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Research Article

Rivaroxaban and Therapeutic Monitoring Application: Will it be a Significant Tool for Management of COVID-19?

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Abstract

Background: SARS-CoV-2 is considered one of the most widely spreaded viral infections globally. Expectation of second wave incidence of Corona virus 2019 is high. Direct inhibitors of factor Xa might be proposed as an adjuvant therapy for coagulation disorders associated with COVID-19.

Aim: Development of a bioanalytical assay for detection of rivaroxaban in human plasma and its pharmacokinetics application including bioavailability studies and monitoring of therapeutic levels to ensure its clinical outcomes as an adjuvant therapy in management of Corona virus 2019 disease.

Method: A protein precipitation technique was used for drug extraction from the plasma by using a mobile phase of 10 mM ammonium acetate pH4.7 and acetonitrile 20:80 V/V at flow rate 0.6 ml/min, ESI positive mode, and m/z 436→144.8, 338→296.2 for rivaroxaban and linezolid (as internal standard)respectively. As a clinical application, a bioequivalence study was conducted in a fully replicated crossover design involving 36 volunteers, where, AUC_{0-t}, $AUC_{0-inf,}$ C_{max}, and T_{max} were calculated for the assessment of bioequivalence of test and reference products.

Results: The average recovery of Rivaroxaban was 98.675%. The limit of Quantitation was 0.5 ng/ml, and Correlation coefficient (r^2) was equal to 0.9999. Statistical analysis of the measured parameters showed that there was no significant difference.

Conclusion: The developed LC/MS/MS bioanalytical method proved to be valid for the determination of rivaroxaban in human plasma and its pharmacokinetics application including comparative bioavailability studies which could be applied for therapeutic levels monitoring of rivaroxaban in management of blood coagulation associated with different diseases including COVID-19.

Keywords: Rivaroxaban; COVID-19; Venous Thromboembolism; LC/MS/MS; Factor Xa; Atrial Fibrillation

Introduction

Corona virus 2019 infection is progressively spreading and has a global remarkable effect on health state and the economic process [1]. As of 13 September 2020 the estimated patients suffering from COVID-19 infection reached 28,637,952 cases, with mortality rate of 3.20% [2].

This disease resulted from SARS-CoV-2 virus infection, where, by the end of 2019 several cases of pneumonia with an unknown etiology were observed in china and was thought that seafood market in Wuhan city in China is the source of infection. Route of transmission of SARS-CoV-2 is through spreading of respiratory droplets, sneezing, coughing, and inhalation of aerosol particles lead to viral penetration of upper the respiratory tract and lungs [3].

Rivaroxaban is acting by direct inhibition of factor Xa (activated factor X) with a dose-dependent effect on prothrombin time and is used in management of deep-vein thrombosis or pulmonary embolism, prevention of recurrence after initial treatment, and prophylaxis of venous thromboembolism in patients undergoing hip or knee replacement surgery [4].

Rivaroxaban is marketed in the form of Tablets, as a Rivaroxaban 2.5 mg, 10 mg, 15 mg and 20 mg under brand name Xarelto® Tablets. Following a knee and hip replacement surgeries, rivaroxaban is indicated for prophylaxis of venous thromboembolism in a 10 mg once per day dose for 2 weeks. In case of deep-vein thrombosis and pulmonary embolism rivaroxaban is given as an initial dose of 15 mg twice per day for a period of 21 days, followed by a dose of 20 mg once per day. Prophylaxis of recurrent deep-vein thrombosis, recurrent pulmonary embolism, systemic embolism, atherothrombotic events in patients with coronary artery disease [5].

In a clinical trial of people at high risk of ischaemic events, rivaroxaban in addition to aspirin showed a statistically significant relative risk reduction of 24% in major cardiovascular events in comparison to aspirin and reduction of 42% for stroke and 22% for CVS death. It was concluded that rivaroxaban plus aspirin contributes in reduction of cardiovascular event risks in comparison to aspirin alone and that the greatest effect is for ischaemic stroke [6].

