HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF PREGABALIN AND ETORICOXIB BY RP HPLC METHOD

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Abstract

Pregabalin S-(3)-amino methyl hexanoic acid, is a structural analogue of -amino butyric acid (GABA). The intention of this study is to develop an analytical method for the quantification of pregabalin and etoricoxib simultaneously in dosage form using RP HPLC analytical method. The pregabalin and etoricoxib were quantified on Zobrax (Eclipus) C18 with Orthophosphoric acid (0.1%), and methanol 50:50 vol/vol ratio was employed as mobile phase. The amounts of pregabalin and etoricoxib were quantified by using a detector fixed at 220nm. A selective specific method was developed for the quantification of Pregabalin and etoricoxib in pharmaceutical formulations. The HPLC quantifiable technique developed was successfully validated to existing ICH limit guidelines and was confirmed as robust, specific, accurate, selective, precise, sensitive, and linear. The method linearity was assessed for the range of 25 to 150 µg/ml with r^2=0.999. The precision and accuracy were found to be well within RSD of Not more than 2.0%. The RP HPLC method developed for the quantification of pregabalin and etoricoxib simultaneously in dosage form was found to be precise, specific, robust, and accurate.

Keywords: Pregabalin, Etoricoxib, RP HPLC, analytical method validation

Introduction

Pregabalin S-(3)-amino methyl hexanoic acid, is a structural analogue of -amino butyric acid (GABA). They constitute an important group of compounds that are used in the treatment of epilepsy and neuropathic pain[1]. Pregabalin (PRB) is a first-line medicinal drug that significantly eliminates the complaints of many sorts of neuropathic aches (fibromyalgia, peripheral diabetic neuropathy, post-herpetic neuralgia, Chemotherapy-persuaded neuropathic aches in cancer sufferers) with high extent of safety and success [2-4]

Etoricoxib (ECB) is a specific cyclooxygenase-2 antagonist with a lesser risk of gastrointestinal complications [5]. Etoricoxib is used in the treatment of inflammation and aching at joints and muscles of patients aged 16 and up who are impaired from rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis [6-7]. In gout, ECB can be administered for a brief length of period [8]. ECB operates by modulating the cyclooxygenase-II enzyme, which contributes to manufacturing a substance recognized as prostaglandin [9]. In individuals with persistent low back pain, ECB provided much superior pain alleviation. In patients experiencing acute gout and women experiencing primary dysmenorrhoea, ECB demonstrated equal effectiveness [10-11].

For neuropathic discomfort, a combo of pregabalin and etoricoxib is better effective over monotherapy. In the current study, RP HPLC analytical method is presented for the quantification of pregabalin and etoricoxib simulations. The developed assay method for pregabalin and etoricoxib estimation was validated for the analytical method validation parameters as per the international conference on harmonization requirements and the data presented.

Experimental

Instruments

The Shimadzu liquid chromatograph (Shimadzu Scientific Instrument Inc., Kyoto, Japan) instrument consists of a solvent delivery module LC-10 AD, UV detector, an autosampler, online degasser, Rheodyne injector (model 7125, USA) valve, system controller connected with desktop computer preloaded with Shimadzu chromatographic software (LC Solution, Release 1.11SP1) was used. The absorbance spectra were assessed using an UV–Visible spectrophotometer (Model UV-1601PC) with a quartz cell with 1.00cm path length. A sonicator (PCI analytics) was used to

degas the mobile phase. A stationary phase (HPLC Zobrax (Eclipus) C18 150x 3.0mm), was used, with the operating condition at room temperature.

Materials

Pregabalin and Etoricoxib were obtained as gift sample from M/S Divis Pharmaceuticals, Hyderabad, India. The HPLC grade Acetonitrile from Rankem chemicals, HPLC grade Methanol from Fischer scientific, analytical reagent grade triethyl amine, and glacial acetic acid from Merck chemicals were purchased. The ultra-pure water was prepared using a Milli-Q Academic system (Millipore, USA).

Standard solutions preparation

Accurately weighed 37.5 mg of Pregabalin, 30 mg of Etoricoxib was and transferred in to 50 ml volumetric flasks separately. 3/4th of the diluent was added to both of these flasks and sonicated for 10 minutes. Flasks were made up of diluents and labeled as Standard stock solutions 1and 2. (750µg/ml of Pregabalin and 600µg/ml of Etoricoxib)

Chromatographic conditions

The chromatographic evaluation was carried out on Zobrax (Eclipus) C18 150x 3.0mm connected with a guard cartridge (4 mm × 3 mm i.d., 5 μm). The mobile phase consisted of Buffer : methanol in the ratio of 50 : 50. The wavelength 220 nm was identified for enhanced sensitivity for molecules with less concentration and to decrease the mobile phase background. The sample injection volume of 10 μl was followed. The HPLC system was maintained in a laboratory controlled through air-condition facility (20$\pm$2 °C).

