PhD Thesis

In vitro-In vivo Studies on Drug Interaction Between Ketotifen and Commonly Prescribed Drugs

by

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THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACY

In vitro-In vivo Studies on Drug Interaction Between Ketotifen and Commonly Prescribed Drugs

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DECLARATION

I, Mohammed Aktar Sayeed, hereby declare that the thesis work entitled "*In vitro – In vivo* Studies on Drug Interaction Between Ketotifen and Commonly Prescribed Drugs" submitted to the Jahangirnagar University in partial fulfillment of the requirement for the degree of Doctor of Philosophy (Ph.D) in Pharmacy described in this thesis has been carried out by me under the supervision of Professor Dr. Md. Sohel Rana, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.

I further declare that no part of the thesis results of this work was submitted to any Universities or Institutes for any other degree and the work is original.

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APPROVAL CERTIFICATE

This is to certify that Mohammed Aktar Sayeed, Ph.D research student bearing Examination Roll Number 148, Registration Number 1317, Session 2008-2009, Department of Pharmacy, Faculty of Biological Science**s,** Jahangirnagar University, Savar, Dhaka worked under my supervision and completed the thesis work entitled "*In vitro- In vivo* Studies on Drug Interaction Between Ketotifen and Commonly Prescribed Drugs" submitted to the Jahangirnagar University in partial fulfillment of the requirement for the degree of Doctor of Philosophy (Ph.D) in Pharmacy.

The candidate has fulfilled all the terms and conditions of Ph.D research including dissemination of the result of his research study into two seminars (on 28th September 2013 and 26th April, 2014) held in the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.

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Dedicated with respect

To

My parents, my teachers

&

The subjects of my research and those patients who are suffering from drug-drug interactions.

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ABSTRACT

The objectives of the present study were to investigate the drug-drug interactions between ketotifen fumarate and some commonly prescribed drugs. Their interactions were identified and confirmed by UV, IR, DSC and HPLC followed by TLC. The *in vitro* results were correlated with *in vivo* model to see whether the desired drug concentration could attain into the blood stream or not. Finally attempts have been taken to find out the effects of these complexes on the liver and kidney. Each of the drugs absorption was analyzed in the UV-VIS region. The spectra of pure drugs as well as their 1:1, 1:2 and 2:1 mixtures of ketotifen & paracetamol, ketotifen & domperidone, ketotifen & desloratidine, ketotifen & amoxicillin, ketotifen & metformin, ketotifen & chlorpheniramine, ketotifen & theophylline, ketotifen & salbutamol and ketotifen & diclophenac sodium were studied at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4. At both gastric and intestinal pHs, a sharp breakdown was observed in the curves. Similarly when ketotifen was mixed with metformin, a sharp change was observed in the curve at pHs 0.4, 1.2, 2.8 & 7.4 which indicated drug-drug interactions, whereas the absence of such particular breakdown in the curve of ketotifen and metformin mixture at pHs 2.0, 6.0 and 6.8 revealed the absence of drug interactions. Again when various concentrations comprising $1x10^{-5}$M to $9x10^{-5}$M of ketotifen were interacted with chlorpheniramine, sharp changes in the curve were observed at pHs 0.4, 6.0 and 6.8, which demonstrated the presence of drug-drug interactions. On the other hand, the absence of breakdown in the curve of ketotifen and chlorpheniramine mixture at pHs 1.2, 2.0, 2.8 and 7.4 revealed the absence of drug interactions. The stability constant values ($k = 1x10^{-2}$) for the particular interaction was determined by graphical representation of Ardon's plot. The stability constants of ketotifen & paracetamol (5.67, 6.36, 7.21, 14.84, 17.97, 38.35, 24.79 at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8, 7.4 respectively), ketotifen & domperidone (77.89, 84.27,

10.01, 28.95, 60.23, 21.85, 114.82 at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8, 7.4 respectively), ketotifen & desloratidine (14.54, 14.07, 16.73, 5.56, 17.49, 5.16, 3.06 at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8, 7.4 respectively), ketotifen & theophylline (6.8, 7.4, 3.5, 4.8, 9.6, 5.7, 18.4 at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8, 7.4 respectively) and ketotifen & amoxicillin (19.8, 13.7, 2.30, 35.7, 14.1, 8.3, 51.6 at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8, 7.4 respectively) were found to be relatively higher at gastric and intestinal pHs. This reflects that there might be relatively stronger complex formation due to interaction between the mentioned drugs. But relatively low stability constant values were seen when the interaction occured between ketotifen & chlorpheniramine (0.7256, 0.2895, 1.0683, 1.6807, 1.6827, 0.0507, 0.2834 at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8, 7.4 respectively), ketotifen & diclofenac (0.068, 0.057 at pHs 6.8, 7.4 respectively) and ketotifen & metformin (0.03, 0.55, 0.05, 0.01, 0.09, 0.01, 0.77) at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8, 7.4 respectively). If the formation constant is reasonably favorable, two straight lines of different slopes that intersect at a mole ratio corresponding to the mixing ratio in the complex are obtained. In the IR study, the possible interaction between ketotifen fumarate and amoxicillin trihydrate showed characteristic peaks. The peaks of C-Cl (696.33 cm^{-1}) was shifted to higher wave number at 703.08 cm^{-1} and C-O-C group at 1082.11 cm^{-1} was shifted to higher wave number in the complex at 1099 cm^{-1} (C-O-C stretching); The peaks of alkenes at 3040 cm^{-1} was shifted to 3056 cm^{-1} (=CH). Simultaneously the peaks of isocyanates (-N=C=O) at 2270 cm^{-1} and carboxylic 3336.99 cm^{-1}(O-H) were shifted to lower wave numbers in the complex at 2263cm^{-1} and 3327 cm^{-1}. On the other hand the interaction between ketotifen fumarate and diclofenac sodium showed that the peaks of acid phosphines at 839.07 cm^{-1} (P-H stretching) was shifted to higher wave number in the complex at 854.5 cm^{-1}, the peaks of alkyl halides of 1386.88 cm^{-1}, amides of 1575.91 cm^{-1}, amides of 1652.1 cm^{-1}, aromatic group having wave number of 2970.5 cm^{-1} were showed characteristic peaks in

the complexes at 1354.09 cm^{-1}, 1587.48 cm^{-1}, 1647.28 cm^{-1} and 2957 cm^{-1} respectively. Similarly in case of ketotifen & salbutamol interactions showed characteristic peaks at the peaks of C-H (aromatic group), C-O-C (ether group), C-C (ketone) and O-H (carboxylic acid group) at 838.11 cm^{-1}, 1085 cm^{-1}, 1201.7 cm^{-1}, 2925.17 cm^{-1} were shifted to higher wave numbers in the complexes at 855.47 cm^{-1} (C-H stretching), 1099.47 cm^{-1} (C-O-C stretching), 1224.85 cm^{-1} (C-C stretching) and 2937.71 cm^{-1} (O-H). Similarly the interaction between ketotifen and theophylline showed that the peaks of phosphine oxides group at 1180 cm^{-1} (P=O stretching) and alkenes group at 1378 cm^{-1} (C-H) were shifted to lower wave numbers in the complex at 1151 cm^{-1} (P=O stretching) and 1362.77 cm^{-1} (C-H stretching). On the other hand, peak of amides of 1559.51 cm^{-1} (N-H stretching) was shifted to higher wave number in the complex at 1588.19 cm^{-1} (N-H). In the DSC study (ketotifen, domperidone and ketotifen & domperidone mixture) ketotifen exhibited sharp endothermic peak at 193.08^{0}C. But the ketotifen-domperidone complex exhibited a sharp new peak at 120.02^{0}C (-1.74 mW/mg). On the other hand, DSC of the samples (ketotifen, metformin and ketotifen & metformin mixture) were performed and observed that ketotifen-metformin complex exhibited a sharp new peak at 123.04^{0}C (-2.61 mW/mg). But the mixture of ketotifen and theophylline exhibited no sharp peak. The R_f values of ketotifen (0.44) and domperidone (0.53) was found to be completely different from ketotifen-domperidone mixture (0.38). The R_f values of ketotifen (0.49) and metformin (0.51) was found to be completely different from ketotifen-metformin mixture (0.39) which conclude the stability of the complex for both mixtures. However in case of HPLC the complex which was formed between ketotifen & theophylline and ketotifen & metformin could have been partly dissociated in aqueous medium used to dissolve the sample for HPLC analysis. The multiple comparison table shows that there is a significant difference in absorbances at various mentioned times (30 minutes, 60

minutes, 120 minutes & 180 minutes) to complete the drug interaction between the group that took the single drug as well as mixtures (ketotifen & metformin, ketotifen & paracetamol, ketotifen & salbutamol, ketotifen & amoxicillin, ketotifen & diclofenac) The results were expressed as mean ± SEM values. A probability value less than 0.05 ($p < 0.05$) was defined to be significant. The results of investigation of hepatotoxicity of combination drug therapy were compared with single drug sample ketotifen. But the groups which receive the combination drug samples ketotifen & metformin (67.5 $\pm$ 1.44 IU/L) and ketotifen & theophylline (68.5 $\pm$ 2.5 IU/L) showed a significant increase in SGPOT, and showed a significant decrease of ATPN levels in ketotifen & theophylline mixture (7.0 $\pm$ 0.07 IU/L) and in ketotifen & metformin mixture (6.13 $\pm$ 0.73 IU/L). The creatinine concentration was found to be 1.4 mg/dl in case of normal control but it was raised to 3.6 mg/dl when mixture of ketotifen and theophylline was administered. Now we can conclude that the patients having motion sickness and patients who had been suffering from diabetes should take a precaution during coadmintration of ketotifen fumarate & domperidone and ketotifen fumarate & metformin hydrochloride.

Chapter-1

Introduction

1.1 Drug-drug interaction

A drug interaction is a situation in which a substance affects the activity of a drug, i.e. the effects are increased or decreased, or they produce a new effect that neither produces on its own. Typically, interactions between drugs come to mind (drug-drug interaction). However, interactions may also exist between drugs & foods (drug-food interactions), as well as drugs & herbs (drug-herb interactions). Patients taking antidepressant drugs such as monoamine oxidase inhibitors should not take food containing tyramine. Hypertensive crisis may occur (an example of drug-food interactions). These may occur out of accidental misuse or due to lack of knowledge about the active ingredients involved in the relevant substances.

Drug interactions may also occur outside the body i.e.; *in vitro*. Some classic examples include that thiopentone and suxamethonium should not be placed in the same syringe and same is true for benzylpenicillin and heparin.

Generally speaking, drug interactions are to be avoided, due to the possibility of poor or unexpected outcomes. However, drug interactions have been deliberately used, such as co-administering probenecid with penicillin prior to mass production of penicillin. Because penicillin was difficult to manufacture, it was worthwhile to find a way to reduce the amount required. Probenecid retards the excretion of penicillin, so a dose of penicillin persists longer when taken with it, and it allowed patients to take less penicillin over a course of therapy.

A contemporary example of a drug interaction used as an advantage is the co-administration of carbidopa with levodopa (available as carbidopa/levodopa). Levodopa is used in the management of Parkinson's disease and must reach the brain in an un-metabolized state to be beneficial. When given by itself, levodopa is meabolized in the peripheral tissues outside the brain, which decreases the effectiveness of the drug and

increases the risk of adverse effects. However, since carbidopa inhibits the peripheral metabolism of levodopa, the co-administration of carbidopa with levodopa allows more levodopa to reach the brain un-metabolized and also reduces the risk of side effects.

Drug interactions may be the result of various processes. These processes may include alterations in the pharmacokinetics of the drug,[1] such as alterations in the Absorption, Distribution, Metabolism, and Excretion (ADME) of a drug. Alternatively, drug interactions may be the result of the pharmacodynamic properties of the drug, e.g. the co-administration of a receptor antagonist and an agonist for the same receptor.

1.2 Types of drug interactions

Drug interactions are often classified as either pharmacodynamic or pharmacokinetic interactions. Pharmacodynamic interactions include those that result in additive or antagonistic pharmacological effects. Pharmacokinetic interactions involve induction or inhibition of metabolizing enzymes in the liver or elsewhere, displacement of drug from plasma protein binding sites, alterations in gastrointestinal absorption, or competition for active renal secretion. The frequency and prevalence of interactions is dependent upon the number of concomitant medications and the complexity of the regimens. The prevalence is also dependent upon other variables, such as patient adherence, hydration and nutritional status, degree of renal or hepatic impairment, smoking and alcohol use, genetics and drug dosing. Additionally, some patients may exhibit evidence of a particular drug interaction, while others with the same drug combination do not.

- Pharmacodynamic drug interaction
- Pharmacokinetic drug interaction

Interactions Resulting from Alterations in Gastrointestinal Absorption

The rate and extent of drug absorption after oral administration may be grossly altered by other agents. Absorption of a drug is a function of the drug's ability to diffuse from the lumen of the gastrointestinal tract into the systemic circulation. Changes in intestinal pH may profoundly affect drug diffusion as well as dissolution of the dosage form. For example, the absorption of ketoconazole is reduced by the co-administration of antacids or H_2 blockers (e.g. ranitidine, famotidine) that reduce the extent to which the ketoconazole tablet is dissolved. Formation of insoluble complexes by a process known as chelation is another mechanism by which a drug interaction may lead to reduced oral absorption. For example, fluoroquinolones (e.g. ciprofloxacin) and divalent metal ions (such as calcium and iron) form an insoluble complex that results in reduced absorption of both the antibiotic and the metal ion. Interactions that decrease the rate of drug absorption may be of little importance, since the overall extent of absorption may remain unaltered.

Interactions Resulting from Alterations in Metabolizing Enzymes

The liver is the major, though not exclusive, site for drug metabolism. Other sites include the kidney and the lining of the gastrointestinal tract. The two main types of hepatic drug metabolism are phase I and phase II reactions. Phase I oxidative reactions are the initial step in drug biotransformation, and are mediated by the cytochrome P-450 (CYP) system. This complex super family of enzymes has been sub classified into numerous enzymatic subfamilies. Phase II reactions occur following Phase I reactions. In this process, drug metabolites are converted into more water-soluble compounds that can be more easily eliminated by the kidneys.

Enzyme Induction

It may result in increased CYP enzyme synthesis, faster drug metabolism, sub therapeutic drug concentrations and the risk for ineffective drug therapy. The rapidity of the enzyme induction is dependent upon the half-life of the inducing drug as well as the rate of synthesis of new enzymes. Examples of drugs that cause enzyme induction are the barbiturates, some anticonvulsants and rifampin.

Enzyme inhibition

It may result from noncompetitive or competitive inhibition of CYP enzymes by a second drug, an effect that may occur rapidly. Examples of hepatic enzyme inhibitors include cimetidine, fluconazole and erythromycin. The result of noncompetitive enzyme inhibition by addition of a second agent is slower metabolism of the first drug, higher plasma drug concentrations, and a risk for toxicity. In the case of competitive inhibition, the metabolism of both drugs can be reduced, resulting in higher than expected concentrations of each drug. A few drugs are metabolized by enzymes found in cells lining the gastrointestinal tract. One of these drugs is cyclosporine. Some foods and other preparations such as grapefruit juice contain certain substances that may inhibit those specific enzymes, resulting in elevated serum cyclosporine concentrations.

Interactions Resulting from Alterations in Protein Binding

Drugs may exist in plasma either reversibly bound to plasma proteins or in the free (unbound) state. The primary drug-binding plasma proteins are albumin and α1-acid glycoprotein. It is free drug that exerts the pharmacological effect. Drugs may compete with each other for plasma protein binding sites, and when this occurs, one drug may displace another that was previously bound to the protein. Displacement of a drug from its binding sites will therefore increase that agent's unbound concentrations, perhaps resulting in toxicity. Some drugs normally exist in a state of high protein binding, often

exceeding 90%. Thus, even a small decrease in protein binding could significantly increase the free concentrations. Drugs which are normally highly protein bound, and which might participate in binding interactions, include anticonvulsants & warfarin.

Interactions Resulting from Changes in Renal Excretion

The majority of renally eliminated drugs are excreted via passive glomerular filtration. Some drugs are eliminated via active tubular secretion, such as penicillins, cephalosporins, and most diuretics. The active secretion may be inhibited by secondary agents, such as cimetidine, non steroidal anti-inflammatory agents and probenecid, with resulting elevations in the serum drug concentrations and reduced urinary drug concentrations. In some cases, the interaction is desirable, while others may lead to adverse therapeutic outcomes.

1.3 Risk factors and management of drug interactions

In general, the more complex a patient's drug regimen, the higher the risk for interactions. CKD patients often take numerous medications. The average age of a dialysis patient is over 60 years and as a group, elderly patients are more prone to experience drug interactions because of reduced hepatic and renal function. Identification of the potential for interactions may enable the clinician to avoid its occurrence. Drugs that require careful dose titration to maintain efficacy and avoid toxicity must be monitored particularly carefully for drug interactions. Most drug interactions can be avoided or managed by substitution of one or more agents or more intense monitoring for the potential result. Other management strategies include separation of doses of interacting agents (e.g. ciprofloxacin and calcium) or prospective adjustment of doses.

1.4 Clinical significance of drug interaction

The effects of a moderate interaction may cause deterioration in the patient's clinical status, resulting in additional treatment, hospitalization, and/or an extended hospital stay.

The effects of a major interaction are potentially life-threatening or can lead to permanent damage. In addition to being clinically significant, the interaction must be reasonably documented in the literature (suspected, probable, or established).

Purposes of study:

1. The prime objective of this study was to elucidate the possible importance of drug-drug interactions (DDIs) as a contributing factor towards drug safety. The main focus of the study was to identify whether there is any interaction between ketotifen fumarate and commonly prescribed drugs (theophylline anhydrous, amoxicillin trihydrate, domperidone, metformin hydrochloride, chlorpheniramine maleate, diclofenac sodium, paracetamol and desloratidine).
2. The purpose of the present study was to investigate the *in–vitro* and *in-vivo* complexation and strength of complexes, which may be formed due to interaction of ketotifen fumarate and commonly prescribed drugs.
3. The specific purpose was to observe and determine the stability of the complexes, which could be formed between ketotifen fumarate and commonly prescribed drugs at pH 0.4, 2, 2.8, 6.8, and 7.4 at the physiological temperature.
4. To compare the *in-vitro* and *in-vivo* interaction study result with the spectrum obtained by Infrared spectral analysis of the chloroform and aqueous extracts of the mixtures of ketotifen fumarate with commonly prescribed drugs.
5. To see the potentiation and attenuation of activity of ketotifen fumarate under this condition. Such a study can possibly open up a new avenue to formulate a new dosage form of the drug chosen, as well as developing better combination system of therapy in the area of their need.
6. Polypharmacy (prescribing many drugs at a time) is a common practice in case of patients undergoing a major operation, hospitalized patients, and also in geriatric

patients. By Differential Scanning Calorimeter (DSC) and Thin Layer Chromatography (TLC) the complex stability of ketotifen fumarate & theophylline anhydrous, ketotifen fumarate & domperidone, ketotifen fumarate & metformin hydrochloride were confirmed along with some intermediary complexes at room temperature at various pHs.

1.5 Drugs selected for the study

1.5.1 Ketotifen fumarate

Ketotifen is a second-generation H_1-antihistamine and mast cell stabilizer. It is an antihistamine drug that is used for the treatment of general allergy symptoms, certain allergic conditions (including conjunctivitis), and the management of asthma. When used for asthma, the drug is not regarded as effective for treating an immediate attack (it is not rapid bronchodilator). Instead over time its use is associated with reduction in the frequency, duration, and severity of attacks. Likewise, ketotifen fumarate will usually supplement an existing asthma medication program, and not replace the prescribing of immediate rescue devices such as an asthma inhaler or nebulizer. Ketotifen fumarate alleviates allergy symptoms by blocking histamine H_1 receptors, a property that is common to drugs of the antihistamine class. Its second and very unique mode of action, however, makes it useful in the treatment of asthma. Ketotifen fumarate increases the concentration of beta-adrenergic receptors in the body (especially beta-2 receptors). Drugs that stimulate beta-2 receptors are commonly prescribed as bronchodilators, used to increase airflow to the lungs and counter the constriction caused by asthma. While potentially efficacious alone, one key therapeutic effect of ketotifen fumarate is to increase the sensitivity of the body to drugs of the beta agonist class. The beta-2 receptor up regulation properties of ketotifen fumarate makes this drug of interest to the body building and athletic communities. This is due to the strong role of the beta-2 receptor in supporting fat loss. Although not a strong fat loss compound by itself, when taken with a beta-2 agonist thermogenic like clenbuterol, ketotifen fumarate may increase thermogenic

potency and noticeably extend the window of active lipolysis. Clenbuterol and other beta-agonist normally have a limited duration of usefulness here because beta 2 adrenergic receptors decrease in number with regular stimulation. Within several weeks of initiating therapy with such a drug, it usually begins to diminish in effectiveness. ketotifen fumarate may extend this time period considerably. The ability of ketotifen to potentiate the effects of beta 2 agonist drug has been demonstrated in a number of clinical studies. The mean elimination half life of ketotifen is 12 hours.[1] The drug may also help relieve the symptoms of irritable bowel syndrome.[2]

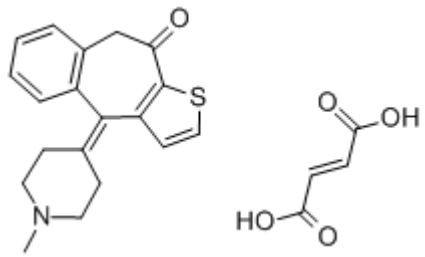


Figure 1.1 Chemical structure of ketotifen fumarate

Table 1.1: Physiochemical properties of ketotifen fumarate

Properties	**Ketotifen fumarate**
Color	White or almost white powder
Odor	Odorless
Solubility	In the form of hydrogen fumerate, it is readily soluble in water. Ketotifen is stable in slightly acidic solution
Molecular formula	$C_{19}H_{19}NOS$
Molecular weight	425.25

Supplied as:

Ketotifen fumarate is an H_1-antihistamine that is available in two versions oral and ophthalmic. Ketotifen fumarate is most commonly supplied in tablet of 1mg. this dosage is usually expressed in terms of the base, so each tablet actually contains 1.38 mg of ketotifen fumarate. It is most commonly sold in as a salt of fumaric acid,

Therapeutic uses:

- To treat conjunctivitis (pink eye), or the itchy red eyes caused by allergies (ophthalmic preparation).
- To aid in the prevention of asthma attacks (oral tablet).

Mechanism of action/Effect:

Ketotifen is a non-bronchodilator anti asthmatic drug which inhibits the effects of certain endogenous substances known to be inflammatory mediators, and thereby exerts anti allergic activity. Ketotifen possesses a powerful and sustained non-competitive histamine (H_1) blocking property. Ketotifen's antihistamine (H_1) effect seems to be distinct from its anti allergic properties. Properties of ketotifen which may contribute to its anti allergic activity and its ability to affect the underlying pathology of asthma include: *In vivo* results: Inhibition of the development of airway hyper reactivity associated with activation of platelets by PAF (Platelet Activating Factor) or caused by neural activation following the use of sympathomimetic drugs or the exposure to allergen; inhibition of PAF-induced accumulation of eosinophils and platelets in the airways; suppression of the priming of eosinophils by human recombinant cytokines and thereby suppression of the influx of eosinophils into inflammatory loci; antagonism of bronchoconstriction due to leukotrienes. *In vitro* results: Inhibition of the release of allergic mediators such as histamine, leukotrienes C_4 and D_4 (SRS-A) and PAF. Ketotifen also (potentially) improves insulin sensitivity within muscle tissue. Ketoifen increases appetite; therefore another side effect is typically weight gain.

Side effects:

Common side effects include

- dry mouth
- appetite stimulation
- weight gain
- dizziness
- CNS stimulation
- Drowsiness

These side effects are commonly associated with strong antihistamine compounds. In rare cases severe allergic reaction on the skin or urinary bladder inflammation called cystitis may occur.

1.5.2 Amoxicillin trihydrate

Amoxicillin is a moderate-spectrum, bacteriolytic, β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics. Amoxicillin is one of the most common antibiotics prescribed for children. Amoxicillin is susceptible to degradation by β-lactamase-producing bacteria, which are resistant to a broad spectrum of β-lactam antibiotics, such as penicillin. For this reason, it is often combined with clavulanic acid, a β-lactamase inhibitor. This increases effectiveness by reducing its susceptibility to β-lactamase resistance. Amoxicillin is used in the treatment of a number of infections, including acute otitis media, streptococcal pharyngitis, pneumonia, skin infections, urinary tract infections, *Salmonella* infections, Lyme disease, and chlamydia infections.[3] It is also used to prevent bacterial endocarditis in high-risk people who are having dental work done, to prevent *Streptococcus pneumoniae* and other encapsulated bacterial infections in those without spleens, such as people with sickle-cell disease, and for both the prevention and the treatment of anthrax.[3] The British Medical Association recommends against its use for infectious endocarditis prophylaxis. These recommendations have not appeared to have changed the rates of

infection.[4] Amoxicillin and amoxicillin-clavulanate are recommended by guidelines as the first-choice drug for bacterial sinusitis, but most sinusitis is caused by viruses, for which amoxicillin and amoxicillin-clavulanate are ineffective.[5,6] Amoxicillin is occasionally used for the treatment of skin infections, such as acne vulgaris.[7] It is often an effective treatment for cases of acne vulgaris that have responded poorly to other antibiotics, such as doxycycline and minocycline.[8]

1.5.3. Domperidone

There is some evidence that domperidone has antiemetic activity.[9] It can be used in patients with Parkinson's disease[10] because, unlike metoclopramide,[11] domperidone does not cross the blood–brain barrier. Domperidone has also been found effective in the treatment of gastroparesis.[12]

1.5.4. Theophylline

Theophylline is a competitive nonselective phosphodiesterase inhibitor,[13] which raises intracellular cAMP, activates PKA, inhibits TNF-alpha[14,15] and inhibits leukotriene[16] synthesis, and reduces inflammation and innate immunity[16]. It is also an nonselective adenosine receptor antagonist,[17] antagonizing A_1, A_2 and A_3 receptors almost equally, which explains many of its cardiac effects. Theophylline can also cause nausea, diarrhea, increase in heart rate, arrhythmias, and CNS excitation (headaches, insomnia, irritability, dizziness and lightheadedness).[18,19] Seizures can also occur in severe cases of toxicity and is considered to be a neurological emergency.[20] It can reach toxic levels when taken with fatty meals, an effect called dose dumping.[21] Theophylline toxicity can be treated with beta blockers. In addition to seizures, tachyarrhythmias are a major concern.[22]

1.5.5 Metformin hydrochloride

Metformin is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function.[23-25]

As of 2010, metformin is one of only two oral antidiabetics in the World Health Organization Model List of Essential Medicines (the other being glibenclamide).[26] When it is prescribed to people with contraindications, but otherwise, there is no significant risk.[27] Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis).[28] The "average" person with type 2 diabetes has three times the normal rate of gluconeogenesis; metformin treatment reduces this by over one third.[29] The molecular mechanism of metformin is incompletely understood: inhibition of the mitochondrial respiratory chain (complex I), activation of AMP-activated protein kinase (AMPK), inhibition of glucagon-induced elevation of cyclic adenosine monophosphate (cAMP) and consequent activation of protein kinase A (PKA), and an effect on gut microbiota have been proposed as potential mechanisms.[30-31] A study in 2001 suggested that activation of AMPK, an enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats,[32] was required for metformin's inhibitory effect on the production of glucose by liver cells.[33] Research published in 2008 further showed that activation of AMPK was required for an increase in the expression of SHP, which in turn inhibited the expression of the hepatic gluconeogenic genes PEPCK and Glc-6-Pase.[34] Metformin is frequently used in research along with AICAR as an AMPK agonist. More recent studies using mouse models in which the genes for AMPKα1 and α2 catalytic subunits (Prkaa1/2) or LKB1, an upstream kinase of AMPK, had been knocked out in hepatocytes have raised doubts over the obligatory role of AMPK, since the effect of metformin was not abolished by loss of AMPK function. The mechanism by which biguanides increase the activity of AMPK remains uncertain; however, research suggests that metformin increases the concentration of cytosolic AMP (as opposed to a change in total AMP or total AMP/ATP).[35] Increased cellular AMP has also been proposed to explain the inhibition of

glucagon-induced increase in cAMP and activation of PKA. Metformin and other biguanides may antagonize the action of glucagon, thus reducing fasting glucose levels.[36] In addition to suppressing hepatic glucose production, metformin increases insulin sensitivity, enhances peripheral glucose uptake (by inducing the phosphorylation of GLUT4 enhancer factor), decreases insulin-induced suppression of fatty acid oxidation,[37] and decreases absorption of glucose from the gastrointestinal tract. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors.[38] The increase in insulin binding after metformin treatment has also been demonstrated in patients with NIDDM.[39] AMPK probably also plays a role, as metformin administration increases AMPK activity in skeletal muscle.[40] AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake. Some metabolic actions of metformin do appear to occur by AMPK-independent mechanisms; a 2008 study found the metabolic actions of metformin in the heart muscle can occur independent of changes in AMPK activity and may be mediated by p38 MAPK- and PKC-dependent mechanisms.[41] Metformin has an oral bioavailability of 50-60% under fasting conditions, and is absorbed slowly. Peak plasma concentrations (C_{max}) are reached within one to three hours of taking immediate-release metformin and four to eight hours with extended-release formulations.[42] The plasma protein binding of metformin is negligible, as reflected by its very high apparent volume of distribution (300–1000 L after a single dose). Steady state is usually reached in one or two days. Metformin has acid dissociation constant values (pKa) of 2.8 and 11.5 and, therefore, exists very largely as the hydrophilic cationic species at physiological pH values. The metformin acid dissociation constant values (pKa) make metformin a stronger base than most other basic drugs with less than 0.01% unionized in blood. Furthermore, the lipid solubility of the unionized species is slight as shown by its low log P value [log (10) of

the distribution coefficient of the unionized form between octanol and water] of -1.43. These chemical parameters indicate low lipophilicity and, consequently, rapid passive diffusion of metformin through cell membranes is unlikely. The log P of metformin is less than that of phenformin (-0.84) because two methyl substituents on metformin impart lesser lipophilicity than the larger phenylethyl side chain in phenformin. More lipophilic derivatives of metformin are presently being investigated with the aim of producing prodrugs with better oral absorption than metformin itself.[43] Metformin is not metabolized. It is cleared from the body by tubular secretion and excreted unchanged in the urine; metformin is undetectable in blood plasma within 24 hours of a single oral dose.[44] The average elimination half-life in plasma is 6.2 hours. Metformin is distributed to (and appears to accumulate in) red blood cells, with a much longer elimination half-life: 17.6 hours (reported as ranging from 18.5 to 31.5 hours in a single-dose study of non-diabetic people).[44]

1.5.6 Diclofenac sodium

Diclofenac sodiumis a nonsteroidal anti-inflammatory drug (NSAID) taken or applied to reduce inflammation and as an analgesic reducing pain in certain conditions. Diclofenac is used to treat pain, inflammatory disorders, and dysmenorrhea.[45] Inflammatory disorders may include musculoskeletal complaints, especially arthritis, rheumatoid arthritis, polymyositis, dermatomyositis, osteoarthritis, dental pain, TMJ pain, spondylarthritis, ankylosing spondylitis, gout attacks, and pain management in cases of kidney stones and gallstones. An additional indication is the treatment of acute migraines. Diclofenac is used commonly to treat mild to moderate postoperative or post-traumatic pain, in particular when inflammation is also present, and is effective against menstrual pain and endometriosis. Diclofenac is also used for the treatment of conditions such as osteoarthritis, actinic keratosis, and acute pain caused by minor strains, sprains, and contusions (bruises). Diclofenac has been found effective against all strains of multidrug-

resistant *E. coli*, with a MIC of 25 micrograms/ml. Therefore, it may have the capacity to treat uncomplicated urinary tract infections caused by *E. coli*.[46] It has also shown effectiveness in treating *Salmonella* infections in mice,[47] and is under investigation for the treatment of tuberculosis.[48] Diclofenac is an antiuricosuric agent.[49]

1.5.7 Chlorphenamine maleate

Chlorphenamine (INN) or chlorpheniramine (USAN, former BAN), commonly marketed in the form of chlorpheniramine maleate, is a first-generation alkylamine antihistamine used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria. Its sedative effects are relatively weak compared to other first-generation antihistamines. Chlorphenamine is one of the most commonly used antihistamines in small-animal veterinary practice. Although not generally approved as an antidepressant or anti-anxiety medication, chlorphenamine appears to have these properties as well.[50,51] Chlorphenamine is part of a series of antihistamines including pheniramine (Naphcon) and its halogenated derivatives and others including fluorpheniramine, dexchlorpheniramine (Polaramine), brompheniramine (Dimetapp), dexbrompheniramine (Drixoral), deschlorpheniramine, dipheniramine (also known as triprolidine with the trade name Actifed), and iodopheniramine. The halogenated alkylamine antihistamines all exhibit optical isomerism, and chlorphenamine in the indicated products is racemic chlorphenamine maleate, whereas dexchlorpheniramine is the dextrorotary stereoisomer.In addition to being an histamine H_1 receptor antagonist, chlorphenamine has been shown to work as a serotonin-norepinephrine reuptake inhibitor or SNRI.[52] A similar antihistamine, brompheniramine, led to the discovery of the SSRI zimelidine. Limited clinical evidence shows that it is comparable to several antidepressant medications in its ability to inhibit the reuptake of serotonin and also norepinephrine (noradrenaline).[53] However, extensive clinical trials of its psychiatric properties in humans have not been conducted. It inhibits serotonin reuptake less than norepinephrine

reuptake,[54] A study performed on Fischer 344/Brown Norway F1 hybrid rats showed that intraventricular administration of Chlorphenamine reduced fear-related behaviors and improved maze performance. It was also noted that long term administration of Chlorphenamine reduced age-related deficits in motor function.[55] Chlorphenamine is combined with a narcotic (hydrocodone) in the product tussionex, which is indicated for treatment of cough and upper respiratory symptoms associated with allergy or cold in adults and children 6 years of age and older.[56]

1.5.8 Paracetamol

Paracetamol or acetaminophen chemically named N-acetyl-p-aminophenol is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). Paracetamol is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients.[57] Though paracetamol is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity. While generally safe for use at recommended doses even small overdoses can be fatal. The ratio between fatal doses and therapeutic doses (the therapeutic index) is much smaller than for other over-the-counter painkillers. According to the US Food and Drug Administration (FOA) as little as 25 percent above the maximum daily dose can cause liver damage when taken over several days. Acute overdoses of paracetamol can cause potentially fatal liver damage. The risk may be heightened by chronic alcohol abuse. Paracetamol toxicity is the foremost cause of acute liver failure in the Western world, and accounts for most drug overdoses in the United States, the United Kingdom, Australia and New Zealand.[58-61] The onset of analgesia is

approximately 11–29.5 minutes after oral administration of paracetamol,[62] and its half-life is 1–4 hours. Paracetamol is the active metabolite of phenacetin, once popular as an analgesic and antipyretic in its own right. However, unlike phenacetin and its combinations, paracetamol is not considered carcinogenic at therapeutic doses.[63] The words *acetaminophen* (used in the United States,[64] Canada, Japan, South Korea, Hong Kong, and Iran) and paracetamol (used elsewhere) both come from a chemical name for the compound: *para*-acetylaminophenol and para-acetylaminophenol. In some contexts, it is simply abbreviated as APAP, for acetyl-para-aminophenol. Paracetamol is approved for reducing fever in people of all ages.[65] Paracetamol can relieve pain in mild arthritis, but has no effect on the underlying inflammation, redness, and swelling of the joint.[66] Regarding comparative efficacy, studies show conflicting results when compared to NSAIDs. A randomised controlled trial of chronic pain from osteoarthritis in adults found similar benefit from paracetamol and ibuprofen.[67,68] Paracetamol is metabolised by the liver and is hepatotoxic; side effects are multiplied when combined with alcoholic drinks, and very likely in chronic alcoholics or patients with liver damage.[69,70] Prolonged daily use increases the risk of upper gastrointestinal complications such as stomach bleeding,[71] and may cause kidney or liver damage.[72-73] Chronic users of paracetamol may have a higher risk of developing blood cancer.[74] However, paracetamol does not help reduce inflammation, while aspirin does.[75] Compared to ibuprofen—whose side effects may include diarrhea, vomiting and abdominal pain—paracetamol has fewer adverse gastrointestinal effects.[76] Paracetamol is generally believed to be safe for use in pregnancy as it does not affect the closure of the fetal ductus arteriosus as NSAIDs can.[77] However, in a study published in October 2010, paracetamol use has been linked to infertility in the subsequent adult life of the male fetus.[78] Reporting on the study, Michelle Roberts writes for the BBC that researchers from Denmark, Finland and France studied

more than 2,000 pregnant women and their babies. They found those women who used more than one painkiller simultaneously, such as paracetamol and ibuprofen, had a seven-fold increased risk of giving birth to sons with some form of undescended testes, or cryptorchidism, compared to women who took nothing. The second trimester - 14 to 27 weeks of pregnancy - appeared to be a particularly sensitive time." Unlike aspirin, paracetamol is generally considered safe for children, as it is not associated with a risk of Reye's syndrome in children with viral illnesses.[79]

1.5.9 Desloratadine

Desloratadine is a tricyclic antihistamine, which has a selective and peripheral H_1-antagonist action. It is an antagonist at histamine H_1 receptors, and an antagonist at all subtypes of the muscarinic acetylcholine receptor. It has a long-lasting effect and in moderate and low doses, does not cause drowsiness because it does not readily enter the central nervous system.[80] Unlike other antihistamines, desloratadine is also effective in relieving nasal congestion, particularly in patients with allergic rhinitis.[81]

1.5.10 Salbutamol sulphate

Salbutamol (INN) or albuterol (USAN) is a short-acting β_2-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma and chronic obstructive pulmonary disease.[82] As a β_2-agonist, salbutamol also finds use in obstetrics. Intravenous salbutamol can be used as a tocolytic to relax the uterine smooth muscle to delay premature labor. While preferred over agents such as atosiban and ritodrine, its role has largely been replaced by the calcium-channel blocker nifedipine, which is more effective, better tolerated and orally administered.[83] Salbutamol has been used in treating acute hyperkalemia on account of its potassium-depleting properties by stimulating potassium inflow in cells.[84,85] Salbutamol has also been trialled in spinal muscular atrophy where it appears to show modest benefits. The drug is speculated to modulate the alternative

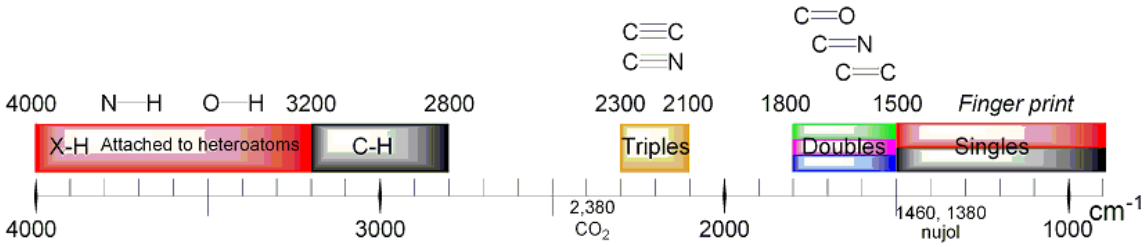
splicing of the gene, increasing the amount of the SMN protein whose deficiency is regarded as the root cause of the disease.[86,87] The most common side effects are fine tremor, anxiety, headache, muscle cramps, dry mouth, and palpitation. Other symptoms may include tachycardia, arrhythmia, flushing, myocardial ischemia (rare), and disturbances of sleep and behaviour.

1.6 Infrared Spectroscopy

Infrared photometry (IR photometry) deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light. It covers a range of techniques, mostly based on absorption spectroscopy. It can be used to identify and study chemicals. A common laboratory instrument that uses this technique is Fourier Transform Infrared (FTIR) spectrometer.

The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near-, mid- and far- infrared, named for their relation to the visible spectrum. The higher-energy near-IR, approximately 14000–4000 cm^{-1} (0.8–2.5 μm wavelength) can excite overtone or harmonic vibrations. The mid-infrared, approximately 4000–400 cm^{-1} (2.5–25 μm) may be used to study the fundamental vibrations and associated rotational-vibrational structure. The far-infrared, approximately 400–10 cm^{-1} (25–1000 μm), lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy. The names and classifications of these subregions are conventions, and are only loosely based on the relative molecular or electromagnetic properties.

Absorption bands



An example of IR spectrum

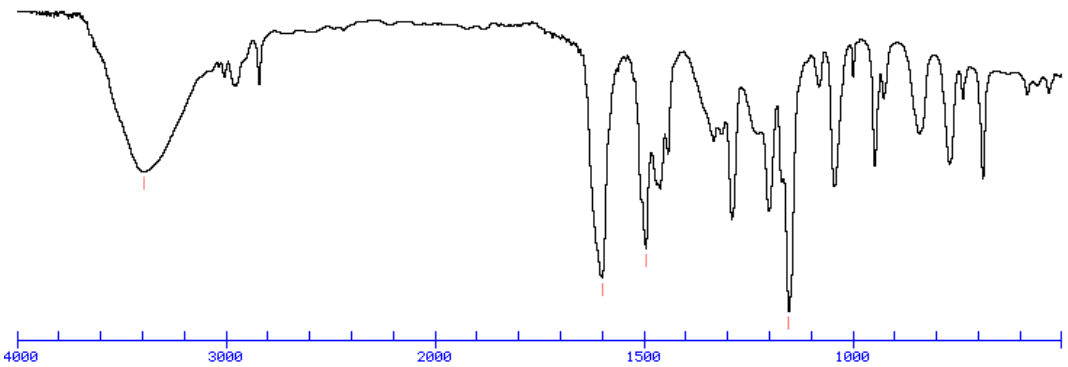


Table for IR absorptions for representative functional groups

Functional group	Molecular motion	Wave number (cm^{-1})
alkanes	C-H stretch	2950-2800
	CH_2 bend	~1465
	CH_3 bend	~1375
	CH_2 bend (4 or more)	~720
alkenes	=CH stretch	3100-3010
	C=C stretch (isolated)	1690-1630
	C=C stretch (conjugated)	1640-1610
	C-H in-plane bend	1430-1290

	C-H bend (monosubstituted)	~990 & ~910
	C-H bend (disubstituted - E)	~970
	C-H bend (disubstituted - 1,1)	~890
	C-H bend (disubstituted - Z)	~700
	C-H bend (trisubstituted)	~815
alkynes	acetylenic C-H stretch	~3300
	C,C triple bond stretch	~2150
	acetylenic C-H bend	650-600
aromatics	C-H stretch	3020-3000
	C=C stretch	~1600 & ~1475
	C-H bend (mono)	770-730 & 715-685
	C-H bend (ortho)	770-735
	C-H bend (meta)	~880 & ~780 & ~690
	C-H bend (para)	850-800
alcohols	O-H stretch	~3650 or 3400-3300
	C-O stretch	1260-1000
ethers	C-O-C stretch (dialkyl)	1300-1000
	C-O-C stretch (diaryl)	~1250 & ~1120

aldehydes	C-H aldehyde stretch	~2850 & ~2750
	C=O stretch	~1725
ketones	C=O stretch	~1715
	C-C stretch	1300-1100
carboxylic acids	O-H stretch	3400-2400
	C=O stretch	1730-1700
	C-O stretch	1320-1210
	O-H bend	1440-1400
esters	C=O stretch	1750-1735
	C-C(O)-C stretch (acetates)	1260-1230
	C-C(O)-C stretch (all others)	1210-1160
acid chlorides	C=O stretch	1810-1775
	C-Cl stretch	730-550
anhydrides	C=O stretch	1830-1800 & 1775-1740
	C-O stretch	1300-900
amines	N-H stretch (1 per N-H bond)	3500-3300
	N-H bend	1640-1500
	C-N Stretch (alkyl)	1200-1025

	C-N Stretch (aryl)	1360-1250
	N-H bend (oop)	~800
amides	N-H stretch	3500-3180
	C=O stretch	1680-1630
	N-H bend	1640-1550
	N-H bend (1^{o})	1570-1515
alkyl halides	C-F stretch	1400-1000
	C-Cl stretch	785-540
	C-Br stretch	650-510
	C-I stretch	600-485
nitriles	C,N triple bond stretch	~2250
isocyanates	-N=C=O stretch	~2270
isothiocyanates	-N=C=S stretch	~2125
imines	R_2C=N-R stretch	1690-1640
nitro groups	-NO_2 (aliphatic)	1600-1530 & 1390-1300
	-NO_2 (aromatic)	1550-1490 & 1355-1315
mercaptans	S-H stretch	~2550
sulfoxides	S=O stretch	~1050

sulfones	S=O stretch	~1300 & ~1150
sulfonates	S=O stretch	~1350 & ~11750
	S-O stretch	1000-750
phosphines	P-H stretch	2320-2270
	PH bend	1090-810
phosphine oxides	P=O	1210-1140

These trends in absorption can be further summarized into the following categories

3600 - 2700 cm^{-1}	X-H
2700 - 1900 cm^{-1}	X=Y
1900 - 1500 cm^{-1}	X=Y
1500 - 500 cm^{-1}	X-Y

Hepatoprotective activity:

Liver is one of the largest organs in human body and the chief site for intense metabolism and extraction. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphate are elevated.[98]

Chapter-2

Materials

2. Materials and Instruments

2.1 Materials

All the chemicals and reagents used in this study were of analytical grade and were stored under optimum storage conditions. The experimental mixtures and solutions were prepared in standard volumetric flasks about one hour prior to recording the data.

2.2 Drugs used in the study

The ketotifen fumarate and other commonly prescribed drugs theophylline anhydrous, amoxicillin trihydrate, domperidone, metformin hydrochloride, chlorpheniramine maleate, diclofenac sodium, paracetamol, and desloratidine were used in this study.

Drugs and reagents for HPLC work:

Working standards of ketotifen fumarate, metformin hydrochloride and theophylline anhydrous with a potency of 99.98, 99.25 and 99.65% respectively were collected from Square Pharmaceuticals Ltd., Pabna, Bangladesh. Potassium dihydrogen phosphate buffer (KH_2PO_4), HPLC grade acetonitrile and methanol were purchased from Active Fine Chemicals Ltd., Dhaka, Bangladesh.

Drugs and chemicals for hepatotoxicity and kidney function test:

The kits used to perform the liver and kidney function test include LTD (Bangladesh), SGPT kits (Human, Germany), SGOT kits (Human, Germany), ALP kits (Human, Germany), Total protein kits (Dutch Diagnostics, Netharland), Bilirubin kits (Human, Germany)

2.3 The λ_{max} values of drugs used in the study

The wavelength for maximum absorption value of the drug used in the experiment is given in the following table.

