In vitro Complexation of Olmesartan Medoxomil with Dapagliflozin, Vildagliptin and Metformin

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ABSTRACT: Drug-drug interactions have been a serious concern for pharmacokinetics, pharmacodynamics and pharmacological profiles of therapeutic agents. The aim of this study was to carry out interactions of olmesartan medoxomil with dapagliflozin, vildagliptin and metformin, which were confirmed by TLC, HPLC and FT-IR. The newly formed complexes showed characteristic thermochemical properties in differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA). In TLC, three spots from the three complexes were found to be different from their precursor drugs. In HPLC chromatograms, the R_t (retention time) of the pure olmesartan medoxomil, dapagliflozin, vildagliptin and metformin were found to be different from their respective complexes. The FT-IR spectra obtained for drug-drug interactions were seen to demonstrate new pattern of peaks compared to pure drugs. The DSC and TGA thermograms of olmesartan medoxomil, dapagliflozin, vildagliptin and metformin were also found to be different from their complexes. All these variations from parent compounds indicated the formation of new complexes.

Key words: Drug-drug interaction, olmesartan medoxomil, dapagliflozin, vildagliptin, metformin, TLC, HPLC, FT-IR, DSC, TGA.

INTRODUCTION

Drug-drug interactions are important reasons of medication errors. It is estimated that drug interaction results ~30% of all adverse drug reactions. In hospitalized patients, it occurs about 30% and for ambulatory patients around 70.3%.[1] It can be defined as the modification of the effects of one drug (i.e., the object drug) by the prior or concomitant administration of another drug.[2] Drug-drug interactions are of serious concern in patients who are receiving multi-drug therapy, that cause an increased risk of health problems even in hospitalized patients.[3]

The extent of the drug interactions is a global problem which increases extensively with increase in population of patient and as the number of medications increases.[4]

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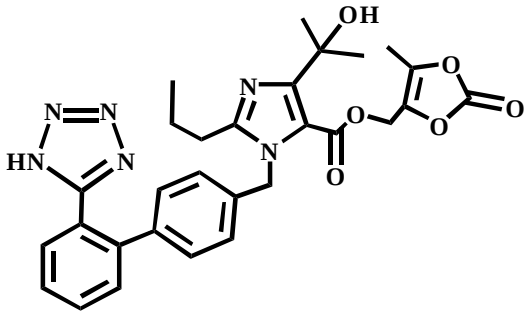
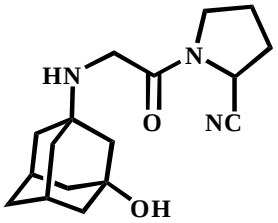
Now-a-days diabetes outbreak is in epidemic form. International Diabetes Federation reports that diabetes affects about 382 million people world-wide and it is estimated that this number will raise to 592 million by 2035.[5] Diabetes is now the leading cause of many serious complications such as cardiovascular, renal and other serious comorbidities.[6] The distinctive properties of diabetes mellitus are chronic hyperglycemia, microvascular (e.g. retinopathy, nephropathy and neuropathy) as well as the macrovascular (e.g. coronary artery disease (CAD), hypertension (HT), atherosclerosis and stroke) pathologies with more than 17.5 million deaths globally which furthermore attributable to cardiovascular complications.[7] Diabetes is undoubtedly one of the most challenging health problems in the 21st century.[8] Hypertension in diabetic patient is also one of the major and common health problems which is frequently difficult to treat and results significant morbidity and mortality. The frequency of hypertension in diabetic people is

probably 1.5-2.0 times more than in the general people.[9] The combined presence of hypertension and diabetes affect same major target organs and responsible for left ventricular hypertrophy and coronary artery disease, decrease in renal function, the development of diabetic retinopathy and cerebral diseases.[10]

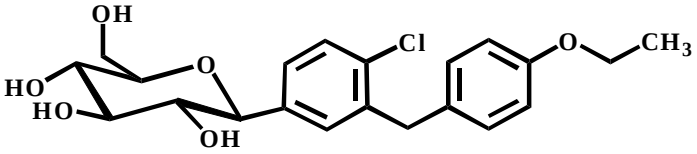
Olmesartan medoxomil, a prodrug, is hydrolyzed to olmesartan during absorption from the gastrointestinal tract. It is a selective AT_1 subtype angiotensin II receptor antagonist antihypertensive agent.[11] Chemically, olmesartan medoxomil is 1H-imidazole-5-carboxylic acid, 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]- (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester. It is practically insoluble in water and sparingly soluble in methanol.[11] The structure of olmesartan medoxomil is shown in figure 1.



