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Isolation and Characterization of Metabolites of Anti-Arrhythmic and Anticonvulsant Drugs

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Abstract: *This research presents the development and validation of stability-indicating RP-HPLC methods for the isolation and characterization of degradation products for Eslicarbazepine acetate (ESA), a third-generation anticonvulsant, and Dofetilide, a potent class III antiarrhythmic agent. The study aimed to establish reliable analytical techniques to monitor drug stability and identify potential impurities formed under stress conditions. Chromatographic separation was achieved using an ACQUITY HPLC BEH C18 column, providing rapid analysis with excellent resolution and sensitivity. Forced degradation studies were performed in accordance with ICH guidelines, exposing both drugs to acidic, alkaline, neutral, oxidative, thermal, and photolytic stress. Results revealed that both ESA and Dofetilide are remarkably stable under most conditions but undergo significant degradation specifically during alkaline hydrolysis. For ESA, the degradation product (DP1) was isolated and identified as 10-hydroxy-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxylic acid through structural elucidation using FT-IR, NMR, and LC-MS. Similarly, Dofetilide's alkaline degradant (DP a) was characterized as 4-(2-((4-aminophenethyl)(methyl)amino)ethoxy)aniline. The developed methods were successfully validated, showing strict linearity in the 10–50 µg/mL range (R 2 =0.999), high precision (%RSD < 1%), and excellent accuracy with recoveries ranging from 99% to 100.5%. These validated stability-indicating methods are suitable for routine quality control, manufacturing analysis, and ensuring the stability of pharmaceutical dosage forms.*

Keywords: Eslicarbazepine acetate, Dofetilide, Forced Degradation, Stability-Indicating Method, HPLC, Metabolites, Validation.

I. INTRODUCTION

1.1 Convulsion:

Convulsion is a neurological disorder which is characterized as a periodic and unpredictable expression of seizures. The electrical impulses discharged by nerve cells in the brain are regarded as seizures. The seizures are produced due to anomalous extreme or synchronous neuronal action in the brain. The cellular alternation in membrane conductances and variation in neurotransmitter affects the neuronal interactions which result in convulsion. Nervous system infection, prenatal factors, a trauma in the head, delay development and genetical issues are some of the factors behind aetiology of seizures. The seizures can be classified as,1,2

a) Partial: Seizure activity starts in one part of the brain
- Simple: Seizure activity while the person is alert
- Complex: Seizure activity with change in awareness of surroundings
- With secondary generalization: Seizure activity begins in one area and spreads

a) Generalized: Seizure activity involves the whole brain
- Absence: Staring and blinking without falling
- Myoclonic: Jerking movements of the body
- Tonic-clonic: Stiffening, falling and jerking of the body

- Tonic: Sudden onset of increased tone in the extensor muscles, falling heavily to the ground
- Atonic: Loss of muscle tone, without the involvement of jerks or contractions of the muscles

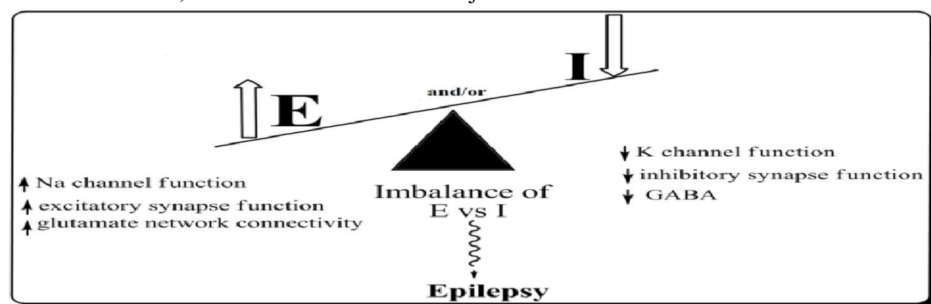

Figure 1.1: Diagrammatic presentation of epileptogenesis (E) excitation, (I) inhibition, (GABA) gamma-aminobutyric acid, (Na) sodium, (K) potassium

Anticonvulsants (antiepileptic drugs/antiseizure drugs) reduce the frequency or severity of seizures. It was found that globally more than 70 million people have been affected by the disease. The epileptic incidence in America, Africa, South-East Asia, Eastern Mediterranean, Europe and Western Pacific was found to be 12.59, 11.2, 99.97, 9.4, 8.23 and 3.66 per 1000 residents respectively. For every 1000 resident in India, the prevalence was found to be 3.0-11.9 yearly. Although, abundant numbers of antiepileptic drugs (AEDs) exists for the treatment of epilepsy, yet nearly 30% of patients have scanty seizure control. This rationalizes the obligation of development of new antiepileptic treatments with sympathetic adverse effect, simplified dose regimens and should be effective and well tolerated. Figure 1.2 and 1.3 illustrate the classification of antiepileptic drugs on the basis of their chemical nature and mechanism of action.4,5

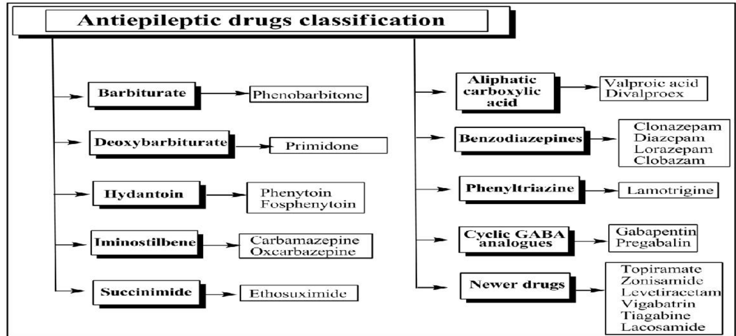

Figure 1.2: Classification of antiepileptic drugs on the basis of chemical nature

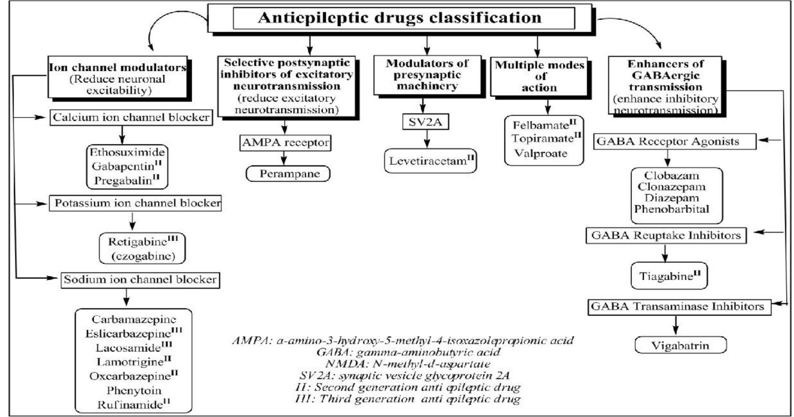

Figure 1.3: Classification of antiepileptic drugs on the basis of mechanism of action

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I. Eslicarbazepine acetate

Eslicarbazepine acetate (ESA) is one of the most prominent anticonvulsant agent developed by the Bial, a Portuguese pharmaceutical company. However, in Europe Bial vends the marketing authorities to Eisai a Japanese company in 2009. ESA is prodrug that is converted to eslicarbazepine (S-licarbazepine: an active metabolite of oxcarbazepine). ESA treats the partial-onset and generalized tonic-clonic seizure by maintaining the steady state of the inactivated voltage-gated sodium channels. The drug obstructs the restore activated state and upholds the repetitive neuronal firing. It defends the generation of seizures in preclinical models of epilepsy. Moreover in nonclinical models of pain ESA proved to be an analgesic agent. The drug is a single third-generation enantiomer which associates to the first generation dibenz[b,f]azepine (carbamazepine) and second generation (oxcarbazepine) anticonvulsants. ESA a voltage-gated sodium channel blocker superior to carbamazepine and oxcarbazepine as it adjust the kinetics and voltage dependence of slow inactivation and fast inactivation of voltage-gated sodium channel was not altered.6,7

Table 1.1: Dose adjustment of eslicarbazepine acetate in different conditions

Condition	Dosage
Partial-onset seizures	400mg orally OD (I seven days) 800-1200mg orally OD (maintenance dose)
With other AEDs for partial-onset seizures	With OXC and CBZ: Avoid the use with other enzyme-inducing AEDs high dose adjustment required.
In renal impairment patients	Creatinine clearance ≥ 50mL/min: No dose adjustment < 50mL/min: 200mg orally OD and 400mg orally OD (maintenance dose)
In hepatic impairment patients	Mild to moderate hepatic impairment : No dose adjustment severe hepatic impairment patients: Avoid use
Pregnancy and lactation	Pregnancy: Might be a teratogen: avoid its use Lactating mothers: excreted into breast milk: avoid its use.
AEDs: antiepileptic drugs, CBZ: carbamazepine, OXC: oxcarbazepine, OD: once daily	

