e-ISSN: 2248-9126
Vol 12 | Issue 2 | 2022 | 17-24. Print ISSN: 2248-9118

Indian Journal of Pharmaceutical Science & Research

www.ijpsrjournal.com

DEVELOPMENT OF ANALYTICAL METHOD FOR SIMULTANEOUS DETERMINATION OF PREGABALIN AND EPALRESTAT BY USING RP-HPLC

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ABSTRACT

The organic phase concentration required for the mobile phase can be estimated by gradient elution method. For aqueous sample mixtures, the best way to start is with gradient reversed phase chromatography. Gradient can be started with 10% organic phase in the mobile phase and organic phase concentration (Methanol & Acetonitrile) can be increased up to 100% within 20-60 min. Separation can then be optimised by changing the initial mobile phase composition and the slope of gradient according to the chromatogram obtained from preliminary run. The initial mobile phase composition can be estimated on the basis of where the compounds of interest were eluted, namely, at what mobile with composition. Changing the polarity of the mobile phase can alter elution of drug molecules. The elution strength of a mobile phase depends upon its polarity, the stronger the polarity, higher is the elution. Ionic samples (acidic or basic) can be separated, if they are present in un-dissociated form. Dissociation of ionic samples may be suppressed by proper selection of pH.

Keywords: Simultaneous Determination, RP-Hplc, Method development.

INTRODUCTION

Knowledge of physicochemical properties of API is invaluable to the method development process [1]. The information should be in support of the drug discovery (organic chemistry synthesis) or from company drug profiles, spectral libraries and reports. Information such as solubility, dissociation constants, partition co efficient, Spectrophotometric properties, fluorescent properties (if any fluorophore present) chromatographic behavior, oxidation-reduction potential, formulation stability studies and solubility studies are all very useful and can expedite the development process. These studies are performed on the drug substance in solution and mixed with the pharmaceutical excipients as part of compatibility studies. The functional groups, which are labile, are identified and susceptibility of the drug to hydrolysis, oxidation, thermal degradation etc. are determined [2].

The purpose of this step is to prepare the sample so that the drug substance can be readily chromatographed and separated from other materials. Thus, it is a step to remove may interference, to enhance the detection of the drug substance as well as to protect or enhance the life of the analytical column [3].

Solid-phase extraction has become a recognized and viable technique for sample preparation methodologies, especially for biological samples; and as an alternative to liquid-liquid extractions by U.S Environmental Protection Agency (EPA) [4].

Drug Profile

Epalrestat

Drug Name: Epalrestat
Class: Synthetic prostaglandin analogue
Chemical Name: 3-Thiazolidineacetic acid,5-(2-methyl-3-phenyl-2-propenylidene)-4-oxo-2-thioxo-,(E,E)-5-((1Z,2E)-2-Methyl-3- phenylpropenylidene) -4-oxo-2-thioxo-3-thiazolidineacetic acid.
Chemical Formula: $C_{15}H_{13}NO_3S_2$
Molecular weight : 319.393g/mol.
Solubility: Soluble in water and organic solvents
Category: Anti glaucoma agent

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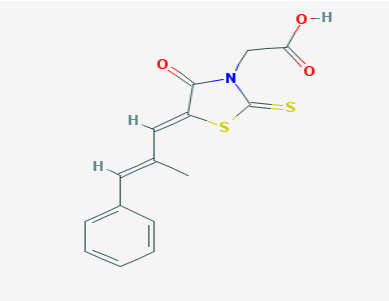
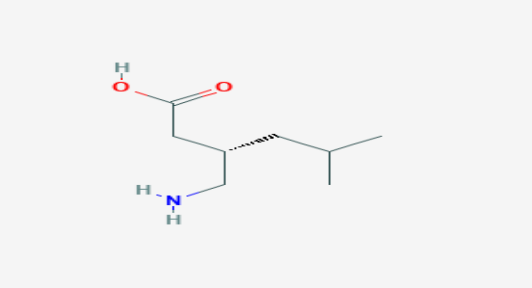
Chemical Structure



