INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF EPALRESTAT AND PREGABALIN IN TABLET DOSAGE FORMS

B. Sivagami [1]*, S. Kavya Lalitha [1], V. Pavan Kumar [1], R. Sireesha [1], R. Chandrasekar [2], M. Niranjan babu [2]
[1]Department of Pharmaceutical Analysis, Seven Hills College of Pharmacy, Venkataramapuram, Tirupati, Chitoor Dist, A. P., India
[2]Department of Pharmacognosy, Seven Hills College of Pharmacy, Venkataramapuram, Tirupati, Chitoor Dist, A. P., India
*Corresponding Author Email: sivagamib_27@rediffmail.com

Article Received on: 20/04/18 Approved for publication: 22/05/18

DOI: 10.7897/2230-8407.09572

ABSTRACT

A simple, Accurate, precise method was developed and validated for the simultaneous estimation of Epalrestat and Pregabalin in Tablet dosage forms. Chromatogram was run through C8 (250 x 4.6 mm, 5µ) Column. Mobile phase contains Potassium Dihydrogen Phosphate Buffer: Acetonitrile taken in the ratio 45:55 was pumped through column at a flow rate of 1 ml/min. The pH was adjusted to 5.4 with Orthophosphoric acid. Temperature was maintained at 25°C. Optimized wavelength selected was 274 nm. Retention time of Epalrestat and Pregabalin were found to be 2.373 min and 2.967 min. % Relative Standard Deviation of the Epalrestat and Pregabalin were found to be 0.1and 0.3 respectively. % Recovery was obtained as 99.85% and 99.42% for Epalrestat and Pregabalin respectively. Limit of Detection, Limit of Quantification values obtained from regression equations of Epalrestat and Pregabalin were 0.24, 0.73 and 0.02, 0.07 respectively. Regression equation of Epalrestat is y = 26994x + 13337, and y = 24913x + 7779 of Pregabalin. Retention time decreased so that the run time decreased. So the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: Epalrestat, Pregabalin, RP-HPLC, Stability, Validation.

INTRODUCTION

Epalrestat is an aldose reductase inhibitor. Aldose reductase reduces glucose to sorbitol. Epalrestat inhibited high glucose-mediated neutrophil endothelial cell adhesion and expression of endothelial adhesion molecules not only through inhibition of a PKC-dependent pathway, but also through increased endothelial production. [1] Chemically Epalrestat is (5-[(1Z, 2E)-2-methyl-3-phenyl propenylidene]-4-oxo-2-thioxo-3-thiazolidine acetic acid. Epalrestat is a carboxylic acid derivative and a noncompetitive and reversible aldose reductase inhibitor used for the treatment of diabetic neuropathy, which is one of the most common long-term complications in patients with diabetes mellitus. It reduces the accumulation of intracellular sorbitol which is believed to be the cause of diabetic neuropathy, retinopathy and nephropathy. It is well tolerated, with the most commonly reported adverse effects being gastrointestinal issues such as nausea and vomiting, as well as increases in certain liver enzymes. 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. It is easily absorbed into the neural tissue and inhibits the enzyme with minimum side effects. [2-5]

Pregabalin 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 pregabalin is (*S*)-3-(aminomethyl)-5-methylhexanoic acid. [6]

Pregabalin binds with high affinity to the alpha2-delta site (an auxiliary subunit of voltage-gated calcium channels) in central nervous system tissues. Although the mechanism of action of pregabalin is unknown, results with genetically modified mice and with compounds structurally related to pregabalin (such as gabapentin) suggest that binding to the alpha2-delta subunit may be involved in pregabalin's antinociceptive and antiseizure effects in animal models. In vitro, pregabalin reduces the calcium-dependent release of several neurotransmitters, possibly by modulation of calcium channel function. Studies also suggest that the descending noradrenergic and serotonergic pathways originating from the brainstem may be involved with the mechanism of pregabalin. Interestingly, although pregabalin is a structural derivative of inhibitory neurotransmitter gamma-aminobutyric acid (GABA), it does not bind directly to GABA or benzodiazepine receptors. The sodium channels, opiate receptors, and cyclooxygenase enzymes are not involved with the mechanism of pregabalin. It is also inactive at serotonin and dopamine receptors and does not inhibit dopamine, serotonin, or noradrenaline reuptake. [7, 8]

