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IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF ALKALINE DEGRADATION PRODUCT OF ESLICARBAZEPINE ACETATE

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ABSTRACT

Eslicarbazepine acetate (ESA) a potent sodium channel blocker and an antiepileptic drug were subjected to various stress conditions as recommended by ICH guideline which includes hydrolytic, oxidative, thermal, and photolytic stress. The degradation studies show that ESA degraded in alkaline hydrolytic condition only while it found to be stable in other stressed conditions. A single degradation product emerged in UPLC studies on Waters Acquity BEH 150 x 2.1 mm, 1.7 µm, C18 column using mobile phase A (0.01M potassium dihydrogen orthophosphate and acetonitrile; 90:10 v/v) and mobile phase B (acetonitrile-water-methanol; 75:5:25 v/v) in the ratio of 50:50 (v/v) at RT 1.83 min with 0.2 ml/min flow rate and 2µl injection volume. The obtain degradation product from Eslicarbazepine acetate was isolated and characterized by IR, ^{1}HNMR and UPLC-MS/MS analytical techniques. IR evaluation exhibited peaks at 3475.73 cm^{-1}, 3363.86 cm^{-1}, 1726.29 cm^{-1} for O–H, COO–H and C=O group, respectively. ^{1}H NMR spectrum showed singlet at 11.865 which indicated the presence of COOH group. UPLC-MS/MS analysis shows molecular ion peak at 255.2 m/z. The recognized peak in the obtained spectrum confirmed the structure of the degradation product as 10-hydroxy-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxylic acid which was formed by alkaline hydrolytic degradation.

Keywords *– Stress degradation, Degradation pathway, Antiepileptic, UPLC, ICH.*

1. INTRODUCTION

The regulatory guidelines necessitate the requirement of drug substance and drug product stability testing data in order to determine the drug stability in various environmental factors. This stability testing data assist the study of molecule intrinsic stability, probable degradation products and postulate the degradation pathways as per International Conference on Harmonization (ICH). It also facilitates the quantification of a drug in presence of degradation products [1-5]. Significantly it is required to isolate and characterize the degradation products formed by analytical techniques [6-8]. The objective of our study is to identify, isolate and characterize Eslicarbazepine acetate (ESA) degradation product formed in stability indicating assay by various

analytical techniques. ESA is a prodrug and an active metabolite of oxcarbazepine. It is a potent third generation antiepileptic agent. ESA use is limited to partial-onset and generalized tonic-clonic seizures. ESA target the voltage-gated sodium channel site [9-13]. Chemically represent as (S)-(-)-10-acetoxy-10, 11-dihydro-5H-dibenz [b, f] azepine-5-carboxamide (Fig. 1) [14]. The stress degradation studies in diverse stress conditions demonstrate that ESA shows degradation in alkaline hydrolysis whereas in acid hydrolysis, neutral hydrolysis, oxidative, thermal, and photolytic stresses it was found to be stable [15-18]. As per our knowledge, no work has been reported so far in the literature on isolation, identification and characterization of degradation products formed in forced degradation condition of Eslicarbazepine acetate.

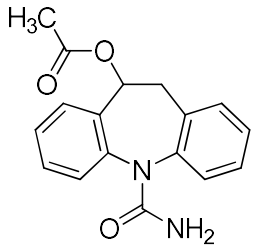

Fig. 1: Structure of Eslicarbazepine acetate

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The standard drug Eslicarbazepine acetate was acquired as a gift sample by Arbro Pharmaceuticals Pvt. Ltd. Hydrogen peroxide (30%), Hydrochloric acid, sodium hydroxide were purchased from SD Fine-chem. Procurement of methanol (MeOH), Acetonitrile (ACN), and HPLC grade water were done from Merck India (Mumbai) and Potassium Bromide (Spectroscopic grade) from Sigma-Aldrich. Analytical grade chemicals and materials were used thought the experiment.

2.2 Instrumentation

2.2.1 Ultra performance liquid chromatography (UPLC)

Waters Acquity system (Waters Corporation, Milford, Massachusetts, USA) was used for UPLC study. The UPLC system was assembled with a solvent manager (binary), sample manager (auto sampler) and a PDA as a detector. In stress degradation studies, for chromatographic separation the column used was Waters Acquity BEH 150 x 2.1 mm, 1.7 µm, C18 column. The Empower software was used to acquired and processed output signal.

