Therapeutic Drug Monitoring of Ticagrelor in Jordanian Patients. Development of Physiologically-Based Pharmacokinetic (PBPK) Model and Validation of Class IV Drugs of Salivary Excretion Classification System (SECS).

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ABSTRACT: Ticagrelor is an orally administered antiplatelet agent that has been approved for the reduction of cardiovascular risk in patients with history of cardiovascular disease. However, Ticagrelor can also cause adverse effects, including dyspnea. Ticagrelor plasma and saliva concentrations of 63 Jordanian patients were measured using a sensitive LCMS/MS method. Ticagrelor was not excreted in saliva as it corresponds to the Class IV salivary excretion classification system. The correlation between plasma ticagrelor concentrations and the occurrence of dyspnea was examined to establish a therapeutic window for plasma ticagrelor. A physiologically-based pharmacokinetic (PBPK) model of ticagrelor was built and validated using previously- published plasma data. The validated model was then used to predict the factors affecting ticagrelor plasma concentrations. A loading dose of 135 mg (1.5 tablet) instead of 2 tablets has been suggested to maintain its therapeutic effect and avoid dyspnea.

KEYWORDS: Ticagrelor; pharmacokinetics; SECS; TDM; Gastroplus; PBPK

1. INTRODUCTION

Ticagrelor is a novel antiplatelet agent which works as a reversible P2Y12 ADP receptor antagonist. It acts directly, without the need for metabolic activation and produces high level of platelet inhibition. Ticagrelor is considered as the first line treatment in a wide spectrum of ACS patients. In December 2010, the European Commission granted approval for use in patients with a history of myocardial infarction or acute coronary syndrome (ACS) for the prevention of future myocardial infarction, including those who are being treated with percutaneous coronary intervention. ACS is a condition where a severe reduction in the blood flow to the heart muscle occurs mostly due to the formation of fatty substance called plaque which builds up in the arteries. This could result in low oxygen supply to the heart muscle followed by a myocardial damage. The aim of using antiplatelet drugs in the treatment of ACS is to prevent intravascular thrombosis and embolization.

Despite successful treatment with ticagrelor, there are some side effects, especially dyspnea in 13.8% of patients. It is usually mild to moderate in intensity. Sometimes reversible, but may worsen and become intolerable, leading to discontinuation of ticagrelor and increasing the risk of ischemic complications. Another antiplatelet agent is considered. Despite the potential impact on treatment outcome, the impact of ticagrelor-related dyspnea on treatment discontinuation and compliance in ACS patients should be considered.

For ACS patients undergoing percutaneous coronary intervention (PCI), ticagrelor is given as oral dose of 180 mg loading dose before the PCI procedure, followed by 90 mg twice as a protocol [3]. The aim of

the study is to investigate the correlation of dyspnea incidence with the high ticagrelor plasma level in ACS patients undergoing PCI. Dyspnea seems most highly correlated with elevated peak concentrations greater than the 0.25 to 1 mg/L [4]. Saliva could be an alternative to blood sample in TDM sampling, since it is easier to collect compared to plasma. Saliva doesn't require filtration steps to isolate the unbound drug. Furthermore, it reflects the free pharmacologically active ingredient in serum and can be measured using standard analytical methods. According to the Salivary Excretion Classification System (SECS), excretion in saliva can be expected for drugs with high effective permeability and low plasma protein binding. According to SECS classification, ticagrelor belongs to class IV compound showing low permeability and high binding to plasma protein. Its excretion in saliva is thus not expected [5]. The need for PBPK studies as a predictive tool has increased because they are not associated with the ethical challenges related to clinical trials in sensitive populations (e.g., cancer patients, pediatrics, pregnant women, etc.). Also due to the cost-effectiveness, robustness, as well as the critical need for PBPK investigation especially for drugs with a narrow therapeutic window and sensitive patient populations. Applications can include pregnancy and organ transplant populations along with obesepatients' surgeries. PBPK models also help in supporting risk assessment studies in animals or humans, simulating steady-state and dynamic drug-drug interactions (DDI), understanding differences in food effects, making PBPK predictions to estimate effective doses, and identifying appropriate doses and dosing regimens in ill and pediatric populations [6]. The global structure of PBPK models is derived from the body anatomy of mammals [7].