The literature review revealed that several analytical methods have been reported for Epalrestat and Pregabalin in UV-Spectrophotometry, RP-HPLC, individually and in combination. This research work implicates the simultaneous estimation of Epalrestat and Pregabalin by RP-HPLC in tablet dosage forms. This present study reports simultaneous estimation of Epalrestat and Pregabalin by RP-HPLC in tablet dosage form. [9-12]

MATERIALS AND METHODS

Materials

Combination of Epalrestat and Pregabalin tablets (PREALDONIL 150MG TABLET), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents were of AR grade obtained from Rankem labs Pvt. Ltd.

Instruments

Electronics Balance-Denver, p^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Epalrestat and Pregabalin solutions.

Methods

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

Preparation of Standard stock solutions: Accurately weighed 15 mg of Epalrestat, 7.5 mg of Pregabalin was transferred to 10 ml volumetric flasks. 3/4 Th of diluents were added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1500µg/ml EPAL and 750µg/ml of PREGA)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (150 µg/ml of EPAL and 75µg/ml of PREGA)

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 100 ml volumetric flask, 50ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (1500 µg/ml of EPAL and 750 µg/ml of PREGA)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (150µg/ml of EPAL and 75µg/ml of PREGA)

Preparation of buffer

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate was taken in a 1000ml Volumetric flask and about 900 ml of milli-Q water was added and degassed to sonicate and finally make up the volume with water then PH was adjusted to 5.4 with dil. Ortho phosphoric acid solution.

0.1%Ortho Phosphoric Acid Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

RESULTS AND DISCUSSION

Determination of λ_{max} and Optimized wavelength: UV scan of the Epalrestat and Pregabalin was done individually and both were overlayed upon each other to get the required wavelength. The wavelength of 274nm was found to be effective in determination of both the drugs at a time. Figure 3&4

Optimized method
Chromatographic conditions

Mobile phase: 0.01N Kh2po4: Acetonitrile (45:55)
Flow rate: 1 ml/min
Column: Discovery C8 (4.6 x 250mm, 5µm)
Detector wave length: 274nm
Column temperature: 25°C
Injection volume: 10µL
Run time:10 min
Diluent: Water and Acetonitrile in the ratio 50:50
Results: Both peaks have good resolution, tailing, Factor, theoretical plate count and resolution.

Observation: Epalrestat and Pregabalin were eluted at 2.373min and 2.967 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines. [13]

VALIDATION

LINEARITY: Six linear concentrations of Epalrestat (37.5-225µg/ml) and Pregabalin (18.75-112.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Epalrestat was y =26994.x + 13337 and of Pregabalin was y = 24913x + 7779 Correlation coefficient obtained was 0.999 for the two drugs. Table 1 Figure 7&8

PRECISION: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % (RSD) Relative Standard Deviation were calculated for two drugs. (% RSD) Relative Standard Deviation obtained as 0.2% and 0.2% respectively for Epalrestat and Pregabalin. As the limit of Precision was less than "2" the system precision was within the limit. Table 2 Figure 9.

REPEATABILITY: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in table 3. Average area, standard deviation and % Relative Standard Deviation were calculated for two drugs and obtained as 0.1% and 0.3% respectively for Epalrestat and Pregabalin. As the limit of Precision was less than "2" the system precision was within the limit. Table 3 Figure 10

INTERMEDIATE PRECISION (DAY_ DAY PRECISION): Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % Relative Standard Deviation were calculated for two drugs and obtained as 0.4% and 0.3% respectively for Epalrestat and Pregabalin. As the limit of Precision was less than "2" the system precision was within the limit. Table 4 Figure 11

ACCURACY: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as

99.85% and 99.42% for Epalrestat and Pregabalin respectively. Table 5 & 6 Figure 12, 13 & 14

SENSITIVITY: Limit of Detection and Limit of Quantification Table 7

ROBUSTNESS: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (40B:60A), mobile phase plus (50B:50A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters passed. (%RSD) Relative Standard Deviation was within the limit. Table 8 Figure 15 & 16

Assay: PREALDONIL 150MG TABLET, bearing the label claim Epalrestat 150mg, Pregabalin 75mg. Assay was performed with the above formulation. Average % Assay for Epalrestat and Pregabalin obtained was 99.80 and 99.24% respectively. Table 9 &10 Figure 17 & 18

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation Table 11 Figure 19-22.

