

A Validated Method for Quantification of Clopidogrel in Human Plasma and Its Application in Comparative Bioavailability Studies

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Abstract

Background: Development of simple, rapid, and sensitive assay for quantification of Clopidogrel in order to investigate its pharmacokinetic parameters in human plasma and its application in comparative bioavailability study of Clopidogrel 75mg Tablets manufactured locally (test) and originally (Reference).

Methods: After extraction of Clopidogrel from human plasma, it was chromatographed with mobile phase consisting of 0.5% formic acid: Methanol (20:80 V/V) at flow rate 0.44ml/min, ESI positive mode, and m/z 321.9→211.9 for Clopidogrel.

The bioequivalence study was conducted in a Two-Way Open-Label, Crossover design involving 24 volunteers. The criteria used to assess bioequivalence of the two products were AUC_{0-t} , AUC_{0-inf} , C_{max} , and T_{max} .

Results: the described method of analysis showed that the average recovery of Clopidogrel from human plasma was 100.312%. The limit of Quantitation was 0.01ng/ml, and the Correlation coefficient (r_2) was equal to 0.999649.

Statistical analysis (ANOVA) of the measured parameters showed that there was no significant difference between the two products.

Conclusion: the LC/MS/MS method presented is direct, simple, reproducible, sensitive, and linear for the determination of Clopidogrel in human plasma, and is adequate for its clinical pharmacokinetic studies, besides the test product was found to be bioequivalent to the reference and both products can be considered interchangeable in medical practice.

Introduction

Clopidogrel is a potent antithrombotic drug that inhibits ADP-mediated platelet activation and aggregation; its antiaggregating effect is attributed to an irreversible inhibition of ADP binding to a purinergic receptor present at the platelet surface [1].

It is available as a generic drug. It is marketed in the form of tablets as a clopidogrel bisulfate under brand name Plavix 75 and 300mg [2]. Clopidogrel is indicated for prevention of thromboembolic diseases [3]. Clopidogrel is very efficient in reducing ischemic cardiovascular events but exposes patients to an increased risk of bleeding, and so the optimal dose and duration determination is important [4].

Clopidogrel is given as a single daily dose of 75 mg. In patients suffering from acute coronary syndrome, clopidogrel treatment is initiated with a single 300-mg loading dose and then continued at 75 mg once a day with acetylsalicylic acid 75 mg to 325 mg daily [5].

Clopidogrel may reduce risk of postoperative myocardial infarction for patients who have a coronary artery stent [6]. It was proven that clopidogrel plus aspirin is more safe and tolerable than any other ADP receptor antagonists plus aspirin, with a notable no increased risk of bleeding. It was proven that a 300mg loading dose of clopidogrel is well tolerated [7].

After single dose administration of 75mg clopidogrel tablet, mean C_{max} , AUC_{last} , AUC_{inf} , $T_{1/2}$ was 4.39±2.58 ng/ml, 11.98±3.87 ng.hr/ml, 12.43±9.94 ng.hr/ml, 6.06±3.87hr respectively. Median T_{max} was equal to 1 hour [8].

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Different analytical methods used for determination of Clopidogrel in biological samples using HPLC-UV methods shows poor quantitation limits LLOQ of 5ng/ml and is not enough for quantitation of clopidogrel in pharmacokinetic application [9].

For more sensitive determination, LC/MS/MS is used for determination of Clopidogrel in human plasma. Following a liquid-liquid extraction, Clopidogrel and internal standard were separated using an isocratic mobile phase on a reversed-phase column and analyzed by mass spectrometry in the multiple reaction monitoring mode using the respective mass to charge ratios, m/z 322/212 for clopidogrel and m/z 264/154 for the internal standard, with a quantitation limit (LLOQ) of 5 pg/ml and a linear dynamic range of 5 pg/ml to 6000 pg/ml [10].

Another rapid and sensitive LC/MS/MS assay developed for determination of clopidogrel in human plasma in which clopidogrel was extracted by single liquid-liquid extraction with pentane using 0.5 mL of human plasma. Chromatographic separations were achieved

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on a C_{18} column. The method was fully validated. The multiple reaction monitoring was based on m/z transition of 322.2 \rightarrow 211.9 for clopidogrel and 264.1 \rightarrow 125.1 for ticlopidine (internal standard). The total run time was 3 min, and the LLOQ was 10 pg/mL. The assay was linear over a concentration range from 10 to 10000 pg/mL with r^2 greater than 0.999 [11].

