IN-VIVO INTERACTION STUDIES OF CHLORAMPHENICOL AND METFORMIN USING REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

Supported by tetfund

[*1]M. A. Garba, [1]**A. Bashir, [1]U. M. Danmusa, [1]R. Bako, [2]M. A. Tanko**
Department of pharmaceutical and medicinal chemistry, Faculty of pharmaceutical sciences, Kaduna State University, Kaduna, Nigeria.
Department Geography, Faculty of Science, Kaduna State University, Kaduna, Nigeria.
*Corresponding author: musagarba.abdullahi26@gmail.com

Received: February 11, 2023 **Accepted:** April 15, 2023

Abstract:

Metformin is mostly prescribed with chloramphenicol in the treatment of diabetic mellitus with gastroenteritis and possible infections. However, the effect of this drug on the pharmacokinetic profile of metformin is yet to be established. The study was aimed at investigating the influence of chloramphenicol when co-administered with metformin on healthy volunteers. It is a one-way single-dose cross-over study in two phases. The subjects acted as their control, and each phase was preceded by an overnight fast. In phase one, metformin was administered to all the volunteers, while in phase two metformin was co-administered with 500 mg chloramphenicol. Plasma glucose levels were determined using the standard glucose oxidase method. Blood samples were collected at 0 - 8.0 and 24 h post-drug administration and stored at – 4 °C before analysis. Plasma samples were acidified with 0.5 ml of HCl and deproteinized with acetonitrile and centrifuged, the supernatant was washed with dichloromethane and injected into the HPLC system. Separation was achieved within 10 min on a Poroshell 120 EC-C18 (4.6mm x 50 mm 2.7 Microns) column with an isocratic mobile phase consisting 0f 0.03M dibasic ammonium phosphate (pH 7) and acetonitrile(10:90) % v/v) at a flow rate of 0.8 ml/min at a detection wavelength of 236 nm, and at ambient temperature. The samples were analyzed for metformin using a reversed phase. The results obtained from the study indicated pharmacokinetic changes though not statistically significant ($P > 0.05$), decreased in the (C_{max}) and AUC_{0-8} of metformin from 1,880.25± 0.45 to 1,710.35±0.4 ng/ml, 6,770.21±0.52 to 5,563.71±0.80 ng/ml/hr respectively and also decrease in the renal clearance by 38 %. The renal clearance of metformin was reduced in a time-dependent manner in the presence of chloramphenicol. This may be concluded that a combination of metformin with chloramphenicol may be clinically safe.

Keywords: RP-HPLC, chloramphenicol, metformin, interactions, human plasma

Introduction

Metformin (N, N -dimethyl biguanide hydrochloride) is the first-line drug for type 2 diabetes and the most commonly prescribed drug for this condition worldwide, either alone or in combination with insulin or other oral antidiabetes patients (Flory and Lipska, 2019). It works by inhibiting the production of hepatic glucose, reducing intestinal glucose absorption and improving 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 (Dumitrescu, *et al.*, 2015). It is also used for other indications such as polycystic ovary syndrome (PCOS) (Gong, *et al.*, 2012).

Metformin 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 (Gong, *et al.*, 2021). It is orally administered in the dose range of 500 mg/b.i.d. or t.i.d. and up to a total of 2,550 mg/day or approximately 35 mg/kg/day. The immediate-release formulation of metformin is rapidly absorbed from the small intestine following an oral dose. It has an onset of action of about 1.5 hours, a half-life in the circulation of about 1.5–4.9 hours, and a duration of action of 16–20 hours (Apampa, 2012). About 20% of a total dose can be absorbed from the duodenum, up to 60% from the jejunum and ileum but only very small amounts from the colon. The rest is excreted in the faeces (Glossmann and Lutz, 2019). It has an absolute oral bioavailability of 40 to 60%, and gastrointestinal absorption is apparently complete within 6 hours of ingestion (Scheen, 1996).