II. Chemistry

Chemically, ESA is (S)-5 carbamoyl-10,11-dihydro-5H-dibenzo[b,f] azepin-10-yl acetate (Figure 1.4) with empirical formula $C_{17}H_{16}N_2O_3$ and molecular weight of 296.32. ESA contains the same chemical moiety as in carbamazepine and oxcarbazepine that is dibenzazepine nucleus with a 5-carboxamide substituent. ESA is class II moiety with high permeability and poor solubility as per the biopharmaceutic classification system. The solubility of ESA at diverse pH in aqueous buffer solutions is <1mg/mL and remains non-ionisable at physiological conditions with log P value (n-octanol/water partition coefficient) of 8.8 (pH 7.5 at 25°C). 7,8

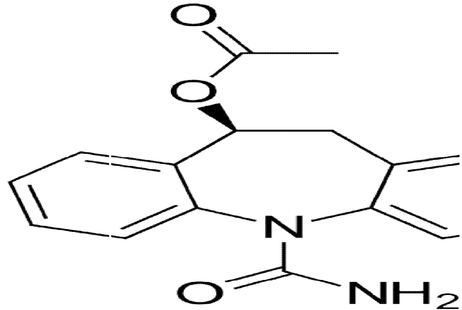

Figure 1.4: Chemical structure of eslicarbazepine acetate

III. Mechanism of action

The target site of ESA is voltage-gated sodium channel (VGSC) which concern with the epileptic discharge generation and propagation. In in vitro studies, it is proved that ESA selectively block the rapid repetitive firing of neurons. In

VGSC ESA comparatively, show high affinity towards inactivated state then deactivated state and by stabilizing the inactivated state ESA restrict the high sodium entry in neuron resulting in the less excitable state.

IV. Pharmacokinetics

In healthy subjects and patients, ESA demonstrates linear and dose-proportional (400-1200 mg/day) pharmacokinetics. ESA achieve steady-state plasma concentration after four to five days (one dose per day). In patient presences of 0.01% ESA were found on oral dose administration in blood and achieve maximal plasma concentration after 14hrs of post-dose peak with half-life of 13-20 h. Due to 90% of the dose recovered in urine ESA show high bioavailability of > 90% with 20 mL/min of clearance and 80-120 mL/min of glomerular filtration rate. ESA exhibits concentration independent plasma protein binding of < 4.0%. In in vitro studies neither warfarin, diazepam, digoxin, phenytoin, and tolbutamide affect ESA nor ESA influence their plasma protein binding. On oral administration, ESA rapidly metabolized by hepatic cells to generate major active metabolite eslicarbazepine (91%) by hydrolysis. The minor metabolism takes place in renal cells to generate R-licarbazepine (5%) and oxcarbazepine (1%). Chiral conversions by oxidation result in R-licarbazepine. ESA prove to have low enzyme-inducing action and hence unlike carbamazepine ESA not metabolized into toxic metabolite. In in vitro studies, it is proved that ESA clinically has no effects on CYP1A2, CYP2A6, CYP2B6, CYP2D6, CYP2E1, and CYP3A4. Whereas the drug shows mild inhibitory action on CYP2C19 and modest activation on UGT1A1- mediated glucuronidation. The studies show that in fresh human hepatocytes ESA exhibits no induction effects on enzyme glucuronidation and sulfation of 7-hydroxy-coumarin. From the systemic circulation, more than 90% of entire two third of unchanged and one-third of glucuronide conjugated metabolites excreted in urine.

V. Safety and toxicity

Several pooled analysis was conducted in several regions to assist the safety and tolerability of ESA. Some commonly reported adverse events include hyponatraemia, convulsion, dizziness, headache, nausea, fatigue, vertigo, somnolence and rash. In carcinogenicity study in mice it was concluded that at 25mg/kg/day, 600 mg/kg/day (female) and 600 mg/kg/day (male) increased the hepatocellular adenomas and carcinomas. In in vitro studies and in vivo mouse micronucleus assay ESA was found to be non-mutagenic and non-clastogenic respectively.

1.2. Arrhythmia:

Cardiac arrhythmia is an abnormality of cardiac rhythm. The heart normally beats at a regular rhythm to supply blood and oxygen to the vital parts of the body. If the beat gets irregular then it generates arrhythmia or dysrhythmia. The heart has a conduction system of sinoatrial (SA) node, internodal and interatrial node, atrioventricular (AV) node, bundle of His and Purkinje fibres. Arrhythmia is caused due to the disturbance of the conduction system in the heart. If the heartbeat is slow (<60 beats per minute) then it is termed as bradycardia when the heartbeat is fast (>100 beats per minute) it is termed tachycardia and irregular heartbeat is known as flutter or fibrillation. Basically, there are two mechanisms behind arrhythmia which includes abnormal impulse formation and abnormal conduction. The arrhythmia can be categorized as:

Bradycardia: Bradycardia is a slow heartbeat. The resting heart rate of fewer than 60 beats per minute is labelled as bradycardia. Bradycardia may be resulted by sinus bradycardia (slowed signal from the sinus node), sinus arrest (normal activity of the sinus node pause) or by AV block or heart block (electrical impulse from the atria to the ventricles blocks. Bradycardia can further categorize as:

- Sick sinus:
- Conduction block:

Tachycardia: The fast heartbeat is term as tachycardia. The resting heart rate of more than 100 beats per minute is defined as tachycardia. It further categorized as:

- Supraventricular tachycardia:
- Atrial fibrillation:

- Atrial flutter:
- Ventricular tachycardia:
- Ventricular fibrillation:
- Premature heartbeats: 10,11

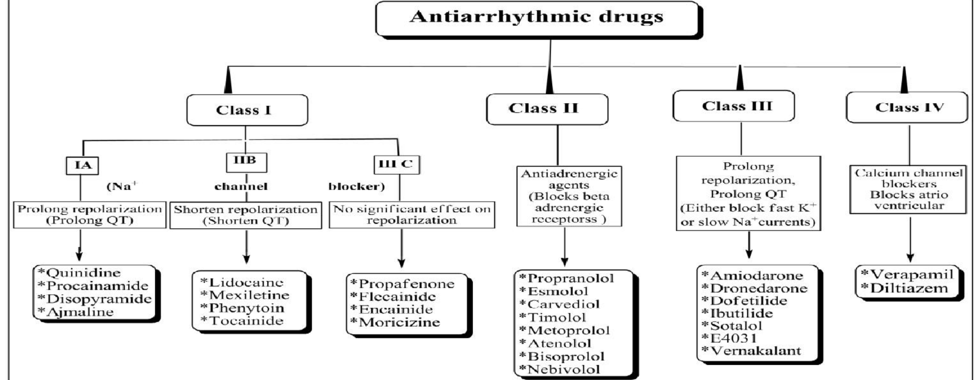

Figure 1.5: Classification of antiarrhythmic drugs

I. Dofetilide

Dofetilide a potent class III antiarrhythmic agent is marketed by Pfizer (Tikosyn). Dofetilide is the choice of treatment for patients of atrial fibrillation and atrial flutter. As per the studies it was found that dofetilide use in post-myocardial infarction patient's does not influence mortality. It is used for suppression of atrial fibrillation in patients with left ventricular dysfunction. Clinically dofetilide in term of chemical cardioversion of atrial fibrillation and sinus rhythm maintenance prove to be more beneficial than other class III agents as it does not have much pulmonary or hepatotoxicity effects. It is labelled as pure class III antiarrhythmic drug as it does not have autonomic or peripheral actions.

II. Chemistry

Chemically dofetilide is N [4-[2-[methyl [2-[4-[(methylsulfonyl) amino] phenoxy]ethyl] amino] ethyl phenyl] methanesulfonamide (Figure 1.6) [124]. Dofetilide empirical formula is $C_{19}H_{27}N_3O_5S_2$ and molecular weight is 441.6. The appearance of dofetilide is white to off powder. Dofetilide found soluble in 0.1M NaOH, acetone, 0.1M HCl and very slightly soluble in propan-2-ol and water. The LogP of the drug is 2.1 (octanol/water partition coefficient) and the distribution coefficient is 0.96 at pH 7.4. The drug is available in 0.125mg, 0.25mg and 0.5mg dosage form.

Figure 1.6: Chemical structure of dofetilide

III. Mechanism of Action

In cardiac action potential the delayed rectier current (Ik) consisit of a fast dormant state (Ikr) and more slow dormant state (Iks) which take place by separate ion channel molecules. Dofetilide selectively block rapidly inactivating component (Ikr) of rectier current and hence it is labelled as exclusive class III antiarrhythmic agent. It causes dose dependent persistence of the action potential interval as well as prolongation of QT and QTc (corrected form of QT) intervals. The prolongation causes enhanced the effective refractory period of cardiac tissues. Due to delayed

repolarization dofetilide enhance the monophasic action potential duration. The drug does not influence the slope of the upstroke of phase 0 depolarization (dV/dTmax) and the resting membrane potential.