2.2.2. Infra-red spectroscopy (IR)

JASCO-FT/IR-470 Plus FTIR spectrophotometer was used for Infra-red spectroscopy of degradation product. KBr (Merck) pellet was used for sample preparation. Previously dried KBr in a hot air oven was triturate with the degradant sample. Pellet was prepared in pellet maker using the pressure of 8-10 tons. During the experiment scanning was carried out in full length spectral range ($4000cm^{-1}$ to $400cm^{-1}$).

2.2.3. Nuclear Magnetic Resonance spectroscopy (NMR)

Bruker Spectro Spin 400 MHz manufactured Bruker (Canton, Massachusetts, USA) was used for NMR analysis. ESA degradation product ^1H NMR was carried out by dissolving the degradant in dimethyl-sulfoxide (DMSO-d6). Chemical shifts were reported on δ scale in ppm units to tetramethyl silane (TMS) as an internal standard.

2.2.4. Mass Spectrometry (Quadrupole-Time of Flight)

Mass spectra were recorded by hyphenated technique LC-MS/MS. In this UPLC was manufactured by Waters Corporation, Milford, Massachusetts, USA and the Quadrupole-Time of Flight mass spectrometer by Micromass MS Technologies, Manchester, UK. A full scan was carried out to detect the ions. MassLynx V 4.1 software was used to process the data.

2.2.5. Other

For the filtration of the mobile phase, Millipore filter assembly with 0.45µm nylon membrane was used, and the pH of the mobile phase was adjusted by microprocessor water proof pH tester (pH tester, Eutech Instruments, Oakton, USA). Hot air oven (Oven universal with thermotech thermostat TIC-4000 N, S.M. Industries, New Delhi, India) was used for thermal studies. The drug was refluxed in a round bottom flask condenser assembly, for hydrolytic degradation studies.

2.3 Preparation of standard stock solution

Weigh accurately 10mg of Eslicarbazepine acetate and transfer into a 10ml volumetric flask and make a stock solution of 1 mg/ml by methanol. Then by diluents, dilute the stock solution and the standard solution of desired concentration was made in stress degradation studies.

2.4 Stress degradation studies

The stress degradation studies were performed to develop the stability indicating property of the drug. The ICH Q1A (R2) guidelines recommend the stress degradation studies under different conditions of acidic hydrolysis, alkaline hydrolysis, neutral hydrolysis, oxidation, dry heat, and photolysis. For stress degradation studies, four samples were made for each stress condition viz., *blank solution* which was stored in ambient condition, *stressed blank solution* which was subjected to stress conditions, *drug solution* that is Eslicarbazepine acetate stored in ambient condition and *stressed drug solution* that is Eslicarbazepine acetate subjected to stress conditions. The stock solution was refluxed at 60˚C for 15 min. with 2N HCl, 0.001N NaOH, 1 ml HPLC grade water and 30% H_2O_2 for acidic, alkaline, neutral and oxidative hydrolytic condition respectively. For thermal stress degradation, the drug solid sample was exposed for 10 days to dry heat condition at 100 ˚C. For photolytic studies, dry powder of drug was exposed for 48 hrs in the daytime (60,000–70,000 lux) to sunlight.

2.5 UPLC method for stress degradation studies

Stress degradation studies and characterization of degradation product was executed on Waters Acquity UPLC system (Waters Corporation, Milford, Massachusetts, USA). The main component of the system includes a solvent manager (binary), sample manager (auto sampler) and a PDA (detector). For UPLC studies chromatographic separation was executed at 30˚C column temperature on C18 column (Waters Acquity BEH 150 X 2.1 mm, 1.7 µm), using 0.01M potassium dihydrogen orthophosphate and acetonitrile; 90:10 v/v (mobile phase A) and acetonitrile-water-methanol; 75:5:25 v/v (mobile phase B) in the ratio of 50:50 v/v at flow rate of 0.2 ml/min. Methanol was used as a diluents and 2µl injection volume was used. 215nm wavelength was used to detect the eluent and data was analysed by Empower software.