The hydrophobicity of metformin is associated with low intestinal and cell membrane permeability, which is recognized as a primary limiting step for metformin oral absorption (Gong, *et al.*, 2021). The half-life of metformin may be prolonged in patients with renal impairment, resulting in a theoretical risk of the rare but fatal lactic acidosis. It has been suggested that this risk may be a consequence of the action of metformin to suppress gluconeogenesis resulting in the inhibition of lactic acid metabolism in the liver, and thus accumulation of lactate (Apampa, 2012) .The intestinal absorption of metformin may be primarily mediated by plasma membrane monoamine transporter (PMAT). However, there is no in-vivo data which indicates the role of PMAT in the disposition and pharmacological effect of metformin (Gong, *et al.*, 2021). Metformin accumulation is a risk factor for fatal lactic acidosis. Therefore, therapeutic drug monitoring of metformin is required as an effort to ascertain that metformin concentration is within the recommended therapeutic range (Wibowo 1996). Large overdoses of metformin can lead to lactic acidosis. Suicide with metformin is rare. Intake of 35 g of metformin has been shown to be lethal (Graham *et al*., 2011).

The present work is aimed at studying the interaction between chloramphenicol and metformin in healthy volunteers using a developed and validated- reverse phase high performance liquid chromatography (RP-HPLC)

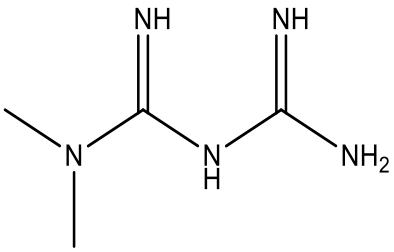

Figure 1: Molecular structure of Metformin.

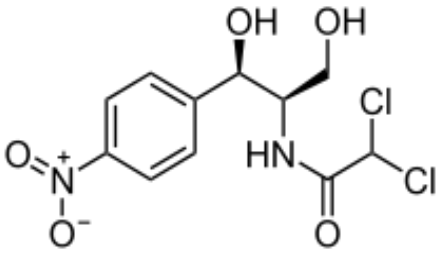

Figure 2: Molecular structure of Chloramphenicol

Materials and methods

Materials

Equipment and reagents

The following equipment and reagents were employed in the course of this study: Digital weighing (OHAUS model EP 64 BY Ohaus Corporation, Switzerland), Centrifuge (Heroes (Labafuge300) D-37520 Ostence Mastance03, Serial N0 40267581, BN: 75003230, HPLC column:, Poroshell 120 EC-C18 4.6 mm X50 mm 2.7 Microns, FTIR machine, HPLC machine (Agilent Technologies Model 1260 Infinity Series, Germany), acetonitrile HPLC grade, Methanol (Sigma – Aldrich ≥ 99.9% U.K), Dipotassium hydrogen phosphate (Buffer) (J.T Baker 99.5% USA), dichloromethane, HPLC grade methanol, HPLC grade water, Tetrahydrofuran THF, Sodium acetate, Hydrochloric acid, metformin HCL (Reference Standard), Acetic acid, Phenytoin (internal standard –Ranbaxy Pharmaceutical Ltd., Lagos),

Methodology

Subjects and ethical clearance

The subjects were diagnosed with diabetes mellitus at the Medical Outpatient Department of Yusuf Dantsoho General Hospital, Tudun- wada, Kaduna, Kaduna State, Nigeria. 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 present 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.

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 60 ± 70 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 (ADA, 2013, Marathe et al., 2000 and PattanaSripalakit *et al*., 2006), 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, 16 and 24 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 4.6mm X50mm 2.7 Microns column, mobile phase acetonitrile(A) / Methanol (B) (10:90) and a UV detector at 236 nm.

Preparation of Mobile Phase

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 and adjusted to P^H 7.2 with 1% acetic acid 1.5ml tetrahydrofuran (THF) was added to the mixture. The solution was filtered by vacuum and sonicated for degassing.

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 before being used for HPLC analysis.

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

Preparation of stock standards and working solutions

Stock solutions (1.0 mg/ml) of metformin 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 µ/ml were prepared in human plasma and vortexed for 1min 0.5 aliquots were transferred into a glass tube and stored at -20^0 C before use.

