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In Vitro Drug Interactions Study of Lisinopril with Metformin

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Abstract : *This study investigates the in vitro interactions of lisinopril with metformin which is important for the possible formulation of polypills comprising both drugs. The study was conducted using the USP apparatus II paddle method. The media used were buffer pH 1.2, 4.5, and 6.8 to simulate gastrointestinal tract pH. Lisinopril tablets (10 mg) were run in the presence of 500 mg metformin tablets and the degree of interaction was established after analyzing the dissolution samples with validated High Performance Liquid Chromatographic methods.*

The results indicated a statistically significant difference in the effect of lisinopril on the dissolution profile of metformin at pH 1.2, 4.5, and 6.8, with a decrease in the percentage of metformin released ($p = 0.0052$; $p = 0.0037$; and $p = 0.0155$, respectively). The effect of metformin on the dissolution profile of lisinopril was only statistically significant at pH 1.2 and 6.8 with an increase in the percentage of lisinopril released ($p = 0.0062$; $p = 0.0036$, respectively), and no statistically significant difference at pH 4.5 ($p = 0.7174$).

Thus, the co-administration of metformin with lisinopril could alter the bioavailability of both drugs. More studies may be conducted in vivo to further ascertain the level of interactions using animal or human model.

Keywords – *Dissolution, Interactions, Lisinopril, Metformin, Polypills*

I. Introduction

Drugs have a great potential for interactions with other drugs, foods, herbs and diseases. The potential for drug-drug interactions (DDI) exists whenever two or more drugs are co-administered. Drug-drug interactions may be beneficial or harmful. Harmful drug-drug interactions are important as they cause 10-20% of the adverse drug reactions (ADR) requiring hospitalization and they can be avoided. Elderly patients are especially vulnerable – with a strong relationship between increasing age, the number of drugs prescribed and the frequency of potential for drug-drug interactions [1].

Although, the medical literature is awash with case reports of adverse drug interactions, only a few are clinically significant. Thus, it is important to anticipate when a potential drug interaction might have clinically significant consequences for the patient so that advice may be given to minimize the risk of harm. This may be achieved by avoiding the combination, making dosage adjustments, spacing dosing times, close monitoring of patient [2].

In co-morbid disease states, the use of multiple drugs is almost inevitable and prescribers resort to the use of a single drug for dual indications, whenever possible, to minimize polypharmacy. Patients also have many concerns when multiple medications are initiated, including prescribing errors, the cost of medications, and possible adverse effects. These worries are not unfounded given that several drugs have been withdrawn from the market in the past several years because of adverse effects from drug interactions [3], [4].

The potential advantages of the polypill are numerous. There will be improved delivery of care by increasing the ease of prescribing, and avoiding multiple steps for dose titration of each drug. There will be improved adherence as individuals would need to take only one pill as against several pills per day [5]. There will be reduced cost as costs of a polypill using generic components are likely to be much lower than the costs of individual drugs. Physicians will spend less time prescribing and monitoring multiple drugs and allocate more time and resources to lifestyle counseling [6].

Polypills have a potential usefulness in the prevention of CVD and the lowering of its incidence [7], [8], [9]. They may minimize side effects while maintaining efficacy [10]. However, some of these assumptions are refuted by recent evidence [6], [7], [11], [12].

The polypills are not without demerits and uncertainties. There is a lack of evidence that a polypill will reduce CVD events and is safe in middle-aged individuals in primary prevention. Pharmaceutical formulation issues may arise since polypills may involve four or five active components. Thus, it will be important to document the bioavailability, pharmacokinetics, possible interactions, and effects on risk factors, for each formulation [6].

There is an increase in the global prevalence of hypertension and diabetes mellitus and a corresponding rise in the co-morbidity [13], [14], [15]. This raises the chances of co-administration of anti-hypertensive and

anti-diabetic therapy. Metformin is the favoured oral anti-hyperglycaemic agent [16], [17] and lisinopril is a first-line drug for the management of hypertension [18]. Besides their major indications, metformin and lisinopril have cardioprotective and renoprotective properties, respectively [16], [17], [19]. Thus, these two drugs are frequently prescribed together. In some cases, other drugs are prescribed alongside which may necessitate the development of polypills. The development of polypills will minimize medication non-adherence. Extensive literature search shows no available data on possible pharmacokinetic interactions between metformin and lisinopril when co-administered.

