IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 6 Ver. II (Nov - Dec. 2015), PP 155-162
www.iosrjournals.org

Development and validation of an RP-HPLC method for related substance and quantitative estimation of eslicarbazepine acetate in bulk drug and pharmaceutical dosage form

Sanjeeva Reddy Kallam[1], Dr. J.Srikanth[2], Dr.K.Vanitha Prakash[3]
[1]*Dr. Reddy's Laboratories Ltd., IDA, Bollaram, Hyderabad, Telangana, India.*
[2]*Suven Nishtaa Pharma Pvt. Ltd., Hyderabad, Telangana, India.*
[3]*S. S. J. College of Pharmacy, Hyderabad, Telangana, India*

Abstract: *A simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography has been developed and validated for the related substance and estimation of Eslicarbazepine acetate in bulk and pharmaceutical dosage form. The chromatographic separations were achieved using a High performance liquid chromatography (Inertsil ODS 3V 150 mm, 4.6 mm, 5µm) employing 0.1% orthophosphoric acid buffer, methanol and acetonitrile in the ratio of 500:250:250 as mobile phase with a 1.5 mL/min flow rate was chosen. Four impurities were eluted within 10 minutes. The column temperature was maintained at 25oC and a detector wavelength of 210 nm was employed. The Eslicarbazepine acetate was exposed to thermal, photolytic, hydrolytic, basic and oxidative stress conditions. The stressed samples were analyzed by the proposed method. High degradation of the analyte was observed when it was subjected to basic conditions. Peak homogeneity data of Eslicarbazepine acetate obtained by photodiode array (PDA) detection demonstrated the specificity of the method in the presence of degradants. The method was validated with respect to linearity, precision, accuracy, ruggedness, and robustness, limit of detection and limit of quantification.*

Keywords: *Eslicarbazepine acetate, RP-HPLC, ICH guidelines, Degradation, Method validation.*

I. Introduction

Eslicarbazepine acetate chemically known as (S)-10-Acetoxy- 10, 11-dihydro- 5H-dibenz [b, f] azepine- 5-carboxamide and is used as an anticonvulsant or antiepileptic drug Literature survey reveals only single pharmacopoeial method is available in Indian Pharmacopoeia except that no pharmacopoeial method is available in United States Pharmacopoeia, British Pharmacopoeia and European pharmacopeia. Present study involves development of a convenient, rapid, and cost efficient and user friendly reversed-phase (RP)-HPLC method with a simple and easily available mobile phase for quantitative estimation and related substance of eslicarbazepine acetate in bulk drug and tablet dosage form within 10 minutes of run time. The optimized method was developed and validated as per International Conference on Harmonisation (ICH) guidelines.

II. Experimental

2.1 Materials and reagents

The reference samples of Eslicarbazepine acetate and its impurities were provided as gift samples from Dr.Reddys laboratories Ltd. HPLC grade methanol, Acetonitrile and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial tablets (Aptiom -400mg dosage) were purchased from the local pharmacy.

2.2 Instrument and Chromatographic Conditions

The HPLC system used for the method development and validation consisted of gradient pumps from Agilent 1260 Technologies, Ultra violet detector from Agilent Technologies. USA, with auto sampler and auto injector. The HPLC system was equipped with data acquision and processing software "EZ Chrome software" Agilent Technologies. USA.

The column used for separation of analytes is Inertsil ODS 3V 150 x 4.6 mm, 5µm column. Mobile phase consisting of 0.1% orthophosphoric acid buffer, methanol and acetonitrile in the ratio of 500:250:250 as mobile phase at a flow rate of 1.5 ml/min. It was filtered through 0.45µm nylon filter and sonicated for 15 min in ultrasonic bath. Sample analyzed at 210 nm at an injection volume of 20 µL.

2.3 Preparation of 0.1% orthophosphoric acid buffer

Accurately taken 1 ml of Orthophospharacid in a 1000mL of milli-Q water and degas to sonicate for 15 minutes. It was filtered through 0.45µm nylon filter.

2.4 Preparation of Mobile phase

Take the above 500mL of buffer solution add 250mL of methanol and 250ml of acetonitrile and degas to sonicate for 15 minutes.