Mechanism of action: Aldose reductase inhibitor used for treatment of diabetic nephropathy [5].
Absorption: It is easily absorbed in to the neural tissue and inhibits the enzyme with minimum side effects.
Metabolism and Elimination: It is an isopropyl ester prodrug, is hydrolyzed by esterases in the cornea to its biologically active free acid. Systemically, Travoprost free acid is metabolized to inactive metabolites via beta-oxidation of the α(carboxylic acid) chain to give the 1,2-dinor and 1,2,3,4-tetranor analogs, via oxidation of the 15-hydroxyl moiety, as well as via reduction of the 13,14 double bond.
Adverse Effects: Nausea, vomiting, diarrhea. Generalized gastric discomfort, cutaneous reactions including erythema, bullae and skin blistering.

Pregabalin
Drug Name: Pregabalin
Class: GABA analogue and gabapentinoid
Chemical Name: (3S)-3-(aminomethyl)-5-methyl hexanoic acid.

Chemical Structure



Chemical Formula: $C_8H_{17}NO_2$
Molecular weight: 159.229g/mol.
PKa value: 4.2 -10.6
Solubility: It is not solvated. It is non -hygroscopic, thermally stable, and soluble in water.
Mechanism of action: Pregabalin binds to auxiliary subunit of voltage gated calcium channels in the central nervous system, potently displacing [^{3}H]-gabapentin. Binding of pregabalin to the alpha$_2$ site is required for analgesic and anticonvulsant activity. Pregabalin reduces the release of several neuro transmitters, including glutamate, noradrenaline [6].
Absorption: Bioavailability is 91%
Metabolism: 98% of the radioactivity recovered in the urine was unchanged pregabalin. The major metabolite is N-methylpregabalin.
Adverse effects: Very common (<10% of patients): dizziness, drowsiness. Common (1-10% of patients): blurred vision, diplopia, increased weight gain, euphoria, vivid dreams, abnormal coordination memory impairment, abnormal walking.

MATERIALS AND METHODS

INSTRUMENTS USED

Name	Manufacturer or Supplier
UV-Visible Spectrophotometer software	Vision Pro
HPLC software	Spin chrome (LC SOLUTIONS)
HPLC	Shimadzu(LC 20 AT VP)
Ultra Sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	Inertsil ODS 3V(250x4.6mm) 5µm
UV-Visible Spectrophotometer	Nicolet evolution 100

METHOD DEVELOPMENT

RP-HPLC Method

A new RP-HPLC method was developed for the determination of Epalrestat and Pregabalin in pharmaceutical formulation. The HPLC method was then validated to indicate that the analytical procedure used is suitable for intended use by using various parameters like specificity, linearity, precision, accuracy, range, robustness, system suitability [7].

Selection of chromatographic conditions:

a. Selection of column:

Based on literature review survey initially different C_{18} column is tried for selected drugs and quality of peaks were observed for the drugs. Finally the column was fixed upon the satisfactory results of various system suitability parameters such as retention time, column efficiency, tailing factor, peak asymmetry of the peaks.

b. Selection of Diluent:

Mobile phase is used as diluent.

c. Selection of mobile phase:

Various trials were taken for selection of mobile phase and further optimization is performed based on the literature survey. For convenience and easy of the study a

single mobile phase along with single chromatographic conditions for the two mentioned drugs were tried.

d. Selection of mode of separation:

The selection of method depends upon the nature of the sample, its molecular weight and solubility. The drug selected in the present study was polar in nature and hence RP-HPLC method was preferred because of its suitability.

e. Preparation of mixed standard solution

15 mg of Epalrestat and 7.5 mg of Pregabalin was weighed accurately and transferred into 100 ml of volumetric flask and dissolve in 50 ml of mobile phase and make up the volume with mobile phase. From above stock solution 15 µg/ml of Epalrestat and 7.5 µg/ml of Pregabalin is prepared by diluting 1ml to 10ml with mobile phase.