In vitro methodologies have been developed to predict drug interaction potential *in vivo* and have become a critical first step in the assessment of drug interactions. Well-executed *in vitro* studies can be used as a screening tool for the need for further *in vivo* assessment and can provide the basis for the design of subsequent *in vivo* drug interaction studies [20].

The objective of this study is therefore to investigate the possible interaction of lisinopril with metformin using an *in vitro* dissolution method.

II. Materials And Methods

2.1 Materials

2.1.1 Instrumentation

Analytical weighing balance (Scout™Pro, Ohaus®, 62 g, 200 g), friabilator test machine (Erweka TA®), hardness tester (Monsanto®), tablet dissolution test apparatus (USP Standards®), HPLC (Agilent® Technologies 1120 Compact LC), eclipse SB – C18 4.6 × 150 mm, 5 µm column (Agilent®), ODS Hypersil RP C18 (125 × 4.6) mm, 5 µm column (Agilent®), T70 UV/VIS Spectrometer (PG Instruments Ltd®), cuvette T70 (PG Instruments Ltd®), pH Meter (pH-012®).

2.1.2 Chemicals and Reagents

Potassium dihydrogen orthophosphate (JT Baker®, USA), distilled water, ammonium acetate (Lab Tech Chemicals®), sodium hydroxide pellets (JT Baker®, USA), glacial acetic acid (Sigma-Aldrich®, Germany), concentrated hydrochloric acid (Sigma-Aldrich®, Germany), methanol (gradient grade for liquid chromatography; Merck KGaA®, Germany), acetonitrile (gradient grade for liquid chromatography; Merck KGaA®, Germany), hexane-1-sulphonic acid sodium salt (ROMIL-SpR®, GB), orthophosphoric acid, 85% (Merck®, Germany).

2.1.3 Test Drugs

Metformin hydrochloride, USP (reference standard), lisinopril, USP (reference standard), diclofenac, USP (reference standard), metformin hydrochloride 500 mg (Glucophage® 500mg) tablets, lisinopril 10 mg (Zestril® 10 mg) tablets. All these drugs had an expiry of not less than one year at the time of study.

2.2 Methods

2.2.1 Physicochemical Properties

The uniformity of weight and friability tests were conducted according to the specifications of the British Pharmacopoeia [21]. Although, hardness is a non-pharmacopoeia test, it was also carried out as it shows the ability of tablets to withstand pressure or stress during handling, packaging, and transportation.

2.2.2 Assay of the Tablets

The assay was done according to the specifications of the British Pharmacopoeia [21].

2.2.3 Buffer Preparations

Buffers of pH 1.2 (0.1 N Hydrochloric Acid) and 4.5 (Acetate Buffer) were prepared according to the standard procedures given in the British Pharmacopoeia [21]. Buffer of pH 6.8 (Phosphate Buffer) was prepared according to the standard procedure given in the United States Pharmacopoeia [22].

2.2.4 Dissolution Studies

This was determined using a 6-compartment dissolution test apparatus (paddle method) containing 900 mL of the dissolution medium, maintained at 37˚C ± 0.5˚C with the paddles set to rotate at 50 rpm. A tablet of lisinopril was put in each of the compartments and the machine operated for 1 hour. Samples were collected at time intervals of 5, 10, 15, 30, 45, and 60 minutes. In all the experiments, aliquots of 5 mL were withdrawn at the specified intervals and replaced with fresh 5 mL dissolution medium to maintain sink conditions. Each withdrawn sample was filtered through a 0.45 µm syringe filter. The filtrate was analyzed with the High Performance Liquid Chromatography (HPLC) coupled with ultraviolet detector. The filtrate (1 mL) was

transferred into the vial. 100 µL of the diclofenac stock solution (100 µg/mL) was added as internal standard to each of the samples prior to the HPLC analysis.

