(Research Article)

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RP-HPLC method development and validation for eslicarbazepine acetate in api

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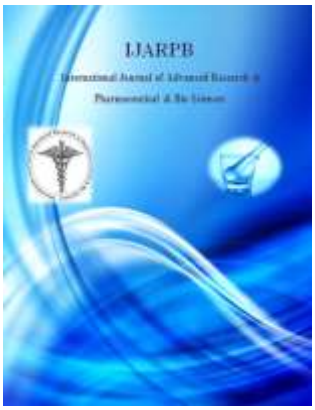

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ABSTRACT

A simple, rapid and precise method was developed for the quantitative determination of Eslicarbazepine Acetate in tablets. The method was based on RP-HPLC. Chromatographic separation was performed on a Inertsil ODS-2, (150 x 4.6mm), 5µm column using a mobile phase of Buffer: Solvent Mixture (Acetonitrile:Methanol: 60:40) in the ratio of 50:50adjusted the pH 2.5 with dilute orthophosphoric acid. The following system conditions were maintained throughout development and validation i.e., flow rate 1ml/min, column was maintained at room temperature and the detected by a UV-wave length at 230 nm.

KEY WORDS: Eslicarbazepine Acetate, RP-HPLC, Method development and validation.

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INTRODUCTION

Eslicarbazepine Acetate [(S)-10-Acetoxy-10, 11-dihydro-5H-dibenz [b,f]azepine-5-carboxamide] is a broad spectrum antiepilepticdrug, chemically different from other anti-convulsants. The mechanism of action of Eslicarbazepine Acetate is inhibition of the release of excitatory neurotransmitters (aspartate and glutamate) and also involvement of the blocking of voltage dependent sodium channels.Eslicarbazepine Acetate is effective for treatment of partial and generalized tonic, clonic seizures as a single drug or as an adjuvant with other anti epileptic drugs[1, 2, 3].

MATERIALS AND METHOD

Materials, reagents, equipment's and instruments

Table: 1 Materials used

Sr.No.	Material Name	Lot. No. / BatchNo.	Mfg.by	Potency
1	ESL Test Sample	ECA/50020111	Ami Life sciences Pvt.Ltd.	NA
2	ESL Working Standard	ECA/WRS/01	Ami Life sciences Pvt.Ltd.	99.75 %

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Table: 2 Chemicals and Reagents used

Sr. No.	Chemical/Reagent	Make	Grade	B.No.
1	Potassium dihydrogen orthophosphate	Merck	AR	MDOM600931
2	Orthophosphoric Acid	Merck	AR	AA1A6100311
3	Acetonitrile	Merck	HPLC	SE1SF61551
4	Methanol	Merck	HPLC	SE1SF61457
5	Purified Water	BTL	HPLC	4093

Table: 3 Equipment used

Sr.No	Equipment	Company Make	Model	Identification No.
1	HPLC	Waters	Alliance	QCI/LC/005
2	HPLC	Waters	Alliance	QCI/LC/026

Table: 4 Column used

Sr.No	Column Name	Serial No.	Manufacturer	In House ID No.
1	Inertsil ODS-2, (150 x 4.6mm),5µm	OHS10077	G.L.Sciences	QC/CL/063

INSTRUMENTS USED

- pH meter (Eutech instruments Ltd)
- Analytical balance (Mettler Pvt Ltd)
- Ultrasonic bath (PCI Pvt Ltd)

PROCEDURE

Buffer Preparation:

1.36g of potassium dihydrogen Orthophosphate was dissolved in l000ml water and pH 2.5 was

adjusted with orthophosphoric acid and mixed well.

Solvent Mixture:

Acetonitrile: Methanol in the ratio of (60:40) v/v was used.

Mobile Phase Preparation:

Buffer: Solvent Mixture in the ratio of (50:50) v/v was used.

Diluent preparation:

Buffer: Methanol in the ratio of (50:50) v/v was used.

Standard Preparation: (50µg/ml)

Accurately 50.0 mg of ESL Working Standard was weighed & transferred into 100 ml volumetric flask. About 60 ml of diluents was added and sonicated to dissolve. The volume was made up to the mark with diluent and mixed. 5 ml of this

Solution was diluted to 50 ml with diluent and mixed.

Sample Preparation: (50µg/ml)

Accurately 50.0 mg of ESL sample was weighed &transferred into 100 ml volumetric flask. About 60 ml of diluents was added and sonicated to dissolve. The volume was made up to the mark with diluent and mixed. 5 ml of this solution was diluted to 50 ml with diluent and mixed.