Table 1: RP-HPLC Optimized Chromatographic Conditions

Mobile phase :	Acetonitrile	methanol
Ratio :	10	90
Column Type	Poroshell 120	
Column dimension	EC-C18 4.6mm X 50mm 2.7 Microns	
Wavelength :	236	
Temperature :	Ambient	
Flow rate :	0.8 mL/min	
Run time :	10 mn	
Injection volume :	2 µL	
pH :	7.0	
Chromatogram :	Metformin	Phenytoin
Retention time (min) :	2.230	4.407

Table 2: Validation Parameters of developed RP-HPLC method for the quantitative analysis of metformin in plasma

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

Table: 3 pharmacokinetics of metformin (mean, n =6) alone and
When co-administered with chloramphenicol in healthy volunteers

	Metformin alone	Metformin + chloramphenicol
Ke(h^{-1})	0.31±0.01	0.47±0.12*
C_{max} ($ngmL^{-1}$)	1,880.25±0.45	1,710.35±0.42
T_{max}(min)	3.0 ±0.19	3.0±0.19
AUC_{0-8}(h ngmLh^{-1})	6,770.21±0.52	5,562.57±0.80
Vd (mL)	1,470.59±0.27	2857.143±0.02*
CL(mLh^{-1})	4425.76±0,24	2857.143 ± 0.26

*Significant difference, ($p<0.05$)

Linearity, LOD and LOQ

Method sensitivity, LOD and LOQ LOD and LOQ for metformin and phenytoin were calculated from the linear regression equation based on a standard deviation of the intercept and the slope using the formula.

$$LOD = \frac{3.3Q}{4S} \quad \text{and}$$

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

Q: the standard deviation of the intercept,

S: the slope of the calibration curve (ICH, 2006)

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. The repeatability of sample application and measurement of peak area was expressed in terms of relative standard deviation (% RSD).

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 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/caffeine (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.

Calibration curve

The peak areas were plotted against the corresponding concentrations to obtain the linear calibration curves Residual analysis was performed to ascertain linearity. A series of concentrations ranging from 0.05 to 5 µgmL^{-1} for metformin.. The calibration Coefficient of Variation and correlation coefficient R^2 (0.995) with a linear regression of equation from the plot is $y = 1.288 x + 0.6412$; where y is the peak area ratios, x is the concentration, 1.288 is the slope while 0.6412 is the intercept were computed with a statistical data package (Figure 3) The results showed a good response of the detector at the concentration used (ICH, 2006, Zhang *et al*., 2002, Bello et al., 2017),

Pharmacokinetic parameters and statistical analysis

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 (t1/2) 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). All these pharmacokinetic parameters were generated with the aid of the Software – Pharm PK software (Joel *et al.,* 2012, Melmed, *et al.,* 2012, Sambo, *et al.,* 2019). 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 $p<0.05$ considered significant as shown in (Table 3).

Results and Discussion

Quality Control of metformin and chloramphenicol was carried out and the result was within an acceptable range. Figures 1 and2 represent the chemical structure of metformin and chloramphenicol, while the Chromatograms of metformin and phenytoin alone respectively represented in Figures 3 and 4 while the obtained chromatogram of metformin and phenytoin spiked with human serum is shown in Figure 4 while chromatogram of metformin and phenytoin is also presented (Figure 5). The constructed calibration curve of metformin in human plasma is presented in (Figure 6). Optimized chromatographic conditions are listed in (Table 1). The validation calibration curve parameters of the developed method are listed in Table 2 of pharmacokinetics parameters of metformin alone and when co-administered with chloramphenicol. Presented in table 3.

This study evaluated the effect of 500 mg chloramphenicol co-administered with 1 g metformin in healthy volunteers. This is to establish the need for concomitant drug intake during the study. Pharmacokinetic changes in $C_{max,}$ AUC, Vd, Cl, were recorded, though most of the parameters were not significantly different in when metformin was co-administered with chloramphenicol. There results indicated an increase in peak plasma concentration of metformin when co-administered with chloramphenicol (C_{max}) from 1,880.25± 0.45 to 1,710.35±0.4 ng/ml ng/mL while the area under the curve (AUC) increased from 6,770.21±0.52 to 5,563.71±0.80 ng/ml/hr which was statistically insignificant at ($P>0.05$). This is in agreement with the findings of (Hills, 1987, Paxton, 1989) , that the high AUC value of metformin in the presence of ciprofloxacin tablet is most likely responsible for the decreased plasma glucose concentration following treatment with the two drugs (Garba *et al.*, 2018).

