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Research Article

Moxifloxacin as an Adjuvant Therapy in Management of COVID-19. What is Behind its Accurate Determination in Human Plasma and Pharmacokinetics Applications?

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Abstract

Background: Corona virus 2019 is considered one of the most widely spreaded pandemics that significantly affected healthcare and economic development worldwide and the probability of development of a second wave of this pandemic is high. Several protocols used fluoroquinolones antibiotics, including moxifloxacin, which are one of the promising adjuvant therapy in management of Corona virus 2019 disease.

Aim: Development of a novel, sensitive and validated LC/MS/MS bio-analytical method for the determination of moxifloxacin in biological fluids and its applications in pharmacokinetics, bioequivalence studies and monitoring drug-plasma levels to assure its effectiveness in management of infections.

Methods: Protein precipitation technique was used for moxifloxacin extraction from human plasma using ciprofloxacin as an internal standard, where, human plasma samples were analyzed using the following chromatographic conditions: eluting solvent consisting of 0.5% formic acid and acetonitrile 45: 55 v/v, pumped at flow rate 0.55 ml/min, ionization mode was set on ESI positive mode, and mass to charge ratio was m/z 402→261, 332→231 for moxifloxacin and ciprofloxacin respectively. Application on bioequivalence study of moxifloxacin generic versus reference product was assessed in 24 healthy subjects, where, AUC_{0-t}, $AUC_{0-inf,}$ C_{max}, and T_{max}. were reported.

Results: The mean recovery of moxifloxacin from human plasma was 80.108%, the lower limit of quantitation was 0.05 ug/ml and the Correlation coefficient (r^2) was equal to 0.9994. Statistical analysis for the pharmacokinetic parameters using analysis of variance (ANOVA) showed a non- significant difference between generic and reference drug products.

Conclusion: The developed LC/MS/MS method proved to be simple, sensitive, selective and valid for the detection of moxifloxacin in human plasma which can be applicable in pharmacokinetic studies, therapeutic drug monitoring in different patient care settings.

Keywords: ANOVA; Corona Virus; COVID-19

Introduction

The in progress epidemic of Corona virus 2019 infection 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].

The infection is caused by SARS-CoV-2 virus. The first cases appeared in Wuhan, China, and researchers suggest that it could be of animal origin especially "bats" due to high identity between SARS-CoV-19 nucleotide with that of bats which is considered as a natural reservoir for many zoonotic diseases like Ebola virus, rabies virus and others. The clinical severity of SARS-COV-2 infection could be asymptomatic, mild, moderate, sever, and critical symp-

toms. You can become infected through breathing if you are very close to a person with Covid-19 disease or touch a contaminated surface and then touch your eyes, nose or mouth [3].

Quinolones are broad-spectrum antibacterial agents that can be used for both Gram-positive and Gram-negative bacterial infections, like mycobacteria. Quinolones act by inhibition of synthesis of nucleic acid through disrupting topoisomerase IV enzymes and DNA gyrase. For more than fifty years, quinolone antibiotics have been recommended due to their high effectiveness, and broad spectrum of activity, high blood level concentrations, and low rate of side effects [4].

Fluoroquinolone antibiotics may be effective against different viral infections like vaccinia virus, papovavirus, HIV, and others. A recent study showed that the fluoroquinolone antibiotics may inhibit replication of SARS-CoV-2 by displaying a higher affinity for binding to viral main protease than chloroquine, so it can be used as adjuvant-therapy in management of COVID-19. An investigational study for the evaluation of the anti-inflammatory effects of besifloxacin, a novel fluoroquinolone under clinical evaluation for treatment of ophthalmic infections, results showed that it has a strong binding action to the Mpro active site more than chloroquine and nelfinavir. Besides, they inhibit pro-inflammatory cytokines leading to inflammatory response suppression. The pharmacokinetic advantages of fluoroquinolones are that they perfuse with higher amounts in lungs and safe compared to other antibacterial drugs [5].