A Cohort study in United States showed that patients initiating oral anticoagulant treatment with rivaroxaban had significantly shorter mean hospital length of stay (LOS) by 1.57 days compared with those treated with warfarin. Patients treated with rivaroxaban incurred significantly lower mean hospitalization costs by $1888 per admission than warfarin-treated ones. In view of rivaroxaban being found to afford the same clinical efficacy as vitamin K antagonist for treating venous thromboembolism (VTE) in clinical trials providing evidence for clinicians to consider the potential for decreasing hospitalization period, costs and simplification of treatment regimens by using newer oral anticoagulants to treat VTE [7].

Concerning the pharmacokinetics, it was found that following oral administration of single dose of rivaroxaban 10 mg tablet, the mean values (C.V.%) of C_{max}, AUC_{o-t} and AUC_{o-inf} was 161.1 (38.7%) ng/ml, 1252 (31.6%) ng.hr/ml and 1268 (30.7%) ng.hr/ml, respectively. The median T_{max} was 2 hour and the mean half life $T_{1/2}$ was 8.721 (55.7%) hr [8]. Another study performed on rivaroxaban 10mg reported that the obtained mean values (CV%) of C_{max}, AUC_{o-t} and AUC_{o-inf} were 215 (25%) ng/ml, 1390 (20%) ng.hr/ml, and 1420 (19.8%) ng.hr/ml, respectively, and the median T_{max}, and mean half life $T_{1/2}$ were 1.8 hour and 8.25 (42.4%)hr respectively [9]. Additionally, a pharmacokinetic study of rivaroxaban recorded that the mean(CV%) of C_{max}, and AUC_{o-t}, median T_{max} and elimination half life $T_{1/2}$ was 161.7 (17.2%) ng/ml, 1201 (21.3%) ng.hr/ml, 3 hours and 10.98 (44.7%) hr respectively [10].

Different methods are investigated and developed for the evaluation of rivaroxaban in pharmaceutical dosage form and biological fluids include high performance liquid chromatography (HPLC) with UV detection [11-13], LC–MS methods which are widely used for quantification of rivaroxaban in plasma [14,15].

LC-MS/MS is the most widely used one for therapeutic drug monitoring and bioavailability studies, due to its high sensitivity and specificity as well as adequate accuracy and precision [16-20] although other methods, such as SALDI-MS, have also been described [17].

Therapeutic drug monitoring [16] and pharmacokinetic studies of rivaroxaban which use LC-MS/MS bioanalytical methods for rivaroxaban determination in biological fluids have been reported in the literature [18,19].

Different bioanalytical methods used for quantification of rivaroxaban in biological samples reported different values for lower limit of quantitation (LLOQ) depending upon the different detection methods and sample treatment as follows; using HPLC-UV method and plasma sample preparation with solid phase extraction showed LLOQ of 10 ng/ml [21], using LC-MS-MS method showed a lower quantitation limits LLOQ of 24 ng/ml [22], and using LC/MS/MS and protein precipitation with acetonitrile showed LLOQ of 1 ng/ml [23].

A reported sensitive, specific and linear valid method for determination of rivaroxaban in biological samples using LC/MS/MS with protein precipitation using methanol, the drug and internal standard were chromatographed on a Kinetex C_{18} HPLC column pumping a mobile phase of 40% ultrapure water containing 0.01% formic acid and 60% methanol , 0.01% formic acid and the flow rate of 0.5 ml/min. The recorded quantitation limit (LLOQ) was of 2 ng/ml and a linear range of 2 ng/ml to 500 ng/ml [24].

The objective of this research was to develop a validated in-house bioanalytical method for the quantitation of rivaroxaban

in human biological fluid which can be introduced for application in bioavailability and pharmacokinetic studies, clinical trials and therapeutic drug monitoring of rivaroxaban in patients to ensure its safety and efficacy. Moreover, investigation of the bioequivalence of rivaroxaban generic product versus reference product.

