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Drug-Drug Interaction Studies of Ibutilide Fumarate with Verapamil and Stability Indicating Method by Chromatographic Techniques

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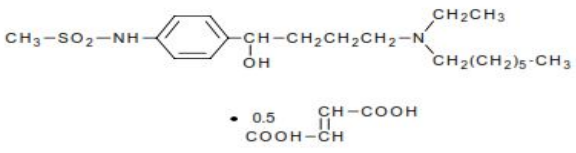
Abstract: ***the aim of the present work is to study interactions of ibutilide fumarate with the antihypertensive drug verapamil at physiological ph 7.4 and temperature 37ºc under simulated condition and using commercially procured pooled male rat liver microsomes upto 4 hours. The work also includes the stability of ibutilide fumarate at various stress conditions as recommended by ich guidelines. The hplc(method 1) and hptlc method was developed and validated for the linearity, precision, robustness, limit of detection and limit of quantitation. The linearity was found between 2-16 mcg/ml and 1-9 mcg/spot , the % rsd was found below 1 for interday and intraday precision, lod was found to be 0.5 mcg /ml and 159 ng/spot and loq was found to be 1 mcg/ml and 482 ng/spot for hplc method 1 and hptlc respectively. The validated hplc methods 1 and 2 were applied for the stability indicating method and interaction studies respectively. The ibutilide fumarate was found to be stable at 40 ºcand the degradants were above 10% with 0.1m hcl, 0.1m naoh, oxidation, light and at 60 ºc. With the increase in time of interaction the ibutilide fumarate concentration varied between 82– 115% with simultaneous variation in the interacting drug verapamil concentration between 25-147% and an additional metabolite peak was found between 43-147% under simulated conditions. The results for the drug ibutilide fumarate with verapamil in the presence of rat liver microsomes containing the cyp3a4 enzymes shows considerable change in the peak area of verapamil . The concentration of ibutilide fumarate shows changes in between 1.156-108% indicating complete binding with the enzymes when administered as a single drug with additional peaks for the metabolites formed. The verapamil concentration changes within 135-185% in presence of enzymes as a single drug. The change in concentration for ibutilide was found to be 71-106% and for verapamil was found to be 97-300% when both the drugs are co administered.***

Keywords: ibutilide fumarate, verapamil, hplc method, hptlc method, drug drug interaction studies, stability indicating method.

I. INTRODUCTION

Ibutilide fumarate was approved by USFDA under the class of antiarrhythmic drug with IUPAC name Methanesulfonamide, N-{4-{4- (ethylheptylamino)-1-hydroxybutyl} phenyl}, (+)(-), (E)-2-butenediote (1:0.5) (hemifumarate salt)(Fig 1). There are no specific pharmacokinetic or other formal drug interaction studies conducted till date for Ibutilide fumarate. The anti hypertensive drug verapamil is an inhibitor of the CYP3A4 enzymes. In the metabolism of Ibutilide there are eight metabolites detected in the urine due to oxidation of the heptyl side chain. The ω- oxidation metabolite has electrophysiological properties similar to Ibutilide but concentration of the metabolite is less than 10% in the plasma.

Figure 1



The drug Ibutilide fumarate is not official in any pharmacopoeia. The formulation available is 0.1mg/ml injection(Corvert injection,

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Pharmacia and Upjohn Company). The Intravenous infusion of Ibutilide results in the dose related prolongation of QT interval[1].
The concomitant therapy of antiarrhythmatic drug with inhibitors of CYP 450 enzymytic activity has to be studied as the inhibitor drug verapamil can alter the distribution and rate of elimination of antiarrhythmatic drug in the plasma thereby increasing the risk of Torsade de pointes[2]. The present research was focused on the co-administration of Ibutilide fumarate with verapamil which can alter the concentration leading to changes in the metabolism of the drug and hence lead to considerable changes in the levels of both the drugs. The safety and efficacy of the drug has not being established in pediatric patients and in nursing women. This research work can be applied for the calculation of dosage for the above group of population.
Before the research work was started the through survey of literature was undertaken to conclude the mechanism of action, pharmacokinetics and stability of the drug Ibutilide fumarate. The property of the drug was analyzed for the accurate results. The few reported methods for analysis of Ibutilide fumarate are UV Visible spectrometry method forming a complex with TPOOO dye[3], HPLC method for trace determinations in pharmaceutical environment[4] , Assay of enantiomers of Ibutilide fumarate in biological fluids[5] and pharmaceutical formulations[6] by HPLC.
The aim of the present work is to study interactions of Ibutilide fumarate with the CYP3A4 inhibitor verapamil at physiological pH 7.4 and temperature 37°C under simulated condition and with rat liver microsomes. The *in vitro* interaction studies forms a base for the metabolic pathway where the subsequent *in vivo* testing can be performed more specifically. So the co administration of Ibutilide fumarate and verapamil has to be taken under consideration in the case of poly pharmacy for treatment of multiple heart diseases.The concentration of Ibutilide was found to have significant variation with the increasing time in the presence of verapamil.
Corvert injection was administered either undiluted or diluted with 0.9% Sodium chloride or 5% Dextrose upto 50 ml. The diluted admixtures were stable for 24 hours at room temperature and 48 hours in refrigeration[1]. The pH of the saline solution is compatible with the blood pH. Till date there are no reported methods for degradation of Ibutilide fumarate. Hence the stability of the drug Ibutilide fumarate were studied under acidic, basic, oxidation, light and elevated temperature conditions. The peaks of the drug and degradants were eluted at separate retention times by HPLC method and at different Rf values by HPTLC method .There are no reported methods for the interactions studies for Ibutilide fumarate with verapamil and stability indicating method.

