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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR ESTIMATION OF PREGABALIN AND EPALRESTAT IN PURE AND PHARMACEUTICAL DOSAGE FORM

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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 Std Azilent 150 x 4.6 mm, 5µ. Mobile phase containing Buffer: Acetonitrile taken in the ratio 45:55 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.1% OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 241 nm. Retention time of Pregabalin and Epalrestatwere found to be 2.930 min and 2.179 min. %RSD of the Epalrestat and Pregabalin were and found to be 0.4and 0.2 respectively. % Recovery was obtained as 98.98% and 99.32% for Epalrestat and Pregabalin respectively. LOD, LOQ values obtained from regression equations of Epalrestat and Pregabalin were 0.02, 0.06 and 0.26, 0.77 respectively. Regression equation of Epalrestat is y = 20545x + 16173, and y = 18476x + 10803of Pregabalin. Retention times were 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.

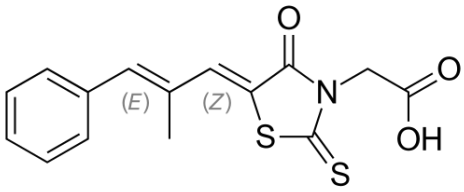
KEYWORDS: Epalrestat, Pregabalin, RP-HPLC.

INTRODUCTION

Liquid chromatography (3) is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase to differing degrees due to differences in adsorption, ion exchange, partitioning or size. These differences will allow the mixture components to be separated from each other by using these differences to determine the time of the solutes through a column. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography and thin layer chromatography (TLC). However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time pressure liquid chromatography began to be used to decreased flow through time, thus reducing separation time of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure debated. High pressure liquid chromatography quickly improved with the development of column packing materials. Additional convenience of on-line detectors became rapidly a powerful separation technique and is today called as High Performance Liquid Chromatography (HPLC).

Epalrestat 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 with. 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. 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.

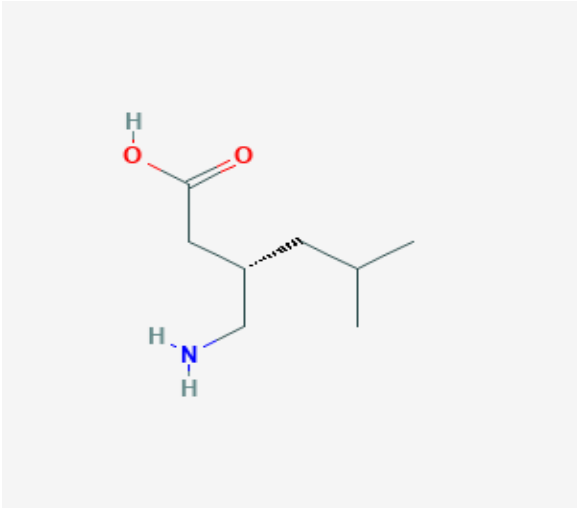
Structure



PREGABALIN

Description: 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. 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.

Structure



EXPERIMENTAL WORK

MATERIALS

Epalrestat and Pregabalin pure drugs (API), Combination Epalrestat and Pregabalin tablets (PREALDONIL 150MG TABLET) received from spectrum lab Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem Ltd.

METHODOLOGY

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

Preparation of Standard stock solutions: Accurately weighed 75 mg of Epalrestat, 75 mg of Pregabalin and transferred to 50ml&50ml volumetric flasks. 3/4 th of diluents was added and solicited for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2.(1500µg/ml EPAL of and 1500µg/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 150µg/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 diluents 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 1500 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 150µg/ml of PREGA).