The objectives of this study are to determine the correlation between ticagrelor plasma concentrations and incidence of dyspnea, to develop a validated method of ticagrelor analysis in plasma and saliva samples using liquid chromatography-mass spectrometry (LC-MS) but also to establish a therapeutic window for ticagrelor in plasma that can be used in therapeutic monitoring of ticagrelor to optimize the dosing regimen, and to determine the variability of key pharmacokinetic parameters of ticagrelor in Jordanian patients. Moreover, to confirm that ticagrelor belongs to class IV of the salivary excretion classification and that it can not be excreted into saliva, and to develop a PBPK model for ticagrelor based on the previously reported data.

2. RESULTS and DISCUSSION

The molecular weight of ticagrelor is 522.6 g/mol [11] and the molecular weight of its active metabolite (AR-C124910XX is the only active metabolite) is equivalent to 478. 5g/mol [12], which is almost equal to the 92% of ticagrelor molecular weight, so because of this similarity in molecular weights, and because of both ticagrelor and its active metabolites have the same side effect of dyspnea [13], which is our research, dyspnea was associated with sustained high plasma concentrations of ticagrelor and its active metabolites. For this reason, and to simplify the calculation, the sum of the concentrations of ticagrelor and the active metabolite for each patient could be considered as the total concentration of ticagrelor in patient plasma responsible for the side effect dyspnea. The Peak levels of total concentration (ticagrelor and its active metabolite) in the plasma was ranged from 274.188 to 2904.976 ng/ml (mean= 1215 ng/ml, standard deviation = 691, CV %= 56.876 %). The trough levels of total concentration(ticagrelor and its active metabolite in the plasma levels) (mean= 535.897 ng/ml, standard deviation = 355 , CV%= 66.25 %).

The results showed that most of the patients had parent and total similar concentrations of more than the upper limit of 1000 ng/ml regardless of dyspnea score (Figure 1). Of the sixty-three patients participated in this study, 9 patients (14%) suffered from severe dyspnea (score 3) and not discharged on ticagrelor post coronary intervention, which is in agreement with reference drug monograph information [3]. The rest of patients have tolerated other dyspnea scores 0, 1& 2.
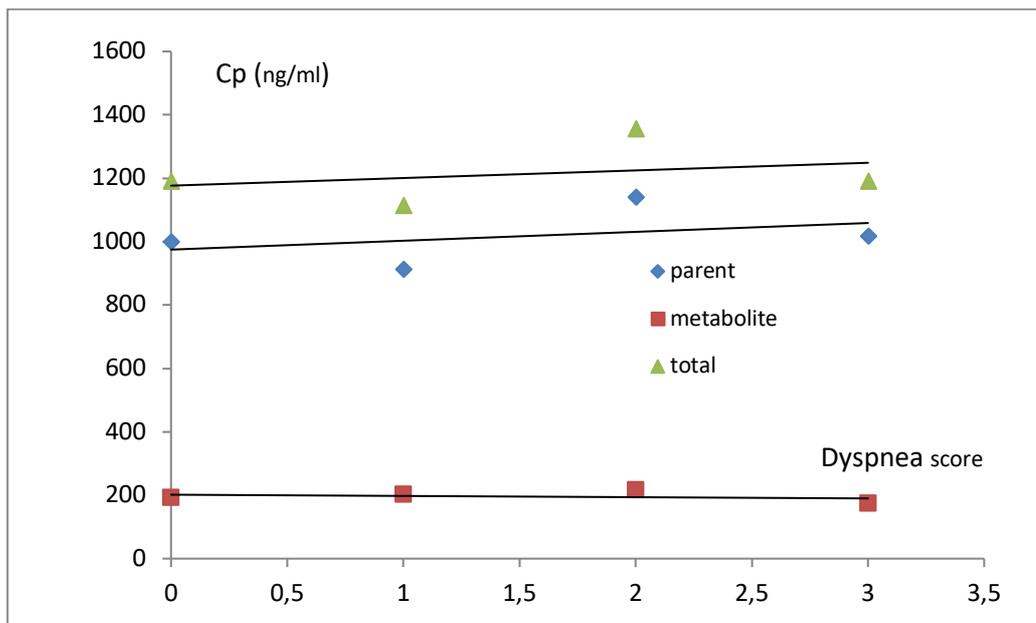
Figure 1: Parent, metabolite and total ticagrelor plasma concentrations in patient versus dyspnea score