For a more linear dynamic range, a fast, sensitive and specific LC/MS/MS method for the determination of clopidogrel in human plasma developed and validated over the range of 10–12000 pg/ml with r^2 of 0.9993. 0.3 mL of plasma Samples were buffered with (buffer pH 6.8), extracted using diethyl ether and 10 μ L of the sample extract was injected onto the LC/MS/MS system. Analysis was performed using a C8 column (temperature controlled to 50 $^{\circ}$ C) using gradient elution at a flow rate of 0.9 ml/min, and run time of 3 min. Detection was achieved in positive electrospray ionization mode. Ion transitions were monitored using MRM for clopidogrel (m/z 322-212) and for 2H3-clopidogrel (m/z 327-217) [12].

This study was performed to investigate the bioequivalence of Clopidogrel between a generic Test Product; Clopidogrel 75mg Tablets and Reference Product. The study protocol called for 24 healthy volunteers. The subjects received One Tablet of test product and One Tablet of reference product, in a randomized fashion with a washout period of Seven days. Twenty-four healthy male volunteers completed the crossover [13]. The bioanalysis of clinical plasma samples would be accomplished through development of an LC/MS/MS method, which was developed and validated in accordance with the international guidelines at DRC [14].

Pharmacokinetic parameters, determined by standard non-compartmental methods, and analysis of variance (ANOVA) statistics were calculated using M-stat software. The significance of a sequence effect was tested using the subjects nested in sequence as the error term. The 90% confidence intervals for the ratio (or difference) between the test and reference product pharmacokinetic parameters of AUC_{0-t} and AUC_{0-inf} and C_{max} were calculated and found to be within the 80.00% to 125.00% confidence limits [15].

Materials and Methods

Chemicals and reagents

1. Water of HPLC grade.
2. Human plasma (Vacsera Blood Bank).
3. Methanol HPLC Grade (Scharlau, Spain).
4. Diethyl ether (Scharlau, Spain).
5. Formic acid (Scharlau, Spain).
6. Ammonium acetate (Scharlau, Spain).

Equipments

Adjustable Pippettes (P200, P1000 and P10000)

1. Disposable plastic pipettes tips - Labtip Yellow (range 5 - 200 μ L) & Labtip Blue (range 200 1000 μ L)
2. Disposable Glass test tubes 120 x 12 mm
3. Vortex mixer (Boeco, Germany).
4. Vacuum pump (Boeco, Germany).
5. PH-meters (Boeco, Germany).

6. Water purifier (Purelab option- R7, and Classic ELGA, U. K.).
7. Sonicator (Crest, U.S.A.)
8. Analytical balance (Sartorius, U.S.A.)
9. Concentrator Plus/Vacufuge[®] plus (Eppendorf, Germany)

LC-MS/MS Components

1. Quaternary pump: Agilent 1200 series, USA
2. Degasser: Agilent 1200 series, USA
3. Autosampler: Agilent 1200 series, USA
4. Mass Detector: Agilent 6410B Triple Quad, USA
5. Data system: Mass Hunter, Agilent.
6. A C_{18} reversed phase column, Phenomenex Luna C_{18} , 4.6 x 50 mm I.D, 5.0 micron.
7. Guard column Phenomenex C_{18} , 4 x 3 mm I.D.

Methods

LC/MS/MS assay

Chromatographic conditions

In house developed chromatographic conditions was used. Mobile phase composition is 0.5% formic acid: Methanol (20:80 V/V). The flow rate was set at 0.44ml/min. Injection volume was set at 10 μ L. MS/MS 6410B detector was operated at ESI positive mode, m/z was 321.9 \rightarrow 211.9 for Clopidogrel, and 264 \rightarrow 154.2 (Internal standard) Ticlopidine. Fragmentor energy was set at 144 for both Clopidogrel, and (internal standard) Ticlopidine. Collision energy was set at 12 for both Clopidogrel, and internal standard.

Clopidogrel stock standard solution

1. Accurately weigh 13.02mg of Clopidogrel bisulfate standard equivalent to 10 mg Clopidogrel base. Transfer into a 100 ml volumetric flask. Add about 80 ml Methanol. Sonicate for 10 minutes. Complete to volume with Methanol. This solution contains 100ug/ml Clopidogrel "Solution A".
2. Transfer 0.1ml of Solution A into a 100ml volumetric flask and complete to volume with water to obtain 100ng/ml "Solution B".

From "Solution B" the following were prepared

Working solutions

In order to fulfill the needs for study validation, the following solutions, based on the corresponding master standard solution, should be prepared in volumetric flask using methanol as a solvent.

