Pharmacokinetic Profiling of Moxifloxacin Gastroretentive Drug Delivery System after Oral Administration

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Abstract
In the present investigation, we have designed a gastroretentive drug delivery system of moxifloxacin for the pharmacokinetic profiling of this system by relating it with moxifloxacin controlled release drug delivery system. A fast, simple, precise, specific and sensitive high performance liquid chromatography (HPLC)-ultraviolet-visible (UV) method was developed and validated for the plasma sample analysis. The C_{18} column was deployed for moxifloxacin separation using the mixture of acetonitrile and 0.5% triethylamine solution. The calibration curve was linear for concentration range of 10–1000 µg/L. A pharmacokinetic study was performed using oral single dose of 5 mg/kg moxifloxacin in healthy white New Zealand rabbits (n = 6) by administrating gastroretentive and controlled release systems respectively. One compartment open model was applied for the estimation of pharmacokinetic parameters after oral administration. The controlled release system of moxifloxacin shows significantly lower value average steady state plasma concentration (884.33±5.27 µg/L) than gastroretentive system (1389.67±11.31 µg/L). The quick attainment of steady state level of drug in plasma indicates higher value absorption rate constant and lower absorption half-life for gastroretentive system. The area under curve for controlled and gastroretentive system were found to be 9.57±0.53 and 27.34±1.32 mg.h/L respectively. The results concluded that gastroretentive system have 2.86 folds greater relative bioavailability and double value of mean residence time in comparison to controlled release system.
Keywords: Bioavailability, controlled release system, gastroretentive, moxifloxacin, pharmacokinetic

Introduction
Moxifloxacin, an antibiotics is chemically 1-cyclopropyl-7-(s, s)-2,8- diazabicyclo (4.3.0)non-8-yl)-6- fluoro-8-methoxy-1,4-dihydro-4-oxo-3 quinoline carboxylic acid. It act by trapping a deoxyribonucleic acid (DNA) drug enzyme complex and inhibiting adenosine tri-phosphate (ATP)-dependent enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. Recently, it is deployed in the treatment of community acquired pneumonia (CAP), chronic bronchitis (CB) and chronic obstructive pulmonary disease (COPD) [1–4]. When the

non-triple bismuth treatment or quadruple treatments are failing in *Helicobacter pylori* infection, then the moxifloxacin is used as second line drug. It have high volume of distribution value and long half-life, suitable for once daily dosing in several infections [5]. It is eliminated majorly by the liver [6].

In recent times, the spectrofluorimetric and high-performance liquid chromatography (HPLC) coupled with mass spectrophotometry (MS) were used for estimation of moxifloxacin level in human serum, human plasma and rat plasma [7–11]. The HPLC method was used for estimation of moxifloxacin in murine plasma & brain tissues [12], murine lung homogenate [13], dog plasma [14], and male camel serum [15]. The HPLC facilitated with fluorescence detector was used various methods by the virtue of its sensitivity. The concentration of moxifloxacin in several samples of respective animal model were estimated using HPLC with fluorescence detector like milk & plasma of lactating goat [16], milk & plasma lactating ewes [17], human plasma [18], human saliva & urine [19, 20], and human dialysate [21]. The simple HPLC methods were deployed for the determination of moxifloxacin level in human cerebrospinal fluid (CSF) & serum [22], human plasma, aqueous and vitreous fluid [23], and human plasma [24–27]. The reverse phase HPLC (RP-HPLC) with fluorescence detector was used for measurement of moxifloxacin level in human plasma & body fluids [28, 29, 31], rat plasma [30], human micro-dialysate [32], and blood plasma [33]. For analysis of moxifloxacin concentration in different body fluid in various animal models several methods were deployed namely- capillary electrophoresis with laser-induced fluorescence [34], square-wave adsorptive voltammetery [35], differential pulse polarography [36], voltammetery [37], capillary electrophoresis with contactless conductivity detection [38], spectrofluorimetery [39, 40], ultra HPLC and ultra performance liquid chromatography (UPLC)-MS/MS [41, 42].

Among the reported methods, HPLC method using fluorescence detector is most commonly used in analysis of biological samples of moxifloxacin. Since, the fluorescence detector is very expensive than UV detector and method required costly chemical for analysis of samples. The HPLC using UV detector was reported but they are very few [43–47]. Thus, the protein precipitation technique was selected because of its obvious advantages like shorter processing time, consumption of less organic solvent, fewer steps, and good cleans up of plasma samples. The suitability of the method was validated for the pharmacokinetic study using rabbit plasma.

