



## International Journal of Pharma Professional's Research



Received on 22 March, 2016; received in revised form, 02 April, 2016; accepted, 18 April, 2016; published 30 April, 2016

### IN - VIVO STUDY ON INTERACTION OF KETOTIFEN FUMERATE WITH THEOPHYLLINE ALONG WITH THEIR IR STUDIES

Md. Khalequeuzzaman<sup>1</sup>, Md. Mominur Rahman<sup>2</sup>, \*Mst. Rozina Parul<sup>1</sup>, Mohammed Aktar Sayeed<sup>2</sup>, Md. Raisul Islam<sup>3</sup>

1. Department of Pharmacy, Gono Bishwabidyalay, Mirzanagar, Savar, Dhaka - 1344.

2. Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

3. Department of Pharmacy, Jahangirnagar University, Savar, Dhaka – 1342.

#### Key words:

Kitotifen fumerate, Theophylline, pharmacological activities & drug interactions.

#### Correspondence to Author:

Mst. Rozina Parul

Department of Pharmacy, Gono Bishwabidyalay, Mirzanagar, Savar, Dhaka – 1344

**E-mail:** rozina.parul@gmail.com

**ABSTRACT:** This is very much important than any other time or ever to know about the medicines we take. If we take several different medicines, suffering from various types of diseases, consult more than one doctor or have certain health conditions, we and our doctors must be aware of all the medicines we take to avoid potential problems, such as drug interactions. The study of Drug interaction between various drugs has been conducted for many years and successful results have also been established. In the continuation of the study of drug interaction we have studied the in vivo interaction between the Kitotifen fumerate and Theophylline along with their IR studies. In-vivo study showed decreased in the plasma concentration of free ketotifen fumarate when given concurrently with Theophylline which may be due to the drug interaction. IR study confirmed that there is an interaction between Ketotifen fumarate and Theophylline with the presence of extra peaks in their combination (aqueous extract 763.84  $\text{cm}^{-1}$ , 1595.2  $\text{cm}^{-1}$ , 2281.89  $\text{cm}^{-1}$ , 2625.23  $\text{cm}^{-1}$ , 2729.39  $\text{cm}^{-1}$ , 3384.25  $\text{cm}^{-1}$  & chloroform extract 703.08  $\text{cm}^{-1}$ , 2115.04  $\text{cm}^{-1}$ , 2161.33  $\text{cm}^{-1}$ , 2232.7  $\text{cm}^{-1}$ , 2657.06  $\text{cm}^{-1}$ , 2701.42  $\text{cm}^{-1}$ ) forms as compared to that of their pure forms. So, we can say that if both ketotifen fumerate with Theophylline administer concurrently, mild complex can be formed after reaction which can ultimately reduce the pharmacological activities of both combinations of drugs.

#### INTRODUCTION:

It is often necessary to take more than one drugs at a time and some degree of drug-drug interaction occurs with concomitant use of drugs. Drug interactions can cause serious harm to the patient,

sometimes causes serious adverse reactions and now a days become an obvious concern for the health care providers. For example, drug interactions, particularly with drugs having a narrow therapeutic range, may have serious

adverse consequences. There are various types of drug interactions such as Drug-drug interactions, Drug-food/beverage interactions i.e. Interactions Resulting from Alterations in Gastrointestinal Absorption, Interactions Resulting from Alterations in Metabolizing Enzymes (Enzyme induction, Enzyme inhibition), Interactions Resulting from Alterations in Protein Binding, Interactions Resulting from Changes in Renal Excretion.

- Drug interactions are very common. There are several reasons:
- Health care providers may not be aware of interactions with drugs they prescribe.
- Several health care professionals may prescribe medications for one patient
- Aging patients have multiple health issues and take many medications
- Drug interactions may not be identified as the cause of unexpected treatment results or side effects
- Health care providers may not know about all medications and supplements their patients are taking.

