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Development and validation of RP-UPLC method for the simultaneous estimation of pregabalin and epalrestat in pharmaceutical dosage form

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Abstract
In our present investigation we have developed an accurate procedure to estimate pregabalin and epalrestat in tablet dosage form. Chromatogram column was used HSS (100mmx2.1mm, 1.8µ). Orthophosphoric acid (0.1%): acetonitrile 65:35 v/v was used as mobile phase and it was pumped through at a flow rate of 0.5 ml/min. Temperature was constant at 25°C. Wavelength was selected at 210 nm. Chromatographic retention time of pregabalin and epalrestat were observed at 1.100 min and 1.841 min. Percentage of recovery was acquired as100.25% and 100.68% for pregabalin and epalrestat. Limit of Detection (LOD) were found 0.02 and 0.11 respectively for pregabalin and epalrestat and limit of Quantitation (LOQ) were found 0.06 and 0.33 for pregabalin and epalrestat respectively. Results of analysis were validated statistically. The results of the study showed that the proposed RP-UPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of pregabalin and epalrestat in tablet dosage form.

Keywords: pregabalin, epalrestat, RP-UPLC, OPA (0.1%)

Introduction

Pregabalin was used to relieve neuropathic pain[1] (pain from damaged nerves) that can occur in arms, hands, fingers, legs, feet, or toes. Pregabalin extended-release tablets are usually taken once daily after an evening meal. Take pregabalin at around the same time(s) every day. The pharmacology of pregabalin requires binding to alpha$_2$-delta subunits[2], including structure-activity analyses of compounds binding to alpha$_2$-delta subunits. Structurally name is (S)-3-(aminomethyl)-5-methylhexanoic acid and mass is 159. Less than 2% of pregabalin is metabolized and it is excreted virtually unchanged in the urine (Fig 1). Epalrestat is used for the treatment of nerve damage due to diabetes called diabetes neuropathy [3]. Structurally name is ((5Z)-5-[(2E)-2-Methyl-3-phenyl-2-propen-1-ylidene]-4-xo-2-thioxo-3-thiazolidineacetic acid and mass is 319. It inhibits the enzyme aldose reductase and reduces the level of sorbitol, which damages the nerves and reversed the renal accumulation of the polyol pathway metabolites of sorbitol and fructose, and increased myo-inositol level[4]. It is soluble in organic solvents such as DMSO and dimethyl formamide (DMF), sparingly soluble in aqueous buffers (Fig 2).In literature,a very fewmethods were developed to estimate combination of pregabalin and epalrestat in tablet dosage form by HPLC [5-11] and only one effort was done by UPLC[12]. In this investigation we have developed an optimized method to estimate such combination in tablet dosage forms are reported for the analysis of combination of various drugs [13].

Experimental

Chemicals and reagents

Epalrestat and Pregabalin pure drugs were supplied by zyduscadila Ltd., Ahmedabad. Acetonitrile, methanol, ortho-phosphoric acid and HPLC grade water were purchased from Rankem, Hyderabad, India. Combination of Epalrestat and Pregabalin tablets (Pre-Aldonil) were purchased from local market.

Instrument

UPLC analysis was performed on WATERS, acquity software: Empower 2, 2695 separation module equipped with auto Sampler and UV detector (2487) and column HSS (100mmx2.1mm, 1.8µ). A manually operating Rheodyne injector with 10 µL sample volume was equipped with the UPLC system. In addition, an electronic analytical weighing balance (Sartorious), digital pH meter (Adwa – AD 1020), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (LABINDIA UV 3000+) were used in this study.

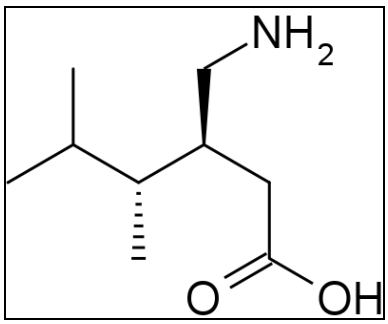

Fig 1: Molecular structure of Pregabalin

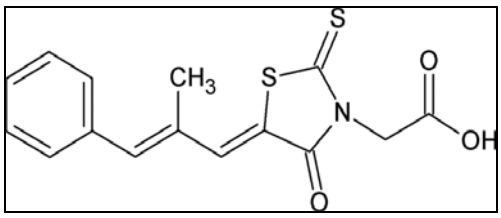

Fig 2: Molecular structure of Epalrestat

Materials and Methods

Selection of wavelength

Suitable wavelength for the UPLC analysis was determined by recording UV spectrums in the range of 200-400 nm for individual drug solutions of pregabalin and epalrestat. Suitable experimental wavelength was closed to 210 nm. At this wavelength both the drugs show good absorbance.