Table 2.1: The λ_{max} value of drug used in the study

Name		Wavelength (λ_{max})
Drug 1	Ketotifen fumarate	300 nm
Drug 2	Theophylline anhydrous	273 nm
Drug 3	Amoxicillin trihydrate	272 nm
Drug 4	Domperidone	284 nm
Drug 5	Metformin hydrochloride	233 nm
Drug 6	Chlorpheniramine maleate	265 nm
Drug 7	Salbutamol sulphate	292 nm
Drug 8	Diclofenac sodium	276nm
Drug 9	Paracetamol	257 nm
Drug 10	Desloratidine	247 nm

2.4 Instruments and equipments used in the study

Table 2.2: Name and sources of instruments and equipments

Name	Source
pH Meter	Cyberscan 500, Hanna, Portugal
UV/VIS Spectrophotometer	UV mini-1240, Shimadzu, Japan
Electronic balance	Shimadzu Corporation, Japan
Digital water bath	Premiere HH- 4
IR Spectrophotometer	IR Affinity-1 A213747 Shimadzu Corporation, Japan
Diffefential Scanning Calorimeter	Shimadzu Serial No: C30454600885SA
Sealer machine	Serial no C302146 shimadzu corporation 01545
HPLC	Shimadzu-UFLC Prominence, equipped with an auto sampler (Model- SIL 20AC HT) and UV-Visible detector (Model- SPD 20A)

2.5 Solvents

Solvents used in this study include distilled water, methanol and chloroform.

2.6 Buffers

Table 2.3: Name and sources of buffer ingredients

Ingredients of Buffers	Source
Sodium hydroxide	Reagent grade, Merck, India
Sodium di hydrogen phosphate (NaH_2PO_4)	Reagent grade, Merck, India
Disodium hydrogen phosphate (Na_2HPO_4)	Reagent grade, Merck, India
Hydrochloric acid	Reagent grade, Merck, India
Sodium chloride	Reagent grade, Merck, India

Chapter-3

Methods

3. Methods:

3.1 Preparation of buffer solutions

3.1.1 Preparation of lower pH buffer solutions (pH 0.4, 2.0 & 2.8):

These buffers were prepared by using sodium chloride (pellets), and conc. hydrochloric acid with the help of pH meter.

3.1.2 Preparation of higher pH buffer solution (pH 6.8 &7.4):

These buffers were prepared by using sodium di hydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) with the help of pH meter.

3.2 Stock drug solutions preparations

3.2.1 Preparation of ketotifen fumarate solution

100 ml of 1×10^{-3} M solution of ketotifen fumarate was prepared as the stock solution by dissolving 0.0425 gm of ketotifen fumarate in 100 ml of distilled water in a 100 ml volumetric flask. To prepare 1×10^{-5} M solution of ketotifen fumarate, 1 ml of 1×10^{-3} M solution was taken in another 100 ml volumetric flask and the volume was adjusted by distilled water up to the mark.

3.2.2. Preparation of other commonly prescribed drugs solution

100 ml of 1×10^{-3} M solution of other commonly prescribed drugs was prepared as the stock solution in a 100 ml volumetric flask. To make it 1×10^{-5} M solution, 1 ml of 1×10^{-3} M solution was taken in another 100 ml volumetric flask and the volume was adjusted by distilled water up to the mark.

The interaction of ketotifen fumarate and other commonly prescribed drugs had been studied by different methods of analysis under different biological pHs (0.4, 1.2, 2.0, 2.8, 6.0, 6.8 & 7.4) at different concentrations. The spectral characteristics and

spectrophotometric analysis of the complexation process had been evaluated. The results obtained from various methods are discussed below.

3.3 Spectral studies of interaction of ketotifen fumarate & other commonly prescribed drugs

In spectral observation analysis, each of the drugs studied showed absorption in UV-VIS region. The molecular species of ketotifen fumarate & other commonly prescribed drugs when separately mixed showed some changes in absorption characteristics of this drug molecule including some shifts in the absorption maxima. Initial detection of complexation of ketotifen with other commonly prescribed drugs was done from the nature of spectra of pure compounds as well as their 1:1, 1:2 and 2:1 mixtures in buffer solution of pH 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 at a fixed concentration ($1x10^{-5}$ M). It is obvious that each compound has its unique molecular structure or electronic configuration which is responsible for absorption of light in the form of ultra-violet or visible form. For this reason the spectrum of any pure compound obtained from UV spectrum will be of one kind that will be totally different from the other compound or the complex of that compound with other compounds. It is because interaction between two compounds may lead to form complex which has different light absorption capacity (due to change in physicochemical and optical properties) and the spectral pattern is altered. Thus alteration in spectral pattern may be regarded as an indicator for primary interaction of drugs.

3.4 Effect of other commonly prescribed drugs on ketotifen fumarate by Job's method of continuous variation at different pH

The molar ratios of the complexes of ketotifen fumarate with other commonly prescribed drugs were estimated by Job's spectrophotometric method of continuous variation.[97] The

observed absorbance values were measured at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 at various concentrations 1 x 10^{-5} M to 9 x 10^{-5} M of ketotifen fumarate with other commonly prescribed drugs at 300 nm. In this method, solutions of different concentrations of ketotifen fumarate and other commonly prescribed drugs were prepared by plotting corrected absorbance against the volume fraction of one reactant. It may be mentioned that drug solutions with identical analytical concentrations are mixed in such a way that total volume and the total moles of reactant in each mixture is constant but the mole ratio of the reactants varies systematically. If the formation constant is reasonably favorable, two straight lines of different slopes that intersect at a mole ratio that corresponds to the combining ratio in the complex are obtained.[88]

3.5 The Ardon's spectrophotometric methods

In the Ardon's spectrophotometric method, [89] concentrations of ketotifen fumarate was varied while keeping the concentrations of other commonly prescribed drugs fixed at 1×10^{-4} M. All the experiments were performed in buffer at pH 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4. The absorbance of solutions having pH 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 were measured at 300 nm using UV-VIS spectrophotometer. For calculations, the Ardon's equation was used. This equation is given below:-

$$\frac{1}{(D-\in_{\mathrm{A}} \mathrm{C})}=\frac{1}{KC(\in_{COM}-\in A)[B]^{N}}+\frac{1}{C(\in com-\in A)}$$

Where,

D = Absorbance of the mixture.

B = Molar concentration of the ketotifen fumarate.

C = Molar concentration of the other commonly prescribed drugs

$\in_{com}$ =Molar extinction co-efficient of the complex.

$\in_A$ = Molar extinction co-efficient of the ketotifen fumarate.

The value of n was chosen as 1, which is an essential condition for validation of the method. The value for 1/ (D- $\in_A$C) was plotted versus 1/[B] to get the straight lines. The concentration of ketotifen fumarate was kept constant 1×10^{-4} M (denoted by C in the equation) & the concentration of interacting species, Other commonly prescribed drugs was varied (denoted by B in the equation). The 1:1 complex gave a straight line in the plots with an intercept and a slope. The stability constant of the complex was given by the relation,

K = intercept / slope

It is to be mentioned that this method is only valid for the systems where 1:1 complexes are found.

Least squares method of plotting

For a series of data having two variables x & y comprising an equation in the form of

Y = mx + C

Where,

y = Dependent variable.

x = Independent variable.

m = Slope of the curve drawn by plotting y against x.

c = Intercept of the curve.

From this equation, a best fitting curve can be obtained by the method of linear regression. This is known as the Least Square Method of plotting.

3.6. Drug interactions were analyzed by IR spectroscopy

3.6.1 Preparation of chloroform extracts for IR study

i. 100 mg of pure powder of ketotifen fumarate was dissolved in 10 ml of distilled water in a 50 ml beaker, similarly 100 mg of pure powder of other commonly prescribed drugs was dissolved in another beaker.

ii. Both the above solutions were mixed in a separate 100 ml beaker with constant stirring.

iii. Then the mixture was transferred to the separating funnel. The interacted drug was extracted in chloroform.

iv. Chloroform was evaporated and the precipitated solid drug product was analyzed by IR spectrophotometer.

3.6.2 Preparation of aqueous extracts for IR study

Another experiment for aqueous extracts were carried out by mixing both the drug solutions together and evaporated. The drug product obtained was also analyzed by IR spectrophotometer.

3.7 Complex formation confirmed by Differential Scanning Calorimeter (DSC)

3.7.1 Preparation of ketotifen standard disk

2.3 mg of ketotifen fumarate was placed in a disk. Then the disk was sealed by using the sealer machine. The ready sealed disk placed into the disk detector and the temperature was raised to 550 ^{o}C for scanning.

3.7.2 Preparation of metformin standard disk

4.8 mg of metformin hydrochloride was placed in a disk. Then the disk was sealed by using the sealer machine. The ready sealed disk placed into the disk detector and the temperature was raised to 550^0C for scanning.

3.7.3 Preparation of ketotifen-metformin standard disk

4.4 mg of previously reacted dry crystalline residue of ketotifen fumarate and metformin hydrochloride mixture was placed in a disk. Then the disk was sealed by using the sealer machine. The ready sealed disk placed into the disk detector and the temperature was raised to 550^0C for scanning. Similarly 2-5 mg of domperidone, theophylline anhydrous and their mixture were allowed to run through the differential scanning calorimeter chamber to identify the complex.

3.8 Stability of the complex identified by Thin Layer Chromatography (TLC)

The stability of the complex after formation was confirmed by thin layer chromatographic technique where ethanol was used as solvent. When the R_f values of the mixtures differ from the R_f values of the pure compounds, indicate the stability of the complex.

3.9 HPLC study

3.9.1 Preparation of mobile phase

To prepare buffer solution of pH 3.1, potassium dihydrogen phosphate (KH_2PO_4) was taken in a 1000 ml volumetric flask. About 500 ml of double distilled water was added into the flask, dissolved the salt and finally water was added up to the mark. Then pH was adjusted to 3.1 by adding dilute phosphoric acid. The mixture was sonicated for 10 minutes and then filtered through a 0.22 µm millipore filter. HPLC grade actonitrile was also filtered and degassed before use into the HPLC system.

3.9.2 Preparation of standard stock solution

Standard solutions of the pure drugs were prepared separately as $1x10^{-3}$M concentration. The drugs were dissolved in aqueous methanol (50:50, v/v) and final volume was made up to the mark of each of the volumetric flask with the same solvent to get the concentration of 1.0 µmole/ml.

3.9.3 Preparation of mixture

From the stock solution ketotifen fumarate & metformin hydrochloride (1:1) and ketotifen fumarate & theophylline anhydrous (1:1) were taken and finally made into 20 µL as injection volume.

3.9.4 Chromatographic conditions

For simultaneous determination of ketotifen, metformin and theophylline by RP-HPLC method, the mobile phase was comprised of potassium dihydrogen phosphate buffer (pH 3.1) and acetonitrile in the ratio of 60:40 (v/v) at a flow rate of 0.7 ml/min. The injection volume was 20 µl for both standard and samples. The run time was set for 15 min. Before analysis, every standard and sample was filtered through 0.45 µm filter tips. The mobile phase was also filtered, sonicated and degassed before use. The column eluate was monitored with a UV detector at 270 nm. All analyses were done at ambient temperature under isocratic condition.

3.10 *In vivo* drug interaction study of ketotifen fumarate with commonly prescribed drugs on rat model

3.10.1 Selection of Animal

Wister rats (150-200 g) of either sex bred were used. The animals were housed under standard conditions, maintained on a 12-h light/dark cycle and had free access to food and water up to the time of experimentation. The animals were acclimatized to the laboratory environment 1h before the experiments.

3.10.2 Preparation of drug solution

20 ml of each drug solutions were prepared according to their corresponding doses. ketotifen fumarate and prescribed drugs solutions formulated in 5% Tween-80 and 0.5% carboxy methyl cellulose in milli-Q water used in the study.

Table 3.4: Doses for administration

Drugs sample	Doses
Ketotifen fumarate	0.1 mg/kg/body wt
Amoxicillin trihydrate	13.75 mg/kg/body wt
Domperidone	5 mg/kg/body wt
Theophylline	150 mg/kg/body wt
Diclofenac Na	10 mg/kg/body wt
Salbutamol	0.1 mg/kg/body wt
Paracetamol	13.75 mg/kg/body wt
Metformin HCl	10 mg/kg/body wt

3.10.3 Methodology

Freshly prepared solutions of ketotifen, metformin and ketotifen fumarate and commonly prescribed drugs were administered as single dose to five groups of rats. The selection of dose levels based on efficacy dose and toxicokinetic doses. The blood samples were collected at 30 minutes, 60 minutes, 120 minutes & 180 minutes to compare the drug interaction between the group that took the single drug as well as mixtures (ketotifen & commonly prescribed drugs)

Group I. Each rat received ketotifen fumarate (0.2 mg/kg),

Group II. Each rat received commonly prescribed drugs,

Group III. They received ketotifen (0.2 mg/kg) & commonly prescribed drugs.

3.10.4 Procedure

1. Five rats were separated into five baskets.
2. The standard drugs and mixtures were administered as per dose for 7 days.
3. Then the rats were sacrificed and blood samples were collected by puncturing the hearts.
4. Required test tubes were taken and marked in numbers for each group.
5. Serum was poured into a small glass tube containing heparin and shaken well to inhibit the coagulation.
6. In each tube 0.5 ml of serum and 1.5 ml of 0.9% NaCl solution were taken for complete precipitation of protein.
7. To sediment the precipitated proteins and to obtain supernatant fluid, the mixture was centrifuged at 4000 rpm for 20 minutes.
8. 1 ml of supernatant fluid was taken by 1 ml pipette into the test tubes and 1 ml of demineralized water were added and mixed well.
9. The supernatant fluids were measured at 300 nm.

3.10. 5 Statistical analysis

The results were expressed as mean ± SEM values for each experiment. Differences in mean values between experimental groups were analyzed by utilizing SPSS one way ANOVA including:

1. Descriptive statistics
2. Levene's test of homogeneity of variance
3. Multiple comparisons
4. Robust tests of equality of means.

3.11 Experimental design for hepatotoxicity and kidney function test

Hepatoprotective activity evaluation

The serum was used for estimation of biochemical parameters (SGPT, SGOT, and total protein) to determine the functional state of the liver. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by a UV kinetic method based on the reference method of International Federation of Clinical Chemistry in which both SGOT and SGPT were assayed based on enzyme coupled system; where keto acids formed by the aminotransferase reacts in a system using NADH.

Experimental animal

Male long Evans rats (*Rattus novergicus*) weighing 130-150 gm were used. They were purchased from the pharmacology laboratory of Jahangirnagar University. Animals were maintained under standard environmental conditions (temperature 27 $\pm$ 1.0^0C, relative humidity 65% and 12 hours light and 12 hours dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments.

Preparation of test materials

1. Normal saline (0.9% NaCl solution) was prepared.
2. Test solution: ketotifen, theophylline, metformin, ketotifen-theophylline mixture, ketotifen-metformin mixture were prepared separately.

Procedure

A total of 30 rats were divided into 6 groups of 5 animals each:

Group I: Each rat received normal saline as vehicle control (5 ml/kg body weight) for seven days

Group II: Each rat received ketotifen as a standard drug sample (10 mg/kg)[100]

Group III: Each rat received commonly prescribed drug as a standard drug sample.

Group IV: Each rat received ketotifen & commonly prescribed drug as a test sample.

Chapter-4

Result and Discussion

4.1 UV spectrum analysis:

In UV spectral observation analysis, each of the drugs absorption studied in UV-VIS region. Initially detected the nature of spectra of pure compounds as well as their 1:1, 1:2 and 2:1 mixtures in buffer solutions at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4.

4.1.1 UV spectral studies of interaction of ketotifen fumarate & theophylline anhydrous

The molecular species of ketotifen fumarate and theophylline anhydrous when separately mixed showed some changes in absorption characteristics of this drug molecule including some shifts in the absorption maxima. Initial detection of complexation of ketotifen fumarate with theophylline anhydrous was done from the nature of spectra of pure compounds as well as their 1:1, 1:2 and 2:1 mixtures in buffer solutions at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 at a fixed concentration ($1x10^{-5}$ M). It is obvious that each compound has its unique molecular structure or electronic configuration which is responsible for absorption of light in the form of ultra-violet or visible form. For this reason the spectrum of any pure compound obtained from UV-spectrum will be of one kind that will be totally different from the other compound or the complex of that compound with other compounds. It is because interaction between two compounds may lead to form complex which has different light absorption capacity (due to change in physicochemical and optical properties) and the spectral pattern is altered. Thus alteration in spectral pattern may be regarded as an indicator for primary the primary interaction of drugs.

The UV spectra of ketotifen fumarate at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 & 7.4 and their 1:1, 1:2 and 2:1 mixtures of ketotifen fumarate with theophylline anhydrous in the same pHs were shown in the figures 4.1 – 4.7.

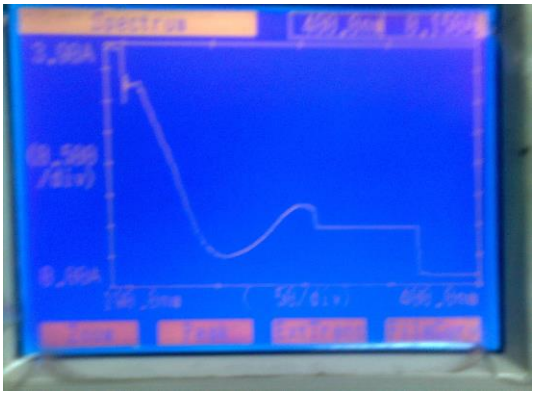


Figure 4.1.1 (Ketotifen fumarate)

Figure 4.1.2 (Theophylline anhydrous)

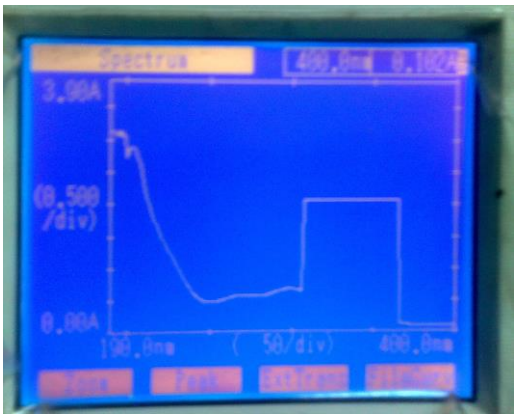


Figure 4.1.3 (Ketotifen : Theophylline=1:1)

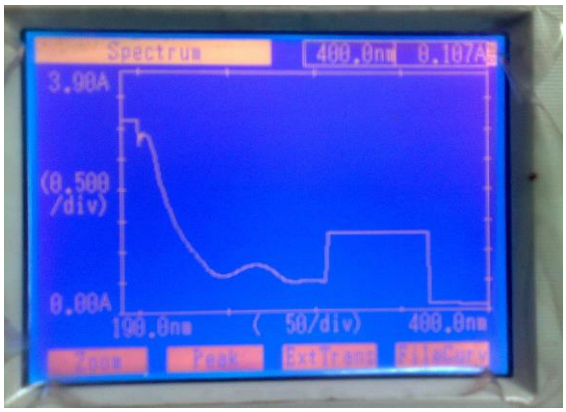


Figure 4.1.4 (Ketotifen : Theophylline=1:2)

Figure 4.1.5 (Ketotifen : Theophylline=2:1)

Figure 4.1: UV spectral studies of ketotifen fumarate and theophylline at pH 0.4.

The spectra of ketotifen and theophylline mixtures (1:1, 1:2) showed completely different absorption characteristics when compared with the spectra of ketotifen and theophylline in single form.

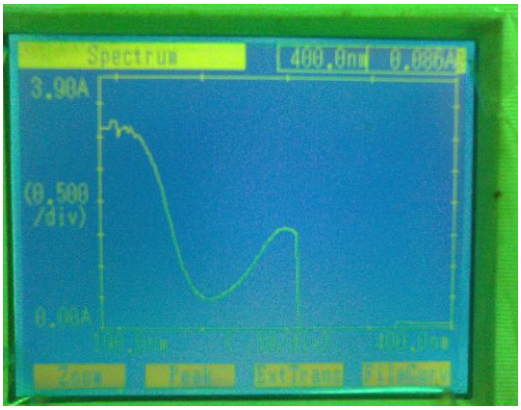


Figure 4.2.1 (Ketotifen fumarate)

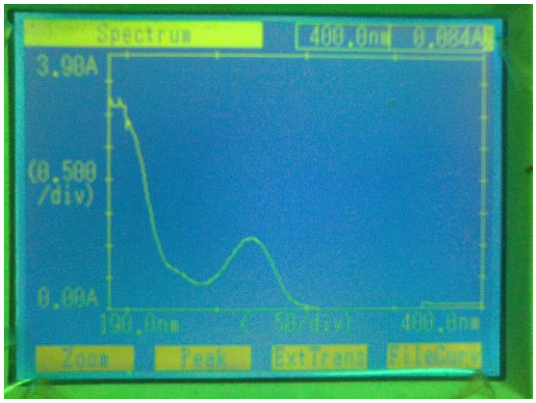


Figure 4.2.2 (Theophylline anhydrous)

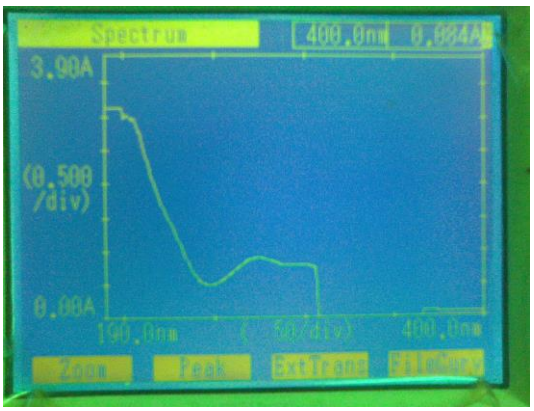


Figure 4.2.3 (Ketotifen : Theophylline = 1:1)

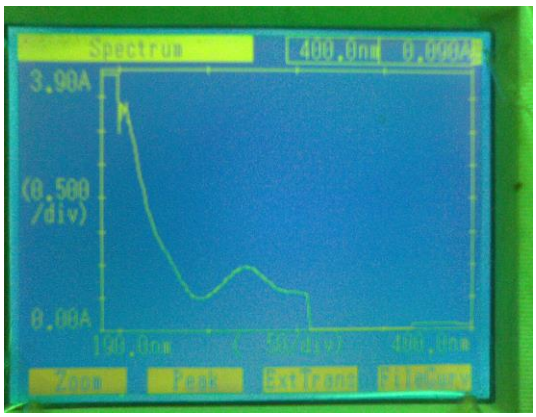


Figure 4.2.4 (Ketotifen : Theophylline = 1:2)

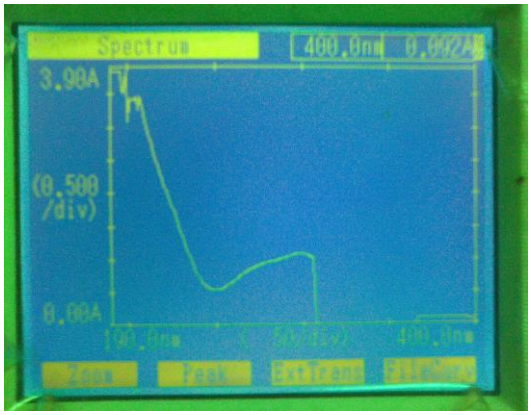


Figure 4.2.5 (Ketotifen : Theophylline = 2:1)

Figure 4.2: UV spectral studies of ketotifen fumarate and theophylline at pH 1.2.

When ketotifen and theophylline were mixed together at a ratio of 1:1 and 1.2 showed some changes in absorption characteristics.

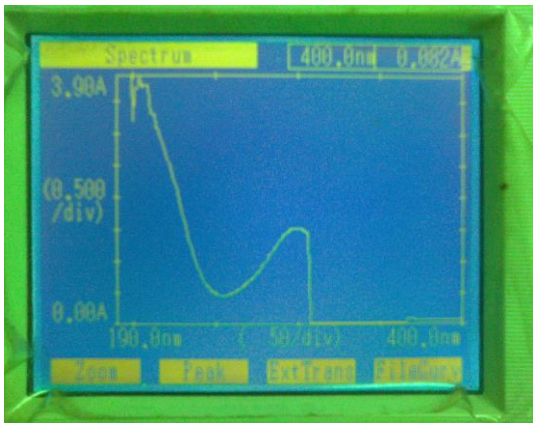


Figure 4.3.1 (Ketotifen fumarate)

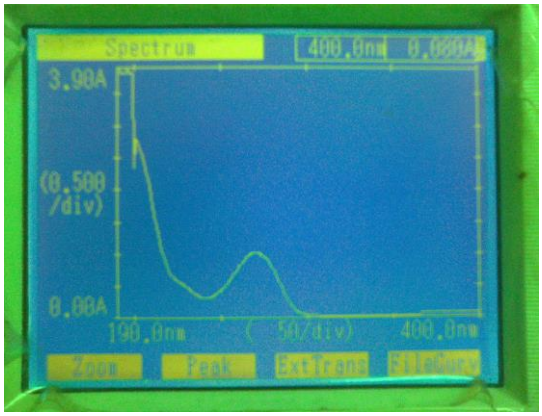


Figure 4.3.2 (Theophylline anhydrous)

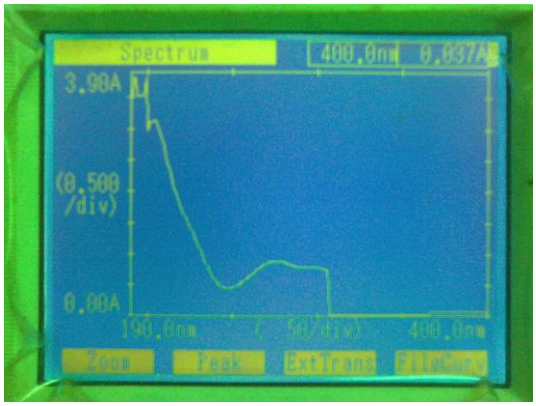


Figure 4.3.3 (Ketotifen : Theophylline = 1:1)

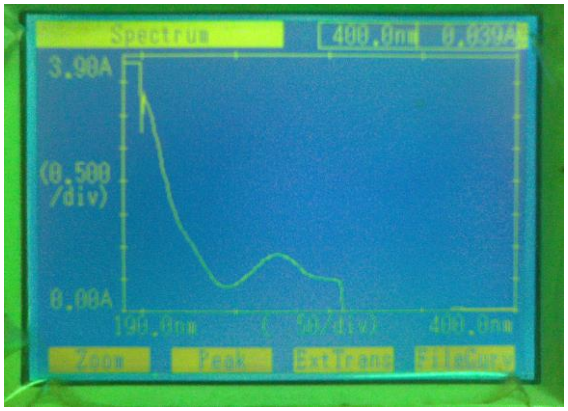


Figure 4.3.4 (Ketotifen : Theophylline = 1:2)

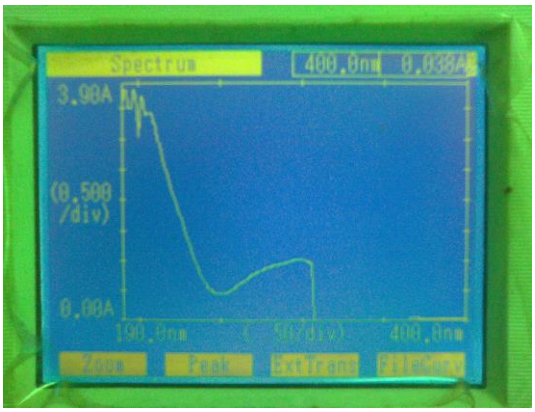


Figure 4.3.5 (Ketotifen : Theophylline = 2:1)

Figure 4.3: UV spectral studies of ketotifen fumarate and theophylline at pH 2.0.

The above mentioned figures showed changes in absorption characteristics, when ketotifen and theophylline were mixed together (1:1, 1.2).

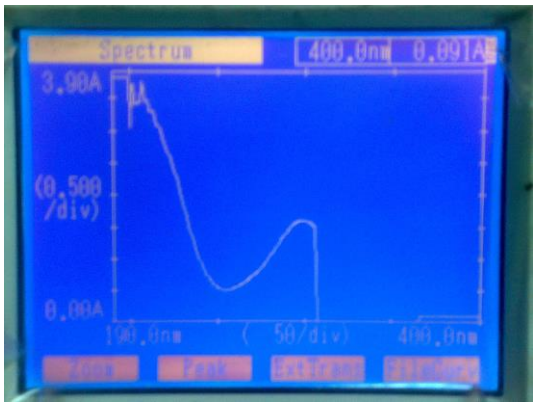


Figure 4.4.1 (Ketotifen fumarate)

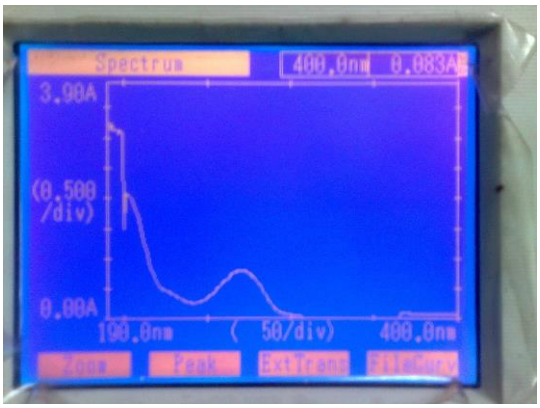


Figure 4.4.2 (Theophylline anhydrous)

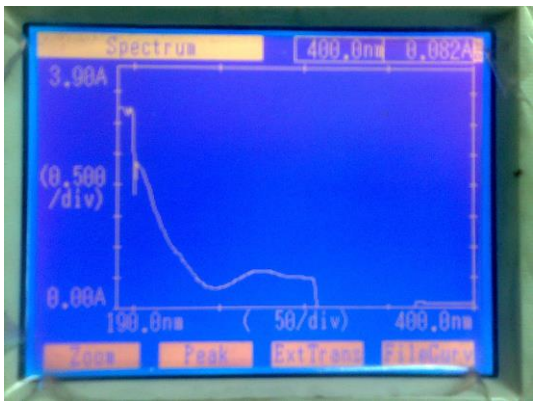


Figure 4.4.3 (Ketotifen:Theophylline=1:1)

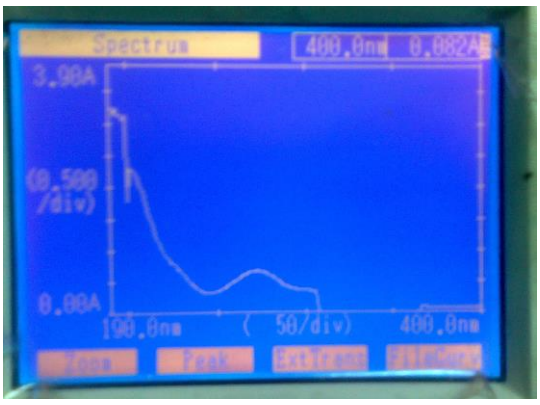


Figure 4.4.4 (Ketotifen:Theophylline=1:2)

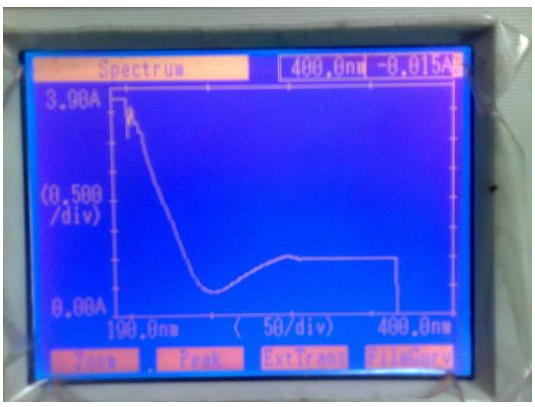


Figure 4.4.5 (Ketotifen :Theophylline=2:1)

Figure 4.4: UV spectral studies of ketotifen fumarate and theophylline at pH 2.8.

The above figures showed some changes in absorption characteristics, when ketotifen and theophylline were mixed together at 1:1, 1.2, 2:1 ratios.

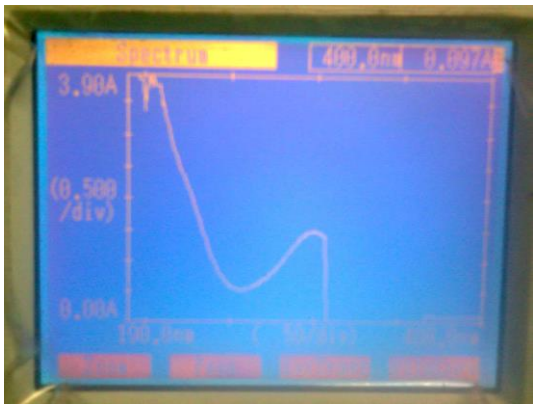


Figure 4.5.1 (Ketotifen fumarate)

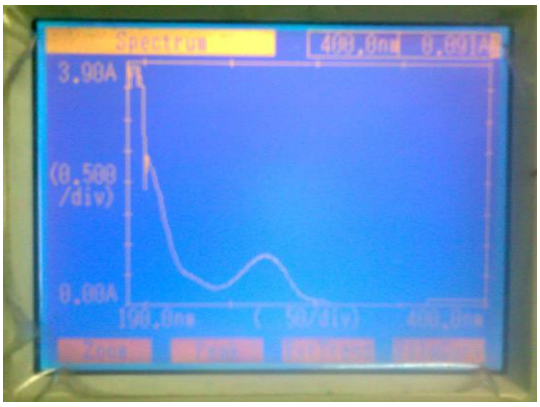


Figure 4.5.2 (Theophylline anhydrous)

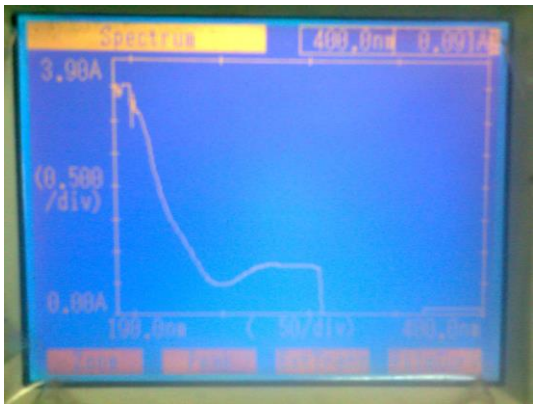


Figure 4.5.3 (Ketotifen :Theophylline=1:1)

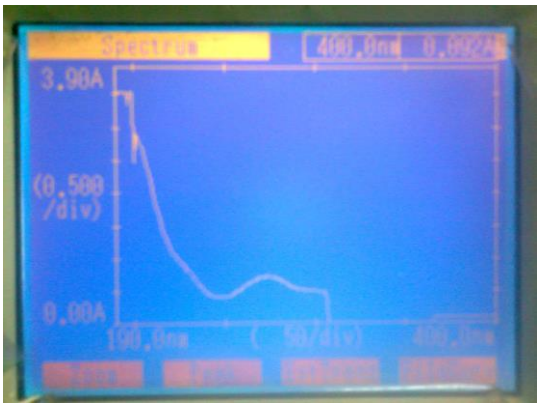


Figure 4.5.4 (Ketotifen :Theophylline=1:2)

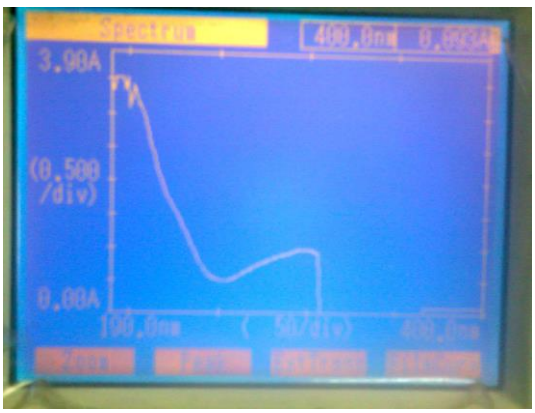


Figure 4.5.5 (Ketotifen : Theophylline=2:1)

Figure 4.5: UV spectral studies of ketotifen fumarate and theophylline at pH 6.0.

Ketotifen and theophylline showed some changes in absorption characteristics, when they were mixed together at 1:1 and 1:2 ratio.

Figure 4.6.1 (Ketotifen fumarate)

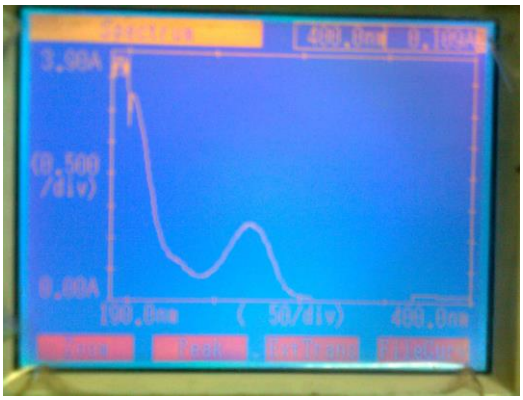


Figure 4.6.2 (Theophylline anhydrous)

Figure 4.6.3 (Ketotifen :Theophylline=1:1)

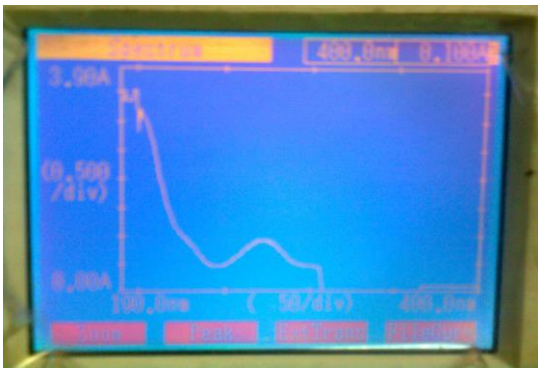


Figure 4.6.4 (Ketotifen :Theophylline=1:2)

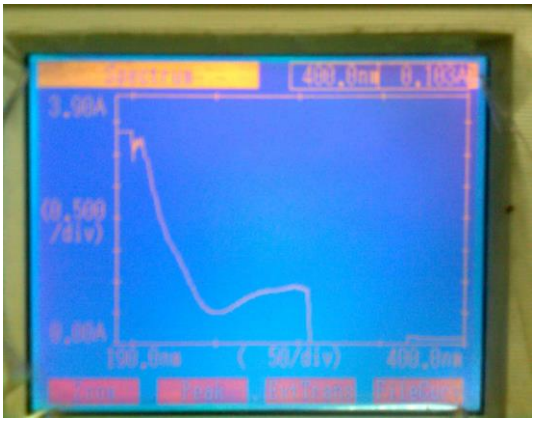


Figure 4.6.5 (Ketotifen :Theophylline=2:1)

Figure 4.6: UV spectral studies of ketotifen fumarate and theophylline at pH 6.8.

The figures (4.6.1 to 4.6.5) showed changes in absorption characteristics.

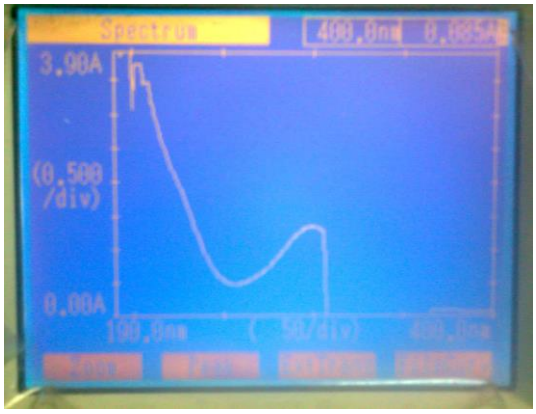


Figure 4.7.1 (Ketotifen fumarate)

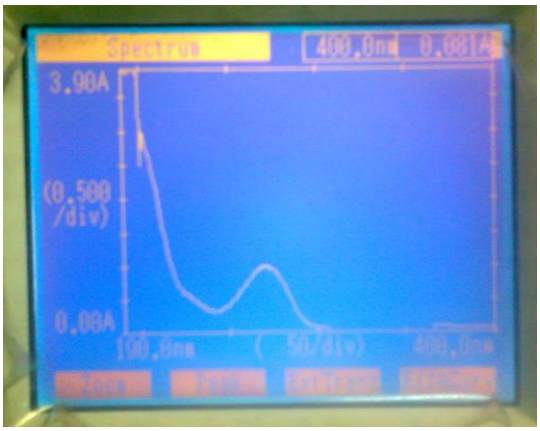


Figure 4.7.2 (Theophylline anhydrous)

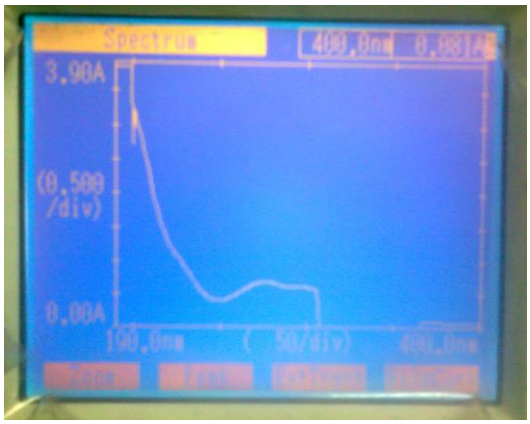


Figure 4.7.3 (Ketotifen:Theophylline=1:1)

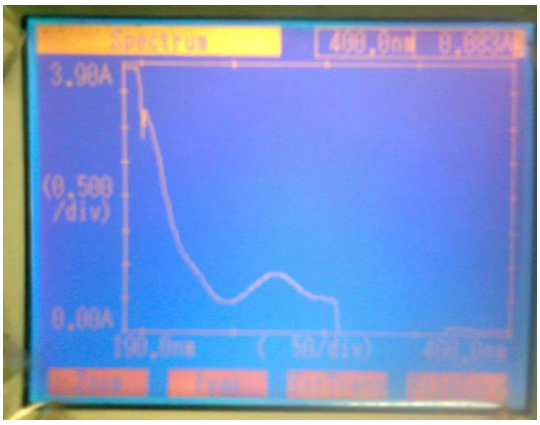


Figure 4.7.4 (Ketotifen:Theophylline=1:2)

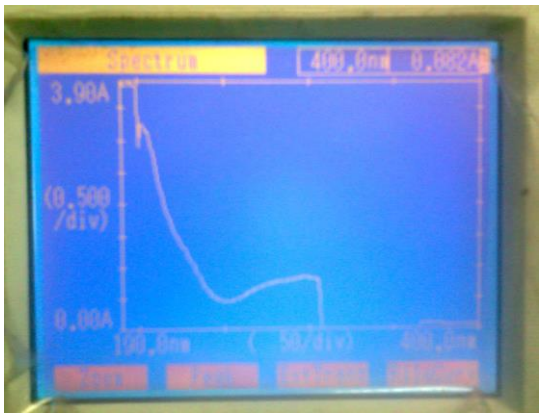


Figure 4.7.5 (Ketotifen:Theophylline=2:1)

Figure 4.7: UV spectral studies of ketotifen fumarate and theophylline at pH 7.4.

When ketotifen and theophylline were mixed together at pH 7.4 showed moderate changes in absorption characteristics as compared to their single form.

4.1.2 UV spectral studies of interaction of ketotifen fumarate & domperidone

The molecular species of ketotifen fumarate and domperidone when separately mixed showed some changes in absorption characteristics of this drug molecule including some shifts in the absorption maxima. Initial detection of complexation of ketotifen fumarate with domperidone was done from the nature of spectra of pure compounds as well as their 1:1, 1:2 and 2:1 mixtures in buffer solutions at pH 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 at a fixed concentration ($1x10^{-5}$) M. At pH 0.4 it was observed that when the spectra of ketotifen and domperidone mixtures (1:1, 1:2 & 2:1) were compared with pure drug samples individually showed changes in their absorption intensities. This is due to the interaction of ketotifen fumarate with domperidone that alter the absorption intensities as complexations occur. Similarly at pH 1.2, 2.0, 2.8, 6.0, 6.8 & 7.4 when the spectra of mixtures of ketotifen fumarate with domperidone (1:1, 1:2 and 2:1) were compared with pure drug samples ketotifen fumarate and domperidone individually showed deviation from the original lines which indicate complex formation as shown in the Figures 4.8 – 4.14.

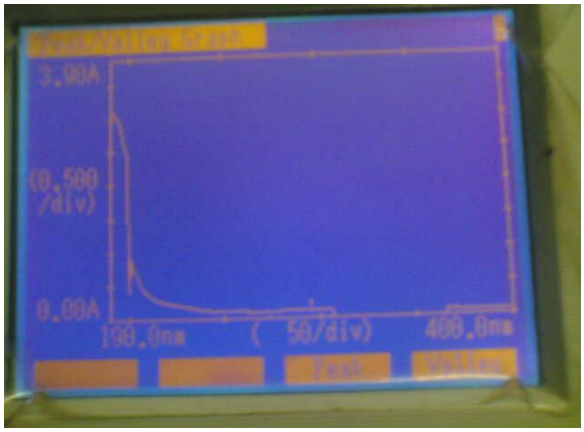


Figure 4.8.1 (Ketotifen fumarate)

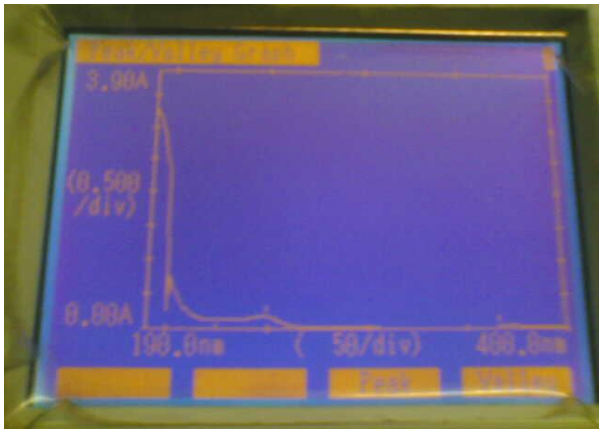


Figure 4.8.2 (Domperidone)

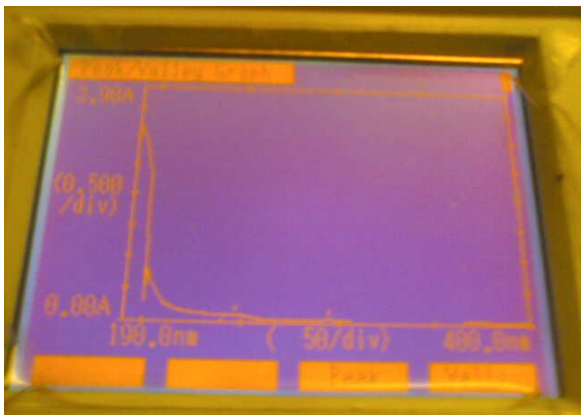


Figure 4.8.3 (Ketotifen : Domperidone=1:1)

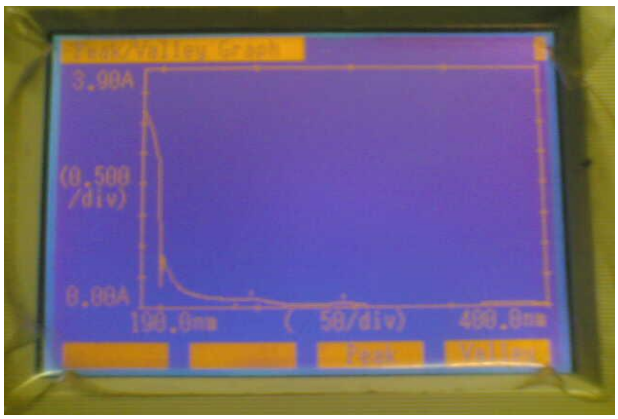


Figure 4.8.4 (Ketotifen : Domperidone=1:2)

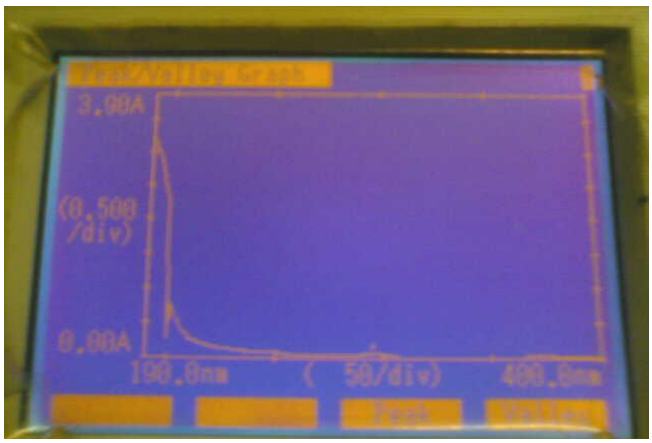


Figure 4.8.5 (Ketotifen : Domperidone=2:1)

Figure 4.8: UV spectral studies of ketotifen fumarate and domperidone at pH 0.4.

