BIOEQUIVALENCE OF TWO ORAL FORMULATIONS OF MOXIFLOXACIN AND ITS ANALYTICAL STUDY BY HPLC-UV METHOD

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ABSTRACT: An HPLC-UV method was developed which permits determination, bioequivalence and stability of moxifloxacin in tablets. The chromatographic separation was achieved on C18 column in isocratic mode (methanol, water and acetonitrile 45:30:25) at pH 4 and eluents were detected at 295 nm. After single oral dose (400 mg) of two formulations (Avelox, Bayer and Staxin, Stand Pharma) of moxifloxacin administered to healthy volunteers, pharmacokinetics parameters were derived from the plasma concentrations curves for both formulations. Both of the formulations were bioequivalent on the basis of pharmacokinetic analysis with no adverse reactions of drug were observed. The present method is effective extraction technique with good reproducible recovery and a limit of quantification of 20 $ng.ml^{-1}$. The method was successfully applied for stability studies of moxifloxacin tablets for six months and has been found stable at accelerated conditions of temperature and relative humidity.

Key words: Bioequivalence; Bioavailability; HPLC; Moxifloxacin; Pharmacokinetics; Stability;

INTRODUCTION

Moxifloxacin, 1-cyclopropyl-7-[(1S, 6S)-2, 8-diazabicyclo[4.3.0] non-8-yl]-6-chloro-8-methoxy-4-oxo-quinoline-3-carboxylic acid, belongs to broad spectrum antibiotics (Ginsburg *et al.,* 2003, Ginsburg *et al.*, 2005, Baum *et al*., 2006) being used for the treatment of several infections of lungs and sinuses, etc, (Nuermberger *et al*., 2004, Wang *et al.*, 2006). As moxifloxacin is a fluoroquinolone, hence it has proficiency to concentrate intracellularly in human phagocytic cells, fibroblasts, and epithelial and endothelial cells (Pascual, 1995). Therefore, intracellular environment will not affect the activity of these compounds against various pathogens (Pascual, 1990, Mor *et al.,* 1994, Walz *et al*., 1997). Pharmacokinetic studies have revealed that moxifloxacin and other fluoroquinolone antibiotics are readily absorbed from gastrointestinal tract and maximum concentration of active ingredient can easily be achieved in blood plasma ($T_{max,}$ 2 h) after oral administration with an absolute bioavailability of about 90% (Ballow *et al.,* 1999).

A number of HPLC methods have been designed and validated for the determination of moxifloxacin from blood plasma by using fluorescence detector (Fuhrmann *et al.*, 2004, Iyer *et al.*, 2006, Landersdorfer *et al.*, 2009). However, a careful survey of literature has revealed that no significant work is reported on the suitable assay determination by HPLC-UV method for moxifloxacin from blood plasma as well as tablet dosage form.

As the most preferable mode for the determination of assays of different drugs in pharmaceutical industries is HPLC coupled with UV-visible detector instead of fluorescence detector, therefore, the present study is focused to develop a novel and facile HPLC-UV method for the determination of moxifloxacin. This newly developed method was validated and successfully applied for the assay determination of moxifloxacin. Bioequivalence studies of two commercially available brands were carried out on blood plasma taken from healthy volunteers after oral administration of the drug.

Additionally, it was noticed that there is no work reported regarding the stability studies of moxifloxacin tablet dosage form at accelerated conditions of temperature and humidity. Therefore, our interests were also focused to check effects of elevated (40°C and 50°C) temperatures and increased humidity (75%) onto the stability of moxifloxacin drug.

MATERIALS AND METHODS

Materials: Moxifloxacin reference standard was gifted by Stand Pharma, Lahore, Pakistan. Avelox and Staxin (400 mg) tablets were taken from the market. All solvents (HPLC grade) and reagents were used as received from Fluka without further purification.

Instrumentation and conditions: The HPLC system comprised of G1311A quaternary pump, G1315B DAD variable wavelength UV detector and 1200 system controller (all from Agilant, Germany). The column used was Shim-Pak ODS 5μm (4.6 × 250 mm) while mobile phase used was a methanol, water and acetonitrile (45:30: 25) mixture. The flow rate, detection wave length, and injection volume were 1.0 $ml.min^{-1}$, 295 nm and 20 μL,

respectively.

Standard preparation: The standard stock solutions of moxifloxacin and benzoic acid were prepared separately by dissolving 100 mg of each standard in 100 ml mobile phase. Both of the solutions (10 ml each) were diluted to 100 ml with mobile phase. The solutions were filtered off through the 0.45-µ filter and degassed before use.