IV. Pharmacokinetics

In fasting condition the bioavailability of dofetilide >90%, the oral bioavailability is unaltered by food and antacid. The maximum plasma concentration occurs approximately at 2-3 hours and achieves steady state plasma concentrations within 2–3 days. The terminal half life of the drug is about 10 hours with 1.5-2.0 accumulation indexes. Dofetilide plasma concentrations are dose proportional. The drug shows 60–70% of plasma protein binding which is not affected by concentration and renal impairment. Dofetilide volume of distribution is 3 L/kg. The intravenous administration does not significantly affect the volume of distribution (2.8-6.3 L/kg). After a single dose administration, approximately 80% of dofetilide excreted by the urine. The excreted form consists of an unchanged form of the drug (80%) and minimally active metabolites (20%). Dofetilide renally eliminates by glomerular filtration and active tubular secretion. Devoid of any chronic renal impairment in patients total dofetilide clearance is proportionate to the creatinine clearance. Dofetilide significantly metabolized by hepatic CYP3A4 enzyme system and show low affinity towards isoenzyme in in vitro studies. So dofetilide might interact with CYP3A4 inhibitors. If the cation transports system alter then the significant amount of dofetilide cleared by the CYP3A4 isoenzyme system.13,14

1.3. Drug Profile:

Table 1.4.1 Drug profile

Drug Name	Eslicarbazepine acetate	Dofetilide
Category	Antiepileptic drug	Antiarrhythmic drug
Structure		
IUPAC	(S)-5 carbamoyl-10, 11 dihydro-5H-dibenzo [b, f] azepin- 10-yl acetate	N[4-[2-[methyl [2-[4-[(methylsulfonyl) amino] phenoxy] ethyl] amino] ethyl phenyl] methane sulfonamide
Molecular Weight	296.32 g/mol	441.56 g/mol
Molecular Formula	C17H16N2O3	C19H27N3O5S2
Appearance	White to pale brown solid	White to off-white solid
Melting Point	177 °C	148 °C
Solubility	Chloroform, Methanol	DMSO, Methanol

II. EXPERIMENTAL WORK

I. Chemicals and reagents

Eslicarbazepine acetate (ESA) gratuitous procured by Arbro Pharmaceuticals Pvt. Ltd. Dofetilide was obtained from Sigma-Aldrich Co. (St Louis, MO, USA). Throughout the study analytical grade chemicals and materials were used. Hydrochloric acid, sodium hydroxide and hydrogen peroxide (30%) were purchased from SD Fine-chem, whereas

acetonitrile (ACN), methanol (MeOH) from Merck India (Mumbai). HPLC grade water (Merck India; Mumbai) was used for the preparation of all the reagents and solutions.

II. Method Development Parameters

Selection of wavelength (λmax):

For the stress degradation studies of eslicarbazepine acetate and dofetilide λmax was found to be 215nm and 230nm respectively. At these wavelengths, the developed method exhibit excellent linearity, hence these wavelength were the choice for analysis.

Optimization of Mobile phase:

The mobile phase is a key component to achieve satisfactory peak resolution and peak sensitivity. An appropriate mobile phase composition not only reduces total analysis time but also increase peak height. Mobile phase strength plays a significant role in deciding the peak symmetries and separations. Qualitative and quantitative composition of aqueous and organic portions in mobile phase helps to achieve the most selective method for chromatographer separation. On the basis of hit and trial method, several combination proportions of various solvents were used for appropriate mobile phase selection for eslicarbazepine acetate and dofetilide analysis. Desirable chromatographic separation and system suitability were achieved by parameters, mentioned in table 3.4.

Eslicarbazepine acetate:

Primarily diverse proportion of water and acetonitrile was used as mobile phase, but it does not gave satisfactory resolution. Later on potassium dihydrogen orthophosphate in different concentrations with water as a buffer was chosen, but the peak shape was unacceptable. However, water substitution with acetonitrile not only declines the retention time but also gives excellent sharp peak. Thus, the choice of buffer was 0.01M potassium dihydrogn orthophosphate with acetonitrile. Subsequent trials were made using different proportions of acetonitrile, water and methanol. However, excellent separation with good resolution and peak shape was achieved using a mixture of acetonitrile-water-methanol in the ratio of 75 :5 :25, v/v. To attain absolute separation and excellent resolution, the chosen ratio of buffer and the mixture of the solution was 50: 50 (% v/v).

Preparation: [Buffer] For 0.01M potassium dihydrogen orthophosphate, accurately weight 136.09mg of potassium dihydrogen orthophosphate and transfer it into reagent bottle. Add 100mL of water and mix well. Take 90mL of above solution and add 10mL of acetonitrile.

[Solvent] Make a mixture of acetonitrile-water-methanol (75:5:25, V/V/V) and mix well.

Take Buffer: Solvent; 50:50 (V/V) mix well and sonicate in an ultrasonic bath to get desire mobile phase.

Dofetilide:

Initially, ammonium acetate buffer was selected in combination with acetonitrile in varying ratios (20:80, 30:70, 10:90) as a mobile phase but the selected mobile phases were not giving good response. To overcome this problem buffer was changed from ammonium acetate to ammonium formate in combination with acetonitrile and observed the response. Different ratios of ammonium formate with acetonitrile were tried (30:70; 10:90; 20:80). The response was good but among all the ratios 10mM ammonium formate: acetonitrile in 20:80 proportions give the best result. So, finally selected mobile phase is acetonitrile: 10mM ammonium formate in the ratio 80:20 (V/V).

Preparation: For 10mM ammonium formate, accurately weight 63.05mg of ammonium formate and transfer it into reagent bottle. Add 100mL of water and mix well. Add acetonitrile to the buffer in the proportion of 80:20 (V/V) respectively to get desire mobile phase. The mobile phase was degassed by ultra sonication before use.

Table 3.4: Optimised chromatographic condition for eslicarbazepine acetate/ dofetilide

Parametrs	Eslicarbazepine acetate	Dofetilide
Mobile Phase	0.01M potassium dihydrogen orthophosphate - acetonitrile (90:10, V/V) [A] Acetonitrile – water - methanol (75:5:25, V/V/V) [B] A:B; 50:50 (V/V)	Acetonitrile : 10mM Ammonium formate 80:20 (V/V)
Diluent	Methanol	Methanol
Flow rate	0.2 mL/min	0.4 mL/min
Injection volume	2mL	2mL

Table 3.5: Separation variables for eslicarbazepine acetate/ dofetilide

Variable	Eslicarbazepine acetate	Dofetilide
Column type	ACQUITY HPLC BEH C18	ACQUITY HPLC BEH C18
Column Dimension	150mm x 2.1mm	150mm x 2.1mm
Particle size	1.7µ	1.7µ
Bonded phase	HPLC BEH column	HPLC BEH column
Column temperature	30 °C	30 °C
Detector	Photodiode array	Photodiode array
Detection wavelength	215nm	230nm

III. Selection of separation variable:

On the basis of the physicochemical properties of the eslicarbazepine acetate and dofetilide, reverse phase chromatography with C-18 column was preferred. For chromatographic separation general preference is ACQUITY HPLC BEH C18 column, as it provides broadest pH range. The ACQUITY HPLC BEH C18 column, packed with 1.7-mm, bridged, ethylsiloxane, hybrid particles bear elevated pressure conditions. It offers excellent retention of the compound by high optimum velocity and small plate height. For superior detection of an analyte, the photodiode array (PDA) detector provides exceedingly sensitive spectral information. PDA operating range is 190 and 500 nm; it is supported by ultraviolet or visible light source. Better resolution, desired retention time and symmetric peak shape was obtained by the following variables:

IV. System suitability testing:

System suitability is basically the determination of instrument performance (e.g., sensitivity, chromatographic retention, theoretical plates, tailing factor, and HETP) by analysis of a reference standard. System suitability test was done to authenticate analytical system working efficiency, accuracy and precision.

Theoretical plates (N) numbers are an indirect determination of peak width at a definite retention time. It is a large number of imaginary separation layers in the chromatographical column which express column efficiency

$N= 16 (tR/W)2$

N= no. of theoretical plates

tR= retention time

W= peak width

As per Center for Drug Evaluation and Research (CDER) guidelines N>2000

HETP (H) is height equivalent to theoretical plate and calculated as:

$H= L/N$

H=HETP

L= Column length in mm

N=No. of theoretical plate

Tailing factor (TF) is defined as the distance from the front slope of the peak (A) to the back slope divided (B) by twice the distance from the center line of the peak (C) to the front slope, with all measurements made at 5% of the maximum peak height.