II. MATERIALS AND METHODS

The solvents used for the methods like methanol, acetonitrile and toluene were procured from Qualigens fine chemicals Ltd. (Mumbai, India). The pure drug Ibutilide was procured from Sigma Aldrich. The precoated aluminium plates of TLC silica gel 60 F_{254} 20 X 20 cm were procured from Ponmani and Co supplier in Coimbatore which is manufactured by Merck, Germany. The rat liver microsomes(pooled vial M9066) was procured from Sigma Aldrich and immediately stored at -70 °C until the studies were done.
The Shimadzu balance was used for weighing. The Jasco V630 Spectrophotometer with matched Quartz cuvettes was used for the UV Spectroscopic method. The HPTLC method was performed using Camag Linomat 5 applicator with 100 µl syringe, Camag UV chamber, Camag TLC scanner (WINCATS software). The HPLC method was performed using Shimadzu LC-2030, *i-prominence* system inbuilt with a auto sampler, temperature control, inbuilt degasser and four mobile phase pumps . The solvents used were filtered through 0.45 µm filter and used for the preparation of solution. The sample solution before placing in the auto sampler was filtered through 0.2µm syringe nylon filter.

A. Theory

The Ibutilide fumarate is a salt of fumaric acid. The previous work done reports the elution of fumaric acid as a seperate peak at 2.627 minutes and the drug peak at 5.7 minutes using buffer at pH 6[4]. The present work was developed and established by using pure form of fumaric acid to identify the peak separately in all the solvents used including the phosphate buffer pH 7.4. In the pH buffer of 7.4 the fumaric acid peak was found to merge with the solvent peak at 1.9 minutes. The peak for the drug was separately obtained by both HPLC and HPTLC methods and was confirmed by the respective Retention time and Rf value.
FDA current statement for *in vitro* studies says that " In vitro studies can frequently serve as a screening mechanism to rule out the importance of a metabolic pathway and the drug-drug interactions that occur through this pathway so that subsequent in vivo testing is unnecessary."
The distribution half life and average half life of Ibutilide fumarate was 1.5 minutes and 6 hours[1]. The half life of verapamil was

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10.46 minutes[10]. The present studies was performed till 4 hours.The rate of metabolism was calculated comparing the peak area at zero minutes with the peak area at specified interval times. Microsomal protein concentrations used are usually less than 1 mg/ml for the specific binding of the drug to their receptors. Excessive concentrations lead to non-specific binding that tends to reduce inhibition response and elevate IC50 values[9] .

B. Preparation of Solution

The fresh stock solution of Ibutilide fumarate with a concentration of 1000 µg/ml was prepared using methanol as the solvent for the HPTLC method. The samples for stability indicating method were prepared with the 0.1 M HCl for acid hydrolysis, 0.1 M NaOH for base hydrolysis, 3% H_2O_2 for oxidation and solution in methanol for elevated temperature and controlled light for photolysis.

C. Preparation of Buffer

The pH 7.4 buffer was prepared by taking 0.6 g potassium dihydrogen orthophosphate, 6.4 g disodium hydrogen orthophosphate and 5.85 g sodium chloride in 1000 ml deionized water and pH was adjusted with 0.1M HCl if necessary. [5]

D. Experimental Conditions

The HPTLC method was developed using Isopropyl alcohol: Methanol:Ethyl acetate in the ratio of 1:8:1 with chamber saturation time of 20 minutes and detection was at 228 nm. The Rf value of fumaric acid and the drug Ibutilide was found to be 0.72 and 0.33 respectively.The linearity was established between 0.4 µg to 10 µg by HPTLC method . The interday precision and intra day precision studies was studied. The LOD and LOQ were calculated statistically.