Master Solution used	Mililitres taken	Final concentration obtained (ng/ml)	Final volume (ml)
"Solution B"	0.01ml	0.1	10
"Solution B"	0.05ml	0.5	10
"Solution B"	0.1ml	1	10
"Solution B"	0.25ml	2.5	10
"Solution B"	0.5ml	5	10
"Solution B"	1ml	10	10
"Solution B"	2.5ml	25	10
"Solution B"	5ml	50	10
"Solution B"	10ml	100	10

Ticlopidine standard solution

Accurately weigh 10mg of Ticlopidine Standard. Transfer into a 100 ml volumetric flask. Add about 80 ml of Methanol. Sonicate for 10 minutes. Complete to volume with Methanol. This solution contains 100ug/ml Ticlopidine "solution A" and from "solution A" transfer 25 ul to a 100 ml volumetric flask and complete volume with methanol to obtain "solution B" 25ng/ml.

Preparation of clopidogrel standard concentrations in human plasma

The standard samples in human plasma were prepared by transferring a 50 ul aliquot of the working standard solutions of Clopidogrel at concentrations ranging from 0.1 to 100 ng/ml to a centrifuge tubes containing 0.5 ml of blank human plasma.

Sample preparation

Volunteers human plasma samples, standard samples (500 ul) were transferred into appropriate centrifuge test tubes 50 ul of the internal standard (Ticlopidine working solution 25ng/ml) were added. Then samples were Vortex-mixed for approximately 1 minute. 1 ml of Acetate buffer pH 6.8 were added. Then samples were Vortex-mixed for approximately 1 minute. 3ml of Diethyl ether were added and Vortex-mix was done for approximately 1 to 2 minutes. Centrifugation of samples was done at 4000rpm for 5 minutes; clear organic supernatant layer was transferred to clean test tube and evaporated till dryness. The remaining residue was reconstituted with 200ul mobile phase and transferred to insert vial for injection and quantitation on LC/MS/MS.

Quantitation

The unknown sample concentration of volunteer human plasma was calculated from the following formula: $Y = aX + b$. Where Y is the peak area ratio, X is the unknown Concentration of Clopidogrel in human plasma samples, a is the slope of the calibration curve, b is the Y-Intercept.

All the previously mentioned procedure was performed stepwise by means of LC-MS/MS instrument using Mass Hunter Quantitative Analysis software, where it substitutes the obtained peak area ratio in the equation and calculates the concentration which is added in the printed output of the LC-MS/MS device for each chromatogram.

Bioequivalence study

Subjects

Twenty-four healthy adult, male volunteers participated in this study were subjected to complete physical examination and neurological assessment, urine analysis and blood (hematology, biochemistry, and serology). None of the volunteers had any history of drug or alcohol abuse, nor did they have any acute or chronic gastrointestinal, cardiac, vascular, hepatic, or renal disease. No concurrent medication was allowed during the course of the study. Subjects did not receive any meals for four hours after study dose administration, neither any beverages drink, nor coffee or tea. At 10:15 a.m. they received a standard meal, and at 2:15 p.m. another meal. The written informed consent for the intended study were reviewed, discussed and then signed by the participant and clinical investigator before the beginning of screening procedure without any obligation on the volunteers to continue if they didn't want to.

Study design

This study was a single-center, open-label, randomized, single-dose study with Two-way crossover design to compare the bioavailability of Clopidogrel between two products, in 24 healthy adult, male volunteers under fasting conditions with a washout period seven days between dosing. The number and disposition of the blood collections as well as the wash out period were designed with respect to pharmacokinetic parameters of Clopidogrel.

Sample collection

The number of blood collections for drug analysis was 17 samples in each study period. The volume of blood taken for the determination of clopidogrel in plasma was 5ml per sample. The following blood samples for the analysis of clopidogrel in plasma were collected at the following intervals: 0 (directly prior to dosing), 10min, 20min, 30min, 45min, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24 and 48 hours after after the administration. Blood samples were collected into tubes containing EDTA disodium as an anticoagulant slightly shaken, and centrifuged at approximately 4000 r.p.m. for 10 minutes. After centrifugation, plasma samples were transferred directly into a 5 ml-plastic tube. These samples were immediately stored at the study site in a freezer at a nominal temperature -80 °C until analysis. The label of the collecting tubes had the study's code number, subject number, study period, and the designated sample number. The total amount of blood loss during the whole study did not exceed 170 ml.

Analysis of plasma samples

A high-performance liquid chromatography coupled with Triple Quad Mass Detector LC-MS/MS was used for the determination of Clopidogrel in human plasma. Samples from 24 subjects (who completed both periods of the study) were analyzed. The bioanalytical method was validated according to the international guidelines.