Chromatographic conditions

Mobile phase: Water (pH 4): Methanol: Acetonitrile
pH: 4
Ratio: 60:20:20
Column: Inertsil ODS, (250×4.6× 5µ)
Temperature: 300C
Wavelength: 239 nm
Flow rate: 1ml/min

Preparation of mobile phase

HPLC grade water has been taken and PH was adjusted to 4 using OPA (60%), Methanol (20 %) and Acetonitrile (20 %) has been mixed together then was degassed and filtered through vacuum filter.

Diluent Preparation

Mobile phase was used as diluent.

Preparation of Epalrestat and Pregabalin solution

15 mg of Epalrestat and 7.5 mg of Pregabalin was weighed accurately and transferred into 100 ml of volumetric flask and dissolve in 50 ml of mobile phase and make up the volume with mobile phase. From above stock solution 15 µg/ml of Epalrestat and 7.5 µg/ml of Pregabalin is prepared by diluting 1ml to 10ml with mobile phase.

Preparation of sample solution

20 tablets (each tablet contains 150 mg of Epalrestat and 75 mg of Pregabalin) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. A weight equivalent to 15 mg of Epalrestat and 7.5 mg of Pregabalin was weighed accurately and transferred into 100 ml of volumetric flask and dissolve in 50 ml of mobile phase and make up the volume with mobile phase. From above stock solution 15 µg/ml of Epalrestat and 7.5 µg/ml of Pregabalin is prepared by diluting 1ml to 10 ml with mobile phase.

METHOD VALIDATION

ACCURACY

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150% [8].

Acceptance criteria: The % recovery of Epalrestat and Pregabalin should lie between 98% and 102%.

PRECISION

Method precision

Prepared sample preparations of Epalrestat and Pregabalin as per test method and injected 6 times in to the column [9].

Acceptance criteria: The % Relative standard deviation of assay preparations of Epalrestat and Pregabalin should be not more than 2.0%.

LINEARITY AND RANGE

Preparation of standard stock solution

Standard stock solutions of Epalrestat and Pregabalin (µg/ml) were prepared by dissolving 15 mg of Epalrestat and 7.5 mg of Pregabalin in 100 ml diluents. Further dilutions are made so as to get 7.5 – 22.5 µg/ml concentrations of Epalrestat and 3.25-10.75 µg/ml of Pregabalin solutions in five levels.

LIMIT OF DETECTION [10]

The minimum concentration at which analyte can be detection can be determined from linearity curvr by applying the following formula.

LOD can be calculated by the formula:

$$\mathbf{LOD = 3.3\ SD/S}$$

Where,
SD - Standard Deviation
S – Slope of calibration curve

LIMIT OF QUANTITATION [11]

Limit of quantitation is the lowest concentration of the substance that can be estimated quantitatively. Limit of quantitation can be determined from linearity curve by applying the following formula;

$$\mathbf{LOQ = 10\ SD/S}$$

Where,
SD - Standard Deviation
S – Slope of calibration curve

ROBUSTNESS

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.
Acceptance criteria: The system suitability should pass as per the test method at variable conditions.

ASSAY OF EPALRESTAT AND PREGABALIN

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.13% and 100.60% for chlorpheniramine **maleate** and Dextromethorphan respectively.

$$\%Assay = \frac{\text{Sample area}}{\text{Std area}} \times \frac{\text{Std dilution}}{\text{Sample dill`n}} \times \frac{\text{Average Wt}}{\text{Label claim}} \times \frac{\text{Potency}}{100}$$

RESULTS AND DISCUSSION

Table 1. Precision

EPALRESTAT			PREGABALIN		
S.No.	**Rt**	**Area**	**S.No.**	**Rt**	**Area**
1	2.917	765.269	1	3.993	2012.580
2	2.920	760.464	2	3.993	2009.765
3	2.923	768.530	3	3.990	2023.634
4	2.923	763.825	4	3.987	2028.946
5	2.917	762.172	5	3.990	2031.510
6	2.930	775.496	6	4.010	2035.765
Avg.	2.921667	765.9593	**Avg.**	3.993833	2023.700
STD	0.004885	5.42459	**STD**	0.008232	10.50642
%RSD	0.0016	0.0070	**%RSD**	0.00206	0.00519