The effect of BMI, alcohol intake, smoking, age, and gender, were also investigated by analysis of variance using Systat V5. A statistically-insignificant effects ($P > 0.05$) of 0.617, 0.89, 0.733 0.085 and 0.052 between (alcohol intake, smoking, age, BMI and gender, respectively) and Cmax of plasma total ticagrelor concentration was observed.

To study salivary excretion of ticagrelor, it is extensively bound to human plasma proteins (> 99%), especially albumin, so the free, unbound fraction in blood is low ($fu < 1\%$). In addition, ticagrelor has low permeability (Peff) with a low absorbed fraction ($Fa < 90\%$). Therefore, ticagrelor is classified as class IV of the salivary excretion classification system (SECS) [5]. This was validated as it was not detected in saliva samples.

In addition, PBPK modeling as shown in Table 1 and Figure 2, the PE% was 24.9 % and 26.2% for Cmax and AUC respectively, which is less than 2-fold difference, indicating good prediction PBPK model. However, the predicted plasma Tmax was similar to the observed plasma Tmax, also indicating good prediction PBPK model.

Table 1: Observed and simulated concentration–time data

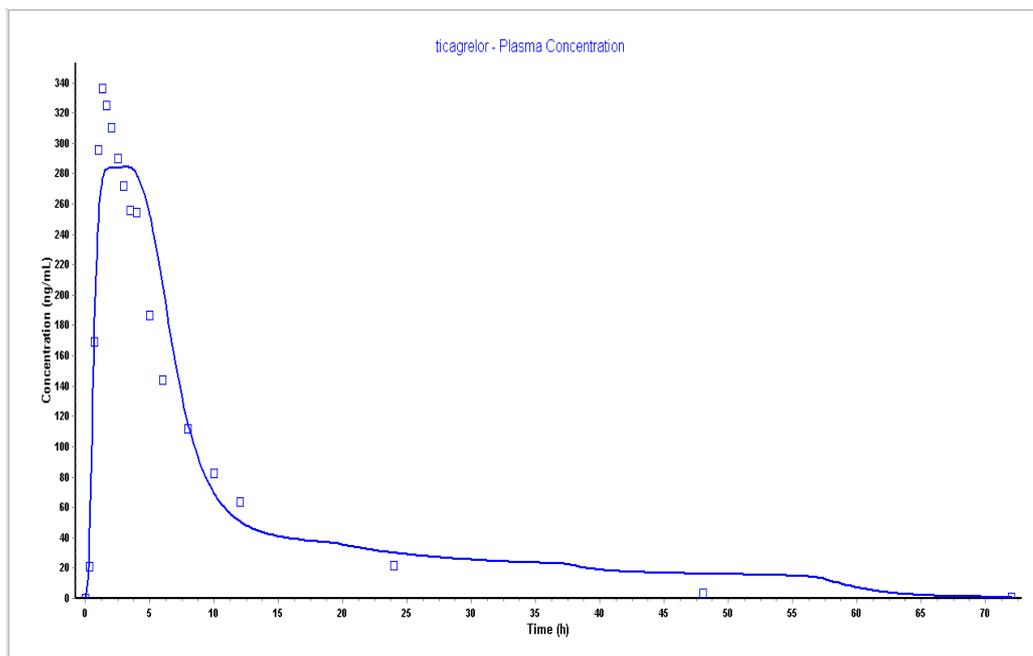| PHARMACOKINETIC (PK) PARAMETERS	OBSERVED PHARMACOKINETIC DATA	SIMULATED PHARMACOKINETIC DATA	%PE
PLASMA CMAX (NG/ML)	373	280	24.9 %
PLASMA TMAX (H)	2.33	2.33	Zero
PLASMA AUC	2800	4350	26.2%