Pharmacokinetic calculations

The following Pharmacokinetic parameters (variables) of clopidogrel were assessed; Maximum plasma concentration (C_{max}), Time point of maximum plasma concentration (t_{max}), Half-life of drug elimination during the terminal phase ($t_{1/2e}$), and terminal rate of elimination (K_e), Area under plasma concentration-time curve from zero to the last quantifiable concentration estimate (AUC_{0-t}), Mean Residence Time (MRT), Area under plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$), Assessment of drug absorption rate ($C_{max}/AUC_{0-\infty}$), Percent of the area measured by AUC_{0-t} relative to the extrapolated total $AUC_{0-\infty}$ [$(AUC_{0-t}/AUC_{0-\infty}) \times 100$].

Statistical analysis of data

Statistical analysis of the determined pharmacokinetic data was performed using statistical computerized program M-Stat software for determination of analysis of variance (ANOVA). Bioequivalence could be demonstrated for Clopidogrel within the prescribed 90% confidence interval of 80.00% to 125.00% for AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} with respect to the parametric method on Ln-transformed data.

Results and Discussion

LC/MS/MS assay

Analytical procedure validation

Chromatogram of Clopidogrel

Clopidogrel was well separated and its retention time was 2.9 min. sharp, and symmetrical peaks were obtained with good baseline resolution and minimum tailing, thus facilitating the accurate measurement of the peak area. The in house developed chromatographic conditions, was nearly in accordance to published literature [10-12] after modifying some conditions.

Linearity and quantitation

Peak area ratios of varying amounts of Clopidogrel in human plasma (Ranging from 0.01 to 10 ng/ml) was highly linear (r^2 was equal to 0.999649). The results of three replicate analysis of Clopidogrel at three different days over one week period were obtained, where the average CV% was 0.999649, which is in accordance with the latest FDA Guidelines [14], and so it could be used for pharmacokinetic and bioavailability studies of Clopidogrel.

Precision and accuracy

To assess the precision and accuracy of the developed analytical method, three distinct concentrations in the range of expected concentrations were evaluated. Precision and Accuracy was assessed at within-day basis, which defines those parameters during a single analytical run; and at between-day basis, which measure the between day variability, possibly involving different analysts, reagents, etc. The results of within day accuracy of Clopidogrel showed an average recovery percentage of 100.312%. The results of between days accuracy of Clopidogrel showed an average recovery percentage of 99.015%, with an average CV% of 0.561%. The results of freeze-thaw stability, short term stability, and long term stability of Clopidogrel in human plasma showed that the average recovery of Clopidogrel was greater than 96% providing that Clopidogrel is stable in the studied condition.

The method used for sample preparation is in accordance with published literature [10-12] which applies liquid- liquid extraction technique for sample preparation.

Bioequivalence study

Clinical observation

All the participating volunteers well tolerated the drug and the procedure adopted in the study. Every sample from the 24 volunteers during each phase was obtained at the proper. No serious adverse event, or unexpected adverse drug reaction occurred during the study. No AE's were observed in either period.

Pharmacokinetic data and assessment of bioequivalence

The assessment of bioequivalence, as a measure of efficacy, was based on the pharmacokinetic parameters derived individually for each participant from the Clopidogrel concentration in plasma. The mean maximum plasma concentration (C_{max}) was 2.247 ± 0.147 ng/ml

and 2.254 ± 0.175 ng/ml, time point of maximum plasma concentration (t_{max}) 0.938 ± 0.169 hr and 0.927 ± 0.188 hr, half-life of drug elimination during the terminal phase ($t_{1/2\alpha}$) 6.385 ± 0.260 and 6.308 ± 0.278 hr, area under plasma concentration-time curve from zero to the last quantifiable concentration estimate (AUC_{0-t}) 9.824 ± 1.695 ng.hr/ml and 10.335 ± 1.909 ng.hr/ml. Moreover, the Mean Residence Time (MRT) 6.682 ± 0.604 hr and 6.694 ± 0.428 hr, Area under plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$) 9.942 ± 1.704 ng.hr/ml and 10.444 ± 1.909 ng.hr/ml for test and reference products respectively.

The results of Clopidogrel pharmacokinetic parameters obtained was nearly in accordance with reported literature which stated that T_{max} for Clopidogrel were found to be 1 hour, C_{max} 3002.99 ± 1832.68 pg/ml, $T_{1/2}$ 6.06 ± 3.87 hours [8,15].