2.5 Diluent:

Water, Acetonitrile (500: 500mL)

2.6 Preparation of Solutions:

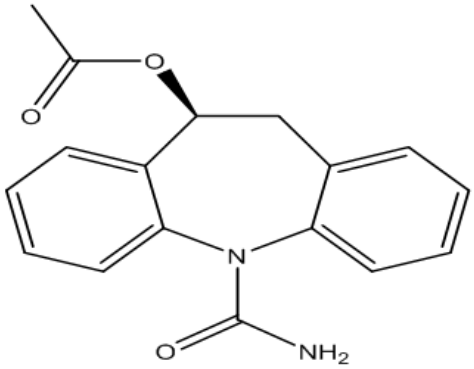
2.6.1 Standard/sample preparation (100µg/mL)**:**

Weight accurately 10mg of Eslicarbazepine acetate standard into a 100ml volumetric flask, add 50mL of diluent, then sonicated for 10min and make up the volume with diluent.

2.6.2 Impurity stock preparation (100µg/mL)**:**

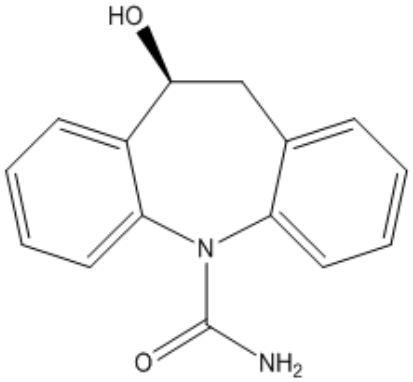
Weight separately each 5 mg of ESA1, (Licarbzepine) Oxcarbazepine, Carbamazepine and Hydroxy desamide into a 50 mL of volumetric flask, add 25 mL of diluent, then sonicated for 10min and make up the volume with diluent.

Figure 1: Structure of Eslicarbazepine acetate



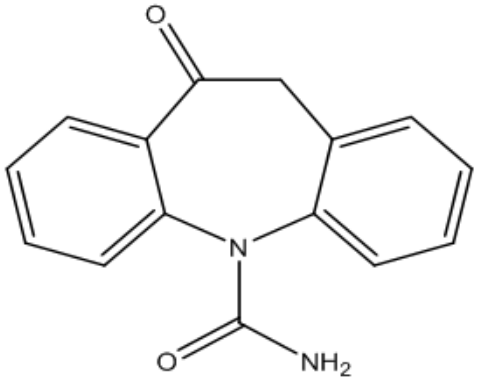
Chemical Name: (S)-10-Acetoxy- 10, 11-dihydro- 5H-dibenz [b, f] azepine- 5-carboxamide
Molecular formula: $C_{17}H_{16}N_2O_3$
Molecular weight: 296.320

Figure 2: Structure of ESA1 (Licarbzepine)



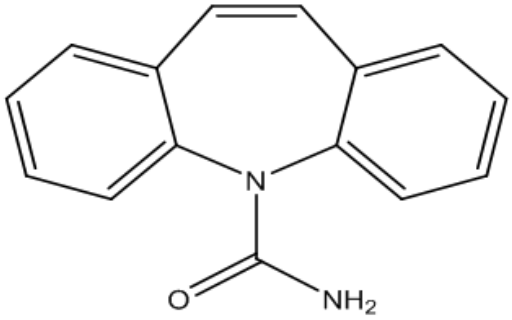
Chemical Name: (S)- (+) - 10, 11-dihydro-10 hydroxy- 5H-dibenz [b, f] azepine- 5-carboxamide
Molecular formula: $C_{15}H_{14}N_2O_2$
Molecular weight: 254.284

Figure 3: Structure of Oxcarbazepine



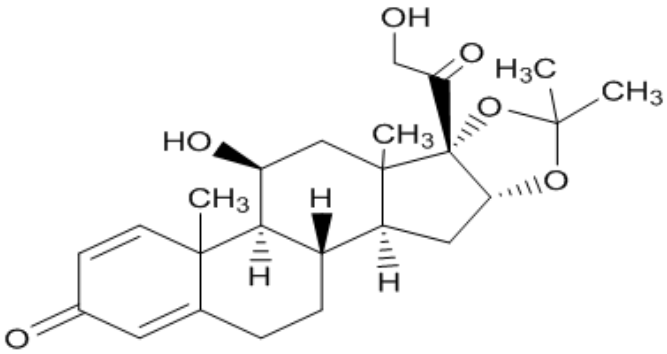
Chemical Name: 10, 11-dihydro-10 oxo- 5H-dibenz [b, f] azepine- 5-carboxamide
Molecular formula: $C_{15}H_{12}N_2O_2$
Molecular weight: 252.268