The HPTLC method developed above was adopted for the stability indicating method with acid, base, hydrogen peroxide, thermal at 40 °C, 60 °C and photolysis. The Rf value for the drug Ibutilide, fumaric acid and the degradants were noted and summarized.

The HPLC method was developed with mobile phase of methanol and water in the ratio of 55:45 The experimental parameters was fixed with a flow rate and injection volume of 0.9 ml/min and 20 µl at 228 nm. The interday precision and intra day precision studies on three consecutive days were studied. The LOD and LOQ were calculated statistically.

The stability indicating method for Ibutilide fumarate by HPLC method were carried out with the same fixed experimental conditions and according to the ICH guidelines under various stress conditions of acid hydrolysis using 0.1 M HCl, base hydrolysis using 0.1 M NaOH, 3% v/v hydrogen peroxide, photolysis with 220 volts light , thermal degradation at elevated temperature of 40 °C and 60 °C. The sampling was done at an interval of every 30 minutes upto 240 minutes.

The HPLC method with fixed experimental conditions of mobile phase consisting of acetonitrile: methanol: water in the ratio of 25:50:25 at a flow rate of 1 ml/minute at 228 nm with a run time of 30 minutes was adopted for the interaction studies to elute both the Ibutilide fumarate and Verapamil at different retention time. The sample injection volume was 20 µl injected using automatic sampler.

E. Method Adopted

The pooled rat liver microsomes(M 9066) are subcellular particles including cytochrome P450 pooled from different male rats(Sprague Dawley) of 8 to 10 weeks. It consists of CYP3A, CYP2C,CYPE1, CYP1A, CYP4A activities. The CYP3A isoenzyme concentration was calculated based on the determination of testosterone 6β-hydroxylase activity. The stock solution of 20 mg/ml of the protein content was diluted further with buffer solution consisting the drug Ibutilide fumarate to give a final concentration of 0.2 mg/ml of protein[7].

The interaction studies by HPLC was carried at a controlled temperature of 37°C at physiological pH of 7.4 under simulated conditions and in the presence of pooled rat liver microsomes containing the CYP 450 isoenzymes. The sampling was performed at an interval of every 30 minutes upto 240 minutes. The sample withdrawn from the buffer solution was further diluted ten times with methanol to stop the interaction between the drug Ibutilide and verapamil. This 20 µl of the diluted sample was injected in the column maintained at 37 °C with a run time of 30 minutes.

The stability indicating method was performed using the above fixed experimental conditions both for HPLC and HPTLC method. The sample withdrawn from the thermostat was diluted ten times with methanol and injected in the column. The results were summarized and calculated for the % of degradation. The change in the peak area for the drug and the separate peaks for the

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degradants were found at different Rf value and retention time.

III. RESULTS

The HPTLC method was developed with linearity in the range of 1 µg to 10 µg and the correlation coefficient of 0.99. The % RSD for interday and Intraday precision studies was found to be less than 1. The LOD and LOQ was 159.37 ng/spot and 482.96 ng/spot respectively.

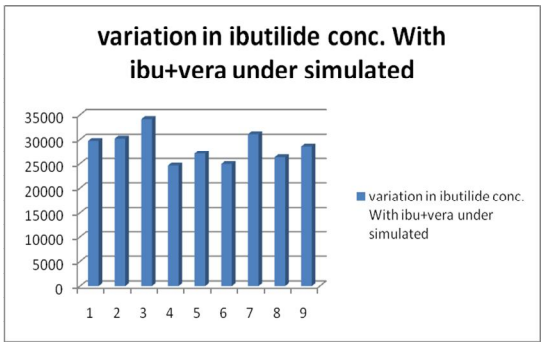
The interaction studies of Ibutilide fumarate with Verapamil under simulated conditions and presence of CYP3A4 enzymes was found to show significant variation in the availability of the drug.

Under simulated conditions the peak of Ibutilide and verapamil were obtained along with the peak of the metabolite eluting after the drug peak of Ibutilide. The metabolite peak was found between 8.189 mins and 10.676 mins. With the increase in time of interaction the Ibutilide fumarate concentration varied between 82– 115% with simultaneous variation in the interacting drug Verapamil concentration between 25-147% under simulated conditions and an additional metabolite peak was found between 43-147%. The results for the drug Ibutilide fumarate with Verapamil in the presence of rat liver microsomes containing the CYP3A4 enzymes shows considerable change in the peak area of verapamil . The concentration of Ibutilide fumarate shows changes in between 1.156-108% indicating complete binding with the enzymes when administered as a single drug with additional peaks for the metabolites formed. The verapamil concentration changes within 135-185% in presence of enzymes as a single drug. The change in concentration for Ibutilide was found to be 71-106% and for verapamil was found to be 97-300%. The peak area at the zero minutes was used for the calculation of peak area changes upto 240 minutes.