Preparation of buffer

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

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

VALIDATION

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Epalrestat (150ppm) and Pregabalin (150ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

PRECISION

Preparation of Standard stock solutions: Accurately weighed 75 mg of Epalrestat, 75 mg of Pregabalin and transferred to 50ml&50ml volumetric flasks. 3/4 th of diluents was added and solicited for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2.(1500µg/ml EPAL of and 1500µg/ml of PREGA)

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Preparation of Sample stock solutions: 5 tablets were

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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 150µg/ml of PREGA)

LINEARITY

Preparation of Standard stock solutions: Accurately weighed 75 mg of Epalrestat, 75 mg of Pregabalin and transferred to 50ml&50ml volumetric flasks. 3/4 th of diluents was added and solicited for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2.(1500µg/ml EPAL of and 1500µg/ml of PREGA)

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (37.5µg/ml of EPAL, and 37.5 µg/ml of PREGA)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of EPAL, and 75 µg/ml of PREGA)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (112.5µg/ml of EPAL, and 112.5µg/ml of PREGA)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (150µg/ml of EPAL, and 150µg/ml of PREGA)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (187.5µg/ml of EPAL and 187.5µg/ml of PREGA)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml. (225µg/ml of EPAL and 225µg/ml of PREGA)

Accuracy

Preparation of Standard stock solutions: Accurately weighed 75 mg of Epalrestat, 75 mg of Pregabalin and transferred to 50ml&50ml volumetric flasks. 3/4 th of diluents was added and solicited for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2.(1500µg/ml EPAL of and 1500µg/ml of PREGA)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Epalrestat, Pregabalin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Epalrestat, Pregabalin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

DEGRADATION STUDIES

Oxidation

To 1 ml of stock solution of Epalrestat and Pregabalin, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 150µg/ml&150µg/ml 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 s solution Epalrestat and Pregabalin, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 150µg/ml&150µg/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 30mins at 60^0c. The resultant solution was diluted to obtain 150µg/ml&150µg/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 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 150µg/ml&150µg/ml solution and10µl 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&1500µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 150µg/ml&150µg/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 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 150µg/ml&150µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION

Optimized method

Chromatographic conditions

Mobile phase **:** 40% OPA (0.1%): 60% Acetonitrile

Flow rate **: 1** ml/min

Column **:** Azilent C18 (4.6 x 150mm, 5µm)

Detector wave length **:** 241nm

Column temperature **: 30**°C

Injection volume **:** 10µL

Run time **: 7** min

Diluent **:** Water and Acetonitrile in the ratio 50:50

Results **:** Both peaks have good resolution, tailing factor, theoretical plate count and resolution.

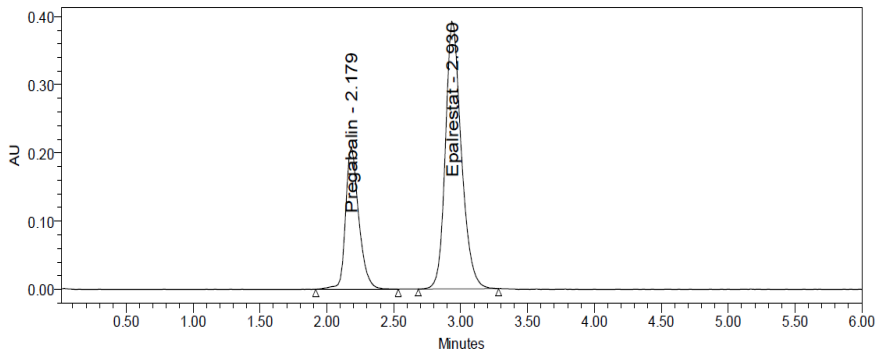

Fig 6.8 Optimized Chromatogram.

Table: 6.1 System suitability parameters 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

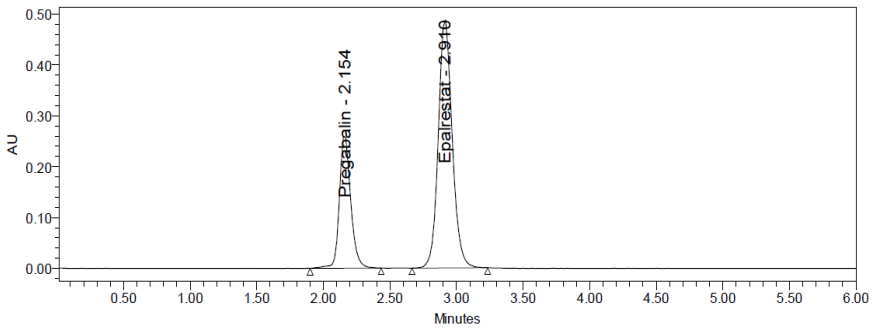

Fig 6.11: System suitability Chromatogram.