Figure 4: Structure of Carbamazepine



Chemical Name: Dibenzo [b,f]azepine-5-carboxamide
Molecular formula: $C_{15}H_{12}N_2O$
Molecular weight: 236.268

Figure 5: Structure of Hydroxy desamide



Chemical Name: (6bS,7S,8bS,11aR,12aS,12bS)-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a,10,10-tetramethyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one
Molecular formula: $C_{24}H_{32}O_6$
Molecular weight: 416.22

2.7 Method Validation

The validation of the method was carried out as per ICH Guidelines**.** The parameters assessed were specificity, linearity, precision, accuracy, stability, LOD and LOQ.

2.7.1 Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including impurities and its degradation products. The specificity of the developed HPLC method for the Eslicarbazepine acetate and its impurities namely ESA1 (Licarbzepine), Oxcarbazepine, Carbamazepine and Hydroxy desamide was carried out in the presence of its degraded impurities. Stress studies were performed for Eslicarbazepine acetate bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of UV light (254nm), heat (80°C), acid (0.5N HCl), base (0.5N NaOH), and Oxidation (3.0 % H_2O_2) to evaluate the ability of the proposed method to separate Eslicarbazepine acetate and its impurities from its degradation impurities. For all degradation studies, period was 48 hours. Related substance, Assay and degradation studies were carried out for stress samples against Eslicarbazepine acetate reference standard.

2.7.2 Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels 50%, 100% and 150 % of test concentration (0.1 mg/mL). The percentage of recoveries were calculated from the slope and Y- Intercept of the calibration curve. The accuracy study of impurities was carried out in triplicate at 50%, 100%, & 150% of specification level (0.1%) to the Eslicarbazepine acetate analyte concentration (100 µg /mL).The percentages of recoveries for impurities were calculated from the slope and Y- Intercept of the calibration curve

2.7.3 Precision

The precision of the assay method was evaluated by carrying out six independent assays of Eslicarbazepine acetate test samples against a qualified reference standard and calculate the %RSD of assay. The precision of the related substances method was checked by injecting six individual preparations of Eslicarbazepine acetate (0.1mg/mL) spiked with 0.1 % of ESA1(Licarbzepine), Oxcarbazepine, Carbamazepine and Hydroxy desamide with respect to Eslicarbazepine acetate analyte concentration. %RSD of area for each impurity of ESA1 (Licarbzepine), Oxcarbazepine, and Carbamazepine and Hydroxy desamide was calculated. The intermediate precision of the method was also evaluated using different analyst and different instrument in the same laboratory.

2.7.4 Linearity

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. The linear regression data for the calibration plot is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance.

2.7.5 Robustness

Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no effect on the peak tailing, peak area and theoretical plates and finally the method was found to be robust.

2.7.6 Limit of Detection & Limit of Quantitation

The LOD can be defined as the smallest level of analyte that gives a measurable response and LOQ was determined as the lowest amount of analyte that was reproducibly quantified. These two parameters were calculated using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations, LOD=3.3×SD/S and LOQ=10×SD/s, where SD = standard deviation, S= slope of the calibration curve.

2.7.7 Solution stability and Mobile phase stability:

The solution stability of Eslicarbazepine acetate in the assay method was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 48 hrs. The same sample solutions were assayed for 6 hrs. Interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solution against freshly prepared reference standard solution for 6 hrs interval up to 48 hrs. Mobile phase prepared was kept constant during the study period. The % RSD for the assay of Eslicarbazepine acetate was calculated during mobile phase and solution stability experiment.

The solution stability of Eslicarbazepine acetate and its impurities in the related substance method was carried out by leaving spiked sample solution in the tightly capped volumetric flasks at room temperature for 48

hrs. Content of impurities - ESA1 (Licarbzepine), Oxcarbazepine, Carbamazepine and Hydroxy desamide were checked in the test solutions.

