin was optimized to give sharp peak with good resolution, plate count and minimum tailing effect.

Table no. 1 Optimized Chromatographic conditions

Parameter	Condition
Mobile phase	OPA (0.1%) : Acetonitrile (52:48)
Flow rate	1 ml/min
Column	BDS C18 (4.6 x 150mm, 5µm)
Detector wave length	240nm
Column temperature	30°C
Injection volume	10mL
Run time	6 min
Diluent	Water : Acetonitrile (50:50)

Analytical 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. Fig no.3

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. See Table no.2

Precision

Precision of an analytical method is usually expressed as the standard deviation. The repeatability studies were carried out by estimating response of Epalrestat 150µg/ml and Pregabalin 75µg/ml 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 same concentrations and the results are reported in terms of relative standard deviation. See Table no.3

Linearity

Linearity test was performed by preparing six different concentrations range from 37.5-225 µg/ml of Epalrestat and 18.75-112.5 µg/ml of Pregabalin from the stock solution. See Table no.4

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 effect on the peak tailing, peak area and theoretical plates and finally the method was found to be robust. See Table no.5

System suitability

The system suitability variables were determined by preparing standard solutions of Epalrestat (150ppm) and Pregabalin (75ppm) and the solutions were injected six times and the variables like peak tailing, resolution and USP plate count were determined. See Table no.6

Limit of Detection and Limit of Quantification

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. See Table no.7.

LOD and LOQ were calculated by using equations,

$LOD = 3.3 \times s/S$ and

$LOQ = 10 \times s/S$,

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

Assay of Epalrestat and Pregabalin

Assay of marketed product PREALDONIL 75MG TABLET, bearing the label claim Epalrestat 150mg, Pregabalin 75mg. Assay was performed with the above formulation. Average % Assay for Epalrestat and Pregabalin gained was 99.73 and 99.32% respectively. See Table no.8

Forced Degradation studies

Stress studies are performed according to ICH guidelines under conditions of hydrolysis (acidic and alkaline), photolysis, oxidation, and thermal studies. See Table no.9

Oxidation

To 1 ml of stock arrangement of Epalrestat and Pregabalin, 1 ml of 10% hydrogen peroxide (H2O2) was included independently. The arrangements were placing for 30 min at 600c. For HPLC think about, the resultant arrangement was weakened to get 150µg/ml&75µg/ml arrangement and 10 µl were infused into the framework and the chromatograms were recorded to survey the steadiness of test.

Acid Degradation Studies

To 1 ml of stock s solution Epalrestat and Pregabalin, 1ml of 1N Hydrochloric corrosive was included and refluxed for 30mins at 600c .The resultant arrangement was weakened to acquire 150µg/ml&75µg/ml arrangement and 10 µl arrangements were infused into the framework and the chromatograms were recorded to survey the steadiness of test.

Alkali Degradation Studies

To 1 ml of stock arrangement Epalrestat and Pregabalin, 1 ml of 1N sodium hydroxide was included and refluxed for 30mins at 600c. The resultant arrangement was weakened to acquire 150µg/ml&75µg/ml arrangement and 10 µl were infused into the framework and the chromatograms were recorded to evaluate the dependability of test.

Dry Heat Degradation Studies

The standard medication arrangement was set in broiler at 105°C for 6 h to contemplate dry warmth corruption. For HPLC examine, the resultant arrangement was weakened to 150µg/ml&75µg/ml arrangement and10µl were infused into the framework and the chromatograms were recorded to survey the strength of the example.

Photograph Stability considers

The photochemical solidness of the medication was additionally considered by uncovering the 1500µg/ml&750µg/ml answer for UV Light by keeping the recepticle in UV Chamber for 7days or 200 Watt hours/m2 in photograph soundness chamber. For HPLC examine, the resultant arrangement was weakened to acquire 150µg/ml&75µg/ml arrangements and 10 µl were infused into the framework and the chromatograms were recorded to evaluate the soundness of test.

