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Stability indicating thin-layer chromatographic determination of eslicarbazepine acetate as bulk drug: Application to forced degradation study

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ABSTRACT

The objective of present work was to develop a validated stability indicating high performance thin layer chromatographic method (HPTLC) for estimation of eslicarbazepine acetate. The stationary phase used was precoated silica gel aluminium plates 60 F-254 with 250 μm thickness. The mobile phase used for separation was toluene: methanol: acetone (6: 2: 2: v/v/v) gave a resolved peak at (R_f value of 0.67 ± 0.02). Eslicarbazepine acetate was subjected to hydrolytic, oxidative, dry heat treatment and photo degradation. The drug was found to degrade in hydrolytic, oxidative, dry heat conditions and was found to be slightly degraded in photolytic conditions. All the peaks of degraded products were separated from the standard drug with significantly different R_f values of its degradants. The drug showed two degradant peaks in acidic medium at R_f value of 0.06 ± 0.02 , 0.17 ± 0.02 . In basic medium three degradant peaks at R_f value of 0.07 ± 0.02 , 0.18 ± 0.02 , 0.70 ± 0.02 . Oxidative hydrolysis showed three degradants which were resolved at R_f value of 0.05 ± 0.020 , 0.16 ± 0.02 , 0.19 ± 0.02 . Dry heat degradation was observed with two resolved peaks at R_f value of 0.77 ± 0.02 , 0.88 ± 0.02 . Slight degradation was shown in photochemical degradation with one degradant at R_f value of 0.51 ± 0.02 . The developed method can effectively separate the drug from its degradation products under accelerated degradation studies; it can be routinely employed as stability indicating method for eslicarbazepine acetate.

Key words: Eslicarbazepine acetate, Thin layer chromatography, Stability indicating method.

INTRODUCTION

Eslicarbazepine acetate (fig.1) is chemically known as (S)-10-Acetoxy-10, 11dihydro-5H-dibenz [b ,f] azepine-5-carboxamide. Its chemical formula is $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_3$ with a molecular weight $296.32 \text{ g mol}^{-1}$. It is a white to off white crystalline solid powder, insoluble in water and soluble in acetonitrile, acetone and methanol. [1-2].

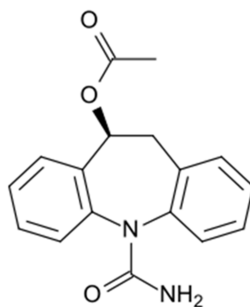


Figure. 1: Chemical structure of eslicarbazepine acetate

Eslicarbazepine acetate is used as an anticonvulsant or antiepileptic drug. It is a prodrug which is activated to eslicarbazepine (S-licarbazepine), an active metabolite of oxcarbazepine. ECA may be used to treat bipolar disorder and trigeminal neuralgia. Eslicarbazepine is a new dibenzazepine antiepileptic agent. It is a high affinity antagonist of the voltage-gated sodium channel. It is closely related to both carbamazepine and oxcarbazepine. Eslicarbazepine has similar affinity to inactivated sodium channels (channels in just activated neurons) as carbamazepine, and greater efficacy in animal models of seizure than oxcarbazepine. It is an effective and reasonably well-tolerated adjunct in patients with suboptimal control of their partial seizures [3-6].

Literature survey reveals various HPLC methods for determination of eslicarbazepine acetate in bulk and formulation, RP-HPLC method development and validation in API, development of liquid chromatographic separation method & validation estimation of (R)- enantiomer [4], development & validation of an RP-HPLC Method for Quantitative Estimation in bulk drug and tablets, enantioselective HPLC-UV method for determination of eslicarbazepine acetate (BIA 2-093) and its metabolites in human plasma, stability indicating HPLC method for the determination of eslicarbazepine acetate and its impurities in bulk drugs and pharmaceutical dosage forms, development & validation of a stability indicating RP-HPLC for determination of eslicarbazepine in eslicarbazepine acetate tablets [7-10]. However to the best of our knowledge, stability indicating HPTLC method has not yet been reported for eslicarbazepine acetate.