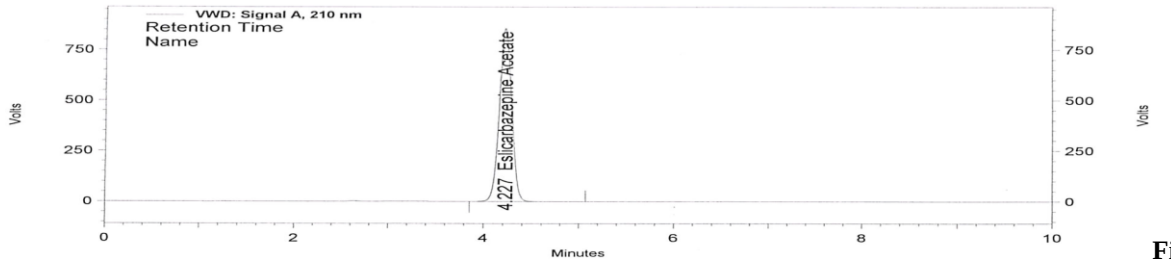


Fig.6. Eslicarbazepine standard chromatogram

Fig.7. Eslicarbazepine spiked chromatogram

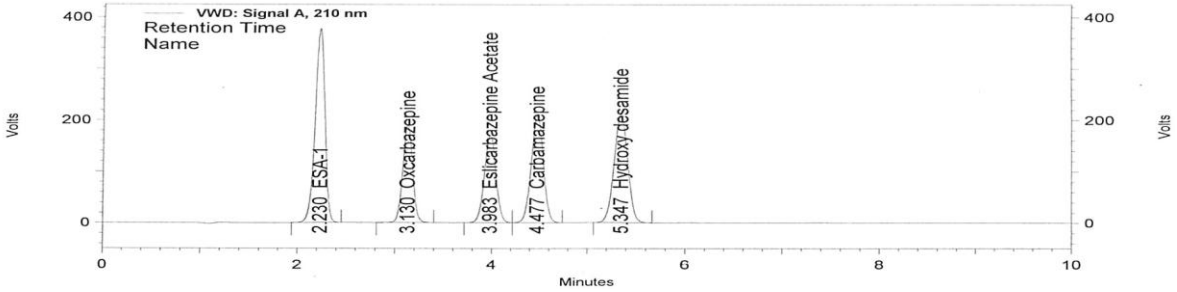


Fig.8. Acid degradation chromatogram

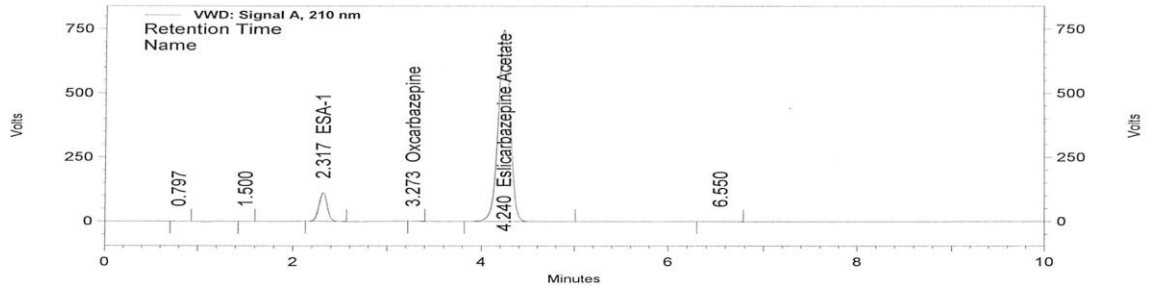


Fig.9. Blank of base (0.5N NaOH) chromatogram

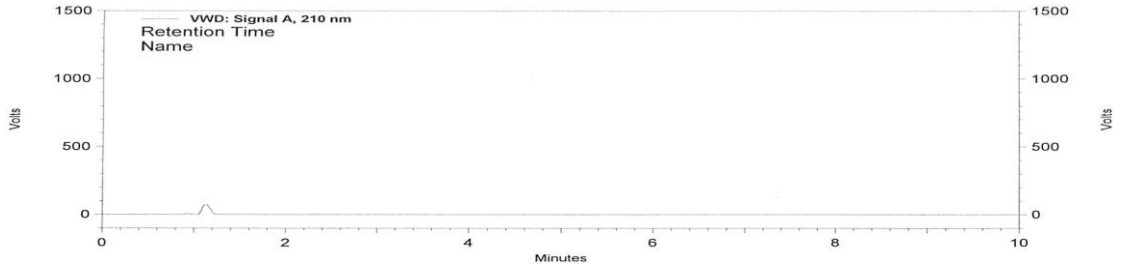


Fig.10. Base degradation chromatogram

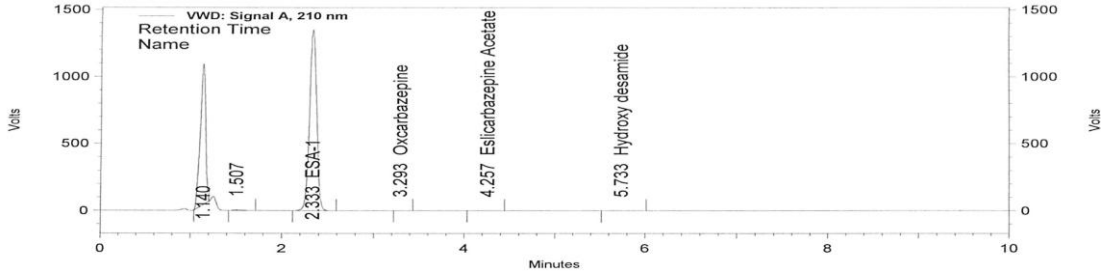


Fig.11. Hydrogen peroxide (H_2O_2) Blank chromatogram

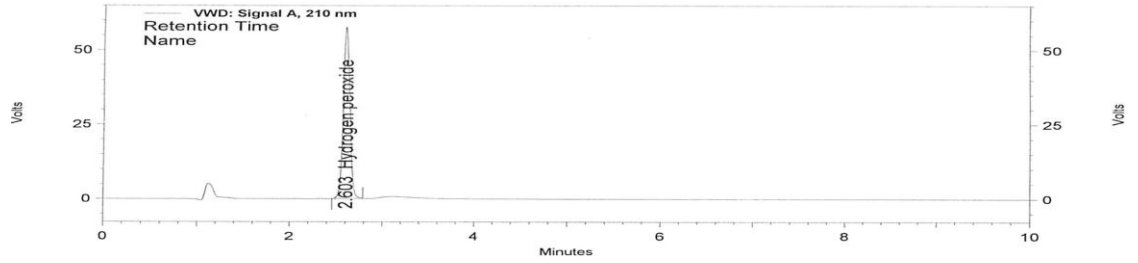


Fig.12. Peroxide degradation chromatogram