The spectra of ketotifen and domperidone mixture (1:1) showed changes in absorption characteristics when compared with the spectra of ketotifen and domperidone in single form.

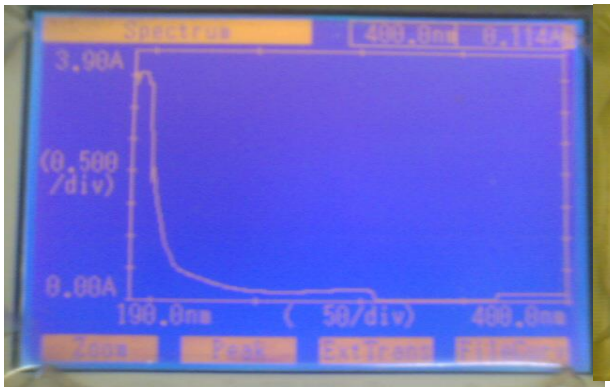


Figure 4.9.1 (Ketotifen fumarate)

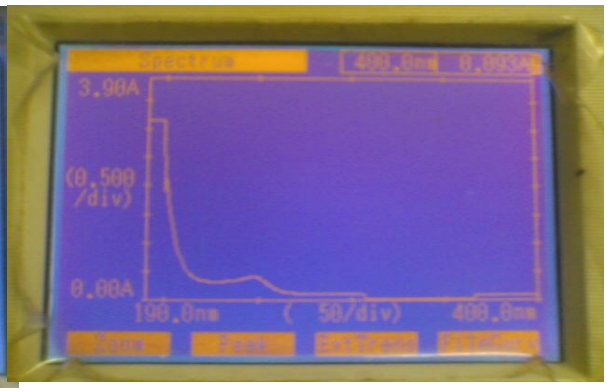


Figure 4.9.2 (Domperidone)

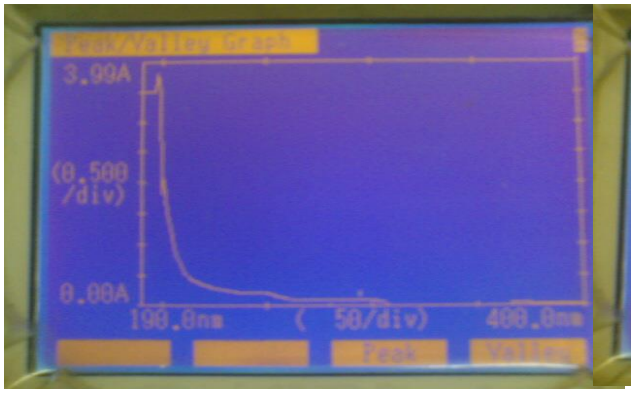


Figure 4.9.3 (Ketotifen: Domperidone=1:1)

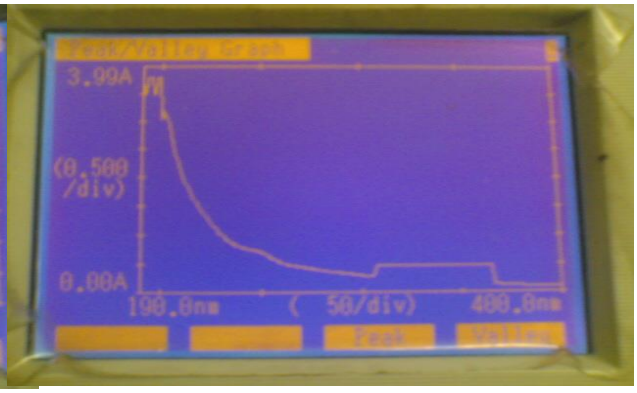


Figure 4.9.4 (Ketotifen : Domperidone=1:2)

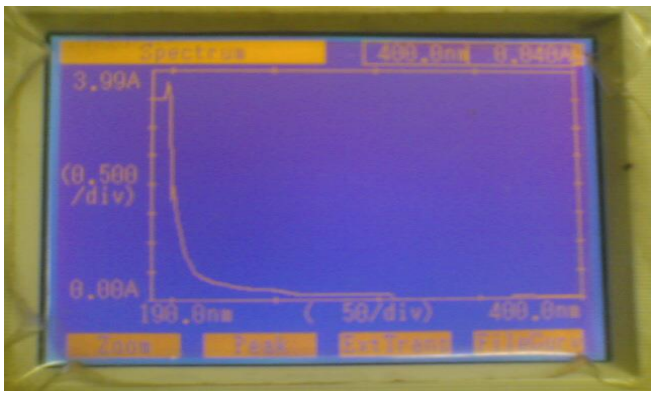


Figure 4.9.5 (Ketotifen : Domperidone=2:1)

Figure 4.9: UV spectral studies of ketotifen fumarate and domperidone at pH 1.2.

Ketotifen and domperidone mixture (1:2) at pH 1.2 showed completely different absorption characteristics when compared with the spectra of ketotifen and domperidone in single form.

Figure 4.10.1 (Ketotifen fumarate)

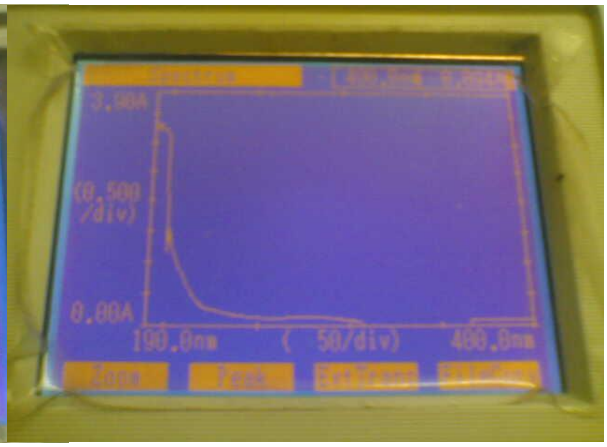


Figure 4.10.2 (Domperidone)

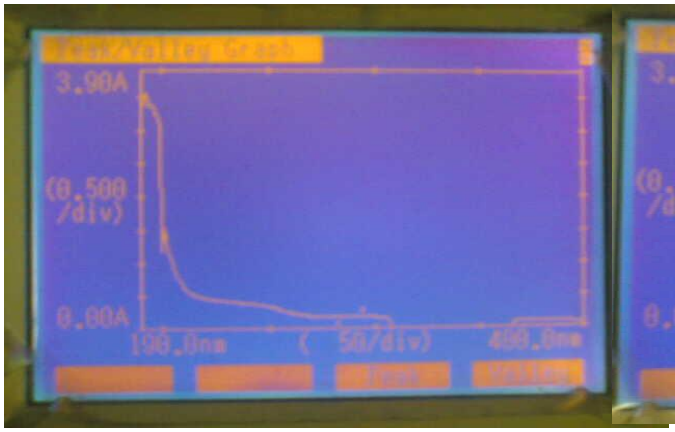


Figure 4.10.3 (Ketotifen : Domperidone=1:1)

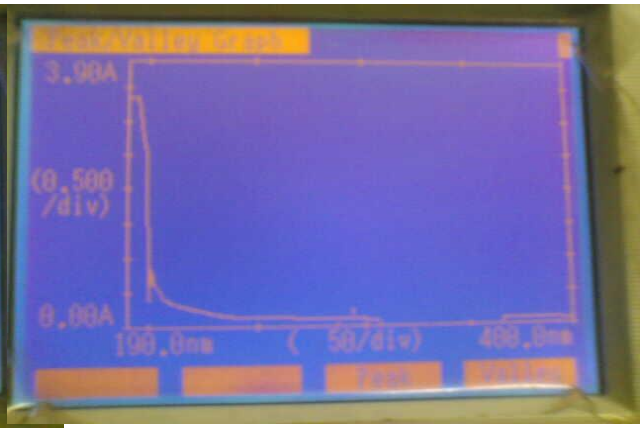


Figure 4.10.4 (Ketotifen : Domperidone=1:2)

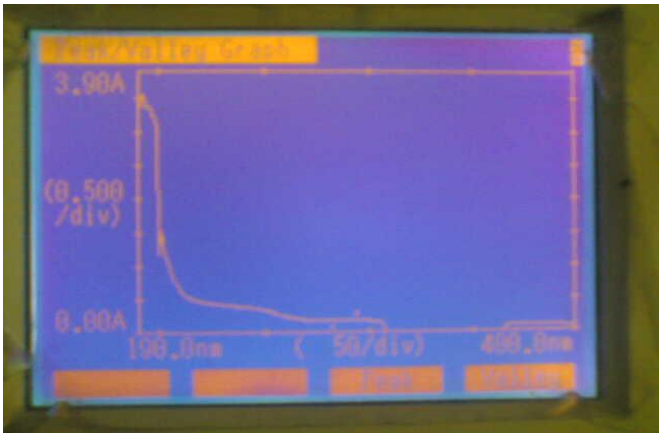


Figure 4.10.5 (Ketotifen : Domperidone=2:1)

Figure 4.10: UV spectral studies of ketotifen fumarate and domperidone at pH 2.0.

The mixtures (ketotifen : domperidone = 1:1,1:2) showed changes in absorption characteristics.

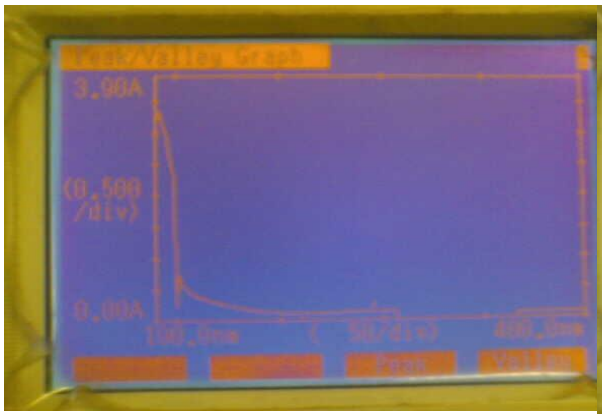


Figure 4.11.1 (Ketotifen fumarate)

Figure 4.11.2 (Domperidone)

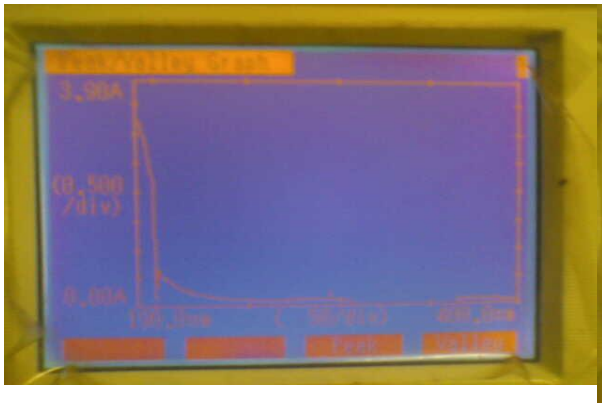


Figure 4.11.3 (Ketotifen : Domperidone=1:1)

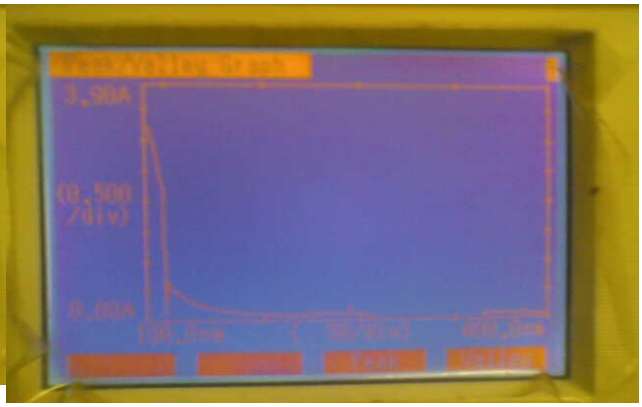


Figure 4.11.4 (Ketotifen : Domperidone=1:2)

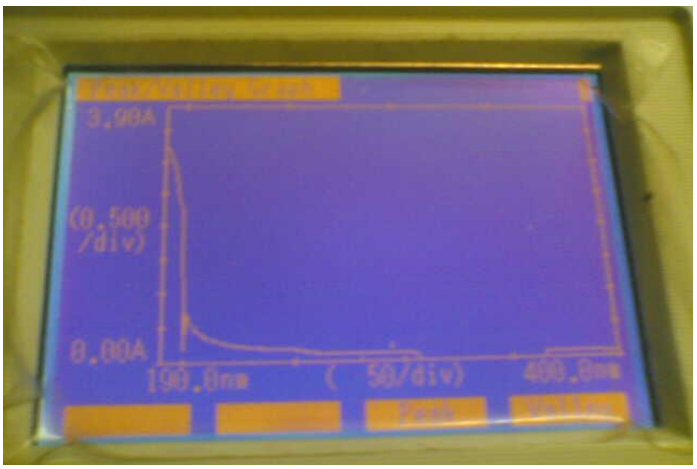


Figure 4.11.5 (Ketotifen : Domperidone=2:1)

Figure 4.11: UV spectral studies of ketotifen fumarate and domperidone at pH 2.8.

When ketotifen and domperidone were mixed together (2:1) at pH 2.8, showed no changes in absorption characteristics.

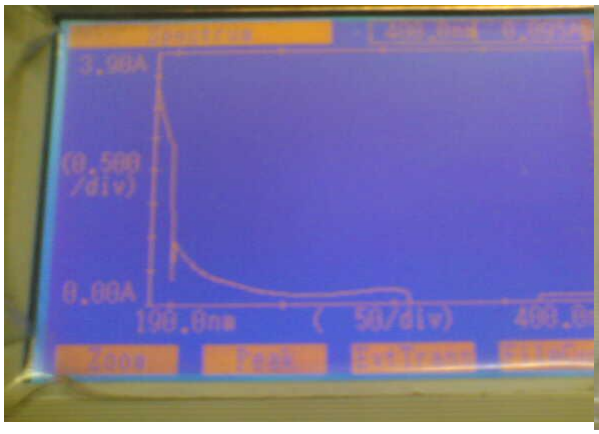


Figure 4.12.1 (Ketotifen fumarate)

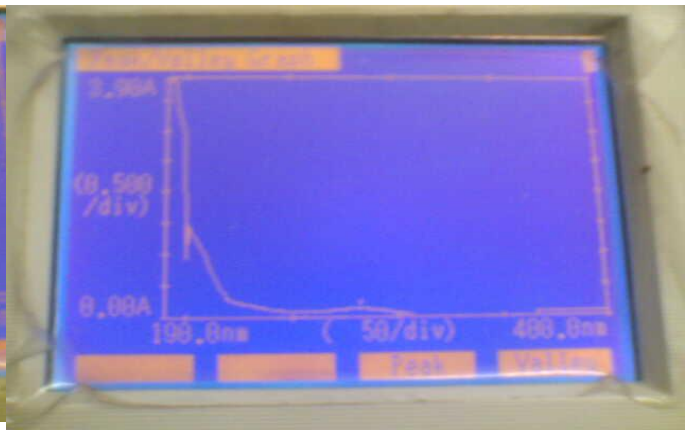


Figure 4.12.2 (Domperidone)

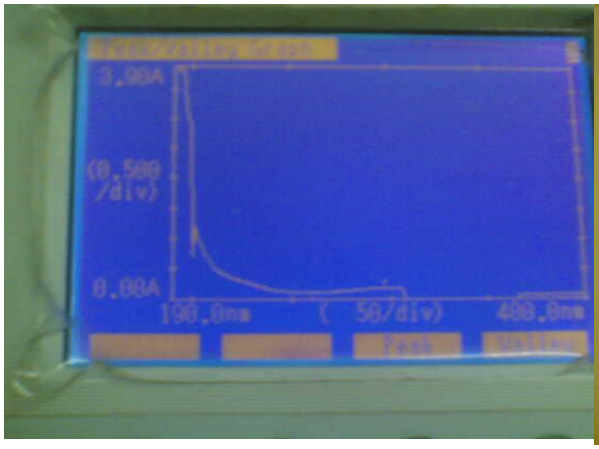


Figure 4.12.3 (Ketotifen : Domperidone=1:1)

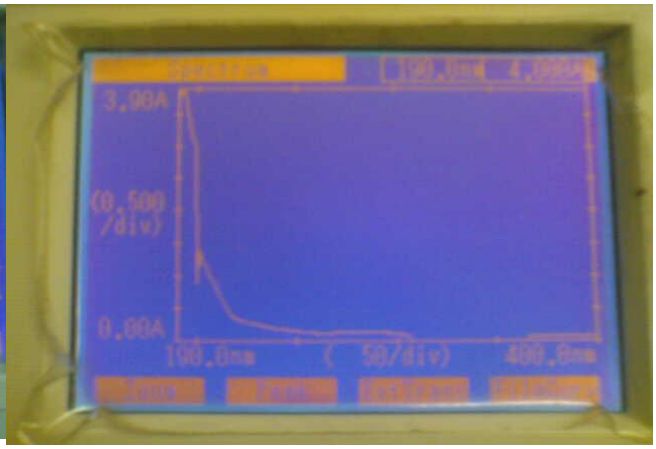


Figure 4.12.4 (Ketotifen : Domperidone=1:2)

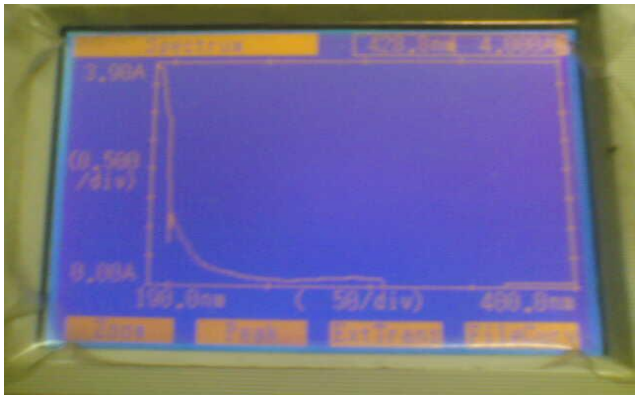


Figure 4.12.5 (Ketotifen : Domperidone=2:1)

Figure 4.12 UV spectral studies of ketotifen fumarate and domperidone at pH 6.0.

When ketotifen and domperidone were mixed together (2:1), showed changes in absorption characteristics.

Figure 4.13.1 (Ketotifen fumarate)

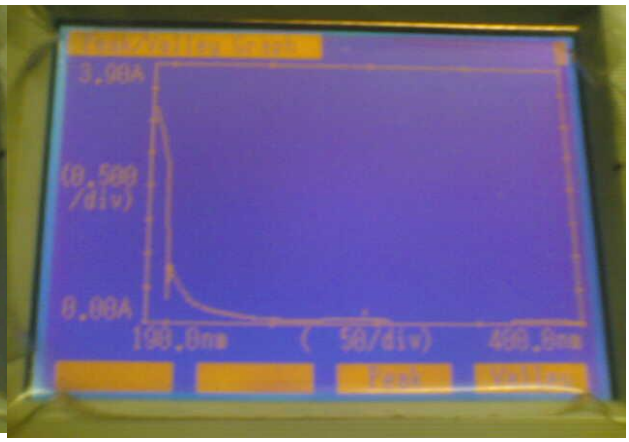


Figure 4.13.2 (Domperidone)

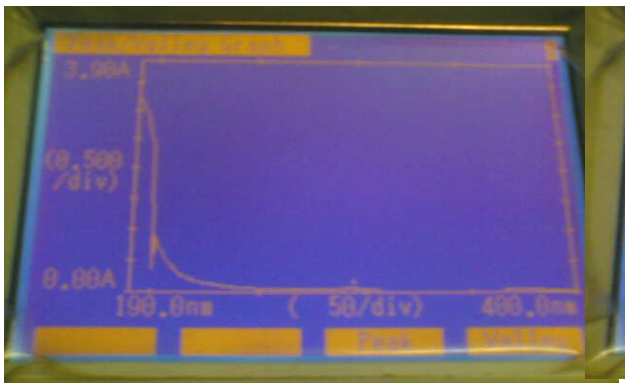


Figure 4.13.3 (Ketotifen : Domperidone=1:1)

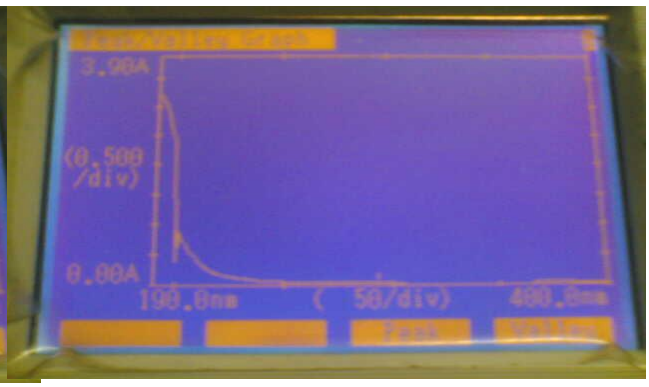


Figure 4.13.4 (Ketotifen : Domperidone=1:2)

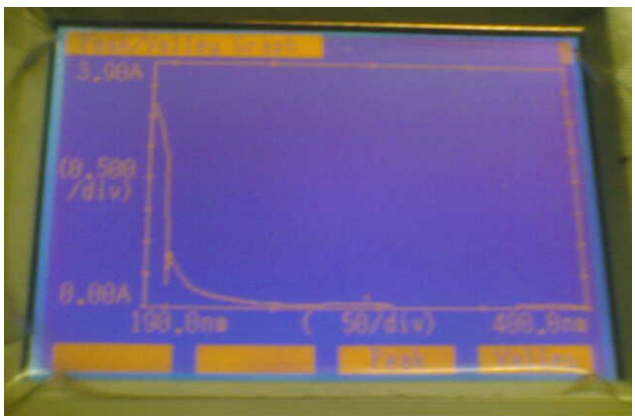


Figure 4.13.5 (Ketotifen : Domperidone=2:1)

Figure 4.13: UV spectral studies of ketotifen fumarate and domperidone at pH 6.8.

The mixtures of the above mentioned drugs at 2:1 ratio, showed changes in absorption characteristics.

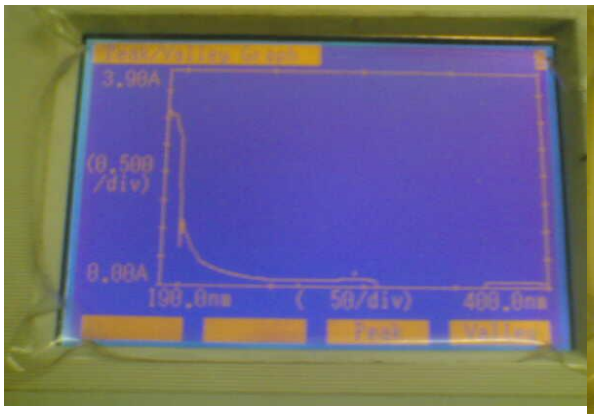


Figure 4.14.1 (Ketotifen fumarate)

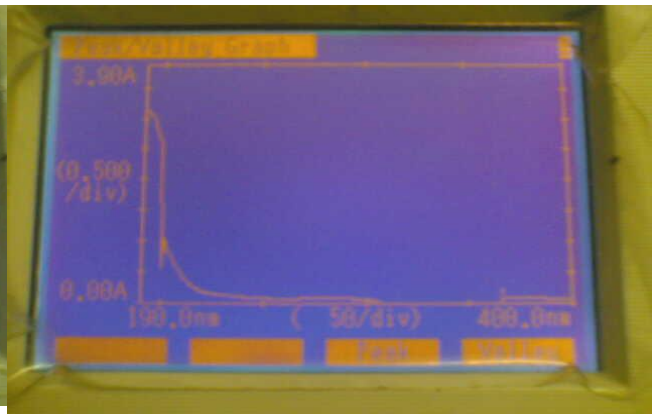


Figure 4.14.2 (Domperidone)

Figure 4.14.3 (Ketotifen : Domperidone = 1:1)

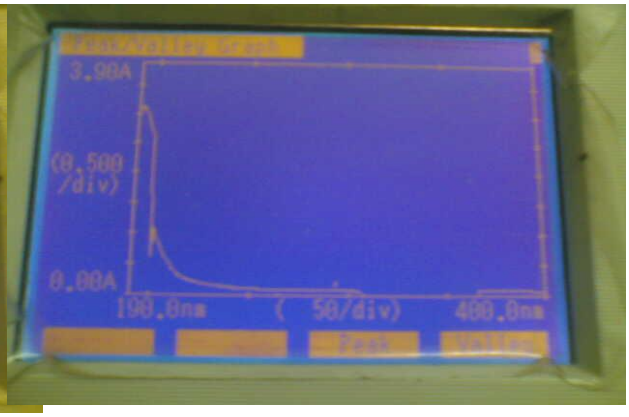


Figure 4.14.4 (Ketotifen : Domperidone = 1:2)

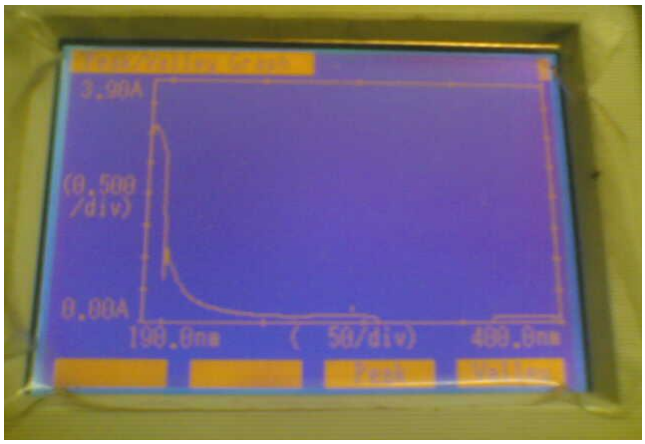


Figure 4.14.5 (Ketotifen : Domperidone = 2:1)

Figure 4.14: UV spectral studies of ketotifen fumarate and domperidone at pH 7.4.

The above figures showed changes in absorption characteristics, when ketotifen and domperidone were mixed together (1:1, 1.2).

4.2. Job's method of continuous variation

4.2.1 Effect of theophylline on ketotifen by Job's method of continuous variation at different pHs

The molar ratios of the complexes of ketotifen with theophylline were estimated by Job's spectrophotometric method of continuous variation. The observed absorbance values were measured at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 at various concentrations of $1x10^{-5}$ M to $9x10^{-5}$ M ketotifen with theophylline at 300 nm. In this method, solutions of different concentrations of ketotifen and theophylline were prepared by plotting corrected absorbance against the volume fraction of one reactant. It may be mentioned that drug solutions with identical analytical concentrations are mixed in such a way that total volume and the total moles of reactant in each mixture is constant but the mole ratio of the reactants varies systematically.

At pHs 0.4, 1.2, 2, 2.8, 6, 6.8 and 7.4 ketotifen forms strong 1:1 and 1:2 complex with theophylline. These 'V' shaped curves indicate the formation of 1:1 and 1:2 complexes of ketotifen with theophylline. These may indicate strong kinetics of complexation between ketotifen and theophylline.

Table 4.1: Values of Job's plot for complexation of ketotifen and theophylline at pH 0.4.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of theophylline ($Mx10^{-5}$)	Absorb. of theophylline (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.88	9	1.108	1.007	0.167
2	0.1	8	1.254	1.061	0.293
3	0.133	7	1.02	0.839	0.314
4	0.198	6	0.971	0.754	0.415
5	0.229	5	0.874	0.629	0.474
6	0.262	4	0.793	0.54	0.515
7	0.303	3	0.658	0.441	0.52
8	0.336	2	0.424	0.391	0.369
9	0.376	1	0.227	0.386	0.217

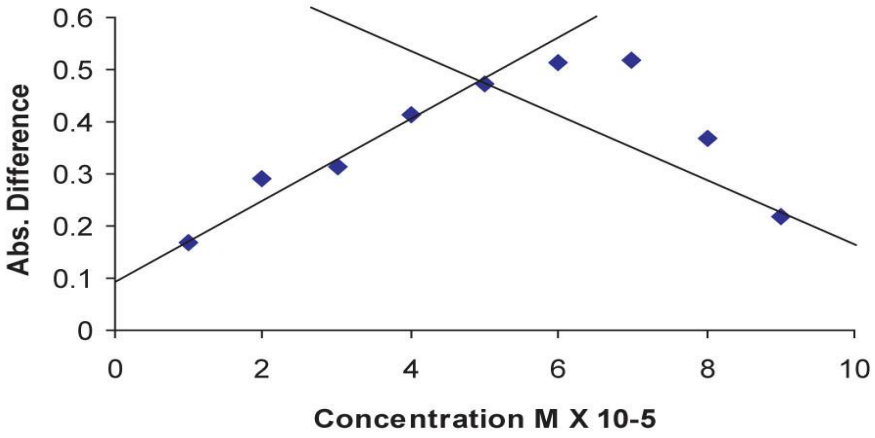


Figure 4.15: Job's plot for complexation of ketotifen and theophylline at pH 0.4.

Table 4.2: Values of Job's plot for complexation of ketotifen and theophylline at pH 1.2.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of theophylline ($Mx10^{-5}$)	Absorb. of theophylline (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.066	9	1.393	1.286	0.173
2	0.129	8	1.297	1.087	0.339
3	0.187	7	1.256	0.96	0.483
4	0.255	6	1.126	0.86	0.521
5	0.307	5	1.018	0.735	0.59
6	0.37	4	0.93	0.651	0.649
7	0.42	3	0.813	0.563	0.67
8	0.482	2	0.591	0.513	0.56
9	0.553	1	0.331	0.551	0.333

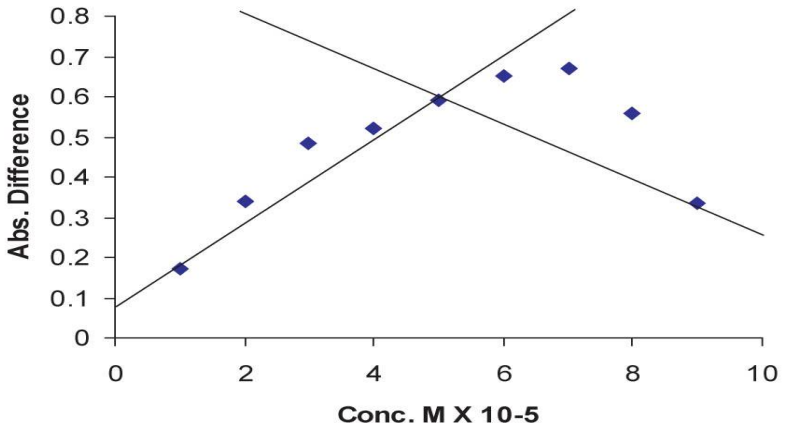


Figure 4.16: Job's plot for complexation of ketotifen with theophylline at pH 1.2.

Table 4.3: Values of job's plot for complexation of ketotifen and theophylline at pH 2.0.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of theophylline ($Mx10^{-5}$)	Absorb. of theophylline (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.065	9	0.831	0.764	0.132
2	0.118	8	0.778	0.617	0.279
3	0.177	7	0.718	0.563	0.332
4	0.236	6	0.685	0.568	0.353
5	0.295	5	0.59	0.528	0.357
6	0.344	4	0.571	0.521	0.394
7	0.401	3	0.454	0.508	0.347
8	0.459	2	0.358	0.508	0.309
9	0.567	1	0.236	0.523	0.28

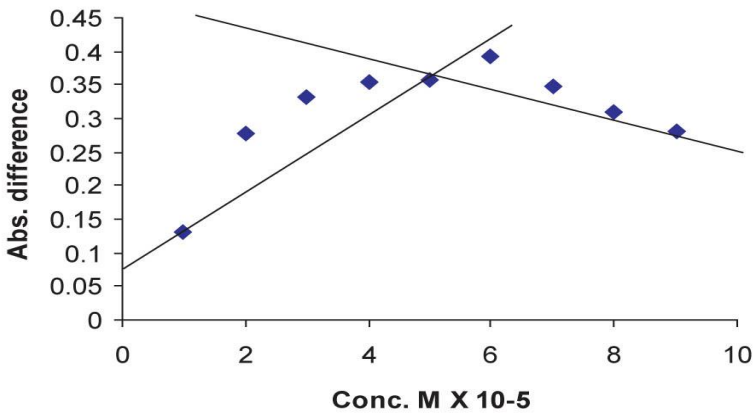


Figure 4.17: Job's plot for complexation of ketotifen with theophylline at pH 2.0.

Table 4.4: Values of job's plot for complexation of ketotifen with theophylline at pH 2.8.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of theophylline ($Mx10^{-5}$)	Absorb. of theophylline (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.078	9	2.926	2.973	0.031
2	0.136	8	2.849	2.672	0.313
3	0.209	7	2.649	2.153	0.705
4	0.271	6	2.514	1.655	1.13
5	0.339	5	2.136	1.265	1.21
6	0.393	4	1.73	0.96	1.163
7	0.461	3	1.295	0.718	1.038
8	0.516	2	0.851	0.581	0.786
9	0.579	1	0.401	0.568	0.412

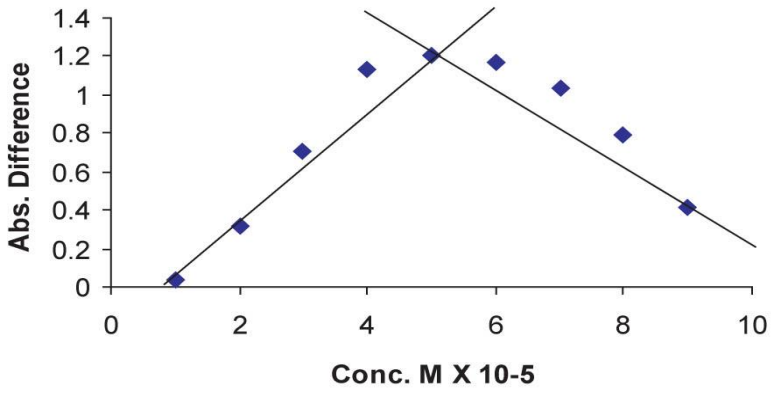


Figure 4.18: Job's plot for complexation of ketotifen with theophylline at pH 2.8.

Table 4.5: Values of job's plot for complexation of theophylline and ketotifen at pH 6.0.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of theophylline ($Mx10^{-5}$)	Absorb. of theophylline (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.052	9	2.89	2.984	-0.042
2	0.1	8	2.78	2.783	0.097
3	0.158	7	2.679	2.224	0.613
4	0.205	6	2.503	1.708	1
5	0.256	5	2.314	1.268	1.302
6	0.31	4	1.886	0.976	1.22
7	0.357	3	1.442	0.672	1.127
8	0.415	2	0.937	0.525	0.827
9	0.486	1	0.481	0.495	0.472

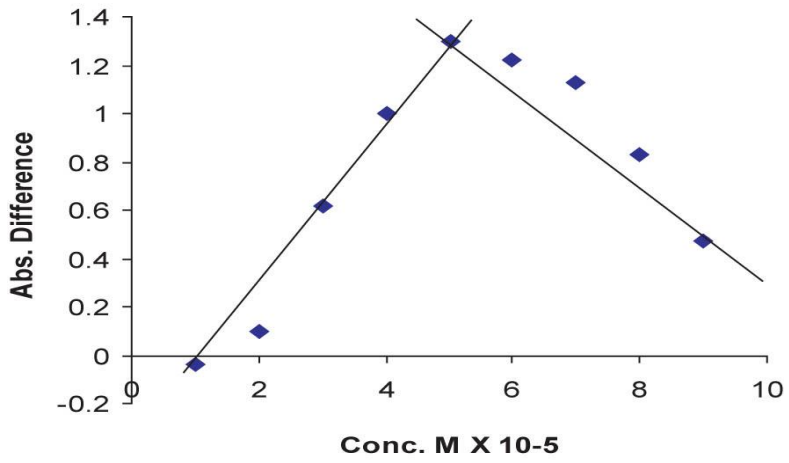


Figure 4.19: Job's plot for complexation of ketotifen with theophylline at pH 6.0.

Table 4.6: Values of job's plot for complexation of ketotifen and theophylline at pH 6.8.

Conc. of ketotifen ($M\times10^{-5}$)	Absorb. of ketotifen (A)	Conc. of theophylline ($M\times10^{-5}$)	Absorb. of theophylline (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.081	9	2.852	2.88	0.053
2	0.148	8	2.776	2.641	0.283
3	0.242	7	2.654	2.151	0.745
4	0.322	6	2.574	1.649	1.247
5	0.4	5	2.162	1.262	1.3
6	0.486	4	1.761	0.985	1.262
7	0.557	3	1.311	0.773	1.095
8	0.638	2	0.821	0.662	0.797
9	0.747	1	0.419	0.745	0.421

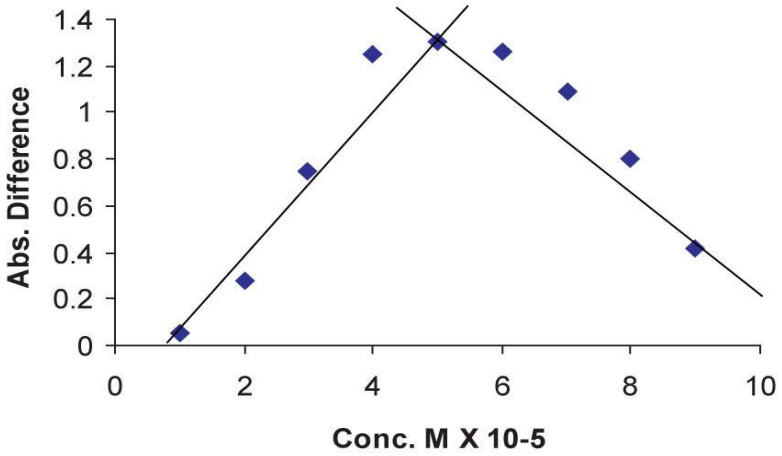


Figure 4.20: Job's plot for complexation of ketotifen with theophylline at pH 6.8.

Table 4.7: Values of job's plot for complexation of ketotifen and theophylline at pH 7.4.

Conc. of ketotifen ($M\times10^{-5}$)	Absorb. of ketotifen (A)	Conc. of theophylline ($M\times10^{-5}$)	Absorb. of theophylline (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.092	9	2.944	2.878	0.158
2	0.151	8	2.847	2.68	0.318
3	0.233	7	2.701	2.148	0.786
4	0.308	6	2.532	1.663	1.177
5	0.378	5	2.155	1.316	1.217
6	0.459	4	1.743	0.962	1.24
7	0.533	3	1.31	0.759	1.084
8	0.611	2	0.842	0.657	0.796
9	0.698	1	0.381	0.661	0.418

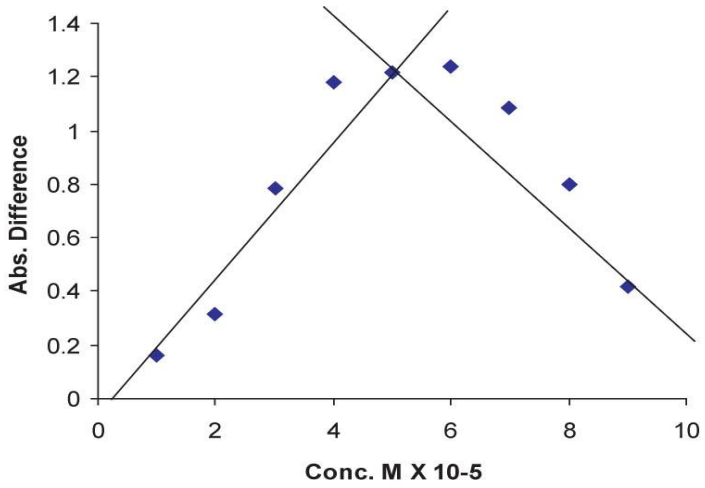


Figure 4.21: Job's plot for complexation of ketotifen with theophylline at pH 7.4.

4.2.2 Effect of desloratidine on ketotifen fumarate by Job's method of continuous variation at different pH

The molar ratios of the complexes of ketotifen fumarate with desloratidine were estimated by Job's spectrophotometric method of continuous variation. The observed absorbance values measured at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 at various concentrations of $1x10^{-5}$ M to $9x10^{-5}$ M ketotifen fumarate with desloratidine at 300 nm**.** In this method, solutions of different concentrations of ketotifen and desloratidine were prepared by plotting corrected absorbance against the volume fraction of one reactant. It may be mentioned that drug solutions with identical analytical concentrations are mixed in such a way that total volume and the total moles of reactant in each mixture is constant but the mole ratio of the reactants varies systematically. At pH 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 forms strong 1:1 complex.

Table 4.8: Values of job's plot for interaction of ketotifen with desloratidine at pH 0.4.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of desloratidine ($Mx10^{-5}$)	Absorb. of desloratidine (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.16	9	0.541	0.305	0.396
2	0.268	8	0.479	0.315	0.432
3	0.419	7	0.426	0.333	0.512
4	0.543	6	0.361	0.375	0.529
5	0.687	5	0.306	0.432	0.561
6	0.826	4	0.211	0.529	0.508
7	0.971	3	0.157	0.62	0.508
8	1.129	2	0.102	0.86	0.371
9	1.229	1	0.059	1.087	0.201

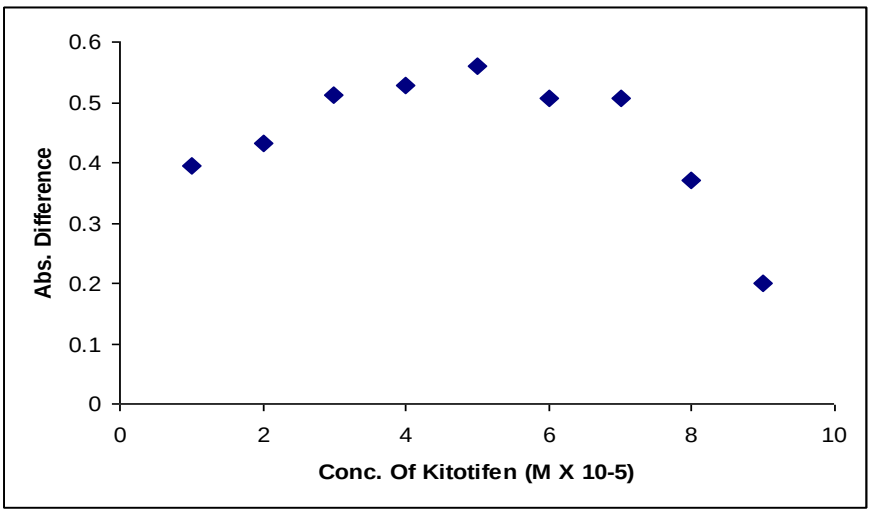


Figure 4.22: Job's plot for complexation of ketotifen with desloratidine at pH 0.4.

Table 4.9: Job's plot for complexation of ketotifen with desloratidine at pH 1.2.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of desloratidine ($Mx10^{-5}$)	Absorb. of desloratidine (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.127	9	0.634	0.352	0.409
2	0.265	8	0.55	0.367	0.448
3	0.365	7	0.495	0.372	0.488
4	0.54	6	0.412	0.397	0.555
5	0.669	5	0.321	0.449	0.541
6	0.795	4	0.273	0.484	0.584
7	1.065	3	0.22	0.843	0.442
8	1.126	2	0.136	0.962	0.3
9	1.201	1	0.084	1.071	0.214

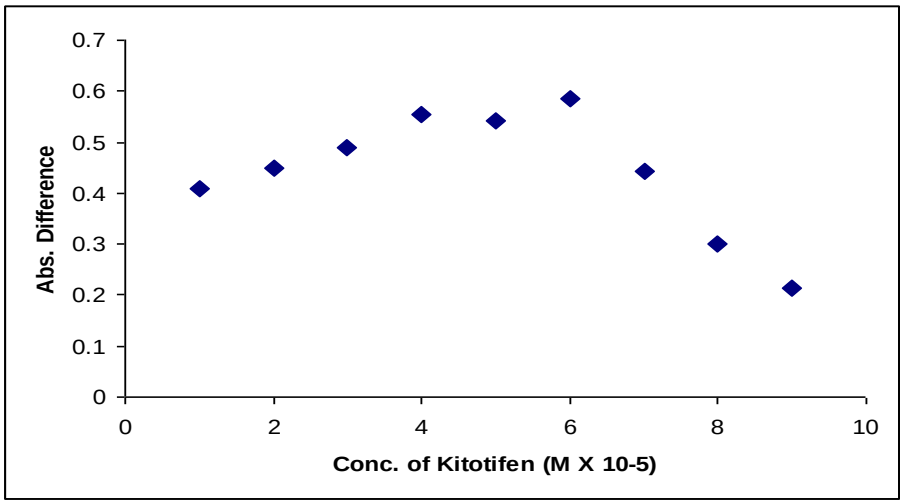


Figure 4.23: Job's plot for complexation of ketotifen with desloratidine at pH 1.2.

