

In vitro Interaction of Metformin with Diclofenac in Aqueous Medium

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ABSTRACT: Combination therapy may be unavoidable and common way for the treatment of disease where two or more drugs are given concurrently. The drugs may exhibit effects independently or may interfere with each other. Metformin is an anti-diabetic drug and diclofenac is a NSAID. The *in vitro* interaction of metformin with diclofenac was studied at room temperature and at different pH conditions. The studies were performed by various UV-Visible spectrophotometric, conductometric and HPLC methods. It was found that metformin formed stable 1:1 complex with diclofenac. The interaction may greatly influence the activity of these molecules.

Key words: Metformin, Diclofenac, interaction.

INTRODUCTION

After receiving the drug orally, it must be dissolved in the GI fluids and then absorption occurs through the membrane into the systemic circulation. The drug is distributed to various parts of the body where it may be stored, metabolized, exert a pharmacological action and excreted. Thus, a drug may come in close contact with foodstuffs and different body components or with other drugs inside the body and it may form a complex with such a species. The area of drug interaction involves and correlates all the disciplines of drug management and health care system relevant to the contemporary practice of medicine.¹⁻³

Knowledge of drug interaction may allow early recognition and prevention of adverse consequences. The most comprehensive understanding of clinically significant drug interaction can be achieved by combining knowledge of the mechanism of drug interaction with recognition of the high-risk patients and the identification of drug with a narrow therapeutic index. Problem arising from the interaction of drugs may be overcome by partial

changes in the molecular pattern, by blocking the reactive site in the molecule, by changing the dosage regimen or by avoiding the combined application of interacting drugs. However, to take any step to manage the interaction problems, the nature of interaction should be known. We should know the possible interaction of a new drug prior to use clinically. For the drugs which are being used conventionally, interaction studies are also very important to detect the problems yet to be found out.⁴

The principal purpose of the present study was to investigate complex formation and to study the nature and strength of complex which could be due to interaction of metformin with diclofenac. Metformin is an anti-diabetic drug. It is the first-line drug of choice for the treatment of type-2 diabetes, particularly in overweight and obese people and those with normal kidney function. Its mode of action is thought to be multifactorial and includes delayed uptake of glucose from the intestinal tract, increased peripheral glucose utilization mediated by increased insulin sensitivity and inhibition of increased hepatic and renal gluconeogenesis. On the other hand, diclofenac is a NSAID. It is commonly used for the reduction of pain, fever, inflammation and stiffness caused by conditions such as osteoarthritis, kidney

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stones, rheumatoid arthritis, psoriatic arthritis, gout, ankylosing spondylitis, tendonitis and the treatment of primary dysmenorrhea. It works by inhibiting both the COX-1 and COX-2 enzymes.⁵

MATERIALS AND METHODS

Drugs and chemicals. The working standard of diclofenac sodium (potency: 98.70%) was a gift from Square Pharmaceuticals Ltd., Dhaka, Bangladesh. The working standard of metformin (potency: 99.27%) was obtained from Beximco Pharma Ltd., Dhaka, Bangladesh. Hydrochloric acid (37%), potassium chloride, orthophosphoric acid, sodium hydroxide, potassium hydroxide, potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate, methanol, ethanol were of analytical grade and purchased from the local suppliers.

Preparation of buffer solutions^{6,7}

pH 1.4. This buffer was prepared by dissolving 6.57g of potassium chloride in demineralized water and added 119.0 ml of 0.1M hydrochloric acid. Then the volume was made up to 1000 ml with the same solvent. pH was adjusted to 1.4 with hydrochloric acid. 250 mL of 0.1M hydrochloric acid was prepared by mixing 2.25 ml of 37% hydrochloric acid with demineralized water.

pH 2.4. This buffer was prepared by mixing 6.7 ml of orthophosphoric acid with 50.0 ml of 4% v/v solution of 2M sodium hydroxide and diluted to 1000 mL with demineralized water. pH was adjusted to 2.4 with sodium hydroxide. 100 ml of 2M sodium hydroxide was prepared by dissolving 8.0g of sodium hydroxide in demineralized water. 100.0 ml of 4% v/v solution of 2M sodium hydroxide was prepared by diluting 4 ml of 2M sodium hydroxide to 100 ml with demineralized water.

pH 7.4. This buffer was prepared by mixing 65.4 mL of 0.02M potassium dihydrogen orthophosphate with 289.7 ml of 0.01M disodium hydrogen orthophosphate and diluted to 1000 ml with demineralized water. 100 ml of 0.02M potassium dihydrogen orthophosphate was prepared by dissolving 0.2722g of potassium dihydrogen orthophosphate in demineralized water and the final

volume up to 100 ml. 500 ml of 0.01M disodium hydrogen orthophosphate was prepared by dissolving 0.710g of disodium hydrogen orthophosphate in demineralized water.