Method validation: Method validation was carried out in the mobile phase as per reported method (Vinod, 2000). The specificity and selectivity of the HPLC system were determined by a separate chromatographic analysis of the excipients mixtures without moxifloxacin; no interfering peaks at the retention times of moxifloxacin and benzoic acid (internal standard) were observed. The performance parameters thus obtained have been given in Table 1. The accuracy and precision were determined by use of quality control sample prepared by adding to the mobile phase in the known amount (lying in the middle range of entire standard curve) of standard, from three concentrations representing the entire range of standard curve; one within three times of the lower limit of quantification (low quality control sample), one near the center (middle quality control) and one near the upper boundary of standard curve (high quality control). Measurements were made as ten replicates at each concentration and mean and coefficient of variance (CV) were thoroughly calculated. Linearity was calculated by preparing ten different concentrations of moxifloxacin along with benzoic acid as internal standard in the mobile phase. Injection volume used was 20 µL of each concentration.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated by preparing the solutions of moxifloxacin and benzoic acid in the mobile phase and were diluted to known concentrations to a final response equal to three times of the signal-to-noise ratio. The LOQ was taken as ten times of signal-to-noise ratio. The specificity of the method was established by using six different samples. The calibration curve in the mobile phase was generated by using six concentrations. The quality control samples were used to accept or reject the run. The replicate measurements were made at three concentrations, one at lower limit of quantification, one in the mid range and another approaching high end of the range.

Study participants: Ten healthy humans (age 18-25 y, median age of 21 with 10 % ideal weight, mean 55 kg and build) were selected. The participants were detailed about the type of study, the safety of the medicine and possible undesirable effects, etc., and written consent was obtained. All the procedures followed were in accordance with the current revision of the Helsinki Declaration. The study was approved by the Ethics Committee, University of Sargodha Medical College and was carried out at District Head Quarter Hospital, which is affiliated with the University of Sargodha.

Study design: The participants were kept on fast for 10 h prior to initiation of study. A single dose of 400 mg of the Avelox and Staxin were administered to the participants along with 240 ml of water. The participants were kept fasting for 5 h after administration of the drug. They were allowed to take water 1 h after administration of the drug. After that standard meals were served throughout the study. There was one week washout phase between two treatments (oral administration of two formulations). Venous blood samples (3-5 ml) from antecubital vein were collected from each participant by using canulas under aseptic conditions. Heparin (Leo, Denmark) was used as an anticoagulant. The blood samples collected in centrifuge test tubes were arranged in order on test tube racks and labeled accordingly with great accuracy. Blood samples were collected just before (blank) and after 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0, 72.0, h of administration of the drug. The blood samples were centrifuged at 3000×g for 3 min. Plasma were separated by using micropipette with sucker and stored in specially capped glass test tubes.

Plasma sample preparation: Plasma samples (250 µL) were transferred to a 2 ml polypropylene vial to which internal standard (50 µL, 4000 $ng.ml^{-1}$) and 1 ml of acetic acid and acetonitrile were added. The samples were centrifuged at 3000×g for 1 min and the aqueous layer discarded. The organic phase was transferred to 2 ml glass vials and the solvent was evaporated to dryness at 40°C under a stream of nitrogen. The residue was re-dissolved in 250 µL of mobile phase, of which 200 µL was transferred into 250 µL glass vials and placed in the auto-sampler for analysis. The injection volume was 20 µL. Sample vials were wrapped in aluminium foil to protect moxifloxacin from light exposure.

Tablet sample preparation: For stability study twenty tablets of moxifloxacin were weighed accurately crushed and ground to a fine powder. The powder was mixed thoroughly to obtain a homogeneous sample. A sample solution was prepared by dissolving the powdered tablets containing about 100 mg of moxifloxacin in 100 ml of mobile phase and filtered separately. Ten ml of solution was diluted to 100 ml with mobile phase. Accurately weighed quantity (100 mg) of benzoic acid was dissolved in mobile phase (100 ml) and diluted to known concentration of about 0.1 $mg.ml^{-1}$. Each solution was filtered through 0.45 µ filter and degassed before use.

Specimen analysis The plasma samples were analyzed by the HPLC method validated above, and concentration of moxifloxacin was determined.

Bioequivalence (pharmacokinetic evaluation) Concentration-time curves were plotted and following parameters were determined. AUC_{0-t}, the area under the

curve from time zero to time t; $AUC_{0-□}$, the area under the curve from time zero to time infinity using the formula $AUC_{0-□} = AUC_{last} + Ct/ke$; $t_{½}$ =0.693/ke, the half-life of the drug; C_{max}, the peak drug concentration; t_{max}, the time to peak drug concentration. Cl=Dose/$AUC_{0-□}$, Clearance. The area under the curve, the concentration-time curves were calculated by the linear trapezoidal method. The terminal rate constant, ke, was determined by regression analysis of at least three data points in the terminal phase. The statistical analysis was performed by use of Statgraphics® 5.1.