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Validation: Specificity

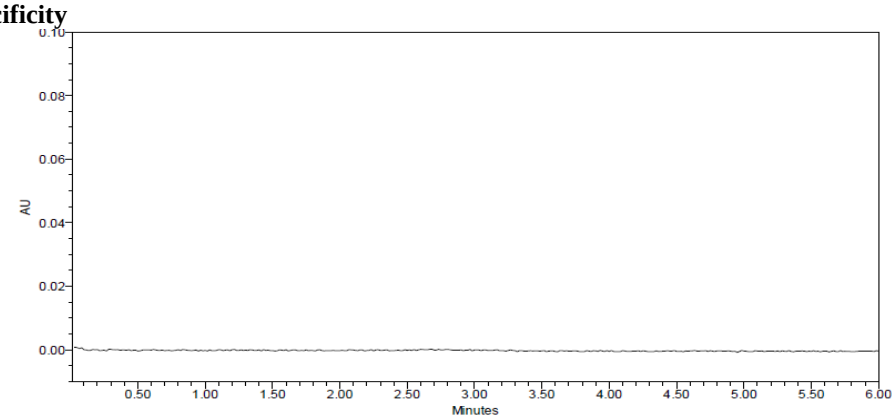

Figure No. 6.12. Chromatogram of blank.

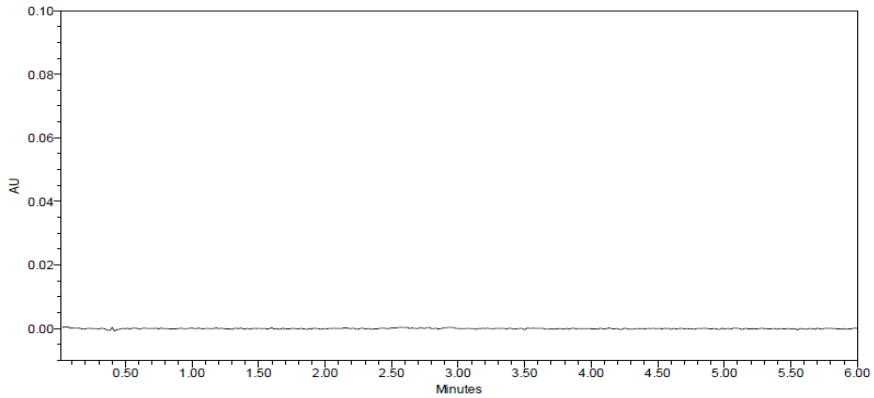

Figure No. 6.13 Chromatogram of placebo.

Linearity

Table 6.2 Linearity table for 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

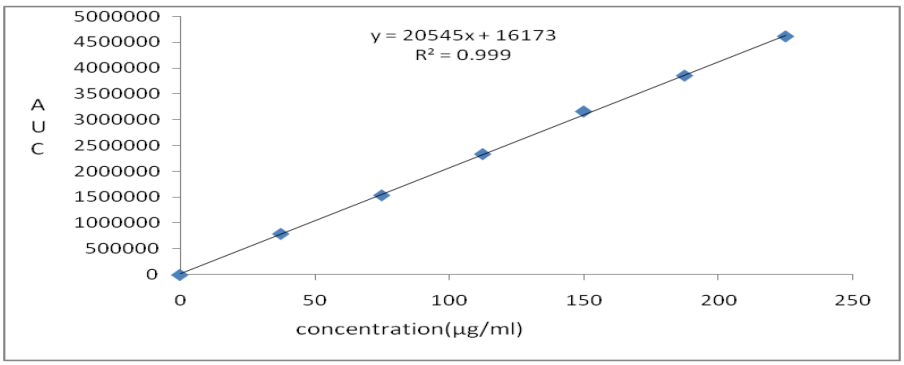

Fig No. Calibration curve of Epalrestat.