TABLE 1 LINEARITY TABLE FOR EPALRESTAT AND PREGABALIN

Epalrestat		Pregabalin	
Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**
0	0	0	0
37.5	1087916	18.75	445889
75	2041248	37.5	966941
112.5	2980983	56.25	1413804
150	4008313	75	1912792
187.5	5145687	93.75	2348127
225	6087067	112.5	2776280

TABLE 2 SYSTEM PRECISION OF EPALRESTAT AND PREGABALIN

S. No	Area of Epalrestat	Area of Pregabalin
1.	4030625	1933592
2.	4013874	1929075
3.	4021592	1934777
4.	4033815	1931264
5.	4034095	1924751
6.	4015891	1925878
Mean	4024982	1929890
Standard Deviation	9060.2	4065.6
%RSD	0.2	0.2

TABLE 3 REPEATABILITY OF EPALRESTAT AND EMPOAGLIFLOZIN

S. No	Area of Epalrestat	Area of Pregabalin
1.	4025748	1918227
2.	4027767	1915135
3.	4018426	1910180
4.	4029788	1929603
5.	4025517	1919061
6.	4022579	1921673
Mean	4024971	1918980
Standard Deviation	4012.0	6525.3
%RSD	0.1	0.3

TABLE 4 INTERMEDIATE PRECISION OF EPALRESTAT AND PREGABALIN

S. No	Area of Epalrestat	Area of Pregabalin
1.	4047726	1949722
2.	4006071	1937131
3.	4010024	1937605
4.	4012275	1934281
5.	4035834	1938768
6.	4008620	1934429
Mean	4020092	1938656
Standard Deviation	17332.7	5708.0
%RSD	0.4	0.3

TABLE 5 ACCURACY TABLE OF EPALRESTAT

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	75	75.09	100.12	99.85%
	75	74.03	98.70	
	75	75.46	100.62	
100%	150	150.30	100.20	
	150	150.35	100.23	
	150	150.52	100.35	
150%	225	224.68	99.86	
	225	223.21	99.21	
	225	223.59	99.38	

TABLE 6 ACCURACY TABLE OF PREGABALIN

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	37.5	36.94	98.51	99.42%
	37.5	36.98	98.61	
	37.5	36.90	98.40	
100%	75	74.16	98.87	
	75	73.67	98.22	
	75	74.69	99.58	
150%	112.5	113.47	100.86	
	112.5	113.39	100.79	
	112.5	113.53	100.91	

TABLE 7 SENSITIVITY OF EPALRESTAT AND PREGABALIN

Molecule	LOD	LOQ
Epalrestat	0.24	0.73
Pregabalin	0.02	0.07

TABLE 8 ROBUSTNESS DATA FOR EPALRESTAT AND PREGABALIN

S.no	Condition	%RSD of Epalrestat	%RSD of Pregabalin
1	Flow rate (-) 1.1ml/min	0.1	0.3
2	Flow rate (+) 1.3ml/min	0.3	0.8
3	Mobile phase (-) 35B:65A	0.1	0.1
4	Mobile phase (+) 45B:55A	0.1	0.5
5	Temperature (-) 25°C	0.1	0.2
6	Temperature (+) 35°C	0.2	0.2

TABLE 9 ASSAY DATA OF EPALRESTAT

S.No	Standard Area	Sample area	% Assay
1	4030625	4025748	99.82
2	4013874	4027767	99.87
3	4021592	4018426	99.64
4	4033815	4029788	99.92
5	4034095	4025517	99.81
6	4015891	4022579	99.74
Average	4024982	4024971	99.80
Standard Deviation	9060.2	4012.0	0.099
%RSD	0.2	0.1	0.1

