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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF EPALRESTAT AND PREGABALIN IN BULK AND TABLET DOSAGE FORM

S. Angala Parameswari* and Gaddam Arunamma

Department of Pharmaceutical Analysis, Krishna Teja Pharmacy College, Tirupathi - 517506, Andhra Pradesh, India.

Keywords:

RP-HPLC, Epalrestat, Pregabalin, Method validation, Stability, Simultaneous estimation

Correspondence to Author:

Dr. S. Angala parameswari

Associate professor,
Department of Pharmaceutical
Analysis, Krishna Teja Pharmacy
College, Tirupathi - 517506,
Andhra Pradesh, India.

E-mail: eswarialagusundaram@gmail.com

ABSTRACT: A New simple, sensitive, precise, accurate and stability indicating RP-HPLC method has been developed for the simultaneous determination of epalrestat and pregabalin in combined tablet dosage form. The chromatogram was achieved with STD Discovery column 250 × 4.6 mm, 5 μ and mobile phase containing 0.1% ortho phosphoric acid buffer and acetonitrile taken in the ratio of 45: 55 was pumped through column at a flow rate of 1 ml/min. temperature was maintained at 25 °C. The effluents were monitored at 244 nm by using PDA detector. The retention time of epalrestat and pregabalin were found to be 2.407 min and 3.272 min. The validation of the method was done according to the ICH guidelines for different analytical parameters. The method was found to be linear in the range of 37.5 - 225 μ g and 18.75 - 112.5 μ g/ml for epalrestat and pregabalin respectively. The assay of marketed formulation was determined and find with 99.22% and 99.07% w/v for epalrestat and pregabalin respectively. The stressed sample was analysed and this proposed method was found to be specific and stability indicating as no interfering peaks of degradation compound and excipients were noticed. So the method was simple and economical that can be applied successfully for simultaneous estimation of both epalrestat and pregabalin in bulk and combined tablet formulation.

INTRODUCTION: Epalrestat was chemically 2-[(5Z)-5-[(E)-2-methyl-3-phenylprop-2-enylidene]-4-oxo-2-sulfanylidene-1, 3-thiazolidin-3-yl] acetic acid, 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. It reduces the accumulation of intracellular sorbitol, which is believed to be the cause of diabetic neuropathy. Pregabalin was chemically (3S)-3-(aminomethyl)-5-methylhexanoic acid.

Pregabalin was used for the management of neuropathic pain associated with diabetic peripheral neuropathy or spinal cord injury and postherpetic neuralgia. Both the drugs are official in IP and USP.

The extensive literature survey revealed that RP-HPLC¹⁻¹⁰ and UV spectrophotometric methods^{11, 12, 13} and UPLC method¹⁴ were available for the determination of epalrestat and pregabalin individually or in combination with other drugs, but no method was reported for simultaneous estimation epalrestat and pregabalin in combined tablet dosage form using RP-HPLC method. The study was thus performed with an aim to develop a simple, economic, sensitive, rapid, accurate, precise and stability indicating RP-HPLC method for the determination of epalrestat and pregabalin in combined tablet dosage form.

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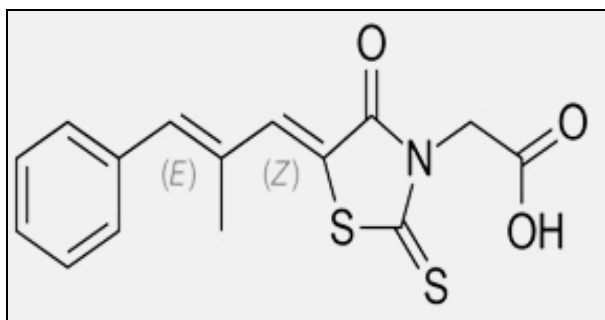


FIG. 1: STRUCTURE OF EPALRESTAT

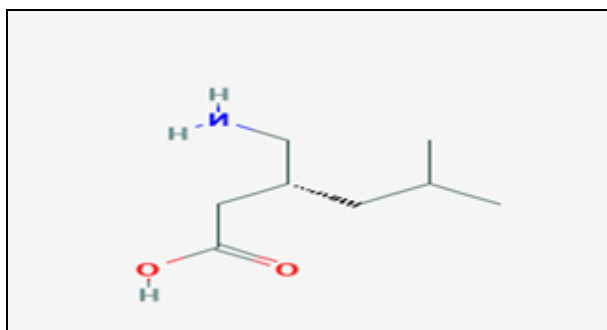


FIG. 2: STRUCTURE OF PREGABALIN

MATERIALS AND METHODS:

Instruments: Waters (2695) PDA detector HPLC using the software Empower 2.