The International Conference on Harmonization (ICH) guidelines entitled stability testing of new drug substances and product requires the stress testing of the drug substance should be carried out to elucidate the inherent stability characteristics of the active substance. An ideal stability indicating method is one that quantifies the drug per se and also resolves its degradation product. HPTLC has become a part of routine analytical techniques in many product development and analytical laboratories due to its advantages. The major advantage of HPTLC is that several samples can be simultaneously using a small quantity of mobile phase unlike HPLC thus lowering the analysis time and cost per analysis with high sample throughput. The uniform particle size (7 μm) of precoated HPTLC plates enables achievement of a greater resolution and an easy reproducible separation. The method of detection does not place any restriction on the choice of the mobile phase and unlike HPLC mobile phases having pH 8 and above can be employed [11-12].

The aim of the present work was to develop an accurate, specific, reproducible stability indicating TLC method for estimation of eslicarbazepine acetate as bulk drug in presence of its degradation products.

MATERIALS AND METHODS

Chemicals and reagents

Gift sample of pure drug eslicarbazepine acetate was procured from CTX Life Sciences Pvt Ltd., Surat, Gujarat, India. All solvents and reagents used for the analysis were of analytical grade and were purchased from (Merck, Mumbai, India).

HPTLC instrumentation

A Camag HPTLC system equipped with Linomat V applicator (Switzerland), TLC Scanner III and integrated software Win-Cats (V 3.15, Camag) was used for the analysis. The standard and the sample solutions were spotted in the form of bands of width 6 mm with a Camag 100 μl sample (Hamilton, Bonaduz, Switzerland) syringe, on silica gel pre-coated aluminum plate 60 F-254 plates (20 \times 10 cm, 250 μm thickness, E. Merck, Darmstadt, Germany) supplied by Anchrom technologist, Mumbai. The plates were prewashed with methanol and activated at 110 $^{\circ}\text{C}$ for 5 min prior to chromatography. The slit dimension was kept at 5 mm \times 0.45 mm with data resolution of 100 μm /m step and the scanning speed was 20 mm/s. The monochromatic band width was set at 254 nm, each track was scanned three times and baseline correction was used.

The mobile phase consisted of toluene: methanol: acetone (6: 2: 2 v/v/v) and 10 ml of mobile phase was used per chromatographic run. Linear ascending development was carried out in a (20 cm \times 10 cm) twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was (20 min) at room temperature (28 $^{\circ}\text{C} \pm 2$) at relative humidity of 60% \pm 5. Each chromatogram was developed over a distance of 80 mm. Following the development, the TLC plates were dried in a stream of air with the help of hair dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed at 254 nm. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis.

HPTLC Method and Chromatographic Conditions

Preparation of standard stock and working standard solutions

For preparation of standard stock and working standard solutions eslicarbazepine acetate (10 mg) was weighed accurately and transferred into a 10 mL volumetric flask and dissolved in methanol. Then mixture was sonicated for 20 min. Volume was made up to the mark with methanol to give the concentration of (200 ng/spot).

Prewashing of plates

Densitometric estimation was carried out on (20 cm × 10 cm) pre-coated silica gel 60 F-254 plates from E. Merck. The plates were pre-washed with methanol, dried and activated for 30 min at 110°C.

Selection of solvent

Methanol was selected as a solvent for preparing drug solutions.

Selection of stationary phase

Identification and separation of eslicarbazepine acetate was carried out on (20 cm × 10 cm), pre-coated silica gel aluminium plates 60 F-254 (250 µm thickness E. Merck, Darmstadt, Germany).

Sample application

The standard and working standard solution of eslicarbazepine acetate were spotted on pre-coated TLC plates in the form of narrow bands of length 10 mm, at 10 mm from the bottom and left margin and 10 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nl/s.

Selection of wavelength

Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis at 254 nm using methanol as a blank solution. The detection wavelength was selected at 254 nm and the spectrum of the drug is in (fig.2).

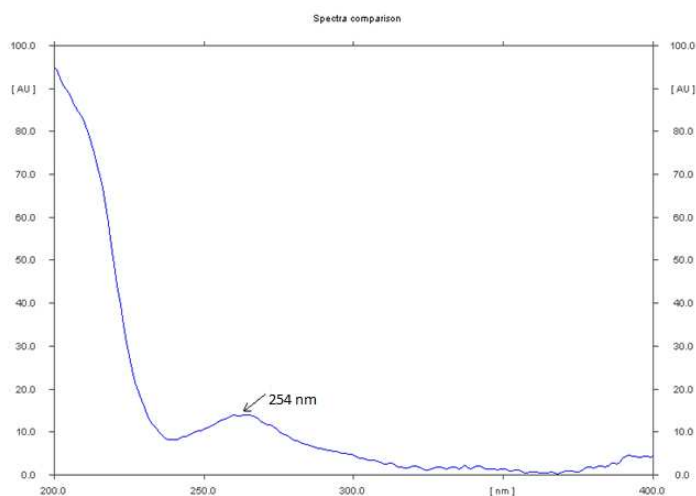


Figure. 2: Spectrum for selection of wavelength (254 nm)

