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Development and Validation of the RP-HPLC Method for Estimation of Eslicarbazepine Acetate in Eslicarbazepine Acetate Tablets

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ABSTRACT

Eslicarbazepine acetate was measured using a practical, easy to use, accurate, precise and reproducible RP-HPLC technology that was developed and validated. Utilizing a mobile phase made up of methanol and water, (Orthophosphoric acid was used to modify the pH of water to 2.5) in 65:35 ratio on Finepak SIL C18T-5 (250 mm× 4.6 mm, 5µm). The separation was accomplished using an isocratic elution method at 0.8 ml/min at 30°C. A Photo diode array detector was used to measure the effluent at 218 nm. In the concentration range of 80 to160 µg/ml, eslicarbazepine acetate demonstrated linearity with correlation value of 0.998. Recovery studies were done to estimate the accuracy of the approach at 80%, 100% and 120% and they were determined to be within the limits furthermore it was demonstrated that the method's accuracy was good. It shows maximum degradation in basic condition than acidic and oxidative condition. No thermal degradation occurred. The developed method on HPLC is precise, specific, repeatable and accurate. The process uses a mobile phase is simple to prepare and is linear over a broad range. Following validation in according with ICH requirements.

INTRODUCTION

Eslicarbazepine acetate, also known chemically as (S)-10-acetoxy-10, 11-dihydro-5H-dibenz [b,f]azepine-5-carboxamide(fig.1) is a potent antiepileptic medication with a broad therapeutic index.[1]It is prodrug that is activated to eslicarbazepine[2]. Epilepsy is most common and dangerous neurological conditions.[3] Epilepsy is a chronic condition that requires ongoing care[4]. ECA is also used as a supporting therapy to treat adult patients with partial onset seizures that are resistant to the treatment[5]. Eslicarbazepine acetate is effective for the treatment of partial and generalized tonic-clonic seizures as a single drug or as an adjuvant with other antiepileptic drug. Eslicarbazepine acetate in pharmaceutical dosage form has been quantified using a few High Performance Liquid Chromatography (HPLC) methods. Present study involves development of a convenient, rapid method on RP-HPLC with a simple and easily available mobile phase for quantitative estimation of eslicarbazepine acetate in bulk drug and tablet dosage form. The optimized method was developed and validated as per International Conference on Harmonization (ICH) guidelines.[6]

Stress degradation studies were conducted to determine the drug's stability indicating feature. Studies on stress degradation conducted in accordance with ICH Q1A (R2) standards included acidic hydrolysis, alkaline hydrolysis, neutral hydrolysis, Oxidation, dry heat and photolysis. According to ICH guidelines, concentrations of acid, base and peroxide should be chosen ascending order[7].

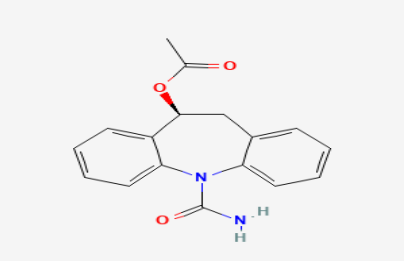

Figure No.1: Structure of Eslicarbazepine acetate

MATERIAL AND REAGENTS

Active Pharmaceutical Ingredient was supplied by Amoli organics Private Limited, Vapi industrial Estate, Pardi, Vapi, and Gujarat. Analytical grade Orthophosphoric acid and merck methanol were the only chemicals used plus Millipore Milli- Q equipment for water filtration was employed to create the water.

Methods

Chromatographic Conditions and Instrumentation: The Finepak SIL C18T-5 column (250 mm × 4.6 mm, 5 µm) was use for optimum separation. The HPLC equipment utilized for method development and validation includes the JASCO-4000 Extrema with ChromNAV software for processing the data equipped with PDA detector and autosampler. Using a 65:35 Methanol: Water (the pH of the water was adjusted to 2.5by Orthophosphoric acid) mobile phase with 0.8 ml/min flow rate. Run time is 10 min prior to being sonicated for 15 minutes in an ultrasonic bath, it was filtered using a 0.45 µm nylon filter. The sample at 218nm was analyzed using an injection volume of 10 µL.

Standard and sample solution preparation of Eslicarbazepine acetate

Curve of calibration methodology Eslicarbazepine acetate, weighing precisely 100 mg was dissolved in the diluents in a volumetric flask with a volume of 100 ml. Eslicarbazepine acetate concentrations of 80, 100, 120, 140 and 160 µg/ml were used to produce the Calibration curve along with a detection wavelength of 218 nm. Procedure for preparing a sample solution for an assay. Eslicarbazepine acetate 100 mg equivalent powder from 20 tablets was precisely weighed and dissolved in diluent in a volumetric flask of 100 ml. To make up the volume, add 50 ml of diluent, sonicate for 10 minutes, and then filter the mixture through 0.45 µm filters.

