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In vitro drug-drug interaction study between Ranitidine Hydrochloride and Bisoprolol fumarate

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Abstract
The current study's objective is to assess the pharmacological interactions between Bisoprolol fumarate and Ranitidine hydrochloride using in vitro model. Using the Job's approach and Ardon's method, a spectral observation research, and the thin layer chromatography method, interactions between bisoprolol fumarate and ranitidine hydrochloride were assessed. UV-VIS spectrophotometers were used for spectral observation in both acidic and basic pH conditions. Different spectra from the individuals and 1:1, 1:2, and 2:1 complexes were obtained in the UV-VIS spectrophotometer. The drug complexes produced a distinct spectrum than its pure component. When Ranitidine hydrochloride is combined with Bisoprolol fumarate at a 1:1 ratio, the spectra suddenly alters as a result of interaction. The stability constant values at various levels of pH (1.2, 2.0, 3.0, 5.5, and 6.4) determined by the Ardon's plots are 0.896, 1.612, 1.200, 1.36, and 0.294. According to the stability constant values, Bisoprolol fumarate and Ranitidine hydrochloride interact in a moderately stable manner. Because of this, the complex displayed fluctuating activity during the test.

Keywords Bisoprolol, Ranitidine, Drug-drug interaction, Job's, Ardon's, UV

Paper type Research paper

1. Introduction
Combination therapy is often necessary to treat coexisting diseases. The purpose of combination therapy is to i) obtain a desired therapeutic effect ii) to treat coexisting disease iii) to broaden the spectrum in case of antibiotic therapy iv) to delay the emergence of malignant cells in cancer chemotherapy vi) to resist the development of microbial resistance to antibiotics vii) to minimize the adverse drug reaction. Combined effects of drugs may cause the following implications i) enhancement of drug effects which includes summation, synergism and potentiation. It may also cause drug antagonism. Incompatibility may also result from such therapy. Incompatibility refers to chemical or physical reactions between multiple drugs administered concurrently.

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Organoleptic properties of drugs can be changed through physical reactions while chemical reactions might bring complete change of participating molecules.

With the advent of new technology and high-throughput screening hundreds of active molecules are being introduced in the market each year. Many of them are INN drugs and the detailed safety profiles of majority of them have not been established. Most of the molecules are synthetic and the chances of adverse drug reactions (ADR) are higher for these candidates. Various types of interactions can be occurred originating from the combined drug formulation to site of action. Chemical interaction may occur due to ionization of different functional groups at various physiological pH which causes increase their reactivity with other drugs or natural metabolites. The chances of formation of new products from the chemical reactions may have new pharmacological activity which might get un-noticed throughout initial phase of a clinical trial. Physical and pharmacological interaction between two active molecules is also of prime concern for healthcare professionals evaluating the safety profile of drugs. Physical interaction can decrease the bioavailability of drugs; sub-therapeutic level of plasma-drug concentration might pose risk for patients in case of antibiotics and drugs having narrow therapeutic indices. Pharmacological interaction of drugs can exert undesirable effects which may be toxic to the patient. Hundreds of drugs may induce synergistic effects when co-administered with other active molecules. Some of the effects increase the activity of second drugs by manifolds resulting in plasma drug concentration beyond maximum therapeutic concentration (MTC) ultimately leading to serious toxicity. It is obvious that most of the drugs have passed through detailed pharmacological assay and clinical trial phases, although there always remains a chance of drug interaction between OTC drugs, vitamins, and food supplements with new prescription drugs. INN drugs are prevalent within the countries of Asian subcontinents prescribed by physicians in hospitals and clinics where strict regulation of rational drug use is not ensured. Poly-pharmacy is a common phenomenon among drug practitioners due to unethical practice and aggressive marketing policies of drug manufacturers which can cause the increase in the risk of potential interactions of any of the types.

Drug-drug interactions should always be evaluated in all areas of therapy. In vitro investigations are typically used primarily to identify the nature and amount of medical interaction. Various approaches are used to evaluate the interaction of the drug particles such as computational approaches have been used to assess experimental data to determine whether the interaction is

chemical, physical, pharmacological, synergistic, additive, or antagonistic. Mechanism-based pharmacodynamic simulation is a useful technique when the mechanisms of drug effects and drug-drug interactions are well documented (Danhof, de Jongh, De Lange, Della Pasqua, Ploeger, & Voskuyl, 2007). It is frequently required to take multiple prescriptions at the same time, and some amount of medication collaboration occurs with attending medication utilization (Kampman & Jarvis, 2015). Drug-drug interactions can lead to serious adverse effects and have resulted in early stages of development abandonment, refusal of licensing, stringent prescribing limits, and drug withdrawal from the market. As a result, regulators, notably the United States Food and Drug Administration, have published guidelines for in vitro and in vivo drug interaction studies to be done through development (Bjornsson et al., 2003a). However, these guidelines do not target the precise patterns of the studies, and regulatory agencies want to unify techniques and research designs to enable for better assessment and comparison of different medications. In vitro drug-drug interaction investigations must always be conducted with high standard and consistency, especially when the results could influence clinical study design. In vitro testing protocols and data documentation should be precise and repeatable, with specialized analytical methodologies and documentation of assay procedures and outcomes (Bjornsson et al., 2003b; Wienkers & Heath, 2005). When applicable, chemicals and reagents should have test notes for percentage quality, as well as authentic standards of metabolites utilized in the building of calibration graph.

Ranitidine is well absorbed with peak concentration within 3 hour after oral administration and varying patient to patient with different health conditions. The bioavailability is well and about 55% due to hepatic metabolism and first pass effect. The kinetics in healthy adult and the AUC was about 2500 nanogram per mL and the median Tmax was about 3 hours. Diet and oral bases of antacids can limit the absorption, clinical investigation reveals the administration of powerful antacid in the test subject can speed up the fasted state which can lead the decrease in absorption of this drug. The Vd is larger than apparent body volume which can measure approximately 1.5/Kg. It can secrete a proportion in breast milk but not readily spread through CSF. The plasma protein binding is common which is around 16% approximately. The major site of metabolism is liver and excretes through urine in the form of anoxide which is less in 4% than dose. The related metabolites of ranitidine include different oxides and desmethyl forms. A portion of metabolite eliminated through feaces in conjugate form.