Optimization of the mobile phase

Various solvent systems like mixture of (a) toluene: methanol (7:3 v/v) (b) water: methanol (6: 3 v/v) (c) toluene: chloroform: methanol (1: 5: 3 v/v/v) and (d) toluene: ethyl acetate: methanol (0.5: 4: 2 v/v/v) were tried to separate and resolve spot of eslicarbazepine acetate from its impurities and other excipients. The mixture of toluene: acetone: methanol (5: 2: 3 v/v/v) resolved eslicarbazepine acetate but there was tailing in the peaks. To improve peak symmetry, the ratio was changed. Finally, the mixture of toluene: methanol: acetone (6: 2: 2 v/v/v) showed well resolved peak with better peak shape. The drug was resolved with ($R_f = 0.67 \pm 0.02$). Pre-saturation of TLC chamber with mobile phase for 20 min assured better reproducibility in migration of eslicarbazepine acetate and better resolution.

Method validation

The developed HPTLC method was validated as per the ICH guidelines Q2 (R1) for linearity, precision, repeatability, accuracy, specificity, robustness, limit of detection (LOD), limit of quantification (LOQ).

Linearity (Calibration curve)

A stock solution of eslicarbazepine acetate 10 mg was prepared by dissolving it in methanol. 10 different concentrations of eslicarbazepine acetate (200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 ng/spot) were applied on the TLC plate. The data obtained were treated by least-square regression analysis method.

The linearity range of eslicarbazepine acetate was obtained by plotting the peak area of eslicarbazepine acetate against its varied concentrations over a range (200–2000 ng/spot).

Precision

The intra and inter-day variations were determined using three different concentration levels 200, 400 and 600 ng/spot of eslicarbazepine acetate (n = 3). The precision of the developed method was evaluated by performing repeatability of the sample application and peak area measurement in six replicates of the same spot. The results are expressed in terms of percent relative standard deviation (% RSD) and standard error (SE).

Repeatability

It is also termed as intra-assay precision. Repeatability of sample application was assessed by spotting (400 ng/spot) of standard drug solution six times on TLC plate at different times on same day by sample applicator, followed by development of plate and recording of the peak areas for six spots.

Recovery and specificity studies

Recovery studies were carried out to determine accuracy of the developed method at 80%, 100% and 120% levels. It was done by mixing known quantity of standard drug (400 ng/spot) with the sample formulation and contents were analysed by the proposed method. The % recovery and % RSD were calculated respectively.

The specificity of the method was ascertained by analysing the R_f values and spectra pattern of reference marker and drug samples. The marketed formulation, Aptiom (eslicarbazepine acetate 600 mg (Sunovion Pharma Co. Ltd) was sonicated (10 mg in 5 ml methanol) for 20 min. The volume was made up to 10 ml by adding methanol. The resulting solution was centrifuged and the supernatant was filtered. The amount of eslicarbazepine acetate was determined by developing the chromatogram (400 ng/spot) in triplicate by maintaining the chromatographic conditions. The spot for eslicarbazepine acetate in formulation was confirmed by comparing the R_f and densitogram of the spot with that of standard.

Robustness

In this study, small changes in the composition and volume of mobile phase and development chamber saturation time were made and their effects on the results were examined. The study was done in triplicate at a concentration 400 ng/spot of eslicarbazepine acetate. The results of peak area are expressed as % RSD and SE.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were experimentally determined by visual detection method recommended in ICH guidelines. To estimate the LOD and LOQ blank methanol was spotted six times. Spotting for LOD was done by taking different concentrations as 200, 400, 600, 800 and 1000 ng/spot and the values were considered with a signal-to-noise ratio of 3:1 and 10:1 respectively.

Accelerated degradation of eslicarbazepine acetate

Accelerated degradation was carried by exposing the drug to different stressed conditions. A drug stock solution of eslicarbazepine acetate (10 mg) was prepared in 10 ml methanol. This drug solution was used for forced degradation to provide an indication of the stability indicating property and specificity of the proposed method.