The experiment was conducted using dissolution medium at pH 1.2, 4.5, and 6.8. Twelve (12) units of tablets were used per buffer. The study was done in two phases. The first phase involved the dissolution of lisinopril (10mg) and metformin (500mg) tablets alone in the three media while in the second phase, lisinopril (10mg) and metformin (500mg) tablets were added respectively for the interaction studies.

2.2.5 Chromatographic Conditions

The Agilent® Technologies 1120 Compact LC High Performance Liquid Chromatography coupled with Ultraviolet detector was utilized. This comprised a binary pump, a degasser, auto-sampler column oven, and variable wavelength detector.

For lisinopril, the chromatographic analysis was performed at ambient temperature with isocratic elution. The mobile phase was prepared by adding 200 volumes of acetonitrile to 800 volumes of a 0.408% w/v solution of anhydrous potassium dihydrogen orthophosphate adjusted to pH 2.0 with orthophosphoric acid (85%) containing 0.125% of sodium hexanesulphonate. The mobile phase was filtered using 0.45µm syringe filter. The pump was set at a flow rate of 1.0 mL/min. A sample volume of 20 µL was programmed for injection by the auto-sampler onto the HPLC and sample elution was monitored at 215 nm. The actual concentration of lisinopril in the sample was determined based on the calibration curve linear regression equation obtained from gradient calibration concentrations of (2 – 20) µg/mL versus peak area.

To monitor metformin, the chromatographic analysis was performed at ambient temperature with isocratic elution. The mobile phase consisted of acetonitrile: methanol: 25mM potassium dihydrogen orthophosphate (13: 7: 80 v/v) with pH adjusted to 5.4. The mobile phase was filtered using 0.45µm syringe filter. The pump was set at a flow rate of 0.8 mL/min. A sample volume of 20 µL was also programmed for injection into the HPLC. The column used was ODS Hypersil RP C18 (125 × 4.6) mm, 5µm and sample elution was monitored at 215 nm. The actual concentration of metformin in the sample was determined based on the calibration curve linear regression equation obtained from gradient calibration concentrations of (50 – 350) µg/mL versus peak area.

2.2.6 Statistical Analysis

The graphs were plotted using the Microsoft Office Excel Software Package while the statistical analysis was done with the GraphPad Prism Software Package using student t-test (paired t-test) at 95% confidence intervals. In all cases, the result becomes significant if p is less than or equal to 0.05 value.

III. Results

Table 1: Physicochemical Parameters of Lisinopril and Metformin Tablets

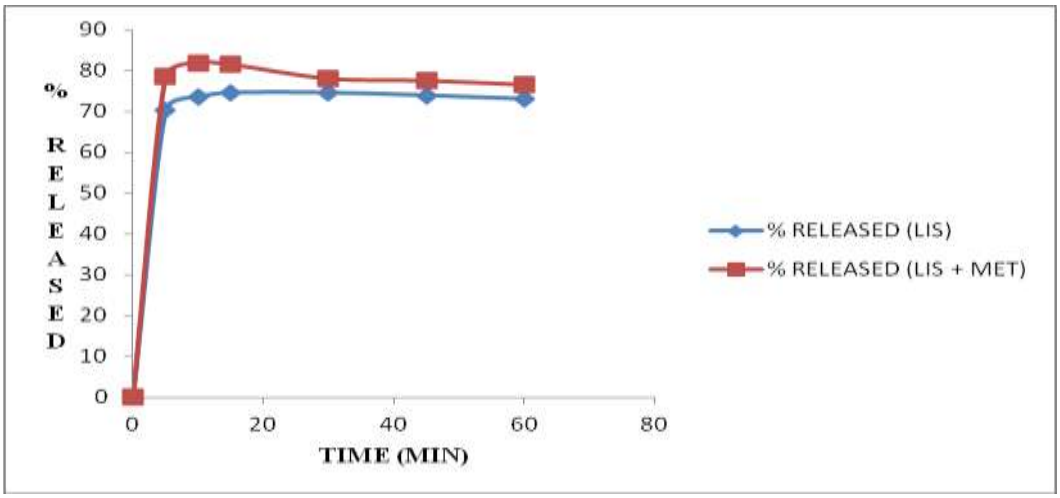
	UNIFORMITY OF WEIGHT (g) (± SD)	**FRIABILITY (%) (± SD)**	**HARDNESS (g) (± SD)**
LISINOPRIL, 10 mg (ZESTRIL®)	0.2138 ± 0.0038	0.0467	3.56 ± 0.2675
METFORMIN, 500 mg (GLUCOPHAGE®)	0.5364 ± 0.0057	0.0374	2.75 ± 0.4859