Fluoroquinolone antibiotics showed to be active against different types of bacterial infections like: *Haemophilus influenzae*, *Mycobacterium tuberculosis, Chlamydia, Mycoplasma* and some atypical mycobacteria [6].

Moxifloxacin, is a fluoroquinolone antibiotic, with a broad spectrum against respiratory tract infections, including Gram-positive and Gram-negative bacterial infections, anaerobic bacteria, and atypical pneumonia infections [7]. It is used for skin, skin structure, and intra-abdominal infections. However, due to safety concerns, its use has now been restricted in the EU to the treatment of acute bacterial rhinosinusitis, acute exacerbation of the chronic obstructive pulmonary disease, or pneumonia only when other therapeutic agents cannot be prescribed or showed an insufficient clinical effect [8].

Moxifloxacin is marketed in different dosage forms including: film coated tablets under brand name Avelox® 400 mg film coated tablets [9], ophthalmic solutions, and intravenous solutions [10]. It is indicated for treatment of rhinosinusitis, pneumonia, exacerbations of COPD, mild to moderate infection of upper reproductive organs which showed insufficient clinical response to other antibiotics or for those patients who cannot use other antibiotics in adults, and recommended daily dose is 400 mg for 5 days up to 21 days according to the medical condition [9].

Incidence of adverse events reported to be more than 5% which included wound infections, diarrhea, nausea, headache, constipation, hypokalemia, and insomnia. Some adverse events make moxifloxacin not preferable for use like hyperhidrosis, tremor, atrial fibrillation, and pleural effusion [11].

After single dose administration of moxifloxacin 400 mg tablet, mean moxifloxacin C_{max}, AUC_{o-t}, and AUC_{o-inf}, was 2.256 ± 0.835 ug/ml, 24.6 ± 6.1 ug.hr/ml, and 28.8 ± 5.7 ug.hr/ml respectively and mean T_{max} was equal to 1.82 ± 1.35 hours [12]. In another study the following data after single dose administration of 400 mg moxifloxacin was obtained, as follows: mean C_{max}, AUC_{o-t} and AUC_{o-inf}, was 1.95 ± 0.46ug/ml, 27.9 ± 4.5 ug.hr/ml, and 29.3 ± 4.7 ug.hr/ml respectively and mean T_{max} was equal to 1.95 ± 0.96 hours [13].

The pharmacokinetics values for reference product in a bioequivalence study performed on moxifloxacin 400mg tablets was reported as follows; mean C_{max}, AUC_{o-t}, AUC_{o-inf}, $T_{1/2}$ and mean T_{max} were 2.98 ± 0.69 ug/ml, 37.44 ± 8.37 ug.hr/ml, 44.64 ± 10.42 ug.hr/ml, 12.81 ± 2.35hr, and 2hr respectively [14]. Also, in a clinical study of moxifloxacin 400mg, the following data were obtained for the reference product; C_{max} (3084.166 ± 631.9647 ng/ml), AUC_{0-t} (35711.043 ± 6959.0461 ng.hr/ml), AUC_{0-inf} (37443.494 ± 6568.4115 ng.hr/ml) and $T_{1/2}$ (11.427 ± 1.9518hr) and Mean T_{max} (1.670hr.) [15].

In a public assessment report of the reference product of moxifloxacin 400 mg, the pharmacokinetics results showed mean C_{max}, AUC_{0-t}, AUC_{o-inf} and mean T_{max} of 2847.7 ng/ml, 31742.1 ng.hr/ml, 32741.1 ng.hr/ml and 1.50hr. respectively [16].

Different analytical methods are investigated and developed for evaluation of moxifloxacin in pharmaceutical dosage form and biological fluids, those methods include; capillary electrophoresis with laser-induced fluorescence [17], square wave adsorptive

voltammetry [18], spectrophotometer [19], spectrofluorimetry [20], HPLC [21-24], ultra HPLC [25].