TF=AB/2AC

TF= tailing factor

A=distance from the front slope of the peak

B= distance from the back slope

C= distance from the center line of the peak

As per Center for Drug Evaluation and Research (CDER) guidelines N$\leq$2.0

V. Method Validation:

As recommended by ICH guideline Q2 (R1) the developed HPLC developed was validated in term of various parameters:

a. Linearity and range:

The solution in the concentration range of 10–50 µg/mL was made by diluting the stock solution 1 mg/mL drug in methanol. The amount of injection was kept constant 2µl and in triplicate the solutions were injected into the HPLC column.

b. Precision:

To inspect the precision of the developed method six injections of five diverse concentrations (10-50 µg/mL) were prepared on the similar day of study and relative standard deviation (% R.S.D.) were calculated to verify the intra-day precision. For the inter-day precision assessment, these studies were carried out on different days.

c. Accuracy:

For the accuracy assessment recovery studies were executed. The decomposed reaction solutions mixture was fortified with five known concentrations of the drug; further recovery of added drug was evaluated.

d. Specificity and Selectivity:

The capability of the analytical method to assess the response of the analyte in the existence of disruptive substances including impurities and its degradation products is specificity. The specificity was estimated by resolution factor of the drug peak from the nearby peak. The purity of each degradation peak will specify the selectivity of the method.

e. Robustness:

The assessment of robustness of the method was carried out by the study of effect of diminutive modification of method parameters such as flow rate (± 10%), column temperature (± 5 ˚C), mobile phase (± 2%), and wavelength of detection (± 5%) and pH of the buffer in the
mobile phase (± 0.2%). In this evaluation, only single parameter is modified and the remaining parameters were kept constant.

f. Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The lowest level of analyte that can be detected and gives a assessable response is termed as LOD, whereas the smallest amount of analyte that can be reproducibly quantified is termed as LOQ. LOD and LOQ were estimated by injecting falling concentration of drug by the following formula, where SD is the standard deviation of the response and S is the slope of the calibration plot..

LOD = 3.3 x SD/S and LOQ = 10 x SD/S

A. Eslicarbazepine acetate (ESA)

I. System suitability testing (SST)

All the separation variables and conditions (Table 3.4 and 3.5) were set. Mobile phase A: B; 50:50,v/v(A: mixture of 0.01M potassium dihydrogen orthophosphate and acetonitrile (90:10, v/v) and B: mixture of acetonitrile-water-

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methanol (75:5:25, v/v) was run to saturate the column. For system suitability testing 6 replicates of reference standards of eslicarbazepine acetate (20µg/mL) were injected and all the SST variables were recorded (Table 3.6).

Table 3.6: SST Parameters of eslicarbazepine acetate for HPLC

S. No	RT	AUC	N	TF	H
1	2.596	105020	10782	1.02	6.861
2	2.634	105178	11100	1.05	6.664
3	2.618	105199	10966	1.03	6.746
4	2.604	105042	10849	1.03	6.818
5	2.628	105075	11051	1.04	6.694
6	2.636	105181	11117	1.05	6.654
Mean	2.619	105115	10977	1.036	6.739
S.D	0.016	79.159	137.752	0.012	0.082
R.S.D.	0.624	0.075	1.254	1.168	1.225

SD: standard deviation; RSD: relative standard deviation; RT: Retention time; AUC: Area under the curve; N: Number of theoretical plates; TF: Tailing factor; H: Height equivalent to theoretical plates Eslicarbazepine acetate standard HPLC chromatogram at 215nm showed retention time (RT) at 2.63min.

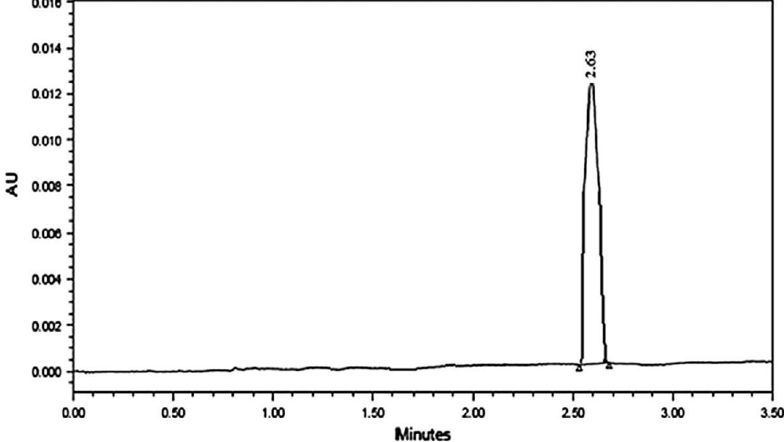

Figure 3.5: Eslicarbazepine acetate standard HPLC chromatogram

II. Stress Degradation

Under various stress conditions HPLC studies on ESA recommended that drug does not degrade under acid hydrolysis, neutral hydrolysis, oxidative, thermal and photolytic stresses. ESA illustrate prominent degradation in alkaline hydrolytic condition. In a mixture of a solution

alkaline hydrolytic condition showed single degradation product. Table 3.7 demonstrates the retention time (RT) and relative retention time (RRT) of the drug and its degradant (DP 1) in a mixture of a stressed sample by HPLC. In 0.001N NaOH, at 60 ˚C after 8 hr of reflux a new peak appeared at RT 1.83 min (0.695 RRT) along with ESA peak at 2.63 min (1.000 RRT). This novel appeared peak is the result of alkaline stress degradation demonstrated as DP 1 at 1.83 min RT. The drug showed stability in other stress conditions. In acidic stress condition, it was observed that at 60 ˚C in 5 N HCl for 24 hr of reflux no degradation occurred. In neutral hydrolysis when drug was refluxed for 2 days in water at 60 ˚C, the drug was found to be stable. Again the drug was found to be stable in oxidative stress condition when refluxed in 30% H_2O_2 at 60 ˚C for 5 days. When exposed to sunlight (60,000–70,000 lux) for 2 days and at 100 ˚C in oven for 30 days, no degradation of the drug was observed suggesting that the drug was stable in photolytic and thermal stress conditions respectively.

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The RT, RRT and resolution of peaks in the chromatograms confirmed the specificity and selectivity of the method (Figure 3.6).

Table 3.7: 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

Figure 3.6: HPLC chromatogram of eslicarbazepine acetate (ESA) and its degradation product (DP 1) in a mixture of stressed solutions

III. Method development and optimization:

ESA stability indicating ability was developed by HPLC, it was recommended that the drug confirm significant degradation in alkaline hydrolytic condition. Primarily, different proportion of acetonitrile and water as mobile phase for stress degradation studies were carried on C18 BEH (150 x 2.1 mm, 1.7 µm) column at 30 ˚C, but the resolution was poor. Afterwards, potassium dihydrogen orthophosphate with water as a buffer was chosen, unfortunately peak shape was not acceptable. However, substituting water with acetonitrile not only lessen the retention time but also gives fine sharp peak. Thus, the choice of buffer was potassium dihydrogn orthophosphate with acetonitrile. Subsequent trials were made using various proportions of acetonitrile, water and methanol. However, best separation with excellent resolution and peak shape was achieved using a mixture of acetonitrile-water-methanol in the ratio of 75:5:25, v/v. To achieve absolute separation and fine resolution, the ratio of the buffer and mixture of solution was 50: 50 (% v/v) chosen. Simultaneous observation with the PDA detector at constant 0.2 mL/min flow rate was carried out at the wavelength of 215nm by C18 BEH (150 X 2.1 mm, 1.7 µm) column and 30˚C column temperature.

IV. Method validation

In accordance with ICH guideline Q2 (R1) the developed method was successfully validated. The method was adequately specific. Figure 3.2 shows that the method remained selective in a mixture of stressed solutions for all components. Table 3.7 shows the RT and RRT of ESA and it's degradation product signifying the selectivity of the method.

By linear regression analysis, the linearity was checked. It was found that the response of the drug was strictly linear in 10-50 µg/mL concentration range (Figure 3.7). The linearity results shown in table 3.8. The equation of regression analysis obtained for ESA: Y= 5098.2 x + 2626.8. The value for correlation coefficient was found to be R2. = 0.999. The LOD and LOQ were found to be 0.382 and 1.15µg/mL respectively. Table 3.9 exemplified absolute residue data for eslicarbazepine acetate and figure 3.8 absolute residue plot for eslicarbazepine acetate. The data obtained from intra-day and inter-day precision studies exemplified in table 3.10. The results specify that the method was adequately precise as the %RSD values for the precision studies were found to be <1%. Excellent recoveries of the drug were obtained at the different added concentrations in the range from 99.153% to 100.566% which has been illustrated in table 3.11. By little alteration of the mobile phase, the developed method was found to be robust. Mobile phase A is

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0.01M potassium dihydrogen orthophosphate and acetonitrile (90:10, v/v) and mobile phase B consisted a mixture of acetonitrile-water-methanol (75:5:25, v/v). The data has been illustrated in table 3.12.