Figure 2. Observed and simulated concentration–time curve: Observed (blue squares) versus predicted (blue line) concentrations of ticagrelor after oral administration of a 90 mg dose Simulations.

The parameter sensitivity analysis (PSA) in Figure 3 shows that permeability and clearance have a major impact on the plasma Cmax of ticagrelor after oral administration. When clearance increases and/or permeability decreases, Cmax decreases.
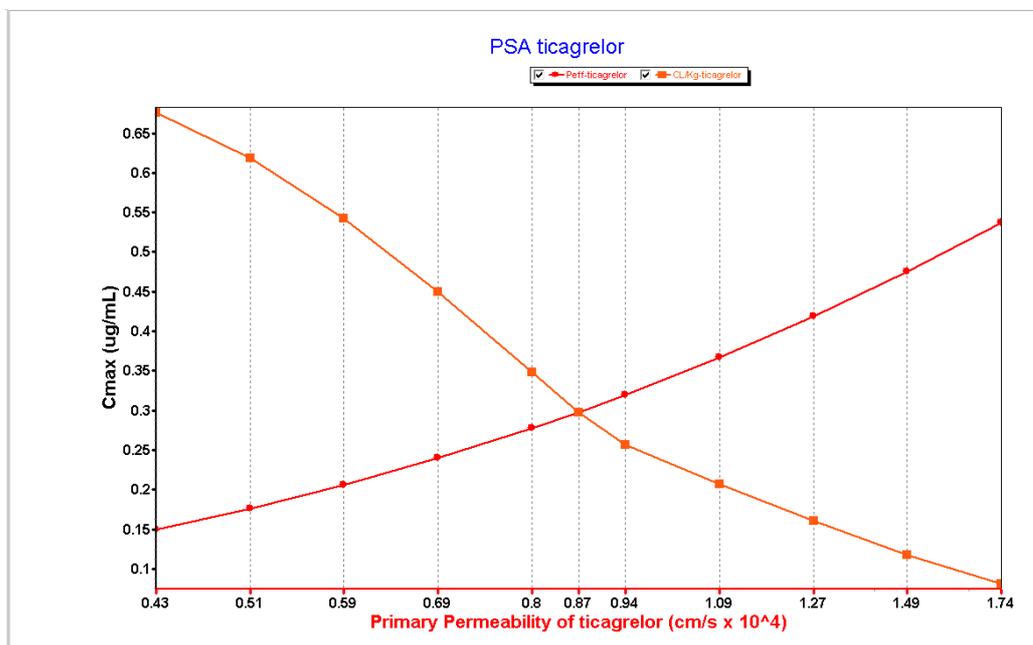

Figure 3: Impact of permeability and clearance on the plasma Cmax of ticagrelor after oral administration of a 90 mg dose

We simulated a loading oral dose of 135 mg followed by multiple oral doses of ticagrelor 90 mg twice daily. The predicted Cmax was 320ng/ml as shown in Figure4, which is accepted in term of inhibition of platelet aggregation [10]. In order to get the maximum efficacy of the treatment with ticagrelor and to avoid ticagrelor dyspnea related side effect, ticagrelor plasma concentration should be maintained in ranged from 0.25 to 1 mg/L [4]. Depending on that, its recommended to adjust the loading dose to 135 mg (1.5 tablet of 90 mg) instead of 180mg (2 tablets of 90mg). A loading dose of 135 mg (1.5 tablet of 90 mg) is predicted to reach plasma concentration of approximately of 320ng/ml which results in adequate inhibition of platelet