Concerning the bioanalytical methods for the determination of moxifloxacin in biological samples LC/ESI-MS/MS for moxifloxacin quantitation in biological fluids as well [26,27]. An HPLC-UV method in which plasma protein precipitation with acetonitrile showed a lower quantitation limits LLOQ of 0.2u g/ml [28]. Another method using HPLC and Fluorescence detector, after subjecting plasma sample to precolumn derivatization with 4-chloro-7-nitrobenzodioxazole (NBD-CI) showed a lower quantitation limits LLOQ of 15 ng/ml [29].

A sensitive method for moxifloxacin quantification in biological samples using HPLC-UV with a quantitation limit (LLOQ) of 0.125 ug/ml and a linear dynamic range of 0.125 ug/ml to 16 ug/ml [30]. An HPLC-FL assay was developed in which the drug was extracted by protein precipitation with acetonitrile showed a linearity range from 0.07 to 2 ug/ml [31]. For obtaining a more sensitivity specific and linear dynamic range, an LC/MS/MS assay developed in which drug extracted by protein precipitation with methanol and acetonitrile mixture, the LOQ was 0.05 ug/mL and the bioanalytical method was linear over the range from 0.05 to 5 ug/mL [32].

In this work, we examine the validity of an in house developed bioanalytical method for quantitation of moxifloxacin in biological fluids using an LC/MS/MS, where, the objective of this study is to introduce a valid, easy, economic and selective bioanalytical method for application in bioavailability and pharmacokinetic studies, clinical trials and therapeutic drug monitoring of moxifloxacin in patient's blood to ensure its safety and efficacy of an adjuvant therapy used in management of COVID-19.

In addition, the validated method can be applied for a comparative bioavailability study of generic versus reference products performed as per guidelines [33] and analysis of plasma samples would be done through the validated LC/MS/MS method in compliance with the international guidelines [34]. Pharmacokinetic calculations will be done using WinNonlin program and statistical analysis (ANOVA) were done using SAS software. Sequence effect was tested and the 90% confidence intervals for AUC_{0-t}, AUC_{0-inf}, and C_{max} were calculated for the ratio or difference between treatments and results showed to be in the limit of 80.00% to 125.00% confidence limits [35].

Methods

LC/MS/MS analytical method

Mass parameters and chromatography

The method was developed in-house as follow: eluting solvent composition is 0.5% Formic acid and Acetonitrile 45:55 V/V. Pump flow rate 0.55 ml/min. 5ul aliquot sample were injected. Mass detector 6410B was operated at electrospray ionization positive mode, m/z was 402→261, 332→231 for Moxifloxacin, and Ciprofloxacin (internal Standard) respectively.

Fragmentor energy was set at 120 for Moxifloxacin, and 110 (internal standard) Ciprofloxacin. Collision energy was set at 25 for Moxifloxacin, and 35 for (internal standard) Ciprofloxacin.

Preparation of solutions

Master standard solution

An accurately weighed 10 mg of moxifloxacin Standard was transferred to a 100 ml volumetric flask. Add approximately 80 ml of methyl alcohol. Place the flask on the sonicator for 10 minutes. The volume was completed with methyl alcohol to obtain a solution containing 100 ug/ml moxifloxacin "Solution A" from which 40 ml was transferred to a 100 ml volumetric flask and makeup the volume with methyl alcohol to obtain a solution of 40 ug/ml "Solution B".

Working solutions

The following serial dilutions, based on the corresponding master standard solution, are prepared in a 10ml volumetric flask. All dilutions are done with methanol.

Solution Used	Mililitres taken	Final concentration obtained (ug/ml)	Final volume (ml)
"Solution B"	0.125 ml	0.5	10
"Solution B"	0.25 ml	1	10
"Solution B"	0.625 ml	2.5	10
"Solution B"	1.25 ml	5	10
"Solution B"	2.5 ml	10	10
"Solution B"	5 ml	20	10
"Solution B"	7.5 ml	30	10
"Solution B"	10 ml	40	10

Ciprofloxacin standard solution

An accurately weighed 10 mg of ciprofloxacin Standard was transferred to a 100 ml volumetric flask and about 80 ml of methyl alcohol was added, sonicate for 10 minutes, complete the volume with methyl alcohol to obtain a solution of 100 ug/ml ciprofloxacin solution (A). From solution (A) transfer 15 ml into a 100 ml volumetric flask and complete to volume with methanol to obtain 15 ug/ml Ciprofloxacin solution (B).

