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Development and Validation of a RP-HPLC Method For the Interaction Studies of Metformin and Clindamycin in Human Plasma

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ABSTRACT

Background: Metformin is mostly prescribed with clindamycin in the treatment of diabetic disease with bone, joint and lower respiratory infections. The interaction of the two drugs is yet known. This study was aimed at developing and validating a simple and reproducible RP-HPLC method for the interaction Studies of Metformin and Clindamycin in Human- Plasma

Methods: The study was designed in two phases. Phase one, all volunteers received 1 g metformin; in phase two, it was co-administered with clindamycin (500 mg). In each phase blood samples were collected at intervals within 24 h post-drug administration. Plasma samples were acidified with HCl, deproteinized with acetonitrile and centrifuged, the supernatant was washed with dichloromethane and injected into the HPLC system.

Results: Chromatographic separation was achieved in 10 minutes with metformin and phenytoin having retention times of 2.230 and 4.407 minutes respectively. The method was precise (3.43% RSD), accurate (% Er of 2.24 and % recovery of 96.52%), with a linear calibration curve (r = 0.995.). LOD and LOQ of the developed method were 0.02 and 0.05 µg/mL respectively. All the parameters were within the acceptable limits.

Conclusion: From the result the developed and validated method was accurate and suitable for routine analysis of metformin in human plasma. It was also concluded that metformin may be co-administered with clindamycin to type 2 diabetic patients with caution to avoid the possible risk of toxicity or therapeutic failure.

Keywords: Clindamycin, Drug-drug interaction, Human plasma, RP-HPLC method.

1.0 INTRODUCTION

Metformin (1-dimethyl biguanide hydrochloride) is the first-line drug of choice for type 2 diabetes and the most commonly prescribed drug for this condition worldwide, either alone or in combination with insulin or other oral anti-diabetes [1]. Metformin inhibits hepatic glucose production, reduces intestinal glucose absorption, and improves glucose uptake and utilization. Besides lowering the blood glucose level, metformin may have additional health benefits, including weight reduction, lowering plasma lipid levels, and prevention of some vascular complications [2]. It is also used for other indications such as polycystic ovary syndrome (PCOS). Metformin is increasingly recognized as a potential anticancer agent due to a reduced cancer incidence in diabetic patients treated with the drug, and recently, patients taking metformin were associated with a reduced risk of COVID-19-related mortality [3]. Metformin is a highly ionized, water-soluble drug that is absorbed, distributed, and eliminated by transporters [4]. It undergoes active tubular secretion in the kidney and is excreted unchanged in the urine. Many high-performance chromatographic (HPLC) methods for the analysis of metformin in plasma were reported in the literature, most of the methods used were either ion pair reagent or cation exchange columns [5], while some requires elaborate sample preparation [6]. Also, other method of determination of metformin by gas chromatography and UV-visible spectrophotometer were reported respectively [7, 8, 9, 10]. Though
these methods are sensitive and reproducible, the reverse phase-HPLC (RP-HPLC) method for the estimation of metformin in human plasma was found to be more suitable [6, 11]. Previously described methods suffered from several limitations including cost and the use of complex extraction procedures which were tedious and time-consuming. Ultra-filtration and column-switching techniques have been suggested to improve specificity and selectivity [6]. The study is to develop and validate a simple, efficient, and economical method for the determination of metformin in human plasma.