Preparation of stock solutions

Metformin. 100 ml stock solution of 1×10^{-3} M was prepared by dissolving 0.0129g of metformin in demineralized water and 1-2 drops of conc. hydrochloric acid was added to dissolve it and finally made the volume was made up to 100 ml with the same solvent. The stock solution was diluted to the desired strength by buffer solutions.

Diclofenac. 100 ml stock solution of 1×10^{-3} M was prepared by dissolving 0.038 g of diclofenac in demineralized water and added 1-2 drops of conc. hydrochloric acid to dissolve it and finally the volume was made up to 100 ml with the same solvent. The stock solution was diluted to the desired strength by buffer solutions.

Drug-drug interaction analysis by observation of absorption spectra. In this procedure, the ultraviolet absorption characteristics of metformin, diclofenac and their 1:1 mixture in solution at different pHs were compared. The concentrations of samples were kept at very dilute levels in each case and the measurements were made using an UV-visible spectrometer (UV-1601, Shimadzu, Japan) equipped with computer and appropriate software program. The stock solutions of the sample were diluted to appropriate levels by buffer of the desired pH and the spectra were recorded between 200-400 nm.

Drug-drug interaction analysis by Job's method of continuous variations. Job's spectroscopic method of continuous variation plots was carried out to confirm the formation of 1:1 complex between the drugs to be studied. In this method, solutions of different concentrations of metformin and diclofenac were prepared using solvent and a continuous variation plots were made by corrected absorbance against the volume fraction of one reactant.

Drug-drug interaction analysis by conductometric titration method. Conductance is an inherent

property of an ionic species. The conductance of species may change in solution due to the interaction with other species. In this method, conductance was changed due to the function of varying molar ratios of the species in a mixture. Conductometric titrations in demineralized water system at pH 7.4 were carried out using a Conductometer (Jenway, Switzerland) to find the molar ratios at which complexation occurred. 35 mL of 0.05M solution of metformin was taken in a 100 mL beaker and was titrated individually with gradual addition of 0.05M solution of diclofenac from a burette. Two titrations were carried out; one was titrated against the other and vice-versa. The conductance at each addition was recorded. Then the conductance was plotted against the molar ratios of the titrants to obtain the conductivity curves. The titration curve showed break at the points of possible interaction.

Drug-drug interaction analysis by HPLC method. High-performance liquid chromatography is a chromatographic technique that can separate a mixture of compounds and is used to identify, quantify and purify the individual components of the mixture. Retention time and absorbance of peak of one species in solution may be changed due to the interaction with other species. In the present study, HPLC analysis of metformin and diclofenac were carried out, using HPLC (Shimadzu, Japan) at pH 7.4 with a concentration of 50 μ g/mL where combination of metformin with diclofenac was of 1:1 molar ratio (50 μ g/ml). Two operations were carried out.

RESULTS AND DISCUSSION

In the present study, various methods of analysis were carried out for the determination of drug-drug interaction of metformin with diclofenac. These methods included the spectrophotometric methods of analysis, spectral characteristics, conductometric titrations, Job's method of continuous variations and HPLC. All of these methods of analysis revealed the formation of complexes among the studied drugs.

Absorption spectral observation. The drugs studied showed absorption in UV-VIS region. The molecular species of diclofenac when mixed with

metformin, showed some changes in absorption characteristics of this molecule (metformin) including some shifts in the absorption maxima. Thus alteration in spectral pattern may be regarded as an indicator for the primary interaction among these drugs. The UV absorption values of the drug and drug mixtures were measured at 200-400 nm. The spectra of metformin alone at different pH conditions showed an absorption maximum at 228 nm. 1 ml of 0.005M metformin and 1 ml of 0.005M diclofenac were mixed and absorbance were measured within the range of 200-400 nm. Before that individual absorbance of 0.005M metformin and diclofenac were measured (Figure 1).

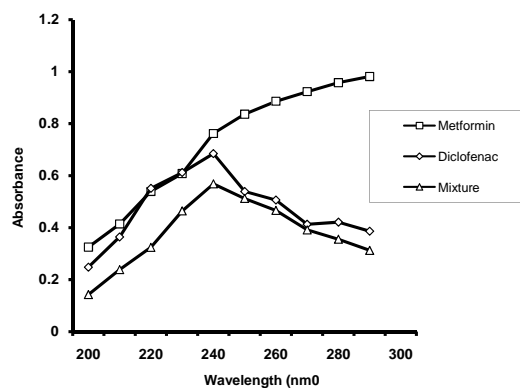


Figure 1. UV spectra of metformin, diclofenac and their mixture at pH 1.4.

