

## ***In vitro* and *in vivo* Interaction Study between Ketotifen Fumarate and Metformin Hydrochloride**

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### **ABSTRACT**

The objectives of the present study were to investigate the drug-drug interactions between ketotifen fumarate and metformin hydrochloride. 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 and metformin, were studied at pHs 2.0, 2.8, and 7.4. When ketotifen was mixed with metformin, a sharp change was observed in the curve at pHs 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 pH 2.0 revealed the absence of drug interactions. The stability constant values ( $k = 1 \times 10^{-2}$ ) for the particular interaction was determined by graphical representation of Ardon's plot. The stability constants of ketotifen and metformin were 0.05, 0.01 and 0.77 at pH 2.0, 2.8, 7.4 respectively. 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°C (-2.61 mW/mg). 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. 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 and metforin ( $67.5 + 1.44$  IU/L) showed a significant increase in SGPT, and showed a significant decrease of ATPN levels ketotifen and metformin mixture ( $6.13 + 0.73$  IU/L). Now we can conclude that the patients who had been suffering from diabetes should take a precaution during co-administration of ketotifen fumarate and metformin hydrochloride.

**Key words:** Stability constant, Job's method, Ardon's mehod, Ketotifen fumarate, Metformin hydrochloride, Hepatotoxicity.

## INTRODUCTION

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 antagonizes histamine at H<sub>1</sub> receptors and reverses isoprenaline-induced beta-adrenoceptor tachyphylaxis, and inhibits both allergen-induced and drug-induced asthma.<sup>2</sup> Clinical trials prove that ketotifen is the drug of choice in the treatment of asthma.<sup>3-4</sup> equivalent to that of disodium cromoglycate, which has an important role in the treatment of asthma.<sup>5</sup> Ketotifen have been found to inhibit anaphylactic histamine release from animal tissues.<sup>6</sup> Metformin acts as an antihyperglycemic agent using in the treatment of type 2 diabetes. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. The major goal of the present study was to elucidate the possible importance of drug-drug interactions (DDIs). The values of stability constants of metformin hydrochloride with ketotifen were determined by using Job's continuous-variation analysis and Ardon's spectrophotometric measurement methods.

## MATERIALS

### Drugs and chemicals

Ketotifen fumarate and metformin hydrochloride were collected from Square Pharmaceuticals Ltd., Dhaka, Bangladesh as a token gift and were used without further purification. Sodium dihydrogen 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.

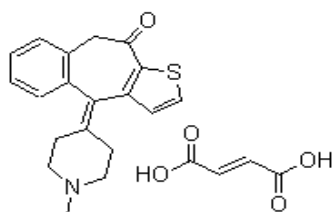


Figure 1: Chemical structure of ketotifen fumarate

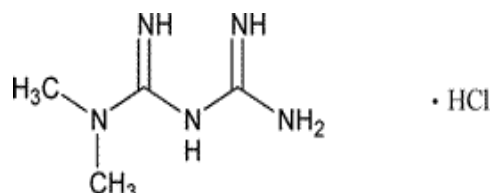


Figure 2: Chemical structure of metformin hydrochloride

### Equipments

UV-Visible spectrophotometer (Model No. UV-1600, Shimadzu, Japan), pH meter (Mettler Toledo, Switzerland), analytical balance (Model No. AL 204-S/01, Mettler Toledo, Switzerland), and a thermostatic water bath (Shimadzu, Japan) were used for

the test. A Dunbuff metabolic shaking incubator (Nickel, Electrical Company, England) was used to shake the drug mixtures to attain equilibrium.

## METHODS

### Preparation of standard solutions

100 ml of  $1 \times 10^{-3}$  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  $1 \times 10^{-5}$  M solution of ketotifen fumarate, 1 ml of  $1 \times 10^{-3}$  M solution was taken in another 100 ml volumetric flask and the volume was adjusted by distilled water up to the mark. Similarly 100 ml of  $1 \times 10^{-3}$  M solution of metformin hydrochloride was prepared as the stock solution by dissolving 0.0165 gm of metformin hydrochloride in 100 ml of distilled water in a 100 ml volumetric flask.

### Absorption spectrum analysis

The absorption characteristics of ketotifen and metformin as well as their 1:1, 1:2 and 2:1 mixtures in the solutions of buffers<sup>7-8</sup> at pH 02.0, 2.8, and 7.4 were compared with those of each interacting species. The concentrations of the sample were measured by using UV-VIS spectrophotometer with a constant temperature ( $25 \pm 1$  °C). The stock solutions of the samples were diluted to appropriate levels, diluted with the buffers ( $1 \times 10^{-5}$  M) at the desired pH and the spectra were recorded between 400 - 190 nm. The spectra were compared with those of the pure samples in each case.