The stability indicating method by HPLC under acid hydrolysis was found with an change in the peak area by 25%, for the base hydrolysis was found to be by 52%, for the thermal degradation at 40 °C was found to be till 102.61%, for the photolysis was found to be upto 115%, oxidation with hydrogen peroxide was found to be upto 92.46% and at 60 °C was found to be upto 45%. Hence the drug was found to be stable under the above stress conditions with the degradation within 10% at 40 °C and above 10% at 60 °C, acid hydrolysis,light and base hydrolysis.The degradants peaks were found to elute at separate retention time.

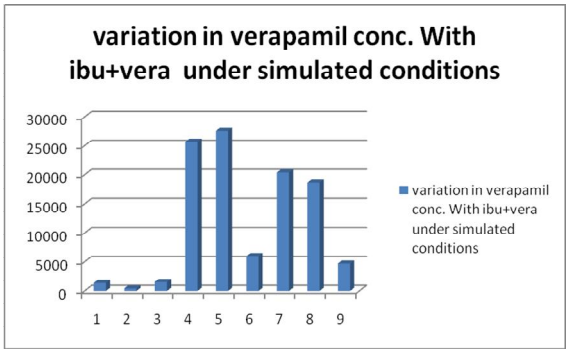
A. Comparison of Interaction studies of Ibutilide fumarate with Verapamil under Simulated conditions and rat liver microsomes