Moxifloxacin standard concentrations in human plasma

Standard serial dilutions of moxifloxacin in human plasma were prepared by transferring a 50 ul aliquot of the serial standard solutions of moxifloxacin at concentrations ranging from 0.5 to 40 ug/ml to a centrifuge tubes containing 0.5 ml of blank plasma.

Sample preparation

Human plasma samples of 500 ul was transferred to an appropriate centrifuge test tubes and 50 ul of internal standard (ciprofloxacin solution 15 ug/ml) was added, the samples were vortex-mix for one minute, then 2 ml acetonitrile was added and vortex-mix the samples for 1 to 2 minutes, centrifugation at 3500 rpm for 5 minutes was done where 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.

Pharmacokinetics applications

Subjects: Twenty-four healthy adult, subjects participated in the bioequivalence study were subjected to complete physical examination, neurological assessment, urine analysis and blood analysis. The selected subjects had neither a history of drug or alcohol abuse nor any acute or chronic gastrointestinal, cardiac, vascular, hepatic, or renal disease. Concurrent medication was not allowed during the time course of the study. Meals, beverages drink, coffee or tea are not allowed for four hours after study dose administration. At twelve p.m. they received a standard meal and at 16:00 o'clock another meal. Before participating in the study, the subjects reviewed and discussed the informed consent with the clinical investigator before the beginning of screening procedure of the study then signed the consent without any obligation on the subjects to continue if they didn't want to.

Study design: This study was a conducted in a Two-way crossover randomized design to investigate comparative bioavailability of moxifloxacin of generic versus reference products in 24 healthy adults male subjects under fasting conditions with a washout period of seven days, the number and disposition of the blood collections as well as the wash out period were designed with respect to pharmacokinetic parameters of moxifloxacin.

Sample collection: The total number of collected blood samples was 17 samples in each study period, blood volume taken was 5 ml per sample. The following sampling intervals were withdrawn at the following intervals: 0 (directly prior to dosing), 15 min, 0.5 hour, 0.75 hour, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 48, and 72 hours after the administration, where, the total amount of blood withdrawn during the whole study did not exceed 170 ml. Blood sample were collected in tubes containing EDTA disodium and centrifuged at approximately 4000 r.p.m. for 10 minutes, plasma samples were separated in a 5 ml-plastic wassermann tube, and the collected samples were kept at a -80°C freezer until analysis.

Plasma samples quantitation: LC-MS/MS technique was used for the analysis and quantitation of moxifloxacin in withdrawn plasma samples.

Pharmacokinetic analysis: The following pharmacokinetic variables were assessed using Winnonlin program; maximum plasma concentration (C_{max}), time 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 the curve from zero to the last quantifiable concentration estimate (AUC_{0-t}), mean residence Time (MRT), area under the 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: Statistical calculations were performed using computer program SAS software for determination of (ANOVA). Bioequivalence could be demonstrated within 90% confidence limit of 80% to 125%.

Results

Analytical procedure validation

Chromatograms of moxifloxacin: Moxifloxacin and ciprofloxacin, the internal standard, showed a good separation with

retention time of 1.1 minutes, sharp and symmetrical peaks were obtained with excellent baseline resolution and minimum tailing, thus facilitating the accurate measurement of the peak response.

Linearity, precision, and accuracy: Peak area ratios of varying amounts of moxifloxacin in plasma (ranging from 0.05 - 4 ug/ml) was linear (r^2 0.9994), the average results of inter-day variation C.V.% were 1.366%, which is in compliance with FDA Guidelines [34].