Table 2: Summary of Dissolution Data of Lisinopril and Metformin Tablets Alone and in Combination with Metformin and Lisinopril Tablets

SAMPLE	**TIME (MIN)**					
	5	**10**	**15**	**30**	**45**	**60**
pH 1.2						
LIS	70.21 ± 4.54	73.47 ± 4.89	74.54 ± 1.49	74.58 ± 1.43	73.9 ± 1.38	73.05 ± 1.44
LIS + MET	78.43 ± 3.57	81.82 ± 0.65	81.42 ± 1.12	77.94 ± 4.92	77.44 ± 2.36	76.45 ± 2.74
MET	50.12 ± 9.41	41.96 ± 4.89	83.34 ± 3.78	107.93 ± 4.29	126 ± 9.49	145.11 ± 3.21
MET + LIS	13.14 ± 1.04	23.79 ± 1.17	35.67 ± 6.43	48.91 ± 9.38	62.54 ± 2.56	69.92 ± 2.60
pH 4.5						

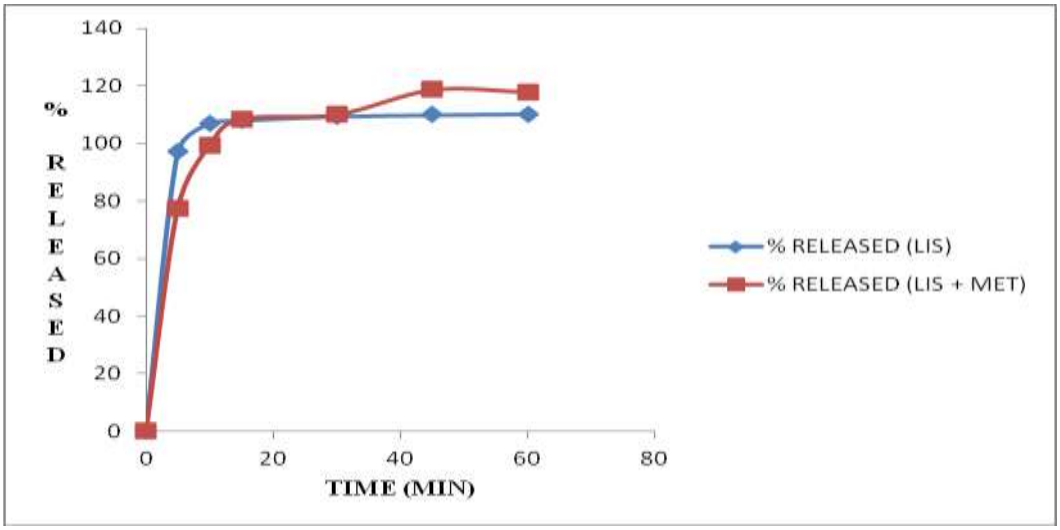
LIS	97.08 ± 4.12	107.02 ± 2.75	108.2 ± 2.65	109.43 ± 2.37	109.97 ± 2.42	110.28 ± 1.91
LIS + MET	77.52 ± 5.52	99.35 ± 3.89	108.54 ± 1.74	110.09 ± 7.12	118.65 ± 0.84	117.93 ± 0.71
MET	47.3 ± 10.97	71.39 ± 7.96	85.19 ± 8.68	103.39 ± 4.31	111.3 ± 10.24	122.78 ± 7.87
MET + LIS	40.46 ± 10.77	49.71 ± 5.77	61.58 ± 5.99	85.92 ± 6.39	94.13 ± 5.20	99.00 ± 1.65
pH 6.8						
LIS	77.69 ± 5.97	83.43 ± 5.81	85.81 ± 5.58	89.39 ± 4.33	83.26 ± 3.96	86.2 ± 4.07
LIS + MET	89.02 ± 4.11	98.83 ± 4.82	95 ± 3.78	94.85 ± 4.04	94.01 ± 2.71	93.78 ± 1.25
MET	24.04 ± 1.75	41.51 ± 4.42	57.01 ± 4.53	79.68 ± 7.16	100.07 ± 3.46	103.88 ± 1.03
MET + LIS	22.72 ± 2.16	36.86 ± 2.56	45.56 ± 4.33	71.62 ± 4.75	87.08 ± 3.31	99.05 ± 5.98