Chemicals and Reagent: Epalrestat and pregabalin pure drugs (API), epalrestat and pregabalin tablets obtained from Spectrum labs, Hyderabad, India. Potassium dihydrogen phosphate buffer, ortho-phosphoric acid are from Rankem chemicals, Hyderabad, India. HPLC grade methanol, acetonitrile and water were procured from Merck specialties private limited, Hyderabad.

Preparation of Mobile Phase: Accurately weighed 1.36 gm of potassium dihydrogen ortho phosphate in a 1000 ml of volumetric flask, add about 900 ml of milli-Q water added and degass to sonicate and finally make up the volume with water then pH adjusted to 5.4 with dil. orthophosphoric acid solution.

Preparation of Standard Solutions: Accurately weighed 15 mg of epalrestat, 7.5 mg of pregabalin was transferred to 10 ml volumetric flasks. 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as a standard solution.

Preparation of Sample Solution: 20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 15 mg

epalrestat transferred into a 100 ml volumetric flask, 50 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluents.

Chromatographic Conditions: The chromatographic column used was STD Discovery column 250 × 4.6 mm, with 5 μm particle size. The flow rate was maintained at 1 ml/min. The detection wavelength of the method was 244 nm. The temperature was maintained at 25 °C. The injection volume was 10 μl. The run time of standard and sample was 10 minutes.

Optimization of RP-HPLC Method: The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of epalrestat and pregabalin. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Methanol, Phosphate buffer pH 5.4 (45:55 v/v) using column Discovery 250 × 4.6 mm, 5m. The results were shown in Fig. 3.

Validation of the RP-HPLC Method: Validation of the optimized method was performed according to the ICH guidelines.

System Suitability: The system suitability parameters were determined by preparing standard solutions of epalrestat (15 μg/ml) and pregabalin (75 μg/ml). 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%. The results were shown in Table 1.

Specificity: Specificity of a method was determined by testing standard substances against potential interferences. There should not find interfering peaks in the blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: Accurately weighed 10 mg of epalrestat, 1.5 mg of pregabalin and transferred to 10 ml volumetric flasks. 3/4th of diluents was added and sonicated for 10 minutes. Volumetric flask was made up with diluents and labeled as a standard stock solution. Each solution was injected in

triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficient on curves for epalrestat and pregabalin. The results were shown in **Table 2**.

Accuracy: Accuracy was carried out by % recovery studies of epalrestat and pregabalin at three different concentration levels (50%, 100%, and 150%). Percentage recovery was calculated from the amount added and the amount recovered. The percentage recovery was within the acceptance criteria, this indicates the accuracy of the method. (Acceptance criteria: % recovery between 98 to 102). The results were shown in **Table 3**.

Precision: The repeatability of the method was verified by calculating the % RSD of six replicate injections of 100% concentration (150 µg/ml of epalrestat and 75 µg/ml of pregabalin) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days. The results were shown in **Table 4**.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae $LOD = 0.78 \sigma/s$ and $LOQ = 0.18 \sigma/s$. The results were shown in **Table 5**.

Robustness: Robustness of the method were verified by altering the chromatographic conditions like flow rate, mobile phase ratio and temperature are made, but there were no recognized change in the result and all are within range as per ICH guidelines. Robustness conditions like flow minus (0.9 ml/min), flow plus (1.1 ml/min), mobile phase minus 55:45 mobile phase plus, temperature minus (25 °C) and temperature plus (35 °C) were maintained and samples were injected in duplicate manner. System suitability parameter was passed. % RSD was within the limit. The result was shown in **Table 6**.

Degradation Studies:

Acid degradation: To 1 ml of stock solution epalrestat and pregabalin, 1ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60 °C. The resultant solution was diluted to obtain 150 µg/ml and 75 µg/ml solutions and 10 µl solutions

were injected into the system and the chromatograms were recorded to assess the stability of the sample. The results were shown in **Fig. 8**.

