Spectroscopic Monitoring Of Metformin And Atorvastatin On Artificial Gastrointestinal Fluid Following Drug-Drug Interaction Rule: An In Vitro Approach

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Abstract: Modern pharmacotherapy provides newer approaches to handle different complicated diseases. With increase in comorbidity, multiple concomitant drug therapy became a rule in different parts of the globe. Diabetes mellitus is associated with several co-morbid conditions, thereby compelling the use of multiple drugs, concomitantly. Polypharmacy, though a need for comorbid and complicated diseases, however, increases the propensity of drug-drug interactions and adverse drug reactions. Objective of the present study was to evaluate drug-drug interaction of Metformin (anti-diabetic) and Atorvastatin (hypolipidemic), one of the commonly prescribed co-medications, using in vitro systems. Artificial gastrointestinal fluids, mimicking conditions in stomach and intestine of human body, were used to screen drug-drug interaction. Metformin and Atorvastatin were dissolved alone and in combination in artificial gastrointestinal fluids and these solutions were then screened through Ultraviolet (UV) spectrophotometric analysis and Fourier Transform Infrared Spectroscopy (FTIR). Concentrations of each drug were altered when co-administered. Metformin concentration in presence of Atorvastatin was lower by 15.4% as compared to Metformin alone in simulated gastric fluid (SGF). On the contrary, Atorvastatin concentration was higher in presence of Metformin by 11.4% in SGF and 23.7% in simulated intestinal fluid (SIF). FTIR study revealed an indication of ionic interaction between Metformin and Atorvastatin in these fluids. Further research for evaluation of different parameters is necessary to achieve therapeutic success with minimum patient suffering.

Index Terms: Artificial gastrointestinal fluid, Atorvastatin, Drug-drug interaction, FTIR, In vitro, Metformin, UV-Vis Spectrophotometry

1 INTRODUCTION
Incredible scientific inventions have gifted mankind with numerous safe and efficacious therapeutic agents that helped to increase the life span of human beings. These broad spectrum drugs have helped in the treatment of various chronic comorbid conditions. Diabetes Mellitus (DM) is a disease of improper metabolism of carbohydrates, proteins, lipids, due to lack of insulin hormone or insensitivity of cells to insulin, causing increased blood sugar level. It is becoming a potential epidemic disease in India with more than 62 million people affected by the disease [1]. Diabetes mellitus is associated with various co-morbid conditions like hyperlipidemia, cardiovascular disorders, etc. Study of prescribing medicines of diabetic patients from various parts of the world reported anti-hyperlipidemics, anti-platelets are often co-prescribed along with the anti-diabetics [2]. Dislipidemia is often associated with diabetes and is found to increase the risk of cardiovascular disorders.
or gas. Covalent bonds in molecules can bend or stretch, on being excited by infrared light. Bending results in a change of the angle between two bonds, whereas stretching causes change in the bond length. Apart, from the movement of these single bonds, molecules also experience vibrational motions that are characteristic of the atoms present in the molecule. Compounds absorb infrared radiations, corresponding to the energies of these vibrations. The band spectrum for a particular compound, therefore, reflects the arrangement of the atoms and molecules in the functional groups of the compound [9]. Therefore, comparison of band spectrum of known compounds with that of the unknown one will give an insight into the structure of the unknown compound [10]. FTIR technique is employed to study the drug-drug interactions. Band spectra are obtained for the individual drugs and the drugs in combination. Band spectrum of the drug combination is compared with that of the individual drugs. Emergence of new bands, disappearance of absorption bands or reduction in the intensity of the band gives a clear indication for drug-drug interactions [11]. Present study tried to evaluate the drug-drug interaction between two most commonly used medicines, Metformin (anti-diabetic agent) and Atorvastatin (hypolipidemic agent) by high precision spectroscopic techniques.

2 MATERIALS AND METHODS

Preparation of Simulated Gastric Fluid:
Artificial gastric fluid, termed as simulated gastric fluid (SGF) was prepared by dissolving sodium chloride, NaCl (3 gm) in about 1450 ml of deionized water and then adjusted pH to 1.2 ± 0.1 with diluted hydrochloric acid, HCl. Finally, the volume of the fluid was adjusted to 1500 ml with deionized water [12].

Preparation of Simulated Intestinal Fluid:
Artificial intestinal fluid, termed as simulated intestinal fluid (SIF) was prepared by dissolving potassium dihydrogen phosphate, KH₂PO₄ (6.805gm) and Sodium hydroxide, NaOH (0.896 gm) in about 450 ml of deionized water and then adjusted pH to 7.4 ± 0.1 with 2N NaOH. Finally, the volume of the fluid was adjusted to 1000 ml with deionized water [13].