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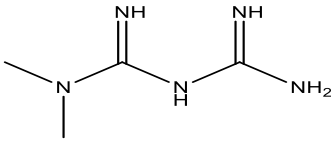

Figure 1: Molecular Structure of Metformin

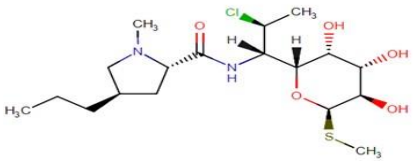

Figure 2: Molecular Structure of Clindamycin

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment and apparatus

Digital weighing balance (OHAUS model EP 64 BY Ohaus corporation, Switzerland), U.V. detector (T80 + U.V/Vis spectrometer by PG instrument Ltd U.K), Centrifuge: Heroes (labafuge300) D-37520 ostence mastance03, serial N0 40267581, BN: 75003230), HPLC column (Zobrax SB-Aq. (C18 4.6 X150 nm id 5nm particle size, Poroshell 120 EC-C18 column), FTIR machine (Agilent technologies Cary 630), HPLC machine (Agilent technologies Model 1260 Infinity Series), HPLC sample bottle (1.5 mL) Agilent technologies Germany.

2.1.2 Chemicals and reagents

Acetonitrile (HPLC grade), Methanol (≥ 99.9%), Methanol, (HPLC grade), Acetic acid, Hydrochloric acid, Dichloromethane (HPLC grade), Distilled water, Tetrahydrofuran (THF), (all solvents were of analar grade obtained from Sigma – Aldrich U.K); Dipotassium hydrogen phosphate (Buffer) from J.T Baker 99.5% USA), Sodium acetate, Metformin HCl (Reference Standard), Phenytoin (internal standard) –(all were obtained from Ranbaxy Pharmaceutical Ltd., Lagos).

2.2 Methods

2.2.1 Preparation of stock standards and working solutions

Stock solutions (1.0 mg/mL) of metformin (analyte) and phenytoin (internal standard) were prepared in HPLC-grade water. They were then diluted with blank human plasma and mobile phase, respectively to produce a working solution of 25µ/mL and, 150 µ/mL respectively. Six working calibration standards in the range of 0.05 – 5.0 µ/mL were prepared in human plasma and vortexed for 1 min, 0.5 aliquots were transferred into a glass tube and stored at -20^0 C before use.

2.2.2 Precision

The precision of the method was determined with the standard and the real sample. The intraday and interday variations for determination of metformin and phenytoin were carried out at standard concentration levels of 0.05, 2.50, and 5.00 μgmL^{-1} respectively. Method repeatability was achieved by repeating the same procedure six times on the same day for intraday precision. The intermediate (interday) precision of the method was checked by performing the same procedure on different days under the same experimental conditions.

2.2.3 Accuracy and recovery

The accuracy of this method was checked by standard addition method, where 80, 100 and 120 % of a pre-analysed 18 µg/mL solution of metformin containing internal standard (IS) and serum was added to the same (18 µg/mL solution) to obtain 32.4, 36 and 39.6 µg/mL solutions of metformin. The mixtures were centrifuged as described under preparation of the calibration curve before finally injecting into the HPLC machine. After obtaining the chromatograms, the metformin content was determined by Subtracting the peak area ratio of metformin/phenytoin (IS) of the pre-analysed unspiked solution (16 µg/mL) from that found in each of the spiked solutions (32.4, 36 and 39.6 µg/mL) and interpolating the final concentrations from the calibration curve. Accuracy was expressed as percentage relative error (% Er) and percentage recovery.

2.2.4 Limit of detection and limit of quantification (LOD and LOQ)

The limit of detection (LOD) was determined by studying the calibration curve using samples containing the drug in the range of LOD. The standard deviation of y intercepts of the regression lines was used as standard deviation. LOD is expressed as:

$$LOD = \frac{3.3Q}{4S}$$

While the limit of quantitation (LOQ) was determined using the expression:

$$LOQ = \frac{10Q}{4S}$$

Where Q in each case is the standard deviation of y-intercepts of the regression lines determined through LINEST function in Microsoft Office Excel 2016, and S is the slope of the calibration curve.

2.2.5 Calibration Curve of Metformin Standard Solution

The calibration curve of metformin was prepared using a blank plasma sample (3.0 mL) spiked with 1.0 ml of each of the different concentrations (0.05 - 5.0 µ/mL) and of the standard metformin and phenytoin in a separate plain collection tube and 1 ml of the thawed plasma was added to each tube and shaken and finally subjected to the extraction procedure earlier developed. The calibration curve was constructed using peak height ratio versus concentrations of metformin. The coefficient of Variation and correlation coefficient R2 (0.998) were computed with a statistical data package. The results showed a good response of the detector at the concentration used.