Neutral Degradation Studies

Stress testing under nonpartisan conditions was considered by refluxing the medication in water for 6hrs at a temperature of 60º. For HPLC think about, the resultant arrangement was weakened to 150µg/ml&75µg/ml arrangement and 10 µl were infused into the framework and the chromatograms were recorded to survey the solidness of the example. For degradation data see Table no.

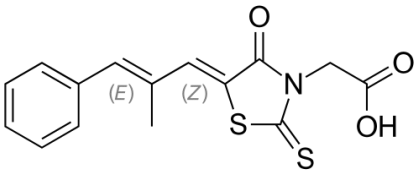


Fig no.1 Structure of Epalrestat

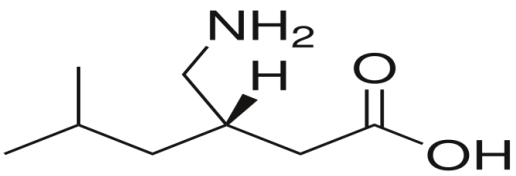
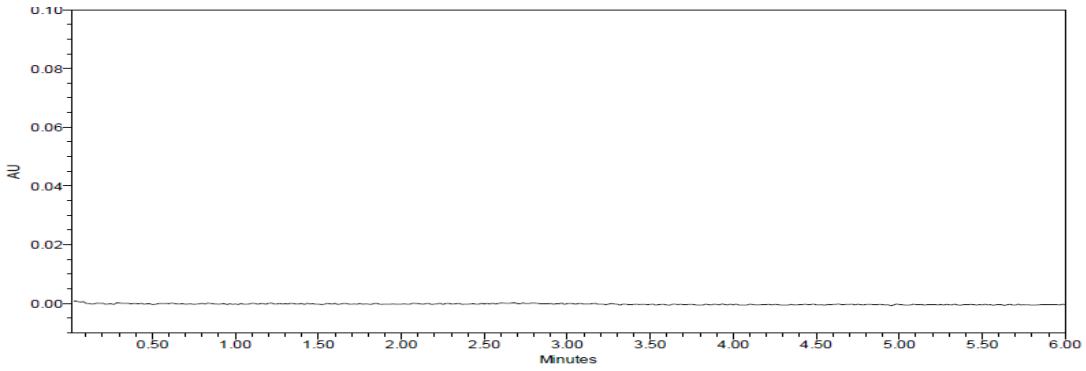
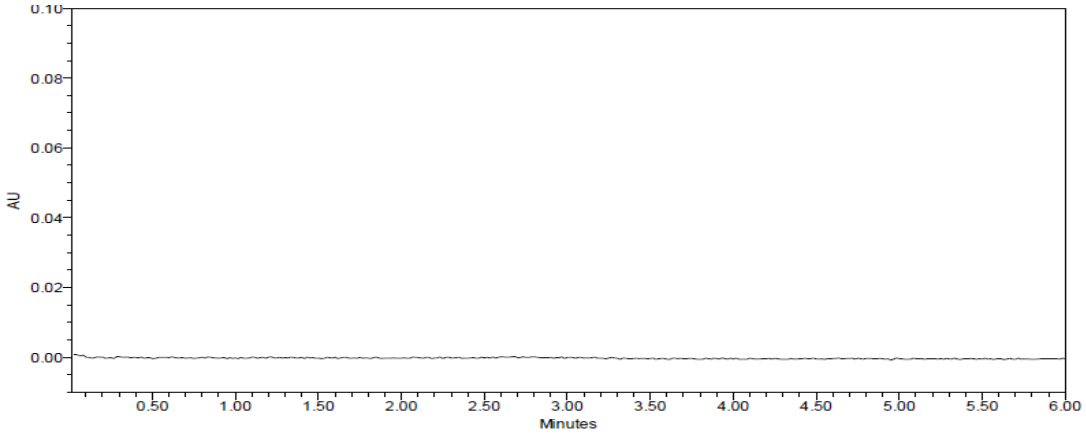