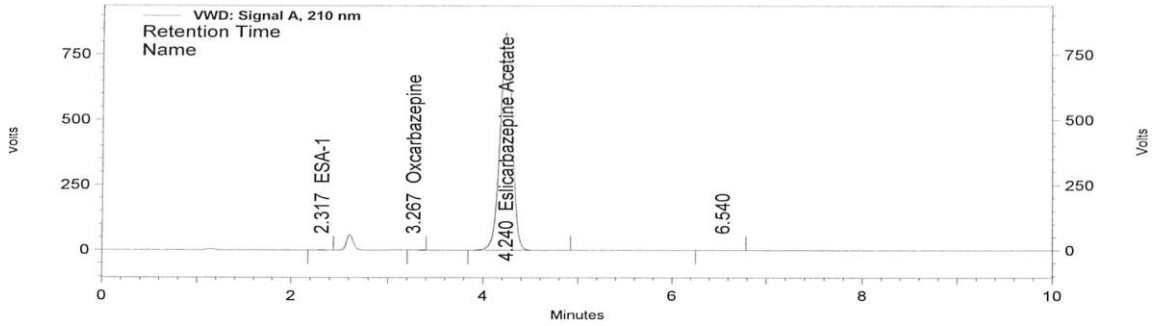


Fig.13. Photo degradation chromatogram

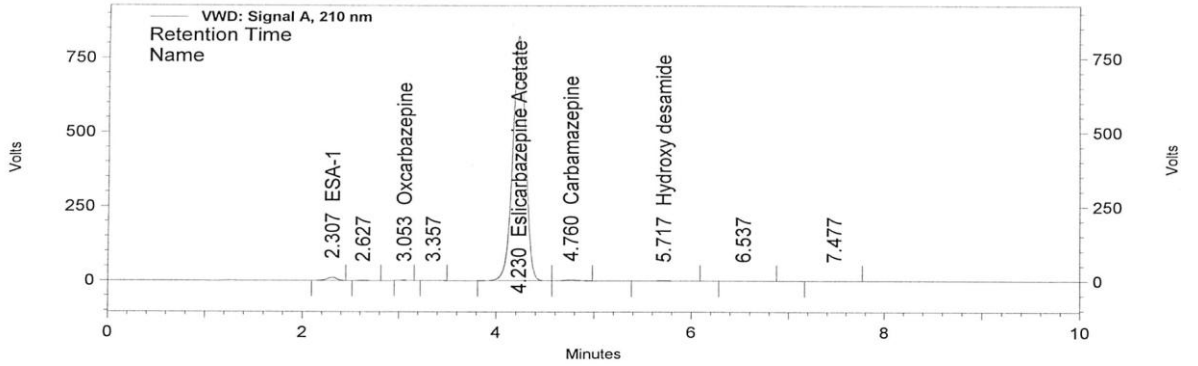


Fig.14. Thermal degradation chromatogram

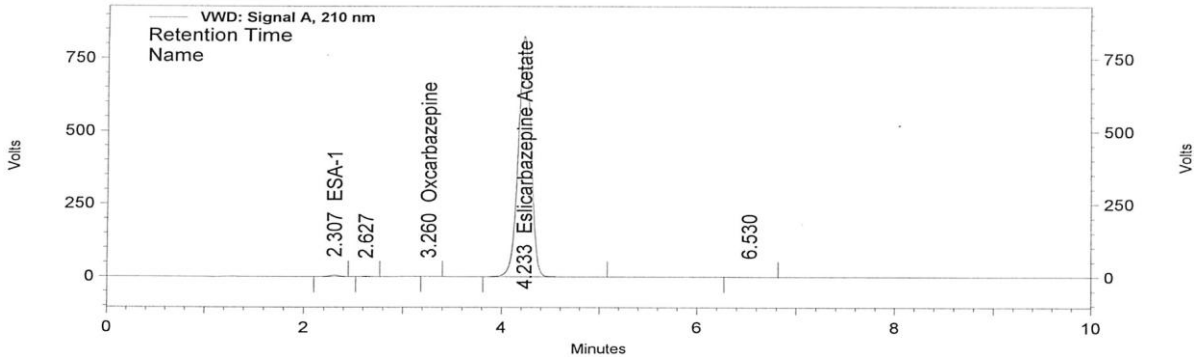


Table 1: Method development and optimized chromatographic conditions

Parameter	Condition
Mobile phase	0.1% orthophosphoric acid buffer, methanol and Acetonitrile in the ratio of 500:250:250
Column	Inertsil ODS 3V, 150 x 4.6 mm, 5µm
Wavelength	210nm
Flow Rate	1.5mL/min
Injection volume	20 µL
Run time	10 min
Diluent	Water, Acetonitrile 500 : 500mL

Table 2: Forced degradation studies

S.No	Stress conditions	Time (hrs)	Assay of Active substance	Total impurities (%	Mass balance Assay impurities (%)
1	Normal	-	100	-	100
2	Acid hydrolysis	48	90.74	9.26	100
3	Base hydrolysis	48	0.06	99.92	99.98
4	Oxidation ($3\%H_2O_2$)	48	99.89	0.11	100
5	Thermal at 80^0C	48	96.88	3.12	100
6	UV light	48	98.23	1.17	99.4

Table3- Limit of Detection and Limit of Quantification

Name	Eslicarbazepine	Licarbzepine	Oxcarbazepine	Carbamazepine	Hydroxy desamide
LOD	2.40	1.59	1.59	1.59	1.59
LOQ	7.29	4.81	4.82	4.81	4.81