2.2.6 Preparation of Mobile Phase

2.2.6.1 Mobile phase A

1.36 g of sodium acetate was dissolved in 500 ml of HPLC water to form a 20 M solution. 90 ml triethylamine was added, the pH of the solution was adjusted to 7.2 with the addition of 1% acetic acid, and 1.5ml tetrahydrofuran (THF) was added to the mixture. The solution was filtered by vacuum and sonicated for degassing.

2.2.6.2 Mobile Phase B

1.36 g of sodium acetate trihydrate was dissolved in 100 ml of HPLC water and adjusted to PH 7.2 with 1% acetic acid. 200 ml of acetonitrile and methanol each were added. The mixture was filtered through a membrane filter and degassed assed before being used for HPLC analysis

2.2.9 Subjects and ethical clearance

The patients diagnosed with diabetes mellitus at the Medical Outpatient Department of Yusuf Dantsoho General Hospital, Tudun- Wada, Kaduna, Kaduna State, Nigeria were the subjects in this study. The diagnosis of diabetes mellitus was made by the presence of classic symptoms of hyperglycemia and a fasting plasma glucose concentration ≥ 130 mg/dL. The ethical clearance for the study was obtained by the proper representation and discussion of various ethical issues with the human ethics committee of Kaduna State Ministry of Health with the reference Number MOH/ADM/744/VOL.1/1160NHREC/17/03/2018 dated 7th MARCH, 2023. All volunteers gave their written informed consent, which was documented and archived.

2.2.10 Study design and blood sampling

The criteria for selecting the participants were based on the National Diabetes Data group's recommendation of 1989 and the selection was done by the practicing Clinicians. Twelve freshly diagnosed diabetic patients with ages ranging from 29.0 ± 4.9 years, weight of 60 ± 7 kg, and height of 162.8 ± 10.6 cm participated in the study. The protocol adopted was a one-way single-dose cross-over study in two periods. Each phase was preceded by an overnight fast. The subjects act as their control. The study was divided into two phases with a washout period of one week between the phases. In phase one, metformin (1 g) alone was administered to all the subjects after overnight fasting. In phase one subjects received a single dose of metformin (1 g) with 150 ml of water [12,13, 14], while in phase two, subjects received metformin co-administered with chloramphenicol (500 mg) in the same manner. Blood samples were collected at different time intervals of 0, 0.5, 1.5, 3.0, 4.0, 6.0, 8.0, 12.0, 16.0 and 24.0 h post-drug administration and stored in an EDTA vacutainer at – 4 ^{0}C before analysis. The concentration of metformin hydrochloride was estimated by injecting 2 µL of deproteinized supernatant liquid into the RP-HPLC on a Poroshell 120 EC-C18 4.6 mm X50mm 2.7 Microns column, mobile phase acetonitrile (A) / Methanol (B) (10:90) and a UV detector at 236 nm.

2.2.10.1 Sample preparation and extraction

Aliquots of 0.5 mL of the calibration curve and, volunteer samples in screw-caped glass were allowed to equilibrate to room temperature. To each tube 100µL of the IS working solution, 50 µl of 1M HCl, and 2 mL acetonitrile were added and vortexed for 15 sec. After the centrifugation, the supernatant was transferred into a cleaned tube containing 2 mL dichloromethane and vortexed for 15 sec. to wash the samples. The mixture was centrifuged for 5 minutes at 4000 rpm at room temperature and the supernatant was then injected into the HPLC machine for the analysis to get the chromatogram of metformin and phenytoin (I.S).