Statistical analysis

The data obtained from measurements of plasma concentration was transformed prior to analysis using natural Ln transformation. Pharmacokinetic parameters, e.g: C_{max} , T_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were analyzed using two-way ANOVA procedure to rule out the possibility of a significant carryover effect. Also 90% confidence interval of 80.00% to 125.00% for AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} with respect to the parametric method on Ln-transformed data should be fulfilled. The results of 2-way ANOVA on C_{max} , T_{max} , AUC_{0-t} and $AUC_{0-\infty}$ for Clopidogrel showed that there was no significant difference between test and reference product.

In this study the point estimate (%) for C_{max} , AUC_{0-t} , $AUC_{0-\infty}$ were 99.759%, 95.289%, 95.414% respectively. The 90% confidence intervals of parametric means of C_{max} , AUC_{0-t} , $AUC_{0-\infty}$ were 97.040% to 102.554%, 90.401% to 100.442%, 90.555% to 100.534% respectively, thus providing a 90% confidence intervals limits lying within FDA acceptance limits (80 % to 125%) [16].

Discussion

The LC/MS/MS method used in this study was simple, of excellent sensitivity, specificity, precision and accuracy. The calibration curve was linear over the concentration range of 0.01 to 10 ng/ml (r^2 was equal to 0.999649), which is in accordance with the latest FDA Guidelines [14], and so it could be used for pharmacokinetic and bioavailability studies of Clopidogrel.

The in house developed chromatographic conditions, was nearly in accordance to published literature [10-12] after modifying some condition. The method used for sample preparation is in accordance with published literature [12] which applied buffered plasma samples using buffer pH6.8 followed by liquid- liquid extraction technique for sample preparation.

The use of crossover designs for bioequivalence studies allows each subject to serve as his own control to improve the precision of comparison. One of the assumptions underlying this principle is that carryover effects (also called residual effect) are either absent or equal for each formulation and preceding formulation.

The mean T_{max} for Clopidogrel were found to be 0.938 ± 0.169 hr and 0.927 ± 0.188 hr, C_{max} 2.247 ± 0.147 ng/ml and 2.254 ± 0.175 ng/ml, AUC_{0-t} 9.824 ± 1.695 ng.hr/ml and 10.335 ± 1.909 ng.hr/ml, $AUC_{0-\infty}$ 9.942 ± 1.704 ng.hr/ml and 10.444 ± 1.909 ng.hr/ml, $T_{1/2}$ 6.385 ± 0.260 and 6.308 ± 0.278 hr for test and reference products respectively.

Pharmacokinetic Parameter	Treatment (Mean ± SD)	
	Test product	Reference product
C _{max} (ng/ml)	2.247±0.147	2.254±0.175
T _{max} (hr)	0.938±0.169	0.927±0.188
AUC _{0-t} (ng. hr/ml)	9.824±1.695	10.335±1.909
AUC _{0-inf} (ng. hr/ml)	9.942±1.704	10.444±1.909
Ke (hr ⁻¹)	0.109±0.004	0.110±0.005
t (1/2) _e (hr)	6.385±0.260	6.308±0.278
MRT	6.682±0.604	6.694±0.428

Table 1: Pharmacokinetic parameters for Clopidogrel tablets of Test and Reference Products.

Pharmacokinetic Parameter	90% Confidence intervals of parametric means		
	Point estimate (%)	Lower limit (%)	Upper limit (%)
C _{max}	99.759	97.040	102.554
AUC _{0-t}	95.289	90.401	100.442
AUC _{0-inf}	95.414	90.555	100.534

Table 2: 90 % C.I for Clopidogrel tablets Test and reference Products.