Estimation by Spectrophotometric Technique:
Metformin tablet (500 mg) and Atorvastatin tablet (40 mg) was dissolved separately in SGF, SIF at a concentration of 1mg/ml. The spectral absorption of Metformin was 233 nm and Atorvastatin was 248 nm [14,15]. Standard curves of the two drugs were plotted against absorbance at different concentrations. Thereafter, Metformin and Atorvastatin were further dissolved separately and in combination with SGF and SIF to prepare solutions of concentration 50 µg/dL. The mixtures were then incubated at 37°C. Samples were withdrawn at time intervals- 0 min, 15 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, and 240 min and the absorbance was measured at 233 nm and 248 nm [12,15]. The molar absorptive values of the two drugs were obtained from the calibration curves. The concentrations of the interacting drugs were calculated from the formulae (1, 2) of Lambert-Beer’s law given below [8]:

\[ A1 = a1 \times C_a \times l + b1 \times C_b \times l \]  
\[ A2 = a2 \times C_a \times l + b2 \times C_b \times l \]

Where: \( C_a, C_b = \) Concentrations of Metformin and Atorvastatin, respectively.
\( a1, a2 = \) Molar absorptive values of Metformin at 233 and 248 nm, respectively
\( b1, b2 = \) Molar absorptive values of Atorvastatin at 233 and 248 nm, respectively
\( l = \) path length of cuvette

3 RESULTS

Estimation by FTIR Technique:
Metformin and Atorvastatin powder were dissolved separately and in combination with SGF and SIF to prepare solutions of concentration 50 µg/dL. The mixtures were then incubated at 37°C for 30 mins. Samples were then subjected to FTIR analysis, following standard protocols. The peaks obtained from the spectrum of the pure compounds were then compared with the characteristic peaks for different functional groups, known from literature [16-18]. The spectrum of the drug mixture was also compared with that of the pure drugs.

Estimation by Spectrophotometric Technique:
Standard curves were plotted with a known concentration of the two drugs in both SGF and SIF were plotted in order to obtain the unknown concentrations of individual drugs in the mixture. The characteristics of these calibration curves were tabulated in table 1. For spectrophotometric analysis, Metformin and Atorvastatin alone and in combination were mixed in SGF. Metformin concentration in SGF alone and Metformin in presence of Atorvastatin were plotted at different time intervals in figure 1a. Metformin concentration in presence of Atorvastatin was estimated from formulae 1 and 2. Metformin concentration in presence of Atorvastatin was found to be lower by an average of 15.4% (± 0.98) as compared to Metformin alone in SGF. Concentrations of Atorvastatin alone and Atorvastatin in presence of Metformin were plotted at different time intervals (Figure 1b). Atorvastatin concentration in presence of Metformin was higher by 11.4% (± 0.78) as compared to Atorvastatin alone. Similarly, Metformin and Atorvastatin concentrations, individual and in combination, were estimated in SIF. Metformin concentration was found to remain unchanged in presence of Atorvastatin in SIF (Figure 2a). However, Atorvastatin concentrations in presence of Metformin in SIF were higher by an average of 23.7% (± 2.22) as compared to Atorvastatin alone in SIF (Figure 2b). Thus in this present study, it was observed that Metformin concentration decreased in presence of Atorvastatin in simulated gastric fluid, however, it remained unchanged in simulated intestinal fluid. On the contrary, Atorvastatin concentration was found to be significantly higher in presence of Metformin in both these fluids. Altered concentrations of these drugs may affect the pharmacokinetics of the drug thereby influencing the drug action.