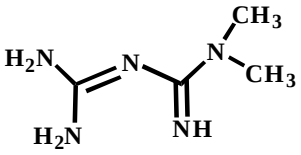
Olmesartan medoxomil, Mol. Wt.: 558.59



Vildagliptin, Mol. Wt.: 305.42



Dapagliflozin, Mol. Wt.: 408.90



Metformin, Mol. Wt.: 129.16

Figure 1.Structure of olmesartan medoxomil, dapagliflozin, vildagliptin and metformin.

Dapagliflozin (Figure 1), a selective sodium-glucose cotransporter-2 inhibitor, reduces renal glucose re-absorption in an insulin-independent manner. It is soluble in ethanol and DMSO. It significantly reduces the development of hyperglycemia. It could improve the insulin sensitivity, reduce β-cell mass and the development of impaired pancreatic function.[12] Vildagliptin (Figure 1) is an oral antihyperglycemic agent of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. It inhibits the inactivation of and glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) by DPP-4, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas.[13] It is soluble in water. Metformin (Figure 1) is a biguanide derived antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, as well as decreasing glucose absorption and increasing insulin-mediated glucose uptake. Another well-known benefit of this drug is modest weight loss.[14]

This paper describes the formation of complexes of olmesartan medoxomil with dapagliflozin, vildagliptin and metformin HCl, namely olmesartan medoxomil-dapagliflozin complex (OD), olmesartan medoxomil-vildagliptin complex (OV) and olmesartan medoxomil-metformin HCl complex (OM). To confirm the formation of new complexes TLC, HPLC and FT-IR experiments were carried out followed by DSC analysis and TGA.

MATERIALS AND METHODS

Materials. Olmesartan medoxomil (potency 99.98%), Dapagliflozin (potency 99.98%), Vildagliptin (potency 99.99%), Metformin HCl (potency 99.97%) were kind gifts from Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh. HPLC grade methanol, acetonitrile and chloroform were purchased from Sigma Aldrich, Germany. Nanopure water was prepared by Opurite (PM00723). TLC plate (HSF-254) was purchased from Merck, Germany.

Methods. The drug-drug complexes of olmesartan medoxomil were synthesized according to mole ratios. Equal volumes of (20 ml) of methanol solution of 0.5 mM drugs were prepared by taking 0.279 g of olmesartan medoxomil, 0.204 g of dapagliflozin, 0.152 g of vildagliptin and 0.082 g of metformin HCl separately and then drugs were added gradually with stirring followed by changing pH and heated at 60^{o}C for 24 hrs. The solutions were then filtered and left at room temperatures.

Analysis of complexes

TLC. To observe the formation of complexes, TLC was carried using methanol-chloroform (40:60) as mobile phase.

HPLC. RP-HPLC was performed on a C_{18} column (5 µm particle size, 25 nm × 46 nm i.d) for estimation of drug content and their related substances. The chromatographic analyses were performed in isocratic mode comprising the mixture of acetonitrile and 15 mM phosphate buffer in the ratio of 60:40 v/v and at a flow rate of 1.0 ml/min. The eluate was monitored through UV detection at 254 nm.

DSC thermogram. DSC thermograms were obtained from the DSC instrument, (model: DSC 60, Shimadzu, Japan). The thermograms were taken in aluminium sealed pan at the temperature range of 30-300^{o}C, where temperature rising rate was 10°C/min in nitrogen gas at a flow rate of 20 ml/min. All the complexes and pure standard drugs were studied.

TGA. TGA were carried out in TGA 50H, Shimadzu, Japan. The thermograms were taken in aluminium pan at the temperature range of 25-600 ^{o}Cwith hold time 5 min. The temperature rising rate was kept at 10 °C/min and the nitrogen gas flow rate was at 10 mL/min. The thermograms of all the complexes and standard drugs were recorded under same conditions.