TABLE 10 ASSAY DATA OF PREGABALIN

S. No	Standard Area	Sample area	% Assay
1	1933592	1918227	99.20
2	1929075	1915135	99.04
3	1934777	1910180	98.78
4	1931264	1929603	99.79
5	1924751	1919061	99.24
6	1925878	1921673	99.38
Average	1929890	1918980	99.24
Standard Deviation	4065.6	6525.3	0.3374
%RSD	0.2	0.3	0.3

TABLE 11 DEGRADATION DATA OF EPALRESTAT

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.67	0.186	0.298
2	Alkali	2.77	0.169	0.297
3	Oxidation	2.00	0.165	0.289
4	Thermal	0.95	0.186	0.298
Degradation Data of Pregabalin				
1	Acid	4.83	0.148	0.296
2	Alkali	2.61	0.150	0.299
3	Oxidation	1.82	0.124	0.294
4	Thermal	0.69	0.148	0.296

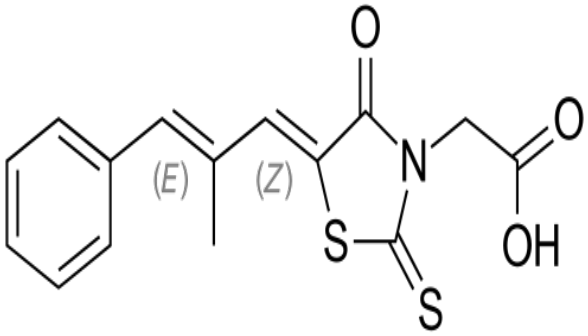

FIGURE 1 STRUCTURE OF EPALRESTAT

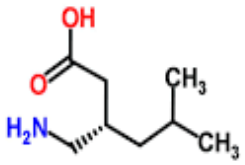

FIGURE 2 STRUCTURE OF PREGABALIN

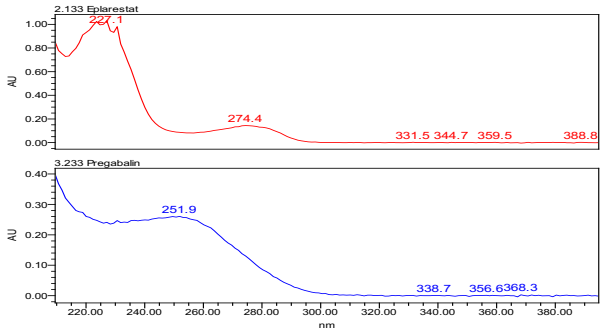

FIGURE 3 INDIVIDUAL UV SPECTRA OF EPALRESTAT AND PREGABALIN

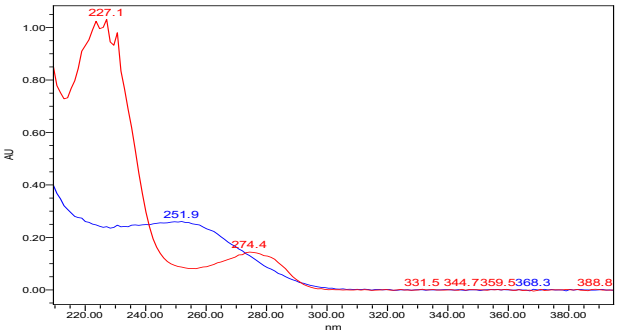

FIGURE 4 OVERLAY UV SPECTRA OF EPALRESTAT AND PREGABALIN

Optimized wavelength selected was 274nm.
λ_{max} of Epalrestat and Pregabalin was 220nm and 229.5nm respectively.
Overlay spectra gave the optimized wavelength for these two drugs.