aggregation [10, 14]. The twice daily dosing regimen of ticagrelor is supported by other study that provides a consistently greater inhibition of platelet aggregation than an equivalent single daily dose [10].
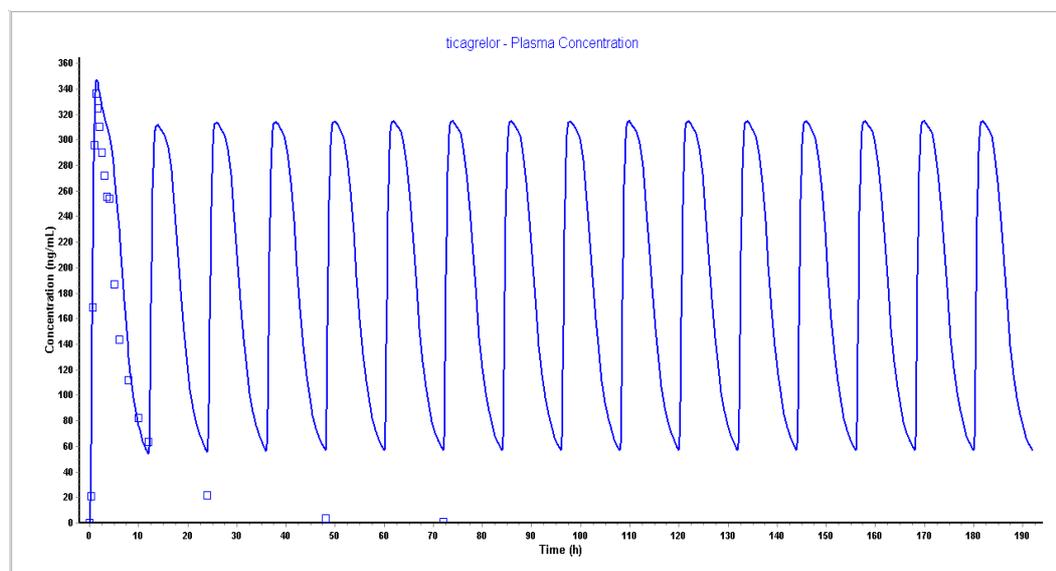

Figure 4: Predicted plasma concentration time after loading dose 135 mg followed by multiple ticagrelor dose of 90 mg per oral twice daily by GastroPlus™ modeling system

3. CONCLUSION

The conclusion is that future work should be initiated to investigate the pharmacodynamic effect of ticagrelor in patients after a 135-mg loading dose compared with a 180-mg loading dose.

4. MATERIALS AND METHODS

4.1 Study designs and human subjects

This TDM study is an observational study that was carried out at Jordan University Hospital (Amman, Jordan) after obtaining the ethical approval from the Institutional Review Board (IRB) (2021/10/15130) of Jordan University on a total of sixty-three patients who were treated with ticagrelor. Patient participation was voluntary after being informed of the study design and purpose. A consent form that included simple description was also signed by all participated patients. The study involved sixty-three Jordanian patients (51 males and 22 females). Those were diagnosed with an acute coronary syndrome and assigned for cardiac catheterization procedure. Ages ranged between 44 to 83 years (mean 59.77 / SD 9.81). In this study, 80.95% of patients were male, while 19.04% of patients were female. The body mass index (BMI) of all patients ranged between 19.11 to 38.57 (mean 28.43 / SD 3.77). Smoker patients made 55.55% while nonsmokers were 44.45%. An oral dose of 180 mg of ticagrelor was given as loading dose for each patient as per the protocol of catheterization procedure before the procedure. Plasma and saliva samples were taken after two hours from the loading dose of 180 mg to measure the peak level (Cmax) and also another two samples were collected for each patient directly before the maintenance dose (90 mg) after 12 hours from the loading dose to measure the trough level (Cmin). The samples were collected then immediately centrifuged at 1500 g for 10 minutes then the plasma samples were obtained and stored at temperature -80 C° until analyzed. The used brand of ticagrelor given in the hospital was (BRILINTA®).

