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 Bihari, 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±0.05 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 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 chemically¹ (S)-10-Acetoxy-10,11-dihydro-5H-dibenz[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 guidelines^{8,9,10}.

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 ratio 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).

$$LOD = 3.3 \text{ SD/Slope}$$

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).

$$LOQ = 10 \text{ SD/Slope}$$

Table 1: System suitability Parameters

System Suitability Parameters	Eslicarbazepine
Linearity range	5-500 µg/ml
Tailing Factor	1.1
Number of theoretical Plates	15649
Retention Time	About 8.0
LOQ	0.25 µg
LOD	0.0825 µg

Table 2: Linearity Data for Eslicarbazepine Acetate

Concentration of Eslicarbazepine Acetate (µg/ml)	Peak Area of Eslicarbazepine Acetate
5	107575
10	199386
25	537875
50	981813
100	1869082
150	2611531
200	3482042
300	5223063
400	6964004
500	8705105

Table 3: Recovery studies of Eslicarbazepine Tablets 400 mg

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Accuracy 50 % -1	20.17	20.12	99.75
Accuracy 50 % -2	20.14	20.11	99.85
Accuracy 50 % -3	20.12	20.12	100.00
Accuracy 80 % -1	32.09	32.09	100.00
Accuracy 80 % -2	32.22	32.21	99.97
Accuracy 80 % -3	32.26	32.21	99.85
Accuracy 100 % -1	40.09	40.03	99.85
Accuracy 100 % -2	40.72	40.76	100.10
Accuracy 100 % -3	40.48	40.48	100.00
Accuracy 120 % -1	48.17	48.15	99.95
Accuracy 120 % -2	48.89	48.85	99.92
Accuracy 120 % -3	48.34	48.30	99.92
Accuracy 150 % -1	60.77	60.76	99.98
Accuracy 150 % -2	60.44	60.28	99.74
Accuracy 150 % -3	60.42	60.44	100.03
Mean		99.93	
SD		0.04	
%RSD		0.04	

Table 4: Precision of developed method at working level

Sample No.	Area
1	652403
2	652203
3	652803
4	654303
5	653403
6	656403
Mean	653586.30
SD	1575.33
% RSD	0.24

Figure 1: Chemical structure of Eslicarbazepine acetate

Figure 2: Linearity graph of Eslicarbazepine acetate

Figure 3a: HPLC Chromatogram of Standard Eslicarbazepine acetate

Figure 3b: HPLC Chromatogram of Eslicarbazepine Sample

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

1. The United States Food and Drug Administration, US Department of Health and Human Services; Prescribing Information 2010:13-14.
2. Subas Chandra Dinda et al (2013). Development and validation of RP-HPLC method for quantitative analysis of Amlodipine besylate in pure and Pharmaceutical Formulations. *Research Journal of Pharmacy*. 6(2): 204-207
3. Gilberto Alves et al (2010). Development and Validation of an HPLC-UV Method for simultaneous quantification of carbamazepine, ox carbamazepine, eslicarbazepine acetate and their main metabolites in human plasma. *Biomedical Chromatography*. 397(4):1605-1615.
4. Amilcar Falcao et al (2011). A chiral liquid chromatography method for the simultaneous determination of oxcarbazepine, eslicarbazepine, R-licarbazepine and other new chemical derivatives BIA2-024, BIA 2-059 and BIA 2-265, in mouse plasma and brain. *Biomedical Chromatography*. 21(11): 34-41
5. Amilcar Falcao et al (2007). Enantioselective HPLC-UV method for determination of eslicarbazepine acetate (BIA 2-093) and its metabolites in human plasma *Biomedical Chromatography*. 21(11): 1127-1134 <http://dx.doi.org/10.1002/bmc.858>
6. Amilcar Falcao et al (2007). A chiral HPLC-UV method for the quantification of dibenz[b,f]azepine-5-carboxamide derivatives in mouse plasma and brain tissue: Eslicarbazepine acetate, carbamazepine and main metabolites. *Biomedical Chromatography*. 34(12): 1391-1401
7. Dhaval Patel et al (2012). RP-HPLC method development and validation for Eslicarbazepine acetate in api. *International Journal of Advanced Research in pharmaceutical and Bio Sciences*. 1(2):84-94.
8. Code Q2 A (R2) (2003)- Stability testing of new drug substances and products; Step-4 Consensus Guideline. ICH harmonized Tripartite Guideline.
9. Kumar N A, Singh S, Bakshi M et al (2000). Guidance on conduct of stress tests to determine inherent stability of drugs. *Pharmaceutical Technology*. 24 (5): 1-14.
10. ICH, Q2 (R1), Validation of Analytical Procedures: Text and Methodology, Step-4 Consensus Guideline, 2005, ICH harmonized Tripartite Guideline.

Cite this article as:

Singh Pradeep Kumar, Subas Chandra Dinda. Development and validation of a stability indicating RP-HPLC method for determination of Eslicarbazepine in eslicarbazepine acetate tablets. *Int. Res. J. Pharm.* 2013; 4(5):178-180