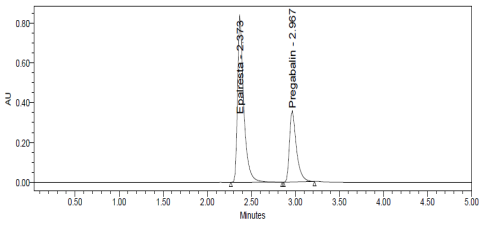

FIGURE 5 OPTIMIZED CHROMATOGRAM

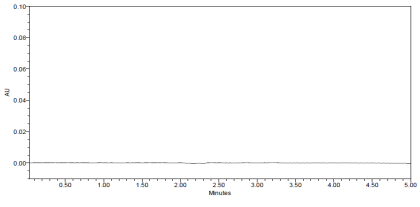

FIGURE 6 CHROMATOGRAM OF BLANK

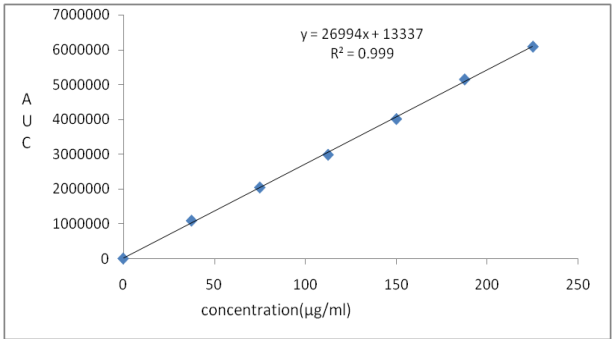

FIGURE 7 CALIBRATION CURVE OF EPALRESTAT

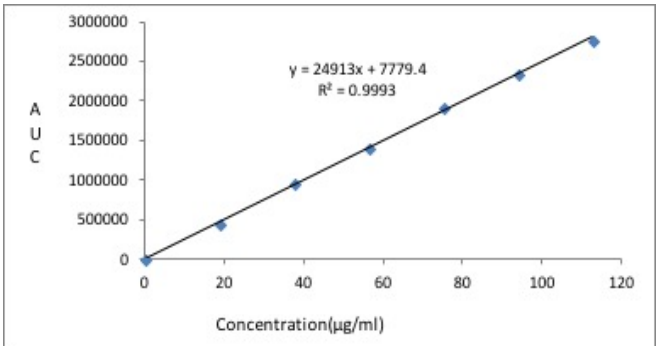

FIGURE 8 CALIBRATION CURVE OF PREGABALIN

SYSTEM PRECISION: LOD & LOQ

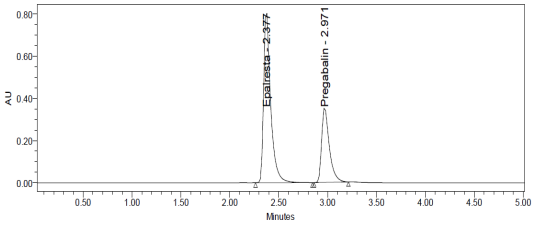

FIGURE 9 SYSTEM PRECISION CHROMATOGRAM

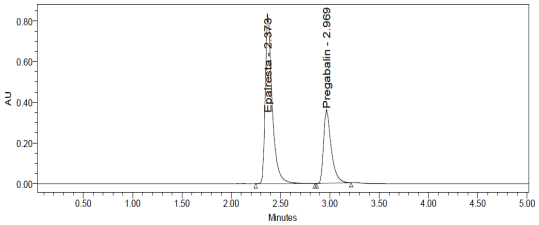

FIGURE 10 REPEATABILITY CHROMATOGRAM

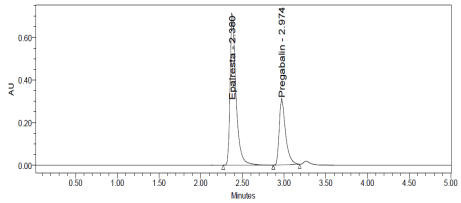

FIGURE 11 INTER DAY PRECISION CHROMATOGRAM

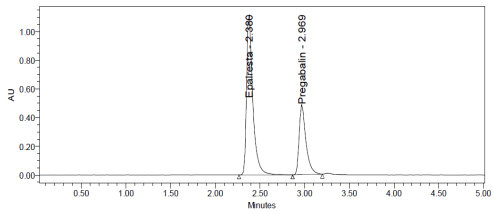

FIGURE 12 CHROMATOGRAM OF ACCURACY 50%

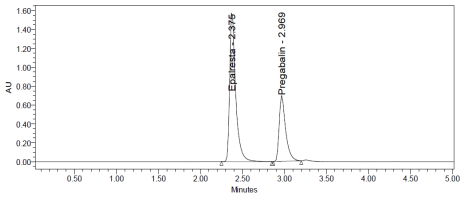

FIGURE 13 CHROMATOGRAM OF ACCURACY 100%

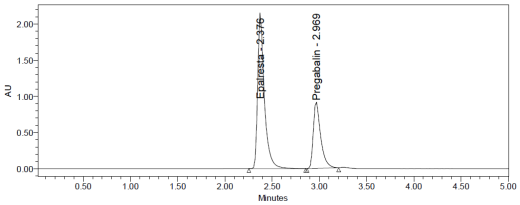

FIGURE 14 CHROMATOGRAM OF ACCURACY 150%

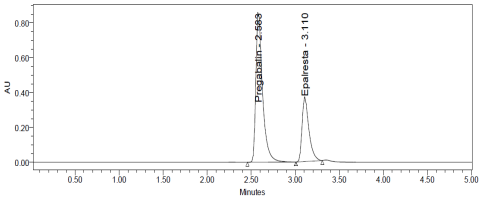

FIGURE 15 FLOW MINUS CHROMATOGRAM

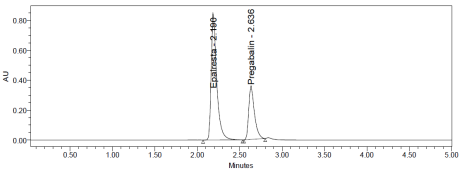

FIGURE 16 FLOW PLUS CHROMATOGRAM

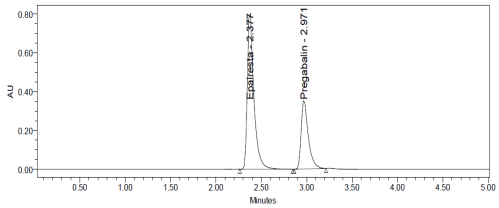

FIGURE 17 CHROMATOGRAM OF WORKING STANDARD SOLUTION

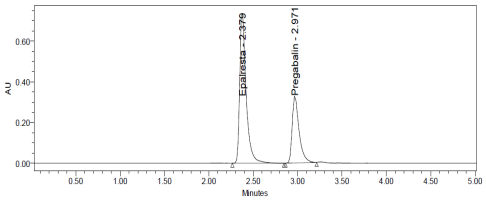

FIGURE 18 CHROMATOGRAM OF WORKING SAMPLE SOLUTION

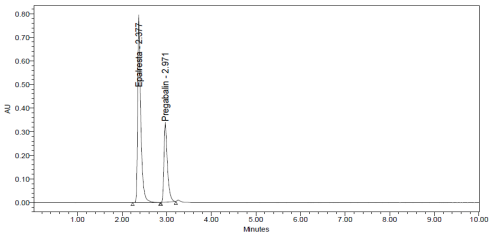

FIGURE 19 ACID CHROMATOGRAM

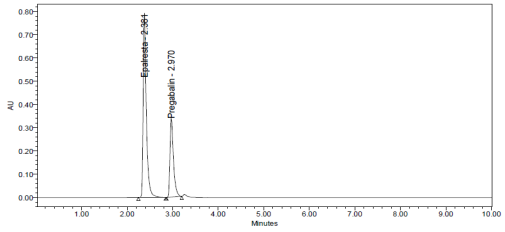

FIGURE 20 BASE CHROMATOGRAM

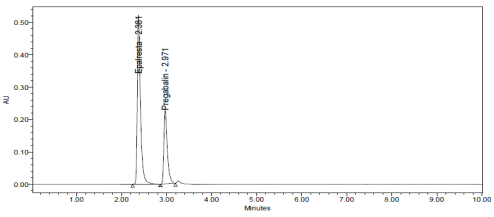

FIGURE 21 PEROXIDE CHROMATOGRAM

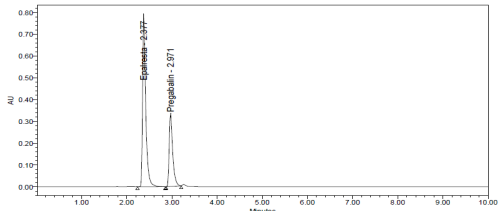

FIGURE 22 THERMAL CHROMATOGRAM