Table 4.10: Values of Job's plot for complexation of ketotifen with desloratidine at pH 2.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of desloratidine ($Mx10^{-5}$)	Absorb. of desloratidine (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.167	9	0.509	0.246	0.43
2	0.318	8	0.444	0.296	0.466
3	0.42	7	0.389	0.301	0.508
4	0.608	6	0.336	0.382	0.562
5	0.773	5	0.295	0.485	0.583
6	0.915	4	0.227	0.592	0.55
7	1.059	3	0.172	0.76	0.471
8	1.218	2	0.115	0.994	0.339
9	1.349	1	0.073	1.227	0.195

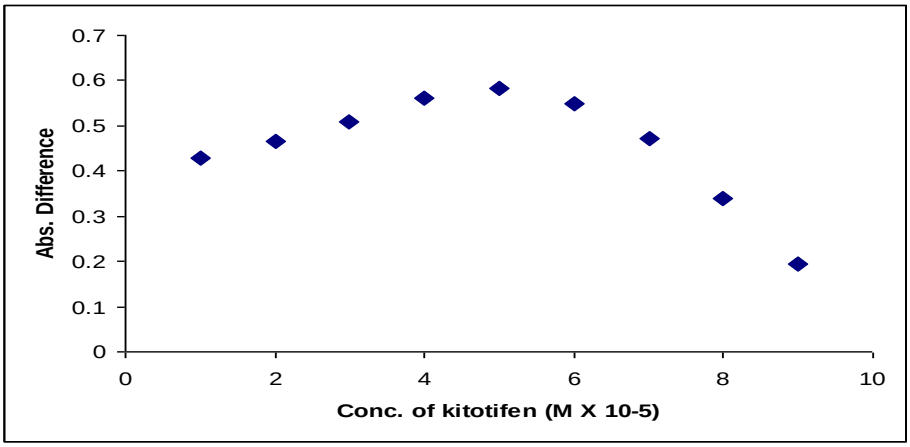


Figure 4.24: Job's plot for complexation of ketotifen fumarate with desloratidine at pH 2.

Table 4.11: Values of job's plot for complexation of ketotifen with desloratidine at pH 2.8.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of desloratidine ($Mx10^{-5}$)	Absorb. of desloratidine (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.137	9	1.15	0.207	1.08
2	0.275	8	1.001	0.238	1.038
3	0.426	7	0.835	0.285	0.976
4	0.588	6	0.707	0.37	0.925
5	0.722	5	0.563	0.449	0.836
6	0.868	4	0.444	0.552	0.76
7	0.993	3	0.333	0.704	0.622
8	1.126	2	0.208	0.824	0.51
9	1.271	1	0.096	1.082	0.285

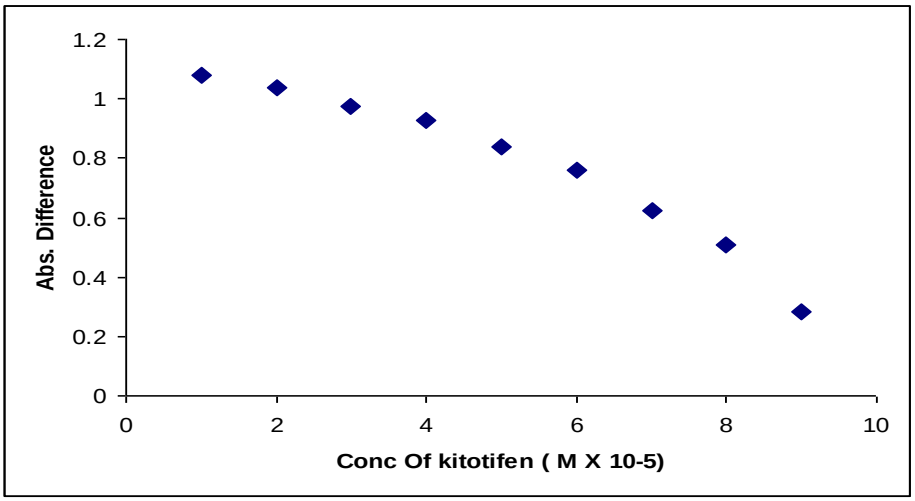


Figure 4.25: Job's plot for complexation of ketotifen with desloratidine at pH 2.8.

Table 4.12: Values of job's plot for complexation of ketotifen with desloratidine at pH 6.0.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of desloratidine ($Mx10^{-5}$)	Absorb. of desloratidine (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.115	9	1.19	0.075	1.23
2	0.295	8	1.17	0.12	1.345
3	0.471	7	1.1	0.135	1.436
4	0.623	6	0.88	0.25	1.253
5	0.702	5	0.702	0.361	1.043
6	0.88	4	0.623	0.52	0.983
7	0.973	3	0.471	0.662	0.782
8	1.19	2	0.34	0.99	0.54
9	1.271	1	0.161	1.123	0.309

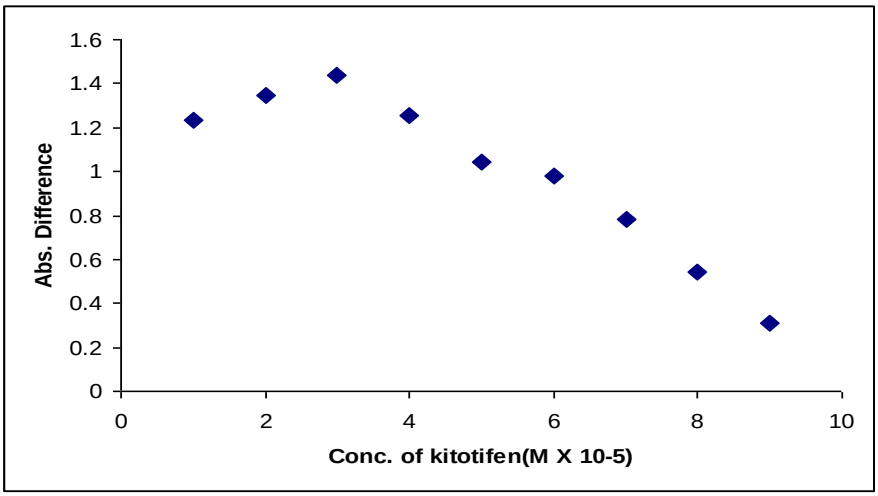


Figure 4.26: Job's plot for complexation of ketotifen fumarate with desloratidine at pH 6.

Table 4.13: Values of job's plot for complexation of ketotifen with desloratidine at pH 6.8.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of desloratidine ($Mx10^{-5}$)	Absorb. of desloratidine (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.123	9	1.198	0.03	1.291
2	0.259	8	1.061	0.069	1.251
3	0.42	7	0.824	0.127	1.117
4	0.528	6	0.745	0.215	1.058
5	0.676	5	0.595	0.323	0.948
6	0.811	4	0.464	0.451	0.824
7	0.931	3	0.353	0.636	0.648
8	1.076	2	0.23	0.806	0.5
9	1.174	1	0.112	1.01	0.276

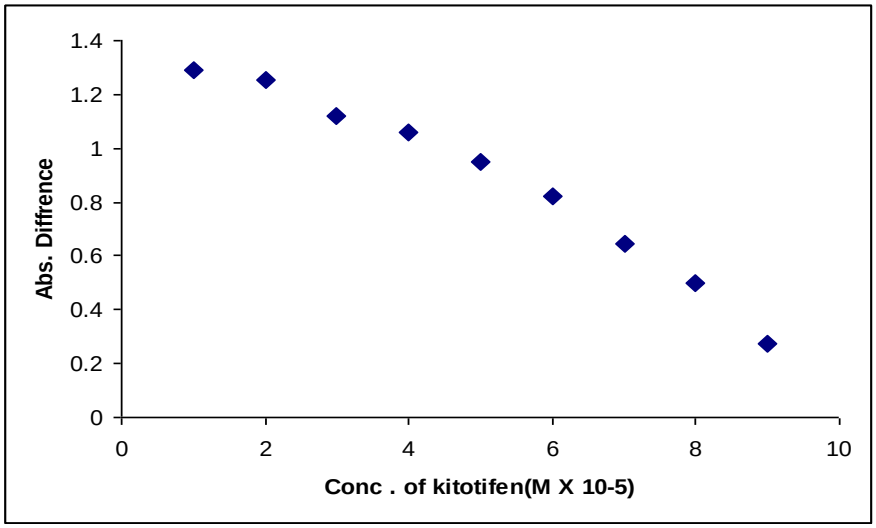


Figure 4.27: Job's plot for complexation of ketotifen fumarate with desloratidine at pH 6.8.

Table 4.14: Values of job's plot for complexation of ketotifen with desloratidine at pH 7.4.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of desloratidine ($Mx10^{-5}$)	Absorb. of desloratidine (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.125	9	1.088	0.026	1.187
2	0.254	8	0.969	0.052	1.171
3	0.38	7	0.854	0.099	1.135
4	0.527	6	0.759	0.208	1.078
5	0.661	5	0.627	0.331	0.957
6	0.782	4	0.48	0.474	0.788
7	0.902	3	0.371	0.625	0.648
8	1.031	2	0.243	0.826	0.448
9	1.132	1	0.14	0.998	0.274

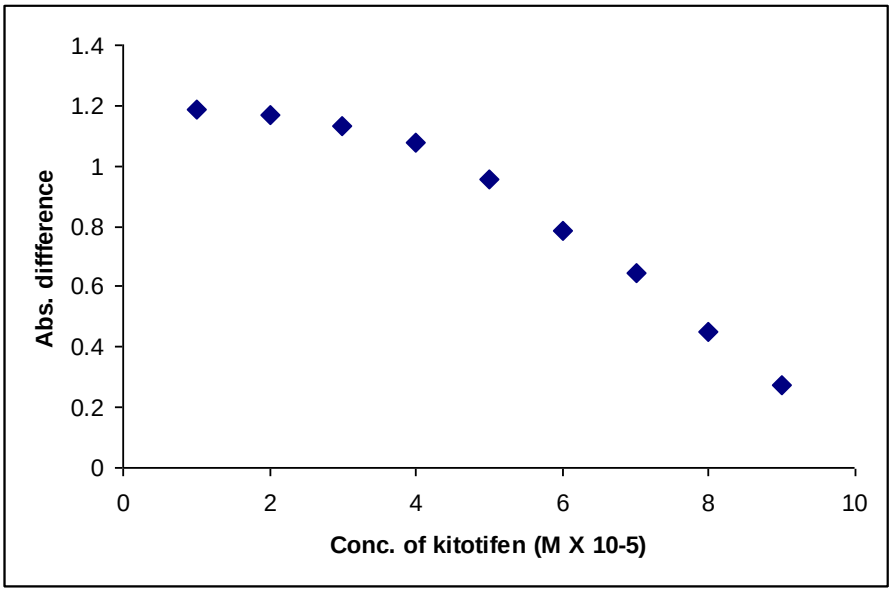


Figure 4.28: Job's plot for complexation of ketotifen with desloratidine at pH 7.4.

4.2.3 Effect of metformin hydrochloride on ketotifen fumarate by Job's method of continuous variation at different pH

The molar ratios of the complexes of ketotifen fumarate with metformin hydrochloride were estimated by Job's spectrophotometric method of continuous variation. The observed absorbance values measured in pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 at various concentrations $1x10^{-5}$ M to $9x10^{-5}$ M ketotifen fumarate with metformin hydrochloride at 300nm. At pH 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 forms strong 1:1 complex.

Table 4.15: Values of job's plot for complexation of ketotifen with metformin at pH 0.4.

Conc. of ketotifen ($Mx10^{-5}$)	Absorb. of ketotifen (A)	Conc. of metformin ($Mx10^{-5}$)	Absorb. of metformin (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.158	9	0.304	0.046	0.416
2	0.285	8	0.287	0.07	0.502
3	0.51	7	0.188	0.132	0.566
4	0.64	6	0.181	0.208	0.613
5	0.71	5	0.178	0.32	0.568
6	0.79	4	0.157	0.435	0.512
7	0.94	3	0.145	0.63	0.455
8	1.07	2	0.121	0.796	0.395
9	1.25	1	0.143	1.005	0.388

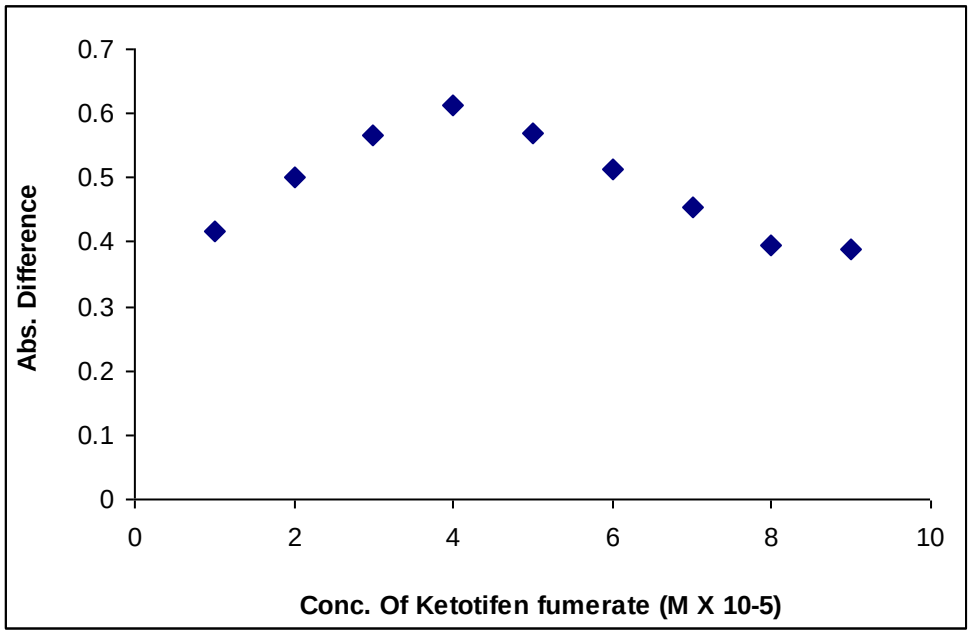


Figure 4.29: Job's plot for complexation of ketotifen with metformin at pH 0.4.

At pH 0.4, various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen were interacted with metformin hydrocloride. The breakdown in the curve at a concentration of $4x10^{-5}$ M indicates the presence of drug interaction.

Table 4.16: Values of job's plot for complexation of ketotifen with metformin at pH 1.2.

Conc. of ketotifen ($M\times10^{-5}$)	Absorb. of ketotifen (A)	Conc. of metformin ($M\times10^{-5}$)	Absorb. of metformin (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.051	9	0.301	0.042	0.31
2	0.26	8	0.184	0.065	0.379
3	0.374	7	0.201	0.127	0.448
4	0.53	6	0.178	0.204	0.504
5	0.611	5	0.188	0.316	0.483
6	0.698	4	0.154	0.431	0.421
7	0.83	3	0.141	0.625	0.346
8	0.931	2	0.135	0.786	0.28
9	1.153	1	0.121	1	0.274

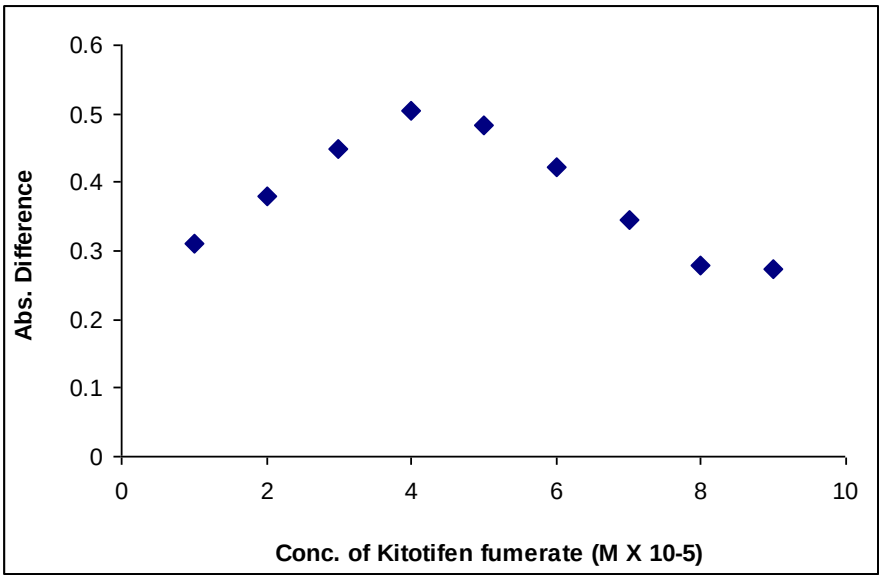


Figure 4.30: Job's plot for complexation of ketotifen fumarate with metformin at pH 1.2.

At pH 1.2, various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen were interacted with metformin hydrchloride. The imperfection of the curve at a concentration of $4x10^{-5}$ M indicates the presence of drug interaction.

Table 4.17: Values of job's plot for complexation of ketotifen with metforminat pH 2.0.

Conc. of ketotifen ($M\times10^{-5}$)	Absorb. of ketotifen (A)	Conc. of metformin ($M\times10^{-5}$)	Absorb. of metformin (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.148	9	0.497	0.027	0.618
2	0.281	8	0.445	0.057	0.669
3	0.413	7	0.419	0.125	0.707
4	0.551	6	0.374	0.218	0.707
5	0.682	5	0.322	0.343	0.661
6	0.819	4	0.286	0.503	0.602
7	0.936	3	0.251	0.691	0.496
8	1.06	2	0.193	0.891	0.362
9	1.197	1	0.027	1.139	0.085

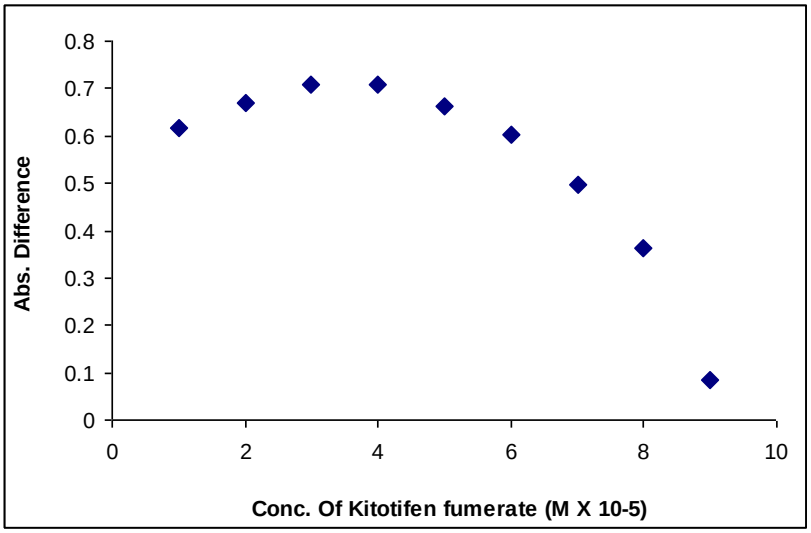


Figure 4.31: Job's plot for complexation of ketotifen fumarate with metformin at pH 2.0.

Ketotifen fumarate and metformin hydrochloride were interacted at various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M at pH 2.0. It is observed that there has no clear cut symptom of drug interaction.

Table 4.18: Values of job's plot for complexation of ketotifen with metforminat pH 2.8.

Conc. of Ketotifen ($M\times10^{-5}$)	Absorb. of Ketotifen (A)	Conc. of Metformin ($M\times10^{-5}$)	Absorb. of Metformin (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.031	9	0.507	0.031	0.507
2	0.272	8	0.455	0.067	0.66
3	0.5	7	0.429	0.135	0.794
4	0.72	6	0.384	0.228	0.876
5	0.75	5	0.332	0.353	0.729
6	0.81	4	0.296	0.513	0.593
7	0.89	3	0.261	0.7	0.451
8	1.009	2	0.203	0.899	0.313
9	1.181	1	0.175	1.149	0.207

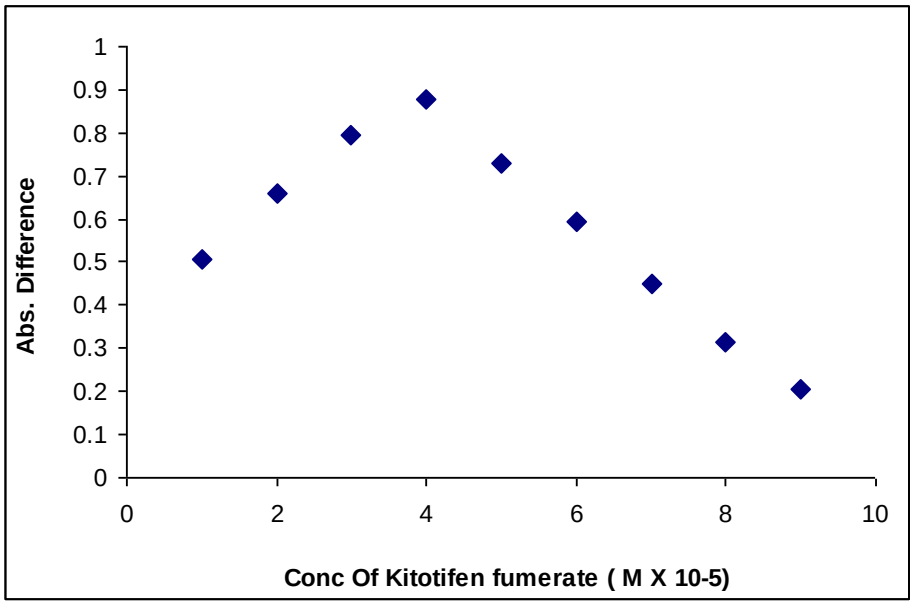


Figure 4.32: Job's plot for complexation of ketotifen fumarate with metformin at pH 2.8.

At pH 2.8, various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen were interacted with metformin hydrochloride. The sharp 'V' shaped angle in the curve at a concentration of $4x10^{-5}$ M indicates the presence of drug interaction.

Table 4.19: Values of job's plot for complexation of ketotifen with metforminat pH 6.0.

Conc. of ketotifen ($M\times10^{-5}$)	Absorb. of ketotifen (A)	Conc. of metformin ($M\times10^{-5}$)	Absorb. of metformin (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.081	9	0.869	0.138	0.812
2	0.34	8	0.779	0.208	0.911
3	0.48	7	0.729	0.248	0.961
4	0.63	6	0.654	0.351	0.933
5	0.72	5	0.551	0.46	0.811
6	0.83	4	0.46	0.612	0.678
7	0.926	3	0.371	0.786	0.511
8	1.087	2	0.28	0.99	0.377
9	1.233	1	0.199	1.211	0.221

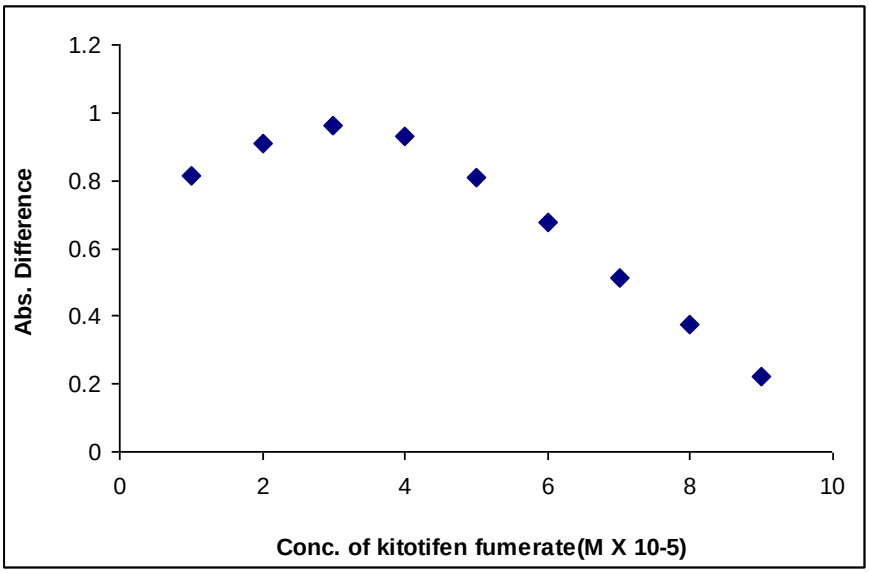


Figure 4.33: Job's plot for complexation of ketotifen fumarate with metformin at pH 6.0.

At pH 6.0, various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen were interacted with metformin. The absence of particular breakdown in the curve indicates that there is no drug interactions happen.

Table 4.20: Values of job's plot for complexation of ketotifen with metformin at pH 6.8.

Conc. of ketotifen ($M\times10^{-5}$)	Absorb. of ketotifen (A)	Conc. of metformin ($M\times10^{-5}$)	Absorb. of metformin (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.198	9	0.769	0.038	0.929
2	0.293	8	0.7	0.108	0.885
3	0.391	7	0.629	0.149	0.871
4	0.526	6	0.554	0.251	0.829
5	0.668	5	0.451	0.36	0.759
6	0.806	4	0.36	0.512	0.654
7	0.941	3	0.271	0.686	0.526
8	1.13	2	0.18	0.89	0.42
9	1.207	1	0.099	1.11	0.196

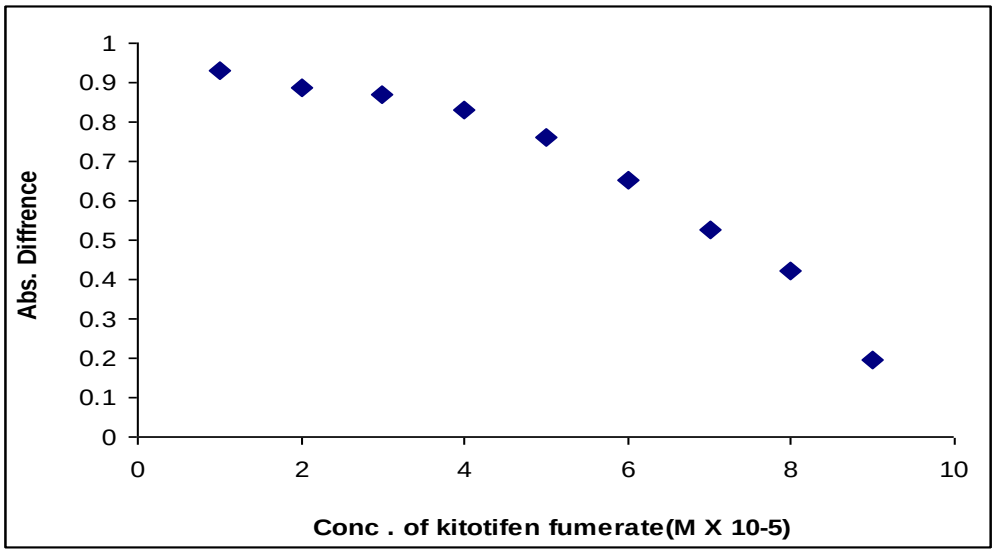


Figure 4.34: Job's plot for complexation of ketotifen fumarate with metformin at pH 6.8.

At pH 6.8, various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen were interacted with metformin. The absence of particular breakdown in the curve indicates that there is no drug interactions happen.

Table 4.21: Values of job's plot for complexation of ketotifen with metformin at pH 7.4.

Conc. of ketotifen ($M\times10^{-5}$)	Absorb. of ketotifen (A)	Conc. of metformin ($M\times10^{-5}$)	Absorb. of metformin (B)	Absorb. of mixture (C)	Absorb. difference D=(A+B)-C
1	0.123	9	1.096	0.204	1.015
2	0.229	8	1.026	0.24	1.015
3	0.321	7	0.928	0.269	0.98
4	0.457	6	0.847	0.371	0.933
5	0.555	5	0.702	0.461	0.796
6	0.681	4	0.684	0.624	0.741
7	0.878	3	0.638	0.825	0.691
8	1.009	2	0.628	1.01	0.627
9	1.121	1	0.423	1.221	0.323

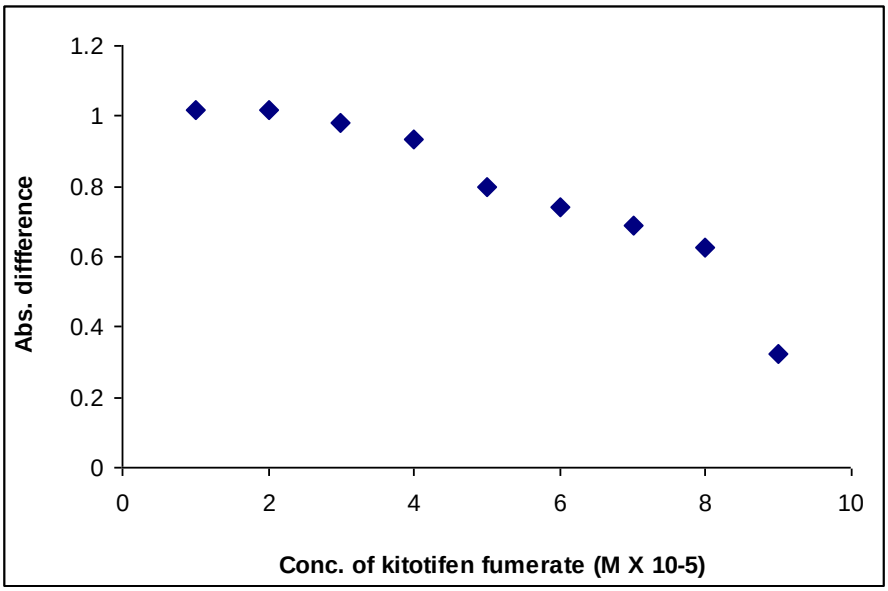


Figure 4.35: Job's plot for complexation of ketotifen with metformin at pH 7.4.

At pH 7.4, various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen were interacted with metformin. The breakdown in curve indicates the presence of drug interaction.

4.2.4 Effect of domperidone on ketotifen fumarate by Job's method of continuous variation at different pH

The observed absorbance values were measured by Job's continuous variation method at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 of various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M. After mixing the absorbance of the solutions were measured at 300 nm. The absorbance's of pure drugs as well as their various concentrations mixture gave immense idea about complexation.

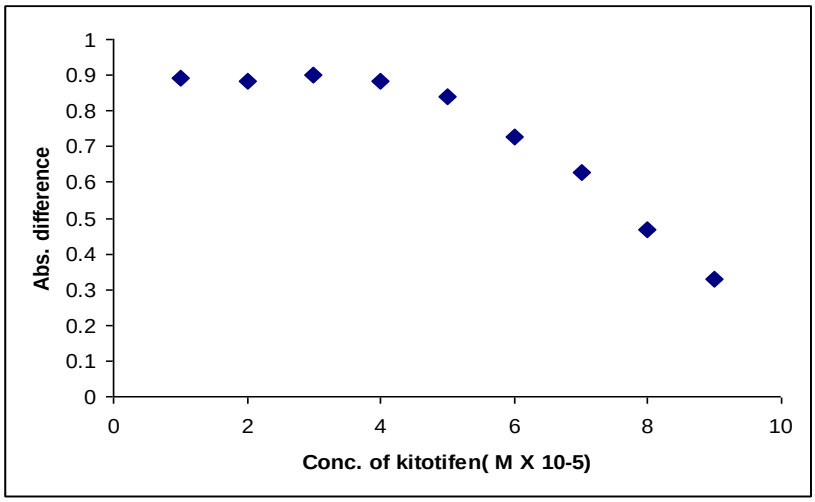


Figure 4.36: Job's plot for complexation of ketotifen with domperidone at pH 0.4.

At pH 0.4, various concentrations comprising $1x10^{-5}$M to $9x10^{-5}$ M of ketotifen fumarate were interacted with domperidone. The presence of particular breakdown in the curve at $3x10^{-5}$ M to $5x10^{-5}$ M concentration of ketotifen indicates the presence of drug-drug interaction.

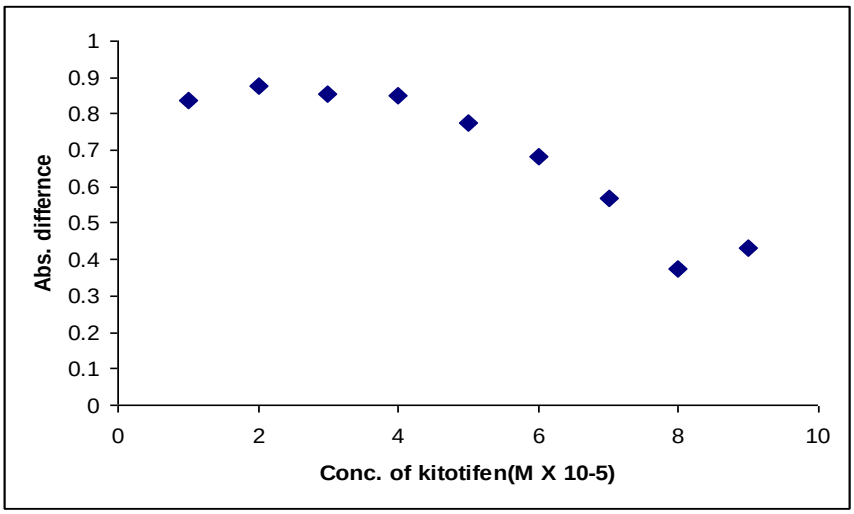


Figure 4.37: Job's plot for complexation of ketotifen with domperidone at pH 1.2.

At pH 1.2, the sharp 'V' shaped angle in the curve at a concentration of $8x10^{-5}$ M indicates the presence of drug interaction.

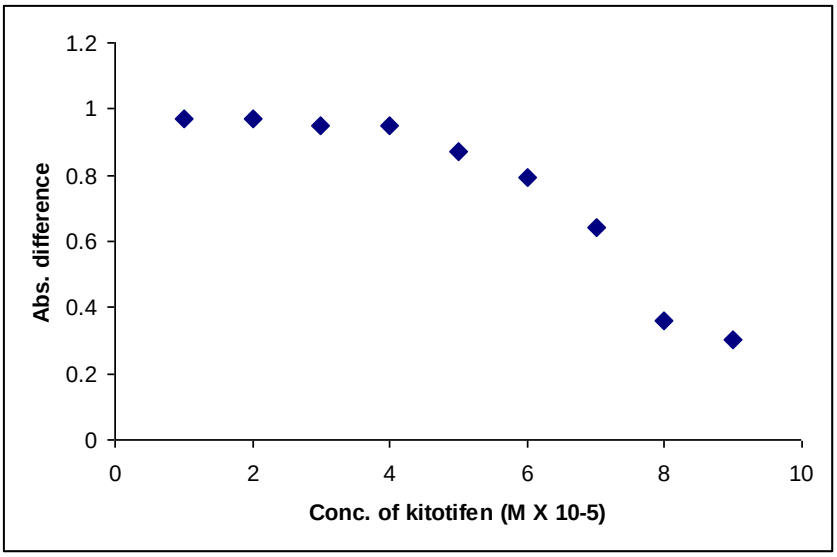


Figure 4.38: Job's plot for complexation of ketotifen with domperidone at pH 2.0.

At pH 2.0, various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen fumarate were interacted with domperidone. The presence of particular breakdown in the curve at $4x10^{-5}$ M and $8x10^{-5}$ M concentrations of ketotifen means the presence of drug-drug interaction.

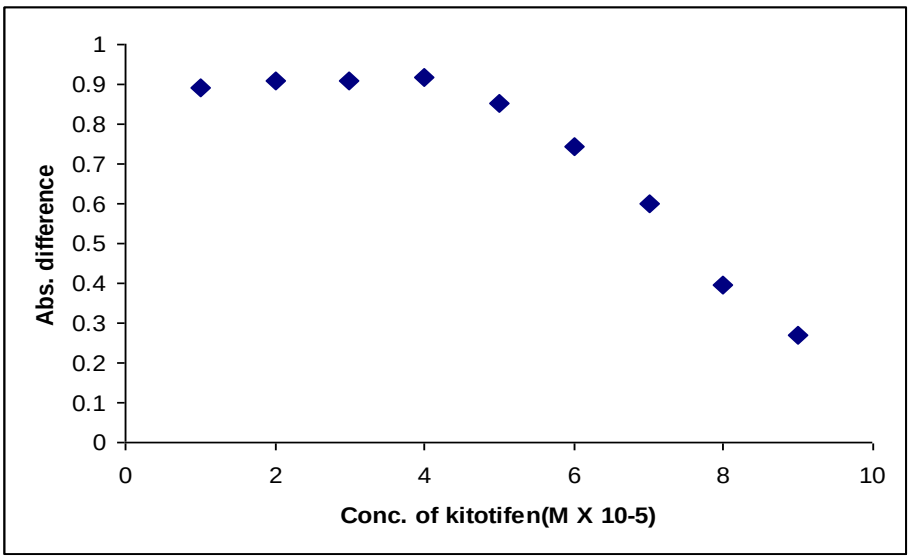


Figure 4.39: Job's plot for complexation of ketotifen with domperidone at pH 2.8.

At pH 2.8, various concentrations, $1x10^{-5}$ M to $9x10^{-5}$ M, of ketotifen fumarate were interacted with domperidone. The presence of particular breakdown in the curve at concentration of ketotifen ($4x10^{-5}$ M), indicates the presence of drug interaction.

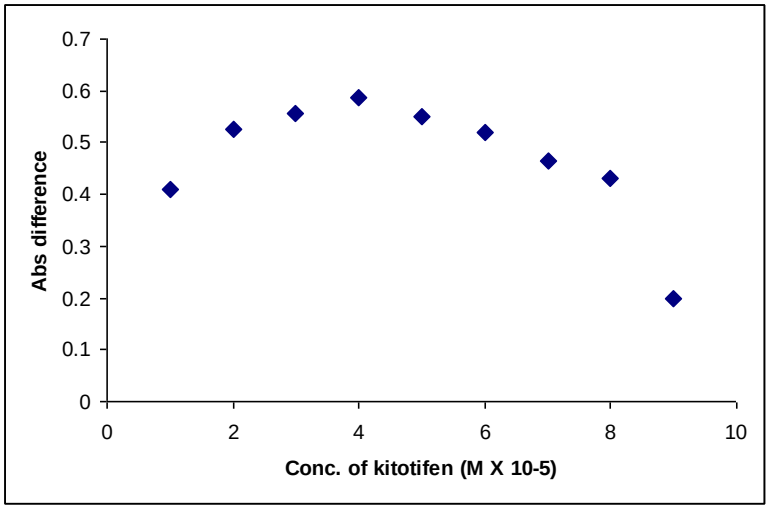


Figure 4.40: Job's plot for complexation of ketotifen with domperidone at pH 6.0.

At pH 6.0, various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen fumarate were interacted with domperidone. The presence of clear breakdown in the curve at $4x10^{-5}$ M and $8x10^{-5}$ M concentrations, indicate the presence of drug interaction.

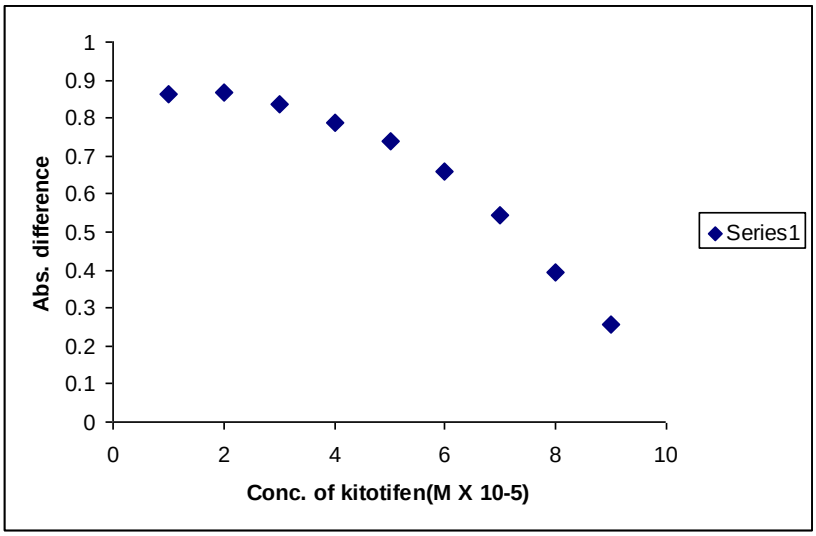


Figure 4.41: Job's plot for complexation of ketotifen with domperidone at pH 6.8.

At pH 6.8, various concentrations, $1X10^{-5}$ M to $9X10^{-5}$ M, of ketotifen were interacted with domperidone. No drug interaction was observed at various concentrations.

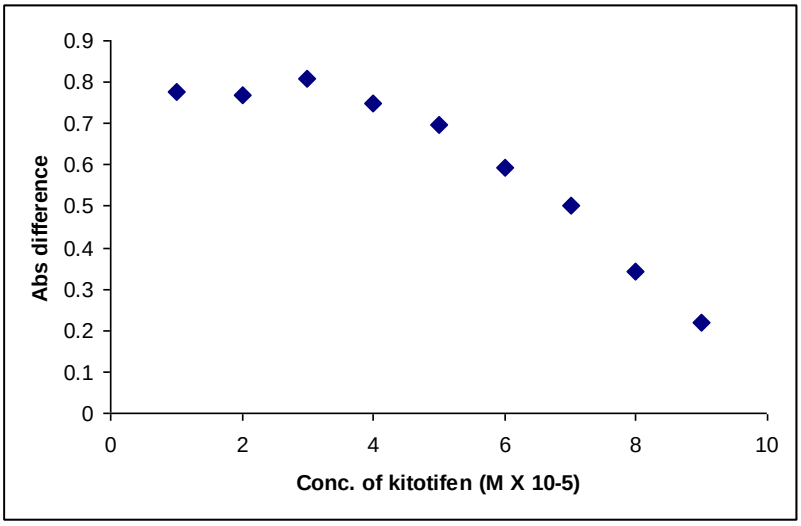


Figure 4.42: Job's plot for complexation of ketotifen with domperidone at pH 7.4.

At pH 7.4, concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M. There is a clear cut interaction observed at $3x10^{-5}$ M concentration when ketotifen was interacted with domperidone.

4.2.5 Effect of chlorpheniramine maleate on ketotifen fumarate by Job's method of continuous variation at different pH

The molar ratios of the complexes of ketotifen fumarate with chlorpheniramine maleate were estimated by Job's spectrophotometric method of continuous variation. The observed absorbance values measured at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4 at various concentrations $1x10^{-5}$ M to $9x10^{-5}$ M ketotifen fumarate with chlorpheniramine maleate at 300 nm.

At pH 0.4, various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen were interacted with chlorpheniramine maleate. The breakdown in the curve at a concentration of ketotifen $4x10^{-5}$ M indicates the presence of drug interaction. But at pH 1.2, 2.0 and 2.8 it is observed that there was no drug interaction occur. On the other hand at high pH (pH 6.0 and 6.8), the breakdown in the curve at a concentrations of ketotifen $4x10^{-5}$ indicate the presence of interaction. At pH 7.4, various concentrations comprising $1x10^{-5}$ M to

$9x10^{-5}$ M of ketotifen were interacted with chlorpheniramine maleate. The absence of particular breakdown indicates the absence of drug interaction.

4.3 Ardon's method

4.3.1 Effect of theophylline on ketotifen using Ardon's method

Ardon's plot confirmed the formation of 1:1 complex of ketotifen and theophylline at pHs 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4, since the method is valid only for 1:1 complexes. This experiment was performed in buffer systems at pHs 0.4, 1.2, 2, 2.8, 6, 6.8 and 7.4. The data for Ardon's plot was given straight lines with intercepts which are presented in figure indicating the formation of 1:1 complexes for all systems.

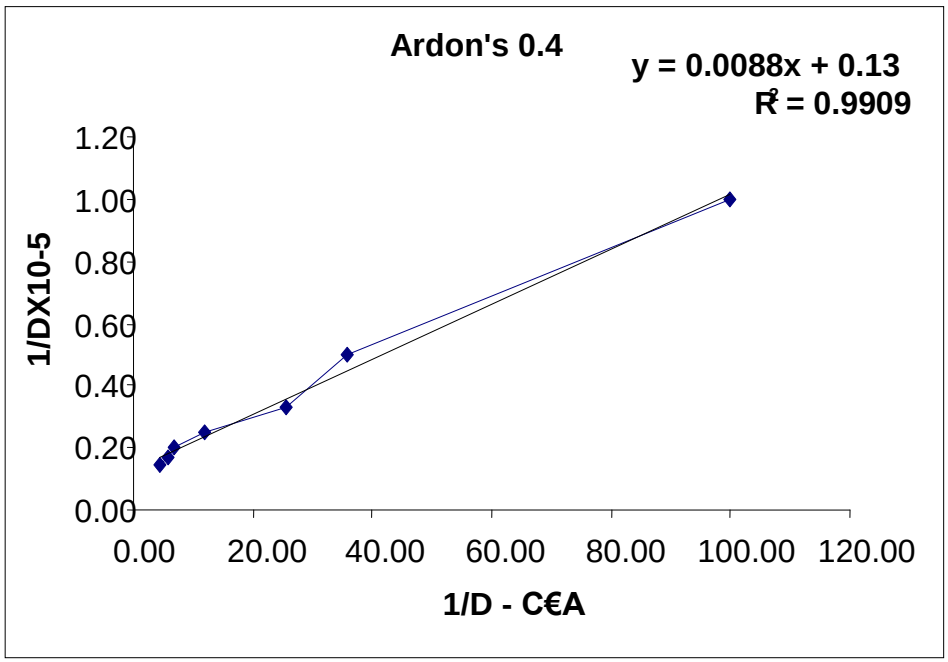


Figure 4.43: Ardon's plot for ketotifen and theophylline at pH 0.4.

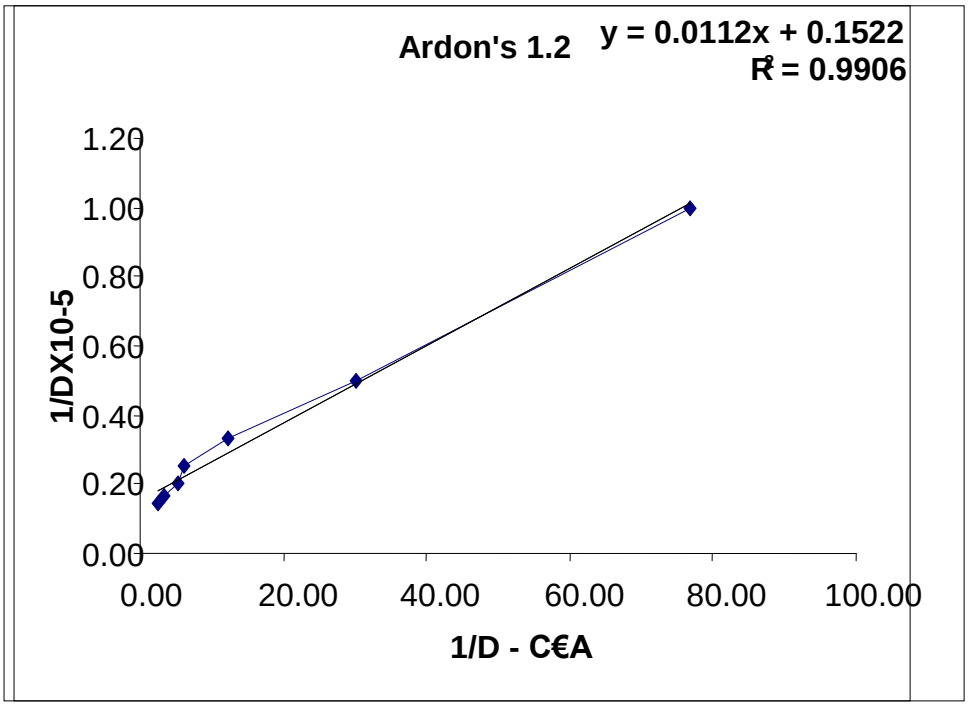


Figure 4.44: Ardon's plot for ketotifen and theophylline system at pH 1.2.