FT-IR. Standard drug and samples pellets were prepared with appropriate quantity of KBr (usually in the ratio of 100: 0.1) by mixing and grinding in an agate mortar. Pellets were made with about 100 mg mixture. FT-IR spectra were recorded with FT-IR 8400S (Shimadzu) spectrophotometer in the range of 4000-400/cm (resolution: 4/cm, number of scans: 30).

RESULTS AND DISCUSSION

Both crystalline and amorphous drug complexes were obtained. To prove the complexation, TLC was carried in methanol-chloroform in the ratio of 40:60. Single spot from the three complexes which were different from their precursor drugs were found (Table 1). Each spot indicated the presence of a new complex.

In HPLC chromatograms, the R_t (retention time) of the pure olmesartan medoxomil, dapagliflozin, vildagliptin, metformin were found to be 3.59 min, 3.80 min, 3.21 min, 2.50 min, respectively. The complexes OD, OV and OM showed R_t at 2.87 min, 9.37 min and 9.77 min, respectively, which were different from the retention times of their parent drugs. These indicated the formation of a new complex for each drug. In DSC, pure olmesartan medoxomil showed melting endotherm at 183.07°C,

dapagliflozin at 69.43°C, vildagliptin at 151.64°C and metformin at 232.30°C. The complex OD showed a broad endothermic peak at 96.69 °C, which was different from the endothermic peaks of its parent drugs e.g. olmesartan medoxomil (183.07°C) and dapagliflozin (69.43 °C) indicating the formation of a new complex. Besides, complexes OV and OM also produced endothermic peaks at 111.97°C and 198.01°C, respectively which were also different from their precursors. These differences also indicated the formation of new complexes as OV and OM (Figure 2).

Table 1. R_f values of olmesartan medoxomil antidiabetic drugs and their complexes on TLC using methanol-chloroform (40:60) as mobile phase.

Item	R_f value
Olmesartan medoxomil (O)	0.8
Dapagliflozin (D)	0.6
Vildagliptin (V)	0.9
Metformin HCl (M)	0.3
OD complex	0.7
OV complex	0.5
OM complex	0.4

TGA thermograms were obtained from pure drugs and their complexes. The overlaid TGA thermograms are shown in figure 3.

The molecular fragmentation pattern of olmesartan medoxomil is shown in figure 4. In TGA for pure olmesartan medoxomil, 20.17% degradation was observed at 243.53 °C which corresponds to first step release of medoxomil group form the drug (i.e. m/e 558.22 to m/e 444.23) (Figure 3).The TGA thermogram was almost similar to molecular fragmentation of olmesartan medoxomil which indicates the single entity of the drug.[15]

The degradation pattern of pure dapagliflozin in TGA was found to be 21.57% and 38.59% at 279.53 °C and 327.85°C, respectively (Figure 3) which supported the breakdown pattern published previously[16] (Figure 5).

The degradation pattern of pure vildagliptin in TGA was also found as 8.18% and 16.41% at 164.83 °C and 243.53°C, respectively (Figure 6) which supported the breakdown pattern published by Kumar *et al.*[17]

Like other above mentioned drugs, pure metformin HCl also showed 20.08% degradation at 251.25 °C for the removal of HCl molecule from the entity, and 51.96% degradation at 300.47 °C for the removal of $-NH_2$-NH group (Figures 3 and 7).[18]