In the present investigation an attempt was made to develop and validate a HPLC method using UV detector to estimate pharmacokinetic profiles of moxifloxacin using plasma samples in white New Zealand rabbits by administrating gastroretentive and controlled release formulations of moxifloxacin.

Materials and Methods

Materials

Moxifloxacin hydrochloride and Ciprofloxacin were kind gift samples from Panacea Biotech Ltd., India and Leben Laboratories, India respectively. Triethylamine, potassium dihydrogen phosphate, Dipotassium hydrogen phosphate and tetrabutyl ammonium hydrogen sulphate solution were purchased from SD Fine Chemicals, India. Acetonitrile and methanol of HPLC grade were purchased from Merck, India.

Methods

Experimental design

The study protocol was approved by Institutional Animal Ethical Committee of Satara College of Pharmacy, Satara (SCOP/IAEC/2019-20/05, Dated 05-01-2020). A two phase cross-over design with one washout periods of 15 days was followed for study. The moxifloxacin controlled release system (CR) was administered orally at single dose of 5 mg/kg body weight using nasogastric tube to healthy white New Zealand rabbits (n = 6). The blood samples (1 mL) were collected through marginal vein of ear at predetermined time (1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h) using a 20 G needle. The blood samples were dripped into a 2 mL heparinized syringe. The blood samples were centrifuged at 1500 rpm for 15 min within 30 min after collection. The plasma was taken out immediately and stored at −40 °C until analysis. The procedure was repeated after a washout period of 15 days by administrating moxifloxacin gastroretentive dosage form (R) orally at single dose of 5 mg/kg body weight to the rabbits (n = 6) [48].

Chromatographic conditions

The chromatographic study was performed using C_{18} column (250 mm × 4 mm, 5 µm particle size, Merck, Germany) by spiking 50 µL of sample at room temperature. The auto sampler vials and column temperature was maintained at 5 °C. The mobile phase was prepared by mixing 1:4 ratio of acetonitrile with tetrabutyl ammonium hydrogen sulphate solution (10 g/L). The concentration of moxifloxacin and internal standard (ciprofloxacin) were determined using Systronics LC 100 Plus WUFENG, with LC 100 UV Detector. The mobile phase flow rate was controlled isocratically at 1 ml/min. The UV wavelength was adjusted at 293 nm [43].

Method validation

The calibration curve was plotted over the concentration range of 10–1000 µg/L using rabbit plasma as a blank. The calibration curve was plotted between peak area ratio and concentration. The coefficient of correlation for six observations should be greater than 0.99 for calibration curve. The stock solutions of 1 mg/ml of internal standard (IS) and moxifloxacin were prepared. The working solutions of moxifloxacin were prepared with concentration, 10, 25, 50, 100, 250, 500 and 1000 µg/L and spiked into plasma. The plasma aliquots (quality control samples) were stored at −40 °C till the analysis of sample. Aliquots of plasma samples, internal standard samples and quality control samples were extracted and accurately 50 µL was spiked into the HPLC system [44].

The peak area of blank plasma sample spiked was compared with different amount of drug and treated as any aliquot, with peak area of the same standards prepared in phosphate buffer. The end point was decided based upon the mean value of six determination (n = 6). The precision in the assay was estimated by calculating standard deviation (SD) of repeated measurement of percentage of mean value. To determine the intra-day precision the mean of six replicate of three standard samples were used for calibration curves, where the value of relative standard deviation (RSD) should be less than 10%. To determine the inter-day precision, the mean of three standard samples were used for calibration curves, where the value of RSD should be less than 10%. The Limit of Quantitation (LOQ) of moxifloxacin in plasma was chosen as concentrations used for lowest concentration on the curve and for which RSD < 15% (LOQ = 10 µg/L). The mean value of recovery should be > 95% [44].