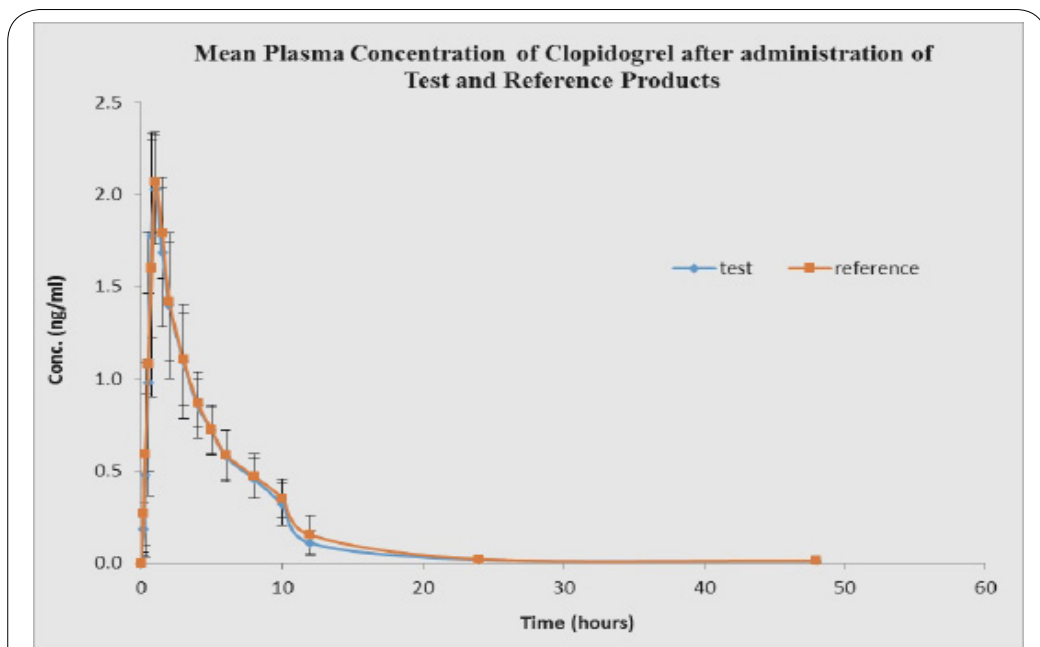


Figure 1: Mean Plasma concentration of Clopidogrel following single dose administration of Clopidogrel tablet test and reference products.

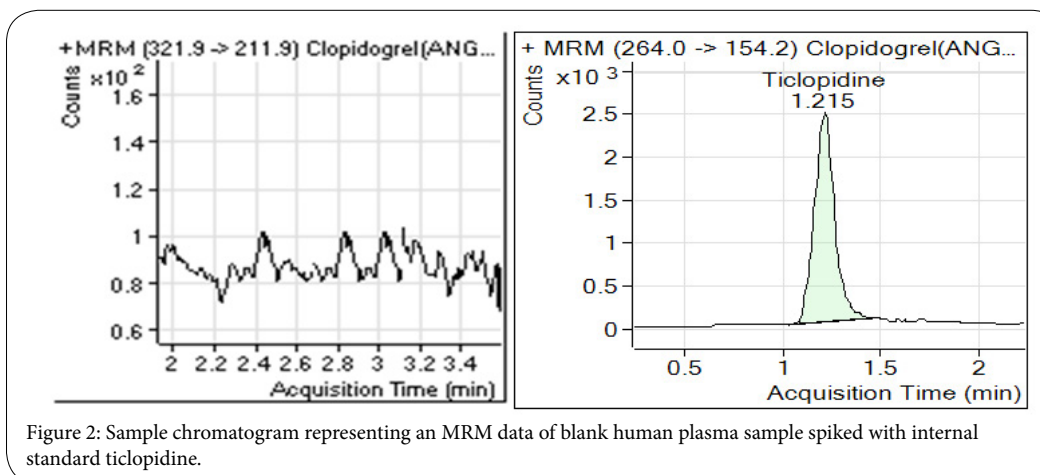


Figure 2: Sample chromatogram representing an MRM data of blank human plasma sample spiked with internal standard ticlopidine.

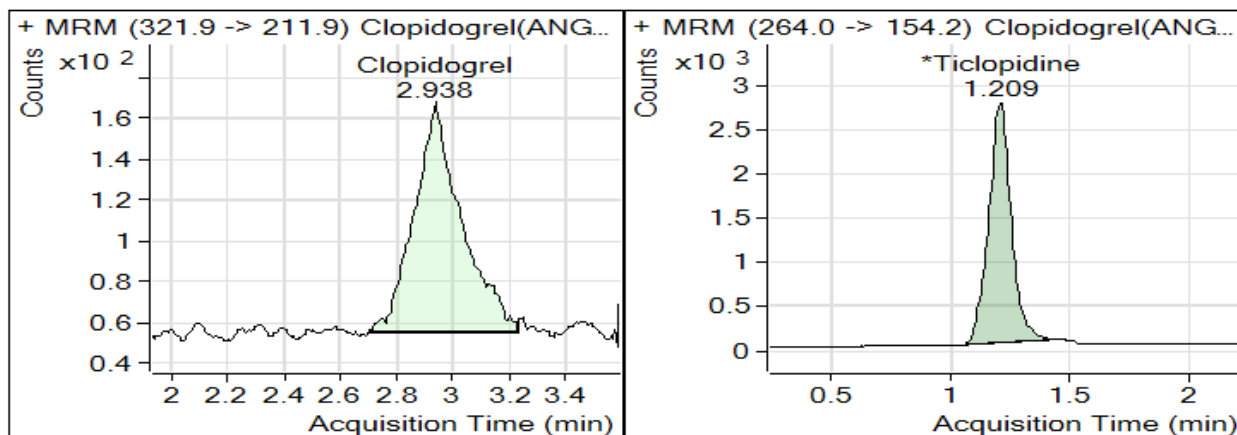


Figure 3: Sample chromatogram representing an MRM data of blank human plasma sample spiked with 0.05ng/ml Clopidogrel and internal standard Ticlopidine.