Acid and base induced degradation

To 1 ml solution of eslicarbazepine acetate 10 ml 1 M HCl and 0.1 M NaOH were added separately. These mixtures were refluxed separately for 4 h at 80°C respectively. One micro liter (200 ng/spot of eslicarbazepine acetate) of resultant solutions were applied on TLC plate and developed.

Hydrogen peroxide induced degradation

To 1 ml solution of eslicarbazepine acetate, 5 ml (H₂O₂) (5% v/v) was added. This solution was heated in boiling water bath for 10 min to remove completely the excess of hydrogen peroxide and refluxed for 4 h at 80°C. One micro liter (200 ng/spot of eslicarbazepine acetate) of resultant solutions were applied on TLC plate and developed.

Dry heat induced degradation

Dry heat degradation of eslicarbazepine acetate was carried out by placing the bulk drug into a hot air oven at 80°C for 2 h. One micro liter (200 ng/spot of eslicarbazepine acetate) of resultant solutions were applied on TLC plate and developed.

Photochemical degradation

10 ml solution of eslicarbazepine acetate was studied by exposing to direct sunlight for 48 h. One micro liter (200 ng/spot of eslicarbazepine acetate) of resultant solutions were applied on TLC plate and developed.

RESULTS AND DISCUSSION**Development of TLC procedure**

The TLC procedure was developed and optimized with a view to quantify the eslicarbazepine acetate content in standard and in test samples. The mobile phase toluene: methanol: acetone (6: 2: 2 v/v/v) was optimized and selected by trial and error method on the basis of resolution with a sharp and well defined peak at $R_f = 0.67 \pm 0.02$ (fig. 3).

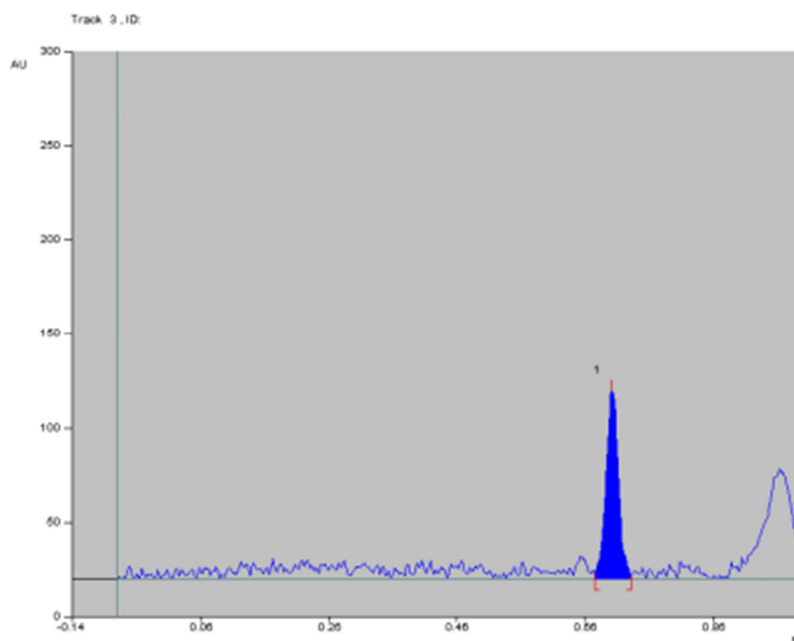


Figure. 3: Chromatogram of eslicarbazepine acetate ($R_f = 0.67$)

Calibration curve

The developed HPTLC method for estimation of eslicarbazepine acetate showed a correlation coefficient ($r^2 = 0.995$) with SD 10.34 and intercept 288.56 in the concentration range of 200–2000 ng/spot (Table 1) with respect to the peak area, (fig. 4) displays the calibration curve of eslicarbazepine acetate at 254 nm.

The linearity of calibration graphs and adherence of the system to Beer's law was validated by correlation coefficient. No significant difference was observed in the slopes and standard curves (ANOVA, $p < 0.05$).