Accelerated stability testing The newly developed method was used for the accelerated stability studies of tablets at 40 °C, 50 °C and 75% relative humidity. The duration of study was six months and was carried out according to ICH (International conference for harmonization) stability study guidelines. In first month, samples were analyzed on daily basis while in second and third months, samples were analyzed weekly. In following two months, samples were analyzed fortnightly. The last analysis was carried out after one month and the analyses were performed by HPLC. Stability study of the active ingredient in the mobile phase was carried out to evaluate the time period that encompasses duration of typical sample preparation, sample handling and analytical run time which was about 30 min. The stability study in mobile phase was extended to 72 h in this work.

RESULTS AND DISCUSSION

Moxifloxacin determination: The determination of moxifloxacin was carried out by using the validated HPLC-UV method. Typical chromatograms showing the separation of moxifloxacin and benzoic acid in the mobile phase have been shown in Figure 1. The LOD and LOQ values for moxifloxacin were 20 and 40 $ng.ml^{-1}$, respectively. Between the days the precision near limit of detection (LOD), in terms of coefficient of variation (CV), ranged from 0.4 to 0.9, and accuracy in terms of percent recovery was found to be greater than 99.51% for moxifloxacin. All performance parameters clearly established the validity of the HPLC method for this study. All results are summarized in Table 1. The method developed was found to posses better performance parameters as compared to the available methods in literature (Stass and Kubitza, 1999, Sevgi, 2007) http:// www. ncbi. nlm. nih. gov/ sites/ entrez? Db = pubmed & Cmd = Search & Term = % 22 Stass % 20H % 22 % 5 B Author % 5 D & itool = Entrez System 2. P Entrez. Pubmed. Pubmed Results Panel. Pubmed_ RV Abstract Pluswhich do not determine the bioequivalence and stability of moxifloxacin tablets. However, the present method is successfully applied to study assay as well as bioequivalence and stability of moxifloxacin tablets.

Bioequivalence (pharmacokinetic evaluation): For bioequivalence study the pharmacokinetic parameters of both formulations were calculated. The pharmacokinetics data following the single oral administration of 400 mg moxifloxacin (both formulations) are given in Table 2. The plasma concentration-time curves are shown in Figure 2 & Figure 3 for Avelox and Staxin respectively. All pharmacokinetics parameters; t_{max}, h; $C_{max,}$ $mg.ml^{-1}$; $t_{1/2,}$ h; $AUC_{0-\infty h}$, $h.mg.ml^{-1}$ and Cl of both formulation were found to be closely related. For the comparison of two sets of data F test was applied and it was found that F < F critical, which clearly indicates that there is no significant difference between the two sets of data.

Stability of moxifloxacin: The stability was monitored by determining concentration of active ingredient in the tablets placed at 40ºC and 50ºC with 75% relative humidity (RH), at various intervals of time. At 40ºC the concentration of moxifloxacin (the active ingredient) dropped slowly according to the equations $y = 25.041e^{-0.0005x}$ with $R^2 = 0.73$. At 50ºC concentration of moxifloxacin (active ingredient) dropped slowly according to the equation $y = -0.0128x+25.092$ with $R^2 = 0.6816$. The % age assay was found to be within the given range of assay after six months at accelerated conditions of temperature and humidity. Assay data of both formulations during six months stability study is g1ven in Table 3. The data obtained at 40ºC and 50ºC

Table 1. Validation parameters of HPLC analysis of plasma

Parameter	Moxifloxacin (Means)	Parameter	Moxifloxacin (Means)
Precision (CV), within day/between days	i) 0.4/0.7 at 20 $ngml^{-1}$ii) 0.5/0.8 at 10 μgml^{-1}iii) 0.4/0.9 at 100 μgml^{-1}	LOQ ($ngml^{-1}$)	40.0
Accuracy (% recovery)	i) 99.75 at 20 $ngml^{-1}$ ii) 99.59 at 10 μgml^{-1} iii) 99.51at 100 μgml^{-1}	Theoretical plates, N	14691
LOD ($ngml^{-1}$)	20.0	Resolution, Rs[a]	3.2
Tailing factor, As	1.1	Capacity factor, k	4.2

[a] Resolution between the adjacent peaks.