### Job's Spectrophotometric method

The molar ratios of the complexes of ketotifen fumarate and metformin hydrochloride were estimated by Job's spectrophotometric method of continuous variation.<sup>9</sup> The observed absorbance values were measured at pHs 2.0, 2.8, and 7.4 at various concentrations  $1 \times 10^{-5}$  M to  $9 \times 10^{-5}$  M of ketotifen fumarate and metformin hydrochloride at 300 nm. In this method, solutions of different concentrations of ketotifen fumarate and metformin hydrochloride 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.<sup>10</sup>

### Ardon's spectrometric method

In the Ardon's spectrophotometric method,<sup>11</sup> concentrations of Ketotifen fumarate was varied while keeping the concentrations of other drugs fixed at  $1 \times 10^{-4}$  M. All the experiments were performed in buffer at pH 2.0, 2.8, and 7.4. The absorbance of solutions having pH 0.4, 1.2, 6.0 and 6.8 were measured at 300 nm using UV-VIS spectrophotometer. For calculations, the Ardon's equation was used. This equation is given below:-

$$\frac{1}{(D - \epsilon_A C)} = \frac{1}{KC(\epsilon_{COM} - \epsilon_A)[B]^N} + \frac{1}{C(\epsilon_{COM} - \epsilon_A)}$$

Where,

D = Absorbance of the mixture.

B = Molar concentration of the ketotifen fumarate.

C = Molar concentration of the other commonly prescribed drugs

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

$\epsilon_{\text{A}}$  = 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  $1/(D - \epsilon_{\text{A}}C)$  was plotted versus  $1/[B]$  to get the straight lines. The concentration of ketotifen fumarate was kept constant  $1 \times 10^{-4}$  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,

$$K = \text{intercept} / \text{slope}$$

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

### **Complex formation confirmed by Differential Scanning Calorimeter (DSC)**

#### **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 °C for scanning.

#### **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°C for scanning.

#### **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°C 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.

### **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.

### **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 norvegicus*) weighing 130-150 gm were used. They were purchased from the pharmacology laboratory of Jahangirnagar University. Animals were maintained under standard environmental conditions (temperature  $27 \pm 1.0^{\circ}\text{C}$ , 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

Normal saline (0.9% NaCl solution) was prepared. Ketotifen, metformin, and ketotifen-metformin mixture were prepared separately as test solutions.

### 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)<sup>12</sup>

**Group III:** Each rat received metformin hydrochloride as a standard drug sample.

**Group IV:** Each rat received ketotifen & metformin hydrochloride as a test sample.

Table 1 - Absorbance of ketotifen fumarate at different pH by using Job's method

Conc. of Ketotifen	Absorbance Difference(D value)		
	pH 2.0	pH 2.8	pH 7.4
1X 10 <sup>-5</sup>	0.618	0.507	1.015
2X 10 <sup>-5</sup>	0.669	0.66	1.015
3X 10 <sup>-5</sup>	0.707	0.794	0.98
4X 10 <sup>-5</sup>	0.707	0.876	0.933
5X 10 <sup>-5</sup>	0.661	0.729	0.796
6X 10 <sup>-5</sup>	0.602	0.593	0.741
7X 10 <sup>-5</sup>	0.496	0.451	0.691
8X 10 <sup>-5</sup>	0.362	0.507	0.627
9X 10 <sup>-5</sup>	0.085	0.66	0.323

## RESULTS

In spectral observation analysis, each of the drugs studied showed absorption in UV-VIS region. The molecular species of ketotifen fumarate and metformin hydrochloride 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 metformin hydrochloride 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 2.0, 2.8, 7.4 . at a fixed concentration ( $1 \times 10^{-5}$ ) M. Continuous variation plot gives information on the relative affinities of the complexes and it also depends on the intrinsic spectral characteristics of each complex. The numeric values of the resulting stability constants were between 0.05 and 0.77 when complexation occurs among the ketotifen and metformin hydrochloride. 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  $123.04^{\circ}\text{C}$  (-2.61 mW/mg). 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. 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 & metforin (67.5 + 1.44 IU/L) showed a significant increase in SGPT, and showed a significant decrease of ATPN levels ketotifen & metformin mixture (6.13 + 0.73 IU/L)

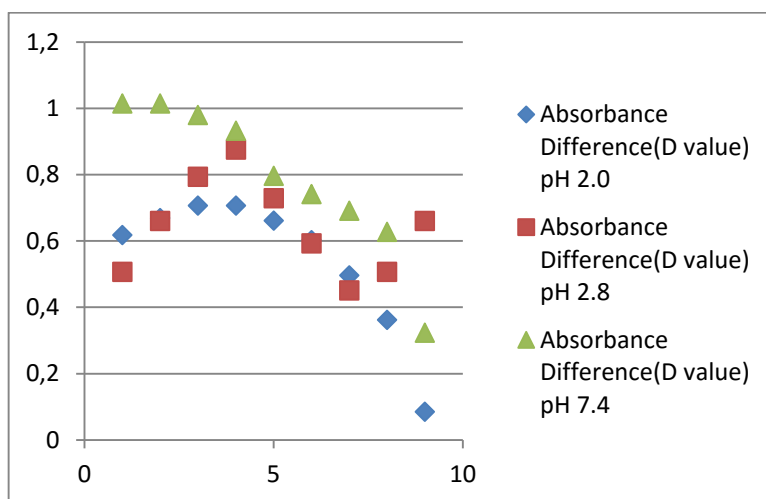


Figure 1 - Job's plot for complexation of ketotifen with metformin at 300 nm.