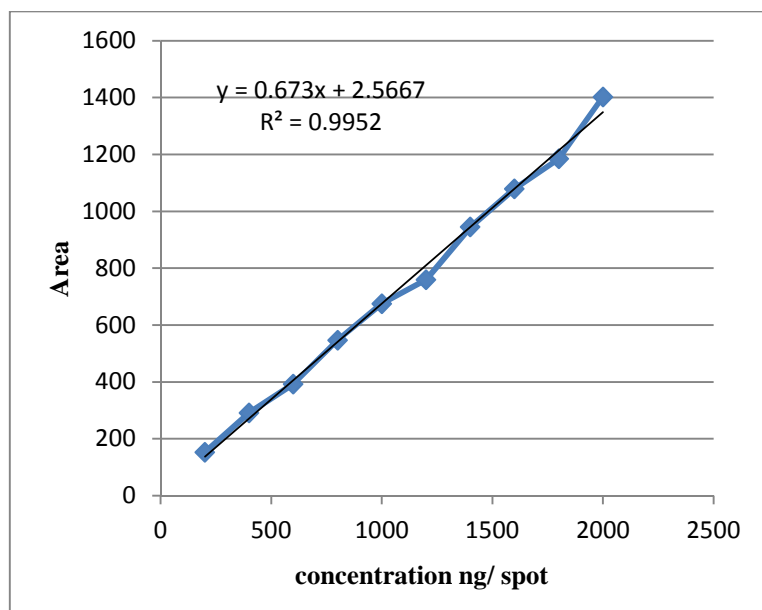


Figure 4: Calibration curve of esliocarbazepine acetate

Table 1: Summary of linear regression and validation data

Parameters	HPTLC
Linearity range (ng/spot)	200-2000
Correlation coefficient (r^2)	0.995
Intra-day precision (n=3)	9.96 ± 0.94
Inter-day precision (n=3)	9.03 ± 0.90
Limit of detection (ng/spot)	200
Limit of quantification (ng/spot)	800
Recovery (n=3) (%)	98.75%
Robustness	Robust
Specificity	Specific

Validation of the method

Precision

The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria which indicated that the method was precise. In precision studies on intra and inter-days, the resultant peak area for esliocarbazepine acetate determined at three different concentration levels (200, 400, 600 ng/spot of esliocarbazepine acetate) showed % RSD (<1.8%) for inter- and intra-day variations which suggested an excellent precision of the method (Table 2).

Table 2: Precision study (n= 3)

Precision	Conc. (ng/spot)	R_r	SD	SE	%RSD
	200	0.67	5.310	1.77	1.390
Intra-day	400	0.67	9.960	3.32	1.772
	600	0.67	15.099	5.03	1.976
	200	0.67	5.008	1.66	1.374
Inter-day	400	0.67	9.032	3.01	1.548
	600	0.67	10.346	3.44	1.334

SD = standard deviation; RSD = relative standard deviation; SE=standard error

Repeatability

The % RSD for repeatability of the drug was found to be (<2). The measurement of peak areas at three different concentration levels showed low value of % R.S.D. (<2). (Table 3). Hence the proposed method for estimation is proved to be repeatable in nature.

Table 3: Repeatability study (n=3)

Conc. (ng /spot)	Area	SD	SE	%RSD
400	583.04	10.482	3.49	1.797
400	582.04	7.261	2.42	1.230
400	578.97	8.136	2.17	1.405

SD = standard deviation; RSD = relative standard deviation; SE=standard error

Recovery and specificity studies

Results of the recovery study showed high efficiency of eslicarbazepine acetate from the samples. The proposed method afforded recovery in the range of 96.25–98.75 % (Table 4). This confirms that the proposed method can be used for the determination of eslicarbazepine acetate in formulations at different concentration levels.

The peak purity of eslicarbazepine acetate was assessed by comparing their respective densitograms at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = (0.32 -0.4) and r (middle, end) = (0.4 -0.48). Good correlation was obtained between standard and sample densitograms of eslicarbazepine acetate.

Table 4: Recovery study (n=3)

Levels	Concentration added (ng/ spot)	Concentration found (ng/ spot)	%Recovery
80	0.32	0.316	97.75
100	0.40	0.395	96.75
120	0.48	0.474	98.75

Robustness of method

The % R.S.D. and SE of the peak areas was calculated for change in mobile phase composition, mobile phase volume, temperature, time from spotting to chromatography and time from chromatography to scanning in triplicate at concentration level of (400 ng/spot) of eslicarbazepine acetate. The deviation obtained by deliberate changes in various parameters % R.S.D (<2) (Table 5) which indicated that the developed HPTLC method was robust.