ForIbutilide fumarate

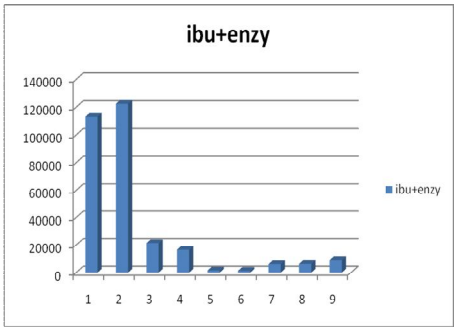


For Ibutilide fumarate

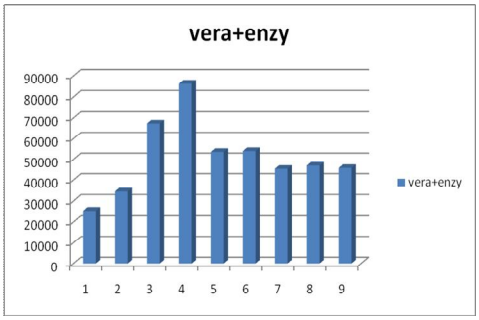
For verapamil



For verapamil



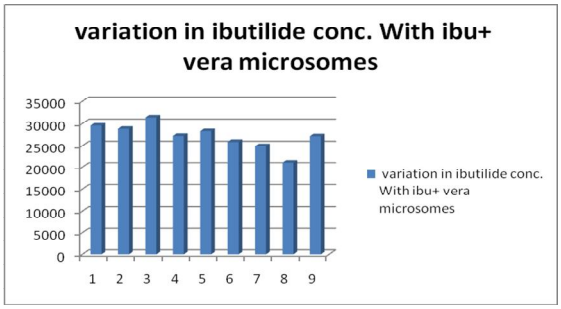
For Ibutilide fumarate

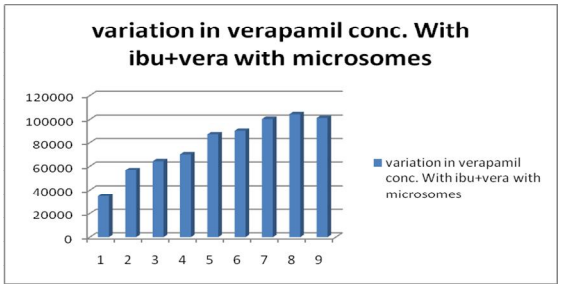


For verapamil

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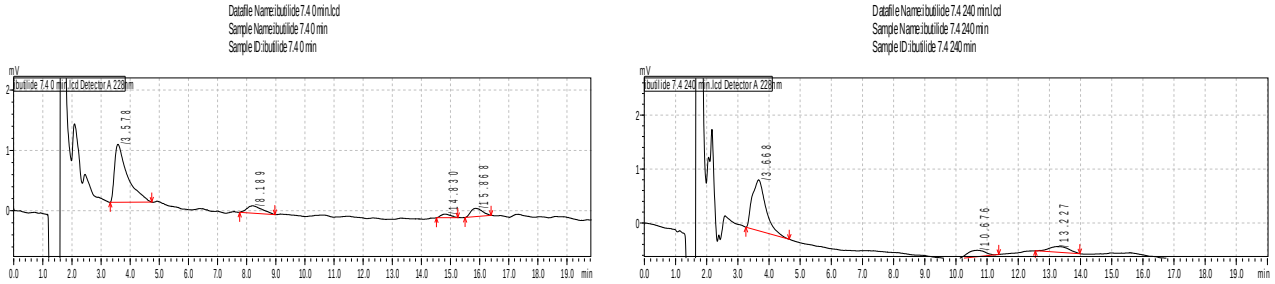
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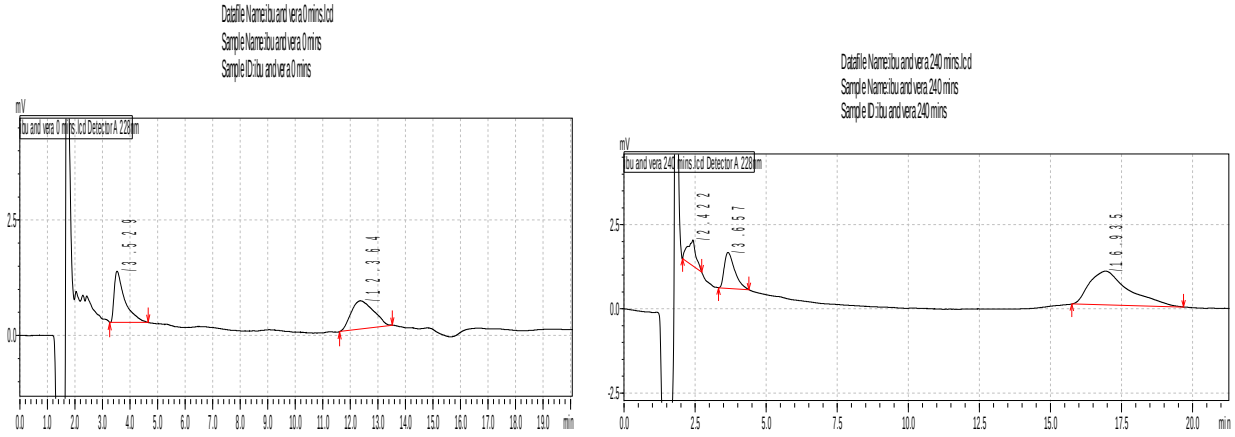