1 ml of 0.005M metformin and 1 ml of 0.005M diclofenac were mixed and absorbance were measured within the range of 200-400nm. Before that individual absorbance of 0.005M metformin and diclofenac were measured as shown in Figure 2.

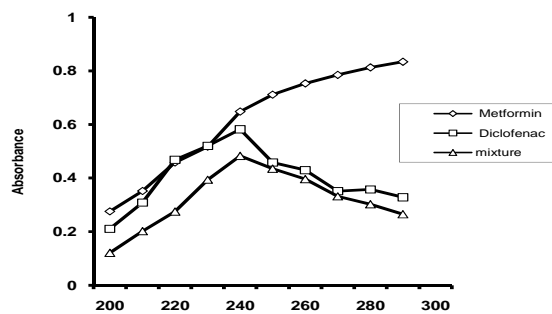


Figure 2. UV spectra of metformin, diclofenac and their mixture at pH 7.4.

From the graphs we found that absorbance of individual metformin and diclofenac varied with the absorbance of their combination. It had been seen that the combination graph possessed lower absorbance than the individual. So, from here we could say that 1:1 mixture of metformin and diclofenac resulted into noticeable changes in the absorption intensities due to interaction. The intensities of absorbance peaks also varied with pH. Change in pH acidic to basic, absorbance shifted to higher wavelength. This ensured that pH change had significant effects on the interaction between these drugs.

Interaction analysis by Job's method of continuous variations. Job's spectroscopic method of continuous variation was carried out to confirm the formation of 1:1 complexes between the drugs to be studied. Job's plots (Figure 3 and 4) gave 'Λ' shaped curve indicating the formation of 1:1 complex for all systems. When metformin was at a very high concentration compared to diclofenac in the mixture, the absorbance decreased larger extent and the difference became negative indicating a stable interaction.

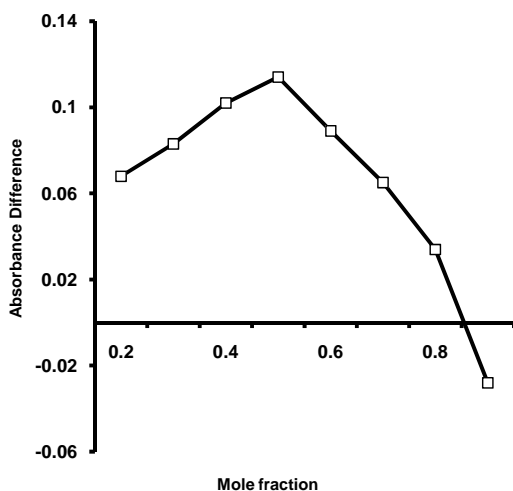


Figure 3. Job's plot for metformin-diclofenac system at pH 7.4.

Conductometric titration. The conductance was plotted versus the molar ratios of the titrants for obtaining conductivity curves (Figure 5).

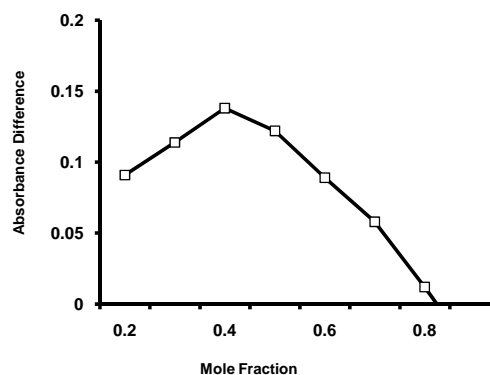


Figure 4. Job's plot for metformin-diclofenac system at pH 2.4.

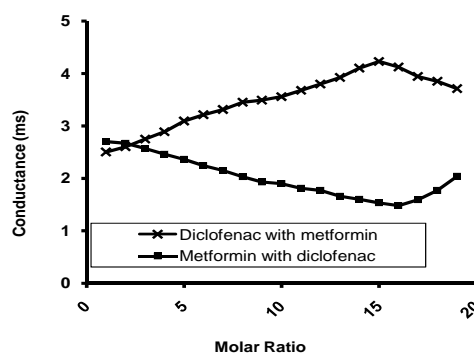


Figure 5. Conductometric titration of metformin with diclofenac at pH 7.4.

When metformin was titrated with diclofenac at pH 7.4, one distinct intersection corresponding to metformin-diclofenac molar ratios of 1:1 was found in the conductivity curve. The reverse titration showed break at 1:1 molar ratio. These indicated that metformin formed stable complex with diclofenac at 1:1 molar ratio through some unstable complex.