Hepatic dysfunction can impair the elimination of drugs which can also change the kinetic profiles of ranitidine. The route of elimination mainly through kidney and also excreted in feaces. The 30% of a single oral dose also measured in the urine in unchanged form which were investigated for 24 hr after oral administration. Elimination half-life estimated about 3 hours which can be altered for different disease conditions and may be longer for IV injection. The half-life is varied from young to elder and also for renal and hepatic dysfunctions. The clearance for ranitidine was calculated about 4-10 mL/min prescribed on FDA information. The other resources estimates plasma clearance is approximately 600 mL/min. Clearance is interrupted in elder patient with hepatic and renal impairment and the dose is always determined at decrease fashion for those disease conditions. The toxicity observed for ranitidine for over-dose above 1g/kg in lower animals which were not found as lethal. IV LD50 values for lower animals were calculated around 80 mg/kg approx. The overdose cases were very rare in case of ranitidine which was reported up to 18g orally and temporary adverse effects were reported which were similar to normal adverse effect. The symptoms of an overdose include tachycardia, bradycardia, nausea, dizziness, vertigo, abdominal discomfort, etc. The Gait disorder and low blood pressure were also been observed. In cases of overdose, the unabsorbed portion from the GI tract can be removed, and patient monitoring and supportive therapy are mandatory.

Ranitidine hydrochloride (RHCl) is a potential H2-receptor blocker that competitively inhibits histamine to interact H2-receptors. The World Health Organization's List of Essential Medicines (21st and 22nd lists) include this well-known antiulcer medication, which has been in use for many years. Gastritis, gastrointestinal reflux disorder (GERD), Recurrent duodenal ulcers, stomach ulcers, Zollinger-Ellison syndrome, and erosive esophagitis are among conditions for which ranitidine is commonly recommended. The recommended adult oral ranitidine dosage is 150 mg twice daily or 300 mg once daily. A 300 mg alternative dose causes plasma variations; consequently, a continuous release dosage form of RHCl is preferred. The kinetics of ranitidine exhibits double peak phenomena within plasma concentration profile. Ranitidine in hydrochloride is a furan derivative containing nitrogen sulphur oxygen which can be easily ionized at different physiological pH. It has also two isomers which can react differently by stereo-specific and sterio-selective fashion.

Ranitidine partially decreases the secretion of HCl stimulated by ingestion of diets and xenobiotics by H2 receptor of parietal cells. It is commonly used in the treatment of GERD and Zollinger-Ellision syndrome

which is caused by gastrin producing tumor. It helps the development of conditions of esophageal tissues which can be damaged by reflux of acid in the esophageal region. The improvement was proved by endoscopic evaluation.

Mechanism: The release of acidity is mainly controlled by gastrin, acetylcholine and histamine. The external signals generated by these three types of receptors. The selective H2 agonist partially stimulates the formation of H+ ions in the lumen of parietal cells. The antagonist bindings to receptors reduces the stimulation by histamine which causes the reduction of the formation of H+ ion which acts as cation in the parietal cell stored in the cannaliculis. The chloride ion then secreted in the stomach through separate channel which cannot be stopped by this mechanism. The inhibition of acid secretion from 55 to 60 minutes after single dose administration and the peak plasma level reaches from 4 hours and the activities lasts up to 10 hours providing partial relief of symptoms related with acidity.

Figure 1
Structure of ranitidine

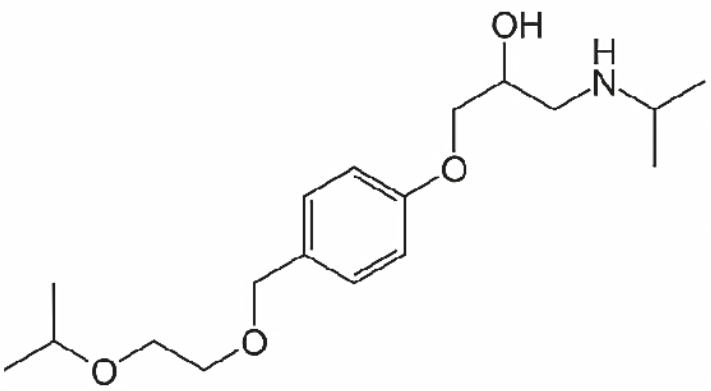

Figure 2
Structure of bisoprolol

Bisoprolol is a second-generation selective beta-1 blocker used to treat cardiovascular disorders like hypertension, chest pain for less blood flow to the heart as well as for heart failure. It is popular now a days for less dose (5 mg) and well tolerated. Both drugs are cheap and are used concurrently for coexisting diseases: acidity and hypertension. At present, the use of ranitidine has been limited because of presence of hazardous impurities in the raw materials. It is also important to find out other problems like incompatibilities and interactions with other substance.

Nowadays, bisoprolol is the most popular beta blocker among the third generation due to its lower dose and tolerability, which improves patient compliance when combined with diuretics. It reduces heart rate and contractility efficiently and causes lower in blood pressure. Different data of clinical studies revealed that bisoprolol sufficiently reduce mortality for cardiac complications and other mortality related to heart failure and cardiac contraction and ejection fraction. The exact mechanism is not fully explainable until now but the common mechanism is assumed to be through the competitive antagonism with adrenaline towards beta-1 receptor on heart which reduces the cardiac output. It is cardiovascular beta-1 adrenergic antagonist established by previous researches and exerts sympatholytic action on efferent division of autonomic nervous system ultimately provides sympatholytic action. It also decreases the output and signaling of the renin-angiotensin system in the kidney, which controls normal blood pressure. Recent studies have also included bisoprolol's additional effects on the central nervous system. It is well dissolved in GI fluids in salt form and absorbed from GIT mainly in passive diffusion and other transport process. The AUC is around 645 g.hr/mL which shows the capabilities of bisoprolol for longer action. The bioavailability of the drug is 91% due to the lower first-pass effect. The rate of absorption is unaffected by food and xenobiotics and state of GI motility. The average peak plasma concentration is attained within 4 hours maximum. The apparent volume distribution for bisoprolol was 3.5 L/kg. The volume of distribution was found similar both in healthy patients and the patients with heart failure. Around 30% Bisoprolol binds with plasma proteins after administration. Close to half of the dose is metabolized by CYP3A4 in the liver into inactive forms. The remaining half of the drug is excreted unchanged into urine. The average plasma half-life of bisoprolol is around 10-12 hours which might be increased to above 18 hours for patient with kidney disease. The body clearance of bisoprolol was calculated to be 14.5 L/h. The patient with hepatic and renal impairment experiences a reduction to 7.8 L/h. The other hepatic condition can also reduce the clearance of bisoprolol. The lethal dose

(LD50) for mouse and rat was 730mg/kg. Signs and symptoms of overdose of beta blockers include cardiac arrhythmia, hypotension, heart failure, ischemia etc. Other symptoms such as bronchoconstriction and hypoglycemia can also occur. The supportive treatment must be initiated for complications associated with over dose. The glucagon therapy has been shown advantageous in case of bradycardia and hypotension with over dose of bisoprolol. The administration of IV glucose is mandatory for hypoglycemia and administration of atropine causes improved patient conditions for bradycardia. Other cardio-supportive therapy along with fluids and TPM therapy are mandatory for heart failure. Isoproterenol was found to be useful for the management of b ronchial insufficiency. Oral administration of IV with or without theophylline or aminophylline is advantageous. Bisoprolol fumarate is not removed adequately by hemodialysis which need some additional investigation.