Table 2. Accuracy

S.No	Spiked level	Pre analysed sample conc. (µg/ml)	Amount added (µg/ml)	Total amount found (µg/ml)	%Recovery	% Mean
1	50 %	7.5	7.51 7.50 7.51 7.51 7.50 7.50	7.52 7.51 7.49 7.50 7.49 7.50	100 100 102 100 102 100	**101**
2	100 %	15	15.00 15.01 15.00	15.1 15.0 14.9	101 100 100	**100**
3	150 %	22.5	22.49 22.50 22.51 22.50 22.49 22.50	22.48 22.49 22.51 22.49 22.50 22.49	98 98 98 99 99 98	**98**

Table 3. Recovery results for Epalrestat

S.No	Spiked level	Pre analysed sample conc. (µg/ml)	Amount added (µg/ml)	Total amount found (µg/ml)	%Recovery	% Mean
1	50 %	3.75	3.75 3.75 3.75 3.75	3.74 3.75 3.76 3.75	100 100 102 100	**101**

			3.75 3.75	3.74 3.75	102 100	
2	100 %	7.50	7.5 7.5 7.5	7.49 7.48 7.51	101 100 100	**100**
3	150 %	11.25	11.25 11.25 11.25 11.25 11.25 11.25	11.24 11.25 11.24 11.23 11.26 11.25	98 98 98 99 99 98	**98**

Table 4. Linearity data of Epalrestat

S.No.	Conc.(µg/ml)	Area
1	7.50	385.279
2	11.25	597.105
3	15.00	768.173
4	18.75	962.233
5	22.50	1145.814

Table 5. Linearity of Pregabalin

S.No.	Conc.(µg/ml)	Area
1	3.75	1014
2	5.625	1519
3	7.50	2023
4	9.375	2530
5	11.25	3038

Table 6. Result of Robustness study

Parameter	Epalrestat		Pregabalin	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	3.480	2.343	4.767	2.093
1.0 ml/min	2.933	2.194	4.030	1.974
1.2 ml/min	2.523	2.107	3.460	1.882
Wavelength				
236 nm	2.927	2.267	4.020	1.921
239 nm	2.940	2.333	4.050	2.000
242 nm	2.940	2.300	4.050	2.000