Accuracy and precision were assessed on within-day and between-day basis at three different drug concentrations in the range of expected ones. The results of intra-day accuracy showed an average recovery percentage of 97.757%, and of inter-day accuracy showed an average recovery percentage of 96.618% with an average C.V.% of 1.366%. The results of stability study in plasma showed that the mean recovery was greater than 95% indicating stability of moxifloxacin in plasma.

Bioequivalence study

Clinical observation: The drug was well tolerated to all participating subjects, the scheduled blood samples of all subjects during the whole study was obtained at the proper time, and neither adverse events nor side effects occurred during the study time course.

Pharmacokinetic data: The calculated pharmacokinetics parameters for the generic and reference products in this study were as follows; mean (C_{max}) was 2.393 ± 0.388 ug/ml and 2.323 ± 0.438 ug/ml, (t_{max}) 1.417 ± 0.504hr and 1.479 ± 0.541hr, ($t_{1/2e}$) 12.872 ± 0.446hr and 12.811 ± 0.609hr, (AUC_{0-t}) 23.074 ± 2.459 ug.hr/ml and 23.615 ± 3.225 ug.hr/ml, ($AUC_{0-\infty}$) 24.019 ± 2.442 ug.hr/ml and 24.556 ± 3.194 ng.hr/ml respectively.

Statistical analysis: Two-way ANOVA were performed for the primary pharmacokinetic parameter, C_{max}, T_{max}, $AUC_{0-t,}$ and $AUC_{0-inf,}$ for detection of significance, if present, between both products and to assure that the 90% confidence limit of 80.00% to 125.00% for AUC_{0-t}, $AUC_{0-inf,}$ and C_{max} was fulfilled or not. In this bioequivalence study the (%) point estimate results for C_{max}, AUC_{0-t}, AUC_{0-inf} were 103.566%, 98.047%, and 98.113% respectively, and the 90% confidence limits of C_{max}, AUC_{0-t}, and AUC_{0-inf} were 97.987% to 109.463%, 93.177% to 103.172%, and 93.477% to 102.979% respectively.

Parameter	T_{max} (hr.)	C_{max} (ug/ml)	AUC_{0-t} (ug.hr/ml)	AUC_{0-inf} (ug.hr/ml)	K_{el} (hr^{-1})	$T_{1/2}$ (hr.)	MRTinf (hr.)
Mean	1.479 ± 0.541	2.323 ± 0.438	23.615 ± 3.225	24.556 ± 3.194	0.054 ± 0.003	12.811 ± 0.609	17.127 ± 1.429
C.V.%	36.596	18.871	13.657	13.008	4.651	4.756	8.345
Range (Median)	1.000-3.000 (1.500)	1.473-3.038 (2.315)	18.465-30.149 (23.855)	19.473-31.064 (24.787)	0.049-0.059 (0.055)	11.818-14.137 (12.619)	14.725-20.335 (16.861)

Table 1: Mean pharmacokinetics of moxifloxacin after administration of single oral dose of reference products to 24 subjects.

Parameter	T_{max} (hr.)	C_{max} (ug/ml)	AUC_{0-t} (ug.hr/ml)	AUC_{0-inf} (ug.hr/ml)	K_{el} (hr^{-1})	$T_{1/2}$ (hr.)	MRT_{inf} (hr.)
Mean	1.417 ± 0.504	2.393 ± 0.388	23.074 ± 2.459	24.019 ± 2.442	0.054 ± 0.002	12.872 ± 0.446	17.153 ± 0.964
C.V.%	35.549	16.196	10.659	10.167	3.494	3.464	5.618
Range (Median)	1.000-3.000 (1.500)	1.740-3.181 (2.272)	17.833-27.831 (23.441)	18.796-28.717 (24.352)	0.051-0.058 (0.054)	12.049-13.674 (12.946)	15.519-18.896 (17.170)

Table 2: Mean pharmacokinetics of moxifloxacin after administration of single oral dose of generic product to 24 subjects.