Chromatographic Conditions

The separation of the drugs was achieved on a HSS column, (100mmx2.1mm, 1.8µ). The mobile phase consists of a mixture of Orthophosphoric acid (0.1%): acetonitrile in the ratio of 65:35 v/v. The mobile phase was set at a flow rate of 0.5 ml/min and the volume injected was 10 µl for every injection. The detection wavelength was set at 210 nm.

OPA (0.1%) buffer preparation

Accurately 1 ml of ortho phosphoric acid (OPA) in a 1000 ml of volumetric flask added about 900ml of milli-Q water added and degasses to sonicate and finally make up the volume with water.

Mobile phase preparation

Mix above buffer 650 ml and 350 ml Acetonitrile and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Preparation of Stock and Working Standard Solution

Accurately weighed and transferred Epalrestat 15 mg, Pregabalin 15 mg and working standards into a 10ml clean dry volumetric flasks respectively, add 5ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1 ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluents.

Preparation of Stock and Working Sample Solution

Five tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to one tablet was transferred into a 10ml volumetric flask and made up with diluents and labelled as sample stock solution. Sample stock solution was filtered by PVDF 0.45um filters. 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluents.

Table 1: Chromatographic conditions

Flow rate	0.5 ml/min
Column	HSS (100mmx2.1mm, 1.8µ)
Detector wavelength	210nm
Column oven	Ambient
Injection volume	5 µl
Run time	3 min

Results and Discussion

Method development

A Reverse phase UPLC isocratic method was developed keeping in mind the system suitability parameters i.e. resolution factor (R_f) between peaks, tailing factor (T) and number of theoretical plates (N), runtime The optimized method developed resulted in the elution of Pregabalin at 1.100 min and Epalrestat at 1.841 min at shown in Figure 3.The total run time is 3 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), area (µV sec), peak resolution (Rf) and peak Tailing factor (T) were evaluated for six replicate injections of the standards at working concentration. Results given in Table 2 were within acceptable limits.

Table 2: System suitability parameters

Parameters	Required limits	Pregabalin	Epalrestat
Retention time (Rt)	% RSD<1%	1.172	1.844
Area (µV sec)	Not less Than 2	356033	617285
peak resolution (Rf)	Not less Than 2000	2300	3924
peak Tailing factor (T)	Not More Than 2	1.31	1.33