LIS – Lisinopril; MET – Metformin



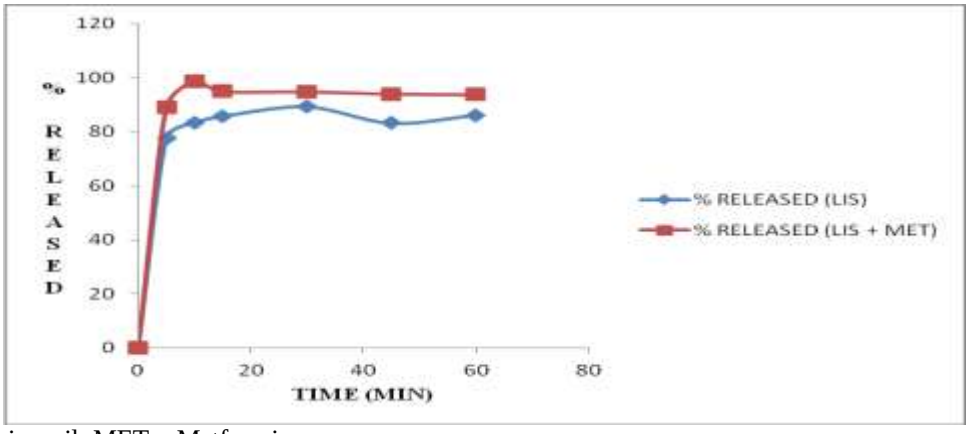
LIS – Lisinopril; MET – Metformin

Figure 1a: Percentage of lisinopril released in the presence of metformin at pH 1.2



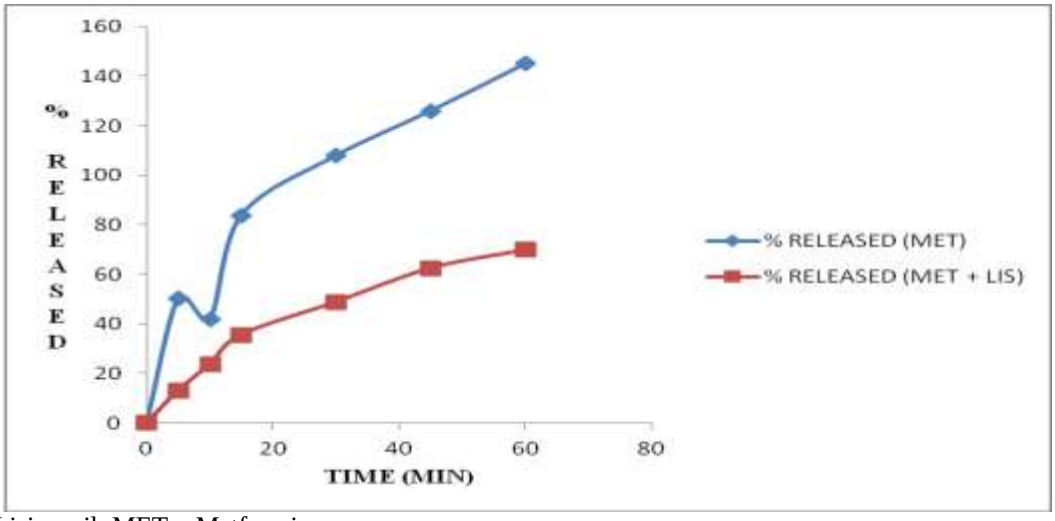
LIS – Lisinopril; MET – Metformin

Figure 1b: Percentage of lisinopril released in the presence of metformin at pH 4.5