HPLC method for drug interaction analysis.

HPLC of metformin: Chromatogram of metformin was taken under following conditions: 10% acetonitrile, 90% phosphate buffer, C_8 column, flow rate 0.7 ml/min, UV 254 nm, injection volume: 20 μ l. The chromatogram was shown in Figure 6.

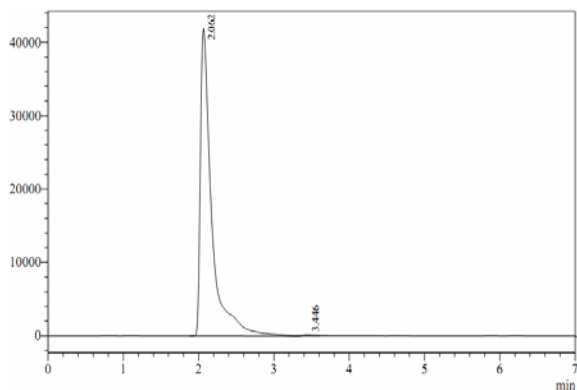


Figure 6. HPLC plot for metformin at pH 7.4.

HPLC of diclofenac: Chromatogram of diclofenac was taken under following conditions: 70% acetonitrile, 30% monobasic sodium phosphate buffer, C₈ column, flow rate 0.7 ml/min, UV 278 nm, injection volume: 20 μ l. The chromatogram is shown in Figure 7.

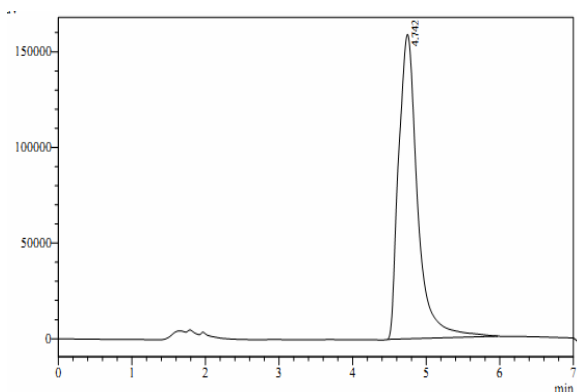


Figure 7. HPLC plot for diclofenac at pH 7.4.

HPLC of diclofenac and metformin: Chromatogram of diclofenac and metformin was taken under following conditions: 90% phosphate, 10% acetonitrile for 5 min, 10% to 70 % acetonitrile for 2 min, 70 % acetonitrile for 10 min, C₈ column, flow rate 0.7 ml/ min, UV 258 and 278 nm, injection volume: 30 μ l. The chromatogram was shown in Figure 8.

From the aforementioned graph, we found that retention time of metformin alone was 2.062 min and diclofenac was 4.742 min. But, in case of the combination of metformin with diclofenac (1 : 1)

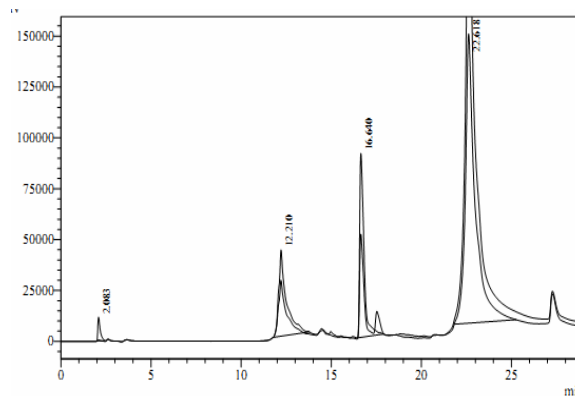


Figure 8. HPLC plot for metformin and diclofenac at pH 7.4.

retention time shifted to 2.083 min. It was little higher than metformin alone and much lower than diclofenac alone. It indicated a possible interaction between metformin and diclofenac. Moreover, if we observed the graph we found appearance of more peaks at times 12.21, 16.46 and 22.618 min indicating different species in the mixture. Thus, it could be inferred that a good number of combination products might resulted due to interaction of metformin with diclofenac. These results are comparable to some of our previous studies⁸⁻¹¹ where simultaneous administration of two or more drugs have been discouraged for the safety of the patient because, in our country, clinical monitorings, even in big hospitals, are rarely performed.

CONCLUSION

In the present work, the studies were performed by various methods including UV-VIS spectrophotometry, conducto-metry and HPLC. The results from all these allowed to conclude that metformin formed stable complex with diclofenac.

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