Oxidative Degradation: 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 °C. For HPLC study, the resultant solution was diluted to obtain 150 µg/ml and 75 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. The results were shown in **Fig. 9**.

Alkali Degradation: To 1 ml of stock solution epalrestat and pregabalin, 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins at 60 °C. The resultant solution was diluted to obtain 150 µg/ml and 75 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. The results were shown in **Fig. 10**.

Thermal Degradation: 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 and 75 µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. The results are shown in **Fig. 11**.

Photo Degradation: The photochemical stability of the drug was also studied by exposing the 1500 µg/ml and 750 µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hrs/m² in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 150 µg/ml and 75 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. The results are shown in **Fig. 12**.

RESULTS AND DISCUSSION: After a number of trials with mobile phases of different composition, and mobile phase containing 0.1% ortho phosphoric acid buffer acetonitrile taken in the ratio of 45:55v/v was selected as mobile phase because of better resolution more no. of Theoretical plates and symmetric peaks. Epalrestat and

pregabalin were found to show appreciable absorbance at 244 nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of epalrestat and pregabalin.

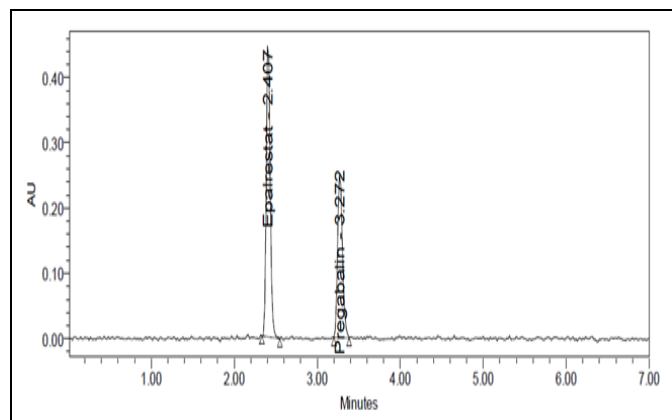


FIG. 3: OPTIMIZED CHROMATOGRAM OF EPALRESTAT AND PREGABALIN

System Suitability: 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.

TABLE 1: SYSTEM SUITABILITY PARAMETERS

Parameter	Epalrestat	Pregabalin
Retention time (min)	2.403	3.265
Theoretical plates (N)	11803	16866
Tailing factor (T)	1.14	1.21

Linearity: Concentration range of 37.5 - 225 µg/ml for epalrestat and 18.75 - 112.5 µg/ml for pregabalin were found to be linear with correlation coefficients 0.999 were within limits. The result was shown in **Fig. 4** and **5**.

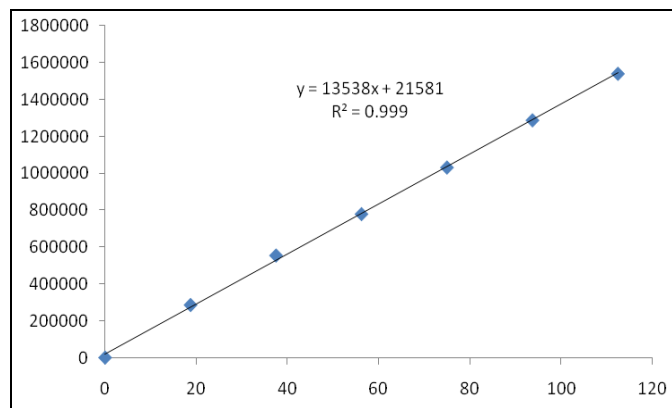


FIG. 4: CALIBRATION CURVE OF PREGABALIN

Accuracy: The Percentage accuracy was a relative standard deviation for accuracy at each level is well

within the limit. Over all the percentage recovery of the relative standard deviation was found to be 99.23% - 100.35 % for all the levels was within the limit.