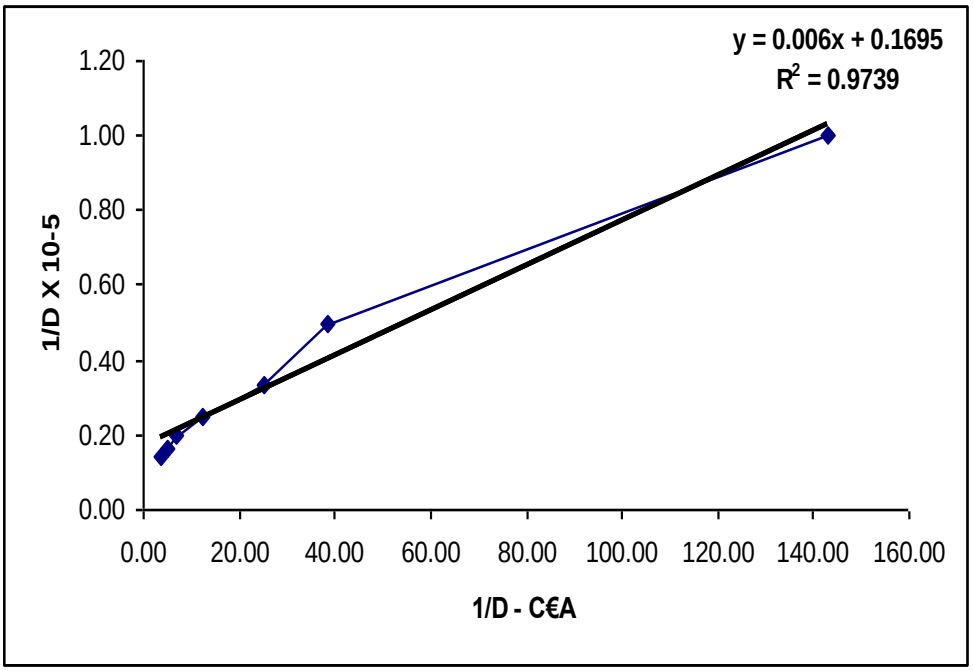


Figure 4.45: Ardon's plot for ketotifen and theophylline system at pH 2.

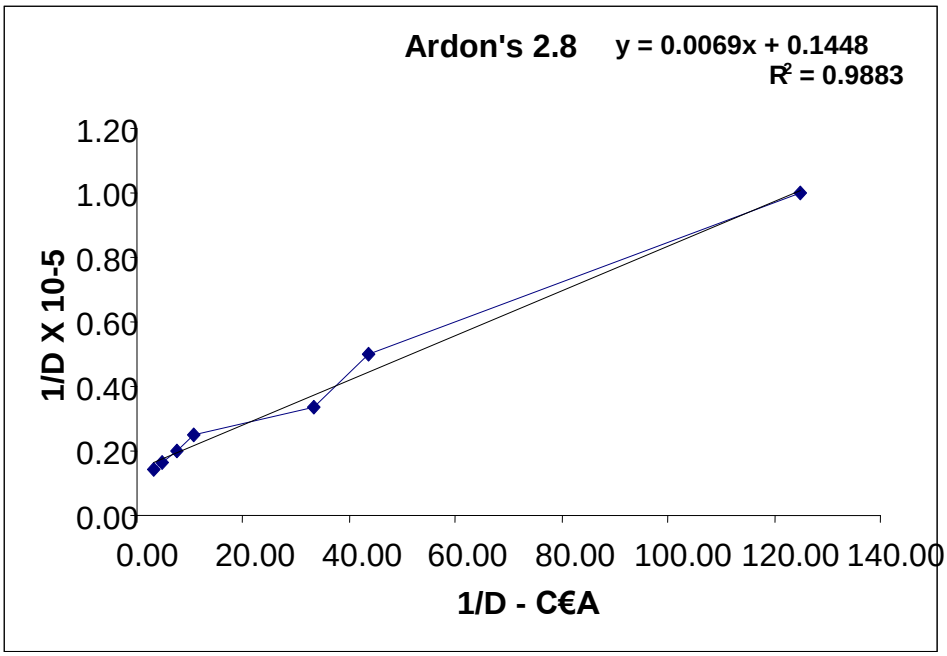


Figure 4.46: Ardon's plot for ketotifen and theophylline system at pH 2.8.

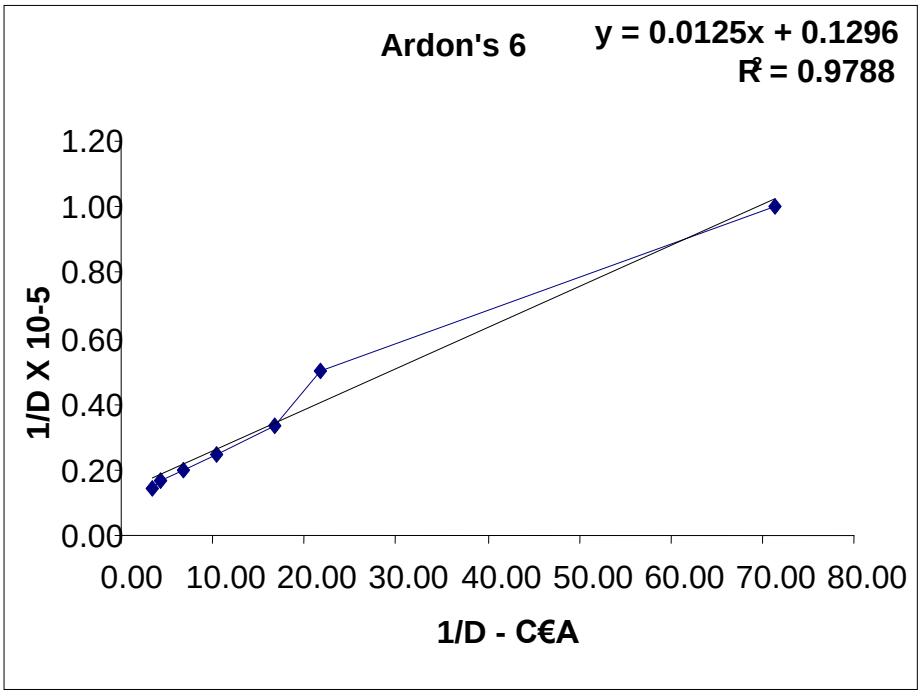


Figure 4.47: Ardon's plot for ketotifen and theophylline system at pH 6.

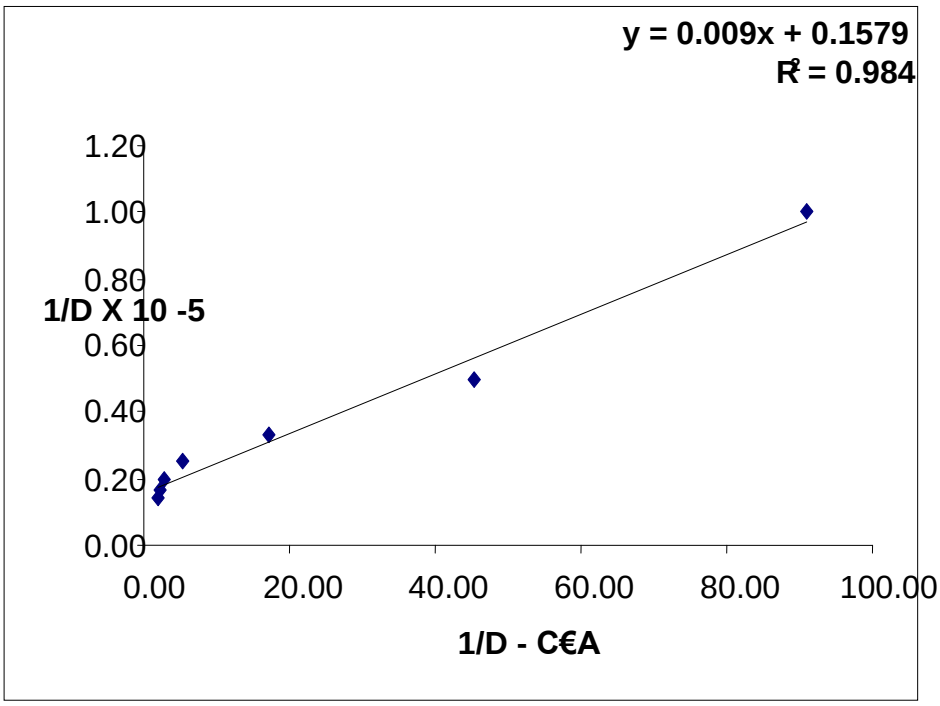


Figure 4.48: Ardon's plot for ketotifen and theophylline system at pH 6.8.

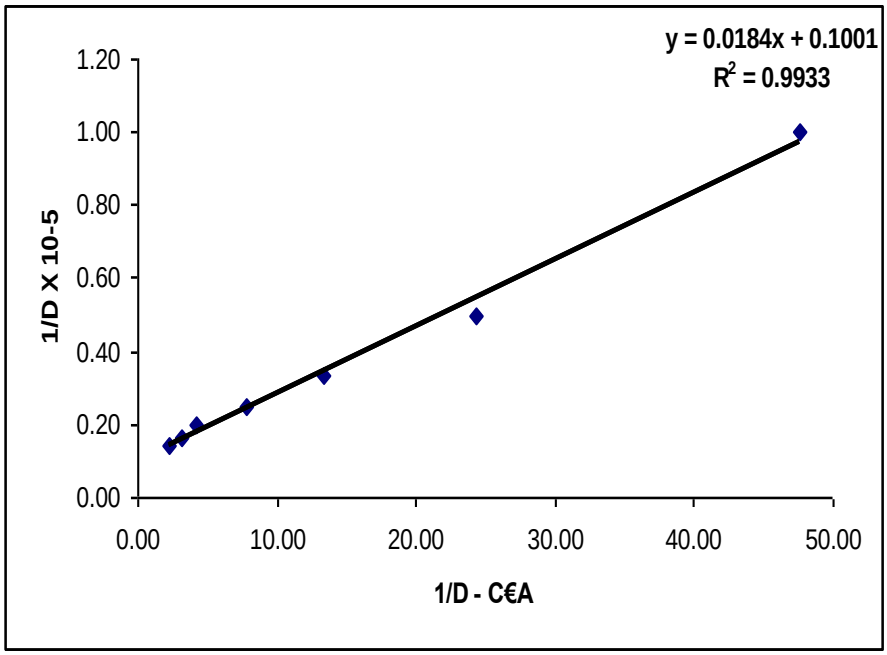


Figure 4.49: Ardon's plot for ketotifen and theophylline system at pH 7.4.

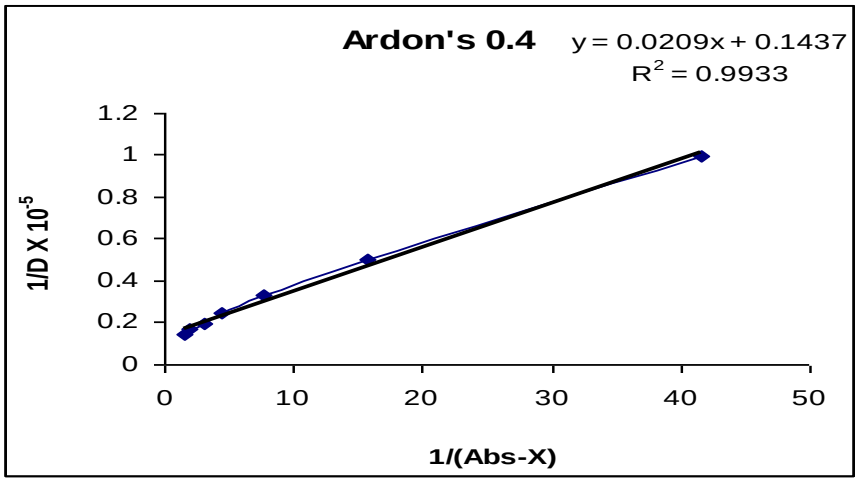


Figure.4.50: Ardon's plot for ketotifen and desloratidine system at pH 0.4.

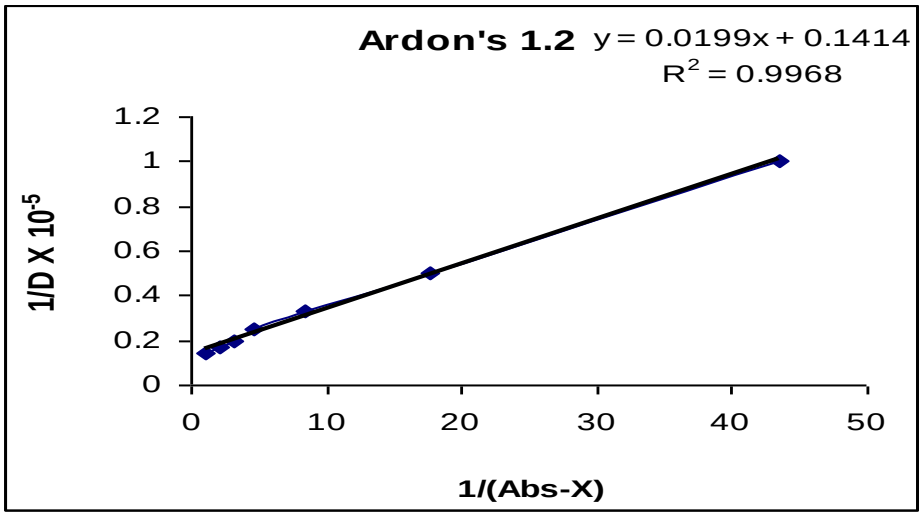


Figure 4.51: Ardon's plot for ketotifen and desloratidine system at pH 1.2.

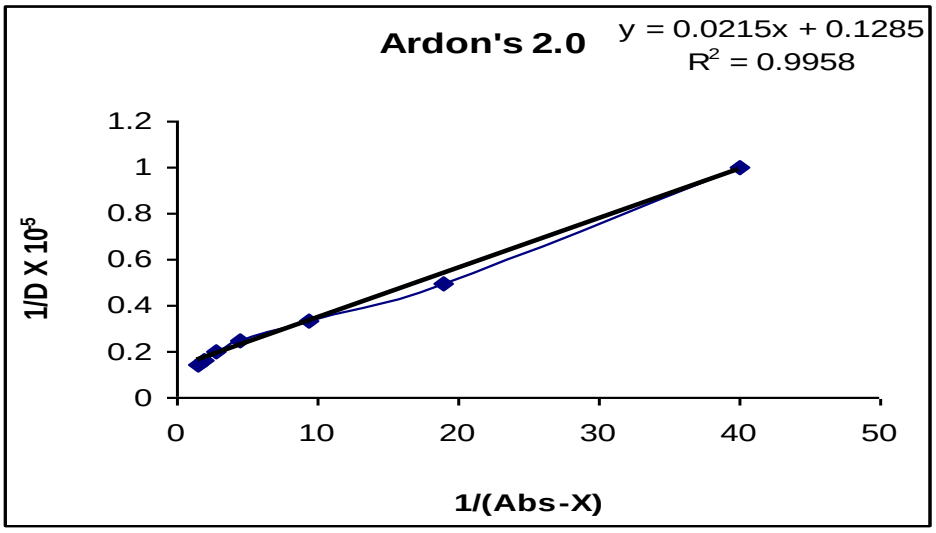


Figure 4.52: Ardon's plot for ketotifen and desloratidine system at pH 2.0.

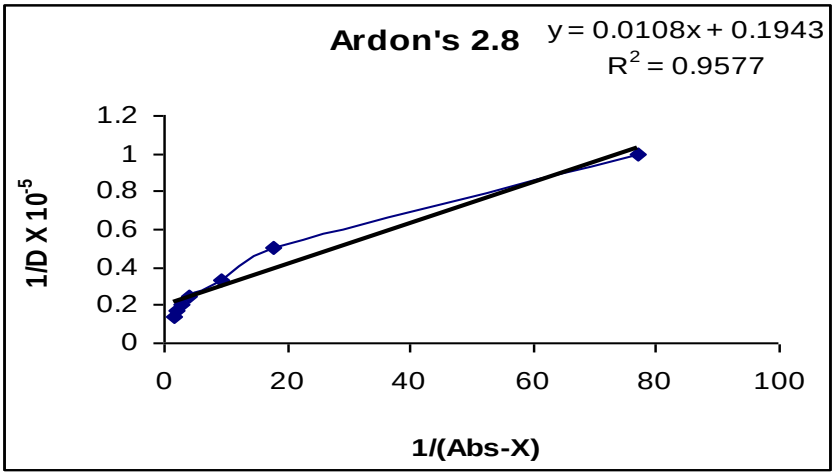


Figure 4.53: Ardon's plot for ketotifen and desloratidine system at pH 2.8.

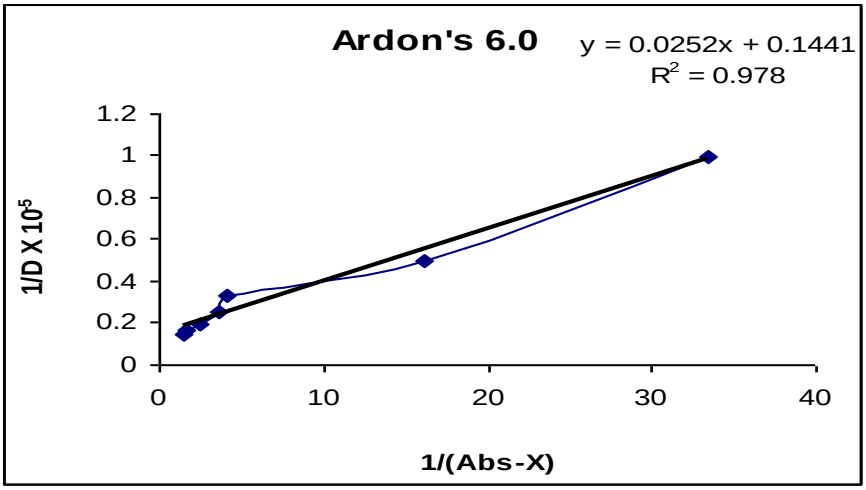


Figure 4.54: Ardon's plot for ketotifen fumarate and desloratidine system at pH 6.0.

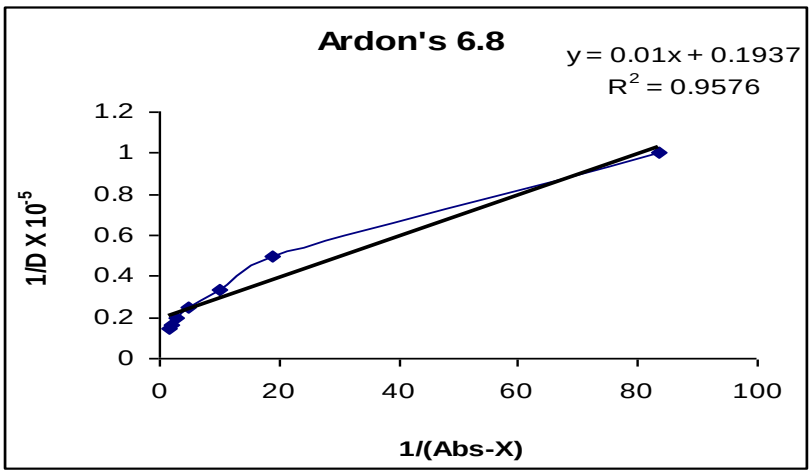


Figure 4.55: Ardon's plot for ketotifen and desloratidine system at pH 6.8.

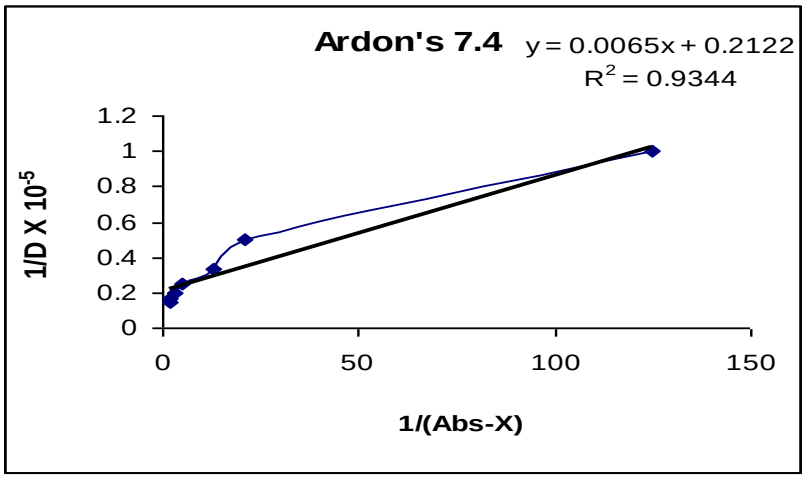


Figure 4.56: Ardon's plot for ketotifen and desloratidine system at pH 7.4.

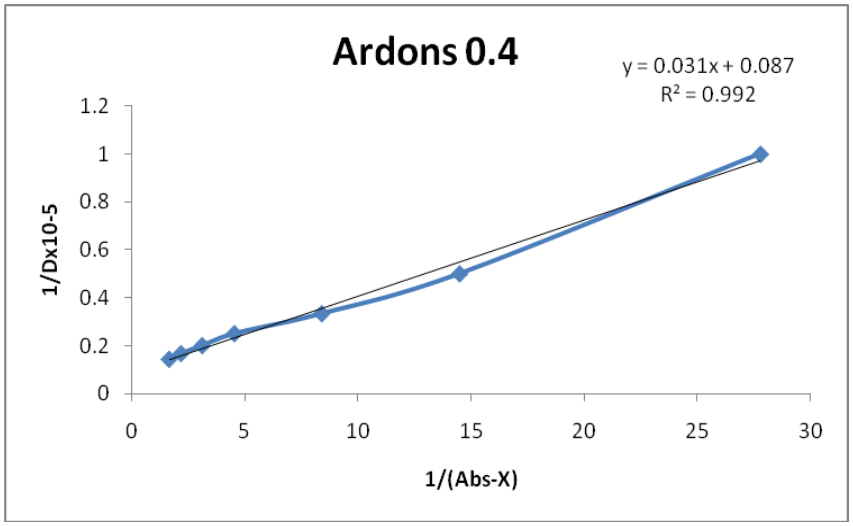


Figure 4.57: Ardon's plot for ketotifen and metformin system at pH 0.4.

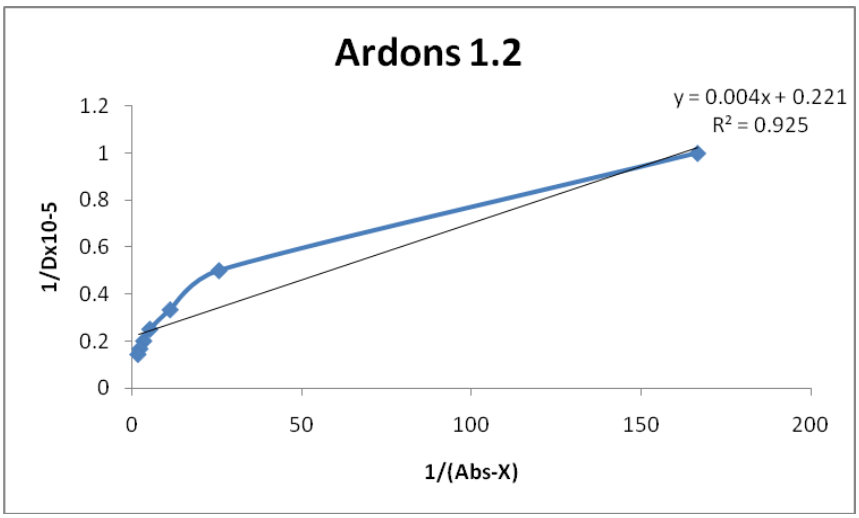


Figure 4.58: Ardon's plot for ketotifen and metformin system at pH 1.2.

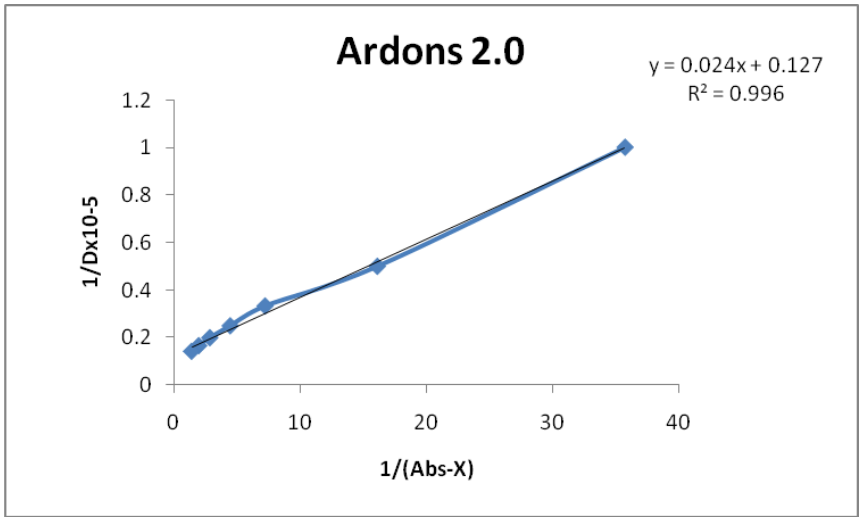


Figure 4.59: Ardon's plot for ketotifen and metformin system at pH 2.0.

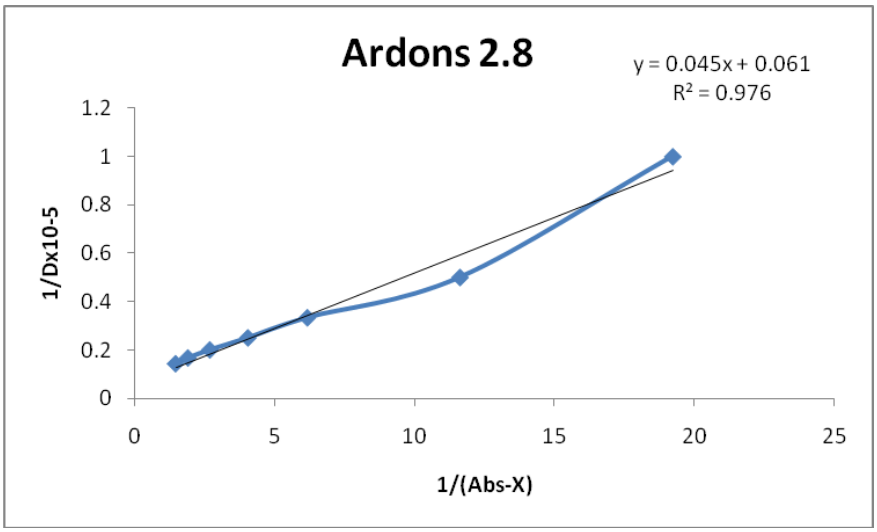


Figure 4.60: Ardon's plot for ketotifen and metformin system at pH 2.8.

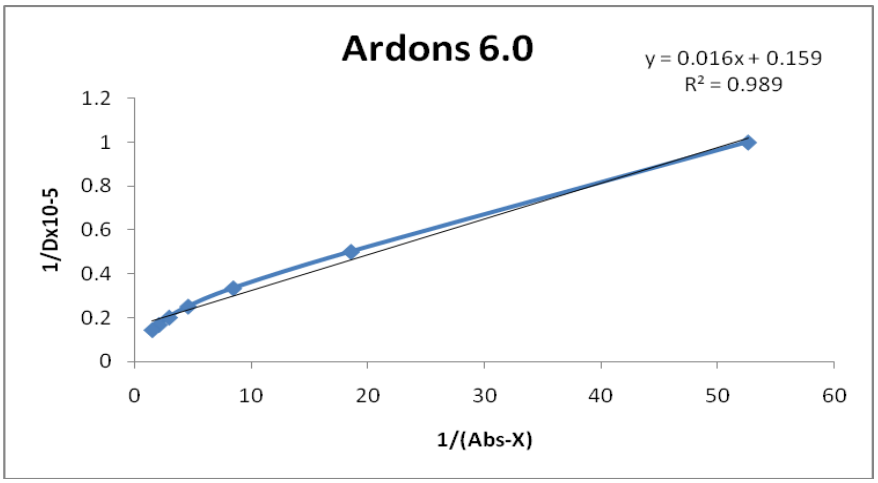


Figure 4.61: Ardon's plot for ketotifen and metformin system at pH 6.0.

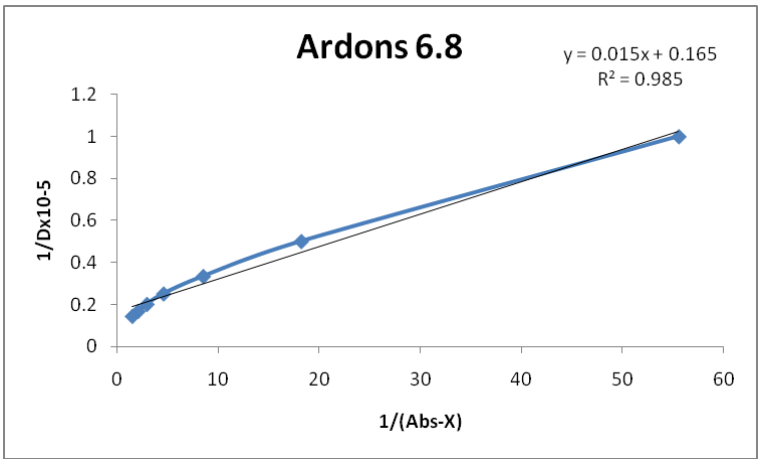


Figure 4.62: Ardon's plot for ketotifen and metformin system at pH 6.8.

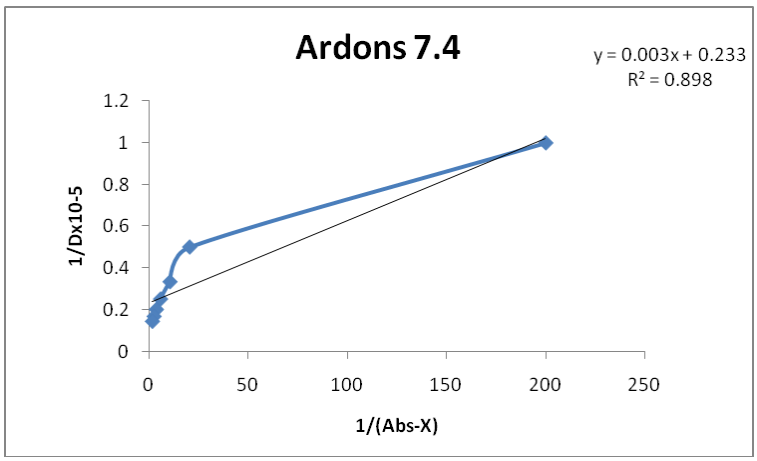


Figure 4.63: Ardon's plot for ketotifen and metformin system at pH 7.4.

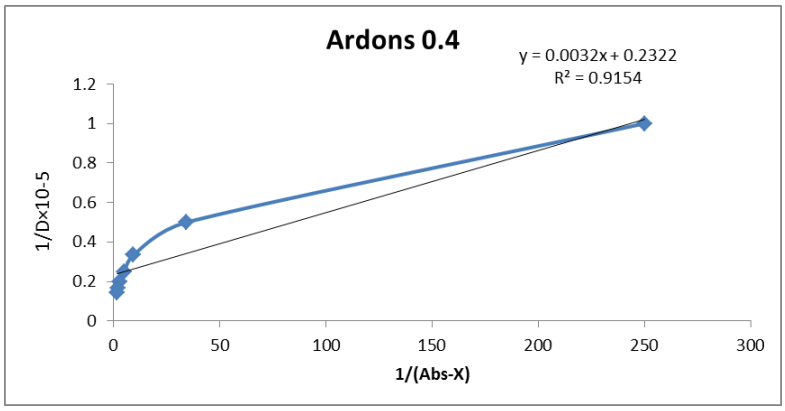


Figure 4.64: Ardon's plot for ketotifen and chlorpheniramine system at pH 0.4.

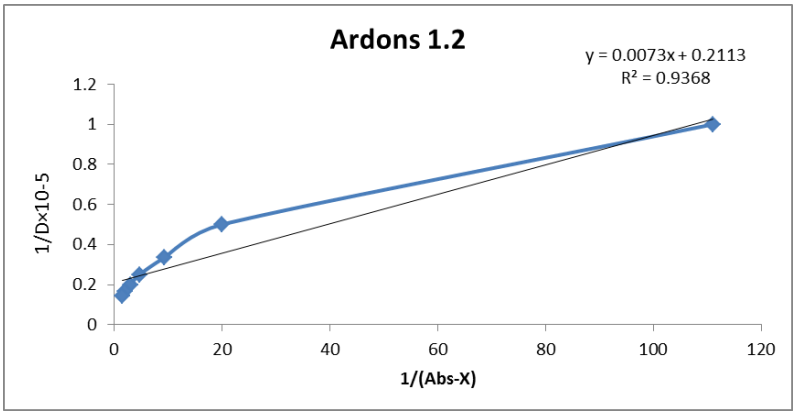


Figure 4.65: Ardon's plot for ketotifen and chlorpheniramine at pH 1.2.

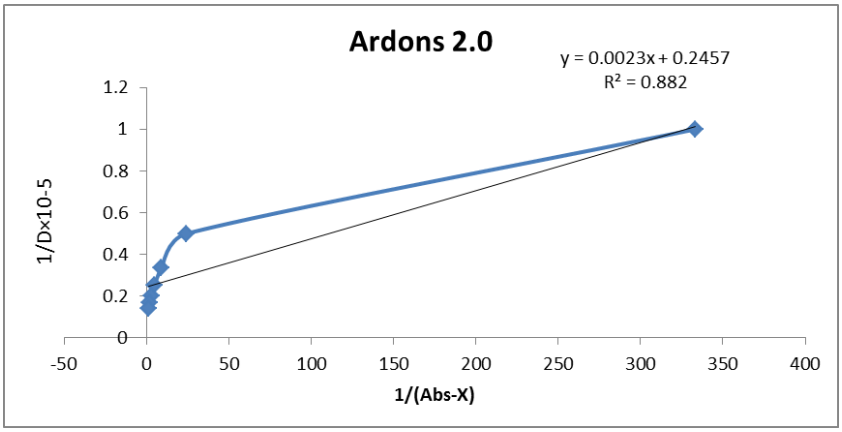


Figure 4.66: Ardon's plot for ketotifen and chlorpheniramine at pH 2.0.

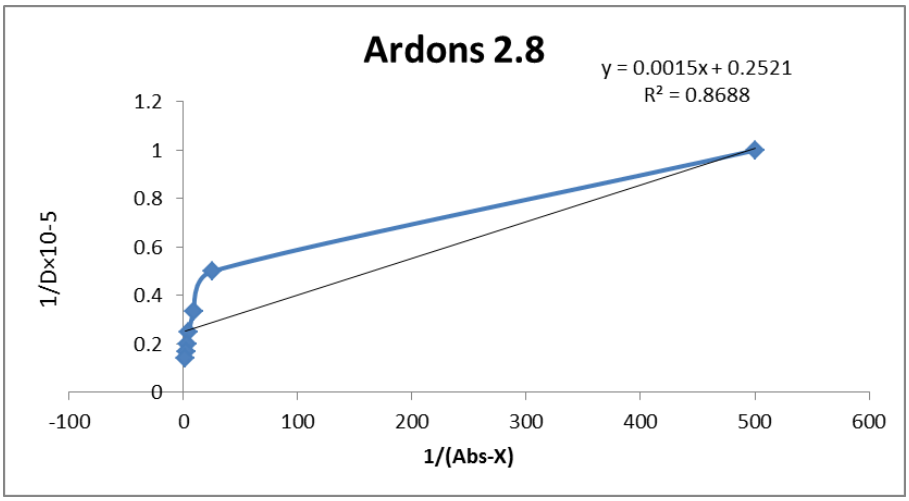


Figure 4.67: Ardon's plot for ketotifen and chlorpheniramine at pH 2.8.

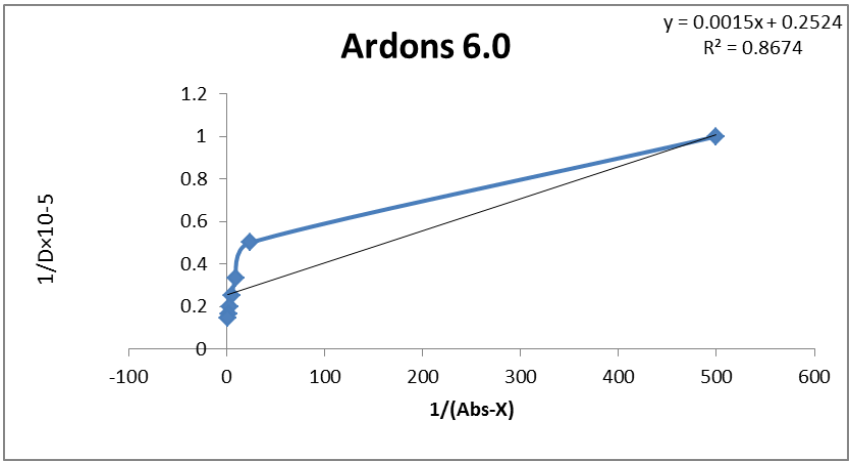


Figure 4.68: Ardon's plot for ketotifen and chlorpheniramine at pH 6.0.

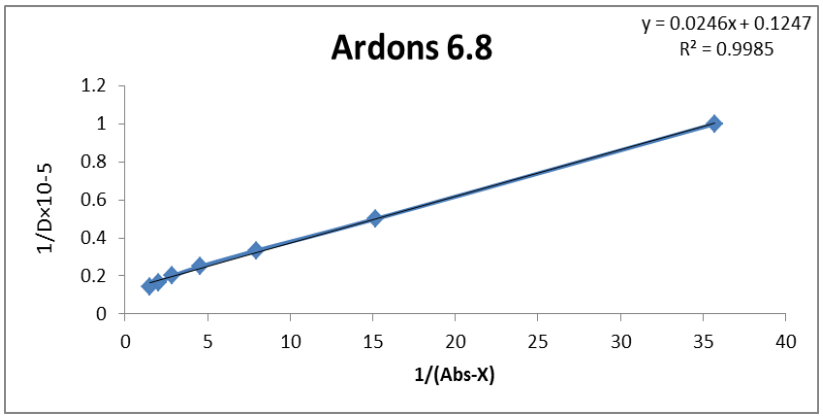


Figure 4.69: Ardon's plot for ketotifen and chlorpheniramine at pH 6.8.

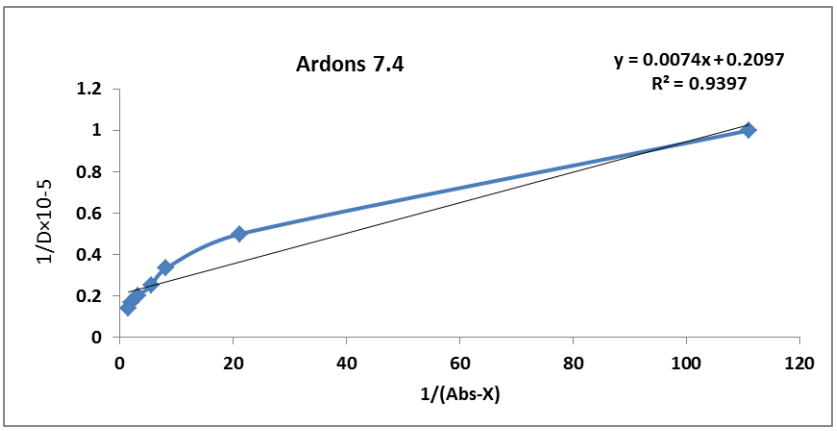


Figure 4.70: Ardon's plot for ketotifen and chlorpheniramine at pH 7.4.

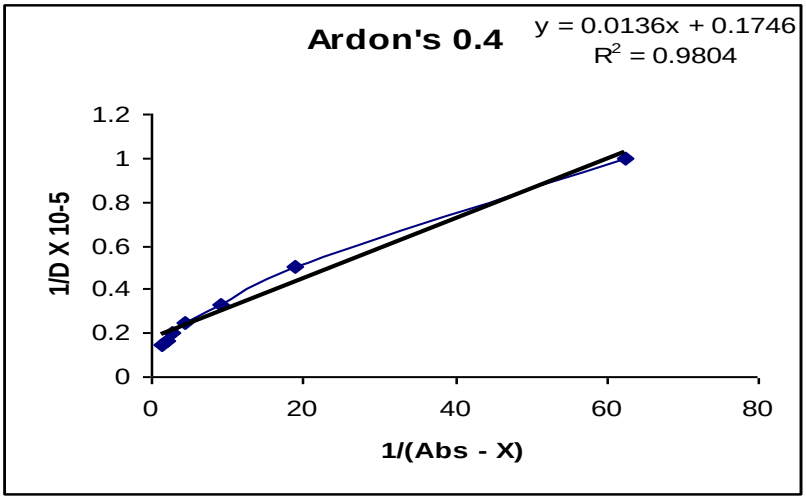


Figure 4.71: Ardon's plot for ketotifen and domperidone at pH 0.4.

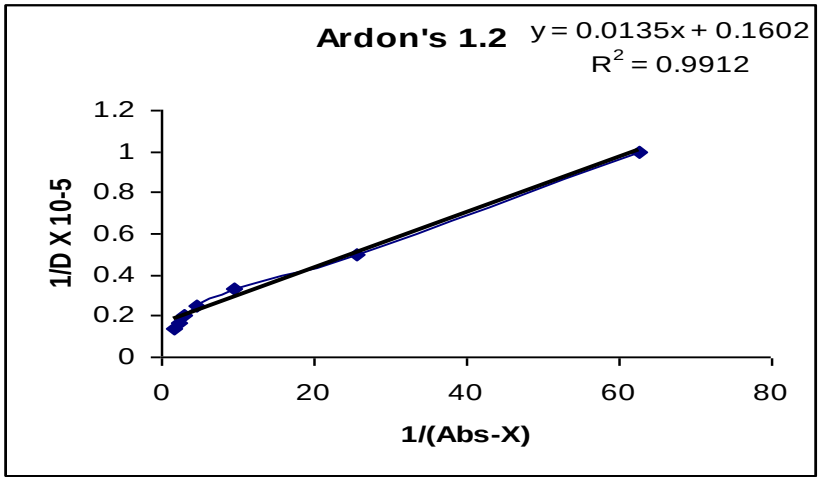


Figure 4.72: Ardon's plot for ketotifen and domperidone at pH 1.2.

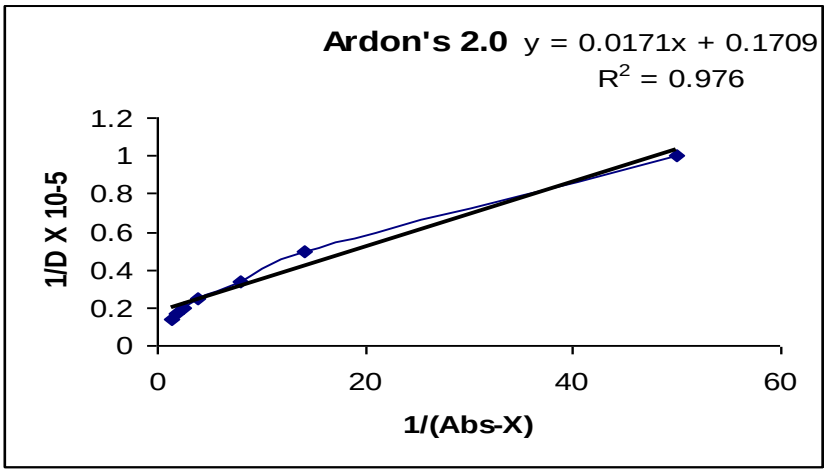


Figure 4.73: Ardon's plot for ketotifen and domperidone at pH 2.0.

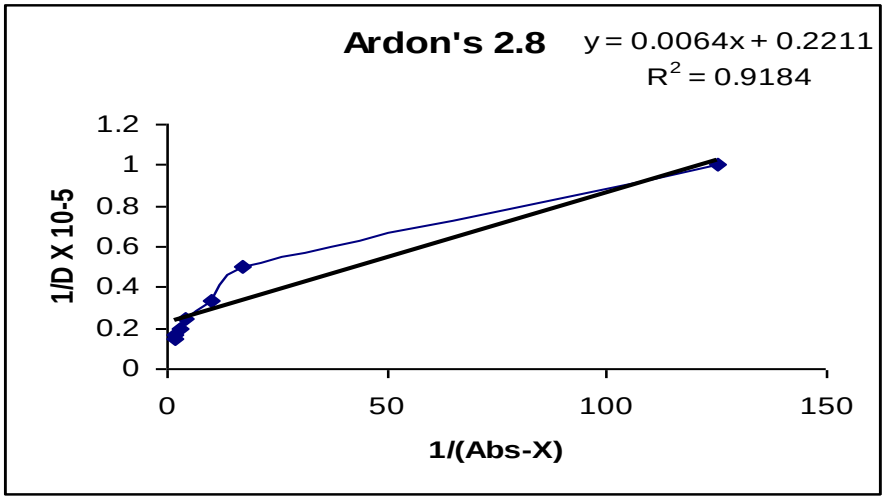


Figure 4.74: Ardon's plot for ketotifen and domperidone at pH 2.8.

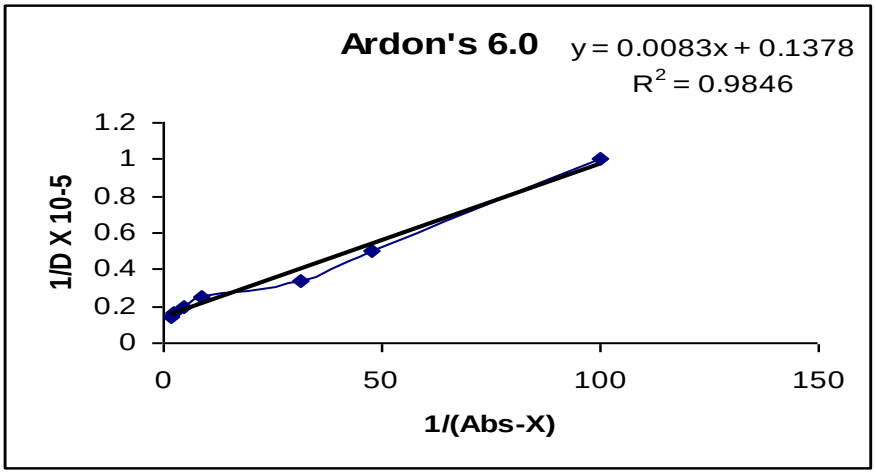


Figure 4.75: Ardon's plot for ketotifen and domperidone at pH 6.0.