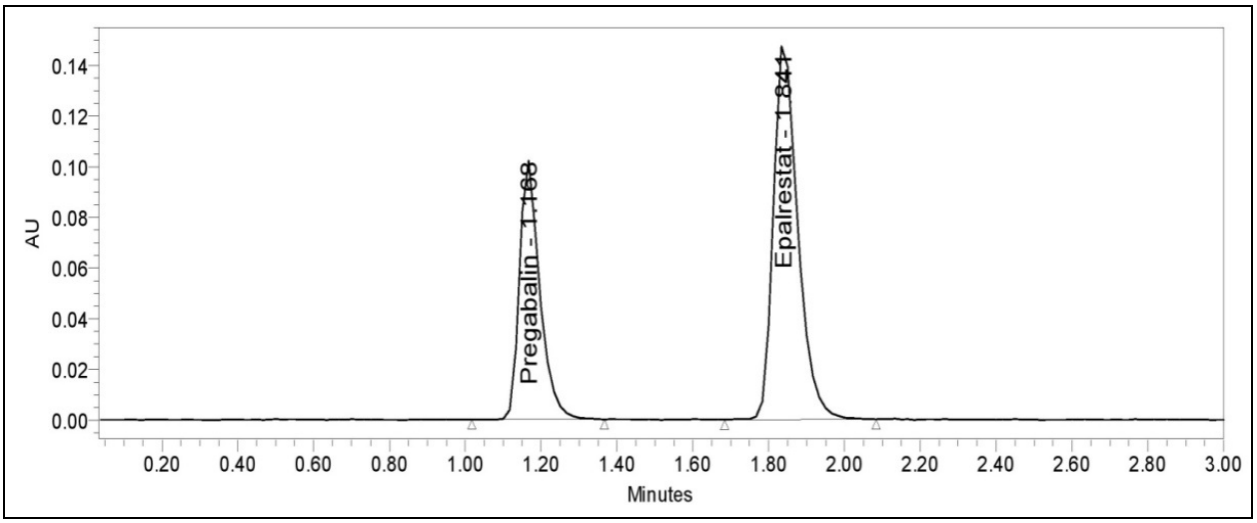

Fig 3: Typical Chromatogram for the Mixture of Standard Solutions

In order to test the applicability of the method developed to a commercial formulation (marketed tablet, Pre-aldonil), was chromatographed and it is represented in figure 3. The sample peaks were identified by comparing the relative retention times with the standard drugs mixture, figure 4. System suitability parameters were ideal for the

chromatographed sample. Integration of separated peak area was done and each drug was determined by using the peak area-concentration relationship obtained in the standardization step. The protocol affords reproducible quantification of the two drugs with error less than 10%, which is the standard level in any pharmaceutical quality control.

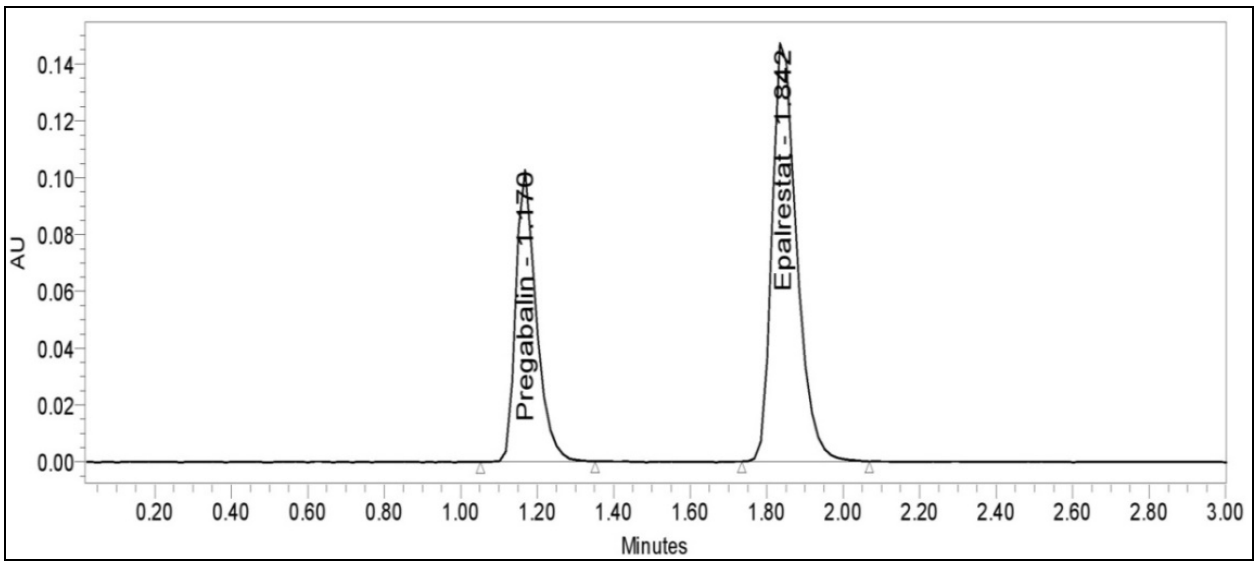

Fig 4: Typical Chromatogram for the Mixture of sample Solutions

Method Validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-UPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines[14] for validation of analytical procedures. The method was validated in terms of parameters like system suitability, selectivity, linearity, accuracy, precision, ruggednessand robustness, limit of detection (LOD) and limit of quantitation (LOQ).

System precision

System Precision Six replicate injections of the mixture of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak areas for the two drugs, which indicates the acceptable reproducibility and thereby the precision of the system.

Repeatability

Six consecutive injections of the sample at working concentration showed % RSD less than 2 concerning peak areas for all the two drugs which indicate the method developed is method precise by the test of repeatability and hence the method should give consistently reproducible results.