Table 3.8: Linearity data for eslicarbazepine acetate (n=6)

Concentration (µg/mL)	AUC	±SD	RSD (%)
10	53453.00	53.254	0.099
20	105132.83	70.844	0.067
30	154759.00	519.991	0.336
40	207171.00	609.537	0.294
50	257341.67	411.715	0.159

AUC: Area under the curve; SD: standard deviation; RSD: relative standard deviation

Table 3.9: Absolute residue data for eslicarbazepine acetate (n=6)

Concentration (µg/mL)	AUC(mean)	Y=mx+c	Absolute residue
10	53453	53608.8	-155.8
20	105132.83	104590.8	542.03
30	154759	155572.8	-813.8
40	207171	206554.8	616.2
50	257341.67	257536.8	-195.13

Figure 3.7: Linearity graph of eslicarbazepine acetate

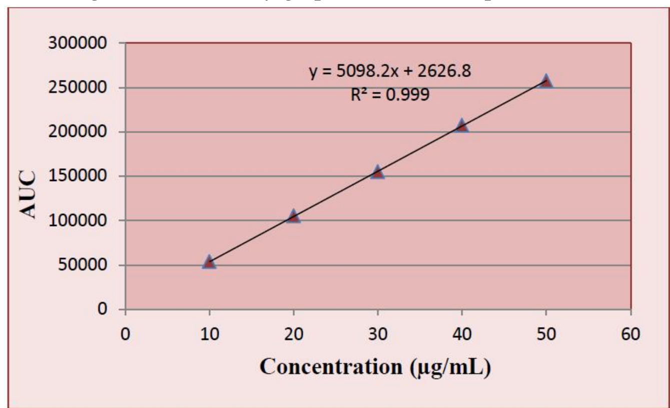

Figure 3.8: Absolute residue plot for eslicarbazepine acetate

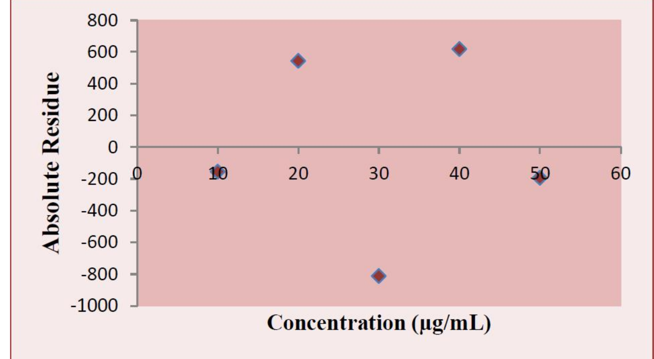

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Table 3.10: Precision data for eslicarbazepine acetate (n=6)

Actual concentration (µg/mL)	Intra-day			Inter-day		
	Measured concentration	±SD	RSD (%)	Measured concentration	±SD	RSD (%)
10	09.958	0.045	0.452	09.817	0.094	0.955
20	20.016	0.169	0.847	20.086	0.075	0.375
30	30.126	0.201	0.667	30.053	0.245	0.814
40	40.210	0.221	0.549	40.131	0.086	0.213
50	50.039	0.330	0.660	50.019	0.124	0.247

Table 3.11: Recovery data for eslicarbazepine acetate (n=4)

Concentration (µg/mL)	Calculated spiked concentration	±SD	RSD (%)	Recovery (%)
10	09.915	0.053	0.529	99.153
20	20.113	0.098	0.487	100.566
30	30.144	0.225	0.748	100.483
40	39.984	0.243	0.608	99.960
50	50.102	0.104	0.207	100.204

Table 3.12: Robustness data for eslicarbazepine acetate (n=4)

Concentration (µg/mL)	Moblie phase A: Moblie phase B (48:52, v/v)			Moblie phase A: Moblie phase B (52: 48, v/v)		
	Measured concentration	±SD	RSD (%)	Measured concentration	±SD	RSD (%)
10	10.125	0.097	0.958	09.968	0.068	0.679
20	20.033	0.119	0.599	19.978	0.127	0.634
30	30.002	0.209	0.698	30.138	0.104	0.345
40	40.213	0.159	0.396	40.110	0.093	0.230
50	49.983	0.278	0.556	50.065	0.155	0.310

SD: standard deviation; RSD: relative standard deviation

V. Degradation product isolation and characterization

Degradation studies on ESA under different stress conditions, recommended that ESA depredate only in alkaline hydrolytic condition. In a mixture of a stress solution, single degradation product appears. 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 the 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, 1H-NMR and mass spectrometry.

VI. IR analysis:

The peaks postulate the presence of the following functional groups:

IR (KBr) ν (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 Figure 3.9.

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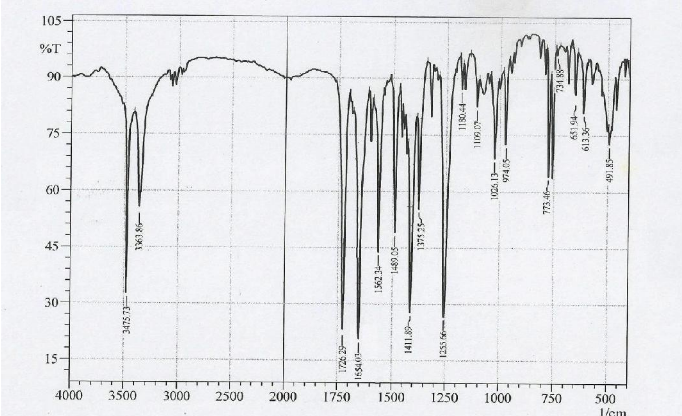

Figure 3.9: IR spectrum of degradation product (DP 1)

3.4.1.5.2 NMR analysis:

ESA degradation product (DP 1) NMR spectra exemplify the following peaks at 400MHz frequency. 1H 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 Figure 3.10.

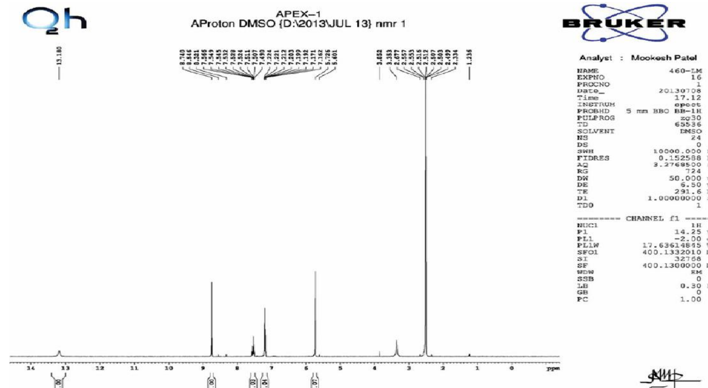

Figure 3.10: NMR spectrum of degradation product (DP 1)

3.4.1.5.3 HPLC –MS/MS analysis:

T The HPLC-MS/MS spectrum of the drug ESA and its degradation product is shown in figure 3.11 and figure 3.12 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.

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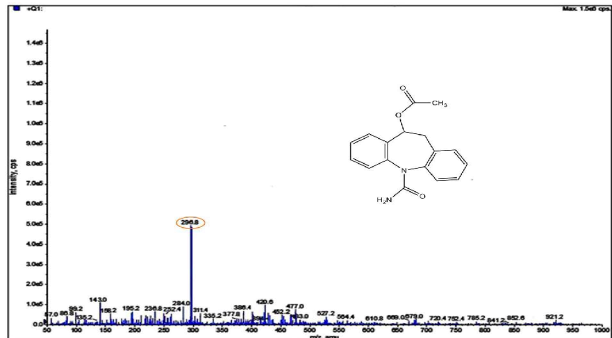
Figure 3.11: Mass spectrum of eslicarbazepine acetate (ESA)

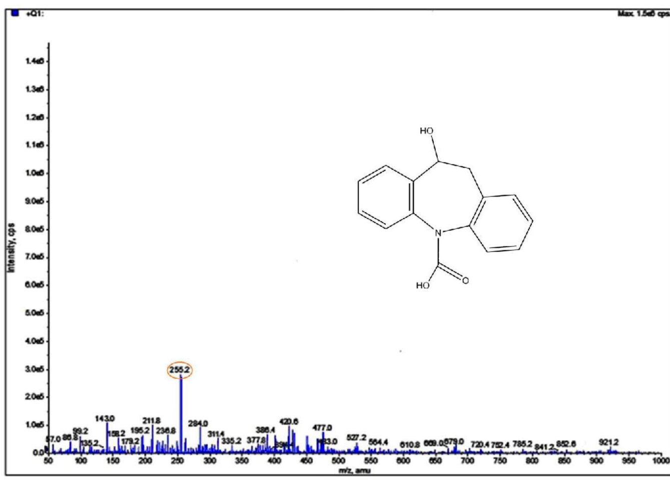
Figure 3.12: Mass spectrum of degradation product (DP 1)

VII. 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 in 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 NH3. 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. Thus, the alkaline hydrolysis of amide results in acid so alkaline degradation product, which can be understood by following mechanism (Figure 3.13):

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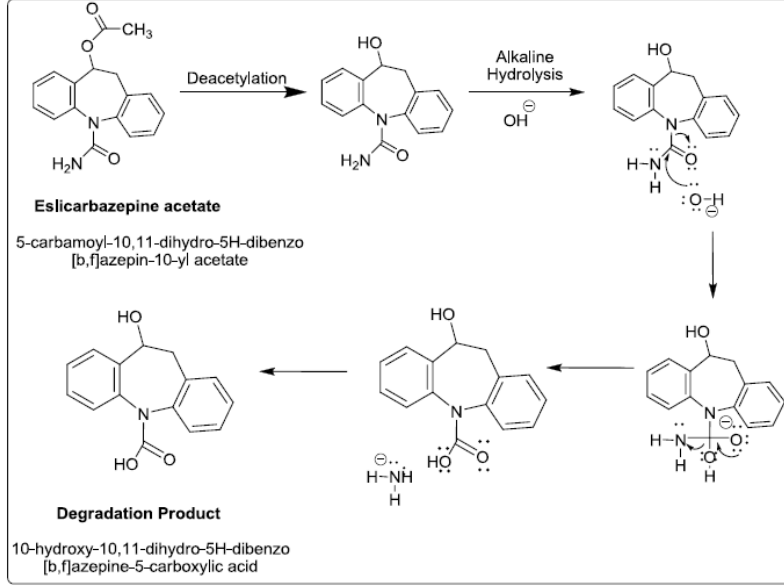

Figure 3.13: A proposed degradation pathway for the formation of degradation product of eslicarbazepine acetate

B. Dofetilide

3.4.2.1 System suitability testing (SST)

Table 3.4 and 3.5 separation variables and conditions were set. To saturate the column mobile phase (Acetonitrile: 10mM Ammonium formate 80:20 (V/V)) was run. Six replicates of reference standards of Dofetilide (20µg/mL) were injected for system suitability testing and all the SST variables were recorded (Table 3.13).