Table: 5 Trials Taken For Optimize Condition

Sr. No.	Trails Taken	Observation	Remarks
1	Mobile Phase: Buffer : Solvent Mixture: (40:60) v/v Flow rate 1.0 ml/min Detector wavelength :230 nm Injection volume:10µL Column:- :- Inertsil ODS-2, (150 x 4.6mm),5µm	Tailing and Retention time High	Not Satisfactory

	Buffer:- pH 2.5 NaH_2PO_4 Buffer		
2	Mobile phase: Buffer : Solvent Mixture: (60:40) v/v Flow rate 1.0 ml/min Column:- :- Inertsil ODS-2, (150 x 4.6mm),5µm Buffer:- pH 2.5 NaH_2PO_4 Buffer	Retention time satisfactory but low theoretical plate	Not Satisfactory
3	Mobile phase : Buffer : Solvent Mixture: (40:60) v/v Flow rate :1.3 ml/min Column :- Inertsil ODS-2, (150 x 4.6mm),5µm Buffer: - pH 2.5 NaH_2PO_4 Buffer	Retention time satisfactory but low theoretical plate.	Not Satisfactory
4	Column: Inertsil ODS-2, (150 x 4.6mm),5µm Flow rate: 1.0 mL/minute Detector : UV Detector wavelength: 230 nm Injection volume: 10µL Run time : 8 minute Diluent : Buffer: Methanol (50:50) v/v Mobile phase: Buffer : Solvent Mixture: (50:50) v/v	Tailing low, Retention time good, USP Plate High, Area High	Satisfactory (Optimize Condition)

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Figure: 1 Chromatogram of standard Eslicarbazepine Acetate

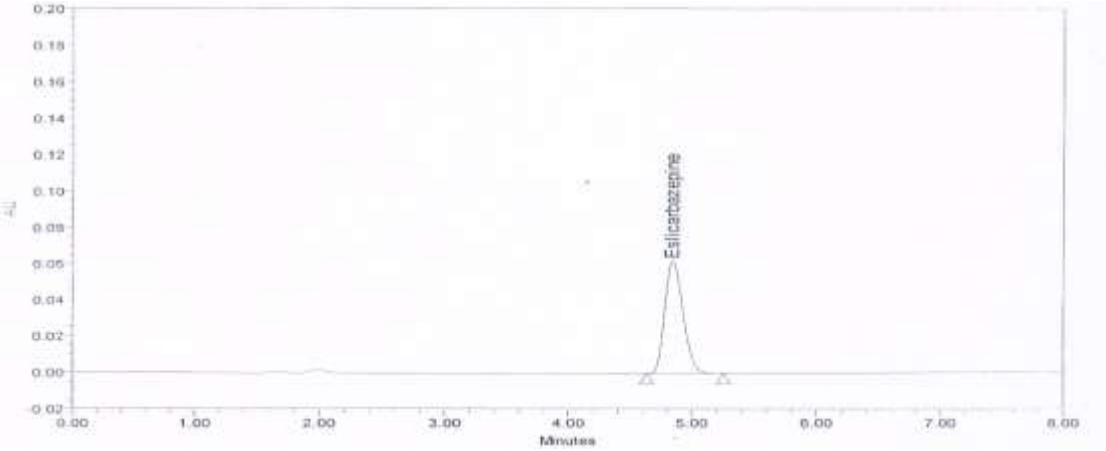
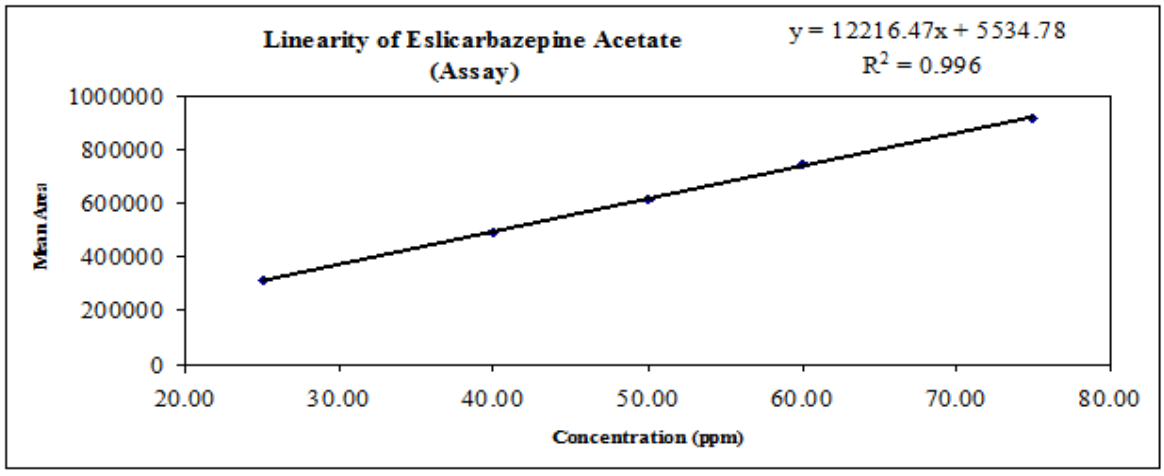