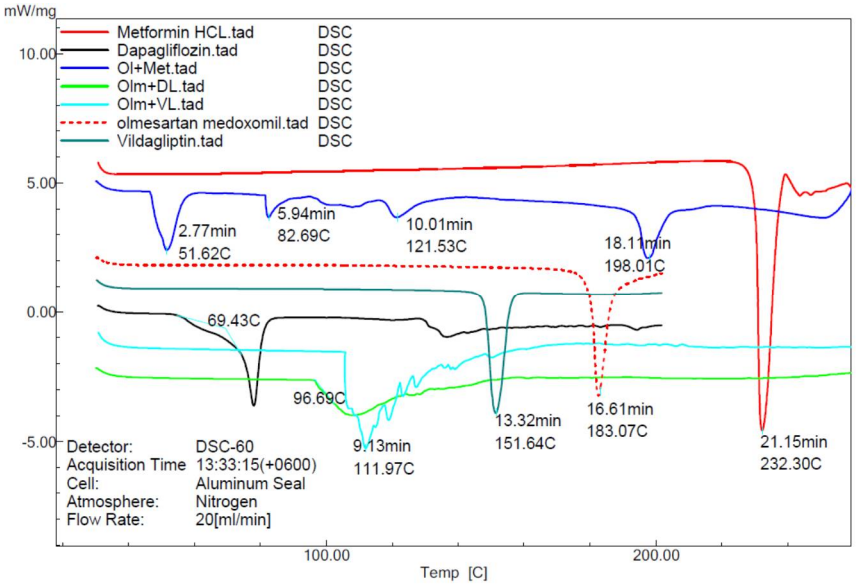


Figure 2. Overlaid DSC thermograms of olmesartan medoxomil, dapagliflozin, vildagliptin, metformin and their complexes.

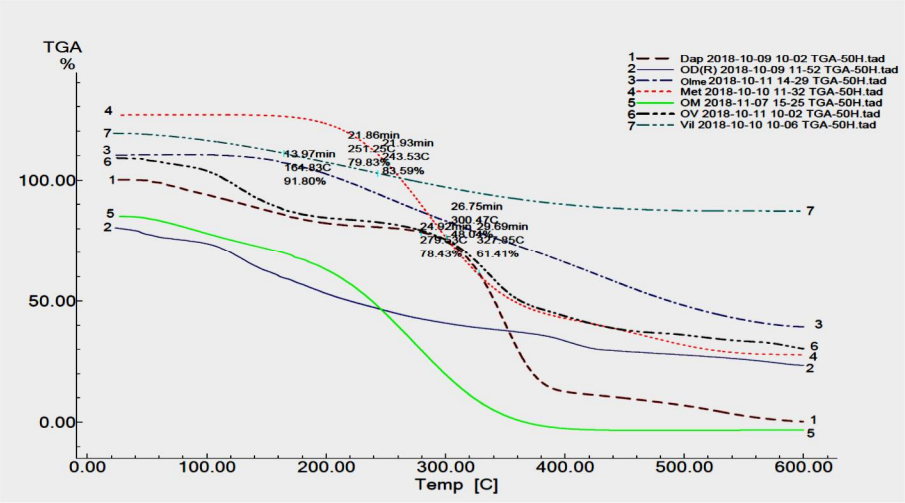


Figure 3.Overlaid TGA of pure drugs and their complexes.

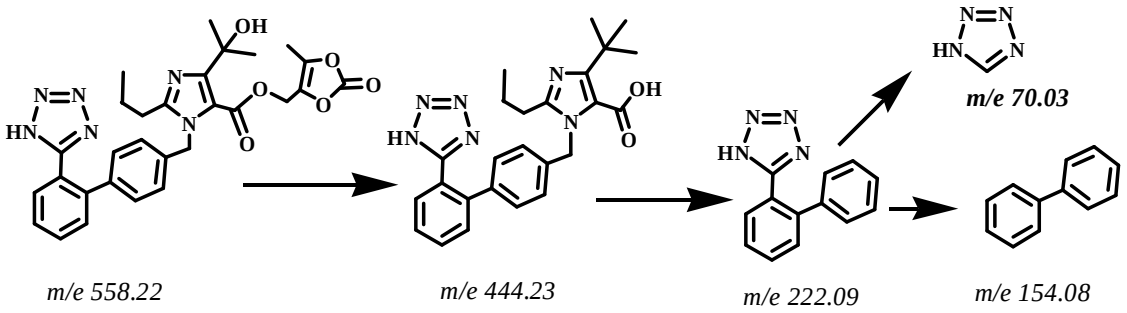


Figure 4. Degradation pattern of olmesartan medoxomil.