Subject	T _{max}	C _{max}	AUC _{0-t}	AUC _{0-inf}	K _{el}	T _{1/2}	MRT _{inf}
A	1.000	2.390	13.754	13.862	0.112	6.212	7.224
B	0.750	2.285	13.439	13.546	0.111	6.220	7.195
C	0.750	2.312	11.115	11.244	0.109	6.378	6.481
D	1.000	2.152	10.387	10.473	0.116	5.981	5.614
E	0.750	2.329	8.495	8.592	0.113	6.159	6.340
F	1.000	2.333	11.844	11.960	0.112	6.174	6.355
G	0.750	2.675	13.202	13.305	0.116	5.978	6.906
H	0.750	2.358	12.704	12.808	0.115	6.010	6.767
I	0.750	2.501	10.293	10.390	0.114	6.092	6.254
J	1.000	2.269	8.613	8.705	0.108	6.396	6.743
K	1.500	2.150	11.624	11.740	0.112	6.202	6.643
L	0.500	2.579	9.544	9.632	0.113	6.124	6.196
M	1.000	2.271	10.526	10.644	0.110	6.299	6.674
N	1.000	2.130	7.089	7.210	0.099	7.006	7.063
O	1.000	2.165	12.183	12.308	0.112	6.196	6.828
P	1.000	2.125	8.419	8.512	0.108	6.426	6.981
Q	1.000	1.915	8.469	8.571	0.108	6.400	6.603
R	1.000	2.036	9.496	9.619	0.106	6.562	7.004
S	1.000	2.346	11.044	11.146	0.117	5.903	5.860
T	1.000	2.059	10.030	10.158	0.102	6.795	7.273
U	1.000	2.063	8.143	8.300	0.102	6.790	7.199
V	1.000	2.198	7.629	7.733	0.106	6.569	6.834
W	0.750	2.290	8.912	9.022	0.109	6.356	6.647
X	1.000	2.175	11.081	11.179	0.113	6.155	6.968
Mean	0.927	2.254	10.335	10.444	0.110	6.308	6.694
SD	0.188	0.175	1.909	1.909	0.005	0.278	0.428
CV%	20.241	7.778	18.473	18.275	4.271	4.411	6.388
Min	0.500	1.915	7.089	7.210	0.099	5.903	5.614
Max	1.500	2.675	13.754	13.862	0.117	7.006	7.273
Median	1.000	2.270	10.340	10.432	0.112	6.216	6.755

Table 3: Pharmacokinetic parameters of CLOPIDOGREL following administration of single oral dose of (Reference product) to 24 Volunteers.

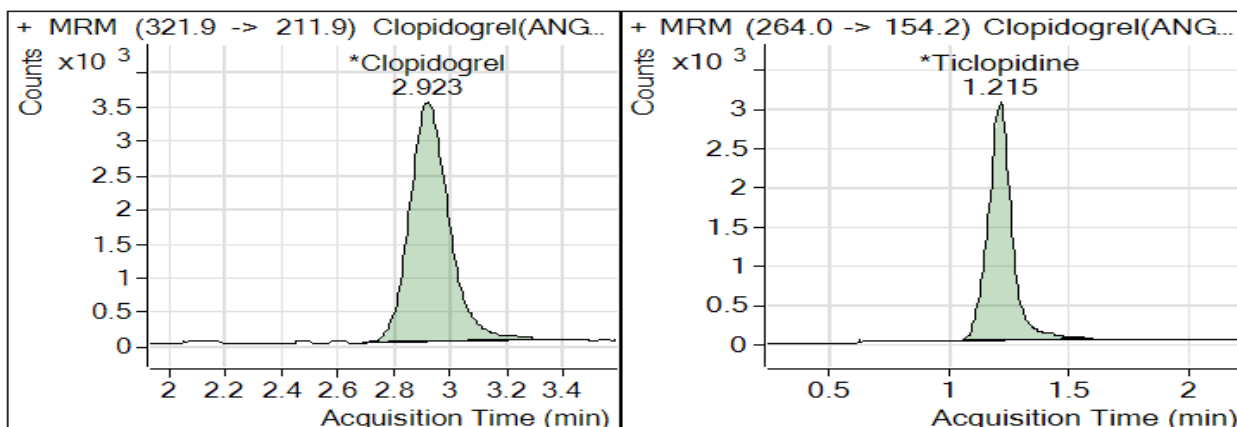


Figure 4: Sample chromatogram representing an MRM data of blank human plasma sample spiked with 5 ng/ml Clopidogrel and internal standard Quetiapine.

