DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF ESLICARBAZEPINE IN ESLICARBAZEPINE ACETATE TABLETS

Singh Pradeep Kumar *, Subas Chandra Dinda
School of Pharmaceutical Education & Research, Berhampur University, Bhanja Bihar, Berhampur, Odisha, India
E-mail: pksmpharm1978@gmail.com

Article Received on: 19/03/13 Revised on: 08/04/13 Approved for publication: 01/05/13

DOI: 10.7897/2230-8407.04536
IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com
© All rights reserved.

ABSTRACT
A simple, precise, rapid and accurate stability indicating reverse phase high performance liquid chromatography has been developed and validated for the estimation of Eslicarbazepine Acetate in tablet dosage form. Separation was carried on a Waters e 2695 HPLC system separation module with Empower 2 software, PDA detector waters 2998 and Symmetry-C18 analytical column (5µm; 250x4.6mm), was operated in isocratic mode using mobile phase A consisting of (Phosphate buffer pH 5.0±0.5 and acetonitrile in the ratio of 90:10) and mobile phase B consisting of acetonitrile and water in the ratio of 80:20) is used in the ratio of 65:35 and at a flow rate of 1ml/min with detection wavelength of 215 nm by an injection volume of 20µl and entire separation was carried out at 35°C column temperature. The linearity was found in the range of 5.0-500.0 µg/ml and showed a correlation co-efficient of 0.9999. The retention time of Eslicarbazepine Acetate was found to be 8.0. This study concluded that the proposed method was found to be accurate, reproducible and consistent which is useful for the routine determination of Eslicarbazepine in tablet dosage form. The method is validated as per ICH guidelines by determining its specificity, accuracy, precision, linearity & range, ruggedness, robustness and system suitability.

Keywords: Eslicarbazepine Acetate, RP-HPLC, Method Development, Validation.

INTRODUCTION
Eslicarbazepine acetate is chemically 1(S)-10-Acetoxy-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide (Fig1), it is an antiepileptic drug. It is a prodrug which is activated to eslicarbazepine (S-licarbazepine), an active metabolite of oxcarbazepine. The literature survey suggested carrying out the separation in reverse phase mode. Very few spectrophotometric methods and HPLC methods are available for estimation of Eslicarbazepine2-7. An attempt has been made to develop a new stability indicating RP-HPLC method for its estimation in tablet dosage form with good accuracy and precision. The method is validated according to the ICH Q2 (R1) and other relevant regulatory guidelines8-10.

MATERIAL AND METHODS
Eslicarbazepine acetate was obtained from Ami Life Sciences, Baroda. Methanol and acetonitrile used were of HPLC grade from E. Merck, India. Potassium dihydrogen phosphate used was of AR grade from S.D. Fine Chem-Limited, India. Ortho phosphoric acid used was of AR grade from E. Merck, India. Triethylamine used was of AR grade from E. Merck, India. HPLC grade water was obtained using millipore water purification system. All volumetric-glassware were pre-calibrated by the manufacturer (Borosil) and were of grade A. Tablets manufactured by Intas Pharmaceuticals Ltd; used for estimation.

METHOD DEVELOPMENT
Preparation of Standard and Sample Solutions

Procedure for Calibration Curve of Eslicarbazepine
Accurately weighed quantity of 100mg of Eslicarbazepine was dissolved in 100ml volumetric flask with the diluent. From this stock solution, concentrations of 5, 10, 25, 50, 100, 150, 200, 300, 400 and 500 µg/ml of Eslicarbazepine acetate and constructed the calibration curve at a detection wavelength of 215nm which was used for estimation (Figure 2 & Figure 3a).

Assay Procedure for Sample Solution preparation
Accurately weighed quantity of equivalent powder of 40mg of Eslicarbazepine from 20 tablets was dissolved in diluent in 100ml volumetric flask and further diluted to fall in working range concentration for the estimation by using the calibration curve (Figure 3b).