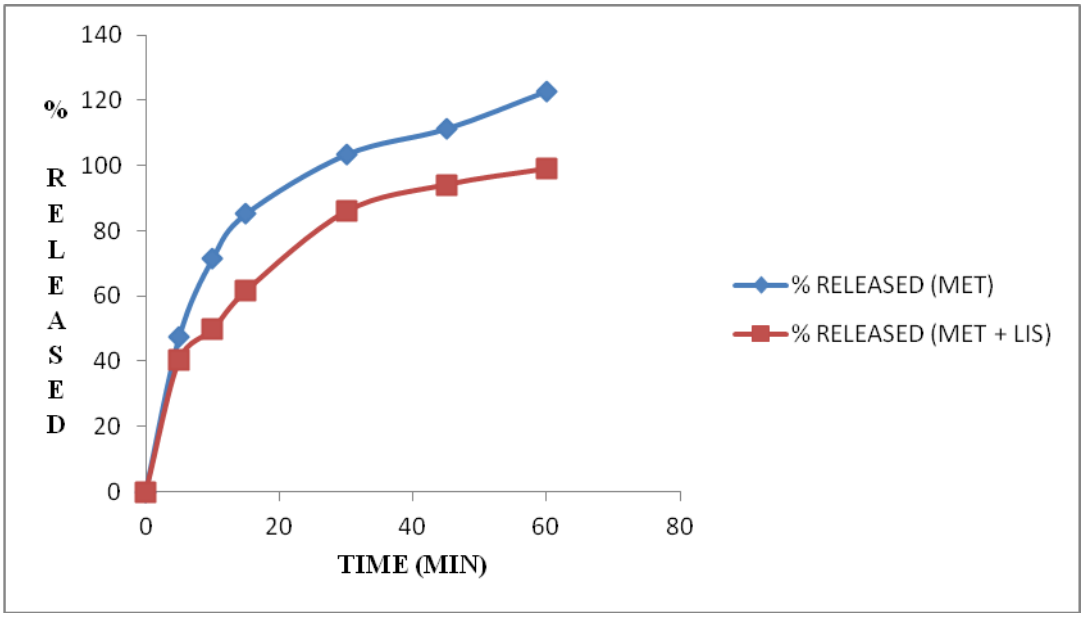
LIS – Lisinopril; MET – Metformin

Figure 1c: Percentage of lisinopril released in the presence of metformin at pH 6.8



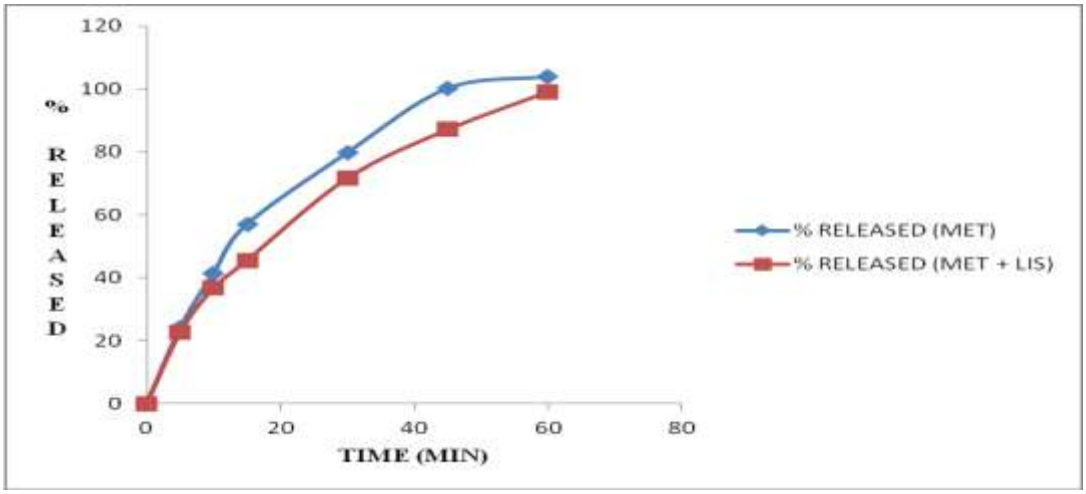
LIS – Lisinopril; MET – Metformin

Figure 2a: Percentage of metformin released in the presence of lisinopril at pH 1.2



LIS – Lisinopril; MET – Metformin

Figure 2b: Percentage of metformin released in the presence of lisinopril at pH 4.5



LIS – Lisinopril; MET – Metformin

Figure 2c: Percentage of metformin released in the presence of lisinopril at pH 6.8

IV. Discussion

TABLE 1 indicates the physicochemical parameters of lisinopril and metformin tablets used for the study while TABLE 2 shows the dissolution data of lisinopril and metformin tablets alone and in combination with metformin and lisinopril tablets respectively.