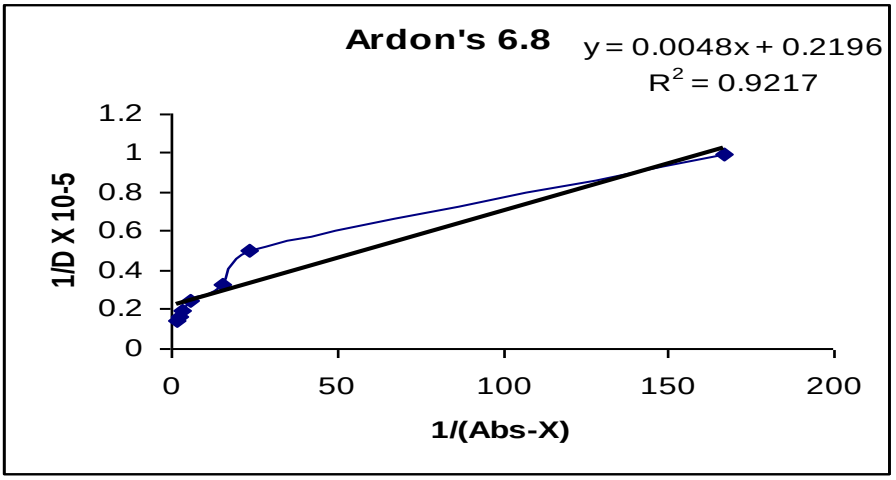


Figure 4.76: Ardon's plot for ketotifen and domperidone at pH 6.8.

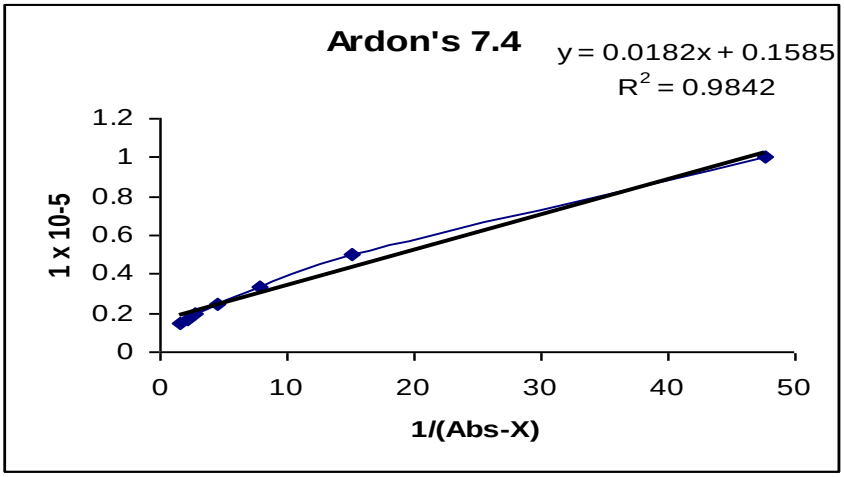


Figure 4.77: Ardon's plot for ketotifen and domperidone at pH 7.4.

4.3.2 Estimation of stability constants

The value of stability constant for the complexation of ketotifen with commonly prescribed drugs at pHs 0.4, 1.2, 2, 2.8, 6, 6.8 and 7.4 were obtained from the spectral data using Ardon's plot. The values for stability constant were calculated from the slopes and intercepts of the straight lines from these plots. It was seen from the Ardon's equation that the values of stability constant was given as [(intercept) / (slope)] of straight line so obtained .i.e. k = (intercept) / (slope). The value of intercept and slope were calculated by least squares method using the following equation,

$$y = mx + C$$

The values of stability constants for the drug-metal system at pHs 0.4, 1.2, 2.0, 2.8, 6, 6.8 and 7.4 are presented in the table given below:

Table 4.22: The stability constant values

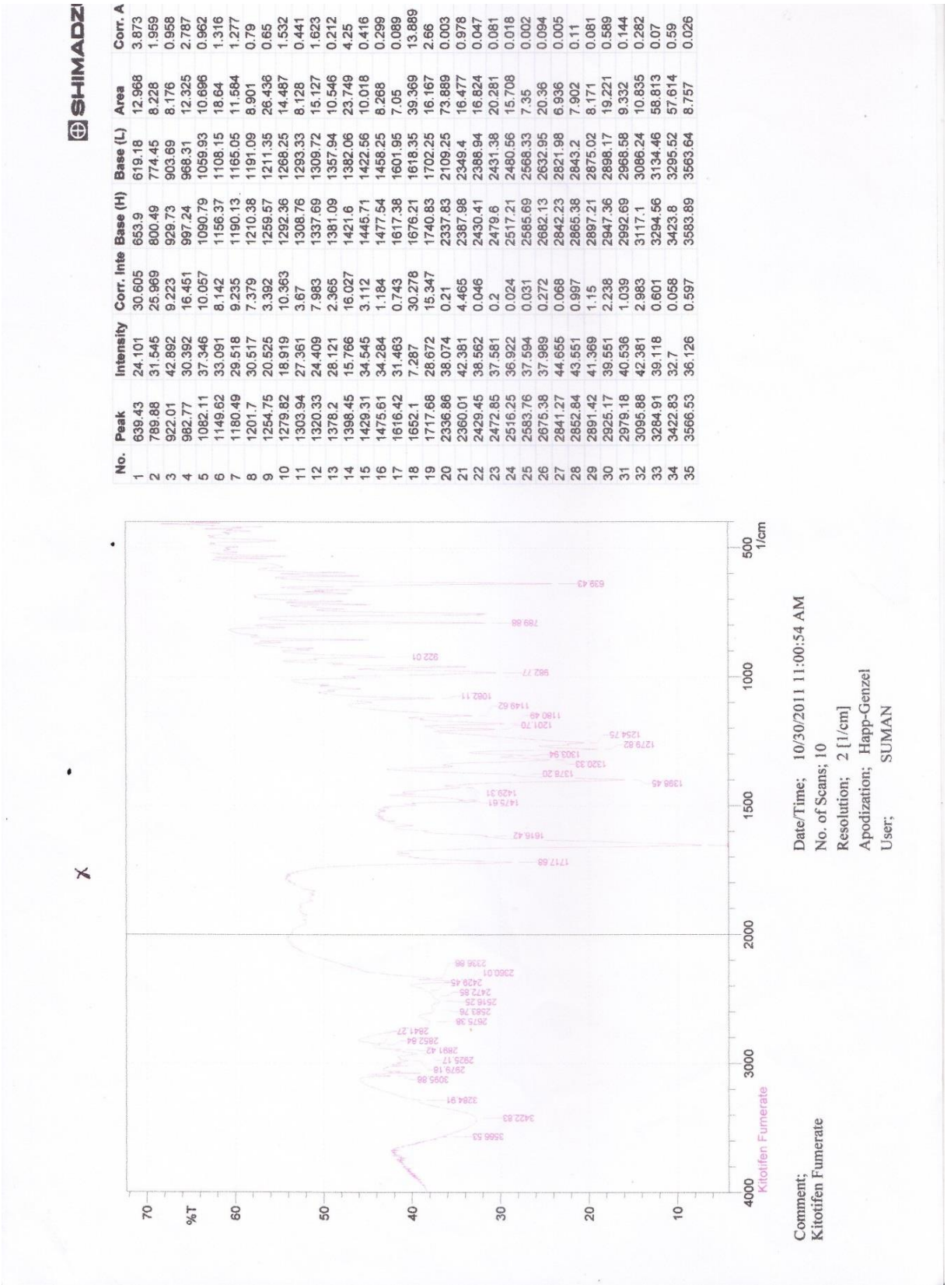
Systems	Stability Constant k ($1x10^{-2}$)						
	At pH 0.4	**At pH 1.2**	**At pH 2.0**	**At pH 2.8**	**At pH 6.0**	**At pH 6.8**	**At pH 7.4**
Ketotifen & chlorpheniramine	0.7256	0.2895	1.0683	1.6807	1.6827	0.0507	0.2834
Ketotifen & domperidone	77.89	84.27	10.0059	28.95	60.23	21.85	114.82
Ketotifen & desloratidine	14.54	14.07	16.73	5.56	17.49	5.16	3.06
Ketotifen & theophylline	6.8	7.4	3.5	4.8	9.6	5.7	18.4
Ketotifen & amoxicillin	19.8	13.7	2.30	35.7	14.1	8.3	51.6
Ketotifen & metformin	0.0281	0.5525	0.0529	0.0136	0.0993	0.01107	0.770
Ketotifen & diclofenac	-	-	-	-	-	0.068	0.057
Ketotifen & paracetamol	5.67	6.36	7.21	14.84	17.97	38.35	24.79

The stability constant values are the concern for drug interaction as well as complex formation between ketotifen with commonly used prescribed drugs. Table 4.22 is showing a good signs of drug interaction between ketotifen and domperidone. Low stability constant values are found for chlorpheniramine, metformin and diclofenac.

4.4 Infrared spectroscopy study (FTIR)

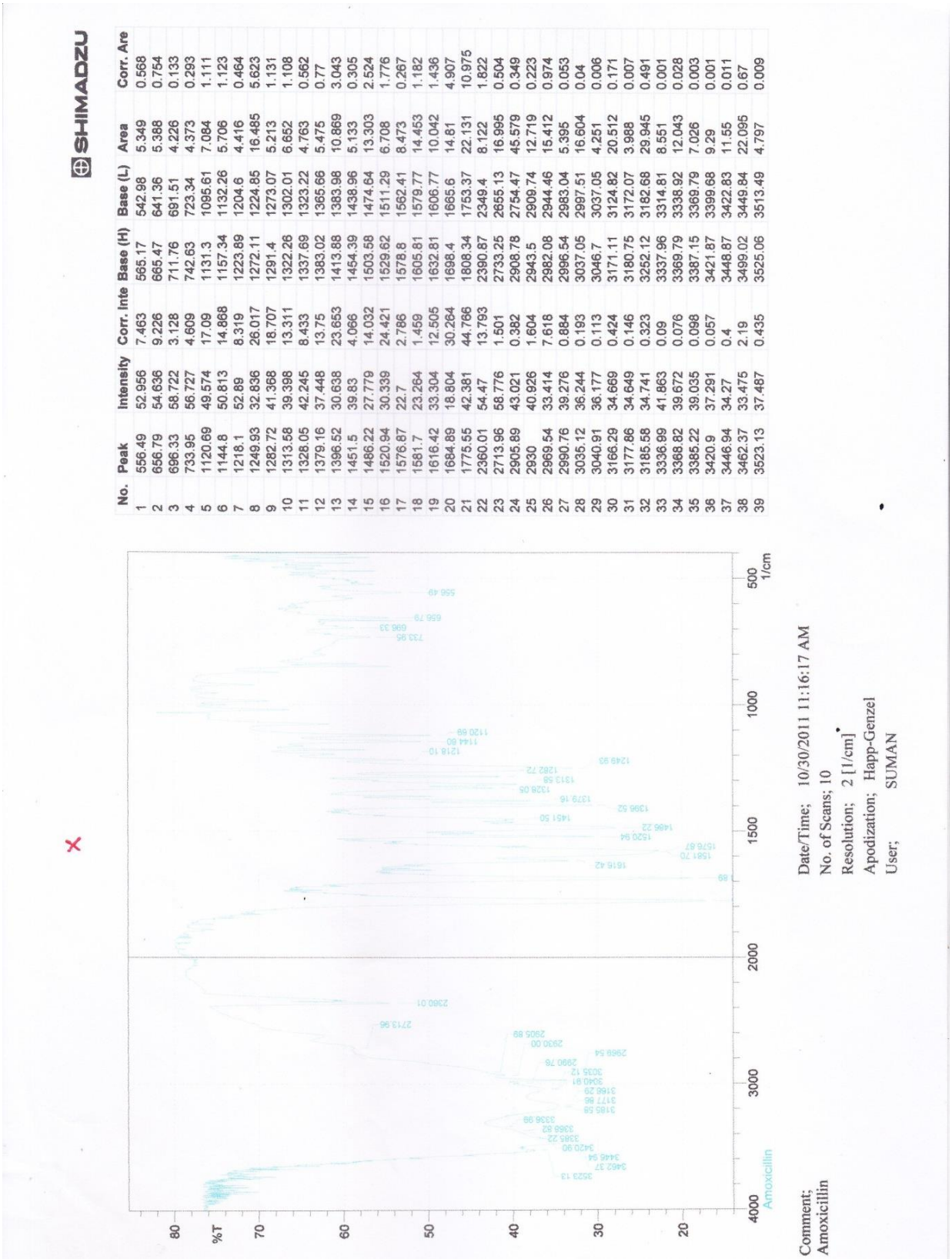
The experiment for both aqueous and chloroform extracts were carried out by mixing both ketotifen fumarate and common prescribed drugs.

4.4.1 IR spectrum of ketotifen fumarate.



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. A
1	639.43	24.101	30.605	653.9	619.18	12.968	3.873
2	789.88	31.545	25.969	800.49	774.45	8.228	1.959
3	922.01	42.892	9.223	929.73	903.69	8.176	0.958
4	982.77	30.392	16.451	997.24	968.31	12.325	2.787
5	1082.11	37.346	10.057	1090.79	1059.93	10.696	0.962
6	1149.62	33.091	8.142	1156.37	1108.15	18.64	1.316
7	1180.49	29.518	9.235	1190.13	1165.05	11.584	1.277
8	1201.7	30.517	7.379	1210.38	1191.09	8.901	0.79
9	1254.75	20.525	3.392	1259.57	1211.35	26.436	0.65
10	1279.82	18.919	10.363	1292.36	1268.25	14.487	1.532
11	1303.94	27.361	3.67	1308.76	1293.33	8.128	0.441
12	1320.33	24.409	7.983	1337.69	1309.72	15.127	1.623
13	1378.2	28.121	2.365	1381.09	1357.94	10.546	0.212
14	1398.45	15.766	16.027	1421.6	1382.06	23.749	4.25
15	1429.31	34.545	3.112	1445.71	1422.56	10.018	0.416
16	1475.61	34.284	1.184	1477.54	1458.25	8.268	0.299
17	1616.42	31.463	0.743	1617.38	1601.95	7.05	0.089
18	1652.1	7.287	30.278	1676.21	1618.35	39.369	13.889
19	1717.68	28.672	15.347	1740.83	1702.25	16.167	2.66
20	2336.86	38.074	0.21	2337.83	2109.25	73.889	0.003
21	2360.01	42.381	4.465	2387.98	2349.4	16.477	0.978
22	2429.45	38.562	0.046	2430.41	2388.94	16.824	0.047
23	2472.85	37.581	0.2	2478.6	2431.38	20.281	0.081
24	2516.25	36.922	0.024	2517.21	2480.56	15.708	0.018
25	2583.76	37.594	0.031	2585.69	2568.33	7.35	0.002
26	2675.38	37.989	0.272	2682.13	2632.95	20.36	0.094
27	2841.27	44.655	0.068	2842.23	2821.98	6.936	0.005
28	2852.84	43.551	0.997	2865.38	2843.2	7.902	0.11
29	2891.42	41.369	1.15	2897.21	2875.02	8.171	0.081
30	2925.17	39.551	2.238	2947.36	2898.17	19.221	0.589
31	2978.18	40.536	1.039	2992.69	2968.58	9.332	0.144
32	3095.88	42.381	2.983	3117.1	3086.24	10.835	0.282
33	3284.91	39.118	0.601	3294.56	3134.46	58.813	0.07
34	3422.83	32.7	0.058	3423.8	3295.52	57.614	0.59
35	3566.53	36.126	0.597	3583.89	3563.64	8.757	0.026

4.4.2 IR spectrum of amoxicillin trihydrate.



SHIMADZU

No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	556.49	52.956	7.463	565.17	542.98	5.349	0.568
2	656.79	54.636	9.226	665.47	641.36	5.388	0.754
3	696.33	58.722	3.128	711.76	691.51	4.226	0.133
4	733.95	56.727	4.609	742.63	723.34	4.373	0.293
5	1120.69	49.574	17.09	1131.3	1095.61	7.084	1.111
6	1144.8	50.813	14.868	1157.34	1132.26	5.706	1.123
7	1218.1	52.89	8.319	1223.89	1204.6	4.416	0.464
8	1249.93	32.836	26.017	1272.11	1224.85	16.485	5.623
9	1282.72	41.368	18.707	1291.4	1273.07	5.213	1.131
10	1313.58	39.398	13.311	1322.26	1302.01	6.652	1.108
11	1328.05	42.245	8.433	1337.69	1323.22	4.763	0.562
12	1379.16	37.448	13.75	1383.02	1365.66	5.475	0.77
13	1396.52	30.638	23.653	1413.88	1383.98	10.869	3.043
14	1451.5	39.83	4.066	1454.39	1438.96	5.133	0.305
15	1486.22	27.779	14.032	1503.58	1474.64	13.303	2.524
16	1520.94	30.339	24.421	1529.62	1511.29	6.708	1.776
17	1576.87	22.7	2.786	1578.8	1562.41	8.473	0.267
18	1581.7	23.264	1.459	1605.81	1579.77	14.453	1.182
19	1616.42	33.304	12.505	1632.81	1606.77	10.042	1.436
20	1684.89	18.804	30.264	1698.4	1665.6	14.81	4.907
21	1775.55	42.381	44.766	1808.34	1753.37	22.131	10.975
22	2360.01	54.47	13.793	2390.87	2349.4	8.122	1.822
23	2713.96	58.776	1.501	2733.25	2655.13	16.995	0.504
24	2905.89	43.021	0.382	2908.78	2754.47	45.579	0.349
25	2930	40.926	1.604	2943.5	2909.74	12.719	0.223
26	2969.54	33.414	7.618	2982.08	2944.46	15.412	0.974
27	2990.76	39.276	0.884	2996.54	2983.04	5.395	0.053
28	3035.12	36.244	0.193	3037.05	2997.51	16.604	0.04
29	3040.91	36.177	0.113	3046.7	3037.05	4.251	0.006
30	3166.29	34.669	0.424	3171.11	3124.82	20.512	0.171
31	3177.86	34.649	0.146	3180.75	3172.07	3.988	0.007
32	3185.58	34.741	0.323	3252.12	3182.68	29.945	0.491
33	3336.99	41.863	0.09	3337.96	3314.81	8.551	0.001
34	3368.82	39.672	0.076	3369.79	3338.92	12.043	0.028
35	3385.22	39.035	0.098	3387.15	3369.79	7.026	0.003
36	3420.9	37.291	0.057	3421.87	3399.68	9.29	0.001
37	3446.94	34.27	0.4	3448.87	3422.83	11.55	0.011
38	3462.37	33.475	2.19	3499.02	3449.84	22.095	0.67
39	3523.13	37.487	0.435	3525.06	3513.49	4.797	0.009

Date/Time; 10/30/2011 11:16:17 AM
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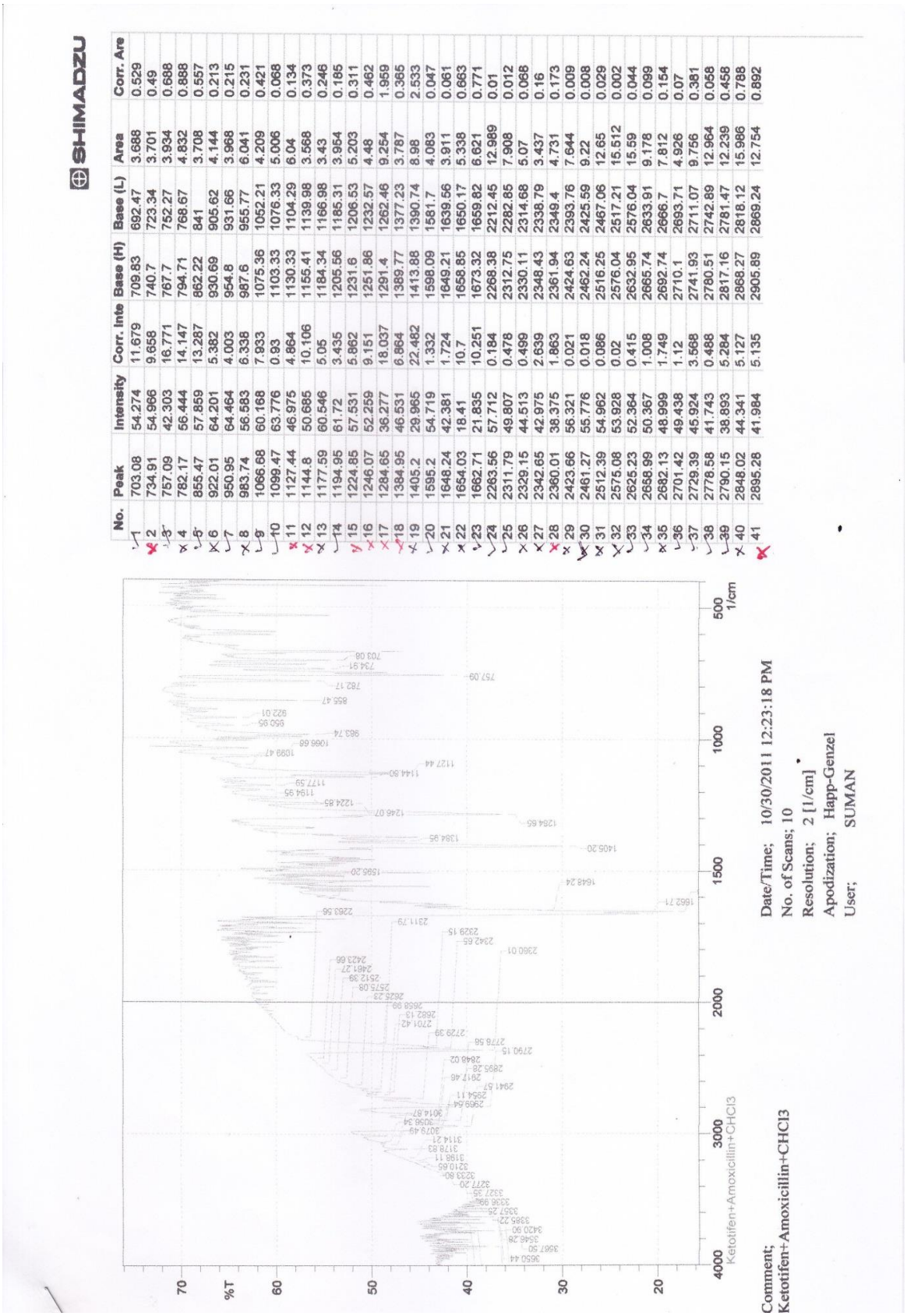
User; SUMAN

Comment;
Amoxicillin

4.4.3 IR spectrum of ketotifen fumarate and amoxicillin trihydrate (chloroform extract).

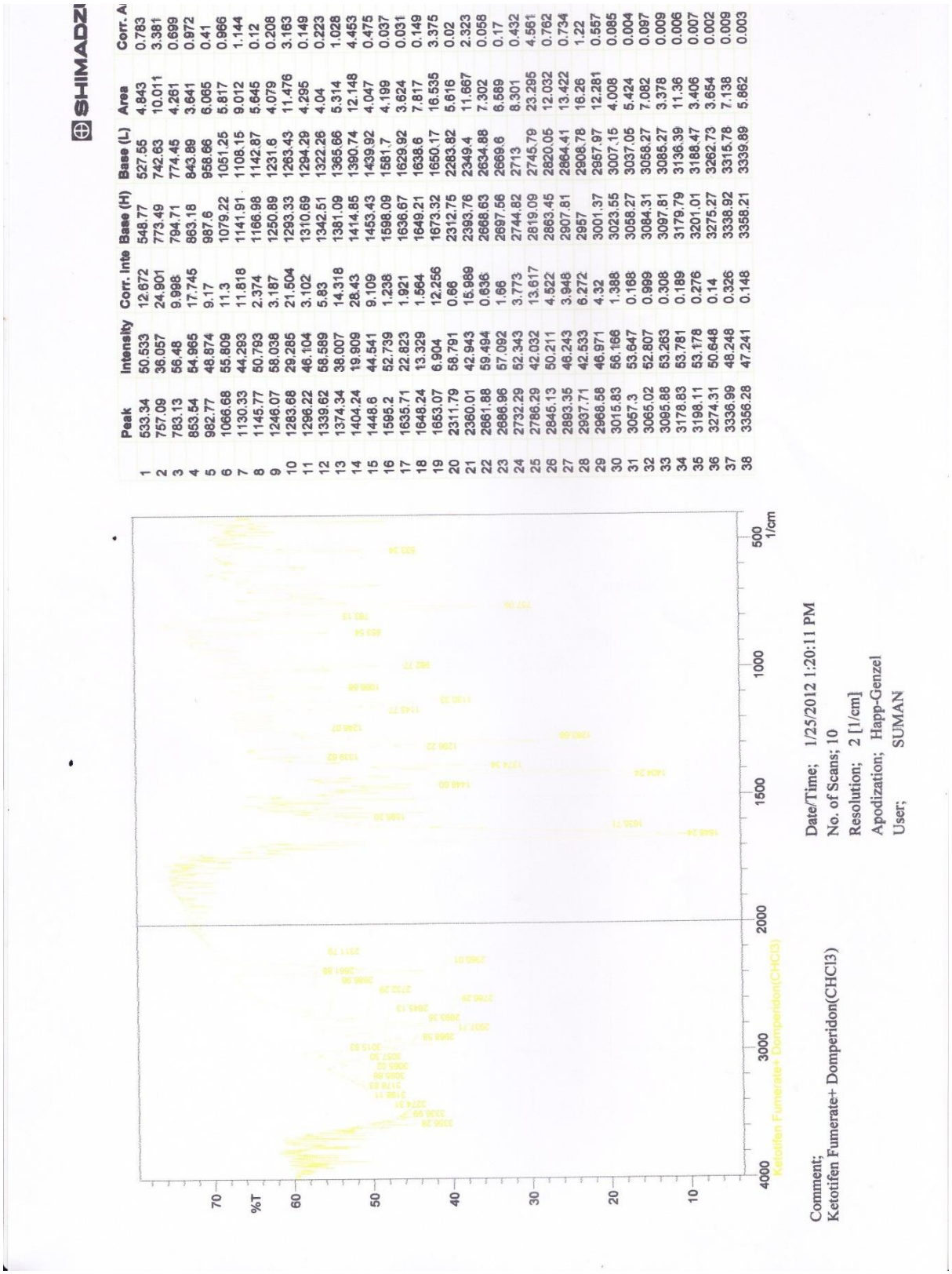


No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	703.08	54.274	11.679	709.83	692.47	3.688	0.529
2	734.91	54.966	9.658	740.7	723.34	3.701	0.49
3	757.09	42.303	16.771	767.7	752.27	3.934	0.688
4	782.17	56.444	14.147	794.71	768.67	4.832	0.888
5	855.47	57.859	13.287	862.22	841	3.708	0.557
6	922.01	64.201	5.382	930.69	905.62	4.144	0.213
7	950.95	64.464	4.003	954.8	931.66	3.968	0.215
8	983.74	56.583	6.338	987.6	955.77	6.041	0.231
9	1066.68	60.168	7.933	1075.36	1052.21	4.209	0.421
10	1099.47	63.776	0.93	1103.33	1076.33	5.006	0.068
11	1127.44	46.975	4.864	1130.33	1104.29	6.04	0.134
12	1144.8	50.685	10.106	1155.41	1139.98	3.568	0.373
13	1177.59	60.546	5.05	1184.34	1166.98	3.43	0.246
14	1194.95	61.72	3.435	1205.56	1185.31	3.964	0.185
15	1224.85	57.531	5.862	1231.6	1206.53	5.203	0.311
16	1246.07	52.259	9.151	1251.86	1232.57	4.48	0.462
17	1284.65	36.277	18.037	1291.4	1262.46	9.254	1.959
18	1384.95	46.531	6.864	1389.77	1377.23	3.787	0.365
19	1405.2	29.965	22.482	1413.88	1390.74	8.98	2.533
20	1595.2	54.719	1.332	1598.09	1681.7	4.083	0.047
21	1648.24	42.381	1.724	1649.21	1639.56	3.911	0.061
22	1654.03	18.41	10.7	1658.85	1650.17	5.338	0.663
23	1662.71	21.835	10.251	1673.32	1659.82	6.621	0.771
24	2283.56	57.712	0.184	2268.38	2212.45	12.989	0.01
25	2311.79	49.807	0.478	2312.75	2282.85	7.908	0.012
26	2329.15	44.513	0.499	2330.11	2314.68	5.07	0.068
27	2342.65	42.975	2.639	2348.43	2338.79	3.437	0.16
28	2360.01	38.375	1.863	2361.94	2349.4	4.731	0.173
29	2423.66	56.321	0.021	2424.63	2393.76	7.644	0.009
30	2461.27	55.776	0.018	2462.24	2425.59	9.22	0.008
31	2512.39	54.962	0.086	2516.25	2467.06	12.65	0.029
32	2575.08	53.928	0.02	2576.04	2517.21	15.512	0.002
33	2625.23	52.364	0.415	2632.95	2576.04	15.59	0.044
34	2688.99	50.367	1.008	2665.74	2633.91	9.178	0.099
35	2682.13	48.999	1.749	2692.74	2666.7	7.812	0.154
36	2701.42	49.438	1.12	2710.1	2693.71	4.926	0.07
37	2729.39	45.924	3.568	2741.93	2711.07	9.756	0.381
38	2778.58	41.743	0.488	2780.51	2742.89	12.964	0.058
39	2790.15	38.893	5.284	2817.16	2781.47	12.239	0.458
40	2848.02	44.341	5.127	2868.27	2818.12	15.986	0.788
41	2895.28	41.984	5.135	2905.89	2869.24	12.754	0.892

SHIMADZU

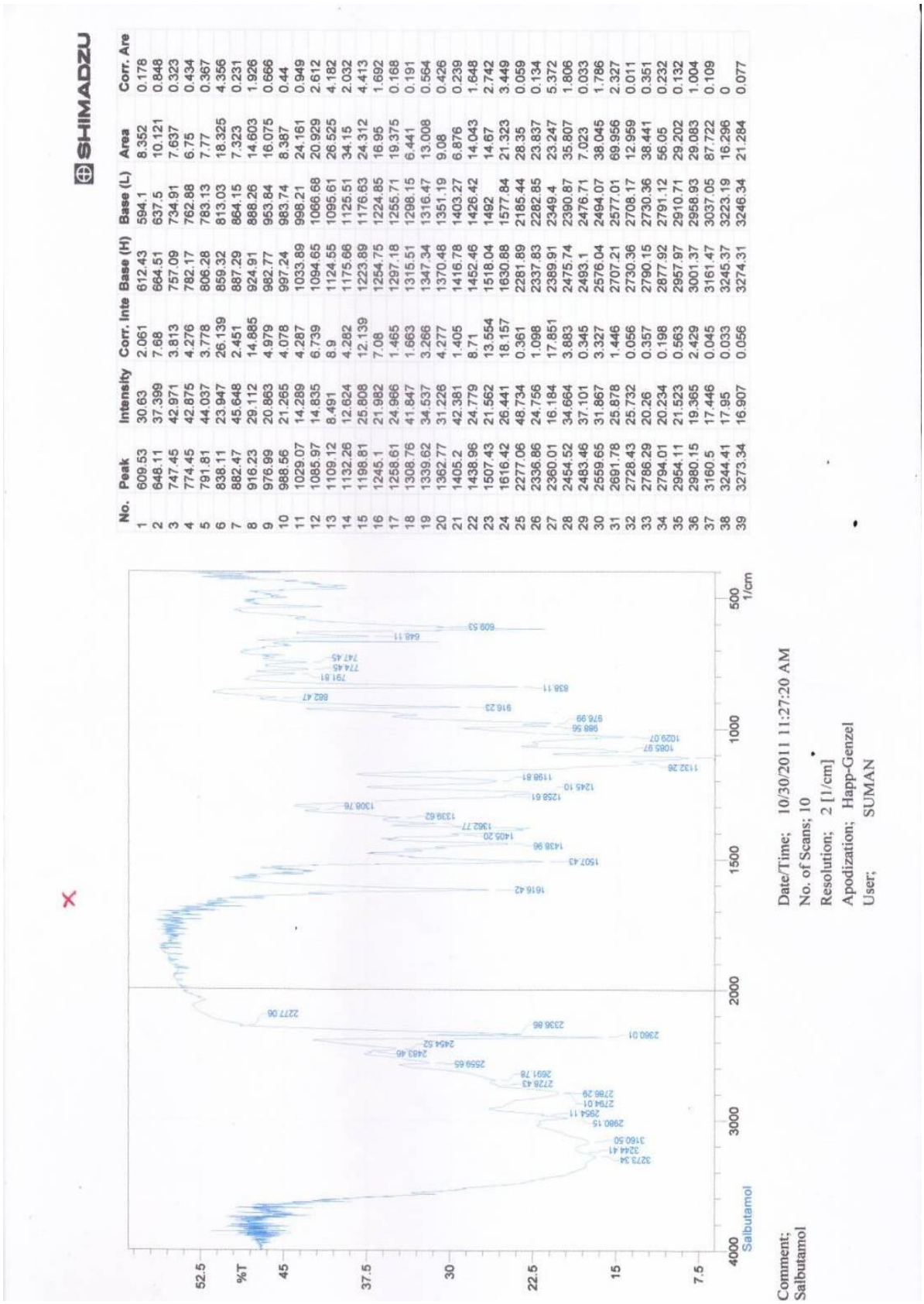
42	2917.46	44.303	0.671	2920.35	2906.85	4.64	0.042
43	2941.57	40.89	3.483	2948.32	2921.32	9.98	0.465
44	2954.11	43.712	1.205	2961.82	2949.29	4.412	0.065
45	2969.54	44.012	3.96	2982.08	2962.79	6.363	0.332
46	3014.87	48.33	2.81	3023.55	2997.51	7.764	0.224
47	3056.34	49.274	0.27	3058.27	3042.84	4.666	0.019
48	3079.49	48.594	0.281	3084.31	3070.81	4.208	0.015
49	3114.21	46.243	2.338	3120.96	3098.78	7.137	0.198
50	3178.83	46.674	0.155	3179.79	3136.39	13.974	0.003
51	3198.11	45.992	0.163	3200.04	3187.51	4.189	0.004
52	3210.65	45.607	0.099	3211.62	3201.01	3.589	0.002
53	3233.8	44.88	0.06	3234.76	3223.19	4.008	0.006
54	3277.2	43.334	0.114	3278.16	3268.52	3.479	0.006
55	3327.35	41.955	0.06	3328.31	3315.78	4.693	0.003
56	3336.99	41.299	0.241	3338.92	3329.28	3.669	0.008
57	3357.25	40.426	0.077	3358.21	3339.89	7.101	0.003
58	3385.22	39.222	0.235	3388.11	3370.75	6.979	0.014
59	3420.9	37.897	0.716	3425.72	3406.44	7.983	0.034
60	3546.28	38.298	2.255	3550.14	3541.46	3.515	0.113
61	3567.5	36.214	4.623	3572.32	3562.68	3.958	0.201
62	3650.44	38.106	6.138	3654.3	3644.65	3.741	0.319
63	3690.95	41.062	3.561	3694.81	3683.23	4.217	0.177
64	3712.17	40.283	4.276	3716.99	3707.34	3.569	0.182
65	3735.31	37.273	5.493	3740.13	3730.49	3.786	0.232
66	3853.94	37.819	5.954	3859.73	3848.15	4.446	0.295

4.4.4 IR spectrum of ketotifen fumarate and domperidone (chloroform extract).



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. A
1	533.34	50.533	12.672	548.77	527.55	4.843	0.783
2	757.09	36.057	24.901	773.49	742.63	10.011	3.381
3	783.13	56.48	9.998	794.71	774.45	4.261	0.699
4	853.54	84.965	17.745	863.18	843.89	3.641	0.972
5	982.77	48.874	9.17	987.6	958.66	6.065	0.41
6	1066.68	55.809	11.3	1079.22	1051.25	5.817	0.966
7	1130.33	44.293	11.818	1141.91	1108.15	9.012	1.144
8	1145.77	50.793	2.374	1166.98	1142.87	5.645	0.12
9	1246.07	58.038	3.187	1250.89	1231.6	4.079	0.208
10	1283.68	29.285	21.504	1293.33	1263.43	11.476	3.163
11	1296.22	46.104	3.102	1310.69	1294.29	4.295	0.149
12	1339.62	58.589	5.83	1342.51	1322.26	4.04	0.223
13	1374.34	38.007	14.318	1381.09	1365.66	5.314	1.028
14	1404.24	19.909	28.43	1414.85	1390.74	12.148	4.453
15	1448.6	44.541	9.109	1453.43	1439.92	4.047	0.475
16	1595.2	52.739	1.238	1598.09	1581.7	4.199	0.037
17	1635.71	22.823	1.921	1636.67	1629.92	3.624	0.031
18	1648.24	13.329	1.564	1649.21	1638.6	7.817	0.149
19	1653.07	6.904	12.256	1673.32	1650.17	16.535	3.375
20	2311.79	58.791	0.66	2312.75	2283.82	5.616	0.02
21	2360.01	42.943	15.989	2393.76	2349.4	11.667	2.323
22	2661.88	59.494	0.636	2668.63	2634.88	7.302	0.058
23	2686.96	57.092	1.66	2697.56	2669.6	8.589	0.17
24	2732.29	52.343	3.773	2744.82	2713	8.301	0.432
25	2786.29	42.032	13.617	2819.09	2745.79	23.295	4.561
26	2845.13	50.211	4.522	2863.45	2820.05	12.032	0.762
27	2893.35	46.243	3.948	2907.81	2864.41	13.422	0.734
28	2937.71	42.533	6.272	2957	2908.78	16.26	1.22
29	2968.58	46.971	4.32	3001.37	2957.97	12.281	0.557
30	3015.83	56.186	1.388	3023.55	3007.15	4.008	0.085
31	3057.3	53.647	0.168	3058.27	3037.05	5.424	0.004
32	3065.02	52.807	0.999	3084.31	3058.27	7.082	0.097
33	3095.88	53.263	0.308	3097.81	3085.27	3.378	0.009
34	3178.83	53.781	0.189	3179.79	3136.39	11.36	0.006
35	3198.11	53.178	0.276	3201.01	3188.47	3.406	0.007
36	3274.31	50.648	0.14	3275.27	3262.73	3.654	0.002
37	3336.99	48.248	0.326	3338.92	3315.78	7.138	0.009
38	3356.28	47.241	0.148	3358.21	3339.89	5.862	0.003

4.4.5 IR spectrum of salbutamol.



SHIMADZU

No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	609.53	30.63	2.061	612.43	594.1	8.352	0.178
2	648.11	37.399	7.68	664.51	637.5	10.121	0.848
3	747.45	42.971	3.813	757.09	734.91	7.637	0.323
4	774.45	42.875	4.276	782.17	762.88	6.75	0.434
5	791.81	44.037	3.778	806.28	783.13	7.77	0.367
6	838.11	23.947	26.139	859.32	813.03	18.325	4.356
7	882.47	45.648	2.451	887.29	864.15	7.323	0.231
8	916.23	29.112	14.885	924.91	888.26	14.603	1.926
9	976.99	20.863	4.979	982.77	953.84	16.075	0.666
10	988.56	21.265	4.078	997.24	983.74	8.387	0.44
11	1029.07	14.289	4.287	1033.89	998.21	24.161	0.949
12	1085.97	14.835	6.739	1094.65	1066.68	20.929	2.612
13	1109.12	8.491	8.9	1124.55	1095.61	26.525	4.182
14	1132.26	12.624	4.282	1175.66	1125.51	34.15	2.032
15	1198.81	25.808	12.139	1223.89	1176.63	24.312	4.413
16	1245.1	21.982	7.08	1254.75	1224.85	16.95	1.692
17	1258.61	24.966	1.465	1297.18	1255.71	19.375	0.168
18	1308.76	41.847	1.663	1315.51	1298.15	6.441	0.191
19	1339.62	34.537	3.266	1347.34	1316.47	13.008	0.564
20	1362.77	31.226	4.277	1370.48	1351.19	9.08	0.426
21	1405.2	42.381	1.405	1416.78	1403.27	6.876	0.239
22	1438.96	24.779	8.71	1452.46	1426.42	14.043	1.648
23	1507.43	21.562	13.554	1518.04	1492	14.67	2.742
24	1616.42	26.441	18.157	1630.88	1577.84	21.323	3.449
25	2277.06	48.734	0.361	2281.89	2185.44	28.35	0.059
26	2336.86	24.756	1.098	2337.83	2282.85	23.837	0.134
27	2360.01	16.184	17.851	2389.91	2349.4	23.247	5.372
28	2454.52	34.664	3.883	2475.74	2390.87	35.807	1.806
29	2483.46	37.101	0.345	2493.1	2476.71	7.023	0.033
30	2559.65	31.867	3.327	2576.04	2494.07	38.045	1.786
31	2691.78	25.878	1.446	2707.21	2577.01	69.956	2.327
32	2728.43	25.732	0.056	2730.36	2708.17	12.959	0.011
33	2786.29	20.26	0.357	2790.15	2730.36	38.441	0.351
34	2794.01	20.234	0.198	2877.92	2791.12	56.05	0.232
35	2954.11	21.523	0.563	2957.97	2910.71	29.202	0.132
36	2980.15	19.365	2.429	3001.37	2958.93	29.083	1.004
37	3160.5	17.446	0.045	3161.47	3037.05	87.722	0.109
38	3244.41	17.95	0.033	3245.37	3223.19	16.296	0
39	3273.34	16.907	0.056	3274.31	3246.34	21.284	0.077

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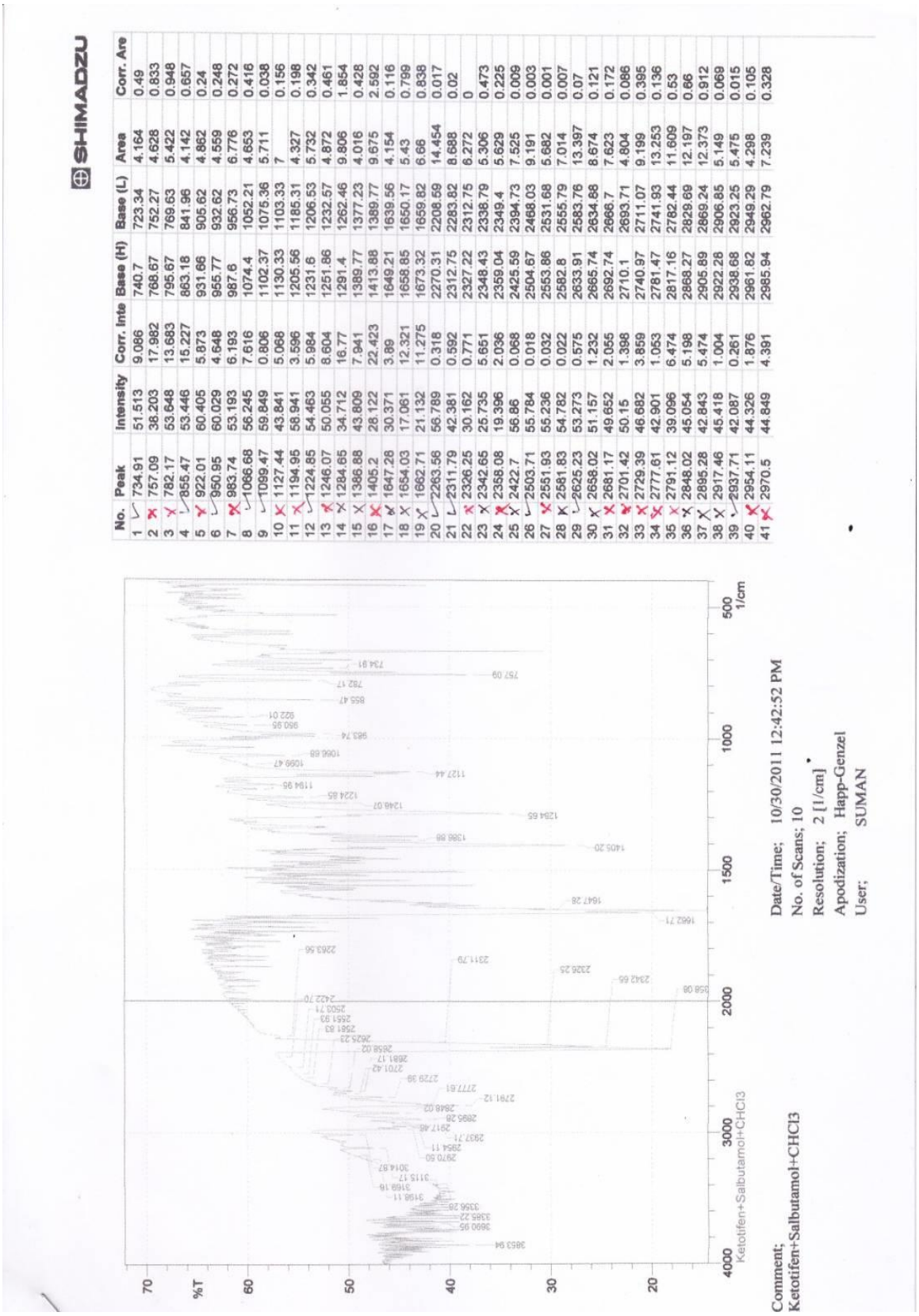
No. of Scans; 10
Resolution; 2 [1/cm]
Apodization; Happ-Genzel
User; SUMAN

Comment;
Salbutamol

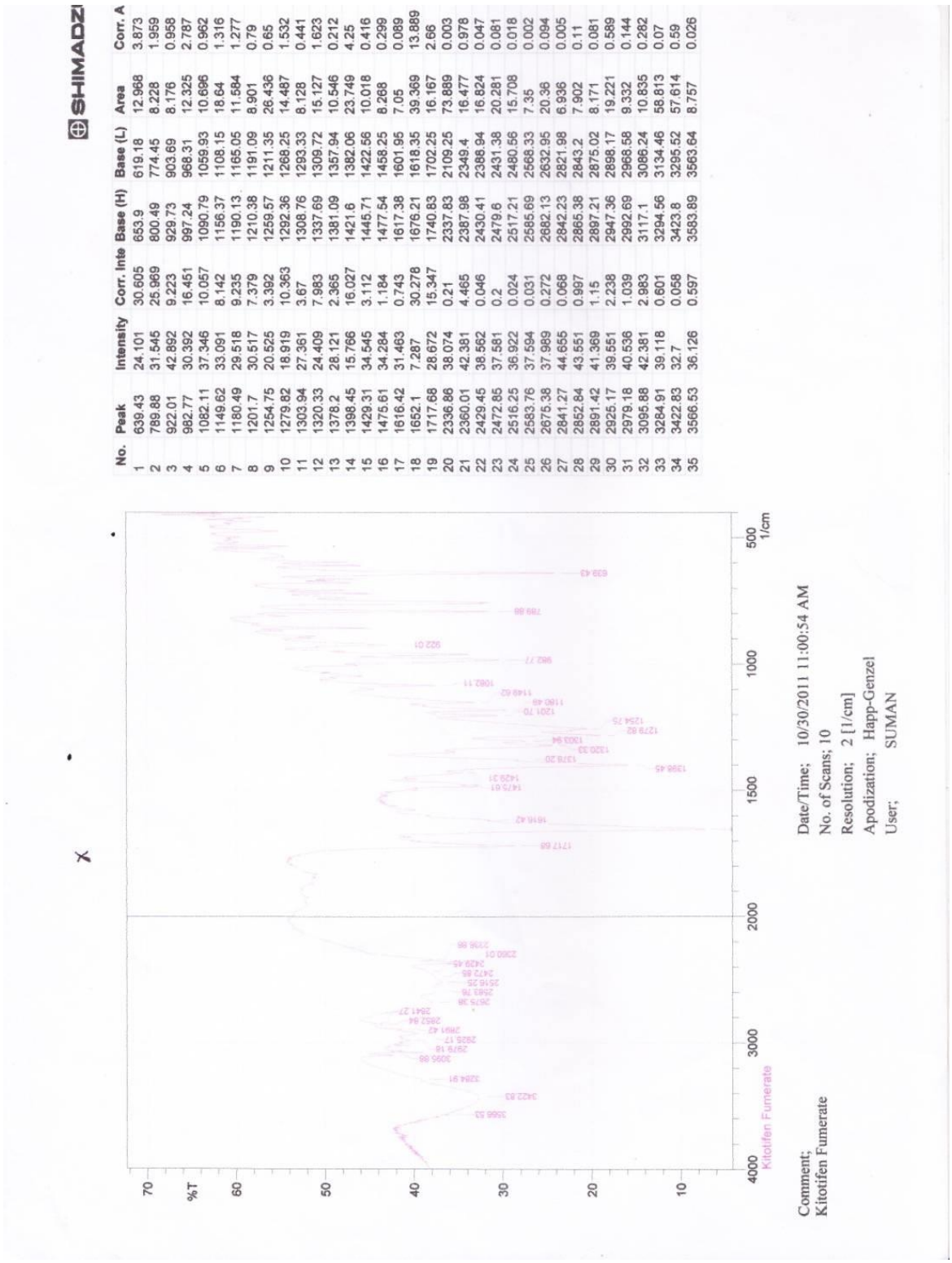
4.4.6 IR spectrum of ketotifen fumarate and salbutamol (chloroform extract).