Parameter	90% Confidence intervals of parametric means		
	Point estimate (%)	Lower limit (%)	Upper limit (%)
C_{max}	103.566	97.987	109.463
AUC_{0-t}	98.047	93.177	103.172
AUC_{0-inf}	98.113	93.477	102.979

Table 3: 90% C.I for moxifloxacin generic and reference products.

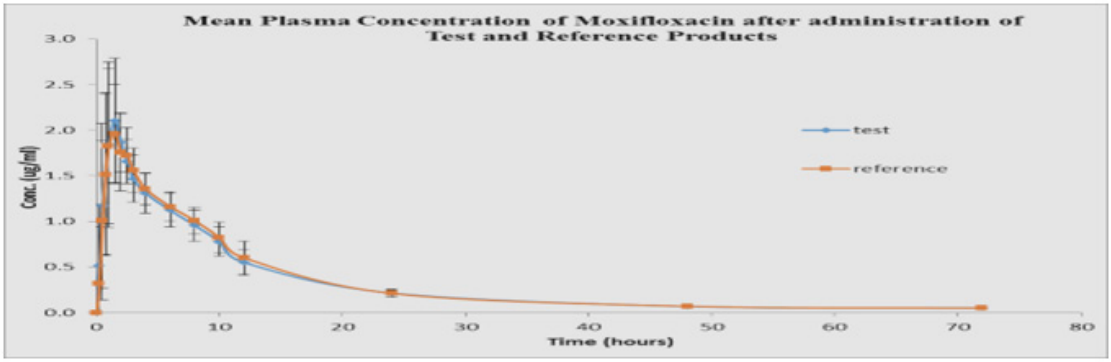

Figure 1: Mean plasma concentration of moxifloxacin following single dose administration of moxifloxacin 400 mg film coated tablet for generic and reference products.

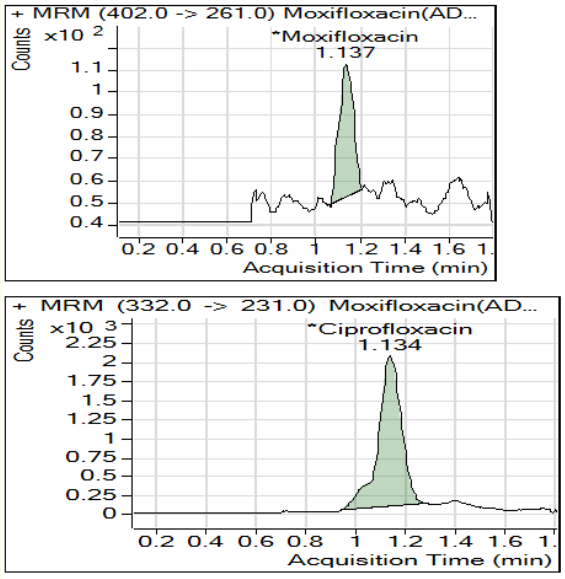

Figure 2: Sample chromatogram - an MRM data of blank plasma spiked with 0.05ug/ml moxifloxacin and internal standard ciprofloxacin.

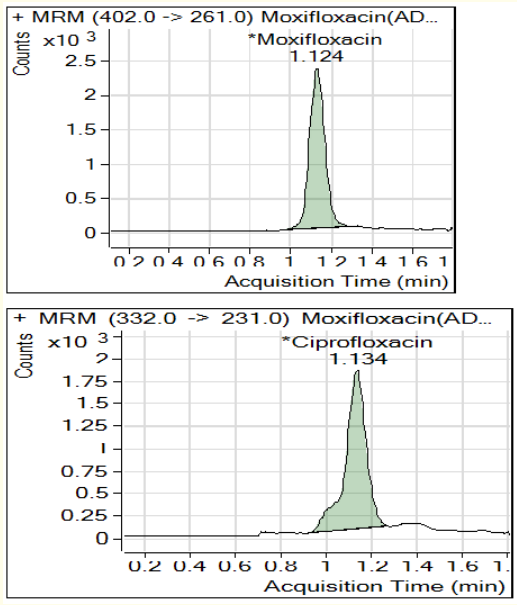

Figure 3: Sample chromatogram - an MRM data of blank plasma spiked with 2 ug/ml moxifloxacin and internal standard ciprofloxacin.