Methods

LC/MS/MS analytical method

Mass parameters and chromatography

The method was developed in-house as follow: eluting solvent composition is 10 mM ammonium acetate pH 4.7: acetonitrile 20:80 V/V. The flow rate was set at 0.6 ml/min. Injection volume was set at 10 ul and MS/MS 6410B detector was operated at ESI positive mode, m/z was 436→144.8, 338→296.2 for rivaroxaban and linezolid (internal Standard) respectively. The fragment or energy was set at 80 for rivaroxaban and 100 for Linezolid and collision energy was set at 25 for rivaroxaban, and 15 for linezolid.

Preparation of solutions

Master standard solution

An accurately weighed 10mg of standard rivaroxaban was transferred to a 100 ml volumetric flask and 80 ml of methyl alcohol was added, the solution was sonicated for 10 minutes and volume was completed with methyl alcohol to obtain a solution containing 100ug/ml rivaroxaban "Solution A".

Then 2.5 ml from "Solution A" was transferred to a 100 ml volumetric flask and completed to volume with methyl alcohol to obtain a solution of 2500 ng/ml "Solution B".

Working solutions

Master Solution used	Mililitres taken	Final concentration obtained (ng/ml)	Final volume (ml)
"Solution B"	0.02 ml	5	10
"Solution B"	0.1 ml	25	10
"Solution B"	0.4 ml	100	10
"Solution B"	1 ml	250	10
"Solution B"	2 ml	500	10
"Solution B"	4 ml	1000	10
"Solution B"	6 ml	1500	10
"Solution B"	8 ml	2000	10
"Solution B"	10 ml	2500	10

Table: All dilutions are done with methanol.

Linezlid standard solution

Ten milligram of standard linezolid was transferred to a 100 ml flask and 80 ml of methyl alcohol was added, then sonication for about 10 minutes was done, and the volume was completed with methyl alcohol to obtain a solution containing 100 ug/ml linezolid solution (A). From solution (A), 1 ml was transferred into a 100 ml volumetric flask and volume was completed with methyl alcohol to obtain 1000 ng/ml Linezolid solution (B).

Preparation of serial dilutions of standard rivaroxaban in human plasma

Standard concentrations of rivaroxaban in human plasma were prepared by transferring a 50 ul aliquot of serial dilutions of standard rivaroxaban concentrations ranging from 2 to 2500 ng/ml to a centrifuge tubes containing 0.5 ml of blank plasma.

Sample preparation

A 500 ul of human plasma samples was added to centrifuge test tubes and 50 ul of internal standard (linezolid working solution 1000 ng/ml) was added and vortex-mix of the samples for approximately one minute. One ml acetonitrile was added and vortex-mix the samples for 1 to 2 minutes, followed by centrifuging at 4500 rpm for 5 minutes and the clear supernatant layer was transferred to a clean vial for quantitation on LC/MS/MS.

Quantitation

The unknown volunteer sample concentration was calculated as per formula: $y = ax + b$. Where Y is the response ratio, X is the unknown concentration of Drug in human plasma samples, a calibration curve slope; b is the Y-Intercept.

Ethics

The bioavailability application was performed according to the Declaration of Helsinki, the study protocol was reviewed by the institutional review board followed by approval from the ethics committee of Drug Research Centre.

Moreover, written informed consent were reviewed, discussed and then signed by the participant and clinical investigator before starting the screening procedure without any obligation on the volunteers to continue if they didn't want to.

Pharmacokinetics application

Study protocol and study design

The study protocol called for 36 healthy volunteers in a fully replicated crossover study design for comparative bioavailability of generic rivaroxaban versus reference products Subjects dosing

was performed as follows; at period 1; volunteers were randomly assigned to one of two treatment sequences (TRTR (sequence 1) or RTRT (sequence 2) as indicated in the randomization scheme, where, subjects in sequence 1 received treatment T at the first dosing period and crossed over to receive treatment R at the second dosing period then received treatment T again in the third dosing period and crossed over to receive treatment R at the fourth dosing period. Subjects in sequence 2 received treatments in the order of R, T, R, and T at the four dosing periods with a washout period of one week between each dosing period.