Subject	T _{max}	C _{max}	AUC _{0-t}	AUC _{0-inf}	K _{el}	T _{1/2}	MRT _{inf}
A	1.000	2.281	13.115	13.287	0.105	6.632	7.630
B	0.750	2.322	12.457	12.599	0.113	6.154	6.264
C	1.000	2.128	10.652	10.778	0.112	6.209	6.223
D	1.000	2.309	11.027	11.140	0.115	6.036	5.942
E	1.000	2.197	7.497	7.589	0.109	6.380	6.025
F	0.750	2.679	11.806	11.901	0.116	5.992	5.910
G	1.000	2.371	11.594	11.715	0.116	5.990	6.122
H	1.000	2.286	9.878	10.029	0.106	6.521	6.984
I	1.500	2.404	11.900	12.025	0.112	6.186	6.208
J	0.750	2.217	7.288	7.379	0.109	6.331	6.279
K	0.750	2.340	8.572	8.684	0.107	6.461	7.092
L	1.000	2.286	12.374	12.488	0.114	6.107	6.400
M	1.000	2.216	8.998	9.115	0.103	6.745	8.096
N	1.000	2.322	8.638	8.799	0.099	6.980	7.383
O	0.750	2.230	9.479	9.633	0.104	6.669	7.300
P	1.000	2.177	9.580	9.678	0.113	6.158	6.040
Q	1.000	2.077	9.889	10.001	0.108	6.435	6.772
R	1.000	1.925	8.393	8.494	0.109	6.377	6.583
S	1.000	2.043	8.500	8.614	0.105	6.624	6.378
T	0.750	2.196	9.061	9.163	0.108	6.415	6.380
U	0.750	2.293	7.486	7.602	0.104	6.684	7.313
V	0.750	2.362	8.198	8.311	0.107	6.490	7.060
W	1.000	2.100	9.460	9.567	0.112	6.172	6.628
X	1.000	2.170	9.924	10.027	0.107	6.493	7.367
Mean	0.938	2.247	9.824	9.942	0.109	6.385	6.682
SD	0.169	0.147	1.695	1.704	0.004	0.260	0.604
CV%	18.018	6.548	17.256	17.140	4.042	4.073	9.043
Min	0.750	1.925	7.288	7.379	0.099	5.990	5.910
Max	1.500	2.679	13.115	13.287	0.116	6.980	8.096
Median	1.000	2.256	9.530	9.656	0.108	6.398	6.491

Table 4: Pharmacokinetic parameters of CLOPIDOGREL following administration of single oral dose of (Test product) to 24 Volunteers.

The results of Clopidogrel pharmacokinetic parameters obtained was nearly in accordance with reported literature which stated that T_{max} for Clopidogrel were found to be 1 hour, C_{max} 3002.99 ± 1832.68 pg/ml, $T_{1/2}$ 6.06 ± 3.87 hours [8,16].

In bioequivalence study 90% confidence interval of 80.00% to 125.00% for AUC_{0-t} , AUC_{0-inf} and C_{max} with respect to the parametric method on Ln-transformed data should be fulfilled. In this study the point estimate (%) for C_{max} , AUC_{0-t} , AUC_{0-inf} were 99.759%, 95.289%, 95.414% respectively. The 90% confidence intervals of parametric means of C_{max} , AUC_{0-t} , AUC_{0-inf} were 97.040% to 102.554%, 90.401% to 100.442%, 90.555% to 100.534% respectively, thus providing a 90% confidence intervals limits lying within FDA acceptance limits (80 % to 125%) [15].

Conclusion

It can be concluded that the bioanalytical method developed for the determination of Clopidogrel in human plasma is valid, sensitive, specific, precise and accurate, and could be used for the determination of drug pharmacokinetic parameters. Besides, results of the bioequivalence study of Clopidogrel tablet (test product) compared to, versus the reference product are bioequivalent, since they deliver equivalent amounts of Clopidogrel to the systemic circulation at the same rate.

Competing Interests

The authors declare that they have no competing interests.

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