2.6 Degradation product isolation and characterization

Degradation studies on ESA under different stress conditions, recommended that ESA depredate only in alkaline hydrolytic condition. In the mixture of a stress solution single degradation product was found. Considerably it is required to isolate and

characterize the degradation product formed. 10 mg/ml of drug solution in 0.001N NaOH is allowed to reflux for 8 hrs at 60˚C. Concurrently the formation of degradation product was monitored by thin layer chromatography. A prominent single spot was observed on TLC plate, which confirms the formation of ESA degradation product. The reaction solution was allowed to cool down. Further, neutralize the reaction solution with HCl acid and add some crushed ice, it gives the precipitated product. The product is allowed to dry completely, and structural elucidation was done by IR, ^{1}H-NMR and mass spectrometry.

3. RESULT AND DISCUSSION

3.1 UPLC analysis

ESA UPLC studies under different stress conditions, recommended that ESA show prominent degradation in alkaline hydrolytic condition and do not degrade under acid hydrolysis, neutral hydrolysis, oxidative, photolytic and thermal stresses. In a mixture of a solution single degradation product was originated in alkaline hydrolytic condition. In a mixture of the stressed sample UPLC analysis demonstrate in Table 1 the retention time (RT) and relative retention time (RRT) of the drug and its degradant (DP 1). 8 hr of reflux in 0.001N NaOH at 60˚C, showed a new peak at RT 1.83 min (0.695 RRT) besides ESA peak at 2.63 min (1.000 RRT). Due to alkaline stress degradation the new peak was emerged at 1.83 min RT which was demonstrated as DP 1. The conformation of specificity and selectivity of the method was done on the basis of RT, RRT and resolution of peaks in the chromatogram (Fig 2).

Table 1: RT and RRT of Eslicarbazepine acetate and its degradation product

PEAKS	RT	RRT
DP 1	1.83	0.695
Eslicarbazepine acetate	2.63	1.000

RT: Retention time; RRT: Relative retention time

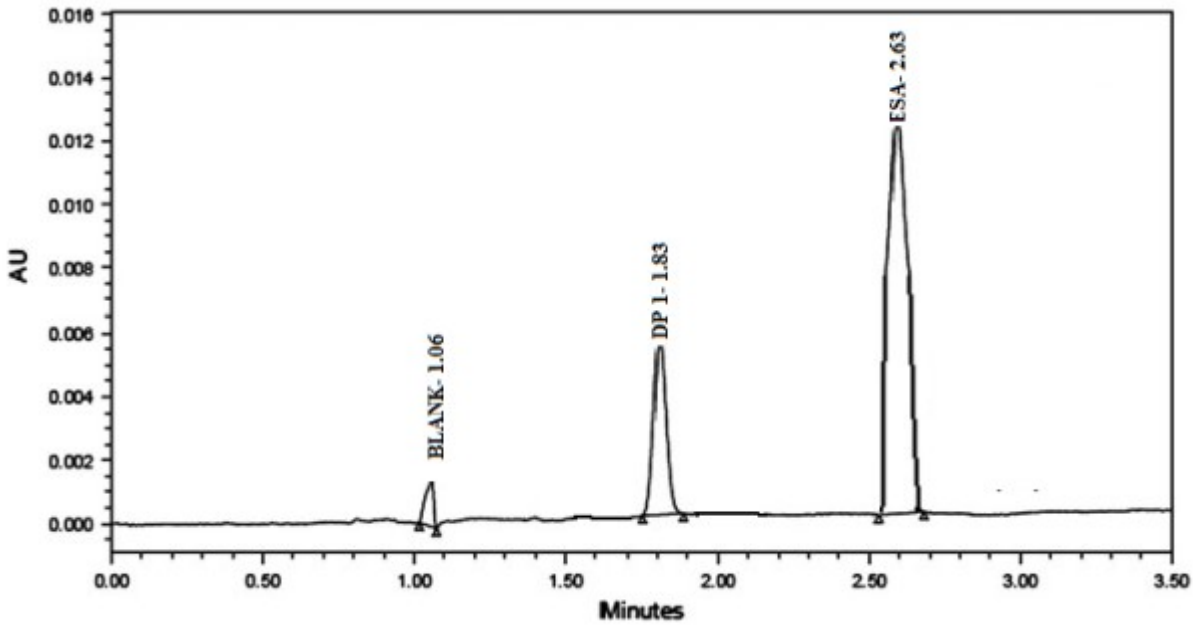

Fig. 2: UPLC chromatogram of Eslicarbazepine acetate (ESA) and its degradation product (DP 1) in a mixture of stressed solutions.