Ketotifen is a benzocycloheptathiophene derivative that has been shown to possess anti-histaminic and anti-anaphylactic properties<sup>1</sup>. It has been demonstrated that it can block in vitro release of mediators from rat peritoneal mast cells<sup>1</sup>. The drug inhibits the release of histamine and leukotriene from basophil and lung tissue, antagonizes histamine at H<sub>1</sub> receptors, inhibits calcium uptake, blocks passive cutaneous anaphylactic reaction, reverses isoprenaline induced beta-adrenoceptor tachyphylaxis, and inhibits both allergen-induced and drug-induced asthma<sup>2</sup>. A number of clinical trials of ketotifen have shown it to have a beneficial effect in the treatment of asthma<sup>3,4</sup> equivalent to that of disodium cromoglycate, which has an established place in the treatment of asthma<sup>5</sup>. Ketotifen, which is useful in the treatment of hay fever and asthma, have been found to inhibit anaphylactic histamine release from animal tissues<sup>6</sup>.

Theophylline are bronchodilators which relax and open the air passages to the lungs to increase the flow of air through them, and thus are used in the treatment and or prevention of symptoms of bronchial asthma and of reversible bronchospasm associated with chronic bronchitis and emphysema. They are used as muscle relaxants, central nervous system and cardiac muscle stimulants. It relaxes and opens air passages in the lungs, making it easier to breathe.

In a research study was undertaken to investigate the effect of theophylline on circulating vitamin B<sub>6</sub> levels in children with asthma. Steady state serum theophylline and vitamin B<sub>6</sub> [pyridoxal 5'-phosphate (PLP) and pyridoxal (PL)] levels were evaluated in these patients. we conclude that theophylline induces a depression of circulating PLP levels in asthmatic children.<sup>7</sup> Another research result shows since depressed vitamin B<sub>6</sub> plasma levels can occur in patients receiving theophylline, we explored a B<sub>6</sub>-theophylline interaction in a rabbit model. B<sub>6</sub> deficiency may contribute to chronic theophylline toxicity; however, pyridoxine administration in the dosage used may not prevent toxicity.<sup>8</sup> Another research result explain on Evaluation of Interaction between Ketotifen Fumerate and Theophylline and their Effects on Protein Binding that concluded if given concurrently, ketotifen and theophylline might form stable complex and hence reduce the pharmacological activities of both drugs.<sup>9</sup>

#### **Objective of this study**

- The prime object of this project was to elucidate the possible importance of drug-drug interactions (DDIs) as a contributing factor towards drug safety.
- The main focus of the project was to identify whether there is any interaction between Ketotifen fumerate (antihistamine) with Theophylline (Bronchodilator).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 fumerate (antihistamine) with Theophylline. The specific purpose was to observe and determine the stability of the complexes, which could be formed between Ketotifen fumerate (antihistamine) with Theophylline. To see the potentiation and attenuation of activity of Ketotifen Fumerate 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.

- 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. In our in vitro and in-vivo study it was found that Ketotifen Fumerate formed complexes with Theophylline along with some intermediary complexes at room temperature and in isotonic environment.

## Materials and Method

### 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.

### Drugs and chemicals

Ketotifen fumerate and Theophylline were collected from Square Pharmaceuticals Ltd., Dhaka, Bangladesh as a token gift and were used without further purification. Sodium di-hydrogen orthophosphate and di-sodium hydrogen orthophosphate, used for the preparation of buffer solutions were purchased from Merck, Germany. Potassium chloride, sodium hydroxide, potassium hydroxide etc. were all of analytical grade.

### Equipments

UV-Visible spectrometer (UV mini-1240, Shimadzu, Japan), pH meter (Cyberscan 500, Hanna, Portugal), Electronic balance (Shimadzu

Corporation, Japan), a thermos tatted water bath (Memmert, German) and IR Spectrophotometer (IR Affinity-1 A213747 Shimadzu Corporation, Japan ) were used for the test.