S.NO	RT	AUC	N	TF	H
1	3.834	108246	10453	1.04	7.077
2	3.828	108204	10420	1.03	7.099
3	3.836	108362	10463	1.05	7.069
4	3.846	108394	10518	1.05	7.033
5	3.818	108212	10365	1.03	7.136
6	3.842	108386	10496	1.04	7.047
Mean	3.834	108300	10453.08	1.041	7.077
S.D	0.010	89.386	54.716	0.008	0.038
R.S.D.	0.261	0.082	0.523	0.861	0.541

Table 3.13: SST parameters of dofetilide for HPLC

SD: standard deviation; RSD: relative standard deviation; RT: Retention time; AUC: Area under the curve; N: Number of theoretical plates; TF: Tailing factor; H: Height equivalent to theoretical plates. Dofetilide standard HPLC chromatogram at 230nm showed retention time (RT) at 3.82min.

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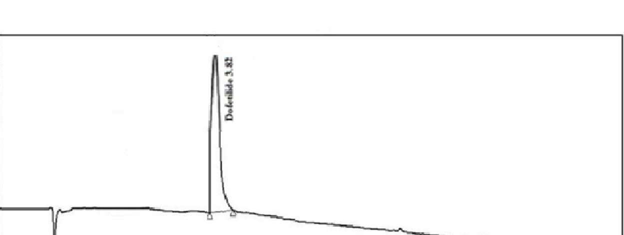


Figure 3.14: Dofetilide standard HPLC chromatogram

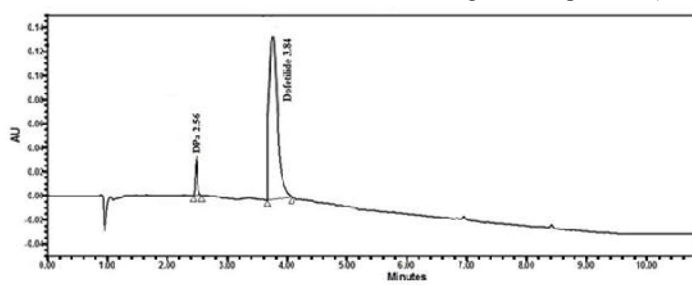
I. Stress Degradation

Dofetilide HPLC studies under different stress conditions, recommended that dofetilide does not degrade under acid hydrolysis, neutral hydrolysis, thermal, oxidative and photolytic stresses. Dofetilide confirmed prominent degradation in alkaline hydrolytic condition at elevated temperature. A single degradation product in a mixture of a solution was found in alkaline hydrolytic condition. In a mixture of stressed sample by HPLC, table 3.14 demonstrates the retention time (RT) and relative retention time (RRT) of the dofetilide and it's degradant (DP a). At 80 ˚C in 0.01N NaOH after 24 hr of reflux, a new peak appeared at RT 2.56 min (0.667 RRT) along with dofetilide peak at 3.84 min (1.000 RRT). This novel emerged peak at 2.56 min RT is due to alkaline stress degradation and demonstrated as DP a. The drug was found to be stable in other stress conditions. In acidic stress condition in 5 N HCl at 80 ˚C for 24 hr of reflux no degradation occurred. The drug was found to be stable in neutral hydrolysis when the drug was refluxed at 80 ˚C for 2 days in water. In oxidative stress condition, the drug was again found to be stable when refluxed for 5 days in 30% H2O2 at 80 ˚C. When the drug was exposed to sunlight (60,000–70,000 lux) for 2 days and at 100 ˚C in the oven for 30 days, no degradation of the drug was observed this signifies that the drug was stable in photolytic and thermal stress conditions respectively.

The RT, RRT and resolution of peaks in the chromatograms confirmed the specificity and selectivity of the method (Figure 3.15).

Peaks	RT	RRT
DPa	2.56	0.667
Dofetilide	3.84.	1.000

Table 3.14: RT and RRT of dofetilide and its degradation product (DPa)



RT: Retention time; RRT: Relative retention time

Figure 3.15: HPLC chromatogram of dofetilide and its degradation product (DP a) in a mixture of stressed solutions

II. Method development and optimization

By HPLC, dofetilide stability indicating ability was established and it was recommended that the drug showed significant degradation in alkaline hydrolytic condition. Primarily, stress degradation studies were carried on C18 BEH (150 x 2.1 mm, 1.7 µm) column at 30 ˚C using various proportion of ammonium acetate buffer with acetonitrile as a

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mobile phase but the selected mobile phases were not giving a good response. Further ammonium formate as a buffer was used and the observed response was good. Different ratios of ammonium formate with acetonitrile were tried (30:70; 10:90; 20:80). The response was good but among all the
ratios 10mM ammonium formate: acetonitrile in 20:80 proportions give the best result. So, finally selected mobile phase is Acetonitrile: 10mM Ammonium formate in the ratio 80:20 (V/V). Subsequently, the flow rate and column temperature were adjusted to get the prominent response using C18 BEH (150 X 2.1 mm, 1.7 µm) column and PDA detector.

III. Method validation

As per the ICH guideline Q2 (R1) the method was successfully validated and proof to be suitably specific. In a mixture of stressed solutions, the method remained selective for all components (Figure 3.15). The RT and RRT of dofetilide and its degradation product signify the selectivity of the method which is illustrated in table 3.14. By linear regression analysis the response of the drug was strictly linear. Figure 3.16 and table 3.15 show a linear response in 10-50 µg/mL concentration range. The equation of regression analysis obtained for dofetilide: Y= 5203.2x+ 4826.6. R2. = 0.999 which is value for the correlation coefficient. Table 3.16 and figure 3.17 exemplified absolute residue data and absolute residue plot for dofetilide respectively. The LOD and LOQ were found to be 1.023 µg/mL and 3.101µg/mL respectively. The developed method was found to be precise as the %RSD values for the precision studies were found to be <1%, intra-day and inter-day precision studies data shown in table 3.17. The result shows excellent recovery of the drug at the various added concentrations in the range from 99.722% to 100.389% (Table 3.18). The developed method was found to be robust by the little change in mobile phase (Table 3.19).

Table 3.15: Linearity data for dofetilide(n=6)

Concentration (µg/mL)	AUC	±SD	RSD (%)
10	58478.00	53.457	0.086
20	108300.67	89.386	0.082
30	158950.83	541.679	0.340
40	212170.67	316.131	0.148
50	268613.17	474.589	0.176

AUC: Area under the curve; SD: standard deviation; RSD: relative standard deviation

Concentration (µg/mL)	AUC(mean)	Y=mx+c	Absolute residue
10	58478	56856	1622
20	108300	108886	-586
30	158950	160916	-1966
40	212170	212946	-776
50	268613	264976	3637

Table 3.16: Absolute residue data for dofetilide(n=6)

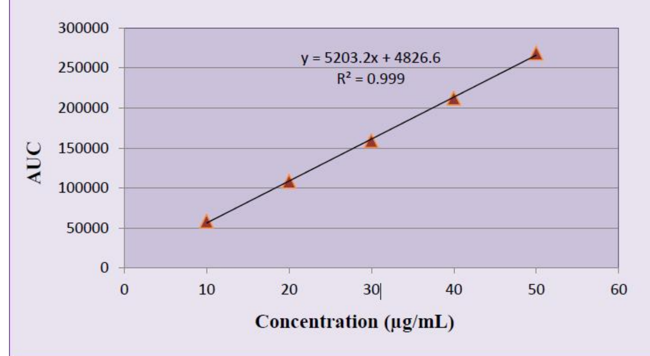

Figure 3.16: Linearity graph of dofetilide

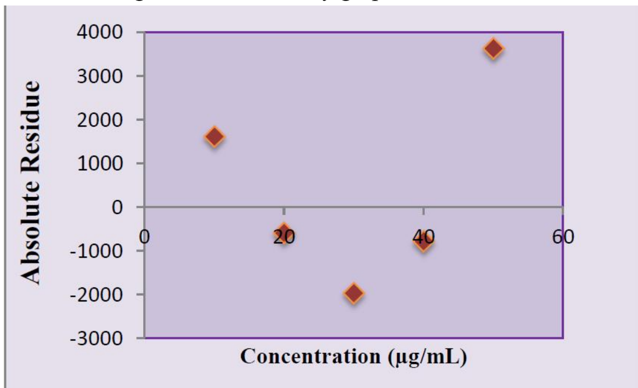

Figure 3.17: Absolute residue plot for dofetilide

Table 3.17: Precision data for dofetilide (n=6)