Method Validation

The recommended technique was validated in accordance with ICH requirements for parameters like specificity, precision, accuracy, linearity, LOD and LOQ.

System Suitability Parameters

Theoretical plate numbers (N), Tailing factor (T), and Percentage relative standard deviation (% RSD) were examined using standard chromatograms after six replicates of a standard eslicarbazepine acetate solution were injected into the system.

Linearity and range

The solution was diluted in methanol from 1 mg/ml to 80-160 µg/ml. From Eslicarbazepine acetate stock solution, linearity test solutions are created at five different concentration levels, ranging from 80 to 160 µg/ml. Utilizing linear regression analysis, the linearity was

identified. The regression analysis equation for eslicarbazepine acetate was Y= 34345x – 61610 for the Range under consideration, and the calibration curve is linear. Eslicarbazepine Acetate was supposed to have correlation coefficients of 0.998.

Specificity

The capacity of the instrument to measure or identify the analyte definitively in the Sample matrix, contaminants, precursors, or degradation product is defined. Specificity is defined by the ICH guidelines as the ability to assess the analyte definitively in the Presence of components that may be present, such as contaminants, degradation products and matrix Components. The particular analytical procedure's lack of specificity may be compensated by additional supporting analytical techniques.

Accuracy

The closeness of the test results to the true value, which can be measured in percent recovery, is referred to as accuracy. The recovery investigation was carried out of three different levels, namely 80, 100, and 120 percent. The outcomes of the recovery experiment were tabulated and determined to be acceptable.

Precision

The degree of scatterness between a series of measurements acquired from multiple sampling of the same homogeneous sample in specific conditions is referred to as the precision of an analytical method. Intraday Precision (repeatability) refers to the use of the same analytical procedure by the same analyst and instrument over short period of time whereas interday precision (intermediate precision refers to the use of same analytical procedure by the different analysts on different day in the same laboratory conditions. On the same day six injections of Eslicarbazepine acetate 120ppm.

Robustness

The method's robustness was tested under a variety of conditions, including changes in flow rate, Temperature, wavelength, and pH of the mobile phase. This deliberate change in the method had no effect on peak tailing, peak area, or theoretical plates, and method was ultimately determined to be robust. The approach's robustness by looking at the effects of tiny changes in method parameters.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The following is the methodology for determining the LOQ and LOD. Linear solution are used to determine LOD and LOQ ranging from 80 µg/mL to 160 µg/mL the technique of computation is based on the response's Standard Deviation (SD) and the slope (S) using the formula and the plot;

$$LOQ= SD/S \times 10$$

$$LOD= 3 \times SD/S$$

The obtained LOD and LOQ values for the Eslicarbazepine acetate are 2.5 and 7.6 µg/ml.

Degradation Studies

The following degradation behavior was suggested by the HPLC investigation on ESA under various stress situations.

Acid degradation: When ESA drug material was exposed to 0.1 N HCL for 48 hours at room temperature, a little deterioration was observed. At room temperature there was a 23.71 Percent deterioration.

Base degradation: ESA medicinal compound was very sensitive to base degradation in basic Solution when it was subjected to 0.1 N NaOH at room temperature for 15 minutes, it is degraded by 29.59 percent.

Oxidative condition (stress degradation using hydrogen peroxide)

The ESA medicinal ingredient was subjected to 6 % H2O2 at room temperature for 48 hours.

There was a 21.14% deterioration.

Thermal degradation: ESA remained thermally stable. The drug material did not degrade after being exposed to 105°C for seven days. (Table no. 3.7)

Assay Method Development

$$\% \text{ Assay} = (At/As) \times (Ws/Ds) \times (Dt/Wt) \times (P/100) \times (\text{Avg, weight/Label Claim}) \times 100$$

At= Average area counts of sample preparation

As= Average area counts of standard preparation

Ws= Weight of working standard taken in mg

Wt= Weight of sample taken in mg

Dt= Sample dilution

Ds= Standard dilution

P= Purity of standard

3. RESULTS AND DISCUSSION

Table No. 3.1: Optimized Chromatographic Conditions

Equipment	HPLCJASCO-4000 Extrema
Column	Finepak SIL C18T-5 (250mm×4.6mm,5µm)
Wavelength	218nm
Detector	PDA detector
Flow rate	0.8ml/min
Column temperature	30ºC
Injection volume	10ul
Run time	10min
Retention time	6.8 min
Diluent	Methanol
Mobile phase	Methanol: water (pH2.5) (65:35V/V)

System suitability parameters:

Table No. 3.2: System Suitability Parameters

System suitability parameters	Eslicarbazepine Acetate
Linearity range	80 to 160 µg/ml
System Factor	1.01
Number of Theoretical Plates	2272
Retention Time	6.8 min
LOD	2.5 µg/ml
LOQ	7.6 µg/ml

Specificity

Injection of Standard and Sample solution and blank were analyzed by direct comparison method to see that diluents or excipients peaks are not interfering with the drug peak.