### The $\lambda_{\max}$ value of drug used in the study

The wavelength for maximum absorption value of the drug used in the experiment is given in the following table 1:

**Table 1: The  $\lambda_{\max}$  value of drug used in the study**

Name	Wavelength ( $\lambda_{\max}$ )
Ketotifen fumerate	300nm
Theophylline	272nm

### Animals for In- Vivo Study

Mice (15-32gm) 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. Experiment was conducted during the light period.

### Preparation of drugs solutions

20ml of each drug solutions were prepared according to their corresponding doses.

Doses: Ketotifen fumarate: 0.1mg/kg

Theophylline: 150mg/kg

### Methodology

#### For Ketotifen fumarate + Theophylline

48 fresh mice were separated for the experiment which were kept in fasting condition overnight for 12hrs. Mice were divided into four groups, 12 mice in each group.

#### Group I

They were kept as control group for the experiment. They were administered normal saline 0.4ml p.o each and the time was noted as 0min. 3 mice were sacrificed after 30mins, blood sample were collected and their absorbances were taken at 300 nm. Similarly every 3 mice were sacrificed after 60mins, 120mins & 180mins and the same procedure were followed as above.

**Group II**

They were administered ketotifen fumarate solution 0.4ml p.o each and the time was noted as 0 min. 3 mice were sacrificed after 30 mins, blood sample were collected and their absorbances were taken at 300nm. Similarly every 3 mice were sacrificed after 60mins, 120mins & 180mins and the same procedure were followed as above.

**Group III**

They were administered Theophylline solution 0.4ml p.o each and the time was noted as 0 min. 3 mice were sacrificed after 30mins, blood sample were collected and their absorbances were taken at 272nm. Similarly every 3 mice were sacrificed after 60mins, 120mins & 180mins and the same procedure were followed as above.

**Group IV**

They were administered mixture of ketotifen fumarate + Theophylline solution (1:1) 0.4ml p.o each and the time was noted as 0min. 3 mice were sacrificed after 30mins, blood sample were collected and their absorbances were taken at 300nm and 272nm. Similarly every 3 mice were sacrificed after 60mins, 120mins & 180mins and the same procedure were followed as above.

**For Infrared Analysis**

The interaction between the drug were analysed by IR spectrum analyses

- Group I (control group i.e no drug used)

Absorbances at 300nm	30min	60min	120min	180min
	0.064±0.134	0.0663±0.006	0.077±0.006	0.3623±0.263

- Group II (Ketotifen fumarate)

Absorbances at 300nm	30min	60min	120min	180min
	1.143±0.027	0.852±0.031	0.7193±0.018	0.788±0.018

- Group III (Theophylline)

Absorbances at 284nm	30min	60min	120min	180min
	0.263±0.014	0.261±0.007	0.266±0.004	0.521±0.021

- Group IV (Ketotifen fumarate+ Theophylline)

Absorbances at 300nm	30min	60min	120min	180min
	0.51±0.016	0.157±0.075	0.137±0.026	0.178±0.007

Absorbances at 272nm	30min	60min	120min	180min
	0.261±0.007	0.132±0.031	0.123±0.009	0.121±0.021

**For analysis of drug interaction between Ketotifen fumarate & Theophylline**

- 100mg of pure powder of Ketotifen fumarate was dissolved in 10ml of distilled water in a 50ml beaker, similarly 100mg of pure powder of was dissolved in another beaker
- Both the above solutions were mixed in a separate 100ml beaker with constant stirring
- Then the mixture was transferred to the separating funnel
- The interacted drug was extracted in chloroform.
- Chloroform was evaporated and the precipitated solid drug product was analysed by IR spectrophotometer
- Another experiment for aqueous extract of interacted drug product was also analysed by IR spectrophotometer