2.2.11 *Pharmacokinetic Parameters*

The pharmacokinetic parameters were determined for the two phases of the study. The highest plasma concentration observed and the corresponding time was defined as the C_{max} and T_{max} values, respectively. The elimination rate constant (Ke) was obtained by linear regression from the best-fit slope of the terminal log-linear decay in plasma concentrations versus time profile. The half-life ($t_{1/2}$) was obtained as 0.693/Ke. The area under the plasma concentration curve to the last quantifiable concentration (Ct) at time t (AUC0-t) was determined by linear trapezoidal integration. The AUC extrapolated to infinity (AUC0-∞) was calculated as AUC0-t + Ct/Ke Pharmacokinetic parameters such as maximum plasma concentration (C_{max}), Time to reach maximum plasma concentration (T_{max}), Total

body clearance (Cl), Volume of distribution (VD), Area under the curve from zero hours to last measurable concentration (AUC0-t), Area under the curve (from zero hours to infinity (AUC0-∞), Area under the Moment curve from zero were generated with the aid of the Software – Pharm PK software [15,16. 17].

2.3 Statistical analysis

Data were expressed as mean ± SEM. Graph Pad Prism Version 7.02 software Windows (San Diego California, USA) was used for data analysis using Wilcoxon (matched-pairs) signed rank test with $p<0.05$.

3. RESULTS

Table 1 represents the Optimized chromatographic conditions of the method while Table 2 represents the validation parameters of the developed method. Chromatograms obtained are presented in Figures 3, 4 and 5 with the retention time of 2.230 and 4.407 respectively. The calibration curve for the RP-HPLC method is shown in Figure 6. The results of the validation parameters for the RP-HPLC method and the Comparison of pharmacokinetics of metformin alone and when co-administered with clindamycin in healthy volunteers are shown in Table 3.

Table 1: Optimized Chromatographic Conditions

Mobile phase :	A	B
Ratio :	10	90
Column Type	Poroshell 120 EC-C18.	
Colum Dimension	(4.6mm x 50 mm 2.7 Microns)	
Wavelength :	236	
Temperature :	Ambient	
Flow rate :	0.8 ml/min	
Run time :	10 MIN	
Injection volume :	2 µl	
pH :	7.0	
Chromatogram :	Methanol	Phenytoin
Retention time (min) :	2.230	4.407
Signal Rate	0.1 min	

Table 2: Validation parameters of the developed method

Parameters	Values
Limit of detection (LOD)	0.02µ/ml
Limit of quantification	0.05
Accuracy(percentage recovery)	96.52%
Accuracy(% E_R)	2.24 %
Precision (% CV)	3.43 %
Specificity (percentage recovery)	96.52 %
Robustness (percentage deviation)	6.21 %