Table4- Linearity data

Name	Eslicarbazepine	Licarbzepine	Oxcarbazepine	Carbamazepine	Hydroxy desamide
Linearity (n=3)					
Intercept	4098573.96	-1595.10	-1044.35	-4419.37	-623.78
Slope	122359597.92	310129.87	272655.19	339805.05	429581.73
r	0.99980	0.99997	0.99847	0.99948	0.99974

Table5- Accuracy data

Name	Eslicarbazepine	Licarbzepine	Oxcarbazepine	Carbamazepine	Hydroxy desamide
Accuracy %Recovery	98.45-99.65	98.78-99.12	98.56-99.21	98.14-98.99	99.08-99.54

III. Results And Discussion

To establish and validate an efficient method for analysis of Eslicarbazepine and its impurities in bulk and pharmaceutical formulations, preliminary tests were performed. Different chromatographic conditions were employed for the analysis of the Eslicarbazepine acetate in both bulk and pharmaceutical dosage form. Finally the analysis was performed by using 0.1% Orthophosphoric acid Buffer: methanol : acetonitrile in the ratio of 500:250:250 % v/v at a flow rate 1.5 mL/min. Samples were analyzed at 210nm at an injection volume of 20 µL and separation was carried by using Inertsil ODS 3V, 150 x 4.6 mm, 5µm column. The proposed method was optimized to give very sharp peaks with greater resolution (Fig 7).The optimized conditions were given in table 1.

Forced degradation studies were performed to establish the stability indicating property and specificity of the proposed method. Degradation studies were carried out at 48 hours under conditions of acid hydrolysis, base hydrolysis, thermal, oxidation, and photolysis and the drug substances were observed high degradation in base (0.5N NaOH) comparative remaining in all conditions. Thermal degradation conditions were performed by the drug sample at 80^oC.Acid and base hydrolysis was performed by exposing the drug substances with 0.5N HCl & 0.5N NaOH at 25^oC for 48 hours and it was showed slight (9%) degradation in acid condition and maximum degradation (99.9%) observed in basic condition of Eslicarbazepine acetate and its impurities. And also very slight degradation observed in thermal, photolytic hydrolysis. And there was no degradation occurs under under oxidation (3% H_2O_2) conditions. The results of forced degradation studies were given in table.2

Precision was evaluated by a known concentration of Eslicarbazepine and its impurities was injected six times and corresponding peaks were recorded and % RSD was calculated and found within the limits. The low % RSD value was indicated that the method was precise and reproducible.

Accuracy of the method was proved by performing recovery studies on the commercial bulk and formulation for Assay and related substances at 50%, 100% and 150%, level. Recoveries of Eslicarbazepine acetate and its impurities namely ESA1 (Licarbzepine), Oxcarbazepine, Carbamazepine and Hydroxy desamide ranges from 98.1% to 99.7% in proposed method and the results were shown in the (Table 5).

Linearity was established by analyzing different concentrations for assay 25%, 50%, 100%, 150%, 200% and 250% level of Eslicarbazepine acetate. The calibration curve was plotted with the area obtained versus concentration of Eslicarbazepine acetate, for Related substance 25%,50%, 75%, 100% and 150%, level of impurities namely ESA1 (Licarbzepine),Oxcarbazepine, Carbamazepine and Hydroxy desamide, In the present study concentrations were chosen ranging between 0.5-3 µg/mL of Eslicarbazepine acetate and its impurities. The linear regression data for the calibration plot is indicative of a good linear relationship between

peak area and concentration over a wide range. The correlation coefficient was indicative of high significance and the results were shown in the (Table 4)

Robustness of the method is the ability of the method to remain unaffected by small deliberate changes in parameters like flow rate, mobile phase composition and column temperature. To study the effect of flow rate of the mobile phase it was changed to 0.1 units from 1.5 mL to 1.4 mL and 1.6 mL. The effect of column temperature also checked by changing temperature to ± 5^{o}C. This deliberate change in the above parameters has no significant effect on chromatographic behavior of the samples. LOD and LOQ of Eslicarbazepine acetate and its impurities were evaluated based on relative standard deviation of the response and slope of the calibration curve. The detection limits were found to be 25 µg/mL and 250µg/mL of Eslicarbazepine acetate respectively. The quantitation limit were found to be 25µg/mL for Eslicarbazepine acetate and its impurities. The results were given in the (Table 3).