**Result & Discussion****In-Vivo Study Ketotifen fumarate and Theophylline**

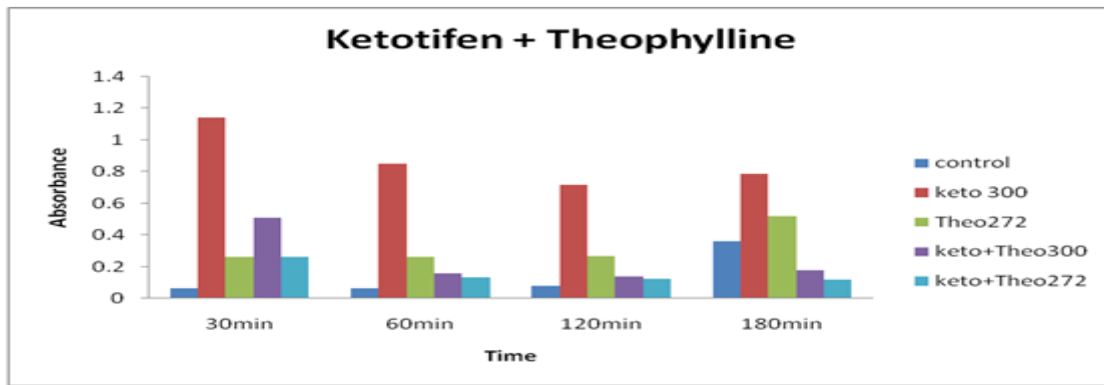


Fig. 1: Graph for Ketotifen fumarate + Theophylline interaction