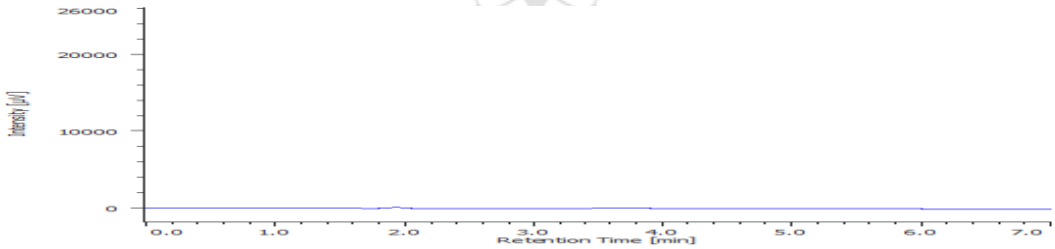

Figure No. 3.1: Chromatogram of Blank

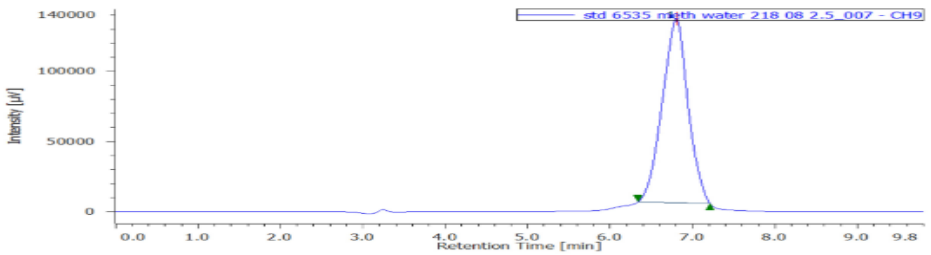

Figure No. 3.2: Chromatogram of Standard

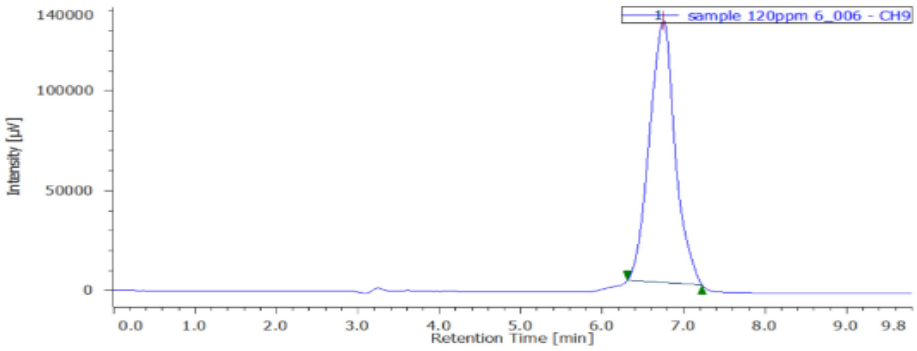

Figure No. 3.3: Chromatogram of Sample

Linearity

The response of Eslicarbazepine acetate was found to be linear in the concentration range of 80 to 160 µg/ml. The correlation coefficient was found to be 0.9983.

Table No 3.3: Linearity of Eslicarbazepine acetate

Concentration of Eslicarbazepine acetate in (µg/ml)	Peak Area
80	2090458
100	2830509
120	3578791
140	4172540
160	4853937