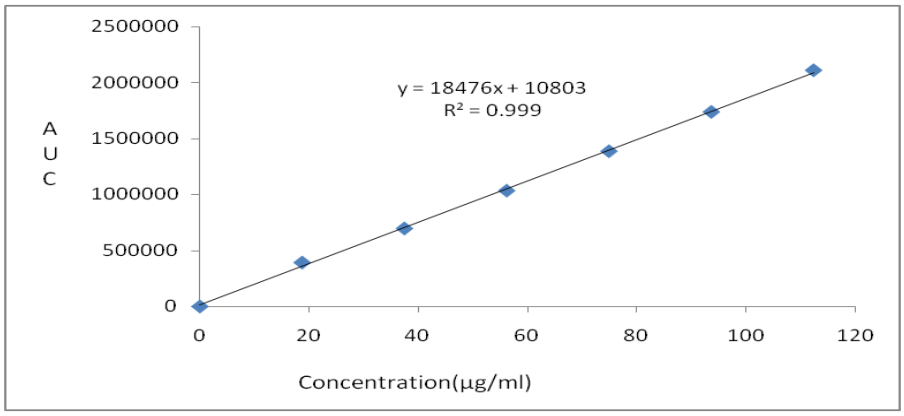

Fig No. Calibration curve of Pregabalin.

Discussion: 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 =20545.x + 16173 and of Pregabalin was y = 18476x + 10803 Correlation coefficient obtained was 0.999 for the two drugs.

PRECISION

System Precision

Table 6.3: System precision table of Epalrestat and Pregabalin.

S. No	Area of Epalrestat	Area of Pregabalin
1.	3105736	1300458
2.	3146003	1316596
3.	3145407	1320473
4.	3159858	1314421
5.	3146567	1321248
6.	3145825	1322787
Mean	3141566	1315997
S.D	18417.3	8222.1
%RSD	0.6	0.6

Repeatability

Table 6.4 Repeatability table of Epalrestat and Pregabalin.

S. No	Area of Epalrestat	Area of Pregabalin
1.	3115267	1310152
2.	3145891	1311046
3.	3152013	1310287
4.	3140181	1312589
5.	3142258	1309030
6.	3140106	1304812
Mean	3139286	1309653
S.D	12592.2	2647.1
%RSD	0.4	0.2

Intermediate precision (Day_ Day Precision)

Table 6.5 Intermediate precision table 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

Accuracy

Table Accuracy table of Epalrestat.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	75	74.08	98.77	98.98%
	75	74.39	99.18	
	75	74.62	99.49	
100%	150	150.49	100.33	
	150	147.25	98.17	
	150	148.32	98.88	
150%	225	223.30	99.25	
	225	221.78	98.57	
	225	220.86	98.16	

Accuracy table of Pregabalin.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	37.5	37.04	98.78	99.35%
	37.5	37.54	100.10	
	37.5	37.34	99.56	
100%	75	73.67	98.22	
	75	74.06	98.74	
	75	75.16	100.22	
150%	112.5	111.51	99.12	
	112.5	112.15	99.69	
	112.5	112.23	99.76	

Sensitivity

Table: Sensitivity table of Epalrestat and Pregabalin.

Molecule	LOD	LOQ
Epalrestat	0.20	0.62
Pregabalin	0.18	0.56

Robustness

Table 6.8: Robustness data for Epalrestat and Pregabalin.

S.no	Condition	%RSD of Epalrestat	%RSD of Pregabalin
1	Flow rate (-) 0.7ml/min	1.3	1.3
2	Flow rate (+) 0.9ml/min	1.3	1.3
3	Mobile phase (-) 50B:50A	0.4	0.2
4	Mobile phase (+) 40B:60A	0.7	0.8
5	Temperature (-) 25°C	1.8	1.8
6	Temperature (+) 35°C	0.3	0.6