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Epalrestat and Pregabalin in Tablet dosage form. Retention time of Epalrestat and Pregabalin were found to be 2.373min and 2.967 min. % RSD of the Epalrestat and Pregabalin were found to be 0.1and 0.3 respectively. % Recovery was obtained as 99.85% and 99.42% for Epalrestat and Pregabalin respectively. LOD, LOQ values obtained from regression equations of Epalrestat and Pregabalin were 0.24, 0.73 and 0.02, 0.07 respectively. Regression equation of Epalrestat is y = 26994x + 13337, and y = 24913x + 7779 of Pregabalin. Retention time decreased so that the run time also decreased. So the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

REFERENCES

1. https://www.medicineindia.org/pharmacology-for-generic/2902/epalrestat
2. Charles P. Taylora, Timothy Angelottib, Eric Faumanc. Pharmacology and mechanism of action of pregabalin: The calcium channel _2—_ (alpha2—delta) subunit as a target for antiepileptic drug discovery. Epilepsy Research 2007; 73; 137-150.
3. S. R. Sharma and Nalini Sharma. Epalrestat an aldose reductase inhibitor, in diabetic neuropathy: An Indian perspective Ann Indian Academy of Neurology. 2008; 11(4): 231-235.
4. Kaori Yama, Keisuke Sato, Natsuki Abe, Yu Murao, Ryosuke Tatsunami, and Yoshiko Tampo**.** Epalrestat increases glutathione, thioredoxin, and heme oxygenase-1 by stimulating Nrf2 pathway in endothelial cells Redox Biology. 2015; (4): 87-96.
5. Okayama N, Omi H, Okouchi M, Imaeda K, Kato T, Akao M, *et al,*. Mechanisms of inhibitory activity of the aldose reductase inhibitor, epalrestat, on high glucose-mediated endothelial injury: neutrophil-endothelial cell adhesion and surface expression of endothelial adhesion molecules. Journal of Diabetes Complications. 2002; 16(5):321-6.
6. Michael A. Rogawski and Charles P. Taylor Calcium Channel α_2–δ Subunit, A New Antiepileptic Drug Target Epilepsy Research. 2006; 69(3): 183-272.
