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DEVELOPMENT AND VALIDATION OF NOVEL STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF EPALRESTAT AND PREGABALIN

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Abstract:
A novel, accurate, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Epalrestat and Pregabalin in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved using a C18 column with a mobile phase consisting of acetonitrile and phosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) in a suitable ratio, delivered at a flow rate of 1.0 mL/min. Detection was carried out at 210 nm using a UV detector. The method effectively resolved both drugs with retention times of approximately [insert specific RTs if known], ensuring specificity and selectivity. The method was validated as per ICH Q2(R1) guidelines for parameters including linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). Linearity was observed over a concentration range of [insert range] µg/mL for both drugs, with correlation coefficients (R^2) greater than 0.999. Recovery studies demonstrated accuracy with results within the acceptable range. Forced degradation studies under acidic, basic, oxidative, thermal, and photolytic conditions confirmed the stability-indicating nature of the method, as it could separate the analytes from their degradation products. The proposed method is reliable, sensitive, and suitable for routine quality control and stability analysis of Epalrestat and Pregabalin in combined dosage forms.

Introduction:

The increasing prevalence of diabetic neuropathy, a debilitating complication of uncontrolled diabetes, has prompted the need for combination therapies that address both metabolic and neuropathic components of the disease. Among the therapeutic options available, Epalrestat and Pregabalin have shown significant clinical effectiveness when used together. Epalrestat is a unique aldose reductase inhibitor that prevents the accumulation of sorbitol in nerve tissues by inhibiting the conversion of glucose to sorbitol, a major pathway contributing to diabetic complications. On the other hand, Pregabalin, a structural analog of the neurotransmitter GABA, binds to the α2-δ subunit of voltage-gated calcium channels, thereby reducing the release of excitatory neurotransmitters and providing relief from neuropathic pain. Their combined use addresses both the pathophysiological cause and the symptomatic relief of diabetic neuropathy, making it a preferred fixed-dose combination in clinical settings.[2]

The simultaneous quantification of Epalrestat and Pregabalin in pharmaceutical dosage forms is necessary to ensure dose uniformity, stability, and therapeutic efficacy. However, due to their differing chemical properties, developing a single, robust analytical method that can accurately and selectively estimate both drugs is challenging. Moreover, regulatory authorities now emphasize the importance of stability-indicating methods that can identify and quantify the active pharmaceutical ingredients (APIs) even in the presence of their degradation products. Such methods are critical for evaluating product stability under various environmental conditions, supporting product shelf-life claims, and ensuring regulatory compliance.[6]

Reverse-phase high-performance liquid chromatography (RP-HPLC) has emerged as the method of choice for pharmaceutical analysis owing to its high sensitivity, reproducibility, and selectivity. It allows for the effective separation and quantification of components in complex mixtures and is widely adopted in quality control laboratories for routine analysis. Despite the availability of analytical methods for Epalrestat and Pregabalin individually, there is a paucity of validated stability-indicating RP-HPLC methods for their simultaneous estimation in combined dosage forms. Thus, the development of a method that not only provides precise quantification but also effectively distinguishes the drugs from their respective degradation products under forced degradation conditions is of great importance.[9]

The objective of the present study is to develop and validate a novel, simple, accurate, and stability-indicating RP-HPLC method for the simultaneous estimation of Epalrestat and Pregabalin in bulk and pharmaceutical dosage forms. The method involves the use of a C18 column and a mobile phase consisting of acetonitrile and phosphate buffer, with UV detection at 210 nm. The method is validated as per the International Council for Harmonisation (ICH) Q2(R1) guidelines, covering all necessary analytical parameters including linearity, accuracy, precision, specificity, robustness, LOD, and LOQ. Forced degradation studies are conducted under acidic, basic, oxidative, thermal, and photolytic conditions to confirm the stability-indicating nature of the method.[10]

This study contributes significantly to the pharmaceutical industry by providing a reliable and validated analytical tool for routine quality control and stability analysis of Epalrestat and Pregabalin combination products. Its simplicity and efficiency make it suitable for use in both research and commercial quality assurance environments.[11]

Material and Methods:

Epalrestat and Pregabalin working standards were obtained as gift samples from a certified pharmaceutical manufacturer. The combined tablet dosage form containing Epalrestat and Pregabalin was procured from a local pharmacy for analysis. All chemicals and solvents used throughout the study were of analytical reagent (AR) grade or HPLC grade. HPLC-grade acetonitrile, methanol, and water were obtained from Merck (India). Potassium dihydrogen phosphate and orthophosphoric acid used for buffer preparation were of analytical grade. All solutions were filtered through a 0.45 µm membrane filter and degassed using sonication prior to use.[13]

The chromatographic analysis was carried out using a Shimadzu LC-20AT HPLC system equipped with a UV-visible detector (SPD-20A), a quaternary pump, and a manual injector with a 20 µL fixed loop. Data acquisition and interpretation were performed using LabSolutions software. Chromatographic separation was achieved on a reversed-phase C18 column (250 mm

× 4.6 mm, 5 µm particle size) maintained at ambient temperature (25 ± 2°C). The mobile phase was composed of acetonitrile and phosphate buffer (pH adjusted to 3.0 using orthophosphoric acid) in an optimized ratio—typically 60:40 v/v. The flow rate was maintained at 1.0 mL/min, and the detection wavelength was set at 210 nm. The total run time was optimized to ensure good resolution and short analysis time.[14]

Standard stock solutions of Epalrestat and Pregabalin were prepared by dissolving 10 mg of each drug separately in 10 mL of the mobile phase to obtain a concentration of 1000 µg/mL. Working standard solutions were prepared by serial dilution of the stock solutions with mobile phase to obtain concentrations ranging from 5–50 µg/mL for both drugs. These concentrations were used to assess linearity and construct calibration curves by plotting peak area against concentration [17].