IV. Conclusion

A new RP-HPLC method has been developed for related substance and estimation of Eslicarbazepine acetate and its impurities namely Licarbzepine, Oxcarbazepine, Carbamazepine and Hydroxy desamide in bulk and pharmaceutical dosage form. The developed method was validated and it was found to be simple, sensitive, precise, and robust and it can be used for the routine as well as stability analysis of Eslicarbazepine acetate, in both bulk and pharmaceutical dosage forms. The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study stability of Eslicarbazepine acetate under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations. Finally it was concluded that the method is simple, sensitive, cost effective and has the ability to separate all impurities from degradation products found in the bulk and dosage form.

Acknowledgements

We would like to thank Dr. J. Srikanth from Suven Nishtaa Pharma Pvt Ltd, Hyderabad, India and Dr.K.Vanitha Prakash from SSJ College of Pharmacy, Hyderabad, India, for giving good support.

References

[1] ICH Q2 (R1), Validation of Analytial procedures, Text and Methodology, International Conference on Harmonization. Geneva. 2005. p. 1-17.

[2]. Fortuna A, Sousa J, and Alves G, Development and validation of an HPLCUV method for the simultaneous quantification of carbamazepine, oxcarbazepine, eslicarbazepine acetate and their main metabolites in human plasma.Analytical and Bioanalytical Chemistry. 2010, 397(4), 1605-15.

[3] Pr. Thacker S, Patel D, RP-HPLC method development and validation for eslicarbazepine acetate in api, International journal of Advanced Research in Pharmaceuticals & Bio sciences. 2012; Vol.2 (2):84-94

[4] Demirkaya F, Kadioglu Y, Determination of carbamazepine using RP-HPLC method in pharmaceutical preparations, Journal of Pharmaceutical Science. 2005, 30:78-82.

[5] Mudigonda S, Narashima RA, Shakil S, Mukkanti K, Stability indicating HPLC method for the determination eslicarbazepine acetate and its impurities in bulk drug and pharmaceutical dosage form., Journal of Liquid Chromatography Related Technologies 2012, 35:1550-64.

[6] M Sing,L Kumar, P Arora,SC Mathu,PK Saini,RM Singh,GN Singh, Development and validation of an RP-HPLC method for quantitative estimation of eslicarbazepine acetate in bulk drug and tablets,Indian journal of pharmaceuitical sciences. 2013: 75 (6): 736-739

[7] Rajendra P.Singh, Jorgej.Asconape,A Review of Eslicarbazepine for the Adjunctive Treatment of Partial – Oneset Epilepsy, Journal of central nervous system diseas. 2011, 3: 179–187.

[8] Ravi Prasad Pandit, Bhuvaneswari Kotamarthi, Rajani Kompally and Anu Radha, FT-IR Spectroscopic Assay Method for Eslicarbazepine Acetate in Bulk and Tablet Formulations, International Journal of Pharmaceutical Chemistry.2015, 05 (07): 255-259

[9] Singh Pradeep kumar, Subas Chandra Dina, Development and validation of stability indicating method for determination of Eslicarbazepine acetate in Eslicarbazepine tablets, International research journal of pharmacy.2013,4 (5), 178-180

[10] Saji Thomas, Saroj kumar Paul, Subhash Chandra joshi,et al.Identification synthesis and characterization of an unknown process related impurity in Eslicarbazepine acetate Active pharmaceuitical ingredients by LC/ESI-IT/MS,$_{1}$H,$_{13}$C and $_{1}$H - $_{1}$H cosy NMR.Journal of pharmaceuitical analysis.2014,4 (5), 339-344

[11] Ana Fortuna, Joana Bicker, Gilberto Alves, A chiral HPLC-UV method for quantification of dibenz (b,f) azepine- 5- carboxamide derivativies in plasma and brain tissue: Eslicarbazepine acetate, carbazepine and main metabolites. Journal of separation science. 2011, 34(12), 1391-1401

[12] Ahmet H. Oztiryaki, Patricio Soares-da-Silva, Comments on the Eslicarbazepine Acetate Section of the Article 'Therapeutic Drug Monitoring of the Newer Anti-Epilepsy Medications'. Journal of Pharmaceuticals 2010, 3, 3629-3632