Actual concentration (µg/mL)	**Intra-day**			**Inter-day**		
	Measured concentration	±SD	RSD (%)	Measured concentration	±SD	RSD (%)
10	10.002	0.019	0.195	09.957	0.045	0.452
20	20.011	0.102	0.511	20.037	0.074	0.373
30	30.107	0.034	0.113	30.074	0.121	0.404
40	40.147	0.186	0.463	40.108	0.139	0.348
50	50.203	0.057	0.113	50.114	0.057	0.114

SD: standard deviation; RSD: relative standard deviation

Table 3.18: Recovery data for dofetilide(n=4)

Concentration(µg/mL)	**Calculated spiked Conc.**	**±SD**	**RSD (%)**	**Recovery (%)**
10	09.972	0.048	0.487	99.722
20	19.987	0.098	0.490	99.935
30	30.086	0.169	0.562	100.289
40	40.155	0.113	0.283	100.389
50	50.118	0.033	0.066	100.236

SD: standard deviation; RSD: relative standard deviation

Table 3.19: Robustness data for dofetilide(n=4)

Concentration (µg/mL)	Acetonitrile: 10mM Ammonium formate (90:10, v/v)			Acetonitrile: 10mM Ammonium formate (70:30, v/v)		
	Measured concentration	±SD	RSD (%)	Measured concentration	±SD	RSD (%)
10	9.984	0.095	0.953	10.050	0.077	0.772
20	20.120	0.046	0.229	19.953	0.097	0.489
30	29.998	0.103	0.344	30.159	0.067	0.225
40	40.175	0.152	0.378	40.067	0.167	0.417
50	50.220	0.043	0.087	50.160	0.096	0.191

SD: standard deviation; RSD: relative standard deviation

IV. Degradation product characterization

Dofetilide showed prominent degradation in alkaline hydrolytic condition at elevated temperature and remain stable under acid hydrolysis, neutral hydrolysis, oxidative, thermal and photolytic stresses. The mixture of stressed solutions was subjected to LC–MS analyses to characterize the degradation product. The parent ion peak of degradation product (DP a) was observed at m/z 285.184 having a mass variation of 156.4 Da from the parent ion of the drug which was observed at m/z 441.564. This indicate the loss of methane sulphonamide group from the drug which results in DP a. The DP a was proposed to be 4-(2-((4 aminophnethyl) (methyl) amino) ethoxy)aniline generated by alkaline hydrolysis of the methane sulphonamide group in the drug. Although sulphonamides are moderately stable nitrogen protective groups but the sulphonamides of less basic aryl amines can be cleaved by basic hydrolysis. The alkaline hydrolytic product (DP a) of dofetilide formation mechanism can be understood by the following figure 3.18:

Figure 3.18: A proposed degradation pathway for the formation of degradation product of Dofetilide

The HPLC-MS/MS spectrum of the drug dofetilide and its degradation product (Dp a) is shown in figure 3.15 and figure 3.16 respectively. Dofetilide show prominent molecular ion peak at 441.564 m/z. Further, in another mass spectrum, the degradation product peak was observed at 285.184 m/z..

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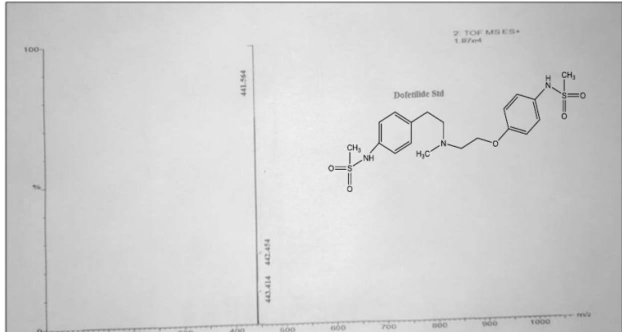

Figure 3.19: Mass spectrum of dofetilide

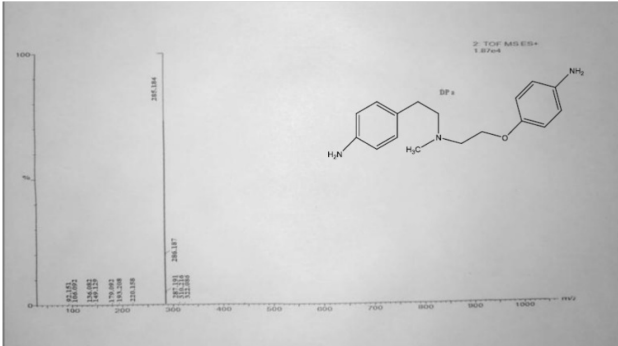

Figure 3.20: Mass spectrum of degradation product (DP a)

III. CONCLUSIONS

Forced degradation studies of eslicarbazepine acetate/dofetilide anticipate the development of various generic products of the drug and avoid identical studies to save time and funds in drug discovery and development process.

A. Eslicarbazepine acetate

The study proved that eslicarbazepine acetate, its metabolite (M3) and degradation product (DP1) were potent anticonvulsant agents. As compared to conventional HPLC methods, the developed method was found to be more acceptable in term of rapid chromatographic separation, excellent resolution, high analysis and good sensitivity. During complete stress testing and method validation, the ICH guidelines have been followed. Stress degradation studies exhibit that eslicarbazepine acetate degrades in alkaline hydrolytic condition. In a mixture of a stress solution, one distinct degradation product appears. Whereas, the drug shows stability in acid hydrolysis, neutral hydrolysis, oxidative, thermal and photolytic stress conditions. The formed degradation product was isolated from the mixture of stressed sample and further characterized by HPLC, FT-IR, NMR and LC-MS. Analytical techniques assist the degradation pathway development. All method validation parameters showed acceptable results with respect to specificity, selectivity, linearity, precision, accuracy and robustness. Thus the developed stability indicating method can be utilized for regular analysis to check the sample stability and to manufacture the drug and its combination pharmaceutical dosage forms.

B. Dofetilide

The study proved that dofetilide, its metabolite (Mc) and degradation product (DP a) were potent antiarrhythmic agents. The HPLC method for the forced degradation study of dofetilide its metabolite (Mc) and degradation product (DP a) gave more satisfactory results, in term of rapid chromatographic separation, excellent resolution, high analysis and

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good sensitivity. The developed method was validated as per the ICH guidelines and gave acceptable results with respect to specificity, selectivity, linearity, precision, accuracy and robustness. Studies showed that the dofetilide degrade under alkaline hydrolytic condition only and found to be stable in acid hydrolysis, neutral hydrolysis, oxidative, thermal and photolytic stress conditions. Analytical techniques assist the development of forced degradation pathway of the drug. Thus these studies facilitate the sample stability routine analysis and formation of combinational dosage forms.

REFERENCES

[1]. D.J. Chong, A.M. Lerman. Practice update: Review of anticonvulsant therapy. Current Neurology and Neuroscience Reports, 16 (2016) 39.

[2]. M. Tabaeizadeh. Review of antiepileptic drugs. JAMA Neurology, 73 (2016) 896.

[3]. P. Ferranaelli. Seizures, Epilepsy and other episodic disorders in adults in Michael Donaghy (Ed) Brain’s diseases of the nervous system, Oxford University Press New York. (1984) 651-710.

[4]. A. Chakraborti, M.F. Shaikh, A. Vezzani, J.M. Abdullah. Experimental models of epilepsy and related comorbidities. Frontiers in Pharmacology, 10 (2019) 179.

[5]. URL-https://www.epilepsysociety.org.uk/seizure-triggers#.WRVOd-uGPDc [Accessed on 25.05.19].

[6]. R.S. Fisher, J.H. Cross, C. D'Souza, J.A. French, S.R. Haut, N. Higurashi. Instruction manual for the ILAE 2017 operational classification of seizure types. Epilepsia, 58 (2017) 531–542.

[7]. R.S. Fisher. The new classification of seizures by the international league against epilepsy 2017. Current Neurology and Neuroscience Reports, 17 (2017) 48.

[8]. C.E. Stafstrom. Recognizing seizures and epilepsy: insights from pathophysiology. Epilepsy. Chichester, UK: John Wiley & Sons, Ltd. 17 (2014) 3-9.

[9]. C.E. Stafstrom, L. Carmant. Seizures and epilepsy: an overview for neuroscientists. Cold Spring Harbor perspectives in medicine, 5 (2015) a022426.

[10]. A. Singh, S. Trevick. The epidemiology of global epilepsy. Neurologic Clinics, 34 (2016) 837-47.

[11]. T. Dua, H.M. de Boer, L.L. Prilipko, S. Saxena. Epilepsy care in the world: results of an ILAE/IBE/WHO global campaign against epilepsy survey. Epilepsia, 47 (2006) 1225-31.

[12]. S. Amudhan, G. Gururaj, P. Satishchandra. Epilepsy in India I: Epidemiology and public health. Annals of Indian Academy of Neurology, 18 (2015) 263-77.