The biological half-life (2.5-3 hours) of the drugs also offers itself to the design of a sustained release formulation (Tehseen, Rao, & Hadi, 2013). It has also been shown that treating gastric diseases with an H2-receptor antagonist such as ranitidine or famotidine in combination with antacids improves local transport of these medications to the parietal cell wall receptor (Gaginella & Bauman, 1983). Drugs having a small absorption window, those that are unstable in the intestinal pH, or those that have low solubility at high pH are prime candidates for gastroretentive drug delivery systems (GRDDS). The delivery system's commercial potential for a wide range of medicinal medicines has been extensively researched. GRDDS provides clinical therapies for both acute and chronic conditions (Malik, Garg, Goyal, & Rath, 2015). Hypertension is a chronic disorder that necessitates long-term care, and managing it by using the patient-friendly dosage forms would be therapeutically beneficial. Antihypertensives of several kinds have proven to be promising candidates for GRDDS formulation (Chawra, Tanwar, Gilhotra, & Singh, 2018). Elevated blood pressure (BP), also known as hypertension, is one of the leading risk factors, accounting for 10.8 million deaths worldwide in 2019 and affecting over 1.3 billion people (data from 2010) (Thoenes, Neuberger, Volpe, Khan, Kirch, & Böhm, 2010). Furthermore, less than one in every five antihypertensive patients achieves effective blood pressure control. Chronic antihypertensive therapy frequently consists of a combination of two or more treatments with complimentary mechanisms of action that target various BP control systems (Carey et al., 2018; Waeber, 2001). Combining angiotensin converting enzyme inhibitor (ACEI) with a calcium channel blocker (CCB) or thiazide or thiazide-like diuretics, or combining angiotensin receptor blocker (ARB) with

either CCB or thiazide or thiazide-like diuretics, has recent times been suggested for antihypertensive effect (Morales et al., 2021).

The primary goal of this effort was to elucidate drug candidate's interactions (DDIs) especially the chemical complexation as a factor to pharmacovigilance because one of these medications has limited therapeutic index (TI) and any possible interaction could result in harmful or sub-therapeutic effects. The primary objectives of this work was to identify the DDIs in addition to determining the stability of the combinations that could develop following interaction between ranitidine and Bisoprolol fumarate at different pH levels. Job's continuous-variation assessment, Ardon's spectrophotometric measuring and thin layer chromatography methods were used to obtain the Rf values and identify the spots whether there is a formation of new complex other than parents or not.

2. Materials and methods

2.1 Literature review

A survey of the literature revealed no particular studies of the interaction between these two medicines. However, several pharmacokinetics and other interaction studies have been conducted between Bisoprolol Fumarate and Amlodipine Besylate (Kasagić-Vujanović, Jančić-Stojanović, Rakić, & Ivanović, 2015), Vildagliptin (Chowdhury, Kabir, Hossain, Chowdhury, & Chakrabarty, 2017), Sildenafil citrate (Rokonujjaman, Salam, & Sultan, 2015), and metformin (Rokonujjaman, Salam, & Sultan, 2015). In vivo drug interaction between bisoprolol with cimetidine and rifampicin has been reported. Interaction studies have also been conducted between Ranitidine and diclofenac (Kenawi, Barsoum, & Youssef, 2005), cetirizine (Kenawi, Barsoum, & Youssef, 2005), triamterene (Muirhead, Bochner, Somogyi, & Therapeutics, 1988), and theophylline (Skinner, Lenert, & Blaschke, 1989). A number of interactions through pharmacokinetic and pharmacodynamics process has been documented for β-adrenoceptor blocking agents. Metoprolol and carvedilol undergoes drug metabolism and are at greater risk for drug interactions than bisoprolol (Brodde & Kroemer, 2003). The plasma level of Dabigatran was found to be significantly higher when co-administered with bisoprolol (Nehaj, Sokol, Ivankova, Mokan, & Mokan, 2020). Bisoprolol was also found to interact and thus lower the concentration of p-glycoprotein inhibitor significantly (Bachmakov, Werner, Endress, Auge, & Fromm, 2006).

2.2 Drugs and chemicals

Ranitidine (Albion Laboratories Ltd., Chittagong, Bangladesh), Bisoprolol fumarate (Virgo Pharmaceuticals Ltd., Dhaka, Bangladesh), and other analytical grade chemicals were procured from local traders via Taj Scientific Ltd, Chittagong, Bangladesh.

2.3 Equipment

The test was conducted using a UV-visible spectrophotometer (Peak, C-7200, USA), pH meter (Hanna-HI981.7, USA), analytical balance (Model No. LF 224 DR, Shinko Denshi Co Ltd, Japan), and a controlled water bath (Bionics scientific technologies (P) LTD, BST/UNB-10, India). A Dubnoff Metabolic Shaking Incubator (Nickle Electrical Companies, USA) was used to shake the drug mixtures to attain equilibrium.

A UV spectrometer is an analytical tool that is often used to find out how much of a certain wavelength of UV and visible light is absorbed or passed through an analyte sample and then compared to a reference sample to find the concentration and wavelength of maximum absorption (lmax). This technique is often mentioned as UV-spectroscopy. The composition of the solute in the sample solution also influences the absorption property of materials. This technique provides valuable information about the analytes and widely used in laboratories. The light property of the source and wavelength of the radiation varies with different procedures and designs of analysis. Shorter wavelengths of light carry more energy, while longer wavelengths carry less. The energy in the light source is inversely proportional to the supplied wavelength. Specific amount of energy is to be needed to stimulate electrons to transfer from lower to higher energy state which can detect as absorption of energies. Electrons in different environments and regions around nucleus of a substance need to get different energy to transfer from lower to higher energy state that's why the absorption of radiation occurs for different wavelength for different materials. We can see the spectrum in the visible region, which is approximately 380 nm, and the color will range from violet to 780 nm, resulting in a red color. UV-spectroscopy uses shorter wavelength than the visible region which is approximately 100 nm so light can be explained by its wavelength characteristics. In UV-visible spectroscopy, it is useful to identify different components and analytes by locating the distinct wavelength corresponding to lmax.