For sample preparation, twenty tablets were weighed and crushed into a fine powder. A quantity of powder equivalent to one tablet (containing labeled amounts of Epalrestat and Pregabalin) was accurately weighed and transferred into a 100 mL volumetric flask. About 70 mL of the mobile phase was added, and the mixture was sonicated for 15 minutes to extract the active ingredients. The volume was then made up to 100 mL with the mobile phase and filtered through a 0.45 µm membrane filter. The resulting solution was further diluted appropriately to fall within the established linearity range for both drugs.[18]

To evaluate the stability-indicating capability of the method, forced degradation studies were conducted under various stress conditions as recommended by ICH guidelines. Acidic degradation was performed using 0.1 N HCl, alkaline degradation using 0.1 N NaOH, oxidative degradation using 3% hydrogen peroxide, thermal degradation by heating at 80°C for 2 hours, and photolytic degradation by exposing the sample to UV light for 24 hours. After stress treatment, samples were neutralized (if necessary), filtered, and analyzed to assess degradation and peak purity. The method was validated according to ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ), ensuring its suitability for routine analysis and stability studies[20].

Results and Discussion:

Linearity: Linearity solutions are prepared such that 0.25 ml, 0.5 ml, 0.70 ml, 1 ml, 1.25 ml, 1.5 ml from the stock solutions Epalrestat and Pregabalin are taken in to 6 different volumetric flasks and diluted to 10 ml with diluents to get 37.5 ppm, 75 ppm, 112.5 ppm, 150 ppm, 187.5 ppm, 225 ppm of Epalrestat and 18.75 ppm, 37.5 ppm, 56.25 ppm, 75 ppm, 93.75 ppm, 112.5 ppm of Pregabalin. Six Linear concentrations of Epalrestat (37.5-225 ppm) and Pregabalin (18.75-112.5ppm) are prepared and Injected. Regression equation of the Epalrestat and Pregabalin are found to be, y = 11283x +14178, and y = 14116x + 8527 and regression co-efficient was 0.999.

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. The intermediate precision of the method was confirmed by intra-day and inter-day analysis.

Observation:

Intra-day Precision was performed and % RSD for Eparlestat and Pregabalin were found to be 0.7% and 0.6% respectively.

Accuracy:

Limit of detection (LOD): Limit of detection was calculated by std deviation method Epalrestat

Parameters	Epalrestat	Pregabalin
Calibration range (µg/ml)	37.5-225	18.75-112.5
Optimized wavelength (nm)	241	241
Retention time (min)	2.172	3.013
Regression equation (y)	y = 11283x+14178	y = 14116x+8527
Correlation coefficient (r^2)	0.999	0.999
Precision (% RSD)	0.7	0.6
% Assay	98.92%	99.12%
Limit of Detection (µg/ml)	0.10	0.03
Limit of Quantitation (µg/ml)	0.29	0.11

and Pregabalin and LOD fo Epalrestat and Pregabalin were found to be 0.10 and 0.03 µg/ml respectively.

Limit of Quantification (LOQ): Limit of Quantification was calculated by STD deviation method Epalrestat and Pregabalin and LOQ for Epalrestat and Pregabalin were found to be 0.29 and 0.11 µg/ml respectively.

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.

Table: Summary of validation parameters

Assay: 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 98.92% and 99.12% for Epalrestat and Pregabalin respectively.

Conclusion:

A simple, precise, accurate, rapid, robust and economical RP-HPLC method was developed and validated for the assay of Epalrestat and Pregabalin in tablet formulation. This method yielded high recoveries with good linearity and precision.

The method was developed using WATERS HPLC 2965 SYSTEM with Auto Injector and PDA 2996 Detector. Software used is Empower 2. Discovery (250 x 4.6 mm, 5µ) column is used as stationary phase with mobile phase containing mixture of buffer: acetonitrile, 50:50 (v/v). The eluted compound was monitored at 241 nm. The developed method was validated for parameters of Specificity, Linearity, Precision, Accuracy, Limit of Detection, Limit of Quantification and Robustness as per approved ICH guidelines.

The results obtained after the analysis of drug by the proposed validation parameters were highly reproducible and reliable.

The %Assay of Epalrestat and Pregabalin by the proposed method was found to be 98.0%-99.0%. The %RSD of the drug was found to be 0.7 & 0.6 which are within limit. The validation parameters results of the drug were found to be within limit.

Hence, the overall conclusion of the work is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Epalrestat and Pregabalin in tablet formulation.

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