Linearity

Standard solutions of pregabalin and epalrestat of different concentrations level (25%, 50%, 75%, 100%, 125% and 150%) were prepared in triplicate. Calibration curves were constructed by plotting the % concentration levels of drugs versus corresponding mean peak area. The results show an excellent correlation exists between mean peak area and % concentration levels of drugs within the concentration range of Epalrestat (1500 µg/ml) and Pregabalin (750 µg/ml) and the results were given in table 3 and figure 5-10. The correlation coefficients of pregabalin and epalrestat are greater than 0.999, which meet the method validation acceptance criteria and hence the method developed is said to be linear in the range of Pregabalin (750 µg/ml) and Epalrestat (1500 µg/ml).

Table 3: Linearity of the pregabalin and epalrestat

Pregabalin			Epalrestat		
S. No.	**Concentration**	**Peak area (mv)***	**S. No.**	**Concentration**	**Peak area (mv)***
1	37.5	96105	1	37.5	170479
2	75	191074	2	75	317399
3	112.5	292912	3	112.5	504348
4	150	380676	4	150	657576
5	187.5	474924	5	187.5	829128
6	225	575982	6	225	984026

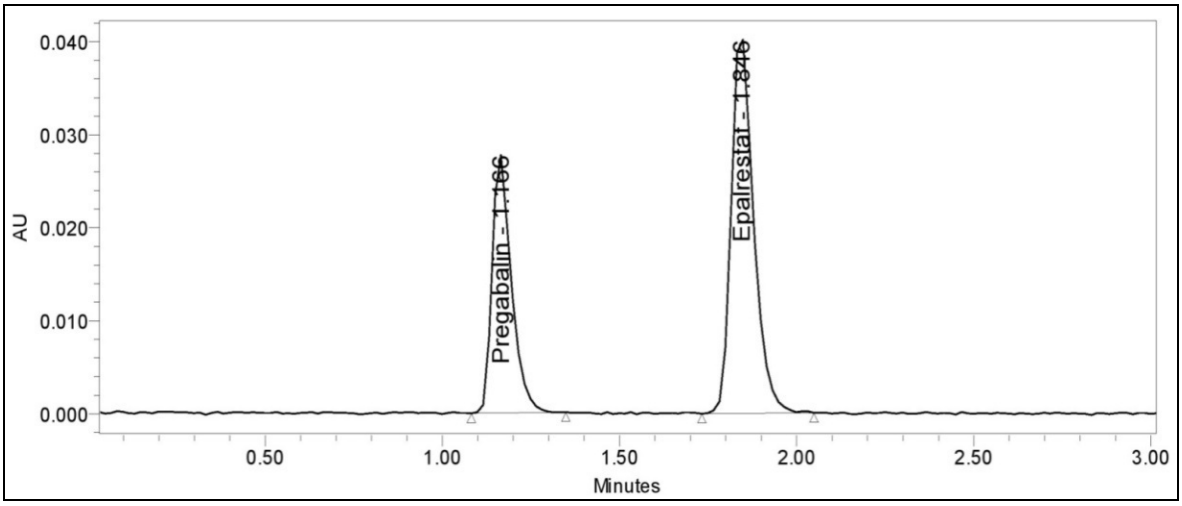