2.4 Preparation of standard solutions

100 ml of 1×10-4 M stock solution of Ranitidine hydrochloride (MW

314.404) was prepared by dissolving 0.031 gm of Ranitidine hydrochloride in 100ml of buffer solution and 100 ml of 1×10-4 M stock solution of Bisoprolol fumerate (MW 325.443) was prepared by dissolving 0.044gm of Bisoprolol fumerate in 100ml of buffer solution were dissolved in distilled water to prepare the stock solutions.

2.5 In vitro studies

2.5.1 Absorbance spectra analysis

The absorption spectroscopy is widely used to determine the formulas and nature of complex ions in medium and to elucidate stability constants. It is important that to conduct such an analysis, either or both of the drug candidates should absorb light that the reactant of product is able to participate in a standard equilibrium without producing an absorbing species.

The three most common methods employed for studies of complex ions are (1) the method of continuous variations [also termed as Job's method], (2) the mole-ratio methods, and (3) the slope-ratio method.

In the absorption spectrum study, spectrums of Bisoprolol fumerate and Ranitidine hydrochloride and their various mixtures of solutions were documented at acidic and basic pH (1.0, 3.0 and 6.8). These two medications were combined in 1:1, 1:2, and 2:1 ratios. The concentration was kept at 1×10-4 M for each solution. The usual solution's spectral range was 200-400 nm. The UV spectrophotometer was used to conduct the experiment.

2.5.2 Job's spectrophotometric method

It is a kind of method of continuous variations, known as Job's method, utilized to determine the stoichiometry of a chemical complex. This method uses series of dilutions of solutions where total moles of molecules in each solution is same. If (nM)i and (nL)i are, respectively, the moles of chemical species and ligand in solution i, then

ntotal = (nM)i + (nL)i

The relative amount of ligand and chemical species in each solution is expressed as the mole fraction of ligand, (XL)i, and the mole fraction of chemical species, (XM)i,

(XL)i = (nL)i/ntotal

(XM)i = 1 – (nL)i/ntotal = (nM)i/ntotal

The concentration of the chemical–ligand complex in any solution is determined by the limiting reagent, with the greatest concentration occurring when the metal and the ligand are mixed stoichiometrically. If we monitor the complexation reaction at a wavelength where the chemical–ligand

complex absorbs only, a graph of absorbance versus the mole fraction of ligand will have two linear branches—one when the ligand is the limiting reagent and a second when the chemical is the limiting reagent. The intersection of these two branches represents a stoichiometric mixing of the chemical and the ligand. We can use the mole fraction of ligand at the intersection to determine the value of y for the chemical–ligand complex MLy.

y = (nL/nM) = (XL/XM) = (XL/1 –XM)

In the job's continuous variation approach (Job, 1928), the absorbance of Bisoprolol fumerate (1×10-4M) and Ranitidine hydrochloride (1×10-4M) was measured using an ultraviolet spectrophotometer at varied pH levels (1.2, 2.0, 3.0, 5.5, and 6.4). The mole fraction was held constant in this case. The absorbance of the mixtures was measured at various molar ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The absorbance of solutions were measured using a UV-VIS spectrophotometer at 532 nm. The absorbance of the combination at various mole fractions was subtracted from the sum of the individual drug readings. After then, the data were plotted versus the mole fraction. The presence of complexation between two medications was indicated by the breakage of the curve in the plot.

2.5.3 Ardon's spectrometric method

The concentration of Bisoprolol Fumerate was changed while the concentration of Ranitidine hydrochloride remained constant in this procedure (Sayeed, & Rana, 2013; Sayeed, Sahaban, Khalequeuzzaman, & Islam, 2013). All of the stages were carried out at various pH levels, including 1.2, 2.0, 3.0, 5.5, and 6.4. At 313.5 nm, the absorbance was measured. The Ardon's equation was utilized, which is shown below-

$$1/ [D-\epsilon_{A}C] =1/KC (\epsilon_{com}-\epsilon A) [B] +1/C (\epsilon_{com}-\epsilon A)$$

Where,

D=Absorbance of the mixture

B=Molar concentration of the drug Bisoprolol fumerate

C=Molar concentration of the drug Ranitidine hydrochloride

ϵ_{com}=Molar extinction co-efficient of the complex

ϵ_{A}=Molar extinction co-efficient of the drug Bisoprolol fumerate

The value is chosen as 1, which is essential condition for the validation of the method. The stability of the complex is calculated from Ardon's plot which was given by the relation, K=intercept/slope.

It should be noted that this strategy is only applicable to systems with 1:1 complexes.

2.5.4 Thin Layer Chromatography method

The first approach proposed for complexation study is thin layer chromatography (TLC). Thin Layer Chromatography technology is useful to separate a complex that has been formed as a result of interaction between two species. TLC typically employs a plate covered with silica gel, which serves as the stationary phase. The plate has been "spotted" with sample solution then placed in a jar with the appropriate solvent. Because of capillary action the solvent rises along the plate and elutes the species at different rates depending on the individual polarity. Multiple solvents are employed in order to get higher resolution of separation. The separation process is determined by the components' respective affinity for the stationary and mobile phases. Compounds travel along the surface of the stationary phase when the TLC plate is run in solvent. Compounds with higher affinity for the stationary phase move more slowly through the plate. Furthermore, molecules with a higher affinity for the mobile phase move faster than others. Distinct spots were visualized and identified with the help of a handheld UV light (long and short wave) as well as with the use of iodine chamber. The Rf values thus calculated were used to confirm the identity of the chemical species.