4.2 Assay methodology

4.2.1 Chemicals and reagents

The used Chemicals and reagents for plasma samples analysis: Ticagrelor (source: ClearSynth), Deshydroxyethoxy Ticagrelor-D7 (I.S) stock solution (source: ClearSynth), Ticagrelor Active Metabolite solution, HPLC grade Methanol and Acetonitrile (source:VWR), Formic acid (source: analytical grade),

Ammonium Acetate buffer (source: Fisher), human plasma Deionized water and blank human plasma. The same chemicals and reagents used for saliva samples analysis in addition to DMSO (Dimethylsulfoxide) (source: Scharlau) and blank human saliva.

4.2.2 Chromatographic conditions

The analysis was carried out using LC-MS/MS system (Applied Biosystems Q-Trap 4500 mass spectrometer detector (MDS Sciex, Canada)(for plasma samples) composed ofAgilent HPLC instrument (Agilent Zorbax XDB-C18 5µm (4.6*150) mmcolumn) while the column temperature maintained at 25 °C and the auto samplertemperature was set to 5.0 °C. Forsaliva samples the analysis was carried out using LC-MS/MS Triple Quad 6500+ mass spectrometer in multiple reactions monitoring (MRM) mode using electro spray with positive ionization and the chromatographic separation employing C18 column. The mobile phase consisting of a mixture of Acetonitrile with Ammonium Acetate buffer(50mM) in a ratio of 90:10 (V/V) and 2000 µl Formic acid per 1.0L with flow rate of the1.0 ml/min (for plasma and saliva samples).

4.2.3 Preparation of stock, intermediate and calibrators

Ticagrelor stock solution was prepared with a final ofconcentrationapproximate of 500.00µg/ml. and Deshydroxyethoxy Ticagrelor-D7 (I.S) Stock solution prepared in approximate of 0.25 µg/ml. While for ticagrelor active metabolite stock solution prepared in approximateof 0.50µg/ml (diluting solvent is methanol-deionizedwater (50:50, v/v). while the working solutions were prepared in concentration of 50.0 µg/ml for ticagrelor, 50.0µg/ml for ticagrelor Active Metabolite working solution and ticagrelor-working solution was 12.9 µg/ml. Standard calibration curves were done by preparing nine concentrations in plasma plotted as standard curve points which contained a final concentration equivalent to (0.00, 0.00), (0.00, 0.00), (2.50, 2.50), (5.00, 5.00), (10.00, 10.00), (50.00, 50.00), (100.00, 100.00), (300.00, 300.00), (900.00, 900.00), (1700.00, 1700.00) and (2500.00, 2500.00) ng/ml of ticagrelor and ticagrelor active metabolite; respectively.

Additionally, blank and zero samples were prepared to confirm the absence of interferences (i.e. the lower limit of quantitation (LLOQ) response was at least five times the response of the blank) at the retention times of the analyte and the IS. The blank sample was a plasma sample and the zero sample was a plasma sample that was spiked with the IS only before being extracted. For ticagrelor in saliva, stock and working solutions of the analyte (Ticagrelor) and its internal standard (Ticagrelor-d7) were prepared and used during the analysis of samples ,The procedure involves dilution of saliva samples with the mobile phase after addition of the internal standard (Ticagrelor-d7).A set of 10 non-zero standards with calibration range (0.500-500.00) ng/ml and quality controls with concentrations (1.500, 75.00 225.00 and 375.00) ng/ml were prepared and analyzed within the run. The total run time was around 3.5 minutes, it showed good linearity over the range (0.500-500.000) ng/ml. Blank and zero samples were prepared to confirm the absence of interferences (i. e. the lower limit of quantitation (LLOQ) response was at least five times the response of the blank) at the retention times of the analyte and the IS. The blank sample was saliva sample that was prepared and extracted without being spiked with ticagrelor and IS. Whilst, the zero sample was a saliva sample that was spiked with the IS only before being extracted.