SHIMADZU

No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	734.91	51.513	9.086	740.7	723.34	4.164	0.49
2	757.09	38.203	17.982	768.67	752.27	4.628	0.833
3	782.17	53.648	13.683	795.67	769.63	5.422	0.948
4	855.47	53.446	15.227	863.18	841.96	4.142	0.657
5	922.01	60.405	5.873	931.66	905.62	4.862	0.24
6	950.95	60.029	4.648	955.77	932.62	4.559	0.248
7	983.74	53.193	6.193	987.6	956.73	6.776	0.272
8	1066.68	56.245	7.616	1074.4	1052.21	4.653	0.416
9	1099.47	59.849	0.806	1102.37	1075.36	5.711	0.038
10	1127.44	43.841	5.068	1130.33	1103.33	7	0.156
11	1194.95	58.941	3.596	1205.56	1185.31	4.327	0.198
12	1224.85	54.463	5.984	1231.6	1206.53	5.732	0.342
13	1246.07	50.055	8.604	1251.86	1232.57	4.872	0.461
14	1284.65	34.712	16.77	1291.4	1262.46	9.806	1.854
15	1386.88	43.809	7.941	1389.77	1377.23	4.016	0.428
16	1405.2	28.122	22.423	1413.88	1389.77	9.675	2.592
17	1647.28	30.371	3.89	1649.21	1639.56	4.154	0.116
18	1654.03	17.061	12.321	1658.85	1650.17	5.43	0.799
19	1662.71	21.132	11.275	1673.32	1659.82	6.66	0.838
20	2263.56	56.789	0.318	2270.31	2208.59	14.454	0.017
21	2311.79	42.381	0.592	2312.75	2283.82	8.688	0.02
22	2326.25	30.162	0.771	2327.22	2312.75	6.272	0
23	2342.65	25.735	5.651	2348.43	2338.79	5.306	0.473
24	2358.08	19.396	2.036	2359.04	2349.4	5.629	0.225
25	2422.7	56.86	0.068	2425.59	2394.73	7.525	0.009
26	2503.71	55.784	0.018	2504.67	2468.03	9.191	0.003
27	2551.93	55.236	0.032	2553.86	2531.68	5.682	0.001
28	2581.83	54.782	0.022	2582.8	2555.79	7.014	0.007
29	2625.23	53.273	0.575	2633.91	2583.76	13.397	0.07
30	2658.02	51.157	1.232	2665.74	2634.88	8.674	0.121
31	2681.17	49.652	2.055	2692.74	2666.7	7.623	0.172
32	2701.42	50.15	1.398	2710.1	2693.71	4.804	0.086
33	2729.39	46.682	3.859	2740.97	2711.07	9.199	0.395
34	2777.61	42.901	1.053	2781.47	2741.93	13.253	0.136
35	2791.12	39.096	6.474	2817.16	2782.44	11.609	0.53
36	2848.02	45.054	5.198	2868.27	2829.69	12.197	0.66
37	2895.28	42.843	5.474	2905.89	2869.24	12.373	0.912
38	2917.46	45.418	1.004	2922.28	2906.85	5.149	0.069
39	2937.71	42.087	0.261	2938.68	2923.25	5.475	0.015
40	2954.11	44.326	1.876	2961.82	2949.29	4.298	0.105
41	2970.5	44.849	4.391	2985.94	2962.79	7.239	0.328

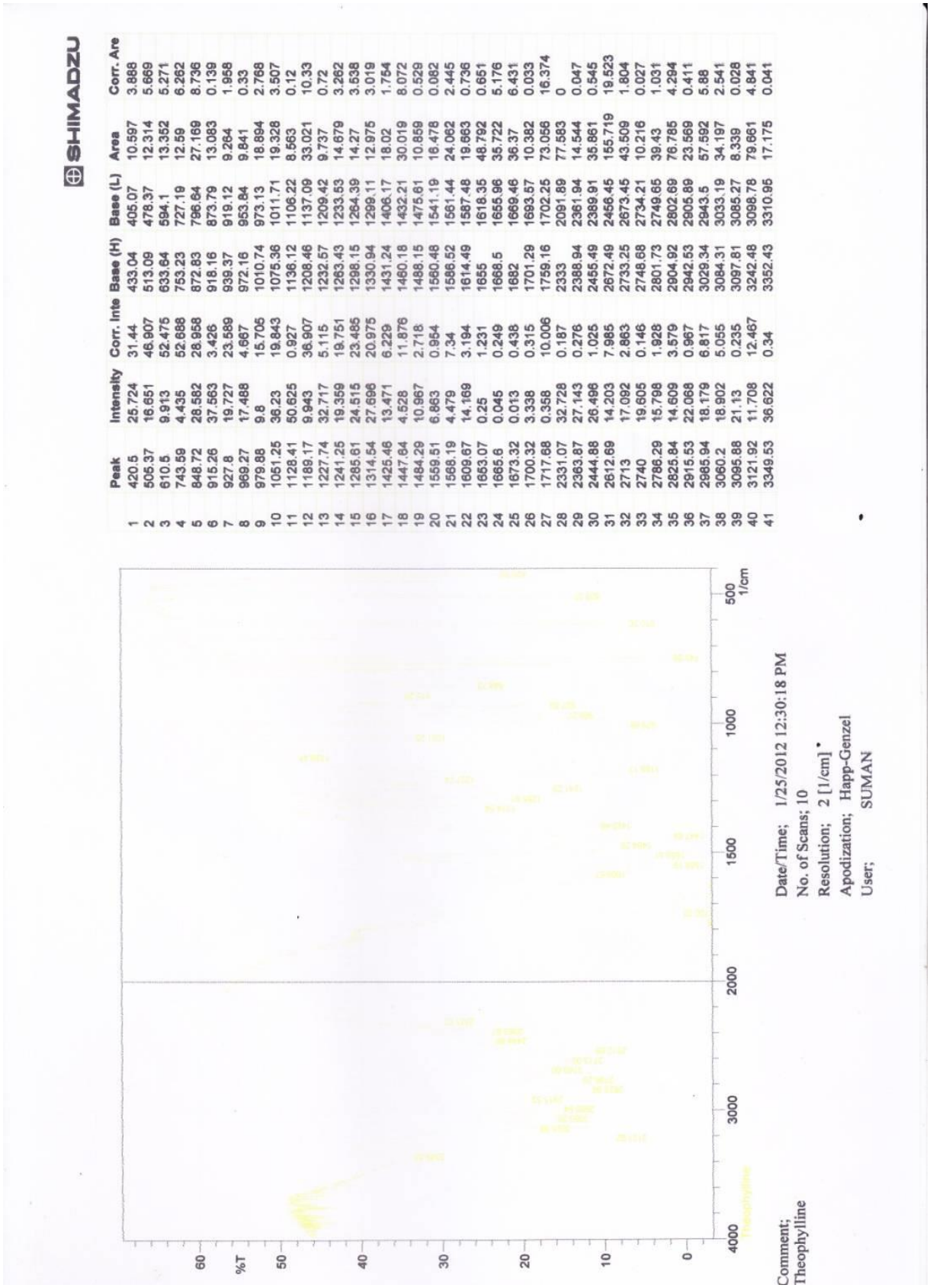
4.4.7 IR spectrum of ketotifen fumarate.



SHIMADZU

No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. A
1	639.43	24.101	30.605	653.9	619.18	12.988	3.873
2	789.88	31.545	25.969	800.49	774.45	8.228	1.959
3	922.01	42.892	9.223	929.73	903.69	8.176	0.958
4	982.77	30.392	16.451	997.24	968.31	12.325	2.787
5	1082.11	37.346	10.057	1090.79	1059.93	10.696	0.962
6	1149.62	33.091	8.142	1156.37	1108.15	18.64	1.316
7	1180.49	29.518	9.235	1190.13	1165.05	11.584	1.277
8	1201.7	30.517	7.379	1210.38	1191.09	8.901	0.79
9	1254.75	20.525	3.392	1259.57	1211.35	26.436	0.65
10	1279.82	18.919	10.363	1292.36	1268.25	14.487	1.532
11	1303.94	27.361	3.67	1308.76	1293.33	8.128	0.441
12	1320.33	24.409	7.983	1337.69	1309.72	15.127	1.623
13	1378.2	28.121	2.365	1381.09	1357.94	10.546	0.212
14	1398.45	15.766	16.027	1421.6	1382.06	23.749	4.25
15	1429.31	34.545	3.112	1445.71	1422.56	10.018	0.416
16	1475.61	34.284	1.184	1477.54	1458.25	8.268	0.299
17	1616.42	31.463	0.743	1617.38	1601.95	7.05	0.089
18	1652.1	7.287	30.278	1676.21	1618.35	39.369	13.889
19	1717.68	28.672	15.347	1740.83	1702.25	16.167	2.66
20	2336.86	38.074	0.21	2337.83	2109.25	73.889	0.003
21	2360.01	42.381	4.465	2387.98	2349.4	16.477	0.978
22	2429.45	38.562	0.046	2430.41	2388.94	16.824	0.047
23	2472.85	37.581	0.2	2479.6	2431.38	20.281	0.081
24	2516.25	36.922	0.024	2517.21	2480.56	15.708	0.018
25	2583.76	37.594	0.031	2585.69	2568.33	7.35	0.002
26	2675.38	37.989	0.272	2682.13	2632.95	20.36	0.094
27	2841.27	44.655	0.068	2842.23	2821.98	6.936	0.005
28	2852.84	43.551	0.997	2865.38	2843.2	7.902	0.11
29	2891.42	41.369	1.15	2897.21	2875.02	8.171	0.081
30	2925.17	39.551	2.238	2947.36	2898.17	19.221	0.589
31	2979.18	40.536	1.039	2992.69	2968.58	9.332	0.144
32	3095.88	42.381	2.983	3117.1	3086.24	10.835	0.282
33	3284.91	39.118	0.601	3294.56	3134.46	58.813	0.07
34	3422.83	32.7	0.058	3423.8	3295.52	57.614	0.59
35	3566.53	36.126	0.597	3583.89	3563.64	8.757	0.026

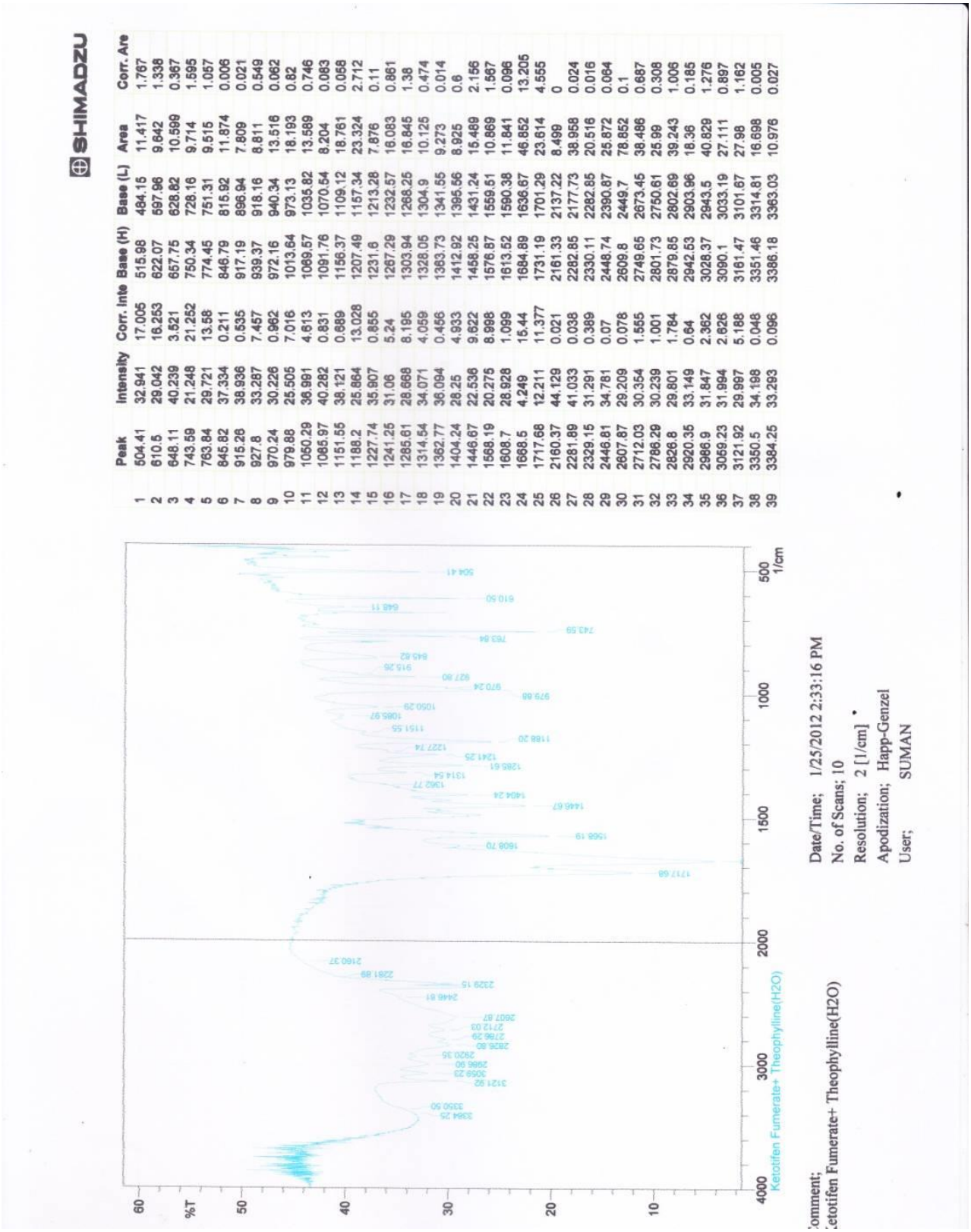
4.4.8 IR spectrum of theophylline.



SHIMADZU

	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	420.5	25.724	31.44	433.04	405.07	10.597	3.888
2	505.37	16.651	46.907	513.09	478.37	12.314	5.669
3	610.5	9.913	52.475	633.64	594.1	13.352	5.271
4	743.59	4.435	52.688	753.23	727.19	12.59	6.262
5	848.72	28.582	28.958	872.83	796.64	27.169	8.736
6	915.26	37.563	3.426	918.16	873.79	13.083	0.139
7	927.8	19.727	23.589	939.37	919.12	9.284	1.958
8	969.27	17.488	4.667	972.16	953.84	9.841	0.33
9	979.88	9.8	15.705	1010.74	973.13	18.894	2.768
10	1051.25	36.23	19.843	1075.36	1011.71	19.328	3.507
11	1128.41	50.625	0.927	1136.12	1106.22	8.563	0.12
12	1189.17	9.943	36.907	1208.46	1137.09	33.021	10.33
13	1227.74	32.717	5.115	1232.57	1209.42	9.737	0.72
14	1241.25	19.359	19.751	1283.43	1233.53	14.679	3.262
15	1285.61	24.515	23.485	1298.15	1264.39	14.27	3.538
16	1314.54	27.696	20.975	1330.94	1299.11	12.975	3.019
17	1425.46	13.471	6.229	1431.24	1406.17	18.02	1.754
18	1447.64	4.528	11.876	1460.18	1432.21	30.019	8.072
19	1484.29	10.967	2.718	1488.15	1475.61	10.859	0.529
20	1559.51	6.863	0.964	1560.48	1541.19	16.478	0.082
21	1568.19	4.479	7.34	1586.52	1561.44	24.062	2.445
22	1609.67	14.169	3.194	1614.49	1587.48	19.663	0.736
23	1653.07	0.25	1.231	1655	1618.35	48.792	0.651
24	1665.6	0.045	0.249	1668.5	1655.96	35.722	5.176
25	1673.32	0.013	0.438	1682	1669.46	36.37	6.431
26	1700.32	3.338	0.315	1701.29	1693.57	10.382	0.033
27	1717.68	0.358	10.006	1759.16	1702.25	73.056	16.374
28	2331.07	32.728	0.187	2333	2091.89	77.583	0
29	2363.87	27.143	0.276	2388.94	2361.94	14.544	0.047
30	2444.88	26.496	1.025	2455.49	2389.91	35.861	0.545
31	2612.69	14.203	7.985	2672.49	2456.45	155.719	19.523
32	2713	17.092	2.863	2733.25	2673.45	43.509	1.804
33	2740	19.605	0.146	2748.68	2734.21	10.216	0.027
34	2786.29	15.798	1.928	2801.73	2749.65	39.43	1.031
35	2825.84	14.609	3.579	2904.92	2802.69	76.785	4.294
36	2915.53	22.068	0.967	2942.53	2905.89	23.569	0.411
37	2985.94	18.179	6.817	3029.34	2943.5	57.592	5.88
38	3060.2	18.902	5.055	3084.31	3033.19	34.197	2.541
39	3095.88	21.13	0.235	3097.81	3085.27	8.339	0.028
40	3121.92	11.708	12.467	3242.48	3098.78	79.661	4.841
41	3349.53	36.622	0.34	3352.43	3310.95	17.175	0.041

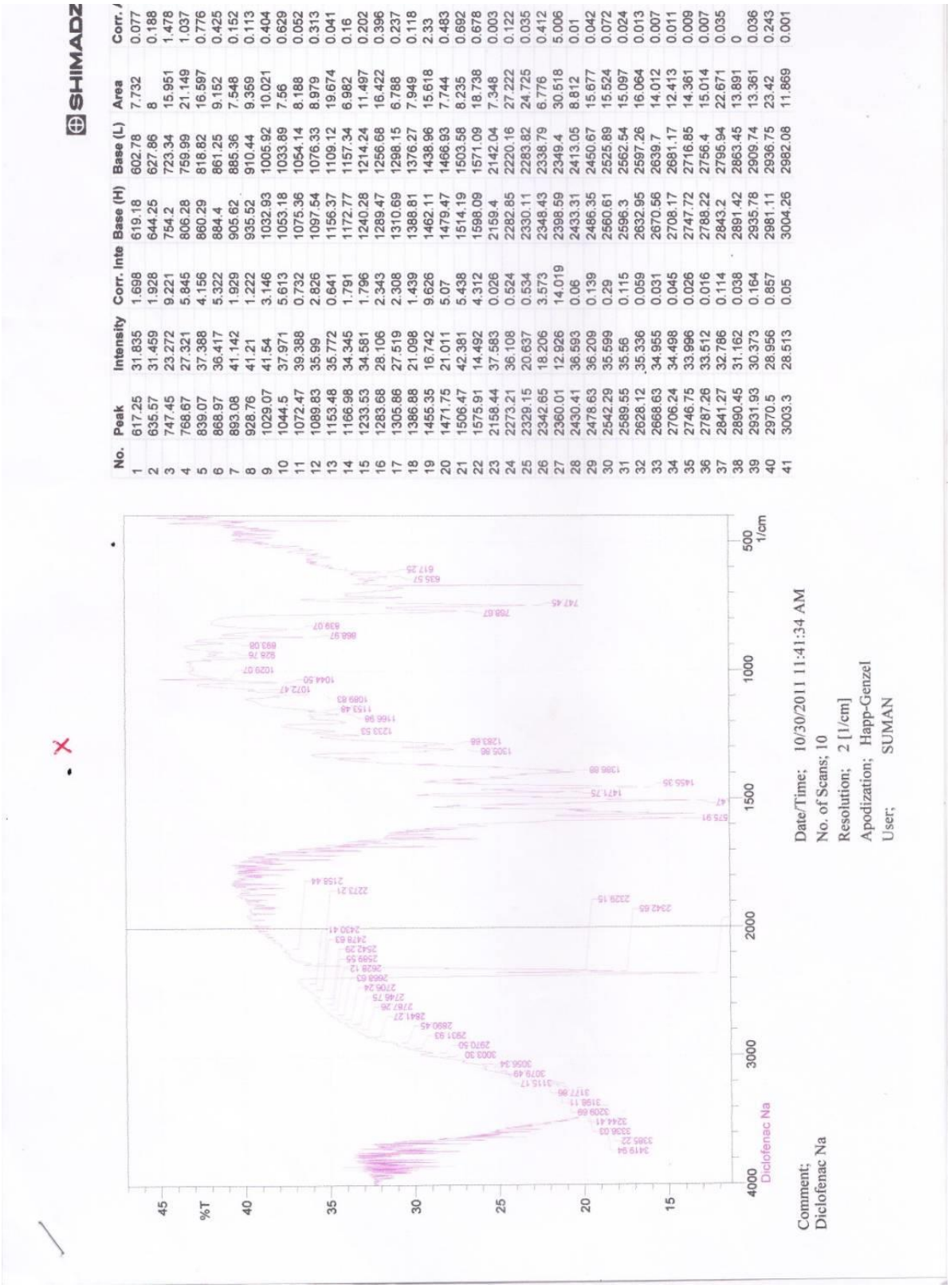
4.4.9 IR spectrum of ketotifen fumarate and theophylline (aqueous extract).



SHIMADZU

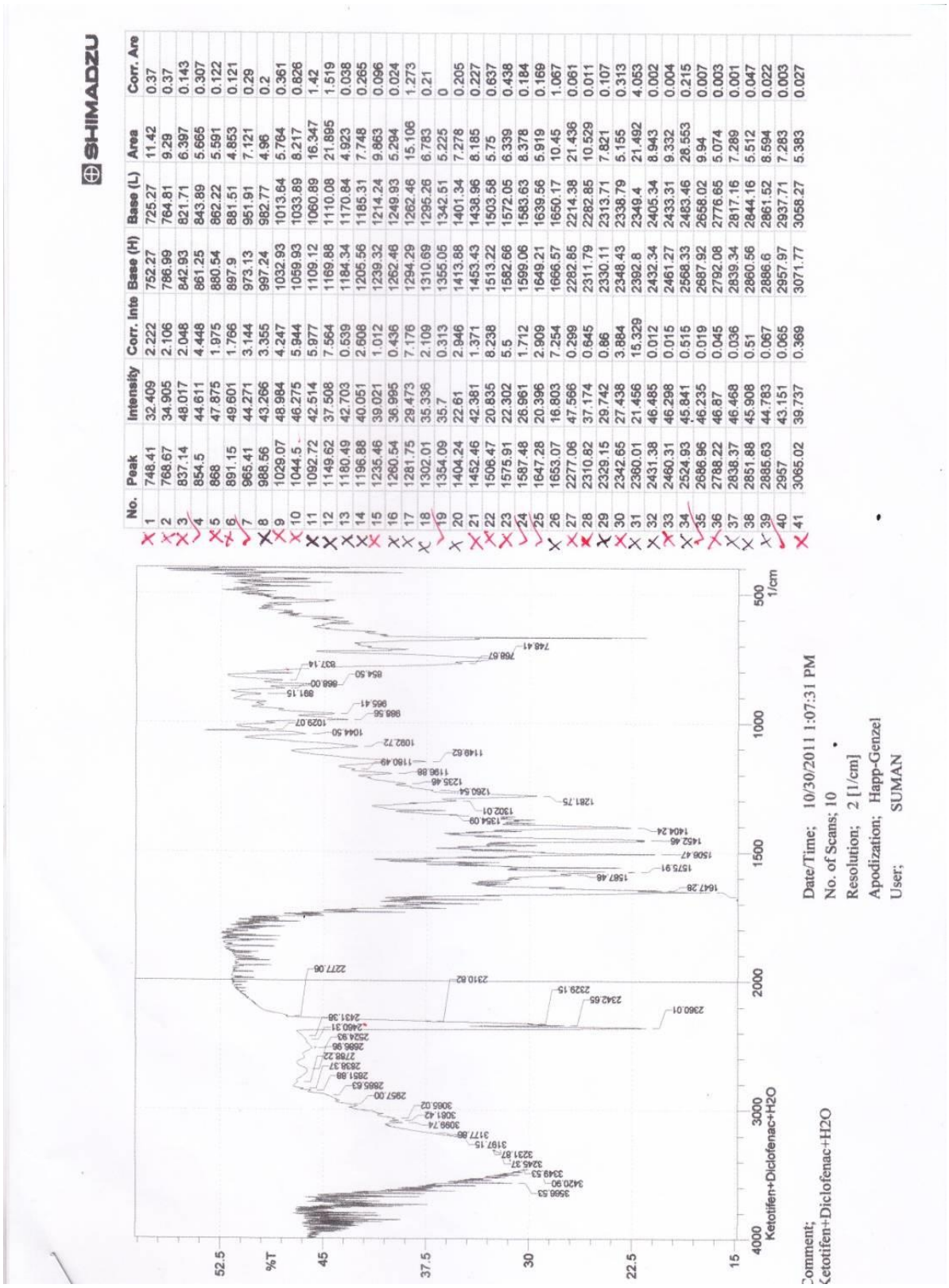
	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	504.41	32.941	17.005	515.98	484.15	11.417	1.767
2	610.5	29.042	16.253	622.07	597.96	9.642	1.338
3	648.11	40.239	3.521	657.75	628.82	10.599	0.367
4	743.59	21.248	21.252	750.34	728.16	9.714	1.595
5	763.84	29.721	13.58	774.45	751.31	9.515	1.057
6	845.82	37.334	0.211	846.79	815.92	11.874	0.006
7	915.26	38.936	0.535	917.19	896.94	7.809	0.021
8	927.8	33.287	7.457	939.37	918.16	8.811	0.549
9	970.24	30.226	0.962	972.16	940.34	13.516	0.062
10	979.88	25.505	7.016	1013.64	973.13	18.193	0.82
11	1050.29	36.991	4.613	1089.57	1035.82	13.589	0.746
12	1085.97	40.282	0.831	1091.76	1070.54	8.204	0.083
13	1151.55	38.121	0.689	1156.37	1109.12	18.761	0.058
14	1188.2	25.864	13.028	1207.49	1157.34	23.324	2.712
15	1227.74	35.907	0.855	1231.6	1213.28	7.876	0.11
16	1241.25	31.06	5.24	1267.29	1232.57	16.083	0.861
17	1285.61	28.668	8.195	1303.94	1268.25	16.845	1.38
18	1314.54	34.071	4.059	1328.05	1304.9	10.125	0.474
19	1362.77	36.094	0.456	1383.73	1341.55	9.273	0.014
20	1404.24	28.25	4.933	1412.92	1385.56	8.925	0.6
21	1446.67	22.536	9.622	1458.25	1431.24	15.489	2.156
22	1568.19	20.275	8.998	1576.87	1559.51	10.869	1.567
23	1608.7	28.928	1.099	1613.52	1590.38	11.841	0.096
24	1688.5	4.249	15.44	1684.89	1636.67	46.852	13.205
25	1717.68	12.211	11.377	1731.19	1701.29	23.614	4.555
26	2160.37	44.129	0.021	2161.33	2137.22	8.499	0
27	2281.89	41.033	0.038	2282.85	2177.73	38.958	0.024
28	2329.15	31.291	0.389	2330.11	2282.85	20.516	0.016
29	2446.81	34.781	0.07	2448.74	2390.87	25.872	0.084
30	2607.87	29.209	0.078	2609.8	2449.7	78.852	0.1
31	2712.03	30.354	1.555	2749.65	2673.45	38.486	0.687
32	2786.29	30.239	1.001	2801.73	2750.61	25.99	0.308
33	2826.8	29.801	1.784	2879.85	2802.89	39.243	1.006
34	2920.35	33.149	0.64	2942.53	2903.96	18.36	0.185
35	2986.9	31.847	2.362	3028.37	2943.5	40.829	1.276
36	3059.23	31.994	2.626	3090.1	3033.19	27.111	0.897
37	3121.92	29.997	5.188	3161.47	3101.67	27.98	1.162
38	3350.5	34.198	0.048	3351.46	3314.81	16.698	0.005
39	3384.25	33.293	0.096	3386.18	3363.03	10.976	0.027

4.4.10 IR spectrum of diclofenac sodium.



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr.
1	617.25	31.835	1.698	619.18	602.78	7.732	0.077
2	635.57	31.459	1.928	644.25	627.86	8	0.188
3	747.45	23.272	9.221	754.2	723.34	15.951	1.478
4	768.67	27.321	5.845	806.28	759.99	21.149	1.037
5	839.07	37.388	4.156	860.29	818.82	16.597	0.776
6	868.97	36.417	5.322	884.4	861.25	9.152	0.425
7	893.08	41.142	1.929	905.62	885.36	7.548	0.152
8	928.76	41.21	1.222	935.52	910.44	9.359	0.113
9	1029.07	41.54	3.146	1032.93	1005.92	10.021	0.404
10	1044.5	37.971	5.613	1053.18	1033.89	7.56	0.629
11	1072.47	39.388	0.732	1075.36	1054.14	8.188	0.052
12	1089.83	35.99	2.826	1097.54	1076.33	8.979	0.313
13	1153.48	35.772	0.641	1156.37	1109.12	19.674	0.041
14	1166.98	34.345	1.791	1172.77	1157.34	6.982	0.16
15	1233.53	34.581	1.796	1240.28	1214.24	11.497	0.202
16	1283.68	28.106	2.343	1289.47	1256.68	16.422	0.396
17	1305.86	27.519	2.308	1310.69	1298.15	6.788	0.237
18	1386.88	21.098	1.439	1388.81	1376.27	7.949	0.118
19	1455.35	16.742	9.626	1462.11	1438.96	15.618	2.33
20	1471.75	21.011	5.07	1479.47	1466.93	7.744	0.483
21	1506.47	42.381	5.438	1514.19	1503.58	8.235	0.692
22	1575.91	14.492	4.312	1598.09	1571.09	18.738	0.678
23	2158.44	37.583	0.026	2159.4	2142.04	7.348	0.003
24	2273.21	36.108	0.524	2282.85	2220.16	27.222	0.122
25	2329.15	20.637	0.534	2330.11	2283.82	24.725	0.035
26	2342.65	18.206	3.573	2348.43	2338.79	6.776	0.412
27	2360.01	12.926	14.019	2398.59	2349.4	30.518	5.006
28	2430.41	36.593	0.06	2433.31	2413.05	8.812	0.01
29	2478.63	36.209	0.139	2486.35	2450.67	15.677	0.042
30	2542.29	35.599	0.29	2560.61	2525.89	15.524	0.072
31	2589.55	35.56	0.115	2596.3	2562.54	15.097	0.024
32	2628.12	35.336	0.059	2632.95	2597.26	16.064	0.013
33	2668.63	34.955	0.031	2670.56	2639.7	14.012	0.007
34	2706.24	34.498	0.045	2708.17	2681.17	12.413	0.011
35	2746.75	33.996	0.026	2747.72	2716.85	14.361	0.009
36	2787.26	33.512	0.016	2788.22	2756.4	15.014	0.007
37	2841.27	32.786	0.114	2843.2	2795.94	22.671	0.035
38	2890.45	31.162	0.038	2891.42	2863.45	13.891	0
39	2931.93	30.373	0.164	2935.78	2909.74	13.361	0.036
40	2970.5	28.956	0.857	2981.11	2936.75	23.42	0.243
41	3003.3	28.513	0.05	3004.26	2982.08	11.869	0.001

4.4.11 IR spectrum of ketotifen fumarate and diclofenac sodium (aqueous extract).



SHIMADZU

No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	748.41	32.409	2.222	752.27	725.27	11.42	0.37
2	768.67	34.905	2.106	786.99	764.81	9.29	0.37
3	837.14	48.017	2.048	842.93	821.71	6.397	0.143
4	854.5	44.611	4.448	861.25	843.89	5.665	0.307
5	868	47.875	1.975	880.54	862.22	5.591	0.122
6	891.15	49.601	1.766	897.9	881.51	4.853	0.121
7	965.41	44.271	3.144	973.13	951.91	7.121	0.29
8	988.56	43.266	3.355	997.24	982.77	4.96	0.2
9	1029.07	48.984	4.247	1032.93	1013.64	5.784	0.361
10	1044.5	46.275	5.944	1059.93	1033.89	8.217	0.826
11	1092.72	42.514	5.977	1109.12	1060.89	16.347	1.42
12	1149.62	37.508	7.564	1169.88	1110.08	21.895	1.519
13	1180.49	42.703	0.539	1184.34	1170.84	4.923	0.038
14	1196.88	40.051	2.608	1205.56	1185.31	7.748	0.265
15	1235.46	39.021	1.012	1239.32	1214.24	9.863	0.096
16	1260.54	36.985	0.436	1262.48	1249.93	5.294	0.024
17	1281.75	29.473	7.176	1294.29	1262.46	15.106	1.273
18	1302.01	35.336	2.109	1310.69	1295.26	6.783	0.21
19	1354.09	35.7	0.313	1355.05	1342.51	5.225	0
20	1404.24	22.61	2.946	1413.88	1401.34	7.278	0.205
21	1452.46	42.381	1.371	1453.43	1438.96	8.185	0.227
22	1506.47	20.835	8.238	1513.22	1503.58	5.75	0.637
23	1575.91	22.302	5.5	1582.66	1572.05	6.339	0.438
24	1587.48	26.961	1.712	1599.06	1583.63	8.378	0.184
25	1647.28	20.396	2.909	1649.21	1639.56	5.919	0.169
26	1653.07	16.803	7.254	1666.57	1650.17	10.45	1.067
27	2277.06	47.566	0.299	2282.85	2214.38	21.436	0.061
28	2310.82	37.174	0.645	2311.79	2282.85	10.529	0.011
29	2329.15	29.742	0.86	2330.11	2313.71	7.821	0.107
30	2342.65	27.438	3.884	2348.43	2338.79	5.155	0.313
31	2360.01	21.456	15.329	2392.8	2349.4	21.492	4.053
32	2431.38	46.485	0.012	2432.34	2405.34	8.943	0.002
33	2460.31	46.298	0.015	2461.27	2433.31	9.332	0.004
34	2524.93	45.841	0.515	2568.33	2483.46	28.553	0.215
35	2686.96	46.235	0.019	2687.92	2658.02	9.94	0.007
36	2788.22	46.87	0.045	2792.08	2776.65	5.074	0.003
37	2838.37	46.468	0.036	2839.34	2817.16	7.289	0.001
38	2851.88	45.908	0.51	2860.56	2844.16	5.512	0.047
39	2885.63	44.783	0.067	2886.6	2861.52	8.594	0.022
40	2957	43.151	0.065	2957.97	2937.71	7.283	0.003
41	3065.02	39.737	0.369	3071.77	3058.27	5.383	0.027

Table 4.23: Interacted peaks for ketotifen fumarate and paracetamol.

Ketotifen fumarate Wave number (cm^{-1})	Paracetamol Wave number (cm^{-1})	Ketotifen fumarate and paracetamol (Aqueous extract) Wave number (cm^{-1})	Ketotifen fumarate and paracetamol (Chloroform extract) Wave number (cm^{-1})
639.43	604.71	504.41	757.09
789.88	625.93	756.13	982.77
922.01	685.72	796.64	1089.47
982.77	714.66	836.18	1132.26
1082.11	729.12	855.47	1224.85
1149.62	796.64	924.91	1284.65
1180.49	808.21	964.45	1335.76
1201.7	837.14	984.7	1372.41
1254.75	867.4	1037.75	1406.17
1279.82	1015.57	1082.11	1510.33
1303.94	1032.93	1106.22	1626.95
1320.33	1108.15	1151.55	1643.42
1378.2	1171.81	1227.74	1662.71
1398.45	1226.78	1244.14	2169.05
1429.31	1243.18	1260.54	2217.27
1475.61	1260.54	1321.3	2311.79
1616.42	1328.05	1371.45	2360.01
1652.1	1370.48	1402.31	2508.53
1717.68	1442.82	1507.43	2625.23
2336.86	1506.47	1610.63	2659.95
2360.01	1516.11	1654.03	2730.36
2429.45	1564.34	2224.02	2779.54
2472.85	1610.63	2282.85	2791.12
2516.25	1653.07	2365.79	2807.51
2583.76	2281.89	2514.32	2848.98
2675.38	2494.07	2598.23	2888.53
2841.27	2586.65	2664.08	2917.46
2852.84	2666.7	2709.14	2937.71
2891.42	2714.92	2797.87	2962.79
2925.17	2794.01	2853.81	3015.83
2979.18	2880.81	2927.1	3053.45
3096.88	2925.17	2969.54	3075.63
3284.91	3035.12	3032.23	3095.88
3422.83	3066.95	3161.47	3146.03
3566.53	3109.38	3206.79	3264.66
	3162.43	3242.48	3296.49
	3293.59	3325.42	3328.31
	3325.42		3515.42

Table 4.24: Interacted peaks for ketotifen fumarate and salbutamol.

Ketotifen fumarate Wave number (cm^{-1})	Salbutamol Wave number (cm^{-1})	Ketotifen fumarate and salbutamol (Aqueous extract) Wave number (cm^{-1})	Ketotifen fumarate and salbutamol (Chloroform extract) Wave number (cm^{-1})
639.43	609.53	649.07	734.91
789.88	648.11	791.81	757.09
922.01	747.45	832.32	782.17
982.77	774.45	853.64	855.47
1082.11	791.81	922.01	922.01
1149.62	838.11	963.48	950.95
1180.49	882.47	983.74	983.74
1201.7	916.23	1029.07	1066.68
1254.75	976.99	1037.75	1099.47
1279.82	988.56	1084.04	1127.44
1303.94	1029.07	1117.8	1194.95
1320.33	1085.97	1127.44	1224.85
1378.2	1109.12	1145.77	1246.07
1398.45	1132.26	1198.81	1284.65
1429.31	1196.81	1260.54	1386.88
1475.61	1245.1	1635.71	1495.2
1616.42	1258.81	2275.14	1647.28
1652.1	1308.76	2311.79	1654.03
1717.68	1339.62	2329.15	1662.71
2336.86	1362.77	2342.65	2263.56
2360.01	1405.2	2358.08	2311.79
2429.45	1438.96	2460.31	2326.25
2472.85	1507.43	2513.36	2342.65
2516.25	1518.42	2559.65	2358.08
2583.76	2277.06	2615.59	2422.7
2675.38	2336.86	2692.74	2503.71
2841.27	2360.01	2725.53	2551.93
2852.84	2454.52	2762.18	2581.83
2891.42	2483.46	2851.88	2625.23
2925.17	2559.65	2930.96	2658.02
2979.18	2691.78	2978.22	2681.17
3096.88	2728.43	3079.49	2701.42
3284.91	2785.29	3150.86	2729.39
3422.83	2794.01	3178.83	2777.61
3566.53	2954.11	3327.25	2791.12
	2980.15	3356.28	2846.02
	3160.5		2895.28
	3244.41		2917.46
	3273.34		2937.71
			2954.11
			2970.5

Table 4.25: Interacted peaks for ketotifen fumarate and amoxicillin.

Ketotifen fumarate Wave number (cm^{-1})	**Amoxicillin Wave number (cm^{-1})**	**Ketotifen fumarate and amoxicillin (Aqueous extract) Wave number (cm^{-1})**		**Ketotifen fumarate and amoxicillin (Chloroform extract) Wave number (cm^{-1})**	
639.43	556.49	649.07	2360.01	703.08	2917.46
789.88	656.79	720.44	2521.07	734.91	2941.57
922.01	696.33	736.84	2558.68	757.09	2954.11
982.77	733.95	757.09	2603.05	782.17	2969.54
1082.11	1120.69	790.85	2691.78	855.47	3014.87
1149.62	1144.8	840.04	2705.28	922.01	3056.34
1180.49	1218.1	853.54	2722.64	950.95	3079.49
1201.7	1249.93	964.45	2735.18	983.74	3114.21
1254.75	1282.72	987.6	2749.65	1066.68	3178.83
1279.82	1313.58	1038.71	2794.97	1099.47	3198.11
1303.94	1328.05	1083.08	2808.48	1127.44	3210.65
1320.33	1379.16	1098.51	2837.41	1144.8	3233.8
1378.2	1396.52	1129.37	2853.81	1177.59	3277.2
1398.45	1451.5	1151.55	2891.42	1194.95	3327.35
1429.31	1486.22	1174.7	2900.1	1224.85	3336.99
1475.61	1520.94	1244.14	2918.42	1246.07	3357.25
1616.42	1576.87	1280.54	2928.07	1284.65	3385.22
1652.1	1581.7	1281.75	2961.82	1384.95	3420.9
1717.68	1616.42	1329.98	3012.94	1405.2	3546.28
2336.86	1684.89	1339.62	3049.59	1595.2	3587.5
2360.01	1775.55	1354.09	3065.02	1648.24	3650.44
2429.45	2360.01	1362.77	3079.49	1654.03	3690.95
2472.85	2713.96	1386.88	3178.83	1662.71	3712.17
2516.25	2905.89	1404.24	3198.11	2263.56	3735.31
2583.76	2930	1448.6	3210.65	2311.79	3853.94
2675.38	2969.54	1457.28	3233.8	2329.15	
2841.27	2990.76	1490.07	3255.02	2342.65	
2852.84	3035.12	1507.43	3274.31	2360.01	
2891.42	3040.91	1559.51	3292.63	2423.66	
2925.17	3166.29	1576.87	3327.35	2461.27	
2979.18	3177.86	1595.2	3336.99	2512.39	
3096.88	3185.58	1602.91	3356.28	2575.08	
3284.91	3336.99	1609.67	3367.86	2625.23	
3422.83	3368.82	1617.38	3379.43	2658.99	
3566.53	3385.22	1623.17	3392.93	2682.13	
	3420.9	1635.71	3420.9	2701.42	
	3446.94	1667.28	3446.94	2729.39	
	3462.37	1653.07	3481.66	2778.58	
	3523.13	2311.79	3509.63	2790.15	
		2329.15	3545.32	2848.02	
		2342.65	3566.53	2895.28	

Table 4.26: Interacted peaks for ketotifen fumarate and domperidone.

Ketotifen fumarate Wave number (cm^{-1})	Domperidone Wave number (cm^{-1})	Ketotifen fumarate and domperidone (Aqueous extract) Wave number (cm^{-1})	Ketotifen fumarate and domperidone (Chloroform extract) Wave number (cm^{-1})
639.43	554.56	652.93	533.34
789.88	653.9	687.65	757.09
922.01	689.58	709.83	783.13
982.77	706.94	735.87	853.54
1082.11	734.91	753.23	982.77
1149.62	792.78	793.74	1066.68
1180.49	863.18	853.54	1130.33
1201.7	885.36	924.91	1145.77
1254.75	925.87	978.92	1246.07
1279.82	1002.06	1018.46	1283.68
1303.94	1015.57	1139.98	1296.22
1320.33	1064.75	1183.38	1339.62
1378.2	1185.31	1201.7	1374.34
1398.45	1302.97	1260.54	1404.24
1429.31	1348.3	1339.62	1448.6
1475.61	1384.95	1354.09	1595.2
1616.42	1410.02	1489.11	1635.71
1652.1	1447.64	1675.25	1648.24
1717.68	1457.28	1683.93	1653.07
2336.86	1488.15	2279.96	2311.79
2360.01	1580.73	2310.82	2360.01
2429.45	1624.13	2360.01	2661.88
2472.85	1696.47	2510.46	2686.96
2516.25	1701.29	2544.22	2732.29
2583.76	2280.92	2596.3	2786.29
2675.38	2399.55	2635.84	2845.13
2841.27	2509.5	2687.92	2893.35
2852.84	2525.89	2715.89	2937.71
2891.42	2648.38	2738.07	2968.58
2925.17	2721.68	2772.79	3015.83
2979.18	2763.15	2822.94	3057.3
3096.88	2834.52	2893.35	3065.02
3284.91	2942.53	2953.14	3095.88
3422.83	2957	3022.58	3178.83
3566.53	3025.48	3116.14	3198.11
	3060.2	3177.86	3274.31
	3090.1	3336.99	3336.9
	3127.71	3355.32	3356.28
	3138.32	3385.22	
	3170.15		
	3380.4		

Table 4.27: Interacted peaks for ketotifen fumarate and theophylline.

Ketotifen fumarate Wave number (cm^{-1})	Theophylline Wave number (cm^{-1})	Ketotifen fumarate and theophylline (Aqueous extract) Wave number (cm^{-1})	Ketotifen fumarate and theophylline (Chloroform extract) Wave number (cm^{-1})
639.43	420.5	504.41	610.5
789.88	505.37	610.5	703.08
922.01	610.5	648.11	855.47
982.77	743.59	743.59	982.77
1082.11	848.72	763.84	1036.78
1149.62	915.26	845.82	1127.44
1180.49	927.8	915.26	1189.17
1201.7	989.27	927.8	1225.82
1254.75	979.88	970.24	1246.07
1279.82	1061.25	979.88	1284.65
1303.94	1128.41	1050.29	1362.77
1320.33	1189.17	1085.97	1405.2
1378.2	1227.74	1151.55	1608.7
1398.45	1241.25	1188.2	1653.07
1429.31	1265.61	1227.74	1662.71
1475.61	1314.54	1241.25	1713.83
1616.42	1425.46	1285.61	2115.04
1652.1	1447.64	1314.54	2161.33
1717.68	1484.29	1362.77	2232.7
2336.86	1559.51	1404.24	2265.49
2360.01	1588.19	1446.67	2329.15
2429.45	1609.67	1588.19	2436.2
2472.85	1653.07	1608.7	2622.34
2516.25	1665.8	1668.5	2657.06
2583.76	1673.32	1717.68	2681.17
2675.38	1700.32	2160.37	2701.42
2841.27	1717.68	2281.89	2728.43
2852.84	2331.07	2329.15	2778.58
2891.42	2363.87	2446.81	2790.15
2925.17	2444.88	2607.87	2848.98
2979.18	2612.69	2712.03	2895.28
3096.88	2713	2786.29	2916.49
3284.91	2740	2826.8	2970.5
3422.83	2786.29	2920.35	3014.87
3566.53	2825.84	2986.9	3056.34
	2915.53	3059.23	3064.06
	2985.94	3121.92	3107.46
	3060.2	3350.5	3336.03
	3095.88	3384.25	3355.32
	3121.92		
	3349.53		

Table 4.28: Interacted peaks for ketotifen fumarate and diclofenac.