Fig no.2 Structure of Pregabalin



Blank chromatogram



Placebo Chromatogram Fig no.3

Specificity

Table no.2 Accuracy of Epalrestat & Pregabalin

% Level	Epalrestat				Pregabalin			
	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	75	74.08	98.77	98.98%	37.5	37.04	98.78	99.35%
	75	74.39	99.18		37.5	37.54	100.10	
	75	74.62	99.49		37.5	37.34	99.56	
100%	150	150.49	100.33		75	73.67	98.22	
	150	147.25	98.17		75	74.06	98.74	
	150	148.32	98.88		75	75.16	100.22	
150%	225	223.30	99.25		112.5	111.51	99.12	
	225	221.78	98.57		112.5	112.15	99.69	
	225	220.86	98.16		112.5	112.23	99.76	

Table no.3 Intermediate precision of Epalrestat and Pregabalin

S. No	Area of Epalrestat	Area of Pregabalin
1.	3252623	1241050
2.	3279229	1230421
3.	3296583	1211693
4.	3230020	1226513
5.	3238091	1240509
6.	3251171	1224768
Mean	3257953	1229159
S.D	25274.9	10983.2
%RSD	0.8	0.9

Table no.4 Linearity of Epalrestat and Pregabalin

Epalrestat		**Pregabalin**	
Conc.(µg/mL)	**Peak area**	**Conc.(µg/mL)**	**Peak area**
0	0	0	0
37.5	793975	18.75	392023
75	1538713	37.5	696010
112.5	2335643	56.25	1033486
150	3160302	75	1385625
187.5	3852671	93.75	1736003
225	4611087	112.5	2107476

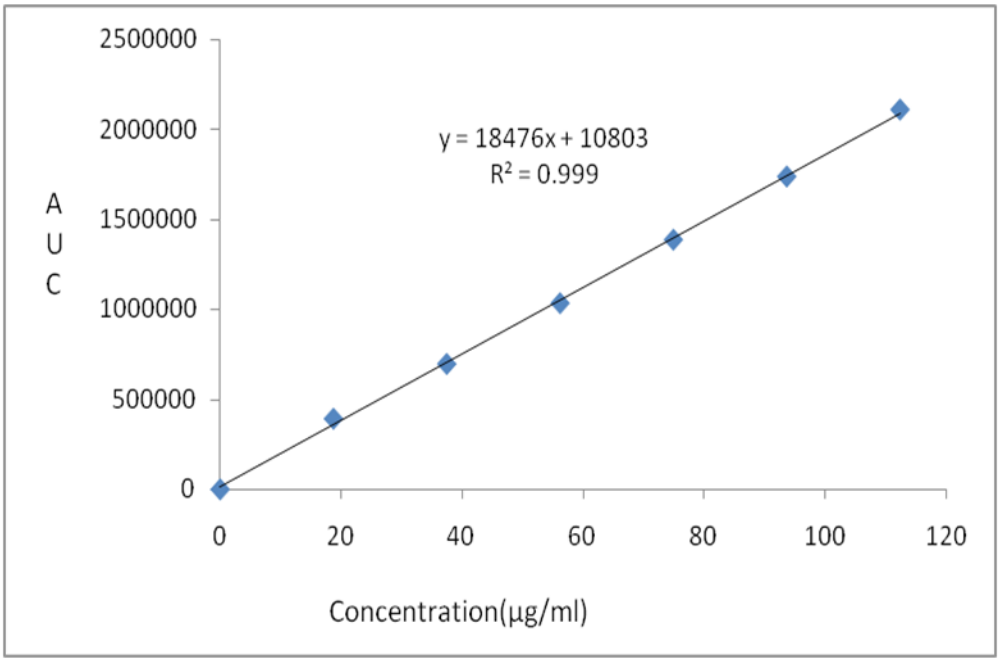


Fig no.5 Calibration curve of Pregabalin

Table no.5 Robustness data of Epalrestat and Pregabalin

S.no.	Condition	% RSD of Epalrestat	% RSD of Pregabalin
1	Flow rate (-) 0.9mL/min	1.3	1.3
2	Flow rate (+) 1.1mL/min	1.3	1.3
3	Mobile phase (-) 57B:43A	0.4	0.2
4	Mobile phase (+) 47B:53A	0.7	0.8
5	Temperature (-) 25°C	1.8	1.8
6	Temperature (+) 35°C	o.3	0.6