**Infrared Study:**

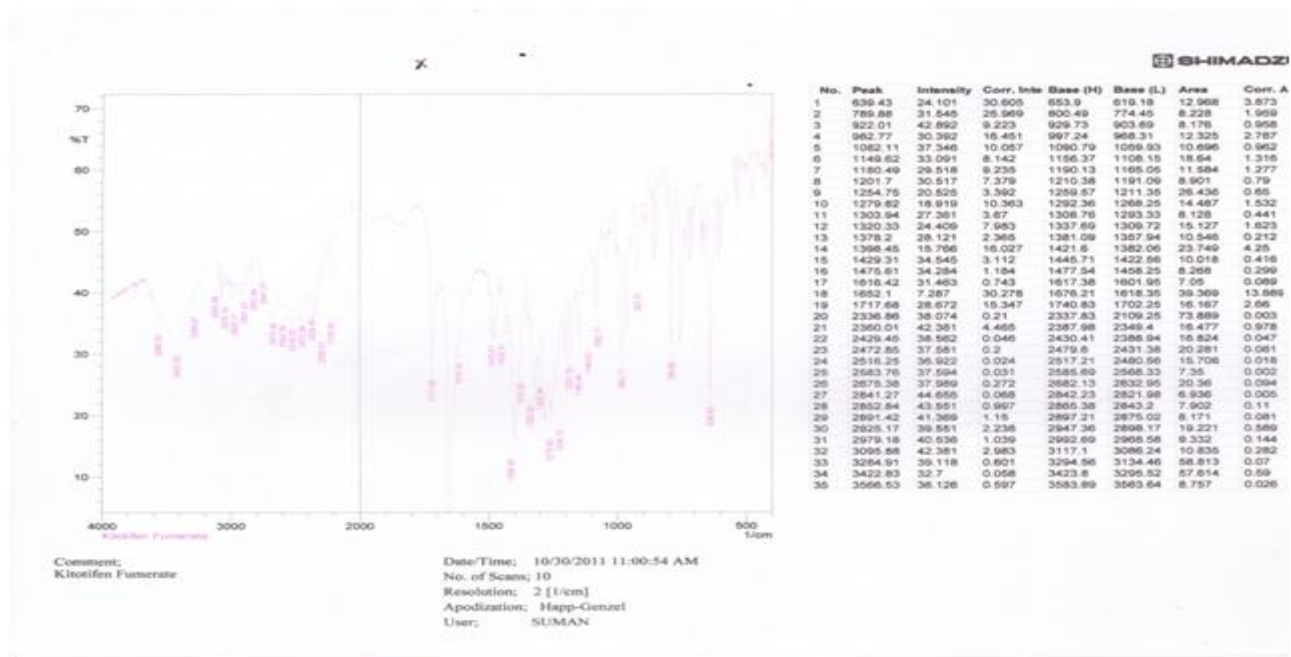


Fig. 2: IR Spectrum of Ketotifen Fumarate

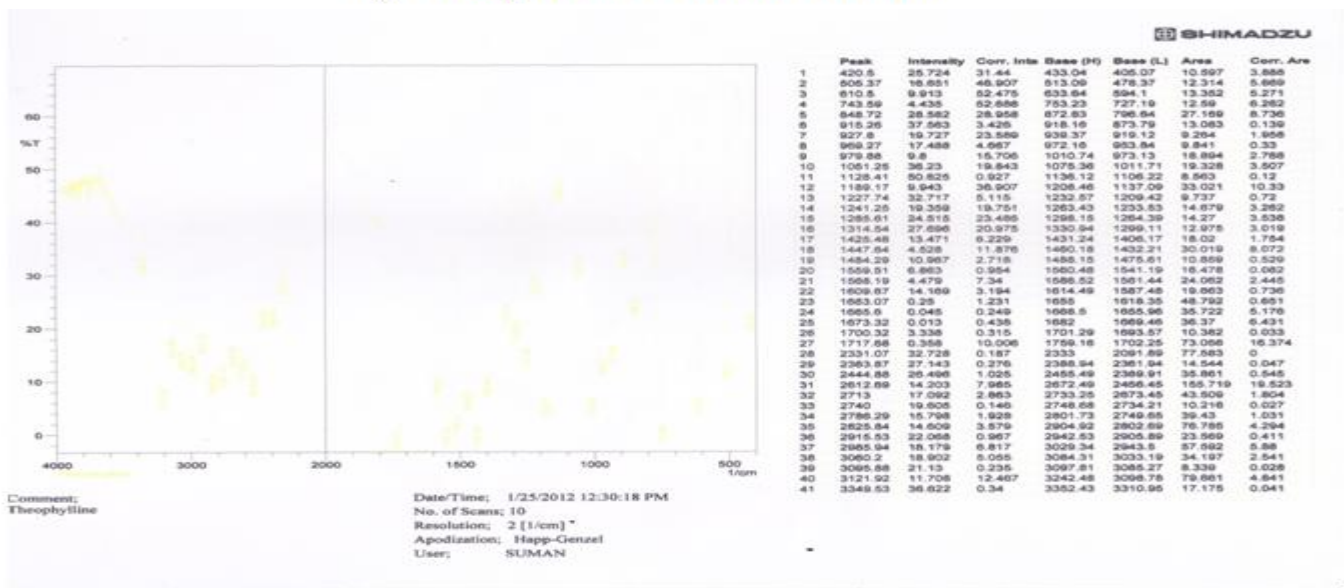