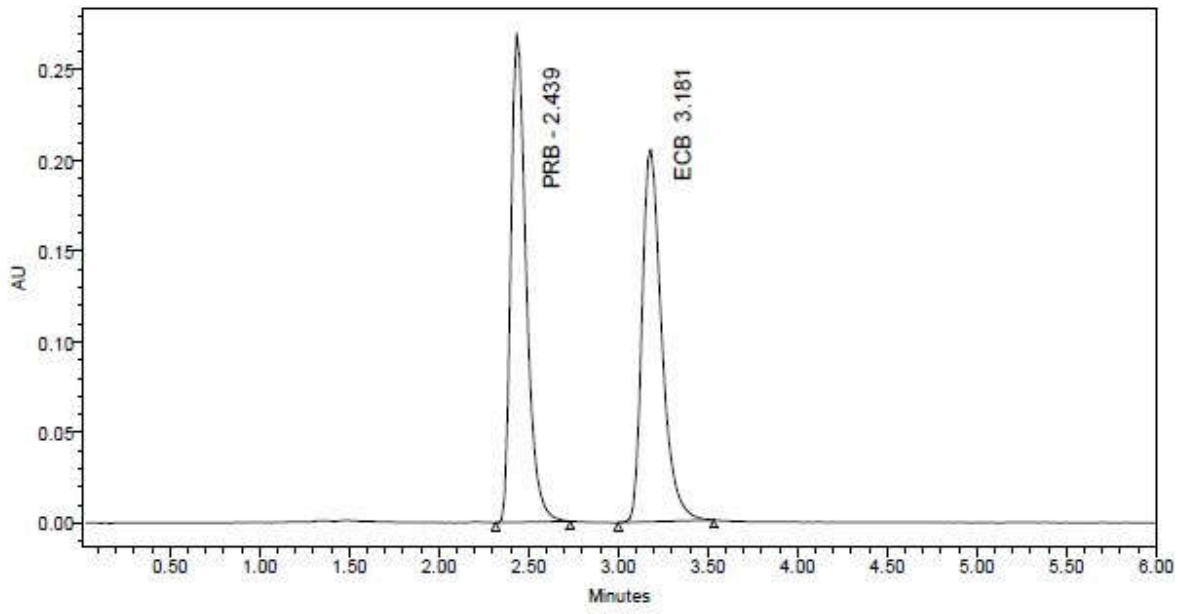
Method validation

The analytical method validation studies were performed using the optimized analytical method for assay estimation of Pregabalin and Etoricoxib in-line with the analytical method validation requirements described by ICH guidelines “Q2B Validation of analytical procedures: methodology” and “Text on Validation of Analytical Procedures”[12-17. Key analytical parameters,including, specificity, accuracy, precision, linearity, detection limit, and quantitation limit were evaluated. Calibration curves were constructed in the range of 0.05 to 1.0% of the target analyte concentration for the limit of detection and quantification. Also, the robustness of

the proposed method was assessed concerning small alterations in the methanol concentrations [18].

Results and discussion

The most frequently used stationary phases in RP-HPLC such as C18, C8, C6 and CN bonded phases. It is known that solutes behave differently in each column yet to be paid different interactions between them and the organic chain bonded to silica, giving rise to differences in separation [19]. Moreover, the optimized mobile phase mixtures for one column will not inevitable are optimum for the other. Thus, the choice of the column that will give desired results is not often straightforward and, because of that, these stationary phases were selected for this study. Columnchemistry, organic modifier, solvent strength, temperature and flow rate were then varied to determine the best chromatographic separation. In this study, the critical chromatographic factors for optimization were based on preliminary experiments and prior knowledge of literature, as well as certain instrumental limitations. Preliminary experiments were carried out to screen the appropriate parameters and to determine the experimental domain. For quantitative estimation of PRB and ECB in bulk drugs and in pharmaceutical dosage form, RP HPLC method was developed and the analytical method validation was completed. The optimized chromatogram was shown in figure 1.



Assay method validation

The last step of the study was to check method validation for specificity, linearity, intra/inter-day precision, and robustness. The optimized HPLC method was specific in relation to the placebo used in the study. All placebo chromatograms showed no interference peaks (figure 4). Excellent linearity was established at five levels in the range of 25-150 µg/ml for PRB and ECB with R^2 of more than 0.999 for all the analytes. The slope and intercept of the calibration curve were 21652X+8679.9 PRB and 19209X+1553.1 for ECB respectively. Since the correlation coefficients are not good indicators of linearity performance of an analytical procedure a one-way ANOVA was performed. For all the analytes, the calculated F- Value (F calc) was found to be less than the theoretical F-value (F *crit*) at a 5% significance level, indicating that there was no significant difference between replicate determinations for each concentration level. The LODs were 1.38 and 0.37 ng/mL and LOQs were 4.20 and 1.11 ng/mL for PRB and ECB respectively. Accuracy (n=9), assessed by spike recovery, was found to be 99.77 and 100.29 for PRB and ECB respectively, which were within acceptable ranges of 100± 2%. The intra and inter-assay precision (n=6) was confirmed since, the %CV was well within the target criterion of ≥2 and ≤ 3, respectively. Robustness study reveals that small changes did not alter the retention times, retention factor, and resolution and therefore it would be concluded that the method conditions are robust.

Conclusion:

In this study, an efficient isocratic reversed-phase high-performance liquid chromatography method was developed, and validated for the simultaneous estimation of the analytes PRB and ECB. This method reduces overall assay development time and provides essential information regarding the sensitivity of various chromatographic factors and their interaction effects on the attributes of separation. The time of analysis, resolution, and quality of the peaks was simultaneously optimized. The validation study supported the selection of the assay conditions by confirming that the assay was specific, accurate, linear, precise, and robust. Therefore, this HPLC method can be used as a routine quality control analysis in a pharmaceutical environment. The results of the study demonstrate the benefit of applying this approach in selecting optimum conditions for the determination of drugs in pharmaceutical formulation and plasma samples. Also, an isocratic stability-indicating HPLC- UV method has been developed for the estimation of PRB and ECB. The proposed method is simple, accurate, precise, and specific, and can separate the drugs from degradation products and excipients found in the pharmaceutical dosage forms. The method is suitable for use in routine analysis of both drugs in bulk API powder or pharmaceutical dosage forms.

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