Table 2 - Stability constant values of ketotifen with metformin at different pHs

System	pH	Stability Constants
Interaction of ketotifen with metformin	pH=2.0	0.05
	pH=2.8	0.01
	pH=7.4	0.77

#### 4.5 Complex confirmation test by Differential Scanning Calorimeter (DSC)

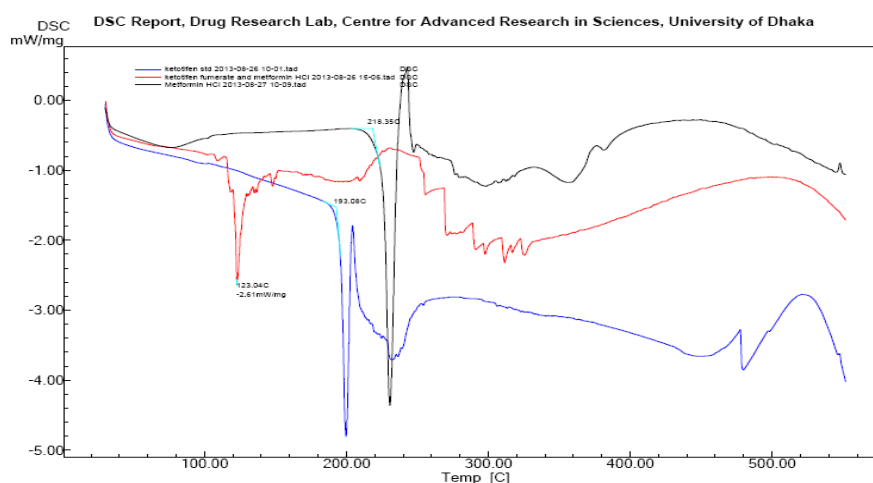


Figure 2: Differential scanning calorimeter study of ketotifen, metformin and ketotifen-metformin mixture.

Table 3: Identification of the stability of the complex (ketotifen-metformin)

Systems	R <sub>f</sub> values	Comment
Ketotifen	0.49	Stable complex is formed
Metformin	0.51	
Mixture of Ketotifen & Metformin	0.39	

Table 4: 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*

## DISCUSSIONS

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 ultraviolet or visible form. Its spectra of alone ketotifen at different pH showed a sharp absorption maximum at 300 nm. When metformin hydrochloride mixed with ketotifen in 2:1 ratio the intensity of the peak of ketotifen change remarkably (absorbance decreases) i.e. absorption characteristics are altered due to interaction but the position of the compound do not shift. The Ardon's plots have been used to evaluate the stability constants and it has been observed that when values of  $1 / (D - C_{eA})$  are plotted against  $1 / \text{Drug}$ , good straight lines are obtained obeying the Ardon's equation. Ketotifen fumarate and metformin hydrochloride were interacted at various concentrations comprising  $1 \times 10^{-5}$  M to  $9 \times 10^{-5}$  M at pH 2.0. It is observed that there has no clear cut symptom of drug interaction. At pH 2.8, various concentrations comprising  $1 \times 10^{-5}$  M to  $9 \times 10^{-5}$  M of ketotifen were interacted with metformin hydrochloride. The sharp 'V' shaped angle in the curve at a concentration of  $4 \times 10^{-5}$  M indicates the presence of drug interaction. At pH 7.4, various concentrations comprising  $1 \times 10^{-5}$  M to  $9 \times 10^{-5}$  M of ketotifen were interacted with metformin. The breakdown in curve indicates the presence of drug interaction. In DSC the ketotifen – metformin complex exhibited a sharp peak which showed interactions between ketotifen fumarate and metformin hydrochloride and confirming the formation of a complex.<sup>91</sup> The stability of the complex after formation was confirmed by thin layer chromatographic technique. When the R<sub>f</sub> values of the mixtures differ from the R<sub>f</sub> values of the pure compounds, indicate the stability of the complex. The results of investigation of hepatotoxicity of combination drug therapy was compared with single drug sample ketotifen fumarate were shown in table 4. But the groups which receive the combination drug samples (ketotifen and metformin) and ketotifen and theophylline showed a significant increase in SGPT, and showed a significant decrease of ATPN levels.



## CONCLUSION

In the Job's spectroscopic method when ketotifen was interacted with metformin a sharp breakdown in the curve was observed at a pH 2.8 & 7.4, indicate the presence of drug interactions. But relatively low stability constant values are seen when the interaction occurs between ketotifen & metformin. In the DSC study ketotifen-metformin complex exhibited a sharp new peak at 123.04°C (-2.61 mW/mg). The stability of the complex after formation was confirmed by thin layer chromatographic technique. More stable complex was formed when ketotifen was interacted with metformin. 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 showed a significant increase in SGPT, and showed a significant decrease of APTN levels in ketotifen & metformin mixture. 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. Coadministration of ketotifen fumarate & metformin hydrochloride should be avoided.

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