Fig.No.1. Chromatogram of Epalrestat and Pregabalin sample

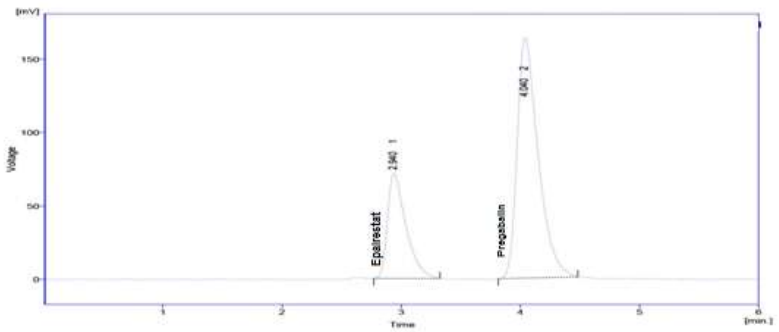


Fig.No.2. Chromatogram of Epalrestat and Pregabalin standard

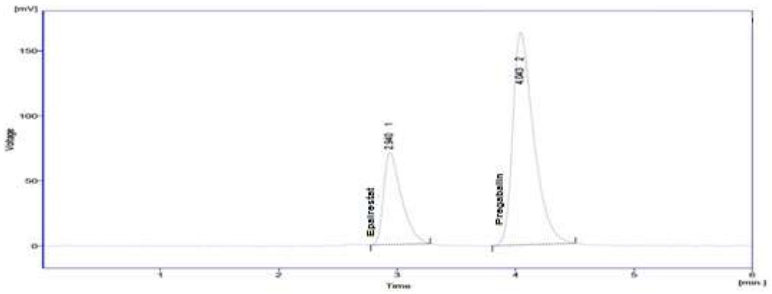


Fig.No.3. Linearity graph of Epalrestat

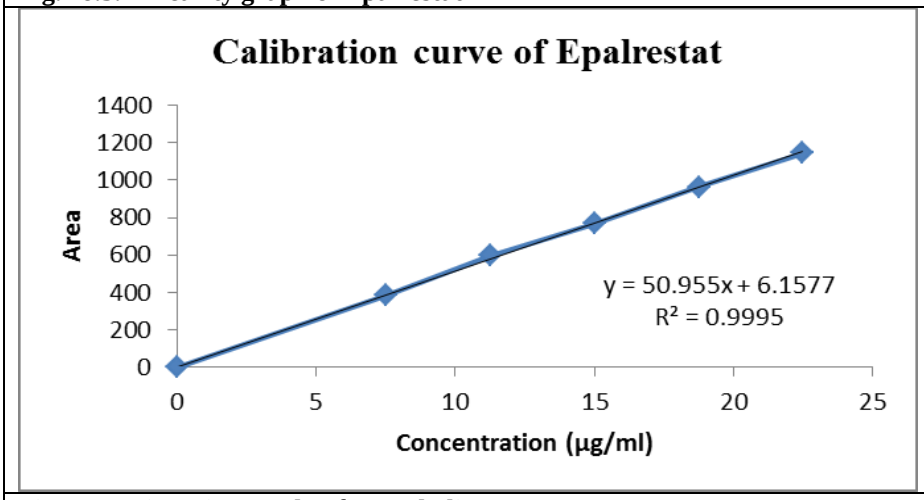
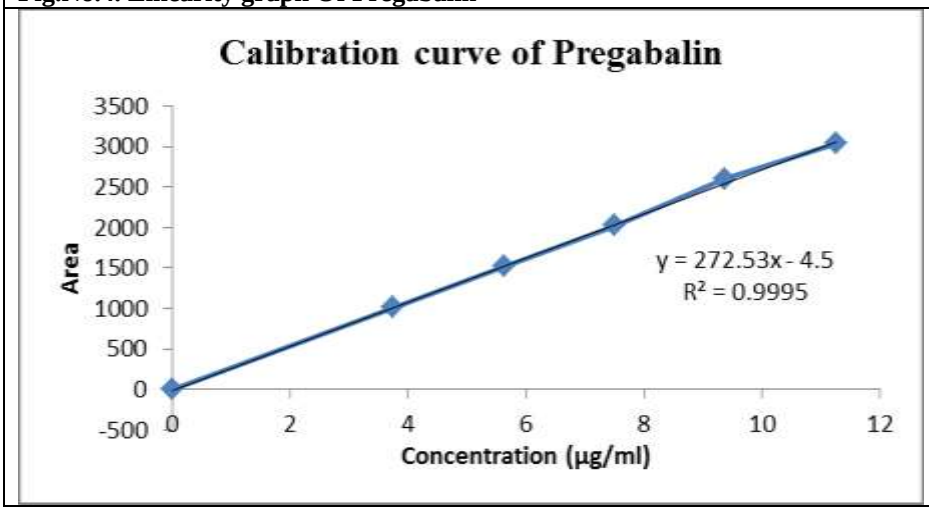


Fig.No.4. Linearity graph Of Pregabalin



In the present work a new method development and validation was carried out for the estimation of Epalrestat and Pregabalin in tablet dosage form by RP-HPLC technique. The solubility of drugs was determined. The appropriate wavelength in UV region has been selected for the measurement of active ingredients in the proposed method.