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3.2. **IR analysis**

The peaks postulate the presence of following functional groups:

IR (KBr) v (cm^{-1}): 3475.73(O–H stretching), 3363.86 (COO–H stretching), 1726.29(C=O stretching). ESA degradation product (DP 1) IR spectrum is shown in Fig. 3.

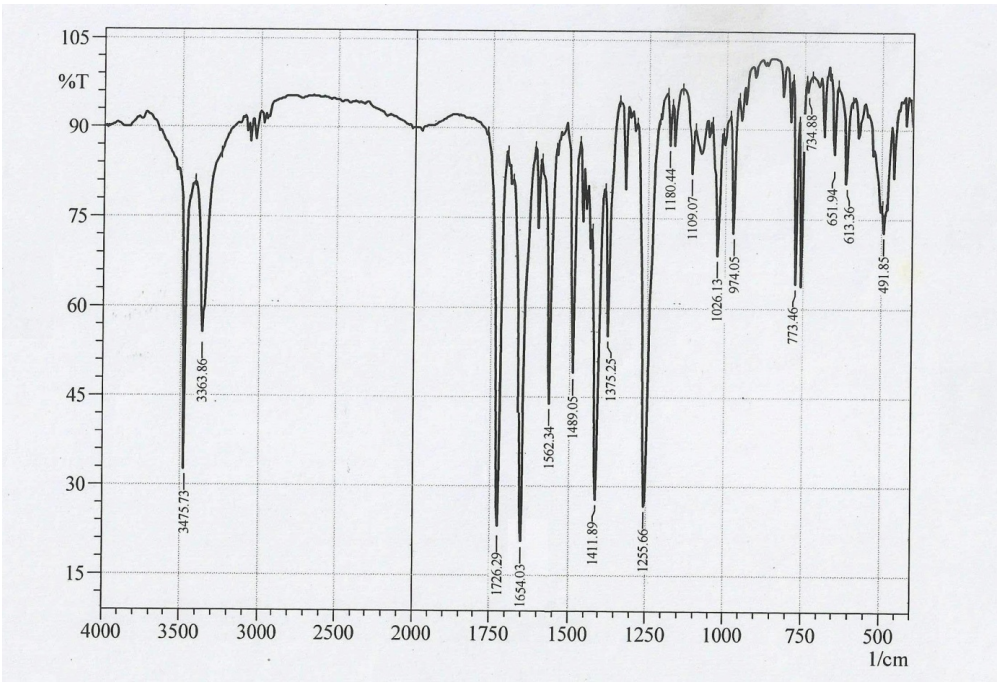

Fig. 3: IR spectrum of degradation product (DP 1)

3.3. **NMR analysis**

ESA degradation product (DP 1) NMR spectra exemplify the following peaks at 400MHz frequency.

^{1}H NMR, 400MHz, δ (ppm): 2.068 (d, 2H of CH_2, J= 1.20), 3.332 (s, 1H of OH), 6.168 (t, H of CH, J= 17.6), 7.231-7.396 (m, 8H of phenyl), 11.865 (s, 1H of COOH). The NMR spectrum of DP 1 is shown in Fig. 4