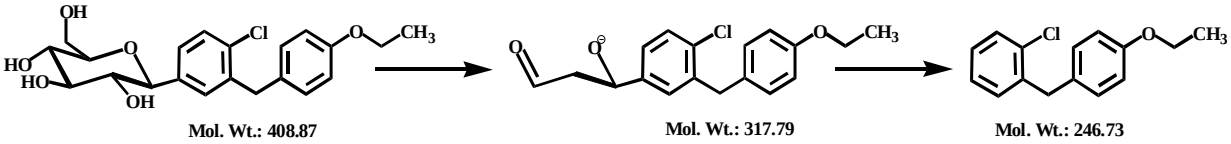


Figure 5. Degradation pattern of dapagliflozine.

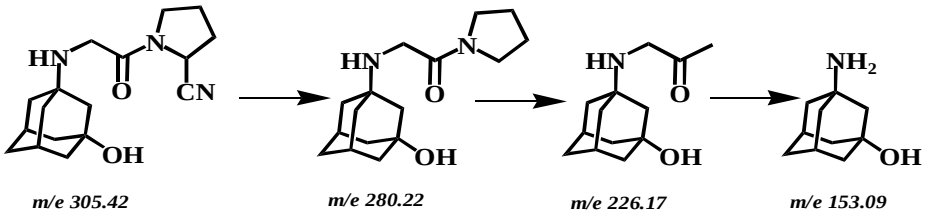


Figure 6. Degradation pattern of vildagliptin.

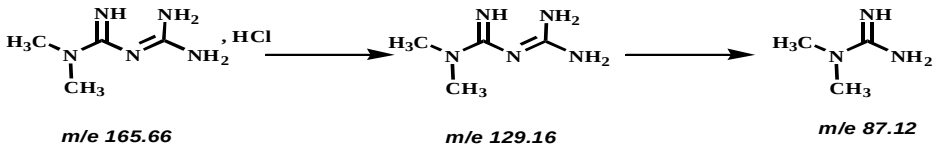


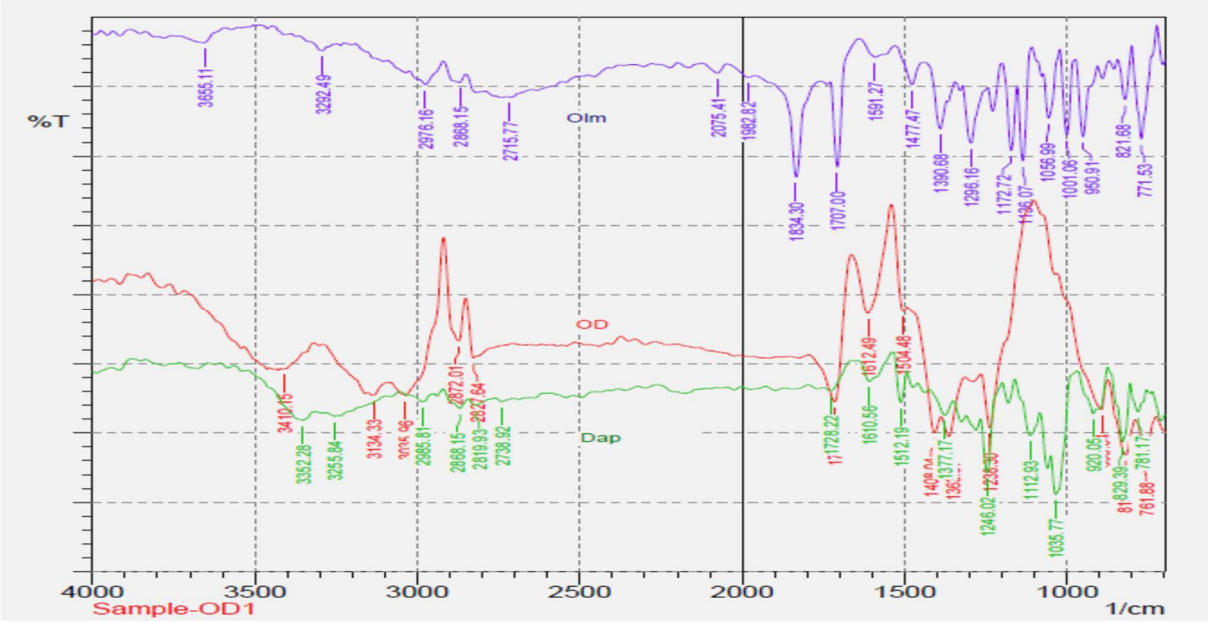
Figure 7. Degradation pattern of metformin HCl.