Method Validation
The proposed method was validated according to ICH guidelines in terms of parameters like Specificity, Accuracy, Precision, Linearity, LOD and LOQ.

System Suitability Parameters
For system suitability six replicates of standard solutions of Eslicarbazepine acetate was injected into the system and studied the suitability parameters like Plate number (N), Tailing factor (T) and Percentage relative standard deviation (%RSD) were studied with the help of standard chromatograms (Table 1)

Linearity and Range
The linearity of calibration curve (analyte to peak area ration Vs concentration) in pure solution was checked over the concentration ranges of 5.0-500.0 µg/ml for Eslicarbazepine acetate. The linearity was evaluated by linear regression analysis, using least square method. The calibration curve was linear in the studied range and equations of the regression analysis obtained for Eslicarbazepine acetate Y: 17241 X + 68219. Correlation co-efficient values for Atenolol found to be 0.9999 (Table 2).

Accuracy
To study reliability, suitability and accuracy of the method, recovery studies were carried out, by adding a known quantity of standard to the placebo. The recovery study was carried out as 50%, 80%, 100%, 120% & 150% level and the contents were determined from respective chromatogram.
From the results obtained we conclude that method was accurate (Table 3).

**Precision**
The precision of the test method was done by performing assay on six replicate determination of sample preparation at test concentration level (as per method of analysis) and calculated relative standard deviation of assay results. Six replicates of from standard solutions were injected and peak areas were obtained and % RSD was calculated (Table 4).

**Limit of Detection**
Limit of detection is the lowest amount of an analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyte can be detected was determined from the linearity curve by applying the formula (Table 1).

**Limit of Quantitation**
Limit of quantitation is the lowest amount of an analyte that can be estimated quantitatively by injecting decreasing amount of the drug with acceptable precision and accuracy under the stated experimental conditions of the method. The minimum concentration at which the analyte can be detected was determined from the linearity curve by applying the formula. The limit of quantitation can be obtained from linearity curve by applying the following formula (Table 1).

**Figure 1: Chemical structure of Eslicarbazepine acetate**
RESULTS AND DISCUSSION
The separation was carried on Waters 2695 Isocratic HPLC system separation module with EMPOWER 2 software, PDA detector waters 2998 and Symmetry-C18 analytical column (5µm; 250x4.6mm), was operated in isocratic mode using mobile phase A consisting of (Phosphate buffer pH 5.0±0.05 and acetonitrile in the ratio of 90:10) and mobile phase B consisting of acetonitrile and water in the ratio of 80:20) is used in the ratio of 65:35 and at a flow rate of 1.0ml/min with detection wavelength of 215nm, by an injection volume of 20µl and entire separation was carried out at temperature 35°C for column. Under the described experimental conditions, sharp peaks that belong to Eslicarbazepine acetate were obtained at retention time about 8.0min. System suitability studies were carried out and Plate number (N), Tailing factor (T) and Percentage relative standard deviation (%RSD) were found and are presented (Table) 1. The Linearity (Table 2) was obtained in the concentration range 5 to 500 µg/ml for Eslicarbazepine acetate with correlation coefficient of 0.9999. The accuracy of the method was determined by performing recovery studies at 50%, 80%, 100%, 120% & 150% were found within the limits (Table 3). The precision of the method was also found to be good (Table 4). The limit of detection(LOD) and Limit of Quantitation (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method (Table 1).

CONCLUSION
The HPLC method developed is accurate, precise, reproducible and specific. The method is linear over a wide range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for quantification of Eslicarbazepine in bulk drug and in tablets. The method developed was then subjected to validation as per ICH guidelines and showed that method is linear, precise, accurate and robust.

ACKNOWLEDGEMENT
The authors are thankful to M/s Panacea Biotec Limited for the support provided during research work.

REFERENCES


Source of support: Nil, Conflict of interest: None Declared