The observed significant decrease ($p<0.05$) in clearance from 4425.76±0,24 to 2857.143 ± 0.26 mL/h with a reduction in the volume of distribution when metformin 1 g was co-administered with 500 mg chloramphenicol, maybe due to the decrease in elimination rate constant (Charles *et al.,* 2009). The mean postprandial glucose level changes were not significant when metformin was co-administered with chloramphenicol. This may be as a result of non-interaction when metformin was co-administered with amoxicillin. This could be concluded that concomitant administration of metformin with amoxicillin had no significant effect.

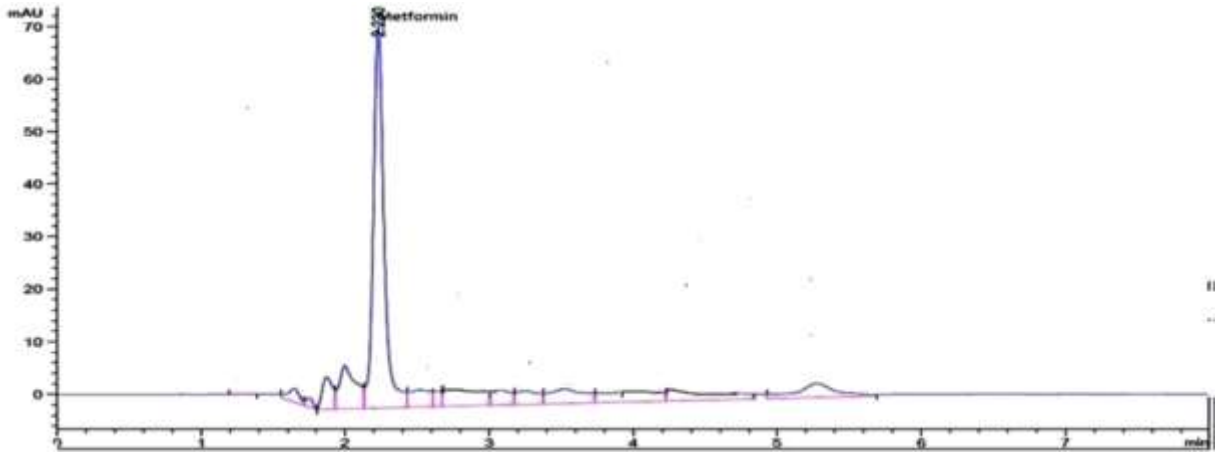

Figure 3 :RP- HPLC chromatogram of metformin alone

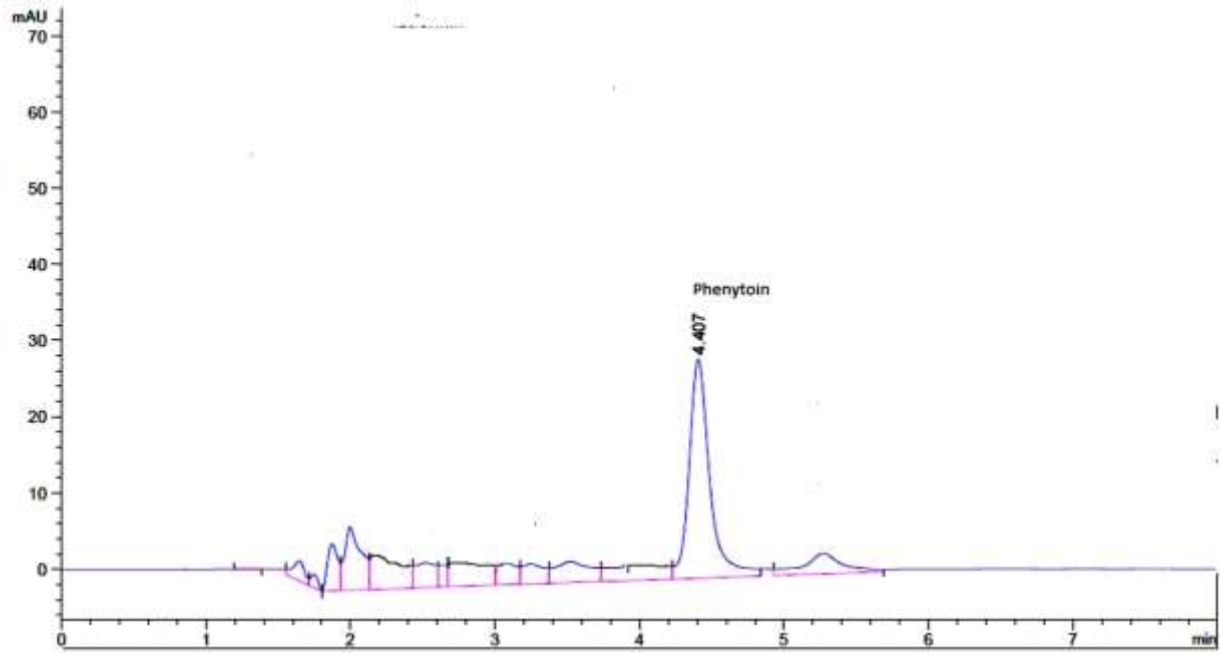

Figure 4:-RP- HPLC Chromatogram of phenytoin alone

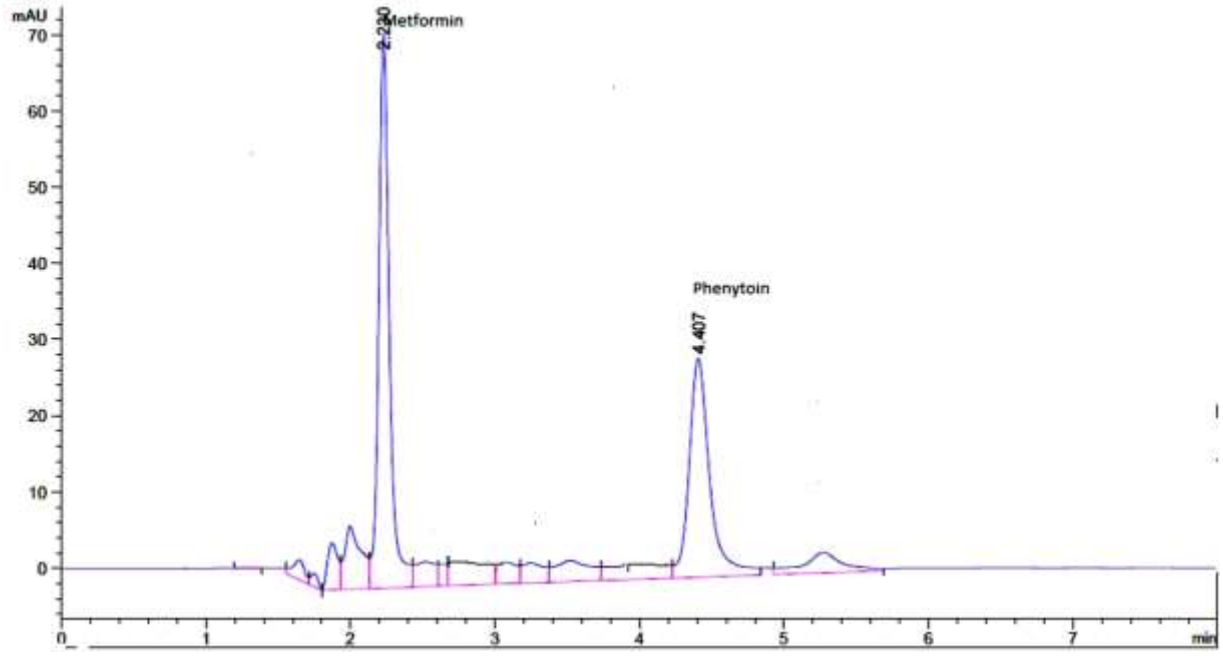

Figure 5: HPLC chromatogram of metformin and Phenytoin spiked with human plasm

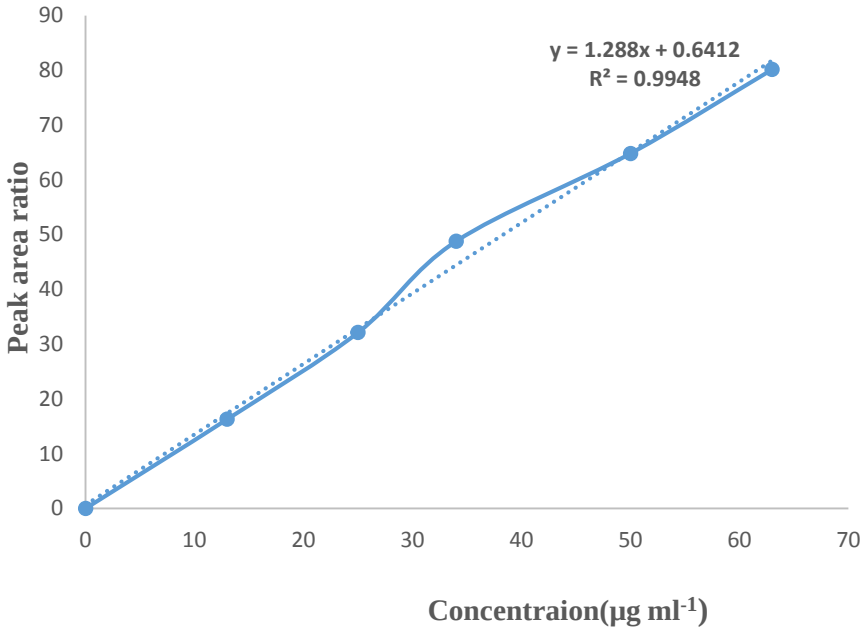

Figure 6: Calibration curve of metformin in plasma using phenytoin as internal standard