Fig 5: chromatogram for linearity 25% level

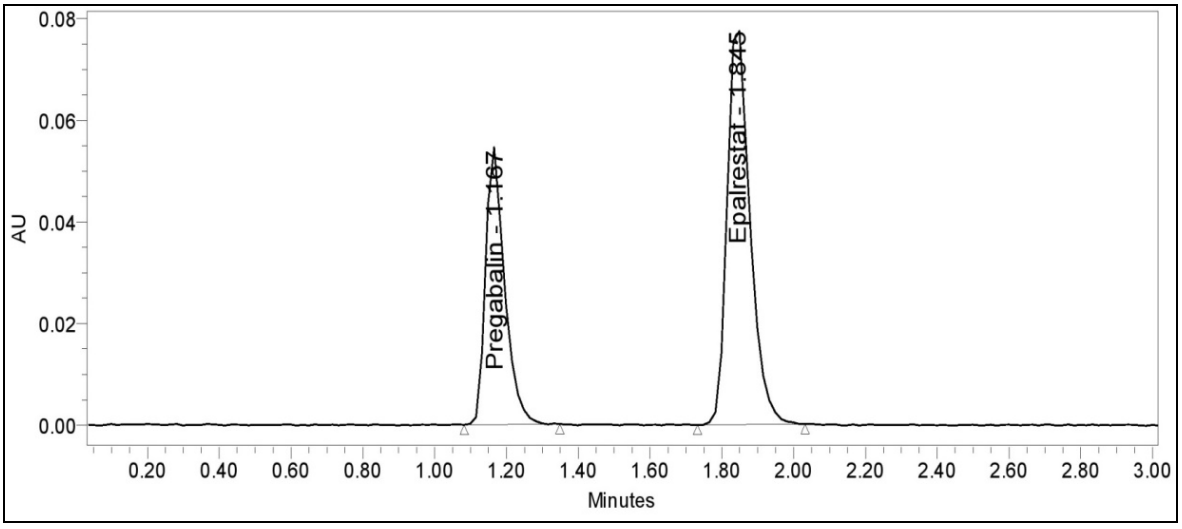

Fig 6: chromatogram for linearity 50% level

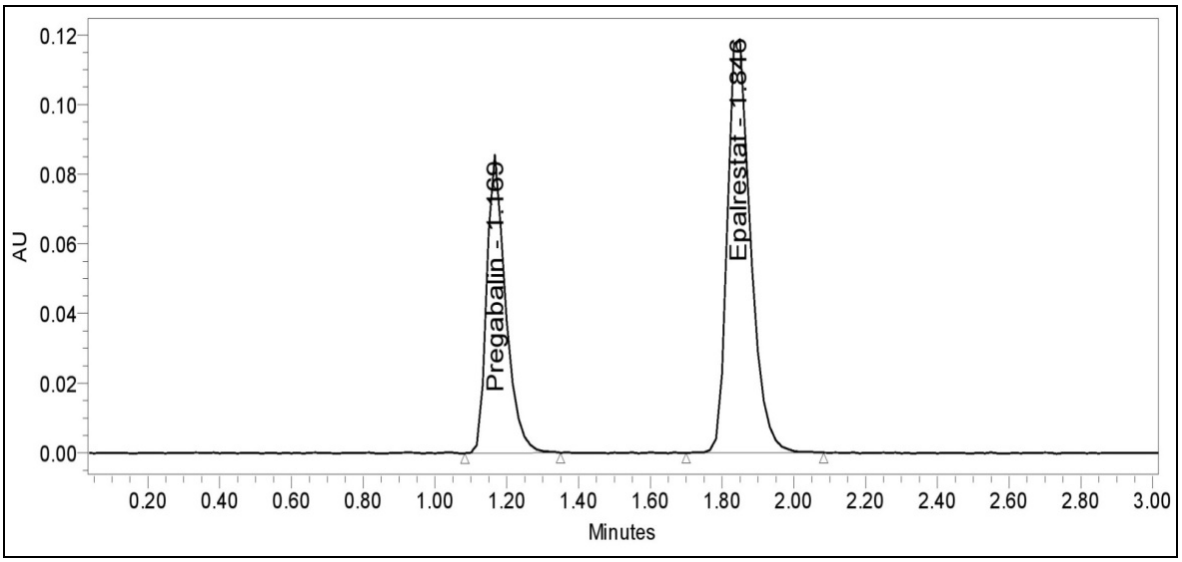

Fig 7: chromatogram for linearity 75% level

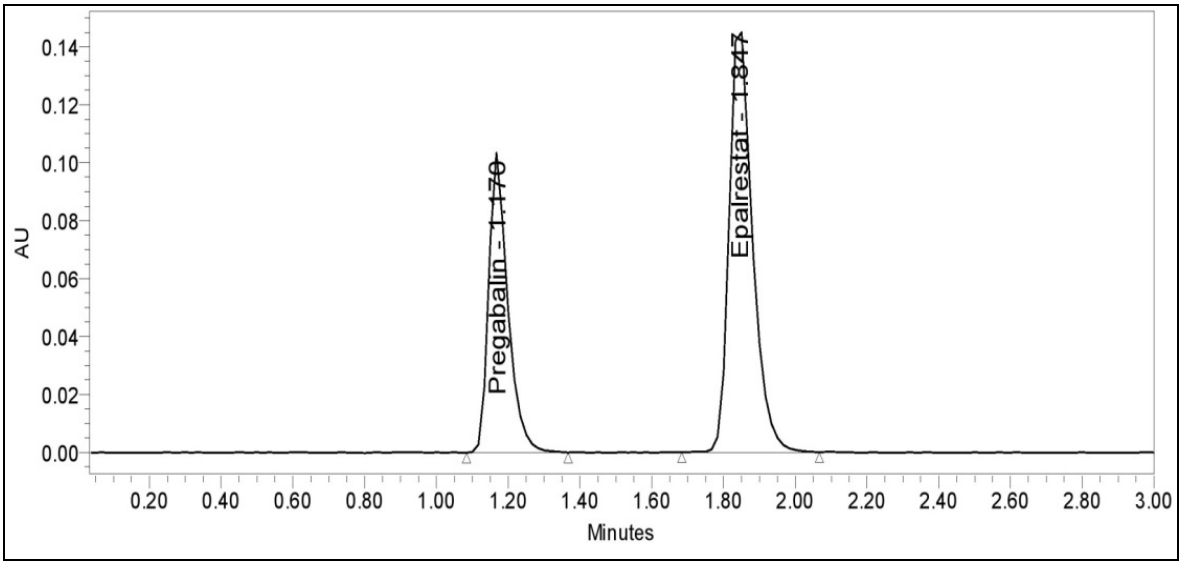

Fig 8: chromatogram for linearity 100% level

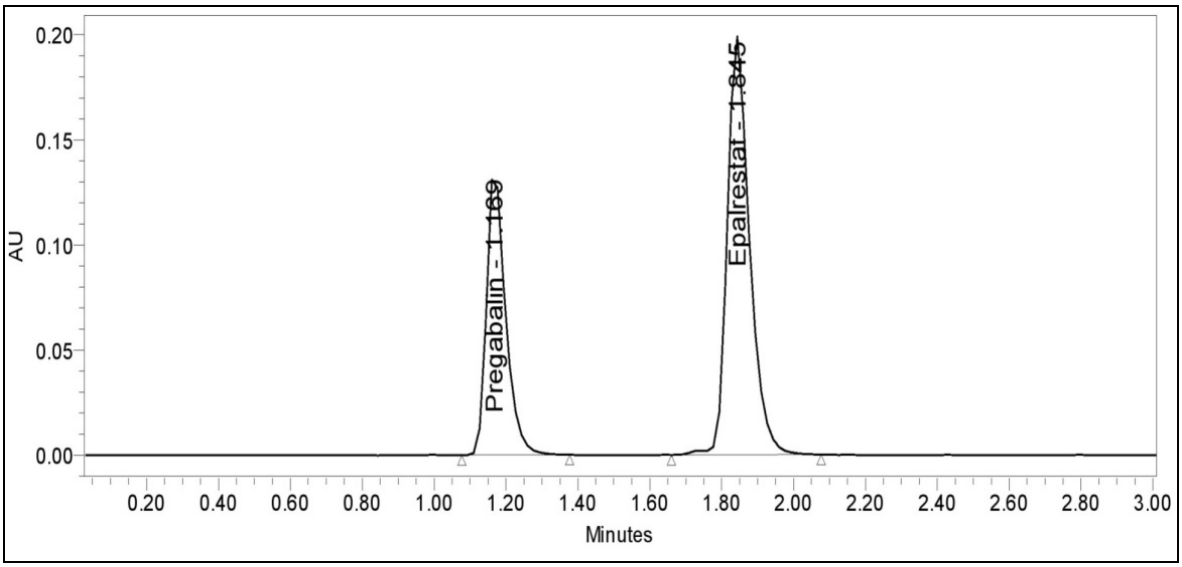

Fig 9: chromatogram for linearity 125% level

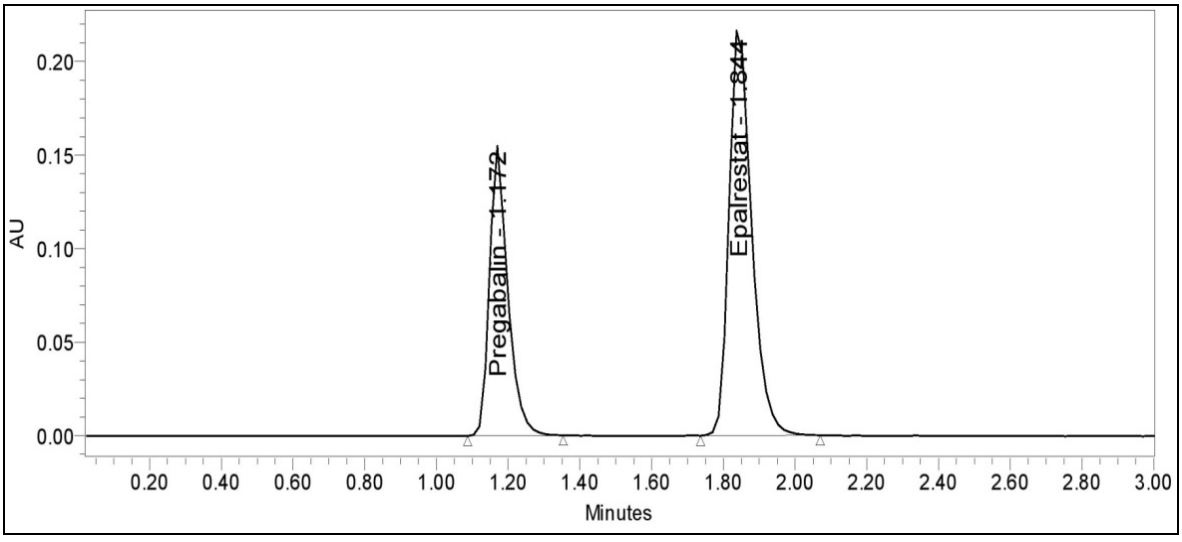

Fig 10: chromatogram for linearity 150% level

Accuracy

Accuracy was determined by means of recovery experiments, by the addition of active drug to preanalyzed sample at different spiked levels (50-150%). At each level, three determinations were performed and results obtained. The accuracy was calculated from the test results as the

percentage of the analyte recovered by the assay. The amounts recovered, values of percent mean recovery were calculated as shown in Tables 3 and 4. The accepted limits of recovery are 98.28 %-101.79 % and all observed data are within the required range that indicates good recovery values and hence the accuracy of the method developed. Mean % of recovery is 100.25% for Pregabalin and 100.68% for Epalrestat.