7. Joshi I, Taylor CP. Pregabalin action at a model synapse: binding to presynaptic calcium channel alpha2-delta subunit reduces neurotransmission in mice European Journal of Pharmacology. 2006; 553(1-3):82-8.
8. Field MJ, Cox PJ, Stott E, Melrose H, Offord J, Su TZ, *et al,*. Identification of the alpha2-delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of Pregabalin. Proc Natl Acad Sci U S A. 2006; 103(46):17537-42.
9. Kasawar GB et al., Development and validation of HPLC method for the determination of Pregabalin in capsules. Indian journal of pharmaceutical sciences 2010; 72 (4): 517-519.
10. P. Janaki Pathi, N. Appala Raju The Estimation of Epalrestat in Tablet Dosage Form by RP-HPLC. Asian Journal of Pharmaceutical Analysis. 2012; 2(2): 49-51
11. Thejo Moorthy Karvadi and B.R. Challa bioanalytical method development and validation of pregabalin in rat plasma by solid phase extraction with HPLC MS/MS: application to a pharmacokinetic study. Journal of Liquid Chromatography and Related Technologies 2014; 37 (1): 130-144.

12. B. Madhu Harika, Y. Rajendra Prasad. Development and Validation of Stability Indicating RP-HPLC method for simultaneous estimation of Epalrestat and Pregabalin in bulk and tablet dosage form. International Journal of Pharmacy 2017; 7(2): 157-164.
13. ICH Harmonised Tripartite Guideline, validation of analytical procedures: Text methodology, Q2 (R1) (2005). International Conference on Harmonization, Geneva, pp: 1-13.

Cite this article as:

B. Sivagami *et al*. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of epalrestat and pregabalin in tablet dosage forms. Int. Res. J. Pharm. 2018;9(5):45-52 http://dx.doi.org/10.7897/2230-8407.09572

Source of support: Nil, Conflict of interest: None Declared