The number and disposition of the blood collections as well as the wash- out period were designed with respect to pharmacokinetic parameters of rivaroxaban.

Subjects

- Thirty-Six healthy adult subjects participated in the bioequivalence study were subjected to general physical examination, neurological assessment, urine analysis and blood analysis. The selected subjects possessed no history of drug or alcohol abuse.
- All subjects didn't have any acute or chronic gastrointestinal, cardiac, vascular, hepatic, or renal disease and concurrent medication was not allowed during the time course of the study. Meals, beverages drink, coffee or tea were not allowed for four hours after study dose administration.
- At 11:00 a.m. they received a standardized breakfast meal followed after four hours by the second meal at 3:00 p.m.

Sample collection

The number of blood samples collected for drug analysis were 17 samples in each study period and volume of blood withdrawn for determination of rivaroxaban in plasma was 5 ml per sample. The following blood samples for the analysis of rivaroxaban in plasma were collected at the following intervals: 0 (directly prior to dosing), 15 min, 30 min, 45 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8 ,10, 12, 24 and 48 hours after the administration.

Blood sample collection was performed into a tubes containing anticoagulant EDTA disodium and centrifuged at approximately 4000 r.p.m. for 10 minutes and plasma samples were separated in a 5 ml-plastic was sermann tube and the collected samples were kept at a -80°C freezer until analysis, where, the total amount of blood withdrawn during the whole study did not exceed 340 ml.

Analysis of samples

Thirty-Six healthy male volunteers completed the crossover [25]. The bioanalysis of plasma samples was performed through a developed LC/MS/MS technique for the quantitation of rivaroxaban in plasma samples. The bioanalytical method was validated to fulfill the requirements of the international guidelines [26].

Pharmacokinetic calculations

The pharmacokinetics parameters were calculated by standard non-compartmental analysis module including; maximum plasma concentration (C_{max}), time point of maximum plasma concentration (t_{max}), half-life of drug elimination during the terminal phase ($t_{1/2e}$), terminal rate of elimination (K_e), area under curve from zero to time (t) (AUC_{0-t}), Mean Residence Time (MRT), area under 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 $AUC_{0-\infty}$ [$(AUC_{0-t}/AUC_{0-\infty})_X 100$].

Statistical analysis of data

Statistical analysis (ANOVA) were calculated using SAS software, where, the 90% confidence interval (C.I.) for the ratio of AUC_{0-t}, AUC_{0-inf}, and C_{max} were found to be within the 80% to 125% confidence limits [27].

Results

Analytical procedure validation

Chromatograms of rivaroxaban

Rivaroxaban and its internal standard were well separated and their retention time was 1 and 0.9 min respectively, and the resulted peaks were sharp and symmetrical with a good baseline resolution and minimum tailing thus facilitating the accurate measurement of peak response. The in house developed chromatographic conditions was in accordance with published literature [21-24] with some modification in extraction and chromatographic conditions.

Linearity, precision, and accuracy

Peak area ratios of varying amounts of rivaroxaban in human plasma (range from 0.5 to 250 ng/ml) was highly linear (r^2 was 0.9999). The average results of interday variation CV% were 0.943% which is in compliance with FDA Guidelines (FDA 2018).

Accuracy and precision was assessed at within-day basis at three drug concentrations in the range of expected concentra-

tions and at between-day basis. The results of intra-day accuracy showed an average recovery percentage of 98.675%. The results of inter-day accuracy showed an average recovery percentage of 100.037% with an average C.V.% of 0.943%. The results of freeze-thaw, short term, and long term stability in human plasma showed that the average recovery of rivaroxaban was greater than 95% providing that both rivaroxaban and linezolid were stable in the studied conditions.