Conclusion

The RP-HPLC developed and validated method used in carrying out interaction studies of metformin and chloramphenicol is very effective and efficient. The method may be used in drug monitoring in clinical practice. The finding from the study also indicated pharmacokinetic changes when metformin was administered alone and when co-administered with chloramphenicol. It is therefore, recommended that patients who are prescribed these two drugs concomitantly should have serum metformin levels monitored or an alternative to chloramphenicol should be considered.

Declarations

The Authors wish to thank the Tertiary Education Fund (TETFUND) for the grand support to Associate Professor M.A. Garba and also to thank the Technologist Mallam Mohamed Kabir Isa and Mr. Silas the Director of the Multi-user science research Laboratory of Ahmadu Bello University, Zaria for their patients and support in carrying out the analysis .I wish to appreciate the contributions of the Directior of admin, and supply, Mallam Zakari, The chairman of health research ethics committee, Dr. Gajere for facilitating the approval of the ethical clearance and all the Staff of the Department of Health and planning Ministry of Health.

Conflict of interest statement

The authors declared that they have no conflict of interest.

References

ADA (American Diabetes Association) (2013). Nutritional Recommendations and principles for an individual with diabetes Mellilus. *Diabetes care .10*:126 – 132.

Apampa B. Pharmacology and safe prescribing of metformin. Nurse Prescribing. 2012;10(12):597-602.

Bello SS, Garba M, Sani FB,Odonula MT and Garba MA (2017). A Study of the influence of Bendrofluazide on Metformin in Type II diabetic patients with hypertension. *International journal of advances in pharmacy, biology and chemistry IJAPBC* – Vol. 6(3), Jul - Sep 2017 ISSN: 2277 – 4688

Charles BG , Precechagoom, Y, Lee TC ,Sheer PA , Flennady VJ and Debuse, N (2009). Population pharmacokinetics of intravenous amoxicillin in very low birth weight infants. *Journal of Pharmaceutical Sciences.* 86(22):1288-12292.