Fig. 3: IR Spectrum of Theophylline



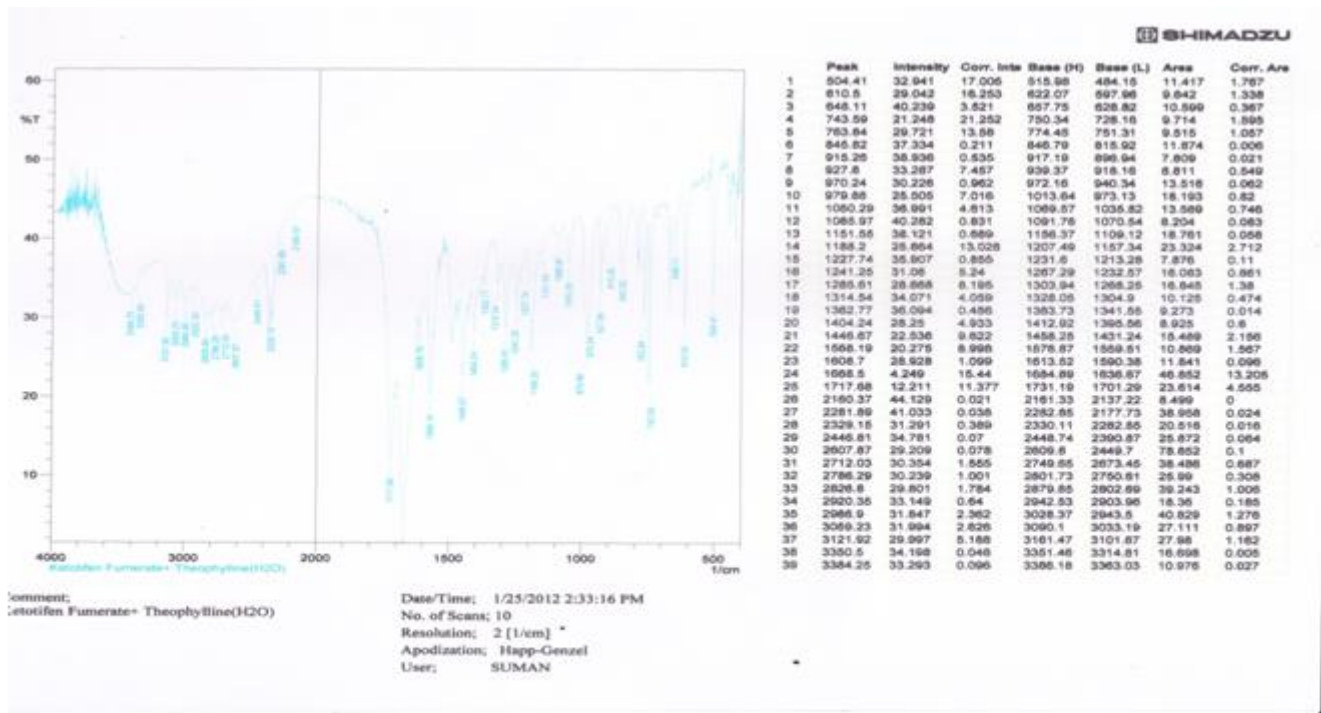


Fig. 4: IR Spectrum of Ketotifen Fumarate + Theophylline (Aqueous Extract)