Discussion

The developed bioanalytical method used in this study was fully validated, where, the standard curve showed linearity from 0.05 to 4 ug/ml and r^2 was equal to 0.9994, which is in compliance with FDA Guidelines [34] and so it could be used for determination of moxifloxacin in human plasma in case of pharmacokinetics and bioavailability studies, and clinical trials. Being a candidate either as standard or adjuvant therapy of several infectious diseases, thus, therapeutic monitoring of moxifloxacin levels in patients is of high importance in assuring its achievement of therapeutic goals in infections including COVID-19 in the potential second wave and avoidance of possible adverse events and therapeutic failure resulting from being in toxic or subtherapeutic levels respectively in patients plasma.

The in-house developed bioanalytical method proved to be valid and selective for the determination of moxifloxacin in human plasma during therapeutic monitoring, bioavailability studies and clinical trials performed in hospitals and during management of infectious diseases including COVID-19. Moreover, the values of the pharmacokinetics parameters obtained in this study for both generic and reference products were in accordance with those reported in the literature [13-16], and also coincide with those reported of T_{max} ranged from 1.5 to 2 hours, C_{max} from 1.95 ug/ml to 3 ug/ml), and $T_{1/2}$ were 12 hr [8-12].

In a study conducted on SARS patients showed that mortality risk in those patients used quinolone antibiotics are the least (3.3%) compared to other antibiotics like beta-lactams (13%), and macrolides (6.7%), besides, the duration of hospitalization and fever are showed to be the least in those treated with quinolones compared to beta-lactams and macrolides [36].

It is worthy to mention that the clinical success for those patient used moxifloxacin in the treatment of pneumonia was 93.5% after 7 to 10 days of treatment [37], suggesting that moxifloxacin may be an effective adjuvant choice for management of COVID-19. Another study on patients treated with arbidol and moxifloxacin showed negative results for SARS-CoV-2 in 69.2 % of severe cases after one week time course treatment indicating that both drugs can help in viral load reduction in management of COVID-19 [38].

These previously evident results supports the superiority of quinolone including moxifloxacin to other antibiotics used as an adjuvant therapy in the management of COVID-19 and thus the use of the in house validated bioanalytical method in this study for detection and investigation of moxifloxacin in human to ensure therapeutic efficacy and safety following drug administration is considered a very useful clinical application.

It is well known that in bioequivalence studies, the 90% confidence interval of 80.00% to 125.00% on Ln-transformed data of C_{max}, AUC_{0-t}, and AUC_{0-inf} should be fulfilled which was in accordance with those obtained in the current study showing that point estimate (%) results were 103.566%, 98.047%, and 98.113% for C_{max}, AUC_{0-t}, and AUC_{0-inf} respectively. The 90% confidence limits were 97.987% to 109.463%, 93.177% to 103.172%, and 93.477% to 102.979% for C_{max}, AUC_{0-t}, and AUC_{0-inf} respectively. Finally, the results of the current study are within the FDA acceptance limits [31].

Conclusion

It was evident that moxifloxacin is a promising choice as an adjuvant therapy in the management and prevention of complications resulted from COVID-19 disease, and thus, therapeutic drug monitoring is an important approach for the achievement of a successful therapeutic treatment due to monitoring of patient's drug levels and avoidance of incidence of subtherapeutic or toxic levels of moxifloxacin during administration. Moreover, the novel bioanalytical LC/MS/MS method developed in this research for the determination of moxifloxacin in human plasma is valid, selective, sensitive, accurate, and precise and its application in the bioequivalence study performed here proved that it is valid to be applied in bioavailability studies, clinical trials, and therapeutic drug monitoring of moxifloxacin in patients. Finally, the results obtained of the bioequivalence study showed that both generic and reference products of moxifloxacin 10mg film coated tablet are bioequivalent.

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