Fig. 1a-c represent the dissolution profiles of lisinopril in the presence of metformin at pH 1.2, 4.5, and 6.8 while Fig. 2a-c show the dissolution profiles of metformin in the presence of lisinopril at pH 1.2, 4.5, and 6.8.

Both the lisinopril and metformin tablets met the BP 2007 specifications for uniformity of weight, friability, and assay by conforming to compendia tolerance tests.

For the dissolution tests, the lisinopril tablets failed to meet the specification at pH 1.2 with percentage released at 30 minutes as 74.58 ± 1.43. The metformin tablets, however, met the BP specifications at all pH ranges.

The formulations to be used for the study must first meet some basic physical and chemical tests which ensure the readiness of the tablets for the dissolution study. The two formulations used for the study passed compendia tolerance tests for uniformity of weight, friability, and assay. The pH 1.2, 4.5, and 6.8, were selected purposely to simulate the stomach, passage between the stomach, and the small intestine respectively [23]. The study revealed that metformin increased the dissolution profile of lisinopril at all pH ranges. At 95% confidence interval, there was a statistically significant difference ($p < 0.05$) in the effect of metformin on the dissolution profile of lisinopril at pH 1.2 ($p = 0.0062$) and pH 6.8 ($p = 0.0036$). However, there was no statistically significant difference at pH 4.5 ($p = 0.7174$).

It is a general belief that only substances in the molecularly disposed form (that is in solution) are transported across the intestinal wall and absorbed into the systemic circulation. An increase in the dissolution profile of lisinopril could signify an increase in the blood pressure lowering effects of the drug and *vice versa*. Our results showed that the co-administration of lisinopril with metformin may alter the bioavailability of lisinopril causing an increase in the effects of lisinopril, especially in an acidic environment (stomach) or in the small intestine. If these drugs must be used together, then the patient should be closely monitored and the BP regularly checked to avoid hypotension. It may be necessary to make dosage adjustments or space dosing times. These should be considered in the formulation of polypills comprising both drugs.

It was also discovered that lisinopril decreased the dissolution profile of metformin at all pH ranges. At 95% confidence interval, there was a statistically significant difference ($p < 0.05$) in the effect of lisinopril on the dissolution profile of metformin at pH 1.2 ($p = 0.0052$), pH 4.5 ($p = 0.0037$), and pH 6.8 ($p = 0.0155$).

An increase in the dissolution profile of metformin signifies an increase in the blood glucose lowering effects of metformin, and *vice versa*. Hence, co-administration of metformin with lisinopril could alter the bioavailability of metformin causing a decrease in the therapeutic effects of metformin. This may have serious implications as the clinical efficacy of metformin may be impaired resulting in poorly controlled blood glucose levels and possible hyperglycaemia.

If these drugs must be used together, then the patient should be closely monitored and the blood glucose levels regularly checked to avoid hyperglycaemia. It may be necessary to make dosage adjustments or space dosing times. These should be considered in the formulation of polypills comprising both drugs.

Although, this study did not attempt to investigate the *in vivo* interactions between lisinopril and metformin, this may be considered in healthy volunteers before definite conclusions can be made. It may also be necessary to consider the possible effects of different strengths of both drugs on interaction studies.

V. Conclusion

On the basis of the *in vitro* interaction results, it is clearly indicated that there was an interaction between lisinopril and metformin and this was found to be statistically significant.

It is strongly recommended that if both drugs are co-administered, then the patient should have the blood glucose levels and blood pressure readings closely monitored. It may also be necessary to make dosage adjustments or space dosing times. In the formulation of polypills, the possible interactions between lisinopril and metformin should be considered.

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