Pharmacokinetic analysis

The plasma concentrations of drug and time data was recorded in case of both the treatments in all study animals. The data was fitted in one compartment open model of drug after oral administration of dosage form. The various pharmacokinetic parameters were determined using Wagner-Nelson method. The non-compartmental model was also applied. The area under concentration-time curve (AUC) and area under first moment curve (AUMC) were calculated using trapezoidal rule. The mean residence time (MRT) is the ratio of AUMC and AUC calculated for both drug delivery system using (Equation 1).

$$MRT = \frac{[AUMC]}{[AUC]} \quad (1)$$

The relative bioavailability (F_r) was calculated using controlled release dosage form (standard) and gastroretentive dosage form (test) using (Equation 2 and 3) [49].

$$Fr = \frac{[AUC]R \times [Dose]CR}{[AUC]CR \times [Dose]R} \quad (2)$$

As, $[Dose]_R = [Dose]_{CR}$

$$Fr = \frac{[AUC]R}{[AUC]CR} \quad (3)$$

Where, $[AUC]_R$ = area under curve for gastroretentive dosage form, $[AUC]_{CR}$ = area under for controlled release system, $[Dose]_{R=}$ dose of moxifloxacin in of gastroretentive system and $[Dose]_{CR}$ = dose of moxifloxacin in of controlled release system.

Statistical analysis

The statistical analysis was carried out using descriptive statistical parameters like mean and standard deviation. The calculation of absorption rate constant, elimination rate constant, elimination half-life and absorption half life, average steady state plasma concentration and total clearance were reported [50].

Results and Discussion

In the present investigation, the concentration of moxifloxacin in rabbit plasma was determined by HPLC-UV method. However, tetrabutyl ammonium hydrogen sulfate is costly ion-pair reagent. Similarly, fluorescence detector is very expensive. The moxifloxacin shows strong ultraviolet absorption at 293 nm, therefore, for economic advantage HPLC-UV method was employed. The internal standard (ciprofloxacin) method was implemented to reduce noise to aspect ratio (error) caused by the instruments and operations. The method was easy to operate, simple in sample preparation, required small volume of samples, and highly sensitive.

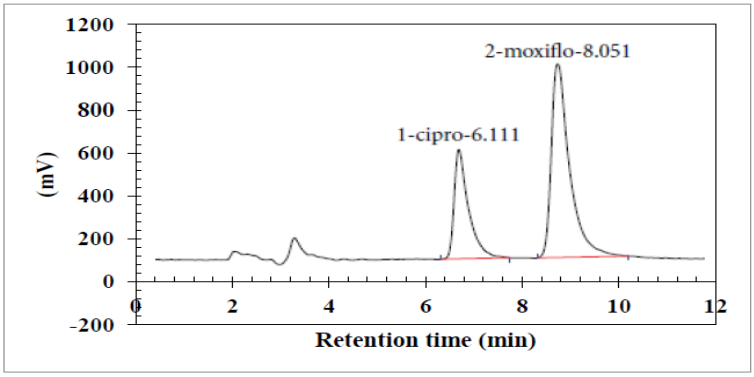

Figure 1 Chromatogram of moxifloxacin and ciprofloxacin in rabbit plasma.

At pH 4.5, separation moxifloxacin is not possible. Hence, the mixture of 0.1% triethylamine (pH adjusted to 4.8 with phosphoric acid)/ acetonitrile in 80:20 ratio was used. The acetonitrile increases the retention of moxifloxacin. The retention times of ciprofloxacin and moxifloxacin were 6.11 and 8.05 min respectively (Figure 1).

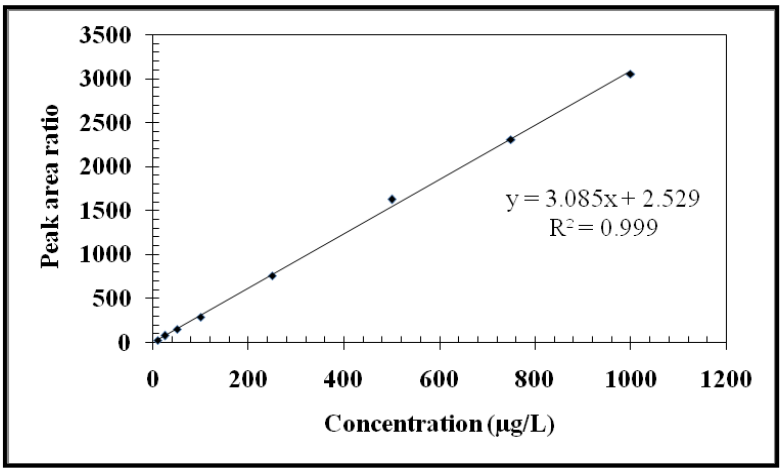

Figure 2 Calibration curve of moxifloxacin.