Table 5: Results of robustness testing

Parameters	SD	SE	%RSD
Mobile phase composition (\pm) 0.1ml	7.261	1.2	1.230
Amount of mobile phase (\pm) 0.1ml	8.136	1.3	1.405
Temperature (\pm) 5°C	9.960	1.6	1.772
Time from spotting to chromatography (\pm) 5 min	9.031	1.5	1.548
Time from chromatography to scanning (\pm) 5 min	10.48	1.7	1.797

SD = standard deviation; RSD = relative standard deviation, SE=standard error

LOD and LOQ

Detection limit and limit of quantification were found to be 200 and 800 ng/spot respectively, which indicate adequate sensitivity of the method. The stronger pronounced effective diffusion of the eslicarbazepine acetate spot at higher R_f value 0.6765 ± 0.02 as against 0.65 ± 0.02 in our proposed method (Table 1).

Stability indicating property

The number of degradation products with their R_f values at % recovery of eslicarbazepine acetate was calculated and listed in (Table 6).

Table 6: Summary of forced degradation study

Acid and base induced degradation

	Stressed Condition	Eslicarbazepine acetate		Degradation Product		Figure
		%	R_f	%	R_f	
1	Acid, 10 ml (1 M HCl reflux for 4 h at 80°C)	90.12 \pm 0.24	0.67	9.88 \pm 0.29	0.06, 0.17	fig.4
2	Base, 10 ml (0.1 M NaOH reflux for 4 h at 80°C)	88.72 \pm 1.01	0.67	11.28 \pm 2.7	0.07, 0.18, 0.70	fig.5
3	Hydrogen peroxide, 10 ml, 5% v/v H ₂ O ₂ (reflux for 4 h at 80°C)	92.24 \pm 2.15	0.67	7.76 \pm 1.12	0.05, 0.16, 0.19	fig.6
4	Dry heat (4 h at 80°C)	95.23 \pm 1.53	0.67	4.77 \pm 1.42	0.77, 0.88	fig.7
5	Photochemical stability (Daylight, 48 h)	98.89 \pm 0.36	0.67	1.11 \pm 2.5	0.51	fig.8

The chromatograms of acid and base degraded products showed additional peaks at R_f value 0.06 and 0.17 in acid induced degradation and 0.07, 0.18 and 0.70 in base induced degradation respectively. The % recoveries of

eslicarbazepine acetate at the level of 90.12% in acid and 88.72% in basic condition suggested that eslicarbazepine acetate undergoes degradation under acidic (fig. 5) and basic conditions (fig. 6).

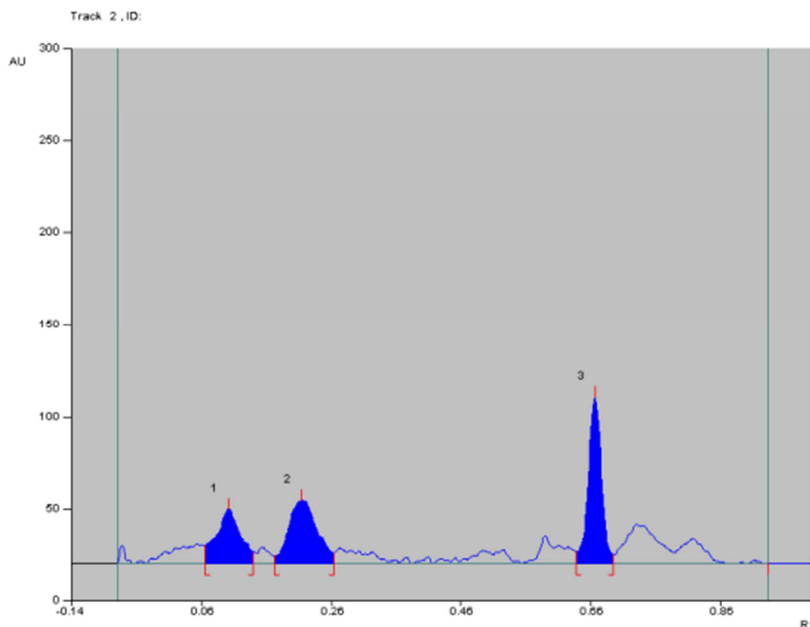


Figure. 5: Chromatogram of acid [1 M HCl (reflux for 4h at 80°C)] treated sample
Peak 1- degradant [$R_f=0.06$], Peak 2 degradant [$R_f=0.17$], Peak 3 – Eslicarbazepine acetate [$R_f= 0.67$]