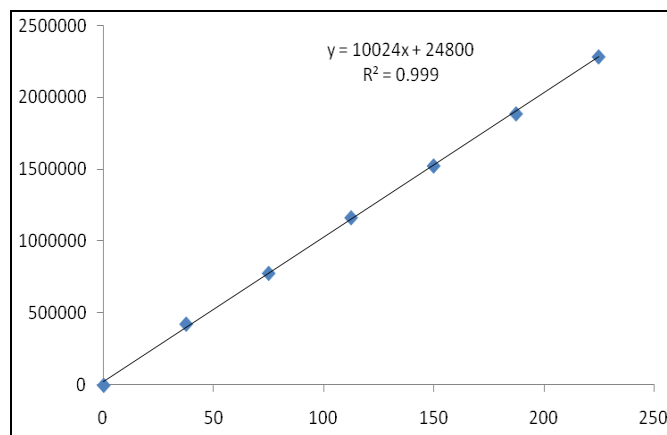


FIG. 5: CALIBRATION CURVE OF EPALRESTAT

TABLE 2: RESULTS FOR LINEARITY

Parameter	Epalrestat	Pregabalin
Y intercept	10024	21581
Correlation coefficient r ²	0.999	0.999
Regression Equation	10024x + 24800	13538x + 21581
Linearity range	37.5 - 225 µg/ml	18.75 - 112.5 µg/ml
LOD	0.78	2.38
LOQ	0.18	0.55

Precision: Percentage relative standard deviation of six results was within the limit. Results shown good degree of precision was found to be 0.7% and 0.3%.

Limit of Detection: Limit of detection of target assay concentration of epalrestat and pregabalin by using formula method 0.78 µg/ml and 0.18 µg/ml within the limits. The results were shown in **Fig. 6**.

Limit of Quantification: Limit of quantification of the target assay concentration of epalrestat and pregabalin by using formula method .38 µg/ml and 0.55 µg/ml were within the limits. The results were shown in **Fig. 7**.

Robustness: To check keeping the ratio of mobile phase constant, the chromatograms of drug solution were recorded with different flow rates such as 0.8 ml/min, 1.2 ml/min the peaks were sharp with good resolution. The parameters like % RSD of peak area, tailing factor and theoretical plates showed were within the limit.

TABLE 3: RESULTS FOR ACCURACY

Recovery level	Epalrestat			Pregabalin		
	Amount added (µg/ml)	Amount found	%Recovery (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery
50%	75	74.42	99.23	37.5	36.85	98.26
100%	150	147.81	98.54	75	74.75	99.66
150%	225	221.37	98.39	112.5	111.59	99.19
Mean recovery			99.07%			99.22%

TABLE 4: RESULTS OF PRECISION

Drug	Interday Precision (% RSD)	Method Precision (% RSD)
Epalrestat	0.7	1.0
Pregabalin	0.3	0.4