Assay

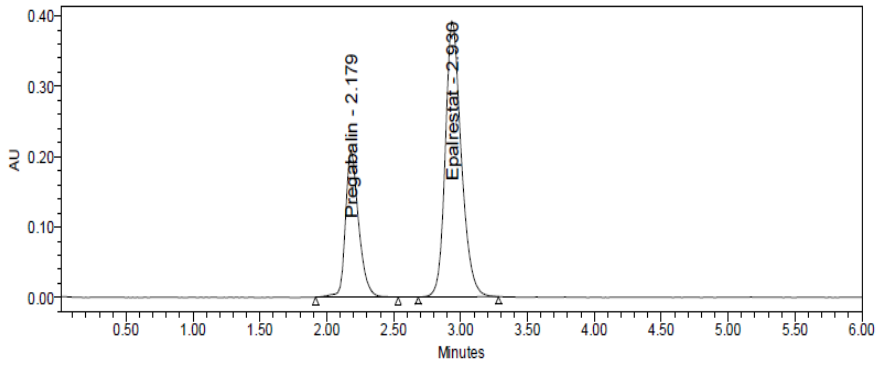

Fig.: Chromatogram of working standard solution.

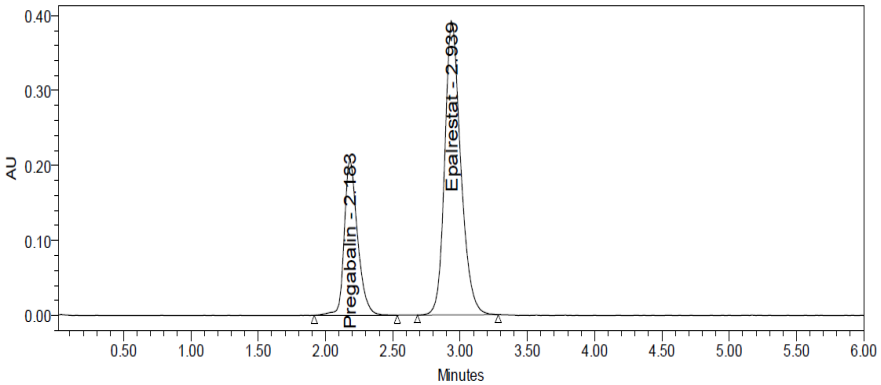

Fig. No: Chromatogram of working sample solution.

DEGRADATION

	Epalrestat			Pregabalin		
S.no	**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

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: Degradation Data of Epalrestat.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.46	0.155	0.373
2	Alkali	2.88	0.140	0.366
3	Oxidation	1.44	0.105	0.374
4	Thermal	0.78	0.120	0.367
5	UV	0.72	0.128	0.361
6	Water	0.74	0.143	0.371

Table Degradation Data of Pregabalin.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.58	0.605	1.474
2	Alkali	2.48	0.988	1.445
3	Oxidation	1.52	0.172	0.976
4	Thermal	0.81	0.158	0.415
5	UV	0.55	0.132	0.454
6	Water	0.49	0.346	0.504

SUMMARY AND CONCLUSION

Parameters		**Epalrestat**	**Pregabalin**	**LIMIT**
Linearity Range (µg/ml)		37.5-225µg/ml	18.75-112.5µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		20545	18476	
Intercept(c)		16173	10803	
Regression equation (Y=mx+c)		y = 20545x + 16173	y = 18476x + 10803	
Assay(% mean assay)		99.73%	99.32%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.6	0.6	NMT 2.0%
Method precision %RSD		0.4	0.2	NMT 2.0%
Accuracy %recovery		98.98%	99.32%	98-102%
LOD		0.20	0.18	NMT 3
LOQ		0.62	0.56	NMT 10
Robustness	**FM**	1.3	1.3	%RSD NMT 2.0
	FP	1.3	1.3	
	MM	0.4	0.2	
	MP	0.7	0.8	
	TM	1.8	1.8	
	TP	0.3	0.6	

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 2.930 min and 2.179 min. %RSD of the Epalrestat and Pregabalin were and found to be 0.4and 0.2 respectively. %Recovery was obtained as 98.98% and 99.32% for Epalrestat and Pregabalin respectively. LOD, LOQ values obtained from regression equations of Epalrestat and Pregabalin were 0.02, 0.06 and 0.26, 0.77 respectively. Regression equation of Epalrestat is y = 20545x + 16173, and y = 18476x + 10803of Pregabalin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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