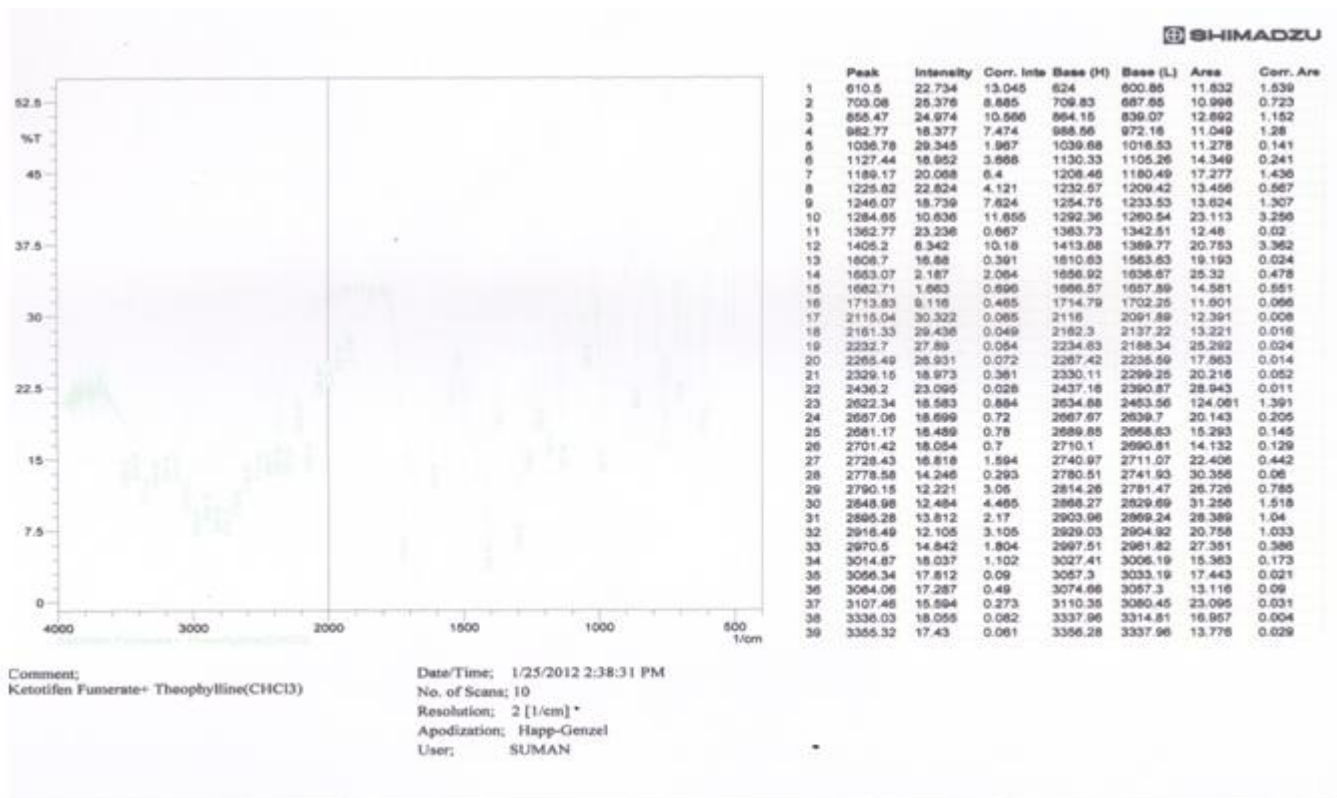


Fig. 5: IR Spectrum of Ketotifen Fumarate + Theophylline (Chloroform Extract)

**Table 2: Interacted peaks for Ketotifen Fumarate and Theophylline**

Aqueous extract Wave No. (cm <sup>-1</sup> )	Chloroform extract Wave No. (cm <sup>-1</sup> )
763.84	703.08
1595.2	2115.04
2281.89	2161.33
2625.23	2232.7
2729.39	2657.06
3384.25	2701.42

**Discussion**

There are some peaks observed in the spectrum of combination of the two drugs Ketotifen Fumarate and Theophylline in both aqueous and chloroformic extracts as compared to that of pure forms. From these extra peaks we confirm that there is an interaction between Ketotifen Fumarate and Theophylline in combination form. By observing the graph obtained by plotting invivo results, we can see that the absorbances of Ketotifen fumarate is decreased when administered in combination with Theophylline and vice-versa. We know that the value of absorbance is directly proportional to the drug concentration from Beer and Lambert's law. Since the absorbance is decreased, the plasma drug concentration also decreases which reflects there might be drug interaction due to which the plasma drug concentration is decreased. Hence we can conclude that there might be drug interaction between Ketotifen fumarate and Theophylline.

There are many extra peaks observed in the IR spectra of interacted product of ketotifen fumarate with Theophylline in the mixture ratio of 1:1 either in aqueous or chloroform extracts, in compared to that of their pure form. The presence of the extra peaks confirmed that there is a interaction between Ketotifen fumarate and Theophylline.

**Conclusion**

From the in-vivo and IR study it is confirmed that there are interaction between Ketotifen fumarate and Theophylline when administered concurrently. Therefore cautions must be taken when these combination drugs are administered to minimize the risk of drug interaction and get maximum therapeutic efficacy of the individual drugs to cure the illness of the patient in its rational use. Further studies can be carried out to determine whether these drug interactions are beneficial or harmful.

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