The linearity calibration curve of moxifloxacin for drug-free plasma over the concentration range of 10–1000 µg/L was determined. The calibration curve was plotted peak area ratio (moxifloxacin/internal standard) versus concentration. It produce straight line with mean regression coefficient and equation 0.999 and $y = 3.085x + 2.529$ respectively (Figure 2). The RSD of repeatability of present method was found to be 1.21 %. Intra-day, inter-day precision RSDs at the six concentrations were 3.17, 3.48 and 2.75 %, while the inter-day RSDs were 3.45, 1.67 and 2.68 %. These results indicated that the validated assay was precise, accurate, and reproducible. The mean percentage extraction recoveries of moxifloxacin at the three concentrations were 79.83 ± 2.34, 82.16 ± 3.21 and 79.69 ± 2.86 % respectively.

The structural similarity and physicochemical properties of ciprofloxacin with moxifloxacin made it suitable to use as internal standard. The ciprofloxacin minimize the error in the drug extraction. The ciprofloxacin have shown good ultraviolet absorption response at 278 nm wavelength. Mean value of the method recovery was 98.85%, which ranged from 96.6 to 101.8%.

According to literature, the moxifloxacin has absorption window in proximal part of gastrointestinal tract (GIT). To confirm the absorption site the study was planned [51]. The plasma concentration of drug (mean ± SD) with respective to the time, after oral administration of controlled release and gastroretentive dosage form were shown in (Figure 3). The most of the pharmacokinetic parameters except absorption rate constant were calculated utilizing one compartment open model unchanged drug in plasma after oral administration of drug using Wagner-Nelson method for both dosage form (Table 1). The quick plasma concentration of moxifloxacin at its maxima from both dosage forms was achieved within 1.2–2 h.

The rapid absorption was confirmed from absorption rate constant and absorption half-life values. Higher the value of absorption rate and lower the value of absorption half-life was the good indicator of better absorption. The rate of absorption is directly proportional to drug

available at absorption site, hence follows a first order kinetics. The value of absorption rate constant was calculated by using method of residual. The tangent touching to y-axis and the terminal linear portion of plasma concentration of drug with respect to time curve was drawn. The residual concentration were determined and the plasma concentration of drug at respective time point was determined and from the slope of line connecting residual line used to calculate absorption rate constant (Ka) by using (Equation 4). The values of absorption rate constants were further used in the calculation of absorption half-life ($t_{1/2(a)}$) of moxifloxacin from respective dosage form using (Equation 5).

Absorption rate constant (K_a) = slope × 2.303 (4)

Absorption half-life ($t_{1/2(a)}$) = 0.693/ K_a (5)

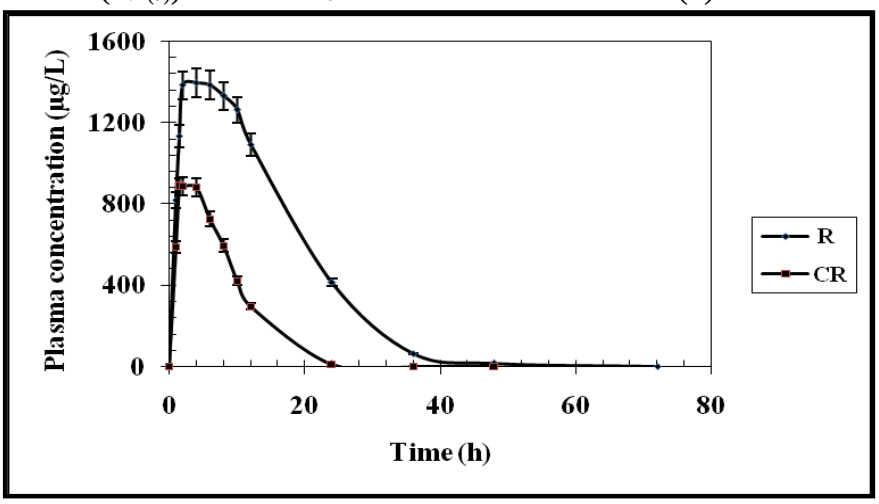

Figure 3 Plasma concentration-time curve.

The elimination rate constant (K) values were determined using feathering technique from the terminal linear portion of respective curves using (Equation 6). Considering the elimination process follows first order kinetics, the elimination half-life was calculated by using (Equation 7).