The drug-drug complexes that were supposed to be formed, however, showed completely different degradation patterns. For OD, the thermogravimetric degradations were found to be 50.10% at 423.92 °C and 56.97% at 598.22 °C which are obviously different from parent pure drugs olmesartan medoxomil and dapagliflozin. These different patterns of degradation indicated the formation of different compound of OD (Figure 3).

For OM complex, 25.31% degraded at 211.31 °C and 81.32% at 592.70 °C. These degradation patterns are also quite different from the parent drugs olmesartanmedoxomil and metformin (Figure 3). These differentiations allowed us to assume the formation of a good complex between olmesartanmedoxomil and metformin.

Like OD and OM, the other complex OV demonstrated degradations at 23.46 % at 183.59 °C, 33.84% at 300.86 ^{o}C and 78.74% at 599 ^{o}C. This pattern suggested a complete different nature of OV from the parent drug olmesartan medoxomil and vildagliptin (Figure 3).

FT-IR is useful in providing information about the presence or absence of specific functional groups. If two pure samples display the same IR spectra, it can be concluded that they are the same compounds. Similarly, any shifts or disappearance of peaks indicate presence of a different compound. The IR spectrum obtained after drug-drug interaction was seen to demonstrate a new pattern of peaks compared to pure drug powder. The characteristic peaks of OH



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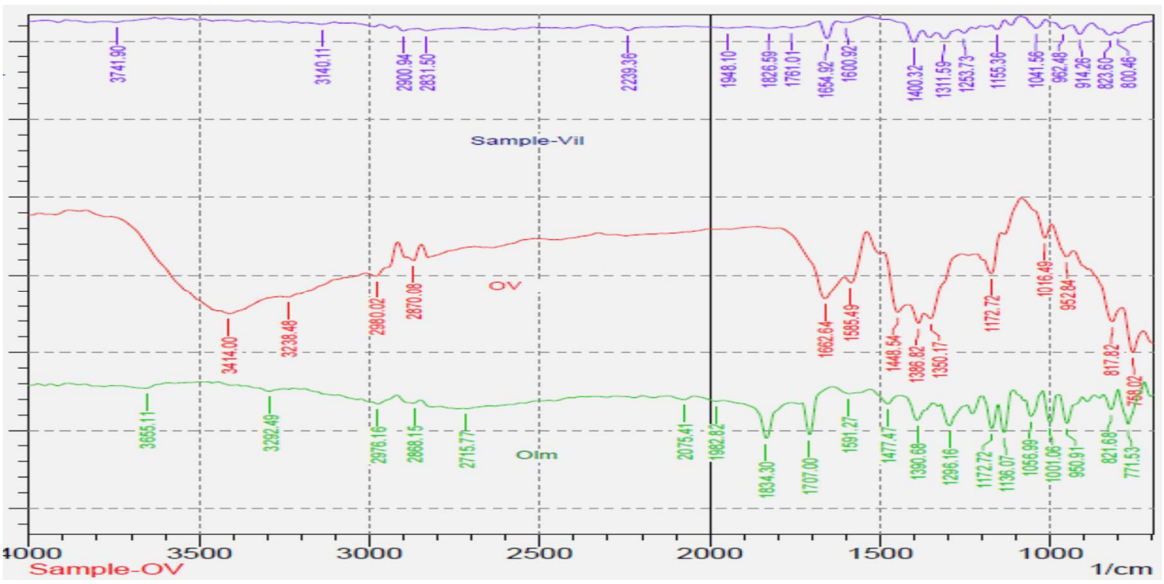

Figure 8. Overlaid FT-IR of olmesartan medoxomil and its complexes: (A) olmesartan medoxomil-dapagliflozin complex, OD, (B) olmesartan medoxomil-vildagliptin complex, OV, (C) olmesartan medoxomil-metformin HCl complex, OM.