Comparative bioavailability (bioequivalence) study

- **Clinical observation:** All the participating volunteers well tolerated the drug and the procedure adopted in the study, every sample from the 36 volunteers during each phase was obtained at the proper time, no serious side effects or unexpected adverse drug reaction occurred during the study.
- **Pharmacokinetics data:** The mean values and standard deviation for the first dose administration of rivaroxaban generic and reference products were: C_{max} 137.445 ± 21.894 ng/ml and 133.508 ± 26.273 ng/ml, AUC_{o-t} 964.628 ± 174.563 ng.hr/ml and 933.791 ± 176.852 ng.hr/ml, AUC_{o-inf} 988.080 ± 176.259 ng.hr/ml and 953.626 ± 174.769 ng.hr/ml respectively.

Concerning the second dose administration of rivaroxaban generic and reference products were as follows; C_{max} 139.094 ± 20.195 ng/ml and 139.200 ± 23.233 ng/ml, AUC_{o-t} 972.712 ± 173.266 ng.hr/ml and 965.532 ± 208.139 ng.hr/ml, AUC_{o-inf} 998.135 ± 177.320 ng.hr/ml and 989.594 ± 213.628 ng.hr/ml respectively.

The results of rivaroxaban pharmacokinetic parameters obtained were nearly in accordance with reported literature which stated that T_{max} were found to be ranged from 1.8 to 3 hours, C_{max} 161ng/ml, and $T_{1/2}$ were 8.25 hr in average [8-10].

Statistical analysis

The results of Two-way ANOVA was undergone on the following pharmacokinetics parameters C_{max}, T_{max}, $AUC_{0-t,}$ and AUC_{0-inf} to investigate the significance between generic and reference products of rivaroxaban showed a non- significant difference.

The 90% confidence interval of 80.00% to 125.00% for AUC_{0-t}, AUC_{0-inf} and C_{max} on Ln-transformed data should be fulfilled. In this study the point estimate (%) results for C_{max}, AUC_{0-t}, AUC_{0-inf} were 100.46%, 100.39% and 100.42% respectively. The 90% confidence intervals of parametric means of C_{max}, AUC_{0-t} and AUC_{0-inf} were 99.82% to 101.10%, 99.81% to 100.97%, and 99.85% to 100.99% respectively, thus providing a 90% confidence intervals limits lying within FDA acceptance limits (80% to 125%) [27].

Subject	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)	K_{el} (hr^{-1})	$T_{1/2}$ (hr)	MRT_{inf} (hr)
Mean	2.347	137.445	964.628	988.080	0.089	7.924	10.108
CV%	16.749	15.929	18.096	17.839	12.411	13.060	14.964
Range	1.50-3.00	105.034-201.605	647.497-1293.370	656.671-1318.655	0.064-0.111	6.265-10.762	7.215-13.980
(Median)	(2.500)	(135.416)	(934.440)	(963.431)	(0.090)	(7.703)	(9.923)

Table 1: Pharmacokinetics of rivaroxaban of generic product administrated at periods I and II.

Subject	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)	K_{el} (hr^{-1})	$T_{1/2}$ (hr)	MRT_{inf} (hr)
Mean	2.264	133.508	933.791	953.626	0.092	7.703	9.703
CV%	20.776	19.679	18.939	18.327	14.747	16.827	23.156
Range	1.00-3.00	90.275-201.403	608.449-1357.512	631.399-1368.523	0.054-0.116	5.985-12.755	6.612-18.826
(Median)	(2.500)	(132.016)	(959.195)	(983.229)	(0.090)	(7.728)	(9.642)

Table 2: Pharmacokinetics of rivaroxaban of reference product administered at periods I and II.

Subject	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)	K_{el} (hr^{-1})	$T_{1/2}$ (hr)	MRT_{inf} (hr)
Mean	2.153	139.094	972.712	998.135	0.089	7.974	10.116
CV%	19.088	14.519	17.813	17.765	16.067	15.220	16.714
Range	1.50-3.00	100.649-196.482	643.700-1354.509	654.110-1383.909	0.064-0.129	5.386-10.769	7.294-15.584
(Median)	(2.000)	(136.004)	(961.659)	(982.995)	(0.089)	(7.763)	(10.032)

Table 3: Pharmacokinetics of rivaroxaban of generic product administered at periods III and IV.