Dumitrescu R, Mehedintu C,Briceag I, Purcărea V, Hudita D.(2015).Metformin-clinical pharmacology in PCOs. *Journal of medicine and life*. :8(2):187.

Flory J, Lipska K. Metformin in 2019.Jama. 2019; 321(19):1926-1927.

Garba MA, Bakare-Odunola MT, Garba M,.Haruna A and Bako R (2018). Effects of metronidazole and amoxicillin on the pharmacokinetics of metformin in type ii diabetic patients. *FUW Trends in Science & Technology Journal*, www.ftstjournal.com e-ISSN: 24085162; p-ISSN: 20485170; April, 2018: Vol. 3 No. 1 pp. 309 – 313

Glossmann HH, Lutz OM.Pharmacology of metformin—An update. *European journal of pharmacology.* 2019;865:172782.

Graham GG, Punt J, Arora M,Day RO, Doogue MP, Duong

J, *et al.*, (2011). *Clinical pharmacokinetics of metformin. Clinical pharmacokinetics*.;50 (2):81- 98.

Gong L, Goswami S, Giacomini KM,Altman RB, Klein TE. (2012) Metformin pathways: *Pharmacokinetics and pharmacodynamics. Pharmacogenetics and genomics*.;22(11):820.

Hills S (1987). Penicillin Antibiotics. In: Antibiotics in Clinical use. Oxford University Press, London. pp 2-4.

International Conference on Harmonization (2006)."Q29(R1): Text on Validation of Analytical Procedures". Federal register CPMP/ICH/381/95.

Jeong Y-S, Jusko WJ. (2021) Metaassessment of metformin absorption and disposition pharmacokinetics in nine species. *Pharmaceuticals*.;14(6):545.

Joel IU, Atul D, Diane TL (2012). PK Function on Microsoft excel. Department of Pharmacokinetics and drug Metabolism. Allergen, Irvine, CA 92606. USA

Lutz OM (2019).Pharmacology of metformin—Anupdate. *European journal of pharmacology*. 865:172782. Melmed S, Polonsky KS, Larsen PR and Kronenberg, H.M. (2012). Williams Textbook of Endocrinology, 12th Edn. Philadelphia: Elsevier/Saunders. pp. 1371–1435.

Marathe PH, Arnold ME, Meeker J, Greene DS, Barbhaiya RH (2000). Pharmacokinetics and bioavailability of a metformin/glyburide tablet administered alone and with food. *Journal of Clinical Pharmacology* 40: 1494-1502.

PattanaSripalakit A.B, PenpornNeamhom .B, AurasornSaraphnhotiwitthaya C (2006). High performance liquid chromatographic method for the determination of pioglitazone in human plasma using ultraviolet detection and its application to pharmacokinetic study *Journal ofChromatography* B; 843: 164-169.

Paxton WJ (1989). Drug absorption and distribution. In: Pharmacokinetics in clinical practice *The New Zealand Medical Journal*. 694: 304-306.

Ptalsky KM (1980).Disease-induced changes in the plasma binding of basic drugs. *Clinpharmacokin*.5; 246- 262.

Sambo GI, Bakare-Odunola MT, Aminu M, Ibrahim AY, Magaji G and Adzu B (2019). Effect of amodiaquine on the pharmacokinetics of glicliazide in diabetic subjects.*African journal of pharmacy and pharmacology*.13 (11) 139-145.

Scheen AJ (1996). Clinical pharmacokinetics of metformin.*Clin Pharmacokinet*;30 (5):359-371.

Wibowo A, Ningrum VD, Izzah N,editors (2018). Stability Test of Metformin Hydrochloride in Human Plasma Using HPLC-UV for the Protocol of Therapeutic Drug Monitoring of Metformin. AIP Conference Proceedings; AIP Publishing LLC.

Zhang M, Moore GA, Lever M, Gardiner SJ, Kirkpatric CM & Begg EJ (2002). Rapid and simple high-performance liquid chromatography assay for the determination of Metformin in human plasma and breast milk. *J. Chromatogr B Analyt Technol Biomed Life Sci.*, 766(1): 175-179.