Table: 6 Data for Optimize Chromatographic condition

Name	RT (minute)	Area (µV*sec)	USP Plate Count	USP Tailing
Eslicarbazepine	4.846	633123	5005	1.23

Figure: 2 Calibration curv of Eslicarbazepine Acetate



RESULTS AND DISCUSSION

Method optimization results

Selection of detection wavelength

Eslicarbazepine Acetate showed absorbance at 230nm. So the wavelength selected for the determination of ESLICARBAZEPINE ACETATE was 230nm.

Selection of proper column

Inertsil ODS-2, (150 x 4.6mm), 5µm

Selection of chromatographic conditions

Optimized chromatographic conditions for estimation of Eslicarbazepine Acetate are finalized as shown in Table Figure shows the chromatogram of standard Eslicarbazepine Acetate at optimized method.

Table: 7 Optimized chromatographic conditions

Column	: Inertsil ODS-2, (150 x 4.6mm),5µm
Flow rate	: 1.0 mL/minute
Detector	: UV
Detector wavelength	: 230 nm
Injection volume	: 10µL
Run time	: 8 minute
Diluent	: Buffer: Methanol (50:50) v/v
Mobile phase	: Buffer : Solvent Mixture: (50:50) v/v

Table: 8 % Assay observed of Eslicarbazepine Acetate

Parameters	ESL
Standard Area	633123
	633112
	633115
	633119
	633120
Mean Area	633118
Sample Area	633098
	633095

Mean Area	633097
%Assay	99.91%

The method for anti epileptic drug by some trial for arising out the condition in which peak for Eslicarbazepine Acetate drug resolved in a single HPLC trial run. In gradient start with gradient of Buffer :(ACN: Methanol: 60:40) in which observed peak but retention time is around 8 min. When increase ACN in gradient system and got a good & sharp peak at 4.86 min. The assay of Eslicarbazepine was done by this method and got 99.17% result.

A simple, precise, accurate, rapid, economicalanalytical method for estimation of Eslicarbazepine Acetate is developed by using RP-HPLC method. The developed method is validateas per ICH guidelines.

The developed method can be used for the analysis of routine quality control test. In the present work the RP-HPLC method for the estimation of Eslicarbazepine Acetate in API form has been developed. The proposed method is simple, precise and accurate and do not suffer from any interferences due to common excipients. The newly developed methods can be used in pharmaceutical industry for routine quantitative estimation of Eslicarbazepine Acetate in API form.

The optimized chromatographic conditions and validation parameters are given below

Table: 9 Result of different parameter

Parameter		**Drug**
Specificity		Specific
Linearity	Regression equation y=mx+c	y = 2216.47x+5534.78
	Slope	12216.47
	Intercept	5534.78
	Correlation coefficient (r^2)	0.9996
Accuracy (Recovery)	Level 1	99.46 ± 0.05
	Level 2	98.42 ± 0.09

n=6	Level 3	99.10 ± 0.03
Precision Method precision (Repeatability) %RSD, n=6		0.61
Intermediate Precision (Ruggedness) %RSD, n=6		0.21
Robustness (% RSD), n=3		< 2
System Suitability		0.4

CONCLUSION

A simple, precise, accurate, rapid, economicalanalytical method for estimation of Eslicarbazepine Acetate form has been developed by using RP-HPLC method.

The developed method it will be validateas per ICH guidelines.The developed method can be used for the analysis of routine quality control sample.

The proposed method shows good agreement with all validation parameters. The optimized method is precise, accurate and robust and so it can be applied as stability indicating for the estimation of Eslicarbazepine Acetate in API form.

In the Specificity There should not be any interference from diluent and blank with main peak.

In the Accuracy (recovery) % recovery is 99.02 and % RSD is 0.36 it meets criteria acoording to ICH Guideline

In the study linearity and range also observed, in which we observed the linear relation between the concentration and the result.

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