3. Results

3.1 Spectral study

The spectrum characteristics of Bisoprolol Fumarate and Ranitidine hydrochloride, as well as their mixes at various ratios, differed. When Bisoprolol fumarate molecules were coupled with Ranitidine hydrochloride, the absorption characteristics of these molecules (Bisoprolol fumarate) changed, incorporating a few variations in the absorption maxima. Because two chemicals form complexes, a new compound with characteristics distinct from the constituent ones is generated. As a result, a shift in the spectrum indicates the creation of complexes and alteration in the above example could be interpreted as a hint for the plausible interaction between these drugs. The UV spectra of Bisoprolol fumarate and Ranitidine hydrochloride, as well as their complexes in various rations and at various pH levels, are presented in Figure 3 & 4

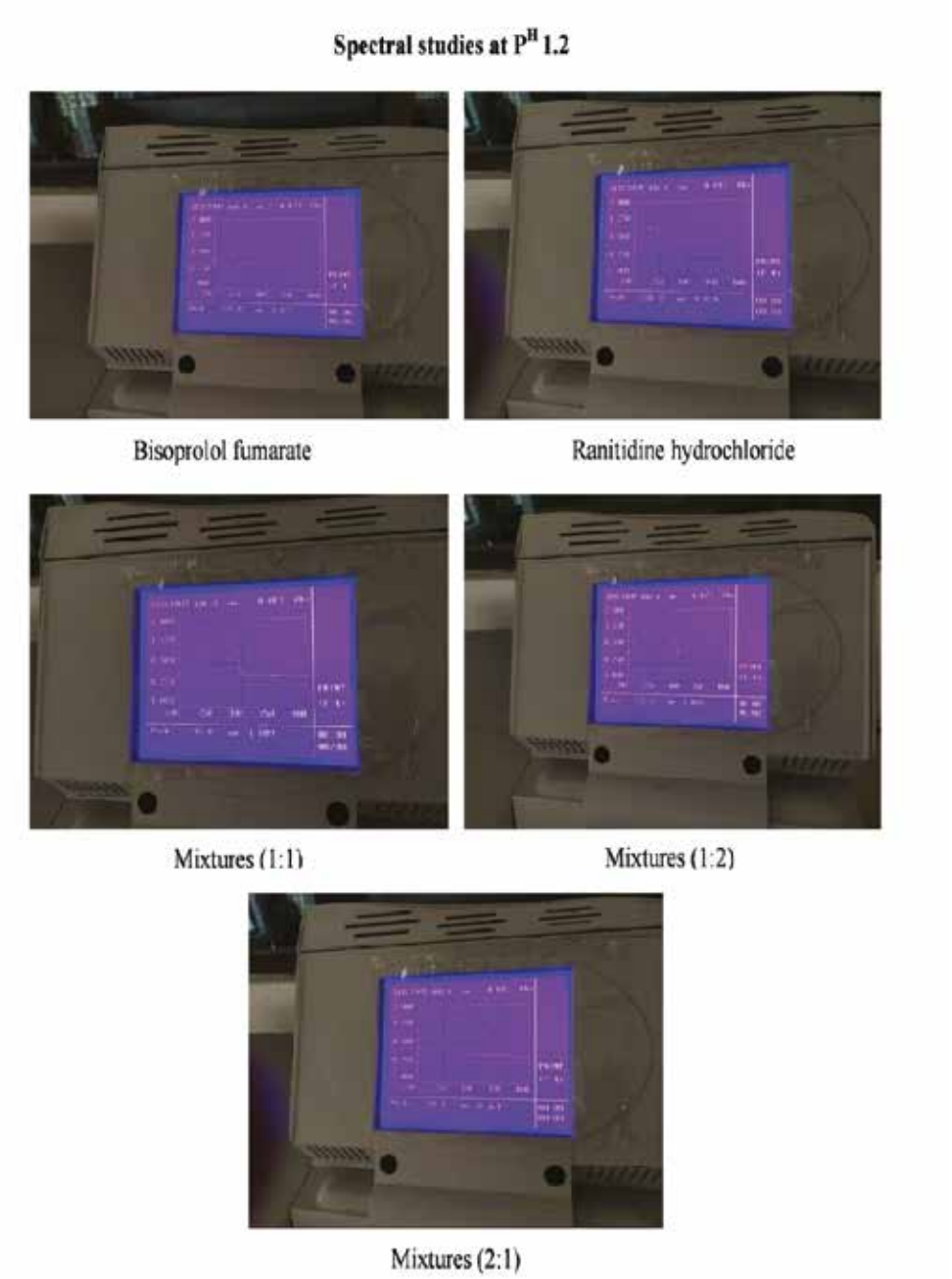

Figure 3
Spectral studies of Bisoprolol fumarate, Ranitidine hydrochloride and their (1:1), (1:2) and (2:1) mixture for P^{H} 1.2