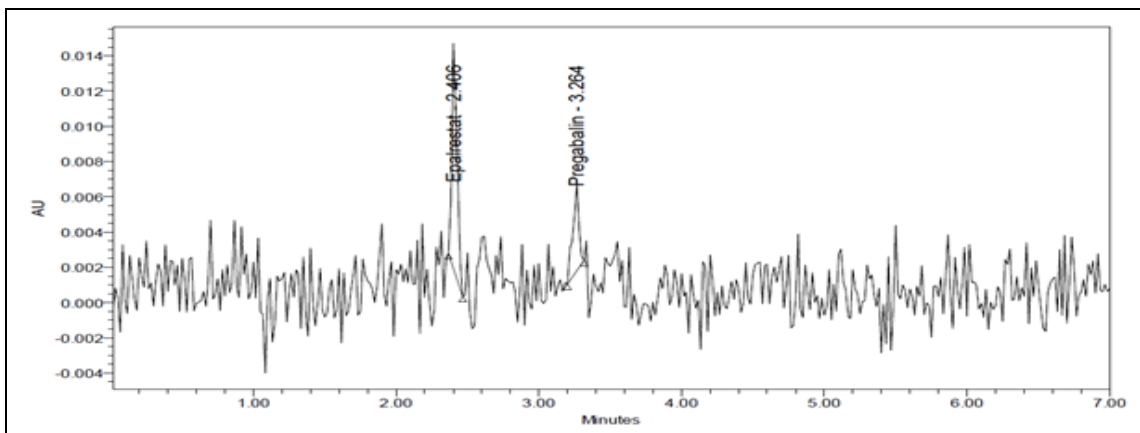


FIG. 6: LOD OF EPALRESTAT AND PREGABALIN

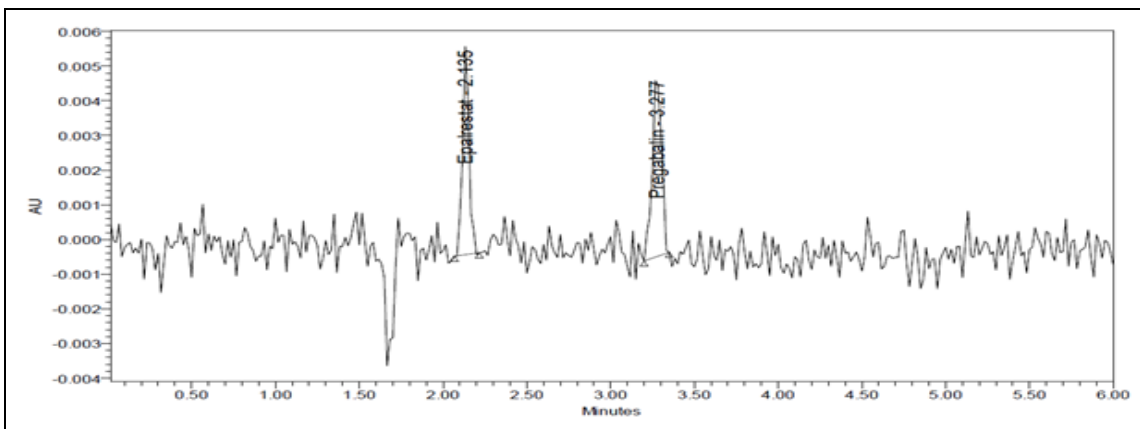


FIG. 7: LOQ OF EPALRESTAT AND PREGABALIN

TABLE 5: RESULTS FOR LOD AND LOQ

S. no	Drug	LOD (µg/ml)	LOQ (µg/ml)
1	Epalrestat	0.78	2.38
2	Pregabalin	0.18	0.55

TABLE 6: RESULTS FOR ROBUSTNESS

Parameters	% RSD	
	Epalrestat	Pregabalin
Detection wavelength at 244		
Flow rate 0.8 ml/min	0.5	0.5
Flow rate 1.2 ml/min	0.6	0.2
Mobile phase 55:45	1.3	1.6
Mobile phase 45:55	0.9	0.2

Forced Degradation Study: Degradation studies indicated the specificity of the developed method in the presence of degradation products. Degradation was carried out in combination of two drugs and purity of drug peaks was confirmed by purity angles. Their combination drug products were

exposed to acid, alkali, oxidative and thermal stress conditions. Then found to be no degradable substances presence and proved that the proposed method was stable towards acid, alkali, peroxide and thermal conditions within the limits.

TABLE 7: RESULTS FOR STABILITY STUDIES OF EPALRESTAT AND PREGABALIN

Parameters	Peak Area		% of degradation	
	Epalretat	Pregabalin	Epalretat	Pregabalin
Acid degradation	1464612	911542	4.94	4.60
Alkaline degradation	1492487	919909	2.99	2.83
Peroxide degradation	1412923	917590	1.57	1.86
Thermal degradation	1428187	914975	0.51	0.69
Photo Degradation	1442653	919230	0.54	0.57