Estimation by FTIR Technique:
FTIR spectroscopy generates characteristic absorption bands for a particular compound, depending on the stretching and vibration of the functional groups present in the compound. Therefore the magnitude of the absorption band is assigned to a particular functional group. The FTIR spectrum of Metformin dissolved in SGF, in figure 3a, showed characteristics peaks (Table 2) representing functional groups, present in the drug. The spectrum of Atorvastatin dissolved in SGF, in figure 3b, also showed characteristics peaks (Table 2) that can be correlated to the functional groups present in the drug. The spectrum of the sample containing the two drugs, in figure 3c, showed peaks at 3209.70 cm⁻¹ corresponding to N-H stretching, and 819.3 cm⁻¹ and 758.44 cm⁻¹, owing to N-H rocking, as was observed in both Metformin and Atorvastatin spectra, with minor shift. Spectrum of the drug combination displayed distinctive peaks at 2368.61 cm⁻¹, 1565.24 cm⁻¹, and 1170.62 cm⁻¹, owing to C=C (alkynes) stretching, C=C (aromatic) stretching and C-F stretching, respectively, also present in the Atorvastatin band spectrum. Peaks at 1642.17 cm⁻¹ and 1513.09 cm⁻¹, corresponded to C=N stretching and N-H bending, respectively, as was found in Metformin spectrum with minor deviation. Peak for C triple bond N stretching at 2152.74 cm⁻¹ was not present in the spectrum of any of the other two pure compounds. Triple bond of carbon is not present in the structure of any of the two drugs. Peak at 1428.22 cm⁻¹ assigned to O-H bending, present in Atorvastatin, was missing in the spectrum of the drug combination. Peak at 992.75 cm⁻¹ was also absent in the band spectrum of the pure drugs. Thus, deviation of the wavenumbers from the characteristic pure compounds along with presence and absence of few bands in the spectrum of the combined solution, indicated there might be changes in the arrangement of the molecules, probably due to some ionic interaction between the two drugs, when administered concurrently. The FTIR spectrum of Metformin dissolved in SIF, in figure 4a, showed characteristics peaks (Table 3) corresponding to functional groups present in the compound. The spectrum of Atorvastatin dissolved in SIF, in figure 4b, also showed characteristics peaks (Table 3) reflecting the structural arrangements present in the compound. Minor shifts were attributed to the presence of the basic medium. The spectrum of the combination of the drugs, in figure 4c, showed peaks at 3002.1 cm⁻¹ that corresponded to N-H stretching and at 915.17 cm⁻¹, 854.45 cm⁻¹, 804.3 cm⁻¹ and 754.44 cm⁻¹ owing to N-H rocking. These peaks were evident in the spectrum of the pure drugs- Metformin and Atorvastatin, with minor shifts. Bands at 1122.48 cm⁻¹ corresponded to C-F stretching as was found in Atorvastatin structure. Various peaks present in the spectra of the two pure compounds were absent in the spectrum of the combination. Peak at 1885.56 cm⁻¹ and 1832.19 cm⁻¹ were absent in the spectrum of the combination. Thus, disappearance of the characteristic peaks along with emergence of the few new peaks in the FTIR spectrum of the drug combination might indicate some ionic interaction between the two drugs when administered concurrently.

4 DISCUSSIONS

Diabetes mellitus is a metabolic disease associated with increased blood glucose levels and multiple other disorders like Dislipidemia. Thus diabetic patients are often found to be co-prescribed with hypolipidemics [2]. Metformin is the most commonly prescribed drugs among anti-diabetic medications [19]. Metformin is a biguanide with plasma half-life of 1.5-3 hrs, excreted unchanged through kidney. It does not undergo metabolism. It decreases blood glucose level in blood by suppressing hepatic gluconeogenesis and glucose output from liver, enhancing glucose uptake by peripheral tissues and retarding glucose absorption [20]. Atorvastatin is the drug mostly prescribed in dyslipidemia in diabetic patients [21]. Atorvastatin is 3-Hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that inhibits cholesterol synthesis. Plasma half-life is 18-24 hours [22]. The concomitant use of Metformin and Atorvastatin has been observed in various surveys around the world [2]. Present study tried to estimate the effect of co-administration of these drugs in artificial gastrointestinal fluids, by various in vitro methods. Spectrophotometric analysis of concomitant use of these drugs in artificial gastrointestinal fluids was found to alter the concentrations of these drugs. Metformin concentration in presence of Atorvastatin was found to be lower by an average of 15.4% (± 0.98) as compared to Metformin alone in SGF. However, in SIF, the concentration of Metformin was found to remain unchanged in presence of Atorvastatin. On the contrary, the concentration of Atorvastatin in combination was found to be higher by 11.4% in SGF and 23.7% in SIF than the drug alone. A study on in vivo model showed both Atorvastatin and Rosuvastatin significantly enhanced the hypoglycemic activity of Metformin. On the other hand, Metformin also enhanced the hypolipidemic activity of the two statins [23]. Another in vitro study by Sultana et al. found that Metformin availability increased in simulated gastric fluid in presence of H₂ receptor agonists- cimetidine and famotidine [24]. Clinical study reported that bioavailability of Metformin was enhanced in presence of anticholinergics [25]. A group of researchers in Gujarat, India, showed that the half-life and volume of distribution of anti-diabetic drug glimepiride decreased in presence of Atorvastatin and Rosuvastatin [26]. Increased or decreased availability of the drug may be attributed to the rearrangement of the chromophoric region, resulting from interaction of the drugs. Change in concentration of one drug due to the effect of another drug may indulge in altered drug action thereby resulting in drug-induced toxicity. FTIR is another in vitro technique used to estimate drug-drug interactions. Interaction between chondroitin sulphate and acelofenac has been tested for estimation of interactions between these two chemicals, with the help of FTIR [27]. Indication of ionic interaction between Metformin and Atorvastatin was also evident from FTIR data in the present study. The drugs when mixed in the artificial gastrointestinal fluids were found to have shift in the wavenumbers and emergence and absence of new peaks were also evident. Ferdous et al., studied the interaction of Metformin and Cefepime (an antibiotic), with the help of FTIR. Displacement of methyl, hydroxyl and amide groups were observed as a result of the interaction of these two drugs [28]. Further investigations are essential to elucidate the nature of interaction between these two drugs and to verify the implication of these drugs in in vivo systems.
5 CONCLUSION
Present study thus tried to evaluate the safety and efficacy of concomitant medication to decipher the effect of drug-drug interactions in vitro systems. Results of the in vitro studies indicated interaction of Metformin and Atorvastatin when mixed together in artificial gastrointestinal fluids. However, the clinical importance of this interaction needs to be evaluated. Therefore, further investigations are essential to estimate the effect of these changes on the action of the drug. The ultimate goal is to optimize the therapeutic regimen in human so that the therapeutic efficacy is increased with minimum risk.