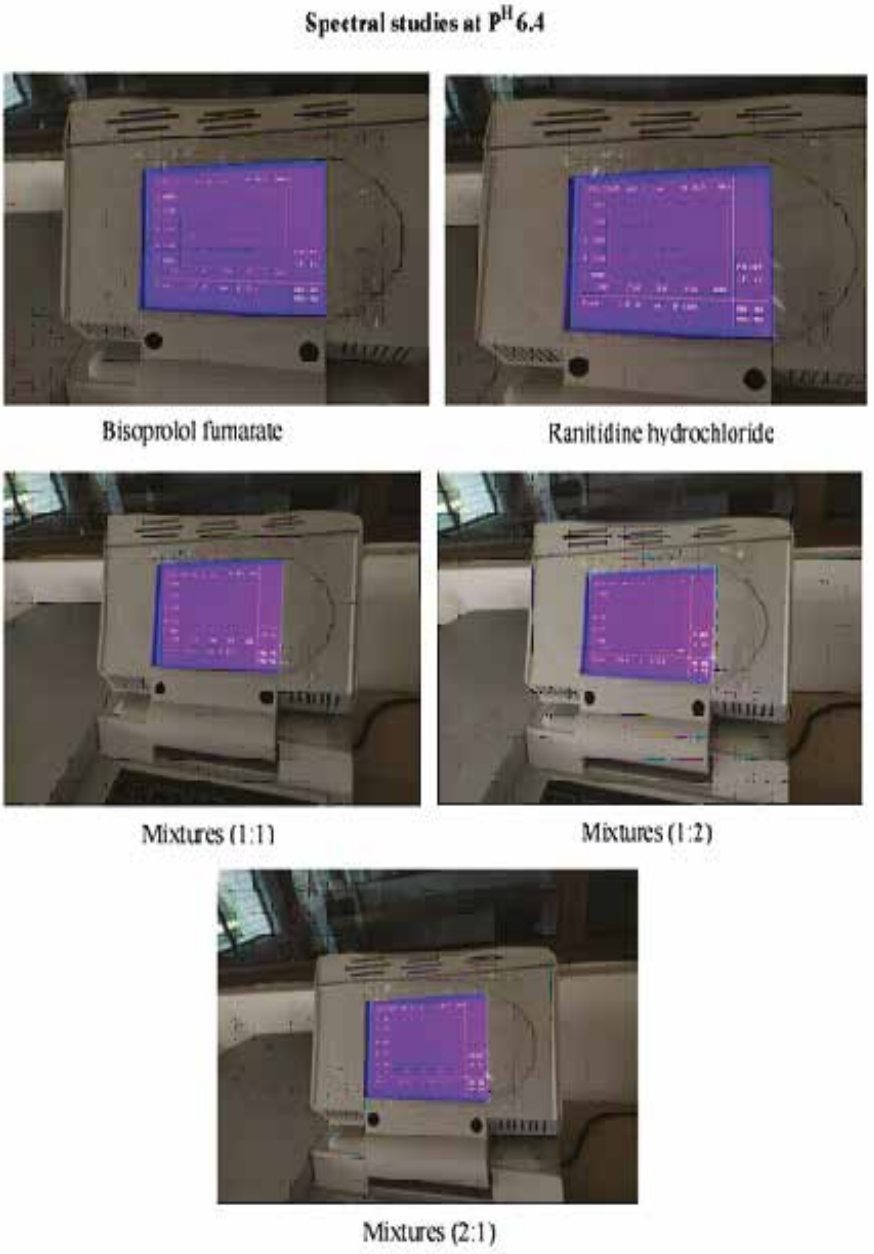

Figure 4

Spectral studies of Bisoprolol fumarate, Ranitidine hydrochloride and their (1:1), (1:2) and (2:1) mixture for PH 6.4

3.2 Job's method analysis

Job's method was used to evaluate the molar percentages of Bisoprolol fumarate complexes with Ranitidine hydrochloride for consistency. Bisoprolol absorbance characteristics were determined at pH (1.2, 2.0, 3.0, 5.5, and 6.4) at various concentrations (0. 1×10-4M to 0.9×10-4 M). The Job's plots at pH 1.2, 2.0, 3.0, 5.5, and 6.4 shown in Figure 5 obtained by plotting absorbance contrasts against the mole portion of the drug. The drug solutions were combined in such a way that the total moles of reactant in each mixture and total volume remained constant, but the reactant mole ratio varied.

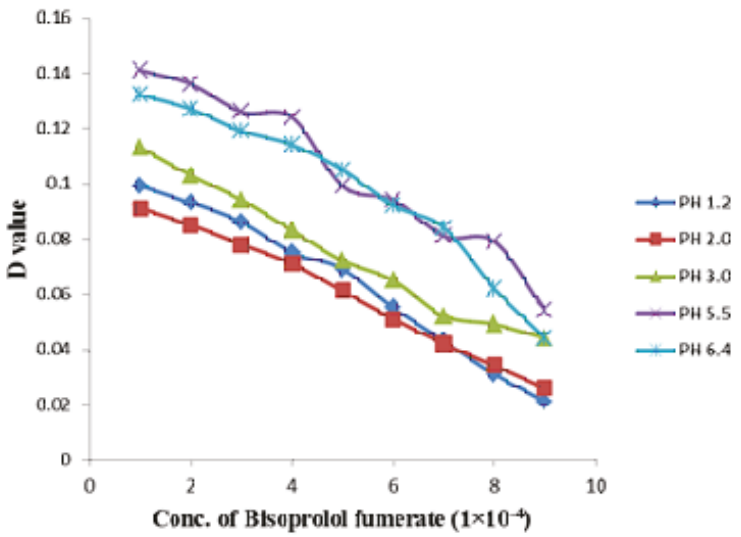

Figure 5

Job's plot for complexation of Bisoprolol fumarate with Ranitidine hydrochloride

Table 1

Values of Job plot for complexation of Bisoprolol fumarate and Ranitidine hydrochloride at pH 1.2, 2.0, 3.0, 5.5 & 6.4

Sl. No.	Absorbance of Mixture				
	pH 1.2	pH 2.0	pH 3.0	pH 5.5	pH 6.4
1	0.099	0.091	0.113	0.141	0.132
2	0.093	0.085	0.103	0.136	0.127
3	0.086	0.078	0.094	0.126	0.119
4	0.075	0.071	0.083	0.124	0.114
5	0.069	0.061	0.072	0.099	0.105
6	0.055	0.051	0.065	0.094	0.092
7	0.043	0.042	0.052	0.081	0.084
8	0.031	0.034	0.049	0.079	0.062
9	0.021	0.026	0.044	0.054	0.044

3.3 Effect of Ranitidine hydrochloride on Bisoprolol fumarate by using Ardon's method

Ardon's plot revealed the establishment of a Ranitidine hydrochloride and Bisoprolol fumarate complex at pH 1.2, 2.0 3.0, 5.5 and 6.4, because the procedure is only valid at these levels for 1:1 complexes. Ardon's data produced straight lines with intercepts that are the diagram in figure 3 depicts the creation of 1:1 complexes for the system at both pH levels.

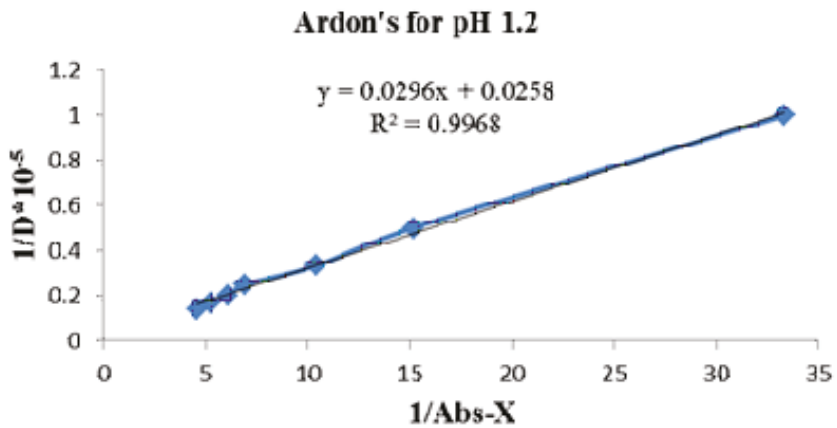

Figure 6

Ardon's plot for complexation of Ranitidine hydrochloride with Bisoprolol fumarate at pH 1.2