B. Representative chromatograms for Interaction studies

Simulated conditions

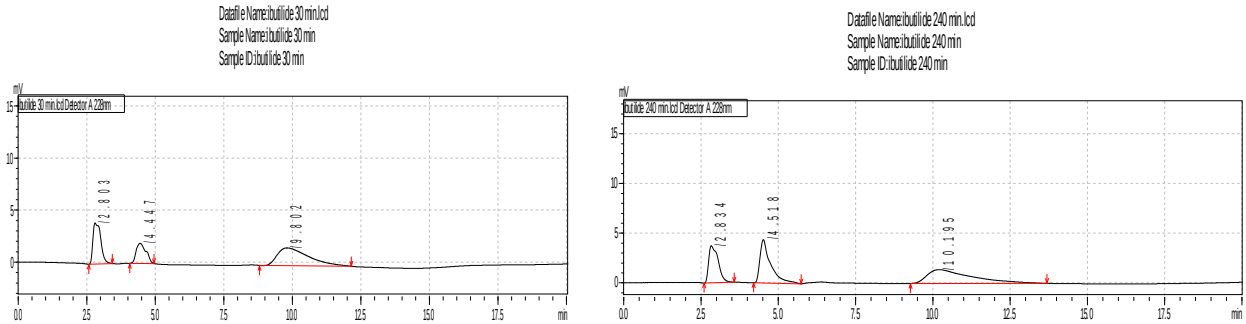


Interaction study in presence of rat liver microsomes



C. Stability indicating method of Ibutilide fumarate

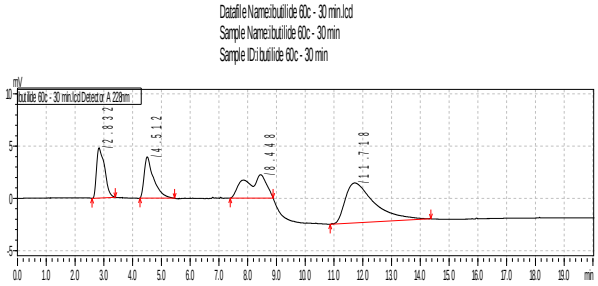
Thermal 40 C

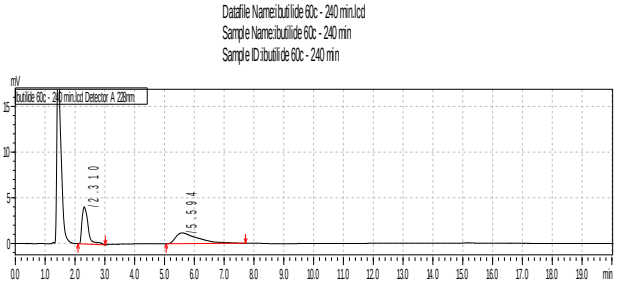


Thermal 60 C

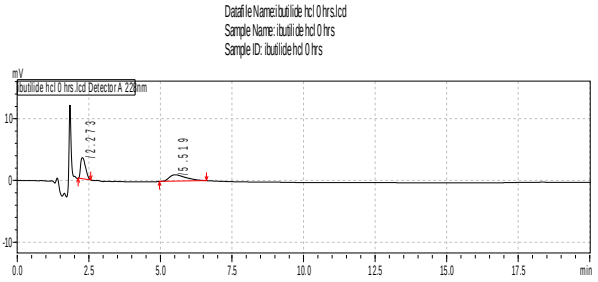
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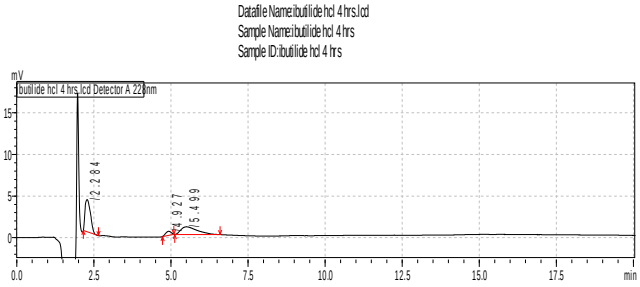
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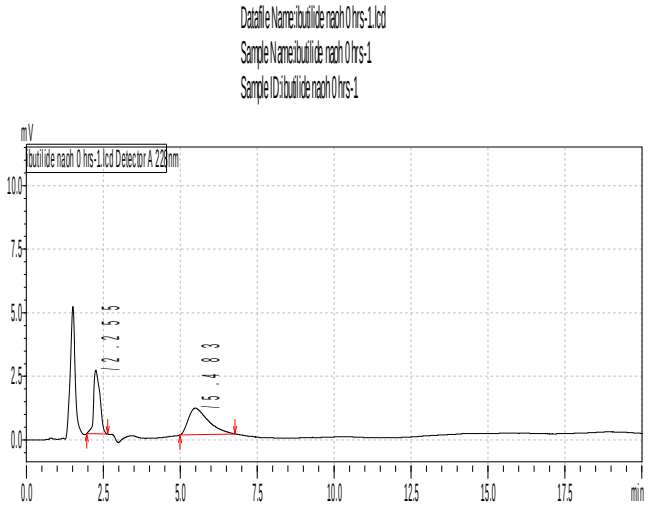