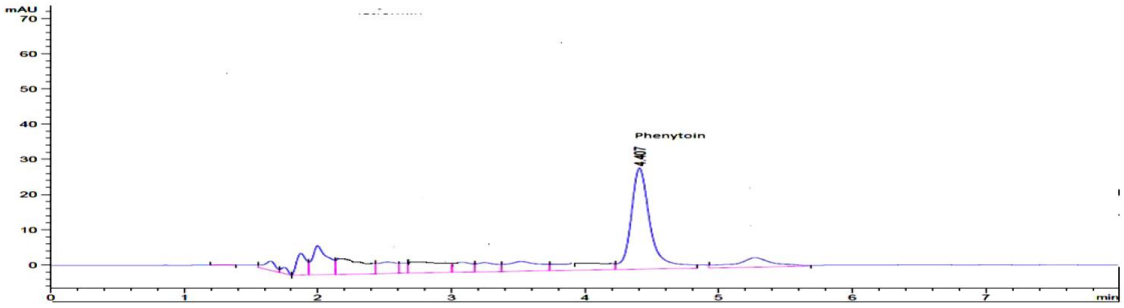

Figure 3: HPLC chromatogram of phenytoin alone

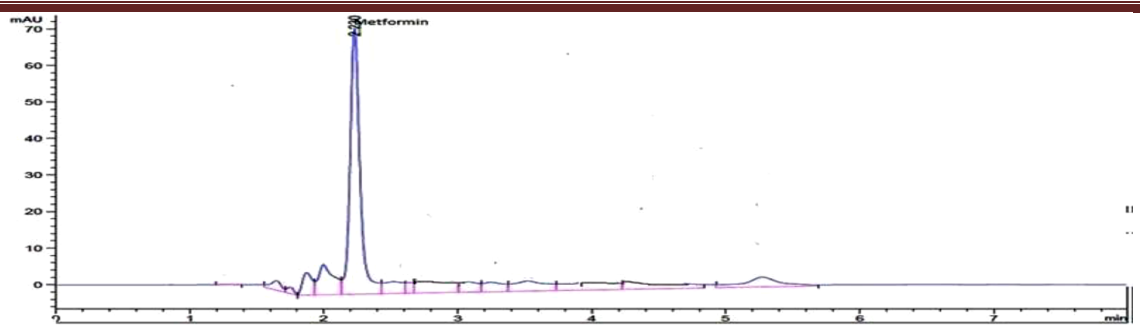

Figure 4: HPLC chromatogram of metformin alone

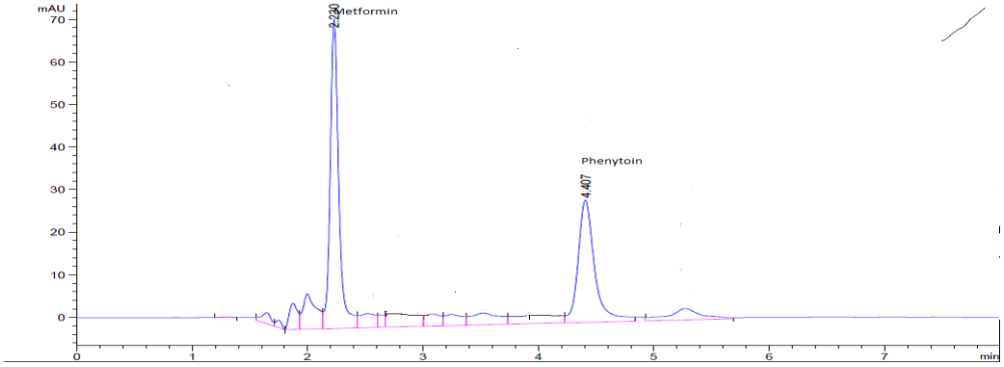

Figure 5: HPLC chromatogram of metformin and Phenytoin spiked with human plasma

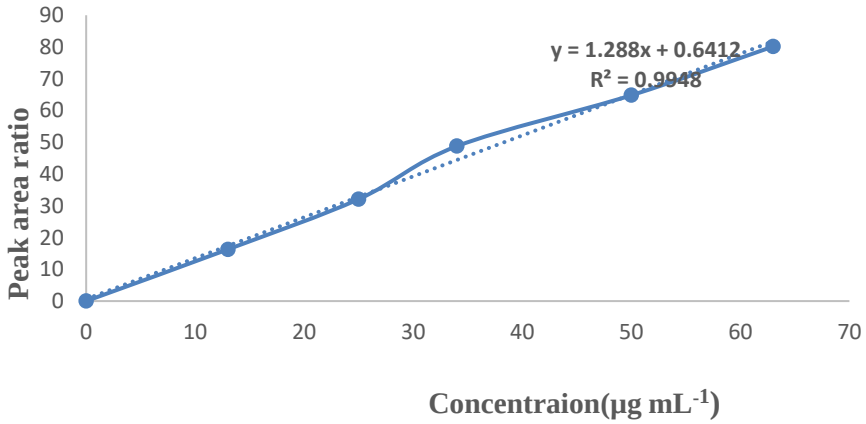

Figure 6: Calibration curve of developed RP-HPLC method for the quantitative analysis of metformin in plasma.