Ketotifen fumarate Wave number (cm^{-1})	**Diclofenac Wave number (cm^{-1})**	**Ketotifen fumarate and diclofenac (Aqueous extract) Wave number (cm^{-1})**	**Ketotifen fumarate and diclofenac (Chloroform extract) Wave number (cm^{-1})**
639.43	617.25	748.41	649.07
789.88	635.57	768.67	750.34
922.01	77.45	837.14	854.5
982.77	768.67	854.5	966.38
1082.11	839.07	868	989.53
1149.62	868.97	891.15	1042.57
1180.49	893.08	965.41	1091.76
1201.7	928.6	988.56	1149.62
1254.75	1029.07	1029.07	1196.88
1279.82	1044.5	1044.5	1260.54
1303.94	1072.47	1092.72	1280.79
1320.33	1089.83	1149.62	1405.2
1378.2	1153.48	1180.49	1448.6
1398.45	1166.98	1196.88	1457.26
1429.31	1233.53	1235.46	1507.43
1475.61	1283.68	1260.54	1576.87
1616.42	1305.86	1281.75	1647.28
1652.1	1386.88	1302.01	1653.07
1717.68	1455.35	1354.09	2275.14
2336.86	1471.75	1404.24	2311.79
2360.01	1506.47	1452.46	2329.15
2429.45	1575.91	1506.47	2342.65
2472.85	2158.44	1575.91	2360.01
2516.25	2273.21	1587.48	2445.84
2583.76	2329.15	1647.28	2645.48
2675.38	2342.65	1653.07	2658.02
2841.27	2360.01	2277.06	2675.38
2852.84	2430.41	2310.82	2734.21
2891.42	2478.63	2329.15	2766.04
2925.17	2542.29	2342.65	2788.22
2979.18	2589.55	2360.01	2840.3
3096.88	2628.12	2431.38	2852.84
3284.91	2668.63	2460.31	2891.42
3422.83	2706.24	2524.93	2928.07
3566.53	2746.75	2686.96	3065.02
	2787.26	2788.22	3176.83
	2841.27	2838.37	3327.35
	2890.45	2851.88	3420.9
	2931.93	2885.63	3462.37
	2970.5	2957	3586.53
	3003.3	3065.02	3650.44

The possible interaction between ketotifen fumarate and amoxicillin trihydrate showed characteristic peaks. The peaks of acid chlorides (C-Cl) at 696.33 was shifted to higher wave number in the complex at 703.08 cm^{-1} and ethers group at 1082.11 cm^{-1} (C-O-C) were shifted to higher wave number in the complex at 1099 cm^{-1} (C-O-C stretching); The peaks of alkenes at 3040 cm^{-1} was shifted to 3056 cm^{-1} (=CH). Simultaneously the peaks of isocyanates (-N=C=O) at 2270 cm^{-1} and carboxylic 3336.99 cm^{-1} (O-H) were shifted to lower wave numbers in the complex at 2263cm^{-1} and 3327 cm^{-1}. Similarly when ketotifen and diclofenac were interacted the peaks of acid phosphines at 839.07 cm^{-1} (P-H stretching) was shifted to higher wave number in the complex at 854.5 cm^{-1}. On the other hand the peaks of alkyl halides (C-F) of 1386.88 cm^{-1}, amides of 1575.91 cm^{-1}, amides of 1652.1, aromatic group having wave number of 2970.5 cm^{-1} were showed characteristic peaks in the complexes at 1354.09 cm^{-1}, 1587.48 cm^{-1}, 1647.28 cm^{-1} and 2957 cm^{-1} respectively. On the other hand the peaks of C-H(aromatic group), C-O-C(ether group), C-C(ketone) and O-H(carboxylic acid group) at 838.11 cm^{-1}, 1085 cm^{-1}, 1201.7 cm^{-1}, 2925.17 cm^{-1} were shifted to higher wave numbers in the complexes at 855.47 cm^{-1} (C-H stretching), 1099.47 cm^{-1} (C-O-C stretching), 1224.85 cm^{-1} (C-C stretching) and 2937.71 cm^{-1} (O-H) when interaction was happening between ketotifen fumarate and salbutamol sulphate. The possible interaction between ketotifen fumarate and theophylline anhydrous showed characteristic peaks. The peaks of phosphine oxides group at 1180 cm^{-1} (P=O stretching) and alkenes group at 1378 cm^{-1} (C-H) were shifted to lower wave numbers in the complex at 1151 cm^{-1} (P=O stretching) and 1362.77 cm^{-1} (C-H stretching). On the other hand the peak of amides of 1559.51 cm^{-1} (N-H stretching) was shifted to higher wave number in the complex at 1588.19 cm^{-1} (N-H) .

4.5 Complex confirmation test by Differential Scanning Calorimeter (DSC)

By studying Jobs continuous variation method as well as Infrared spectroscopy study gave an immense idea about the complex formation between ketotifen fumarate & domperidone; ketotifen fumarate & theophylline and ketotifen fumarate & metformin hydrochloride. However the complex formation was confirmed by the following scanning repots:

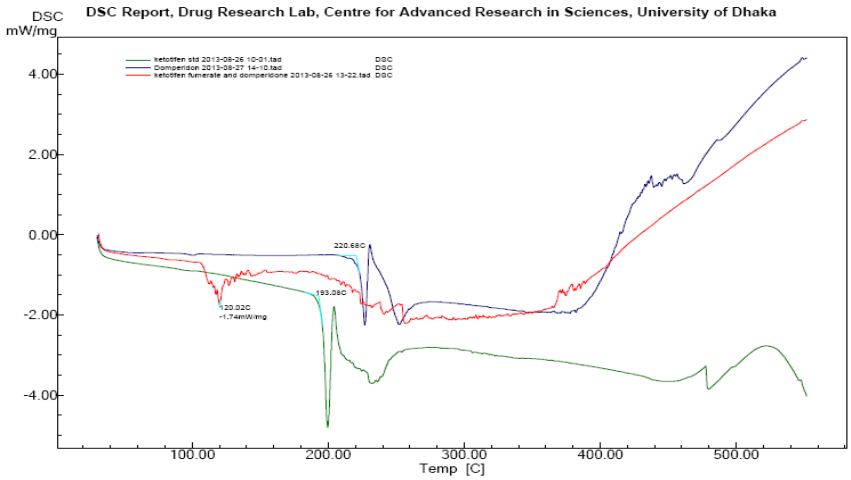


Figure 4.78: Differential scanning calorimeter study of ketotifen, domperidone and ketotifen-domperidone mixture

DSC of all the samples (ketotifen, domperidone and ketotifen & domperidone mixture) were performed to investigate the thermal behavior of the drug. In the DSC study ketotifen exhibited sharp endothermic peak at 193.08^{0}C. Corresponding to the melting of ketotifen fumarate, domperidone showed an endothermic peak at 220.68^{0}C. The ketotifen- domperidone complex exhibited a sharp new peak at 120.02^{0}C (-1.74 mW/mg) which showed interactions between ketotifen fumarate and domperidone.

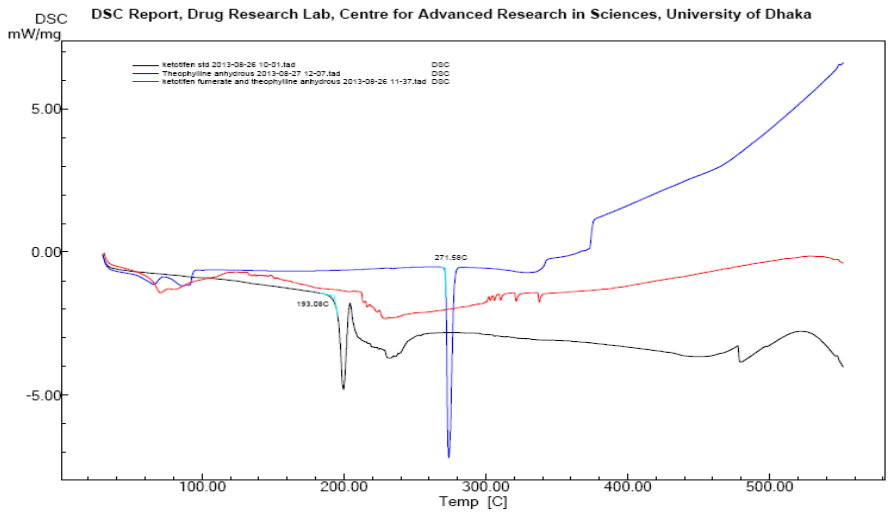


Figure 4.79: Differential scanning calorimeter study of ketotifen, theophylline and ketotifen-theophylline mixture.

Similarly the DSC of all the samples (ketotifen, theophylline and ketotifen & theophylline mixture) were performed to investigate the thermal behavior of the drug. In the DSC study ketotifen exhibited sharp endothermic peak at 192.08^{0}C. Corresponding to the melting of ketotifen fumarate, theophylline showed an endothermic peak at 271.58^{0}C. But the mixture of ketotifen and theophylline exhibited no sharp peak and confirming the absence of complex.

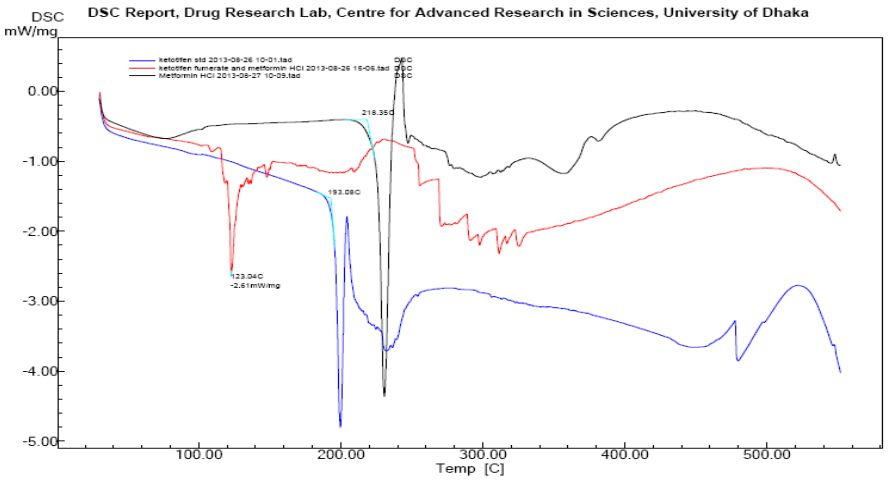


Figure 4.80: Differential scanning calorimeter study of ketotifen, metformin and ketotifen-metformin mixture.

On the other hand DSC of all the samples (ketotifen, metformin and ketotifen & metformin mixture) were performed to investigate the thermal behavior of the drug. In the DSC study ketotifen exhibited sharp endothermic peak at 192.08^0C. Corresponding to the melting of ketotifen fumarate, metformin showed an endothermic peak at 218.35^0C. The ketotifen – metformin complex exhibited a sharp new peak at 123.04^0C (-2.61 mW/mg) which showed interactions between ketotifen fumarate and metformin hydrochloride and confirming the formation of a complex.[91]

4.6 Identification of stability of the complex by Thin Layer Chromatography:

The stability of the complex after formation was confirmed by thin layer chromatographic technique. When the R_f values of the mixtures differ from the R_f values of the pure compounds, indicate the stability of the complex.

Table 4.29: Identification of the stability of the complex (ketotifen-domperidone)

Systems	R_f values	Comment
Ketotifen	0.44	Stable complex is formed
Domperidone	0.53	
Mixture of Ketotifen & Domperidone	0.38	

Table 4.30: Identification of the stability of the complex (ketotifen-theophylline)

Systems	R_f values	Comment
Ketotifen	0.37	Less stable complex is formed
Theophylline	0.53	
Mixture of Ketotifen & Theophylline	0.36	

Table 4.31: Identification of the stability of the complex (ketotifen-metformin)

Systems	R_f values	Comment
Ketotifen	0.49	Stable complex is formed
Metformin	0.51	
Mixture of Ketotifen & Metformin	0.39	

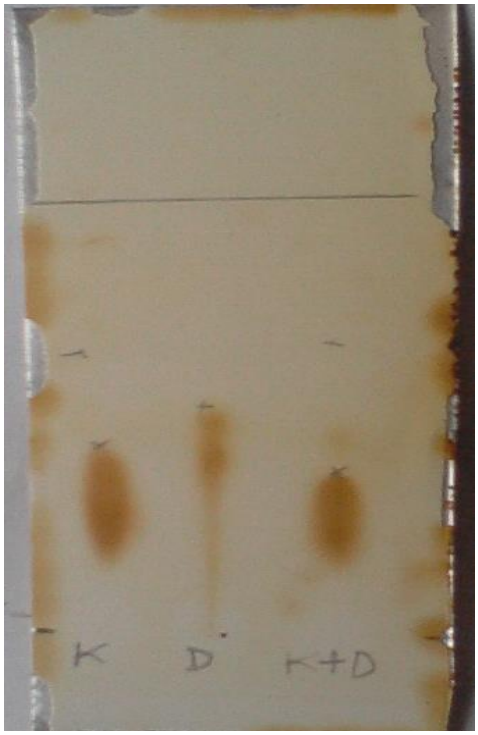


Figure 4.81: TLC plate of ketotifen, domperidone and ketotifen- domperidone complex

Figure 4.82: TLC plate of ketotifen, theophylline and ketotifen- theophylline complex

Figure 4.83: TLC plate of ketotifen,metformin and ketotifen- metformin complex

4.7. Stability of the complex confirmation by HPLC method

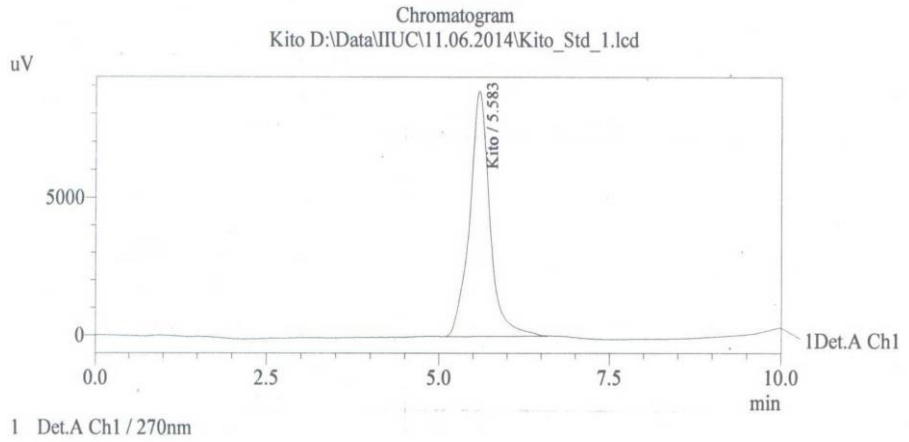


Figure: 4.84: Chromatogram on ketotifen fumarate standard

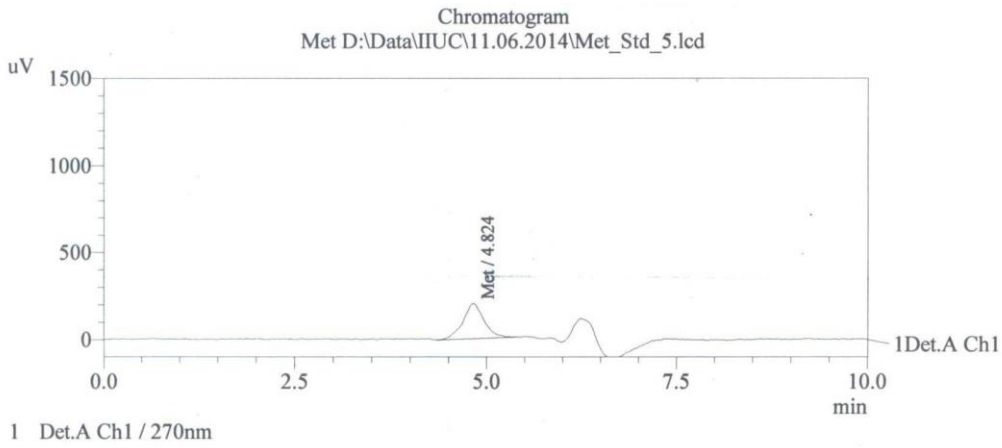


Figure: 4.85: Chromatogram on metformin hydrochloride standard

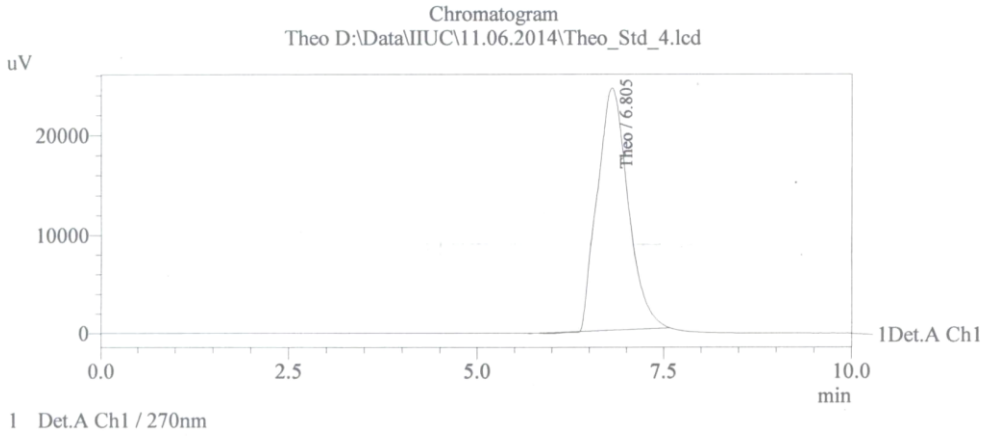


Figure: 4.86: Chromatogram on theophylline anhydrous standard

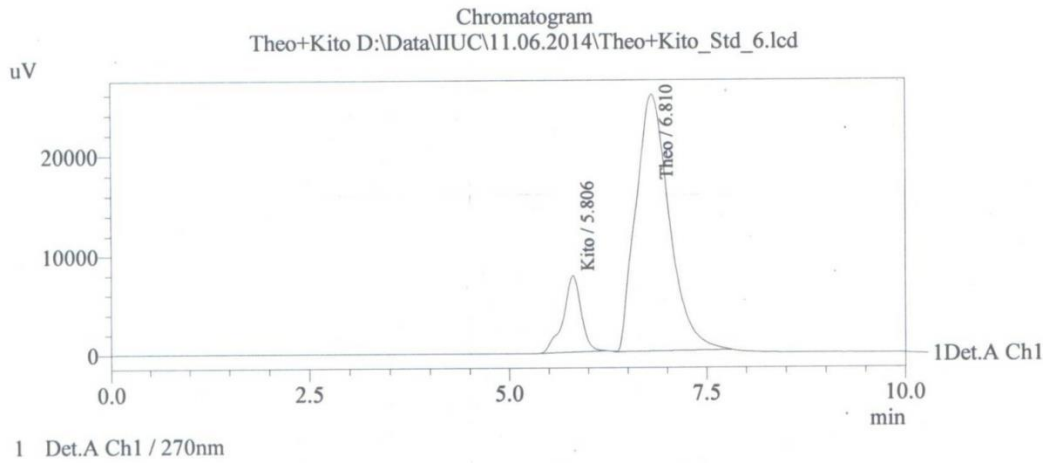


Figure: 4.87: Chromatogram on ketotifen and theophylline mixture

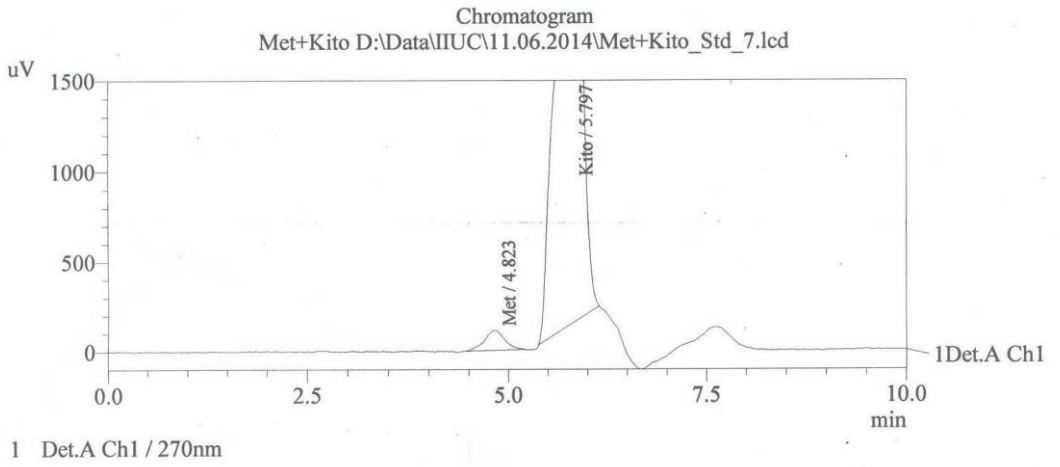


Figure: 4.88: Chromatogram on ketotifen and metformin mixture

The less stable complexes which were confirmed by Jobs continuous variation method as well as determining the stability constant values of ketotifen, theophylline, metformin & their mextures by Ardons spectrophotometric method and finally by IR and DSC methods. However in case of HPLC the complex which was formed between ketotifen &theophylline and ketotifen & metformin could have been partly dissociated in aqueous medium used to dissolve the sample for HPLC analysis. As a result no clear cut difference in R_f values for the complexes was seen. When salt is dissociated, its constituent ions are simply surrounded by water molecules and their effects are visible. However, no transfer or displacement of electrons occurs. Actually, the chemical synthesis of salt involves ionization.[92-95]

4.8 *In-vivo* study

Both the single (ketotifen) and mixtures (ketotifen and commonly prescribed drugs) were administered orally rats. The blood of single drug was measured and compared with the mixtures.

4.8.1 *In vivo* drug interaction study between ketotifen fumarate and paracetamol

The blood samples were collected at 30minutes, 60 minutes, 120 minutes & 180 minutes to complete the drug interaction between the group that took the single drug as well as mixtures (ketotifen & paracetamol), ANOVA [F (2, 6) = 660.440, P=0.000] at time 30. The Tukey post hoc test revealed that the concentration was statistically significant after taking ketotifen (1.1433 $\pm$ 0.04669 nm P= 0.000) and mixture of ketotifen and paracetamol (0.2397 $\pm$ 0.043 nm, P=0.04) compared to the control absorbance (0.0640 $\pm$ 0.023). At time 60, the Tukey post hoc test also revealed that the concentration was statistically significant after taking ketotifen (0.8523 $\pm$ 0.05424 nm P= 0.000) and mixture (0.1523 $\pm$ 0.01834 nm, P=0.046) compared to the control (0.0663 $\pm$ 0.01106). Similarly at time 120, ANOVA [F (2, 6) = 446.469, P=0.000] and at time 180 ANOVA [F (2, 6) = 1082.849, P = 0.000] it is observed that all the results are statistically significant ($p< 0.05$) after taking ketotifen and mixture compared to the control.

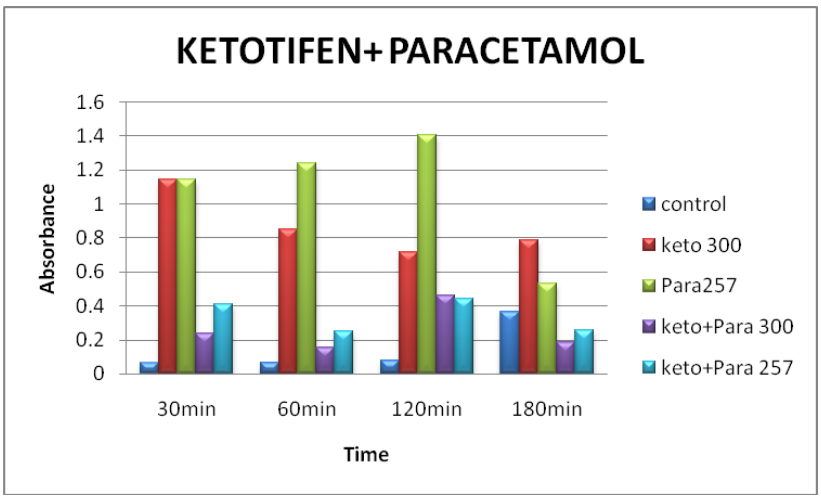


Figure 4.89: Graph for ketotifen fumarate and paracetamol interaction.

4.8.2 *In-vivo* drug interactions study of ketotifen fumarate and salbutamol

The invivo drug interactions study between ketotifen and mixtures (ketotifen and salbutamol) was analysed by ANOVA [F (2, 6)= 154.112, P = 0.000)] at time 30, 60, 120 and 180 minutes. The Tukey post hoc test revealed that the concentration was statistically significant after taking ketotifen (1.1433 ± 0.04669 nm P= 0.000) and mixture of ketotifen and salbutamol (0.8667 ± 0.12503 nm, P=0.012) compared to the control absorbance (0.0640 ± 0.02326). Similarly at time 60, 120 and 180 minutes it is observed that all the results are statistically significant after taking ketotifen and mixture compared to the control.

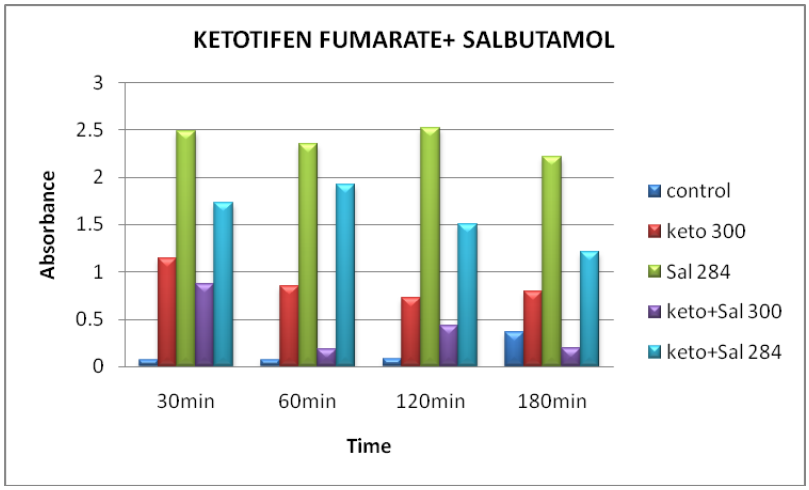


Figure 4.90: Graph for ketotifen fumarate and salbutamol

4.8.3 *In-vivo* drug interactions study of ketotifen fumarate with metformin

The multiple comparison table shows that there is a significant difference in absorbance's at various mentioned times (30 minutes, 60 minutes, 120 minutes & 180 minutes) to complete the drug interaction between the group that took the single drug as well as mixtures (ketotifen & metformin) The results were expressed as mean ± SEM values. A probability value less than 0.05 ($p<0.05$) was defined to be significant.

4.8.4 *In-vivo* drug interactions study of ketotifen with chlorpheniramine

When the standard and mixture of ketotifen and chlorpheniramine are measured at time 30, 60, 120 and 180 minutes it is observed that all the results are statistically significant after taking ketotifen and mixture compared to the control. The results were expressed as mean ± SEM values for each experiment. A probability value less than 0.05 ($p<0.05$) was defined to be significant. Finally from multiple comparisons table, which contains the results of post hoc tests it has been cleared that as the absorbance of drugs given together (ketotifen fumarate & chlorpheniramine) at 1:1 complex, so we can conclude that there is presence of drug interaction.

4.8.5 *In-vivo* drug interactions study of ketotifen fumarate and amoxicillin

After completion of the experiment it is observed that the probability value less than 0.05 ($p<0.05$) was defined to be statistically significant when the drugs given together (ketotifen fumarate & amoxicillin trihydrate) at 1:1 mixture.

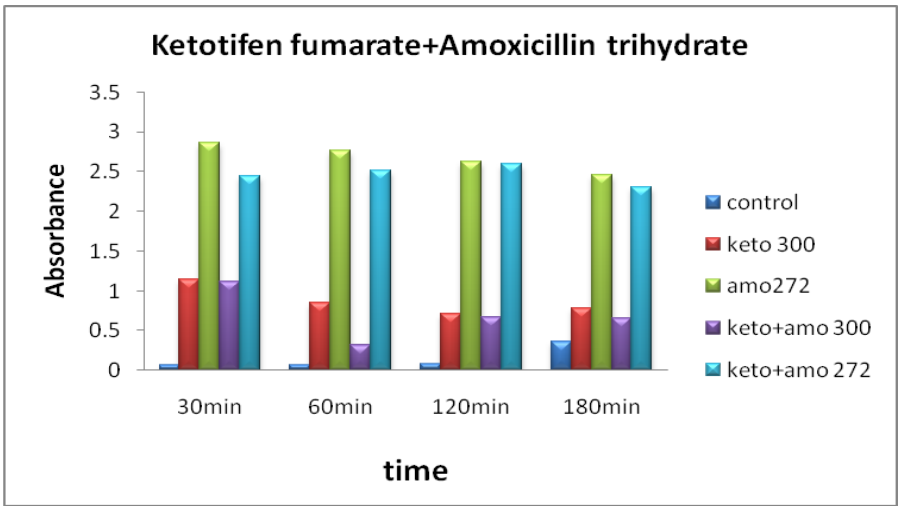


Figure 4.91: Graph for ketotifen fumarate & amoxicillin

4.8.6 *In-vivo* drug interactions study of ketotifen fumarate and diclofenac Na

After completion of the experiment it is observed that as the drugs given in combination form (ketotifen and diclofenac) at a 1:1 mixture the probability value was found to be less than 0.05 ($p<0.05$). So we can conclude that there is presence of drug interaction.

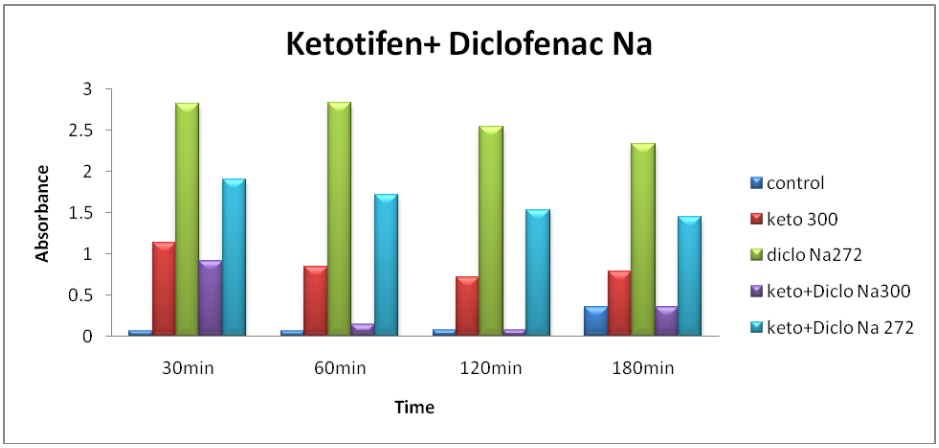


Figure: 4.92: Graph for ketotifen fumarate & diclofenac Na interaction.

4.8.7 *In-vivo* drug interactions study of ketotifen fumarate and theophylline

When the drugs given together (ketotifen fumarate & theophylline) at 1:1 mixture compared to the standard drug samples in single form (ketotifen and theophylline separately), it is observed that the probability value less than 0.05 ($p<0.05$) was defined to be statistically significant.

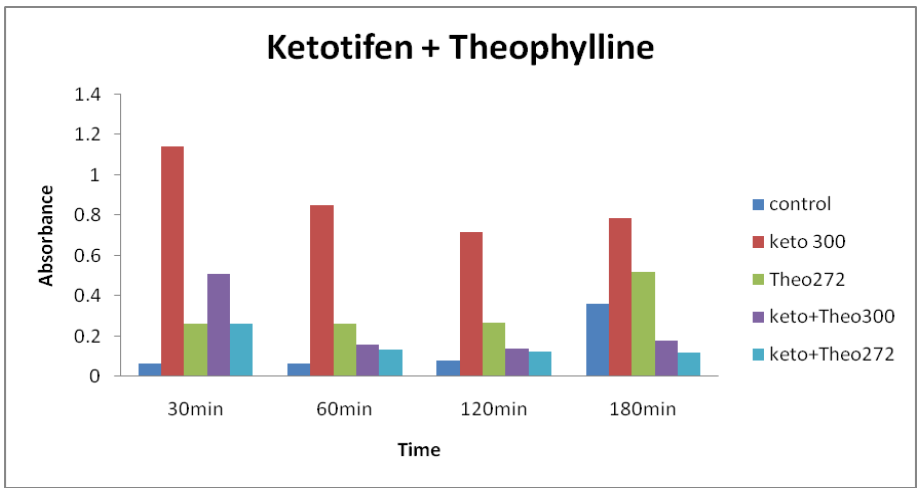


Figure 4.93: Graph for ketotifen fumarate & theophylline interaction.

4.9 Hepatotoxicity and kidney function test

Table 4.32: Evaluation of hepatotoxicity activity

Test samples	Groups	Identification	Dose(mg/kg)	Route of administration
Nnormal saline	I	Normal control group	5 ml/kg	Oral
Ketotifen	II	Standard drug sample	10 mg/kg	Oral
Theophylline	III	Standard drug sample	150 mg/kg	Oral
Metformin	IV	Standard drug sample	10 mg/kg	Oral
Ketotifen+ Theophylline	V	Test sample	10 mg/kg & 150 mg /kg	Oral
Ketotifen+ Metformin	VI	Test sample	10 mg/kg & 10 mg/ kg	Oral

Table 4.33: Effect of drug mixtures on biochemical parameters in albino rats

Treatment	SGPT(IU/L)	SGOT(IU/L)	TPTN(mg/dl)
Normal saline	65.5± 0.0	76.66± 1.35	7.01± 0.18
Ketotifen	65.1± 1.8*	74.66± 5.10*	7.51± 0.34*
Theophylline	64.8± 2.3*	75.66± 2.34*	8.60± 0.25*
Metformin	65.4± 3.3*	76.6± 3.46*	7.01± 0.16*
Ketotifen+ theophylline	68.5± 2.5*	82.56± 4.55*	7.0± 0.07*
Ketotifen+ metformin	67.5± 1.44*	78.31± 5.28*	6.13± 0.73*

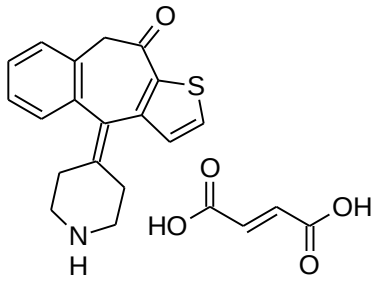
Results of the biochemical estimations are reported as mean ± SEM. Total variation present in a set of data was estimated by one way analysis of variance (ANOVA) by using SPSS software for determining significance.

The results of investigation of hepatotoxicity of combination drug therapy was compared with single drug sample ketotifen fumarate were shown in table 4.32. But the groups which receive the combination drug samples (ketotifen and metformin) and ketotifen and theophylline showed a significant increase in SGPOT, and showed a significant decrease of ATPN levels. The creatinine concentration was found to be 1.4 mg/dl in case of normal control but it was raised to 3.6 mg/dl when mixture of ketotifen and theophylline was administered.

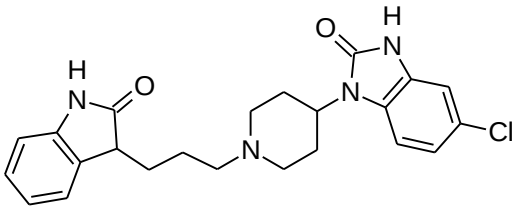
4.10 Possible reactions that occurred during drug interactions

4.10.1 Interaction between ketotifen fumarate and domperidone

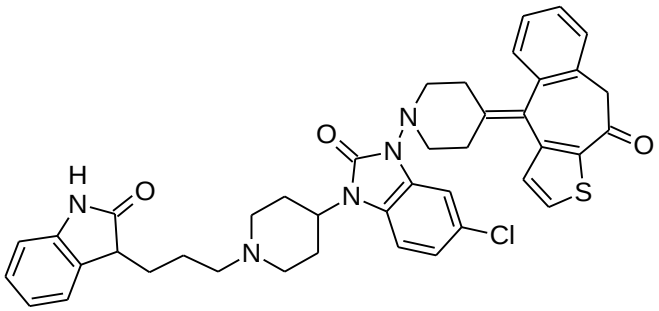
Aliphatic amines are slightly stronger bases than ammonia due to electron releasing properties of R, which develop additional fractional negative charge on nitrogen, making it more reactive for electrophillic attack.[96-98]



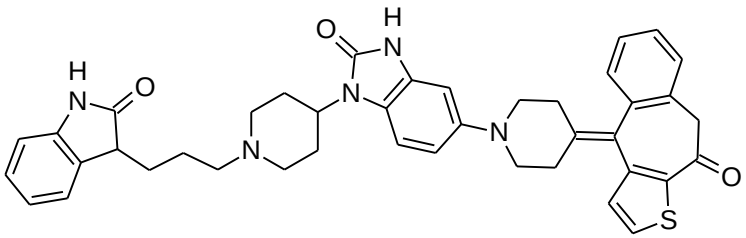
Ketotifen fumarate



Domperidone



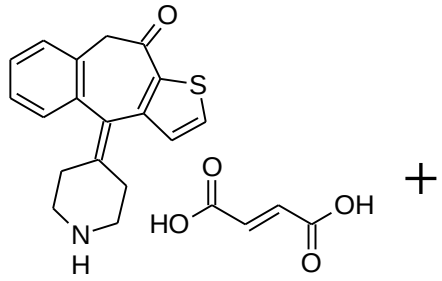
Or



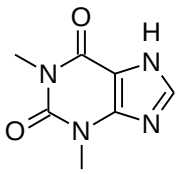
In this reaction an intermediate stage may be formed, which immediately reacts with electron donating agents to complete the addition reaction.

4.10.2 Interaction between ketotifen fumarate and theophylline anhydrous

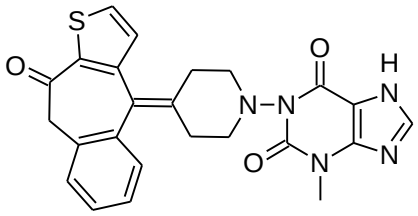
Aliphatic amines are slightly stronger bases than ammonia due to electron releasing properties of R, which develop additional fractional negative charge on nitrogen, making it more reactive for electrophillic attack.[96-98]



Ketotifen fumarate



Theophylline anhydrous



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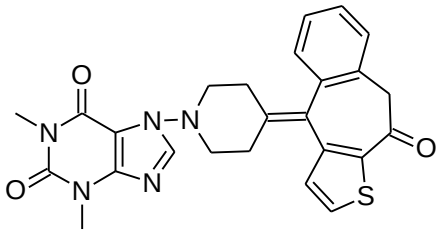
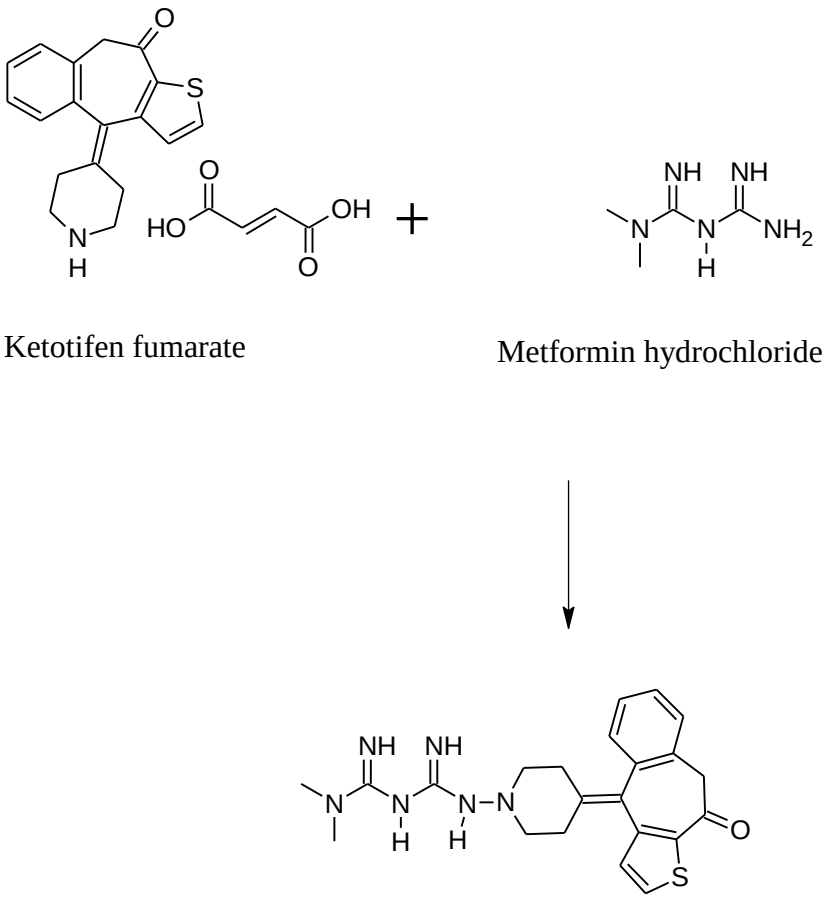


In this addition reaction an intermediate stage may be formed, which immediately reacts with electron donating agents to complete the reaction.

4.10.3 Interaction between ketotifen fumarate and metformin hydrochloride

Among the two possible sites of metformin, the primary amine sites are more reactive than tertiary amine



Ketotifen fumarate

Metformin hydrochloride

These are the possible reactions that occurred during drug interactions between ketotifen & metformin, ketotifen & domperidone and ketotifen & theophylline.

Chapter-5

Conclusion

Conclusion

***In-vitro* study**

Each of the drugs absorption was analyzed in the UV-VIS region. The nature of spectra of pure compounds as well as their 1:1, 1:2 and 2:1 mixtures in buffer solution was studied at pH 0.4, 1.2, 2.0, 2.8, 6.0, 6.8 and 7.4. The molecular species of ketotifen fumarate & theophylline anhydrous when separately mixed showed some changes in absorption characteristics. Similarly when ketotifen fumerate was separately mixed with salbutamol sulphate, domperidone and metformin hydrochloride good results were found. By applying the Job's method we can ensure whether the interaction happening or not. At both gastric and intestinal pH, the presence of sharp breakdown of curve indicates drug interaction, in case of theophylline. But in case of desloratidine sharp changes was observed in the curves at pH 0.4, 1.2, and 2.0, breakdown found at both gastric and intestinal pH. At all pH various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen were interacted with metformin hydrochloride. In the Job's spectroscopic method when ketotifen was interacted with metformin a sharp breakdown in the curve was observed at a pH 0.4, 1.2, 2.8 & 7.4, indicate the presence of drug interactions. Whereas the absence of particular breakdown in the curve when the interaction happening between ketotifen and metformin at pH 2.0, 6.0 and 6.8, indicate the absence of drug interactions. Again when various concentrations comprising $1x10^{-5}$ M to $9x10^{-5}$ M of ketotifen were interacted with chlorpheniramine maleate. When ketotifen was interacted with chlorpheniramine a sharp breakdown in the curve was observed at a pH 0.4, 6.0 & 6.8 indicate the presence of drug interactions. Whereas the absence of particular breakdown in the curve when the interaction occurred between ketotifen and chlorpheniramine at pH 1.2, 2.0, 2.8 & 7.4, indicate the absence of drug interactions. The stability constant values at particular interaction were determined by graphical

representation of Ardon's plot. The stability constant values ($k=1x10^{-2}$) for particular interaction was determined by graphical representation of Ardon's plot. The stability constants of ketotifen & paracetamol, ketotifen & domperidone, ketotifen & desloratidine, ketotifen & theophylline, ketotifen & amoxicillin are relatively higher at gastric and intestinal pH. This reflects that there might be relatively stronger complex formation due to interaction between the drugs. But relatively low stability constant values are seen when the interaction occurs between ketotifen & ketotifen & diclofenac, ketotifen & metformin.

Infrared spectroscopic study

The possible interaction between ketotifen fumarate and amoxicillin trihydrate showed characteristic peaks. The peaks of acid chlorides, ethers and alkene groups were shifted to higher wave number in the complex. Simultaneously the peaks of isocyanates and carboxylic groups were shifted to lower wave numbers. When ketotifen fumarate and diclofenac sodium were interacted the peaks of phosphines, alkyl halides, amides (N-H stretching) and amides (C=O stretching) and aromatic groups showed characteristic change. However ketotifen and salbutamol also showed different peaks of C-H (aromatic group), C-O-C (ether group), C-C (ketone) and O-H (carboxylic acid group) at 838.11 cm^{-1}, 1085 cm^{-1}, 1201.7 cm^{-1}, 2925.17 cm^{-1} were also shifted to higher wave numbers.Similarly ketotifen fumarate and theophylline anhydrous showed characteristic peaks when interacted with each other.

Differential Scanning Calorimeter (DSC) study

In the DSC study (ketotifen, domperidone and ketotifen & domperidone mixture) ketotifen-domperidone complex exhibited a sharp new peak at 120.02^{0}C (-1.74 mW/mg). On the other hand, DSC of the ketotifen, metformin and ketotifen & metformin mixture exhibited a sharp new peak at 123.04^{0}C (-2.61 mW/mg). But the mixture of ketotifen and theophylline no peak was observed.

HPLC study

The complex which was formed between ketotifen & theophylline and ketotifen & metformin could have been partly dissociated in aqueous medium used to dissolve the sample for HPLC analysis.

Identification of stability of the complex by Thin Layer Chromatography:

The stability of the complex after formation was confirmed by thin layer chromatographic technique. More stable complex was formed when ketotifen interacted with donperidone. Less stable complex was formed when ketotifen interacted with theophylline. On the other hand a comparatively stable complex was formed when ketotifen interacted with metformin.

***In-vivo* study**

The multiple comparison table shows that there is a significant difference in absorbances at various mentioned times (30 minutes, 60 minutes, 120 minutes & 180 minutes) to complete the drug interaction between the group that took the single drug as well as mixtures (ketotifen & metformin, ketotifen & paracetamol, ketotifen & salbutamol, ketotifen & amoxicillin, ketotifen & diclofenac) The results were expressed as mean ± SEM values. A probability value less than 0.05 ($p < 0.05$) was defined to be significant. The results of investigation of hepatotoxicity of combination drug therapy were compared

with single drug sample ketotifen fumarate. But the groups which receive the combination drug samples ketotifen & metformin and ketotifen & theophylline showed a significant increase in SGPOT, and showed a significant decrease of ATPN levels in ketotifen & theophylline mixture and in ketotifen & metformin mixture. The creatinine concentration was found to be raised when mixture of ketotifen and theophylline was administered. Now we can conclude that the patients having motion sickness and patients who had been suffering from diabetes should take a precaution during ketotifen administration. Coadmintration of ketotifen fumarate & domperidone and ketotifen fumarate & metformin hydrochloride should be avoided.

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