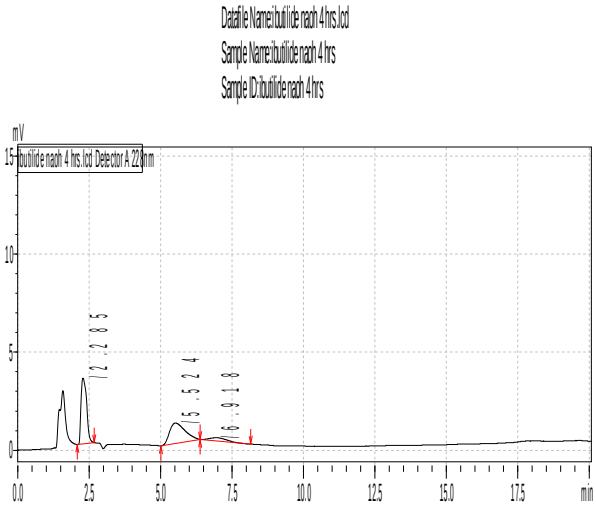
Acid hydrolysis



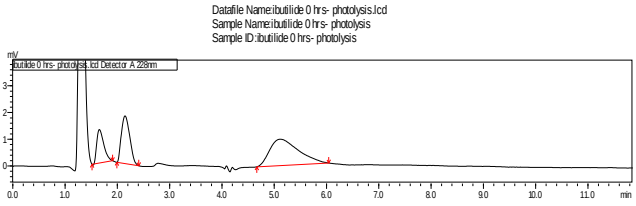


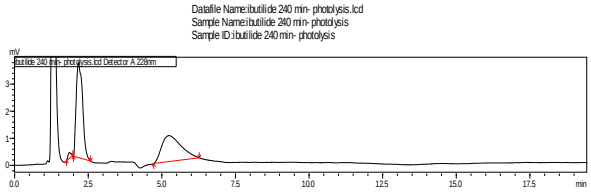
Base hydrolysis





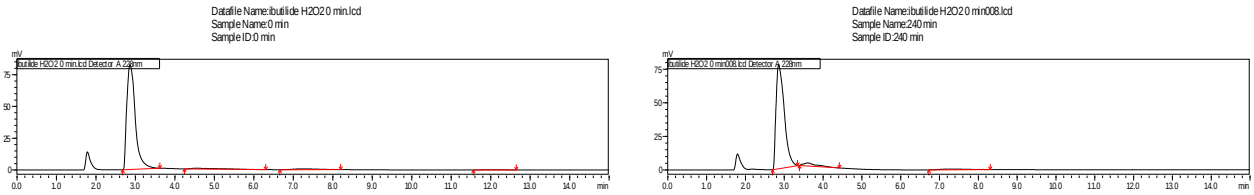
Photolysis – 220 volts light





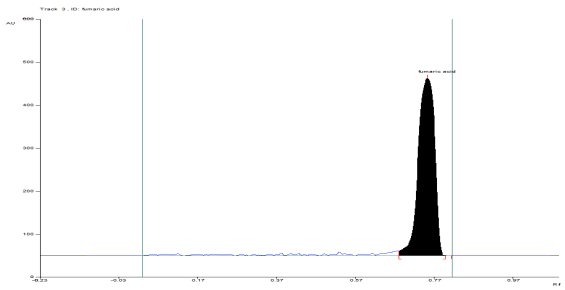
Oxidation – 3% v/v hydrogen peroxide

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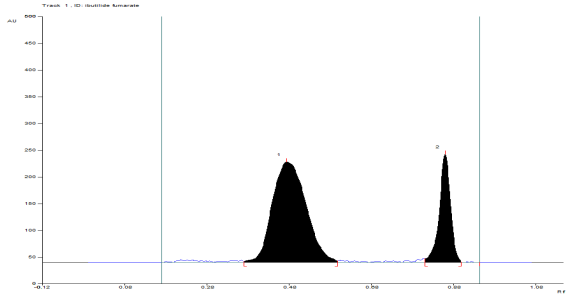


D. Representative HPTLC chromatogram

Fumaric acid



Ibutilide fumarate



IV. DISCUSSIONS

Ibutilide fumarate is used for treatment of atrial flutter and atrial fibrillation. The stability of the drug as specified by the ICH guidelines helps to assess the potency of the drug needed for its activity. The various conditions of elevated temperature, light, oxidation, acid and base were analysed.

The drug was found to be stable at 40 °C . The level of degradants formed due to the stress conditions were more than the specified limit of tolerance for 60 °C, light, oxidation, acid and base hydrolysis. So it is recommended to store the drug under controlled conditions of temperature, light and pH variations. The results suggests the limitations for the exposure of the drug to such conditions under the study conducted can reduce the drug available for the pharmacological activity.

The *in-vitro* drug drug interaction studies establishes changes in the metabolism of both the drugs under the controlled conditions of physiological blood pH of 7.4 under simulated conditions and using rat liver microsomes. Till date there are no specific pharmacokinetics study reporting the particular enzymatic pathway for the metabolism of Ibutilide fumarate. So our research work focuses on the relative changes of the drug concentration in the presence of the drug verapamil which is a moderate inhibitor of CYP3A4 enzymatic pathway under similar physiological conditions of blood and presence of rat liver microsomes containing PYC450 enzymes.

The results of our research work carries indicates the possible interactions when the anti arrhythmatic drugs are co administered with verapamil with a specific metabolic pathway. The distribution half life and average half life of Ibutilide fumarate was 1.5 minutes and 6 hours[(1)]. The half life of verapamil was 10.46 hours[(10)]. The present studies was performed till 4 hours. The steep increase in the verapamil concentration within 4 hours shows the strong binding between Ibutilide fumarate and decrease in the binding with verapamil which results in the three fold drug concentration of verapamil based on the response factor by the drug verapamil. This increase in the concentration of verapamil suggests a specific consideration in the dosage of both the drugs in case of co administration. This research can be used as a base for the clinical studies of Ibutilide fumarate.

V. CONCLUSIONS

The selected class of cardiovascular drug of antiarrhythmatic drugs are very potent in nature with dosage of less than 10 mg. Ibutilide fumarate is available as 1 mg/10 ml injection. The concentration of drug available for its pharmacological actions and the pharmacokinetics depends on the half life of the drug. The method development for the selected classes of cardio vascular drugs using simple and economical mobile phase consisting of water and organic solvents can be adopted for the routine analysis of the drugs.