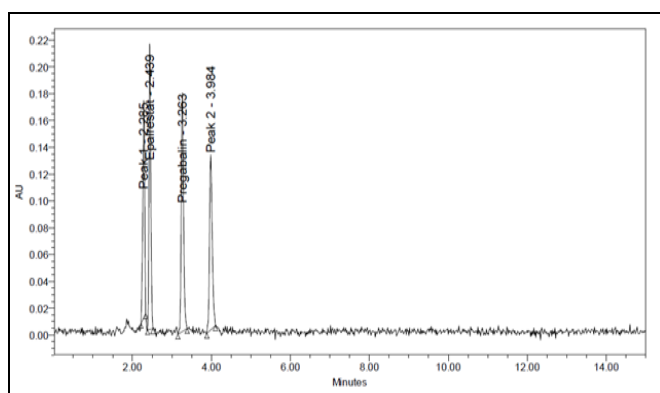


FIG. 8: ACID DEGRADATION

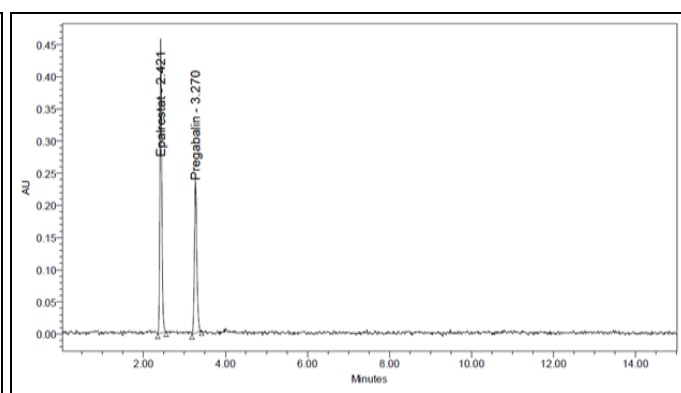


FIG. 9: ALKALINE DEGRADATION

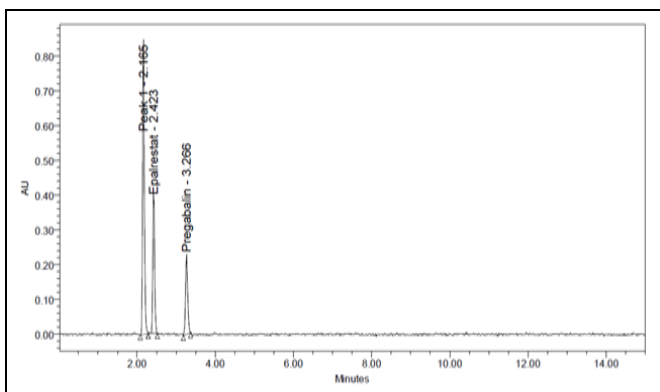


FIG. 10: PEROXIDE DEGRADATION

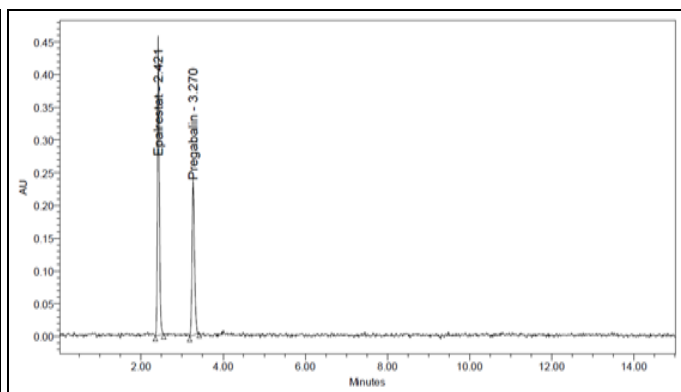


FIG. 11: THERMAL DEGRADATION

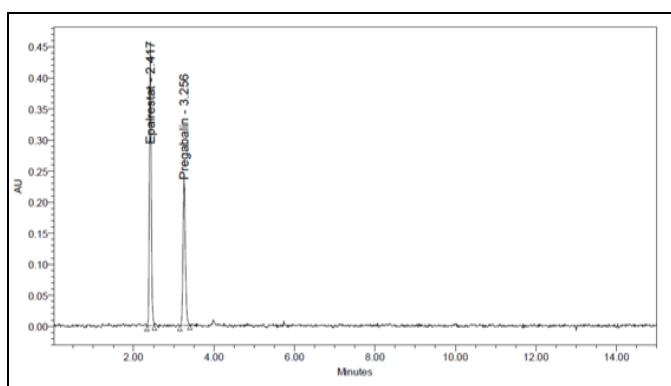


FIG. 12: PHOTO DEGRADATION

CONCLUSION: The RP-HPLC method developed and validated allows a simple and rapid quantitative determination of epalrestat and pregabalin in bulk and tablet dosage forms. All the validation parameters were found to be within the limits according to ICH guidelines.

The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the short retention times allows the analyst to analyze no. of samples in a short period and method was found to be simple, accurate, precise, rugged, robust and stable under forced degradation stress conditions. So the established method can be successfully applied for the routine analysis of the marketed formulations.

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CONFLICT OF INTEREST: Nil

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