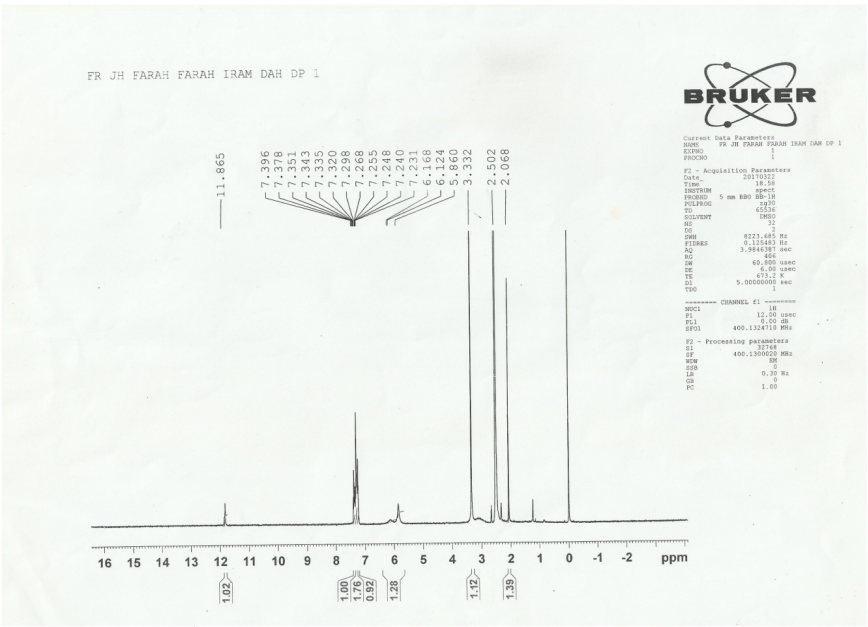

Fig. 4: NMR spectrum of degradation product (DP 1)

3.4 UPLC –MS/MS analysis

The UPLC-MS/MS spectrum of the drug (ESA) and its degradation product (DP 1) is shown in fig 5 and fig 6 respectively. ESA show prominent molecular ion peak at 296.8 m/z. Further in other mass spectrum, degradation product peak was observed at 255.2 m/z.

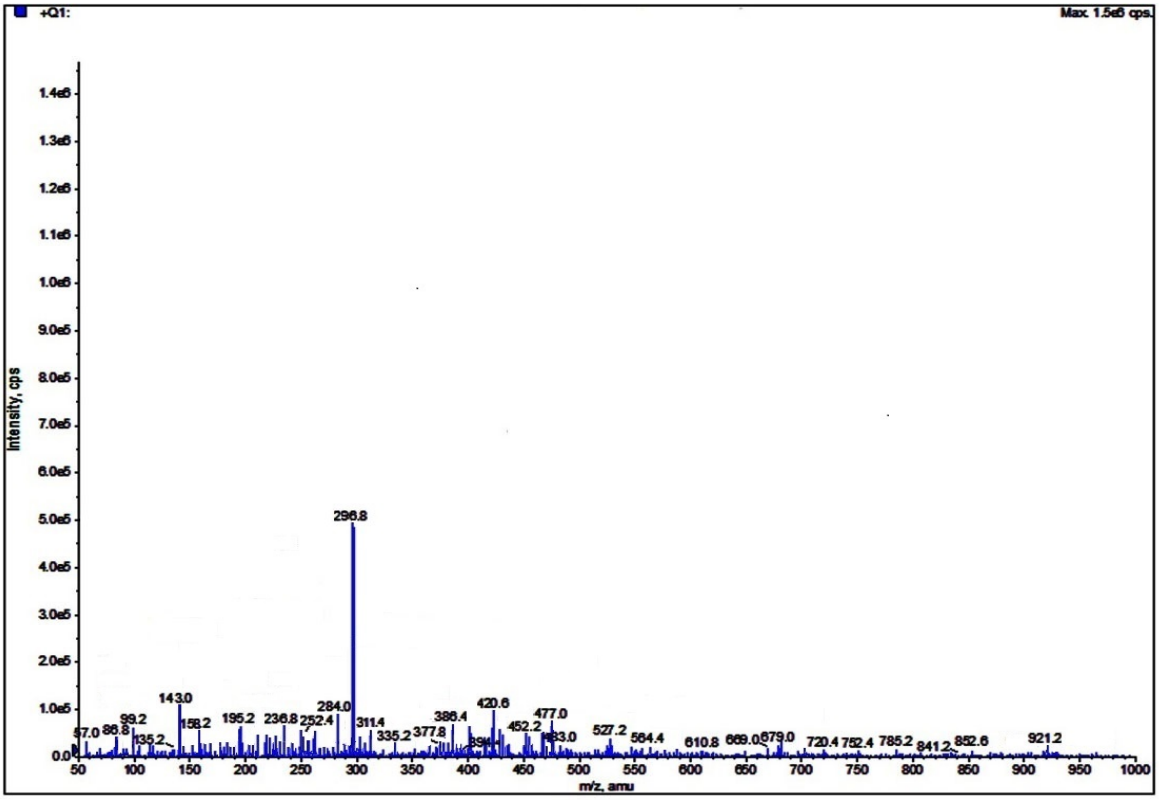

Fig. 5: Mass spectrum of Eslicarbazepine acetate (ESA).