Table 2. Pharmacokinetic data after a single oral dose of 400 mg moxifloxacin (Avelox and Staxin)

Parameter	Avelox[a]	Staxin[a]

t_{max}, h	2 (0.25)	2 (0.20)
C_{max}, mg ml^{-1}	1.4 (0.07)	1.38 (0.06)
$t_{1/2}$, h	4.5 (0.5)	4.45 (0.5)
$AUC_{0-\infty h}$, h. mg ml^{-1}	5.3 (0.05)	5.5 (0.06)
Clearance[b]	72.34 (2)	74.0 (3)

[a] Values are means (± standard deviations)
[b] Total body clearance for extra- vascular administration

Table 3. % assay of Avelex and Staxin during stability (40 °C and 75% RH) for six months

Days	% assay (Avelox) [a]	% assay (Staxin) [a]
0	100.00 (0.5)	99.99 (0.6)
15	99.99 (0.5)	99.99 (0.6)
30	99.90 (0.5)	99.98 (0.6)
45	99.90 (0.5)	99.97 (0.6)
60	99.88 (0.5)	99.97 (0.6)
75	99.87 (0.5)	99.97 (0.6)
90	99.86 (0.5)	99.96 (0.6)
105	99.86 (.5)	99.96 (0.6)
120	99.85 (0.5)	99.95 (0.6)
135	99.84 (0.5)	99.94 (0.6)
150	99.84 (0.5)	99.93 (0.6)
165	99.83 (0.5)	99.92 (0.6)
180	99.83 (0.5)	99.92 (0.6)

[a] Values are means (± standard deviations)

with 75% RH was compared by applying the F-test (a statistical test for the comparison of two sets of data) and was found F < F critical which clearly indicated that there is no significant difference between the two sets of data.

In conclusion, we described here the development of a new, selective, precise and accurate method for the quantification of moxifloxacin in human plasma using HPLC with UV detection and liquid-liquid sample extraction which was applied to a bioequivalence study. Fluoroquinolones have been usually determined using fluorescence detection (Stass, 1998). Although fluorescence detection is usually more sensitive than detection by UV, fluorescence detectors are not as widely available. The method reported here uses a simple and effective extraction technique with good and reproducible recovery and a limit of quantification of 20 $ng.ml^{-1}$. The developed method is suitable for pharmacokinetic studies of moxifloxacin as well. The method was successfully used for the stability study of tablets at accelerated conditions of temperature and humidity. The study was able to demonstrate bioequivalence between the two formulations with a 90% confidence interval. Ahead of the clinical observations carried through during and after the study no adverse effect was observed, showing a good tolerability to the both formulation.

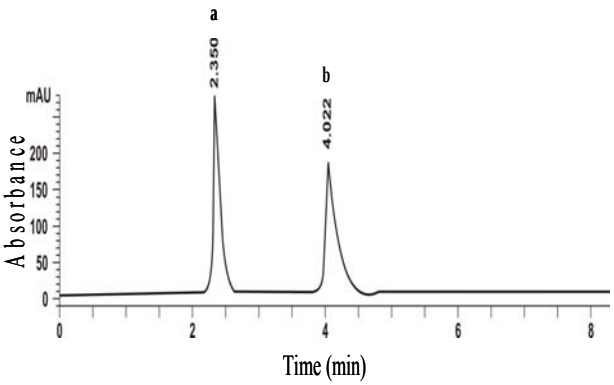

Figure 1. Chromatogram showing moxifloxacin (a) and benzoic acid (b).

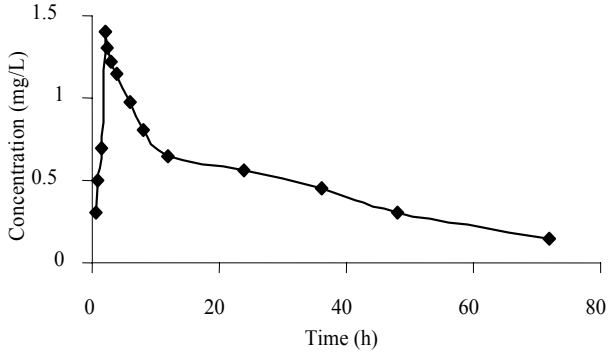

Figure 2. Mean plasma moxifloxacin concentration versus time curve after a single 400 mg dose of Avelox.

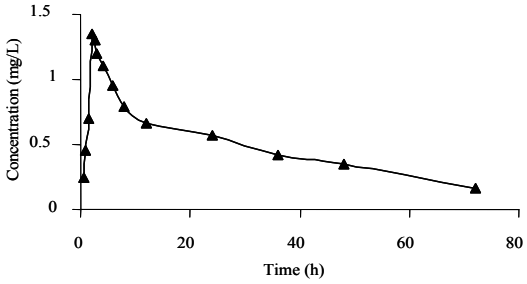

Figure 3. Mean plasma moxifloxacin concentration versus time curve after a single 400 mg dose of Staxin.

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