Elimination rate constant (K) = Slope × 2.303 (6)

Elimination half-life ($t_{1/2}$) = 0.693/ K (7)

The elimination half-lives after oral administration for R and CR dosage forms were found to be 4.25 and 6.79 h respectively. These values of elimination half-lives were higher than the values given in the literature for conventional liquid dosage form indicates both dosage form could prolonged duration of action [52]. The prolongation of half-lives by extravascular (oral) administration may be due to absorption process. The moxifloxacin was well absorbed from gastroretentive dosage form than controlled release dosage form due to residence of dosage form for more period in absorption window. Both the formulations maintained the steady state plasma concentration for more than 10 h. The gastroretentive formulation was found to maintain the plasma concentration consistently above 850 µg/L, for 12 h, producing significant antibiotic effect.

The relative bioavailability of moxifloxacin from gastroretentive dosage form was 2.86 fold than that of controlled release dosage form at the same dose (Equation 1 and 2). The bioavailability results shows the variability of absorption since the R dosage form retained in proximal part of GIT for more than 8 h leads to increased bioavailability than the CR dosage form. The variability in the bioavailability can be associated with two disadvantages: (a)

underexposure of moxifloxacin in animals/human with lower bioavailability which leads to the development of moxifloxacin resistance and (b) overexposure of animal/human with higher bioavailability which produce the risk of side/toxic effects [53]. These situations has been already discussed in literature for few quinolones such as enrofloxacin [54].

Table 1 Pharmacokinetic parameters of moxifloxacin for gastroretentive and controlled release dosage forms

Pharmacokinetic parameter	*R*	*CR*
Absorption rate constant [a] (Ka)	8.71 ± 0.27 h^{-1}	6.48 ± 0.13 h^{-1}
Absorption half-life [a] ($t_{1/2(a)}$)	0.795 ± 0.02 h	0.107 ± 0.01 h
Elimination rate constant [a] (K)	0.163 ± 0.03 h^{-1}	0.102 ± 0.02 h^{-1}
Elimination half-life [a] ($t_{1/2}$)	4.25 ±0.03 h	6.79 ± 0.06 h
Area under curve [a] $[AUC]_{0-72}$	27.34 ± 1.32 mg.h/L	9.57 ± 0.53 mg.h/L
Area under first moment curve [a] $[AUMC]_{0-72}$	327.72 ± 4.91 $mg.h^2/L$	65.65 ± 2.06 $mg.h^2/L$
Mean residence time [a] $[MRT]_{0-72}$	11.97 ± 0.28 h	6.934 ± 0.11 h
Average steady state concentration [a] ($C_{SS\ avg}$)	1389.67 ± 11.31 µg/L	884.33 ± 5.27 µg/L
Steady state plasma concentration time (T_{ss})	2–10 h	1.5–6 h
Total clearance [a] (Cl_t)	0.317 ± 0.012 L/h	0.198 ± 0.009 L/h
Relative bioavailability [a] (F_r)	2.86 ± 0.01 fold	1

Where, [a] indicates the value, as mean ± SD for six determinations (n = 6).

From the literature, in rabbits the apparent volume of distribution of moxifloxacin after intravenous injection administration of drug in 5 mg/kg to rabbits, the volume of distribution (V_d) was reported as 1.95 L. The values of total clearance (Cl_t) for R and CR dosage form were found to be 0.318 L/h and 0.199 L/h respectively. The higher total clearance and elimination rate constant values for gastroretentive dosage form were due to higher plasma concentration.

$$\text{Total clearance } (Cl_t) = V_d \times K \quad (7)$$

This indicates gastroretentive drug delivery system shows better values of pharmacokinetic parameters that supports higher bioavailability. Looking at the anatomical and biochemical similarity between rabbits and human being the present gastroretentive system should undergo clinical trials before bring into the market.

Conclusions

In the present investigation, HPLC-UV method was simple, rapid, precise, and accurate for the determination of moxifloxacin concentrations rabbit plasma. Need of small plasma volume and sensitivity of method make it suitable for preclinical pharmacokinetic study of moxifloxacin drug delivery systems in rabbits. The developed method may be used biomedical analysis of moxifloxacin concentrations in human plasma after validation for therapeutic drug monitoring. The pharmacokinetic parameters obtained after fitting the data in one compartment open model and non-compartmental analysis concluded that the gastroretentive dosage form exhibits higher relative bioavailability and mean residence time than controlled release dosage form.

Acknowledgements
The authors thanks to Panacea Biotech Ltd., India and Leben Laboratories, India for providing kind gift samples of moxifloxacin hydrochloride and ciprofloxacin respectively.

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