stretching of olmesartan medoxomil, dapagliflozin, vildagliptin observed at 3655.11 cm^{-1} and 3352.28 cm^{-1}, 3741 cm^{-1}, respectively which were found to be abolished in the IR spectra of OD and OV, and a single peak was seen at 3410.15 cm^{-1} for OD and 3414.00 cm^{-1} for OV. Likewise, the characteristic stretching peak of -NH_2 of metformin seen at 3742.93 cm^{-1} which was obtained in the downfield at 3232.75 cm^{-1} for OM (Figure 8). These changes the absorption characteristics in the IR spectra indicated the complexation between the drugs.

POSTULATED COMPLEXATION

The postulations for the reactions are shown as follows. In suitable conditions, olmesartan medoxomil and dapagliflozin reacted with each other to obtain new complexes (Figure 9).[19]

According to Neeraj*et al.*[17]olmesartan medoxomil and vildagliptin interacted with one another by releasing water molecule in presence of acid to form new complexes as shown in figure 10.

During interaction of olmesartan medoxomil and metformin in presence of acidic conditions one molecule of water was released to form a complex which was supported by TGA. The postulated reaction is shown in figure 11.

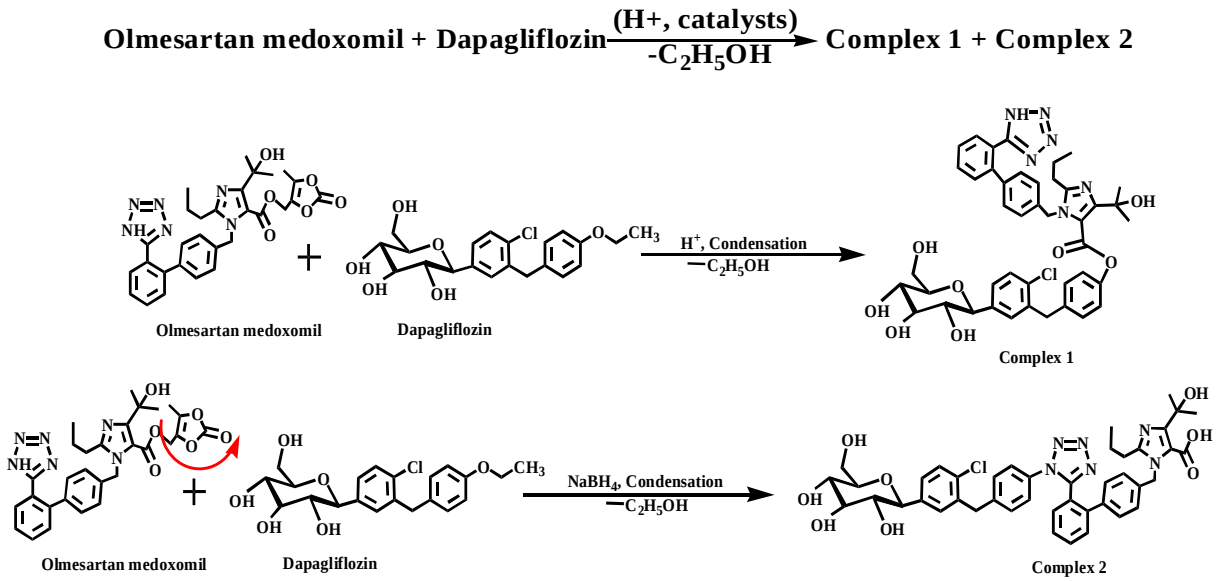

Figure 9. Postulated complexation patterns between olmesartan medoxomil and dapagliflozin.