Subject	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)	K_{el} (hr^{-1})	$T_{1/2}$ (hr)	MRT_{inf} (hr)
Mean	2.153	139.200	965.532	989.594	0.088	8.008	10.117
CV%	19.879	16.690	21.557	21.587	12.783	13.505	17.854
Range	1.00-3.00	101.845-204.951	631.366-1597.792	644.239-1642.105	0.059-0.110	6.308-11.824	7.054-17.184
(Median)	(2.000)	(133.145)	(917.037)	(941.118)	(0.087)	(7.967)	(10.072)

Table 4: Pharmacokinetics of rivaroxaban of reference product administered at periods III and IV.

Pharmacokinetic Parameter	90% C.I of parametric means			90% C.I. of Parametric standard deviation
	Point estimate (%)	Lower limit (%)	Upper limit (%)	Upper limit
C_{max}	100.46	99.82	101.10	0.68
AUC_{0-t}	100.39	99.81	100.97	0.73
AUC_{0-inf}	100.42	99.85	100.99	0.73

Table 5: 90% C.I for rivaroxaban generic and reference products.

Figure 1: Plasma Concentration (Mean ± S.D.) of Rivaroxaban Following Single Dose Administration of Rivaroxaban 10mg Tablet Generic and Reference Products.

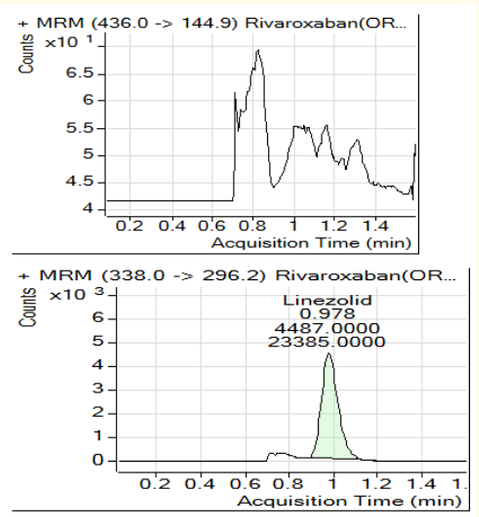

Figure 2: Chromatogram-an MRM Data of Blank Plasma Spiked with Internal Standard Linezolid.

Figure 3: Chromatogram - an MRM Data of Blank Plasma Spiked with 0.5ng/ml Rivaroxaban and Internal Standard Linezolid.

Figure 4: Chromatogram - an MRM Data of Blank Plasma Spiked with 150ng/ml Rivaroxaban and Internal Standard Linezolid.

Discussion

The developed validated LC/MS/MS method used for the detection of rivaroxaban in human plasma was simple, of excellent sensitivity, specificity, precision, accuracy and linear over the range of 0.5 to 250 ng/ml with r^2 equal to 0.9999, which is in compliance with the latest FDA Guidelines [26], thus, could be applied in clinical trials, pharmacokinetics and bioavailability studies. Being a candidate for management and prevention of thrombosis; therapeutic drug monitoring and clinical studies of rivaroxaban is of high importance in assuring its achievement of therapeutic goals for management and prevention of coagulation disorders which is one of the significant symptoms of COVID-19 infected individuals.

The below mentioned clinical studies constitute an evidence of the medical importance and benefits of this study candidate, anticoagulant rivaroxaban, in the medical fields, and thus, requiring an accurate detection of rivaroxaban in human plasma and its clinical applications in therapeutic drug levels monitoring on outpatients and in patients suffering from coagulation disorders in different diseases including COVID-19.

A recent study showed that the rivaroxaban may inhibit and protect different cases including COVID-19 patients from progressing to venous thromboembolism and deep vein thrombosis and that it showed to be superior in lowering the incidence of venous thromboembolism over low molecular weight heparin like enoxaparin [28].