4.2.4 Preparation of Quality control samples

QC samples prepared in plasma contained with a final concentration equivalent to (2.50, 2.50), (7.50, 7.50), (1250.00, 1250.00), (2100.00, 2100.00), (5000.00, 5000.00) ng/ml of ticagrelor and ticagrelor active metabolite; respectively using ticagrelor working solution and active metabolite working solution to give the Low QC, Mid QC and High QC samples. For saliva samples, Quality controls with concentrations (1.500, 75.00, 225.00 and 375.00) ng/ml were prepared

4.2.5 Extraction of ticagrelor from plasma

Preparation of authentic samples (subject, standard curve and QC samples) were prepared by pipetting 250 µl of human plasma samples into their corresponding labeled tubes then each authentic sample Spiked with 25 µl of IS working solution (12.9 µg/ml) excluding sample of blank and 1.0 ml of Acetonitrile (Precipitation solvent) Added then vortex and centrifuge, lastly Inject directly from the supernatant to LC system using an auto sampler.

4.2.6 Bio-analytical method validation

The developed analytical method was validated as per US FDA guidance on bioanalytical method validation (15). The linearity, selectivity, stability and sensitivity tests were performed successfully to validate the analytical method.

The linearity of ticagrelor (in human plasma) was established for the range of 2.50-2500.00 ng/mland the same for ticagrelor active metabolite. While for saliva the linearity range was (0.500-500.000) ng/ml.

As per US FDA guidance, the calibration curve requires a coefficient determination ($r2$) of > 0.98so the calibration curve was plotted using the peak area ratios of ticagrelor and the internal standard versus the concentration of ticagrelor and the internal standard. For validation purpose each run consisted of a double blank, system suitability sample, zero standard while the calibration curve consisting of nine non-zero samples which covered the total range and quality control (QC) samples at three concentrations.

4.2.7 Lower Limits of quantitation (LLOQ)

The analytical method was developed and validated to measure the lowest concentration of the standard curve with acceptable accuracy and precision according the USFDA bioanalytical method validation guidance (15). The LLOQ in plasma was: 7.50 ng/ml. and there was no interference for the matrix on the concentration of LLOQ with range of accuracy percentage of 97.69 - 102 % which the accepted range according the USFDA bioanalytical method validation guidance (15).

4.2.8 Analytical method recovery

According to the US FDA guidance on bioanalytical method recovery, ticagrelor experiment was performed at three extracted low, medium and high-quality control samples and the resulted mean peak areas was compared with the mean peak areas of three un-extracted neat reference solutions, the result of internal standards (ticagrelor, deshydroxyethoxy ticagrelor-D7, ticagrelor active metabolite) recovery tests was 90 % for recovery of ticagrelor in human plasma. Since the precision (CV%) at each level was less than 15% according to the US FDA guidance on bioanalytical method validation so all replicates are accepted.

4.2.9 Selectivity and specificity

Separation of ticagrelor and the internal standard (IS) from the endogenous components like plasma assures that no interferences at the retention time of both ticagrelor and the IS. The peaks were of good shape, completely resolved from the plasma components. The matrix peak was less than 5 % of the peak area of the internal standard which is acceptable limit as per the US FDA guidance on bioanalytical method validation (15). Sample chromatograms are shown in Figures 5A, 5B. Detailed method of analysis is accessed via.
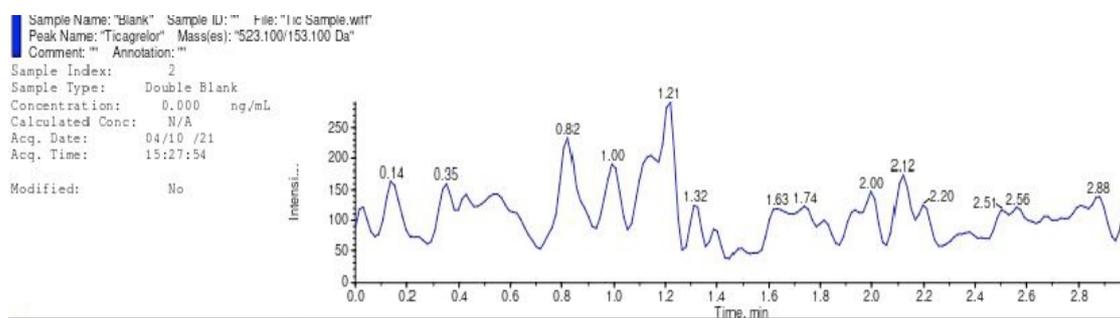