6 REFERENCES
ACKNOWLEDGMENTS

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<p>| Table 1: Characteristics of Standard Curves of Metformin and Atorvastatin in SGF and SIF |</p>
<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Parameters</th>
<th>Metformin in SGF</th>
<th>Atorvastatin in SGF</th>
<th>Metformin in SIF</th>
<th>Atorvastatin in SIF</th>
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<tr>
<td>1.</td>
<td>λmax (nm)</td>
<td>233</td>
<td>248</td>
<td>233</td>
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<td>2.</td>
<td>Slope</td>
<td>0.0238</td>
<td>0.0094</td>
<td>0.0766</td>
<td>0.0108</td>
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<td>3.</td>
<td>Correlation Coefficient</td>
<td>0.9938</td>
<td>0.9991</td>
<td>0.9906</td>
<td>0.9938</td>
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<td>4.</td>
<td>Molar Extinction Coefficient [1/(µg/ml)*cm)]</td>
<td>0.026</td>
<td>0.01</td>
<td>0.072</td>
<td>0.01</td>
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<p>| Table 2: Spectral Positions of FTIR peaks of Metformin in SGF and Atorvastatin in SGF and their corresponding assignments |</p>
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<tr>
<th>Metformin</th>
<th>Wave numbers (cm⁻¹)</th>
<th>Assignments</th>
<th>Wave numbers (cm⁻¹)</th>
<th>Assignments</th>
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<tr>
<td>3494.94, 3208.69</td>
<td>N-H (amine) stretching</td>
<td>3214.34</td>
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<td>1647.17</td>
<td>C=N stretching</td>
<td>2367.61</td>
<td>C=C (alkynes) stretching</td>
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<td>1552.66</td>
<td>N-H bending</td>
<td>1767.83</td>
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<td>1458.15</td>
<td>C-H bending in plane</td>
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<td>1118.38</td>
<td>C=C stretching</td>
<td>1428.22</td>
<td>O-H bending</td>
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<tr>
<td>904.75, 857.45</td>
<td>N-H rocking</td>
<td>787.83</td>
<td>N-H rocking</td>
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</tr>
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</table>

<p>| Table 3: Spectral Positions of FTIR peaks of Metformin in SIF and Atorvastatin in SIF and their corresponding assignments |</p>
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<th>Metformin</th>
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<th>Assignments</th>
<th>Wave numbers (cm⁻¹)</th>
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<td>3007.10</td>
<td>N-H (amine) stretching</td>
<td>3003.1</td>
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<td>2914.52</td>
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<td>2912.52</td>
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<td>1581.45</td>
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<td>O-H bending</td>
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<td>1128.48</td>
<td>C=C stretching</td>
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<td>919.17, 859.45</td>
<td>N-H rocking</td>
<td>787.87</td>
<td>N-H rocking</td>
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</table>

FIGURES

Figure 1: A. Distribution of concentration of Metformin alone and in presence of Atorvastatin in SGF at different time intervals; B. Distribution of concentration of Atorvastatin alone and in presence of Metformin in SGF at different time intervals.
Figure 2: A. Distribution of concentration of Metformin alone and in presence of Atorvastatin in SIF at different time intervals; B. Distribution of concentration of Atorvastatin alone and in presence of Metformin in SIF at different time intervals.

Figure 3: FTIR Spectrum of a) Metformin in SGF, b) Atorvastatin in SGF, c) Metformin + Atorvastatin in SGF
Figure 4: FTIR Spectrum of a) Metformin in SIF, b) Atorvastatin in SIF, c) Metformin + Atorvastatin in SIF