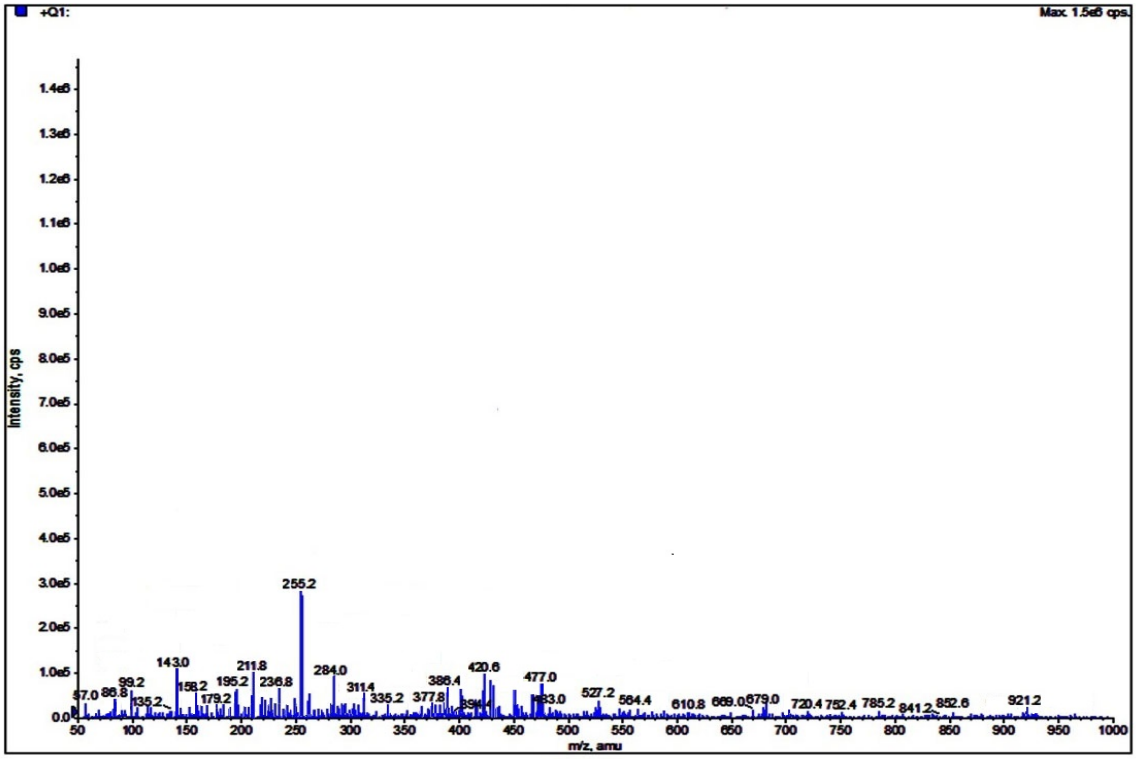

Fig. 6: Mass spectrum of degradation product (DP 1).

3.5 Explanation of degradation pathway of Eslicarbazepine acetate

ESA (5-carbamoyl-10, 11-dihydro-5H-dibenzo[b, f]azepin-10-yl acetate) molecular weight is 296 and the degradation product (10-hydroxy-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxylic acid) molecular weight is 255. On reflux deacetylation of ESA occurred which results into 10-hydroxy-10, 11-dihydro-5H-dibenzo [b, f]azepine-5-carboxamide moiety. Further, under alkaline hydrolysis hydroxide anion is attracted to the electropositive carbonyl carbon atom and delocalization of π bond occurs on an oxygen atom. Further π bond delocalized to N atom and release NH_3. This result in the formation of degradation product 10-hydroxy-10, 11-dihydro-5H-dibenzo [b, f] azepine-5-carboxylic acid with molecular weight 255. Fig 7. exemplified the expected degradation pathway of ESA.