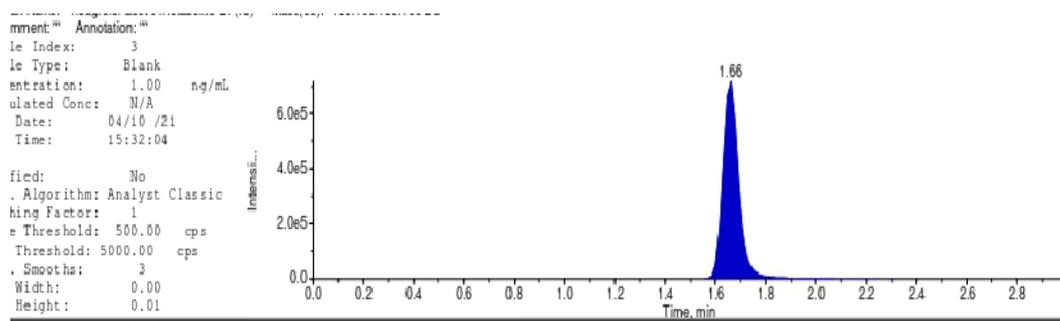**Figure 5 A**: Chromatogram of Human plasma extracted blank sample.

Figure 5 B: Chromatogram of plasma sample

4.3 Data Analysis

4.3.1 PBPK model development

The PBPK model was builtusing GastroPlus™ 9.8to predict ticagrelor plasma concentration, by using a standard plasma concentration versus time for 72 hours after a single 90 mg oral dose of ticagrelor which obtained from ACDIMA Biocenter Amman, Jordan.

PBPK modeling depended on the ratio between the measured concentrations and simulation of virtual human model as predicted by GastroPlus™ 9.8. The mechanistic oral absorption/PBPK model developed for this study to ensure realistic predictions of systemic plasma concentration of ticagrelor and the influenced factors on ticagrelor bioavailability and their effects on ticagrelor Cmax. Ticagrelor characteristic data and pharmacokinetic parameters data were used for the PBPK in GastroPlus modeling as summarized in Table2.

Table 2: Ticagrelor characteristic and pharmacokinetic data used for pbpk modeling

PARAMETER	VALUE	REFERENCE
GENERIC	Ticagrelor	
FORMULA	C23H28F2N6 O4S	[8]
MWT	552.57	[8]
DOSAGE FORM	IR: Tablet	
DOSE/MG	90 mg	
SOLUBILITY	0.005mg/ml	[8]
VOLUME-DISS	250 ml	
REF LOGD	2	[3]
REFLOGD PH	7	[3]
SPECIES	Human	
PKA(ACIDIC)	13.26	[8]
PKA(BASIC)	3.05	[8]
CL (L/HR)	8	*Optimized
PEFF X 10-4 (CM/SEC)	0.8675	*Optimized
T1/2	7.26 h	[9]
VOLUME OF DISTRIBUTION (L/KG)	0.10892	*Optimized
BIOAVAILABILITY	36%	[9]

4.3.2 PBPK model validation

PBPK model was validated by comparing the observed data versus the simulated data. The prediction error percent (PE%) was computed using the following equation:

PE% = [(simulated value –observed value)/ (observed value)] × 100

4.4 Statistical analysis

Microsoft Excel program was used for descriptive statistics, while ANOVA and *t*-testing after log transformation were done using Systat program. P-value of 0.05 was adopted for significant difference.

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