A study included 14,236 atrial fibrillation patients who administered a minimum of one dose of rivaroxaban showed that rivaroxaban was associated with favourable Net clinical benefit (NCB) compared with warfarin. The (NCB) was attributable to lower rates of ischemic events and fatal or critical organ bleeding in patients with atrial fibrillation (AF) [29].

It was reported that after 35 days of treatment the incidence of venous thromboembolism events is lower with rivaroxaban treatment (4.2%) compared to enoxaparin treatment (6.6%), also it showed a higher primary safety outcome without remarkable difference in major bleeding events among patients with pulmonary infections on both rivaroxaban or enoxaparin [30].

Patients with acute medical condition are at high risk to progress a venous thromboembolism for up to 90 days after hospital discharge, moreover, for extending its use as thromboprophylactic agent, rivaroxaban 10 mg is recommended daily for 31 - 39 days was [31].

Advantages of direct acting anticoagulants like rivaroxaban is obvious as it has few drug-drug interactions, although patient renal functions can affect rivaroxaban pharmacokinetics. On the other hand, anticoagulants as warfarin has many drug-drug interactions, drug–food interactions, and drug–herb interactions. Also, Low molecular weight heparin may add for the patient a risk of heparin-induced thrombocytopenia [31]. The previously mentioned advantages of rivaroxaban might justify its superiority as anticoagulant agent over warfarin and low molecular weight heparin in the management of coagulation disorders including COVID-19 disease.

Concerning the second wave of COVID-19 which expected to be characterized, clinically, by higher incidence of coagulation mainly in the gut, lung, muscles and other parts of the body, as one of the most serious clinical presentation in patients, thus, the use of rivaroxaban, as anticoagulant, could be one of the successful candidate for management and prophylaxis of this complication in COVID-19. Moreover, the accurate detection and determination of drug plasma levels in the patients to assure its achievement of therapeutic levels and avoiding incidence of bleeding and possible adverse effects resulting from overdose or toxic levels which might lead to death.

The in house developed chromatographic conditions, is in accordance with published literature [21-24] after some modification in extraction and chromatographic conditions.

As shown previously, the clinical importance of rivaroxaban as an anticoagulant in treatment of deep-vein thrombosis or pulmonary embolism and for the prophylaxis of venous thromboembolism in patients undergoing hip or knee replacement surgery (Kow and Hasan 2020). Besides its favorable Net clinical benefit (NCB), and lower mean hospitalization costs and length of stay (LOS) compared with warfarin in treatment of atrial fibrillation and venous thromboembolism [29].

It is worthy to mention that, the importance of the developed bioanalytical assay is to ensure accurate and precise therapeutic drug monitoring and testing the validity of generic drug products for commercial use and obtaining better clinical outcomes.

In this study, the results of rivaroxaban pharmacokinetic parameters were nearly in accordance with those reported in the literature [8-10].

The results obtained in the bioequivalence study as a clinical application of the developed method showed that the results of the 90% confidence intervals limits were lying within FDA acceptance limits (80% to 125%) [27].

Conclusion

Rivaroxaban could be a candidate for the management of COVID-19 infections as a prophylactic measure of coagulation incidence in CVID-19 patients. Therapeutic drug monitoring is a clinical essential approach for achievement of a successful therapeutic treatment due to monitoring of patient's drug levels and avoidance of potential subtherapeutic or toxic drug levels, thus, an accurate detection of rivaroxaban in patients' plasma is an essential tool for assurance of its efficacy and hence safety.

The fully validated bioanalytical method developed for the quantitation of rivaroxaban in plasma proved to be selective, accurate, precise, economic and ,thus, could be applied in bioavailability and bioequivalence studies, clinical trials, therapeutic and clinical monitoring of rivaroxaban. Finally, the obtained results from the pharmacokinetic application in the bioequivalence study performed showed that both generic and reference products of rivaroxaban 10 mg film coated tablet are bioequivalent.

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