Table no.6 System suitability variables for Epalrestat and Pregabalin

S no	Pregabalin			Epalrestat			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.149	3226	1.15	2.901	3845	1.08	4.4
2	2.154	3380	1.18	2.915	3846	1.08	4.3
3	2.159	3315	1.21	2.910	3832	1.10	4.3
4	2.166	3292	1.19	2.916	3946	1.10	4.4
5	2.169	3314	1.18	2.920	3981	1.08	4.4
6	2.179	2515	1.29	2.930	3054	1.22	3.7

Table no.7 Sensitivity of Epalrestat and Pregabalin

Sample	LOD	LOQ
Epalrestat	0.20	0.62
Pregabalin	0.18	0.56

Table no.8 Assay data of Epalrestat & Pregabalin

S.no.	Epalrestat			Pregabalin		
	Standard area	Sample area	% Assay	Standard area	Sample area	% Assay
1	3105736	3115267	98.96	1300458	1310152	99.36
2	3146003	3145891	99.94	1316596	1311046	99.42
3	3145407	3152013	100.13	1320473	1310287	99.37
4	3159858	3140181	99.76	1314421	1312589	99.54
5	3146567	3142258	99.82	1321248	1309030	99.27
6	3145825	3140106	99.75	1322787	1304812	98.95
Avg	3141566	3139286	99.73	1315997	1309653	99.32
Stdev	18417.3	12592.2	0.4	8222.1	2647.1	0.2
%RSD	0.6	0.4	0.4	0.6	0.2	0.2

Table no.9 Degradation data of Epalrestat & Pregabalin

S.no	Degradation condition	Epalrestat			Pregabalin		
		% Drug Degraded	Purity Angle	Purity Threshold	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.46	0.155	0.373	4.58	0.605	1.474
2	Alkali	2.88	0.140	0.366	2.48	0.988	1.445
3	Oxidation	1.44	0.105	0.374	1.52	0.172	0.976
4	Thermal	0.78	0.120	0.367	0.81	0.158	0.415
5	UV	0.72	0.128	0.361	0.55	0.132	0.454
6	Water	0.74	0.143	0.371	0.49	0.346	0.504

CONCLUSION

A New technique was developed for the simultaneous estimation of the Epalrestat & Pregabalin in Pure and its dosage form. Chromatographic conditions used were mobile phase containing buffer (0.1% OPA): Acetonitrile (52:48), BDS C18 (4.6 x 150mm, 5µm), flow rate 1ml/min at a detection wavelength of 240 nm. Retention time of Epalrestat & Pregabalin were found to be 2.930 min and 2.179 min. %RSD of the Epalrestat & Pregabalin were and found to be 0.4and 0.2 respectively. %Recovery was gained as 98.98% and 99.32% for Epalrestat and Pregabalin respectively. LOD, LOQ values gained from

regression equations of Epalrestat and Pregabalin were 0.20, 0.62 and 0.18, 0.56 respectively. Forced degradation studies were carried out in accordance with ICH guidelines and the results revealed the suitability of the method to study the stability of Epalrestat and Pregabalin under various degradation conditions like acid, base, thermal, oxidative, UV and Photolytic degradations. The developed method was validated and it was found to be simple, sensitive, Precise, robust and it can be used for routine analysis of Epalrestat and Pregabalin in both pure and Pharmaceutical dosage forms.

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