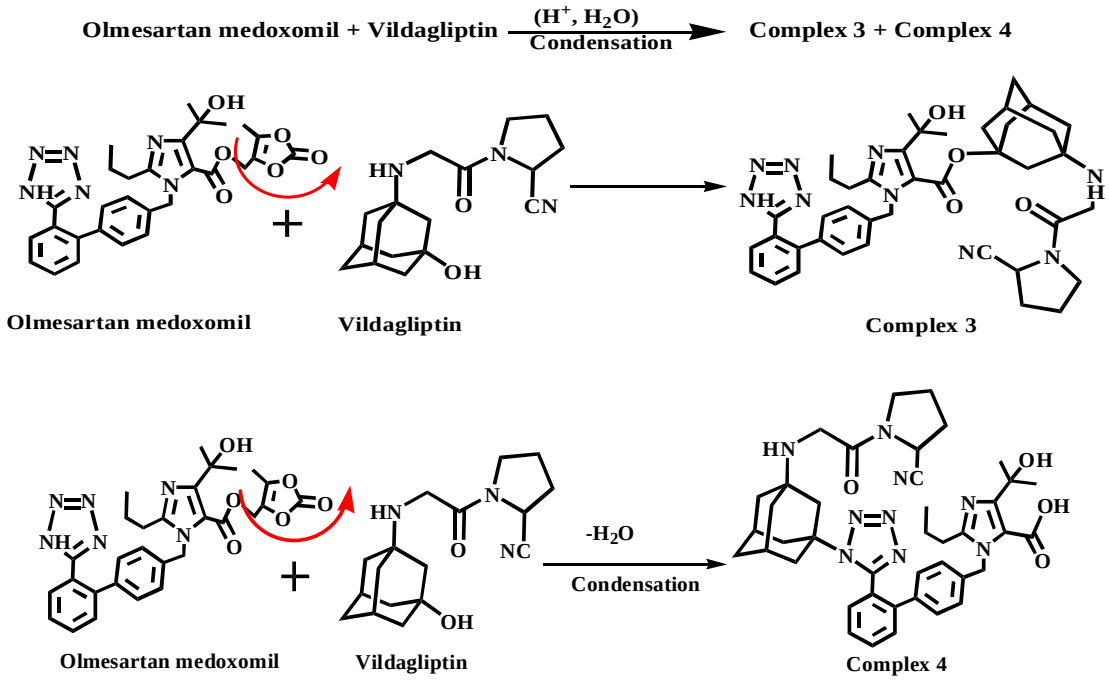

Figure 10. Postulated complexation patterns between olmesartan medoxomil and vildagliptin.

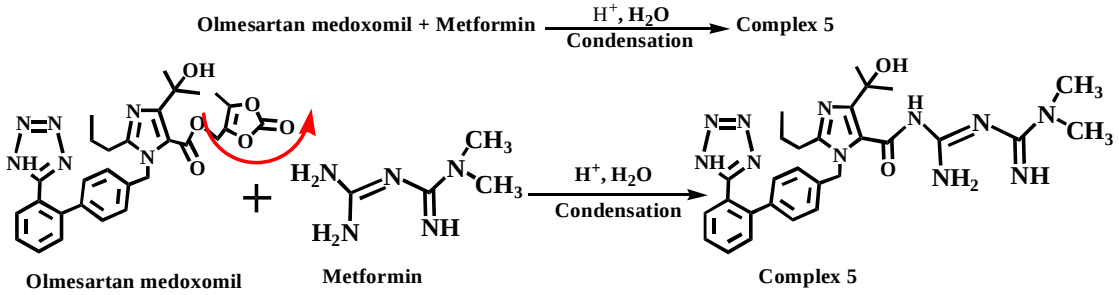

Figure 11. Postulated complexation patterns between olmesartan medoxomil and metformin.

CONCLUSION

Antihypertensive drug olmesartan medoxomil was allowed to react with three antidiabetic drugs namely dapagliflozin, vildagliptin and metformin in acidic condition. It was found that olmesartan medoxomil reacted with these antidiabetic drugs and produced complexes which were justified by studying their thermochemical properties e.g. DSC, TGA and various chromatographic techniques like TLC and HPLC as well as by IR spectrophotometry. From this study, it is obvious that concomitant use of these antidiabetic drugs with antihypertensive drug (olmesartan medoxomil) has a strong probability to produce complexes in the stomach. However, their impacts on the biological activities and/or toxicity profile could not be established at this moment. Further comprehensive studies, including those in *in vivo* model will be required to establish the beneficial and/or untoward effects during concomitant uses of these tested drug molecules.

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