[13]. J. Robertson, S. Baines, E. Emerson, C. Hatton. Service responses to people with intellectual disabilities and epilepsy: A systematic review. Journal of Applied Research Intellectual Disabilities, 30 (2017) 1-32.

[14]. R. Rocamora. A review of the efficacy and safety of eslicarbazepine acetate in the management of partial-onset seizures. Theraputic Advances in Neurological Disorders, 8 (2015) 178–186.

[15]. K.D. Tripathi. Essentials of Medical Pharmacology. Jaypee brothers medical publishers. 7 (2013) 411.

[16]. T. Hanada. The AMPA receptor as a therapeutic target in epilepsy: preclinical and clinical evidence. Journal of Receptor, Ligand and Channel Research, 7 (2014) 39–50.

[17]. M. Bialer, S.I. Johannessen, R.H. Levy, E. Perucca, T. Tomson, H.S. White. Progress report on new antiepileptic drugs: a summary of the thirteenth eilat conference on new antiepileptic drugs and devices (EILAT XIII). Epilepsia, 58 (2017) 181-221.

[18]. E. Bial. Announce partnership agreement for the European commercialization of the novel once daily antiepileptic zebinix; 2009 [Online] Available from: http://www.prnewswire.co.uk/newsreleases/Eisaiandbialannouncepartnershipagreementfortheeuropeancommercialisationofthenoveloncedailyantiepilepticzebinixr153037325.html). [Accessed on 29.02.19].

[19]. A. Serralheiro, G. Alves, A. Fortuna, M. Rocha, A. Falcão. First HPLCUV method for rapid and simultaneous quantification of phenobarbital, primidone, phenytoin, carbamazepine, carbamazepine-10, 11-

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epoxide, 10, 11-trans-dihydroxy-10, 11-dihydrocarbamazepine, lamotrigine, oxcarbazepine and licarbazepine in human plasma. Journal of Chromatography B, 15 (2013) 1-9.

[20]. F.C. Ribeiro, T. Nunes, A. Falcao, C. Neta, R. Lima, S. Tavares, L. Almeida, T. Macedo, P. Soares-da-Silva. Eslicarbazepine acetate (BIA 2-093). Drugs in R & D, 6 (2005) 253-60.

[21]. G. Alves, I. Figueiredo, M.C. Branco M, A. Loureiro, A. Falcao, M. Caramona. Simultaneous and enantioselective liquid chromatographic determination of eslicarbazepine acetate, S-licarbazepine, R-licarbazepine and oxcarbazepine in mouse tissue samples using ultraviolet detection. Analytica Chimica Acta, 596 (2007) 132-40.

[22]. G. Alves, I. Figueiredo, M.C. Branco, A. Loureiro, A. Fortuna, A. Falcao, M. Caramona. Enantioselective HPLC - UV method for determination of eslicarbazepine acetate (BIA 2 - 093) and its metabolites in human plasma. Biomedical Chromatography, 11 (2007) 1127-34.

[23]. A. Falcao, J. Maia, L. Almeida, D. Mazur, M. Gellert, P. Soares - da - Silva. Effect of gender on the pharmacokinetics of eslicarbazepine acetate (BIA 2 - 093), a new voltage - gated sodium channel blocker. Biopharmaceutics & Drug Disposition, 28 (2007) 249-56.

[24]. A. Fortuna, J. Sousa, G. Alves, A. Falcao, P. Soares-da-Silva. Development and validation of an HPLC-UV method for the simultaneous quantification of carbamazepine, oxcarbazepine, eslicarbazepine acetate and their main metabolites in human plasma. Analytical and Bioanalytical Chemistry, 397 (2010) 1605-15

[25]. G. Alves, A. Fortuna, J. Sousa, R. Direito, A. Almeida, M. Rocha, A. Falcao, P. Soares-da-Silva. Enantioselective assay for therapeutic drug monitoring of eslicarbazepine acetate: no interference with carbamazepine and its metabolites. Therapeutic Drug Monitoring, 32 (2010) 512-6.

[26]. A. Fortuna, J. Bicker, G. Alves, A. Falcao, P. Soares - da - Silva. A chiral HPLC - UV method for the quantification of dibenz [b, f] azepine - 5 - carboxamide derivatives in mouse plasma and brain tissue: Eslicarbazepine acetate, carbamazepine and main metabolites. Journal of Separation Science, 34 (2011) 1391-401.

[27]. A.I. Loureiro, C. Fernandes-Lopes, L.C. Wright, P. Soares-da-Silva. Development and validation of an enantioselective liquid-chromatography/tandem mass spectrometry method for the separation and quantification of eslicarbazepine acetate, eslicarbazepine, R-licarbazepine and oxcarbazepine in human plasma. Journal of Chromatography B, 879 (2011) 2611-8.

[28]. M.K. Mone, K.B. Chandrasekhar. Development of liquid chromatographic enantiomer separation methods and validation for the estimation of (R)-enantiomer in eslicarbazepine acetate. Journal of Pharmaceutical and Biomedical Analysis, 54 (2011) 248-51.

[29]. C. Zhang, Z. Zuo, P. Kwan, L. Baum. In vitro transport profile of carbamazepine, oxcarbazepine, eslicarbazepine acetate, and their active metabolites by human P - glycoprotein. Epilepsia, 52 (2011) 1894-904.

[30]. S. Thomas, A. Bharti, P.K. Maddhesia, S. Shandilya, A. Agarwal, S. Biswas, V. Bhansal, A.K. Gupta, P.K. Tewari, C.S. Mathela. Highly efficient, selective, sensitive and stability indicating RP-HPLC–UV method for the quantitative determination of potential impurities and characterization of four novel impurities in eslicarbazepine acetate active pharmaceutical ingredient by LC/ESI-IT/MS/MS. Journal of Pharmaceutical and Biomedical Analysis, 61 (2012) 165-75.

[31]. M. Singh, S. Singh, S. Deshaboina, H. Krishnen, R. Lloyd, K. Holt-Tiffin, A. Bhattacharya, R. Bandichhor. Asymmetric reduction of a key intermediate of eslicarbazepine acetate using whole cell biotransformation in a biphasic medium. Catalysis Science & Technology, 2 (2012) 1602-5.

[32]. M. Srinivas, N.R. Avupati, S. Sait, K. Mukkanti. Stability indicating HPLC method for the determination of eslicarbazepine acetate and its impurities in bulk drugs and pharmaceutical dosage forms. Journal of Liquid Chromatography & Related Technologies, 35 (2012) 1550-64.

[33]. A. Fortuna, G. Alves, A. Almeida, B. Lopes, A. Falcao, P. Soares‐da‐Silva. A chiral liquid chromatography method for the simultaneous determination of oxcarbazepine, eslicarbazepine, R‐licarbazepine and other new chemical derivatives BIA 2‐024, BIA 2‐059 and BIA 2–265, in mouse plasma and brain. Biomedical Chromatography, 26 (2012) 384-92.

[34]. M. Singh, L. Kumar, P. Arora, S.C. Mathur, P.K. Saini, R.M. Singh, G.N. Singh. Development and validation of an RP-HPLC method for quantitative estimation of eslicarbazepine acetate in bulk drug and tablets. Indian Journal of Pharmaceutical Sciences, 6 (2013) 736.

[35]. T. Nunes, J.F. Rocha, A. Falcao, L. Almeida, P. Soares‐da‐Silva. Steady‐state plasma and cerebrospinal fluid pharmacokinetics and tolerability of eslicarbazepine acetate and oxcarbazepine in healthy volunteers. Epilepsia, 54 (2013) 108-16.

[36]. S. Thomas, S.K. Paul, S.C. Joshi, V. Kumar, A. Agarwal, D. Vir. Identification, synthesis and characterization of an unknown process related impurity in eslicarbazepine acetate active pharmaceutical ingredient by LC/ESI–IT/MS, 1H, 13C and 1H–1H COSY NMR. Journal of Pharmaceutical Analysis, 4 (2014) 339-42.

[37]. R.P. Pandit, B. Kotamarthi, R. Kompally, R. Radha. FT-IR Spectroscopic assay method for eslicarbazepine acetate in bulk and tablet formulations. International Journal of Pharmaceutical Chemistry, 5 (2015) 255-9.

[38]. S.R. Kallam, J. Srikanth, K. Vanitha. Development and validation of an RP-HPLC method for related substance and quantitative estimation of eslicarbazepine acetate in bulk drug and pharmaceutical dosage form. Journal of Pharmacy and Bioallied Sciences, 10 (2015) 155-6.

[39]. A.C. Servais, B. Janicot, A. Takam, J. Crommen, M. Fillet. Liquid chromatography separation of the chiral prodrug eslicarbazepine acetate and its main metabolites in polar organic mode. Application to their analysis after in vitro metabolism. Journal of Chromatography A, 1467 (2016) 306-11.

[40]. D.A. Smith, H.S. Rasmussen, D.A. Stopher, D.K. Walker. Pharmacokinetics and metabolism of dofetilide in mouse, rat, dog and man. Xenobiotica, 22 (1992) 709-19.