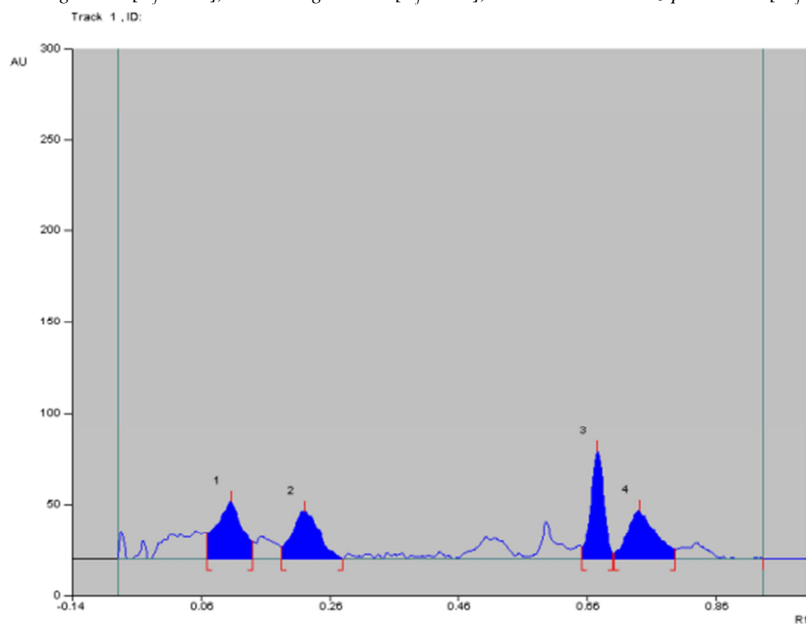


Figure. 6: Chromatogram of base [0.1 M NaOH (reflux for 4 h at 80°C)] treated sample
Peak 1- degradant [$R_f=0.07$], Peak 2- degradant [$R_f=0.18$], Peak 3- Eslicarbazepine acetate [$R_f= 0.67$], Peak 4- degradant [$R_f=0.70$]

Hydrogen peroxide induced degradation

The chromatogram of hydrogen peroxide showed three additional peaks at R_f value 0.05, 0.16 and 0.19 (fig. 7) other than the standard peak of eslicarbazepine acetate. The % recovery of eslicarbazepine acetate at the level of 92.24% suggested that eslicarbazepine acetate showed degradation under oxidative condition.

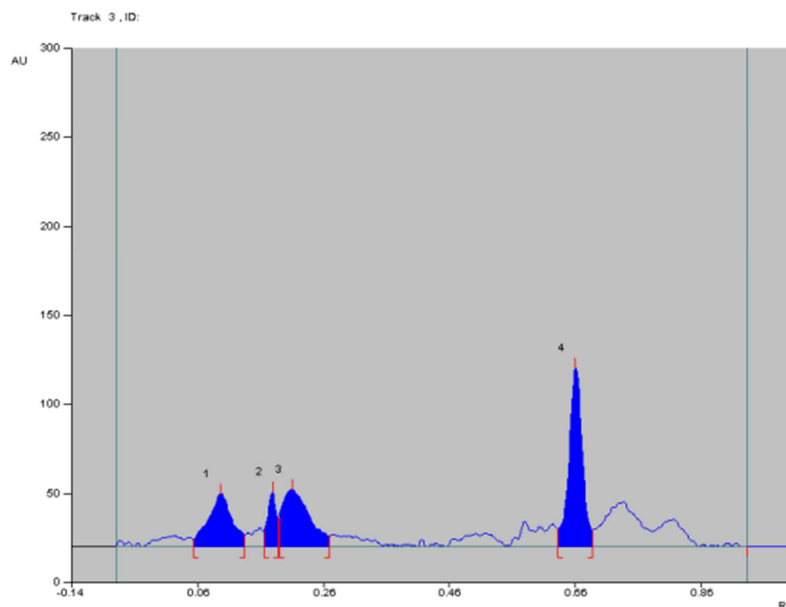


Figure .7: Chromatogram of hydrogen peroxide [5% v/v H₂O₂ (reflux for 4h at 80°C)] treated sample
Peak 1- degradant [$R_f=0.05$], Peak 2- degradant [$R_f=0.16$], Peak 3- degradant [$R_f=0.19$], Peak 4- Eslicarbazepine acetate [$R_f= 0.67$]

Dry heat degradation

The sample degraded under dry heat showed two additional peaks at R_f value 0.07 and 0.88 (fig. 8) other than the standard peak of eslicarbazepine acetate. The % recovery of eslicarbazepine acetate at the level of 95.23% suggested that eslicarbazepine acetate undergoes mild degradation under dry heat condition.