Table 4: Accuracy (recovery) data for pregabalin

% Concentration (at specification level)	Amount spiked (µg)	Amount found (µg)	% Recovery	Mean recovery
50%	75	75.06	100.09%	100.25%
100%	150	150.15	100.10%	
150%	225	226.29	100.57%	

Table 5: Accuracy (recovery) data for epalrestat

% Concentration (at specification level)	Amount spiked (µg)	Amount found (µg)	% Recovery	Mean recovery
50%	75	75.23	100.31%	100.68%
100%	150	151.22	100.82%	
150%	225	227.03	100.90%	

Sensitivity

The sensitivity of measurement of pregabalin and epalrestatby use of the proposed method was estimated in terms of LOQ) and LOD. The limit of detection (LOD) was obtained as 0.02µg/ml for pregabalin and 0.11µg/ml for Epalrestat. The limit of quantitation (LOQ) was obtained as 0.06µg/ml for pregabalin and 0.33µg/ml for Epalrestat. The sensitivity results were given in table 6.

Table 6: Sensitivity Table of pregabalin and epalrestat

Sample	LOD	LOQ
Pregabalin	0.02	0.06
Epalrestat	0.11	0.33

Assay

Pre-Aldonil, bearing the label claim Epalrestat 150 mg, Pregabalin 75 mg. Assay was performed with the above formulation. Average % of assay for pregabalin and epalrestat obtained was 98.87% and 100.5% respectively. The results were given in table 7.

Table 7: Assay Data of Pregabalin and Epalrestat formulation

Drugs in formulation	Label claim	Amount found	Assay %
Pregabalin	75mg	74.9 mg	99.87
Epalrestat	150mg	150.81	100.5

Conclusion

A reverse phase UPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, robustness, limit of detection and limit of quantitation, for the simultaneous quantitative estimation of pregabalin and epalrestat in tablets. A good linear relationship was observed for the two drugs between concentration ranges of 37.5 µg/ml and225 µg/ml. The correlation coefficients were greater than 0.999 for two drugs. The inter day and intraday precision results were goodenough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recoveries after spiking experiments were between 100.25% and 100.68% an indicative of accurate method. Accordingly it can be concluded that the developed reverse phase UPLC isocratic method is accurate, precise, linear, and robust. Accordingly, the method can be used for the routine analysis of pregabalin and epalrestat in tablets.

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