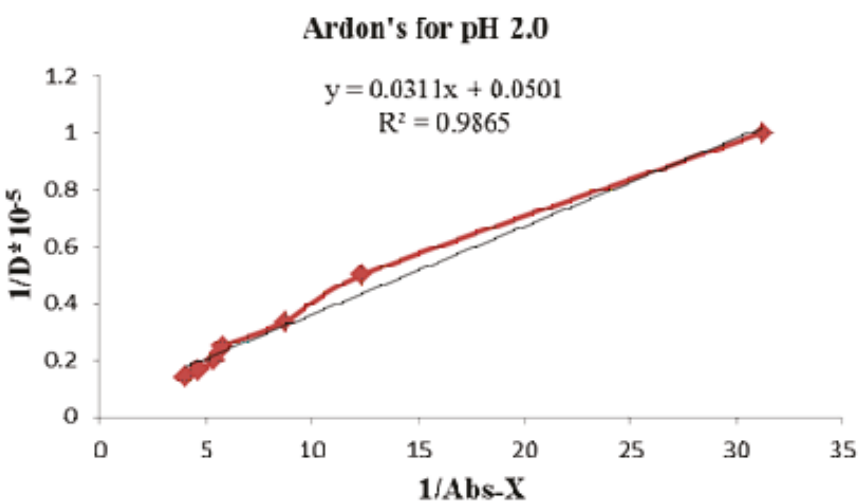

Figure 7

Ardon's plot for complexation of Ranitidine hydrochloride with Bisoprolol fumarate at pH 2.0

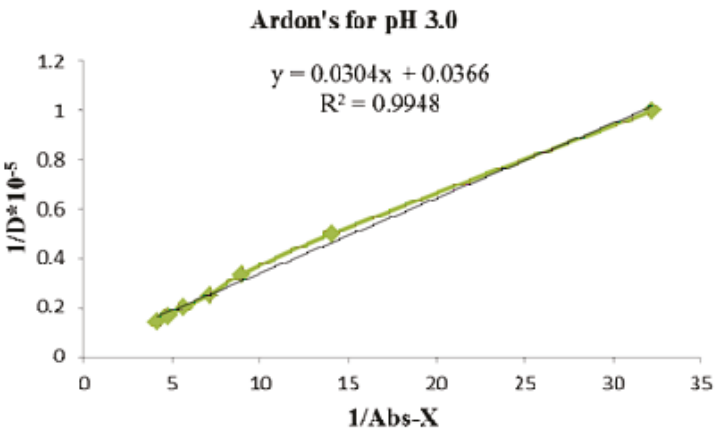

Figure 8
Ardon's plot for complexation of Ranitidine hydrochloride with Bisoprolol fumarate at pH 3.0

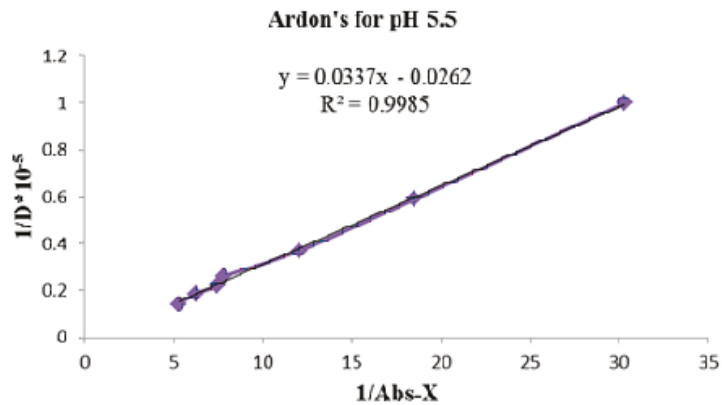

Figure 9
Ardon's plot for complexation of Ranitidine hydrochloride with Bisoprolol fumarate at pH 5.5

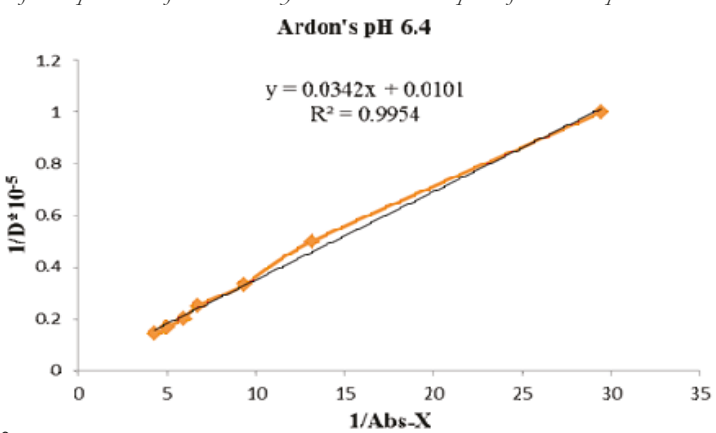

Figure 10
Ardon's plot for complexation of Ranitidine hydrochloride with Bisoprolol fumarate at pH 6.4

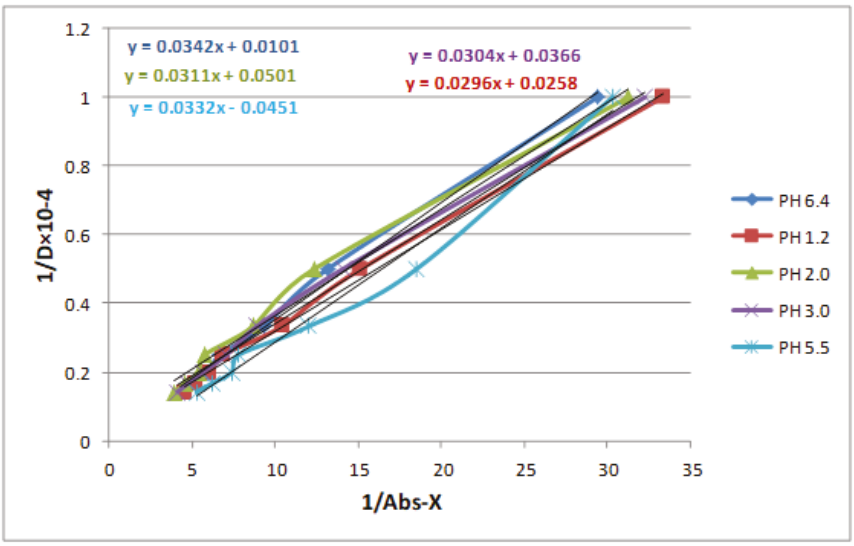

Figure 11

Ardon's plot for complexation of Ranitidine hydrochloride with Bisoprolol fumarate at pH 1.2, 2.0, 3.0, 5.5 & 6.4

3.4 Estimation of stability constant

The Ardon's plot stability constant suggests that Bisoprolol fumarate and Ranitidine hydrochloride have a significant interaction at pH 2.0, 3.0, and 5.5 because their stability constant is more than 1. And it has a little interaction at pH 1.2, but a very slow interaction at pH 6.4 shown in table 2. The following formulae are used to calculate the value of the stability constant-