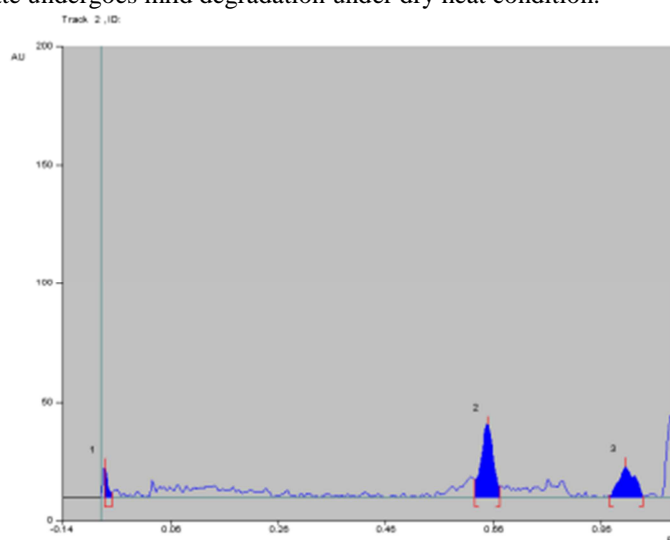


Figure .8: Chromatogram of dry heat [4h at 80°C] treated sample
Peak 1- degradant [$R_f=0.07$], Peak 2- eslicarbazepine acetate [$R_f=0.67$], Peak 3- degradant [$R_f=0.88$]

Photochemical degradation

The sample exposed to photochemical degradation showed additional peak at $R_f = 0.07$ (fig. 9) other than the standard peak of eslicarbazepine acetate. The % recovery of eslicarbazepine acetate at level of 98% which suggests that eslicarbazepine acetate undergoes mild degradation under photochemical degradation.

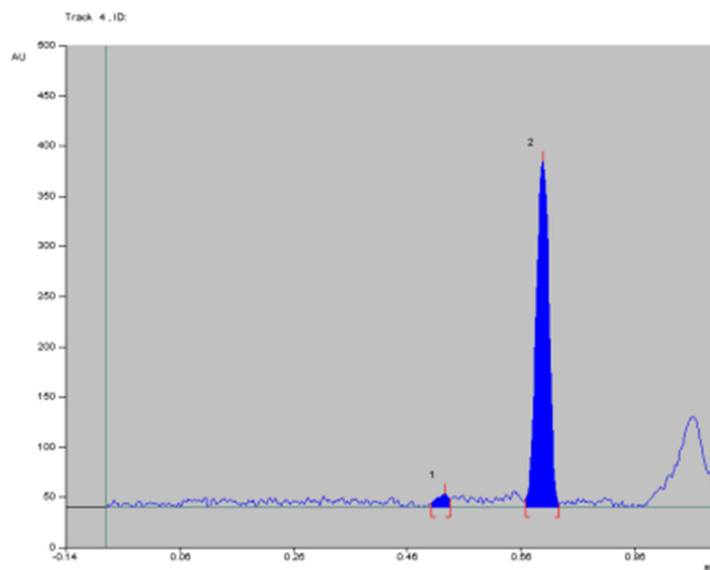


Figure. 9: Chromatogram of photolytic [4h at 80°C] treated sample
Peak 1- degradant [$R_f=0.51$], Peak 2- eslicarbazepine acetate [$R_f=0.67$]

CONCLUSION

The developed HPTLC method enables accurate, precise, specific and stability indicating TLC method for determination of eslicarbazepine acetate. Statistical analysis proves that the method is reproducible for routine analysis of eslicarbazepine acetate in pharmaceutical dosage form without interference from excipients. The developed method was able to separate the drug from its degradants hence can be successfully applied as a stability indicating one.

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