METHOD DEVELOPMENT

For the method development several trials were carried out and reported. These leads to the optimized chromatographic conditions for the estimation of Epalrestat and Pregabalin in tablet dosage form. Initially various mobile phase and stationary phase were tested in an attempt to obtain the best resolution for Epalrestat and

Pregabalin. The mobile phase consisting of water (pH adjusted to 4 with OPA): Methanol: ACN using Silonol ODS 3V (250×4.6×5µ) at a flow rate of 1.0 ml/min was chosen for method development and validation of Epalrestat and Pregabalin by RP-HPLC method. The detection was selected at 239 nm, using reverse phase C_{18} column, the retention time of Epalrestat and Pregabalin were found to be 2.94 min and 3.9 min respectively. The total run time was 6 minutes. A mobile phase consisting water (pH adjusted to 4 with OPA): Methanol: ACN was selected to achieve maximum separation and sensitivity. The flow rate of 1.0 ml/min gave an optimal signal to noise ratio with reasonable separation time.

METHOD VALIDATION

After method development, the validation of the current method has been performed in accordance with ICH guidelines which include accuracy, precision, linearity, range, specificity and robustness.

PRECISION

Method precision: Prepared sample preparations of Epalrestat and Pregabalin as per test method and injected 6 times in to the column.

Acceptance criteria: The % Relative standard deviation of assay preparations of Epalrestat and Pregabalin should be not more than 2.0%.

Observation: Test results for Epalrestat and Pregabalin are showing that the % RSD of assay results are within limits.

ACCURACY

Accuracy of the method was determined by recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%.

Acceptance criteria: The % recovery of analytes should lie between 98% and 102%.

Observation: The percentage mean recovery of Epalrestat and Pregabalin is 99.60% and 98.08 % respectively.

Discussion: The RP-HPLC method developed in the present study has been used to quantify Epalrestat and Pregabalin in tablet dosage form. The average area was taken and % accuracy was calculated. The results are presented in**Table 10& 11**. The observed data was within the required range, which indicates good recovery values and hence the accuracy of the method developed.

LINEARITY AND RANGE

Preparation of standard stock solution

Standard stock solutions of Epalrestat and Pregabalin (µg/ml) were prepared by dissolving 15 mg of Epalrestat and 7.5 mg of Pregabalin dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. Further dilutions are made to get solutions of Epalrestat and Pregabalin.

Acceptance criteria: The relationship between the concentration of Epalrestat and Pregabalin and area of Epalrestat and Pregabalin should be linear in the specified range and the correlation should not be less than 0.999.

Observation: The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Epalrestat and Pregabalin is 0.997 and 0.997. The relationship between the concentration of Epalrestat and Pregabalin and area of Epalrestat and Pregabalin is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

LIMIT OF DETECTION

Limit of detection of 0.3% of the target assay concentration of Epalrestat was found to be 0.72µg/ml shall be 1. Limit of detection of 0.3% of the target assay concentration of Pregabalin was found to be 1.56µg/ml shall be 2.

Observation: The LOD for this method was found to be 0.72µg/ml for Epalrestat and 1.56µg/ml for Pregabalin.

LIMIT OF QUANTIFICATION

Limit of quantification of 0.4 % of the target assay concentration of Epalrestat was found to have S/N Ratio 2.20. Limit of quantification of 10% of the target assay concentration of Pregabalin was found to have S/N Ratio 4.74.

Acceptance Criteria: S/N Ratio value shall be 5 for LOQ solution.

Discussion: The LOD and LOQ were performed, calculated and reported. The values were found to be within the range.

ROBUSTNESS

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria: The system suitability should pass as per the test method at variable conditions.

Observation: From the observation it was found that the system suitability parameters were within limit at all variable conditions.

CONCLUSION

The study was undertaken in order to develop and validate the analytical RP-HPLC method for estimation of Epalrestat and Pregabalin pharmaceutical formulations. An introduction was given to drug profiles under study. Literature survey reveals no analytical methods have been reported for the estimation of Epalrestat and Pregabalin in pharmaceutical formulations. The method was developed and validated by means of accuracy, precision, linearity, LOD and LOQ and robustness as per ICH guidelines. From this work and results a simple, precise, accurate and sensitive RP-HPLC method was developed for the simultaneous estimation of Epalrestat and Pregabalin pharmaceutical dosage form. The results of the study indicate that the proposed HPLC method of analysis can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Epalrestat and Pregabalin.

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