Table 3: Comparison of pharmacokinetics of metformin (mean, n = 6) alone and when co- administered with clindamycin in healthy volunteers (Mean ± S.D, N=6)

	Metformin alone	Metformin +clindamycin
Ke(h^{-1})	0.30 ±0.01	0.15±0.12
C_{max} (ng/ml)	1,880.25 ±0.45	1,282.35±0.4
T_{max}(min)	3.0 ±0.19	1.5±0.17
AUC_{0-8} (h ngmLh^{-1})	6,770±0.52	4,377±0.80
Vd (ml)	1,470.59±0.27	3,45.27±0.02
CL (mlh^{-1})	4,425.76±0.24	5,252.75 ±0.26
t 1/2β(h)	0.301±0.13	0.152±0.14
t1/2α(h)	2.30 ±0.52	4.54±0.32

*Significant difference ($p<0.05$)

4. DISCUSSION

Quality Control of metformin and clindamycin were carried out and the result was within an acceptable range. Figure 1 and 2 represents the molecular structure of metformin and clindamycin. Many Columns were used for trial and error for this method to get the best resolutions, Zorbax X DB-C_{18}, (4.6x150 nm,3.5nm), ZOBRAX SB-Aq. C_{18} (4.6 x150 nm id 5nm), Zorbax X SB-C_{18}, 6x150 nm,3.5nm), was found to elute metformin at a longer time and did not adequately

resolve metformin from the internal standard. However, Poroshell 120 EC-C18 4.6 mm X 50 MM 2.7 Microns was found to elute the analyte in less than two minutes and resolved metformin from the internal standard under the optimized chromatographic conditions Table 1, consisting of a mobile phase A (Sodium acetate, triethylamine, 1% acetic acid, and tetrahydrofuran) and B (Sodium acetate trihydrate, acetic acid, acetonitrile, and methanol) (90:10, v: v), the mobile phase was adjusted to (pH 7.2) at a flow rate of 0.8 ml/min and ambient temperature. Multiple drug prescription is often recommended in patients suffering from diabetes with complications [18]. Despite this growing phenomenon, the influence of diabetes on drug metabolism in the administration of several drugs has not been fully investigated [18]. This study evaluated the effect of 500 mg clindamycin co-administered with 1 g metformin in healthy volunteers. The changes in pharmacokinetic parameters were not statistically significant when metformin was administered alone and with clindamycin [20] , also reported insignificant changes in pharmacokinetics of metformin when co-administered with amoxicillin the changes in pharmacokinetic parameters were not statistically significant [21]. Even though clindamycin does not affect most of the pharmacokinetics parameters of metformin, some changes were observed. The C_{max} decreased from 1,880.25 ±0.45 to 1,282.35±0.4 and AUC_{0-8} from 6,770±0.52 to 4,377±0.80. Although a clear theoretical mechanism is not obvious, and metformin and clindamycin interact with different transporters, thiazides could affect transporter activity through altered ionic balance [22]. The mean postprandial glucose level changes were not significant when metformin was co-administered with clindamycin. This may be a result of non-interaction when metformin was co-administered with clindamycin.

5. CONCLUSION

The developed and validated method reverse-phase high-performance chromatography (RP-HPLC) method in the interaction studies of clindamycin and metformin in human plasma was very effective and efficient. The results of the findings indicated pharmacokinetic changes when metformin was administered alone and co-administered with clindamycin, though not statistically significant ($p>0.05$). It is therefore, recommended that metformin can be co-administered with clindamycin to type II diabetic patients without the risk of side effects.

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Conflict of Interest

No conflict of interest is associated with this work

Contribution of the Authors

Musa Garba Abdullahi - Conceptualization, Supervision and manuscript drafting of the research. Abdulkadir Bashir - Data analysis and manuscript drafting. Umar M. Danmusa - Data collection and manuscript drafting - Aisha Muhammad - Data Analysis and manuscript Drafting. Mahmud Umar - Data analysis and manuscript drafting.

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