The developed and established method can be used for the routine analysis of Ibutilide fumarate in bulk drug and formulations. The stability indicating method was to assess the stability of drugs under the stress conditions which can be applied for the suitable conditions to maintain the potency of the drug Ibutilide fumarate. The interaction studies illustrates the synergistic activity of

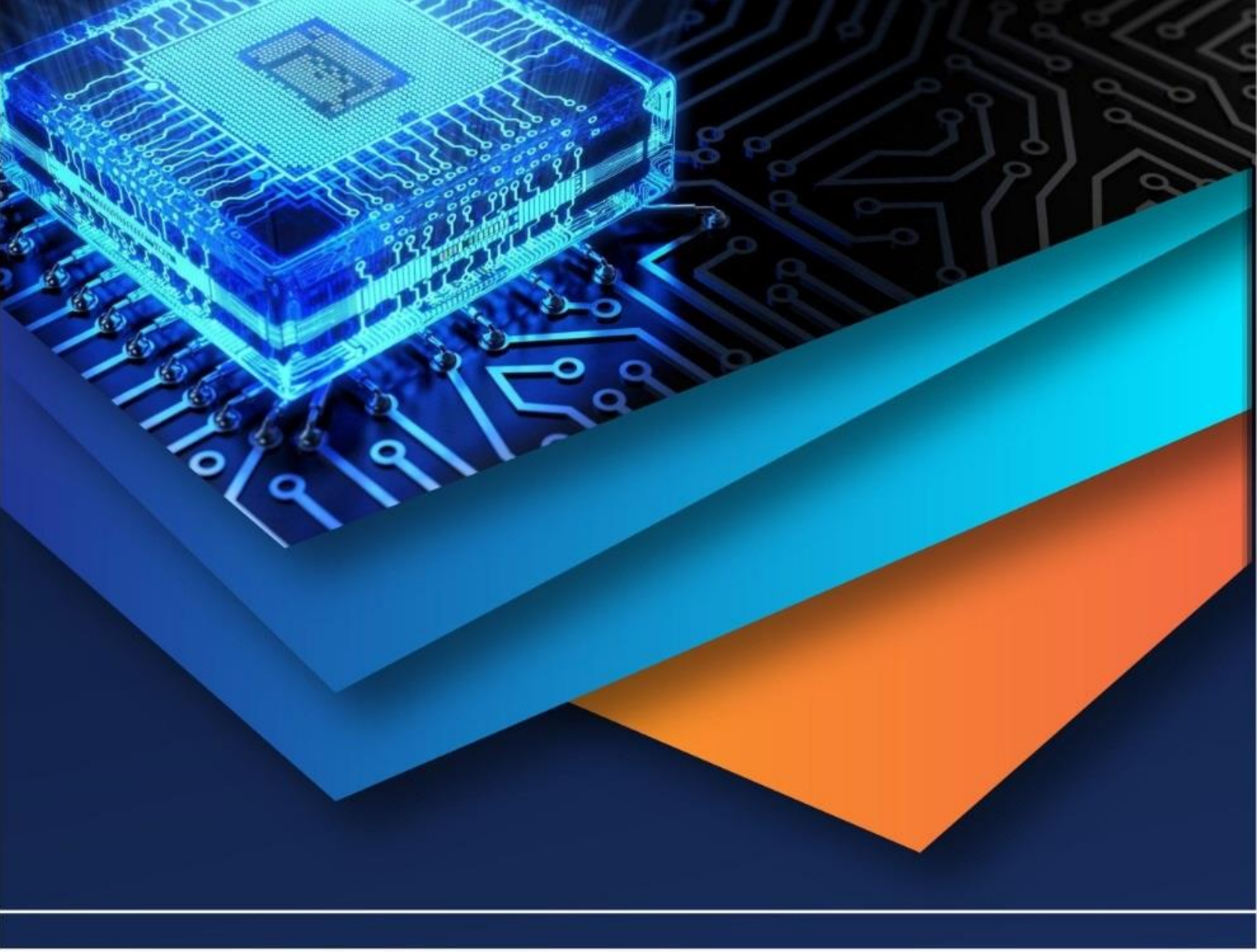
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Ibutilide fumarate when co administered with verapamil which can be further carried over to study the interaction with other calcium channel blockers and for the in vivo studies. The metabolism of Ibutilide fumarate by the liver microsomes enzymes alters the inhibitory activity of the drug by binding with the enzymes thereby the concentration of drug verapamil increases steeply on co administration of the both drugs.

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