Stability constant= c/m

Where, c =intercept of the line

m = slope

Table 2

Stability constant for the complex of Bisoprolol Fumarate and Ranitidine hydrochloride

system	Stability Constant	Stability Constant	Stability Constant	Stability Constant	Stability Constant
Bisoprolol Fumarate+ Ranitidine	At pH 1.2	At pH 2.0	At pH 3.0	At pH 5.5	At pH 6.4
	0.896	1.612	1.200	1.363	0.294

3.5 Determining the interaction between Bisoprolol fumarate and Ranitidine hydrochloride by Thin Layer Chromatography method

The area in the TLC plate is used to evaluate the interaction between Bisoprolol Fumarate and Ranitidine. The result of the TLC plate investigation is presented below-

Figure 12
Running of the solvent throughout TLC plate

Figure 13
Spotting of the TLC in iodine chamber

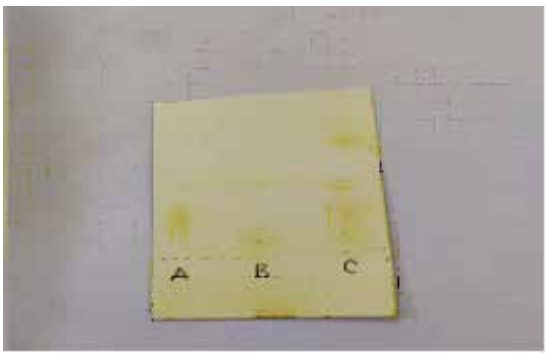

Figure 14
Visualization of the complex on the TLC plate

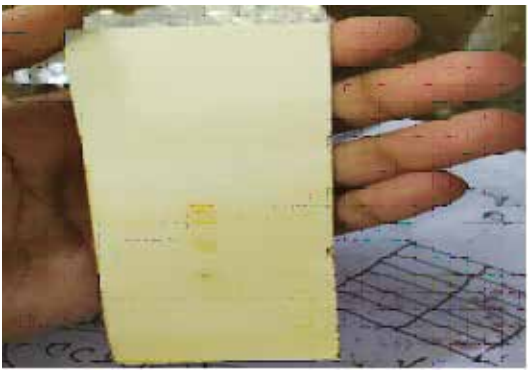

Figure 15
Visualization of drug A, drug B and their complex C on TLC plate

4. Discussion

The study reveals the formation of complexes on TLC plates and from the distinct breakdown of Job's plot at different pH and stability of complexes was evaluated by Ardon's plots which confirmed the chemical interaction between these two molecules.

Drug-drug interactions (DDIs) are one of the most prominent causes of ADRs, and this study found that these complexations may be more frequent in elderly patients as a result of poly-therapy (Gallelli et al., 2010; Gallelli et al., 2002). The frequent use of OTC drugs like ranitidine, in reality, increases the complexity of therapeutic management and, as a result, the possibility of clinically meaningful drug interactions, which can cause the formation of ADRs and either lower or improve clinical effectiveness (Franceschi et al., 2004). For health care providers, the Summary of Product Characteristics (SPCs) is the major source of information about DDIs. Unfortunately, DDI cannot be exhaustively listed. As a result of the limited space in the SPC, information about potential DDIs may be inadequately described. When Ranitidine hydrochloride and Bisoprolol fumarate were separately combined, they showed a few modifications in retention characteristics, including a few changes in the absorption maxima of buffer mixes and their 1:1, 1:2 and 2:1; mixing in buffer solution of pH 1.2, 2.0, 3.0, 5.5 and 6.4 at a different focus (1 X 10-4) M. The change in spectral pattern could be seen as a sign of a link between these drugs. Both compounds has an identical and distinct molecular entity and orientation, and these characteristics are important for each compound's absorption of light in the ultra violet or visible region. All chemicals that generate an ultraviolet-visible spectrum which is characteristically different from another and their complexes in this region.

The reaction of two chemicals to form complexes and generate compounds with properties different from the parent ones is illustrated in figures 1 & 2. As a result, a unique spectrum for the complexed molecules will be observed. As a result, a shift in the spectrum indicates the creation of complexes (Fujita, Yazaki, & Ogura, 1990; LePecq & Paoletti, 1967). In the Job's method study, Bisoprolol fumarate concentrations ranging from 1×10-4 to 9×10-4 combined with Ranitidine hydrochloride at pH 1.2, 2.0, 3.0, 5.5 and 6.4. The occurrence of pharmacological interaction between these two drugs may be indicated by the breakdown of the curve at 4×10-4 and 6×10-4. The Ardon's plots were used to calculate the values of stability constants and it was clearly found that when (1/D×10-4) estimations are plotted against (1/Abs-X), these lines are obtained from the calculations of the Ardon's formula. The Ardon's plot stability constant suggests that Bisoprolol fumarate and Ranitidine hydrochloride have a significant chemical interaction at pH 2.0, 3.0, and 5.5 because their stability constant is more than 1. And it has a minor interaction at pH 1.2, but a very negligible interaction at pH 6.4 shown in table 2. TLC would be an efficient approach for identifying indicator chemicals in crude herbal medications for identification and quality assurance (Kunle, Egharevba, & Ahmadu, 2012). Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) are frequently employed as helpful methods for determining small levels of contaminants. The British Herbal Pharmacopoeia, 1996, placed a strong focus on the use of TLC profiles to define herbal materials, depending on the use of several squirt chemicals and TLC patterns to discover distinctive and active components of herbal products (Yadav & Dixit, 2008). The formation of two or more spots on the TLC plates appears to be a sign of possible drug interaction. It suggests that yet another chemical was formed as a result of the complexation between Bisoprolol fumarate and Ranitidine hydrochloride. The above findings provide evidence for the formation of stable complexes from these two species, indicating that concurrent use of two drugs is limited.

5. Conclusion

Based on the study data shown above, it can be confirmed that the chemical interaction of Bisoprolol fumarate and Ranitidine hydrochloride results a net decrease in the level of free drug concentration, which ultimately results in a decrease in pharmacodynamics towards site of action, and it is evident that one or both drugs will have lower pharmacological activity as sub therapeutic effect. The stability of the complexes formed from the interaction will be a matter of concern if there any additional activity of its own. Therefore,

pharmacological detail will be necessary for the complex if any additional activity to the host which may be harmful or unusual. Careful consideration must take place for the use of ranitidine and other H2 blockers from same generation like famotidine, cimetidine may have similar result with the use along with bisoprolol. When administering these combination drugs as well as other combination therapy this type of study would be necessary to increase the safety profile and eliminate hazardous reaction for new molecules with OTCs.

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