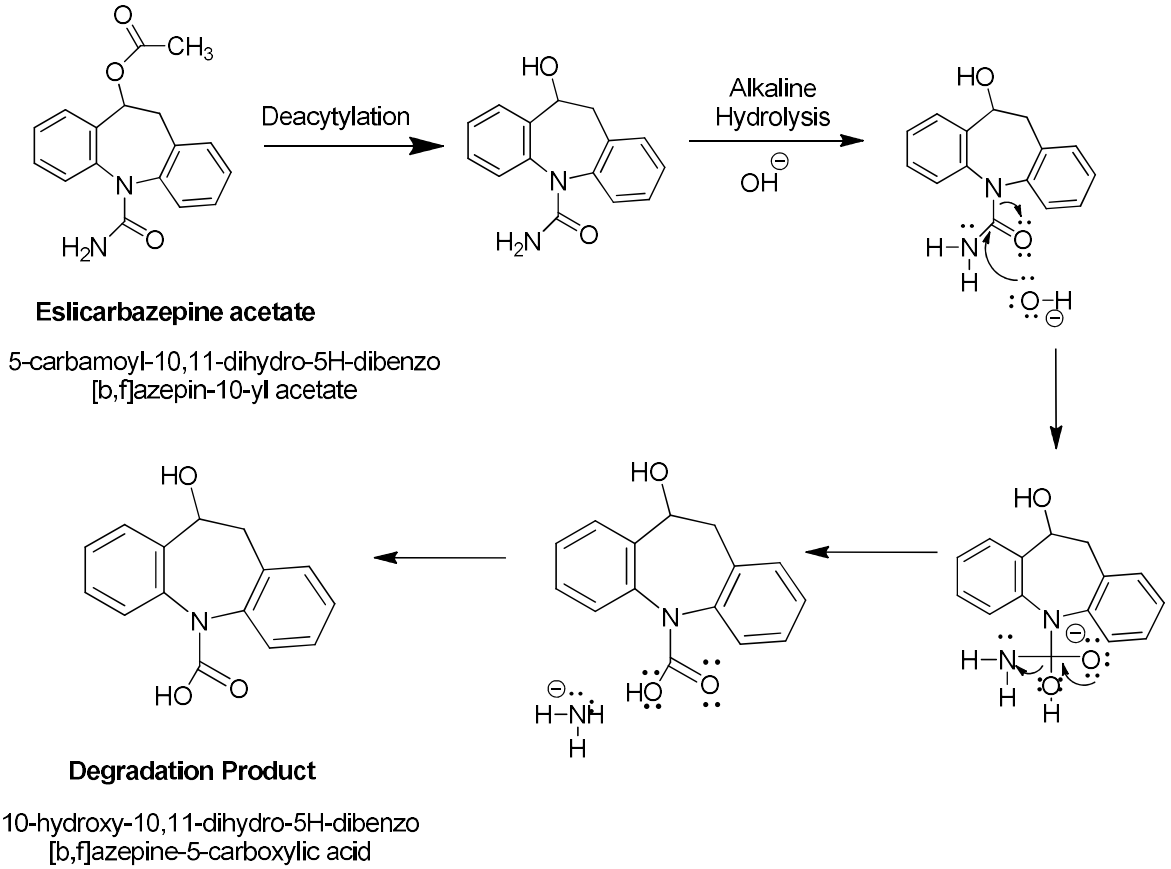

Fig. 7: A proposed degradation pathway for the formation of degradation product of Eslicarbazepine acetate.

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4. CONCLUSION

Eslicarbazepine acetate (ESA) was subjected to various stress degradation conditions, as per ICH guidelines, using UPLC analysis. Analysis results indicated that the drug ESA degraded in alkaline hydrolytic condition resulting into a single degradation product, while it showed stability in other stress conditions. The product was isolated and characterized on the basis of IR, ^{1}HNMR and mass spectroscopy analytical data, and found to be 10-hydroxy-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxylic acid. This analytical study not only confirmed the presences and identification of an unknown alkaline degradation product but also helped in the postulation of a degradation pathway. ESA degradation pathway may facilitate the production of several generic products in near future.

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