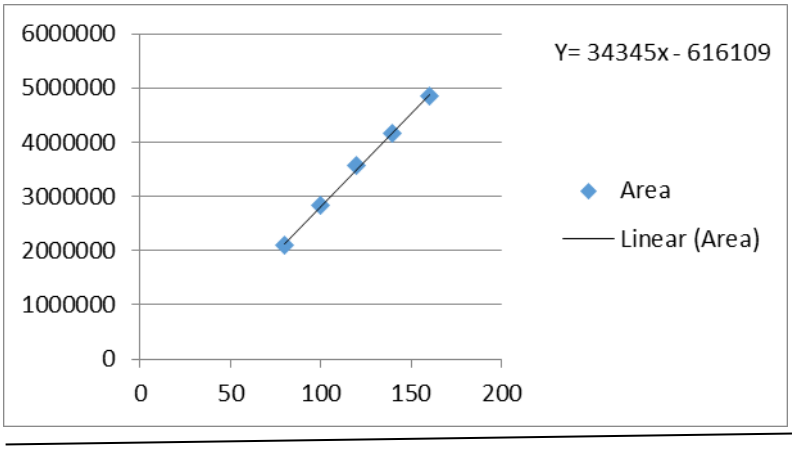

Figure No. 3.4: Standard curve linearity study for Eslicarbazepine acetate

Precision

A. System Precision

Table No. 3.4: System Precision of Eslicarbazepine acetate

Sample ID	Area
Sample-1	3503024
Sample-2	3576298
Sample-3	3554077
Sample-4	3570497
Sample-5	3541661
Sample-6	3542109
Mean	3547944.33±26235.5885
% RSD	0.7395

Method Precision

Table No. 3.5: Method Precision of Eslicarbazepine acetate

Sample ID	Area
Sample-1	3604024
Sample-2	3608298
Sample-3	3654077
Sample-4	3570497
Sample-5	3551661
Sample-6	3632109
Mean	3603444.33±37884.7318
% RSD	1.0513

Accuracy

Table No. 3.6: Accuracy data for Eslicarbazepine acetate

Level	Found (mg)	% Drug	Mean	% RSD
80%	219.09	101.43	100± 0.13	0.134978
	218.52	101.17		
	218.65	101.23		
100%	241.51	100.63	100± 0.29	0.291578
	242.33	100.97		
	240.92	100.38		
120%	257.24	97.44	98± 0.34	0.350484
	256.66	97.22		
	258.43	97.89		

Robustness

Table No. 3.7: Robustness study for Eslicarbazepine acetate

Chromatographic changes		% RSD (Peak area)	% RSD (Retention time)
Flow rate	0.6 ml/min	0.28	0.8
	1 ml/min	0.16	0
Temperature	28	0.36	1.74
	32	0.36	0.26
pH of Mobile phase	2.3	0.15	0.88
	2.7	0.15	0.46
Wavelength	216 nm	0.17	0.38
	220 nm	0.18	0.74

Assay

Table No. 3.8: Assay of Eslicarbazepine acetate

Sr. No	Concentration (µg /ml)	Sample Area	Standard Area
1	120	3522534	2780494
2	120	3573205	3599411
3	120	3551880	3453392
4	120	3589656	3476392
5	120	2879889	3488235
6	120	2867266	3498679
Average		3330738.33	3382767.17
Tablet Average Weight		478 mg	
Standard Weight		100 mg	
Sample Weight		119.8 mg	
Label Amount		400 mg	
Assay		98.22 %	

Degradation studies

Table No. 3.9: Degradation study for Eslicarbazepine acetate

Sr no	Type	Condition	Time	Conc.	Area	% Degradation	Assay value	Assay Degradation
1	Normal	_	_	120	3291965	_	_	_
2	Acid	0.1 N HCl	48 hrs	120	2511511	23.71	98.22	74.51
3	Base	0.1 N NaOH	15min	120	2317830	29.59	98.22	68.63
4	Oxidative	6% H_2O_2	48 hrs	120	2595928	21.14	98.22	77.08

CONCLUSION

The isocratic mode was used with mobile phase composed of methanol: Water, (The pH of water was adjusted to2.5 by Orthophosphoric acid) ina 65:35 ratio on Finepak SIL C18T-5(250x4.6mm i.d., 5µm) under optimized chromatographic conditions. Eslicarbazepine acetate related strong peaks with retention times of roughly 6.8 minutes were produced under the experimental conditions given studies on system suitability were conducted. Eslicarbazepine acetate showed linearity in the concentration range of 80 to160 µg/ml with a correlation value of 0.998. Recovery studies at 80 %, 100 %, and 120%, were used to determine method's accuracy and they were found to be within the limitations. Eslicarbazepine acetate undergoes acid and base hydrolysis by being exposed to 0.1N HCl for 48 hours and 0.1N NaOH at 30°C for 15min. It showed minimal (23.71) degradation in acid condition and maximum (29.59%) degradation in the basic condition. Additionally, No deterioration in thermal degradation was detected. Additionally, there was degradation 6% H_2O_2 oxidation by 21.14%.

The HPLC technique that was created is precise, specific, repeatable and accurate. The process uses a mobile phase that is simple to prepare and is linear over a broad range. These elements make this method appropriate for measuring the amount of Eslicarbazepine acetate in tablets and bulk medications. Following validation in according with ICH requirements.

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