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A simple and sensitive HPLC-fluorescence method for the determination of moxifloxacin in human plasma and its application in a pharmacokinetic study

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A simple sample preparation technique coupled with the specific and sensitive fluorescence detection for the determination of moxifloxacin in human plasma was developed and fully validated. Levofloxacin was chosen as an internal standard. Chromatographic separation was achieved using a Shiseido C18 MGII (250×4.6mm i.d.; 5 µm) column under an isocratic mobile phase comprising of 50 mM potassium dihydrogen phosphate (pH 2.4) – acetonitrile (77:23, v/v) at a flow rate of 1.5 mL/min. Fluorescence detection was optimized for the determination of moxifloxacin in human plasma at an excitation wavelength of 296 nm and an emission wavelength of 504 nm. The total chromatographic run time was 8 min with the retention times of moxifloxacin and internal standard at 6.5 and 3.0 min, respectively. Calibration curves were established over the dynamic range of 20-3000 ng/mL. The analytical method was validated as per US-FDA and EMA guidelines for specificity, sensitivity, linearity, accuracy, precision, recovery, hemolytic effect, lipemic effect, dilution integrity and stability. The validated analytical method was successfully applied in a pharmacokinetic study of a single-dose oral administration of a moxifloxacin 400 mg tablet in Thai healthy volunteers.

1. Introduction

Moxifloxacin (Fig. 1A) is a fourth-generation fluoroquinolone with a broad spectrum of activity against various gram-positive and gram-negative bacteria (Fass 1997). It is used for the treatment of acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, community acquired pneumonia, complicated and uncomplicated skin and skin structure infections, and complicated intra-abdominal infections (Balfour and Wiseman 1999; Ferrara 2007). After an oral single-dose moxifloxacin is readily absorbed from the gastrointestinal tract and achieves a maximal concentration in 2 h (t_{max}) with a bioavailability of approximately 86%. The maximum concentration in plasma (C_{max}) is 2500 ng/mL with an elimination half-life ($t_{1/2}$) ranging from 12 to 16 h (Stass et al. 1998, Stass and Kubitza 1999).

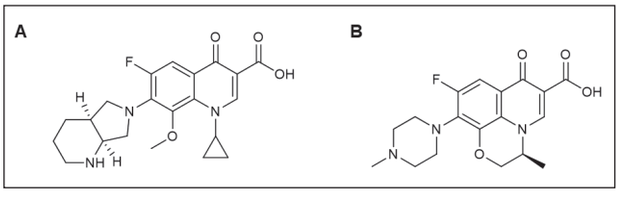

Fig. 1: Chemical structures of (A) moxifloxacin and (B) levofloxacin.

High performance liquid chromatography coupled with different detection techniques including UV spectrometry, spectrofluorometry, capillary electrophoresis and mass spectrometry have been used for quantitative analysis of moxifloxacin in human biological fluids. Sher et al. (2010) developed a liquid chromatography method with UV detection for the determination of moxifloxacin in human plasma. Due to the low sensitivity of the UV detector, they used a time consuming and labor-intensive liquid-liquid extraction technique for sample preparation. However, the sensitivity of the method can be improved only to a LLOQ of 40 ng/mL. Czyrski et al. (2017) developed a simple and fast HPLC-UV method which gave only low sensitivity with a LLOQ of 200 ng/mL. Wang et al. (2013) attempted to improve the sensitivity of the HPLC-UV method by a protein precipitation technique to extract moxifloxacin from study samples. However, the method also requires a pre-concentration step before analysis which may have a negative impact on the stability of the analyte, produces a time-consuming process and leads to a loss of consistency of analyses. Structurally, the detection technique selected for moxifloxacin often depends on a natural fluorescence-sensitive characteristic of the fluoroquinolone molecule that can re-emit light upon light excitation. Consequently, liquid chromatography coupled with fluorescence detection is a suitable and reasonable method with good sensitivity and specificity. Previously, LC-fluorescence methods coupled with complicated sample preparation and separation techniques such gradient elution and on-column focusing (Stass and Dalhoff 1997), pre-column derivatization (Tatar Ulu 2007), or a specific column (Laban-Djurdjević et al. 2006) for fluoroquinolone analysis in biological matrices were reported. Kumar et al. firstly introduced a simple deproteinizing technique and rapid liquid chromatography method with fluorescence detection for the determination of moxifloxacin in plasma and saliva. However, the sensitivity of this method was practically low. The LLOQ values for the assay of moxifloxacin were 125 ng/mL (in plasma) and 250 ng/mL (in saliva) (Kumar and Ramachandran 2009; Kumar et al. 2011). Although LC-ESI-MS/MS methods have also been reported with sensitive and selective benefits (Vishwanathan et al. 2002; Pranger et al. 2010), these techniques are expensive and may therefore not be affordable in several clinical settings.

In the current work, we report a fast and simple HPLC-fluorescence method for determining moxifloxacin in human plasma using trichloroacetic acid in acetonitrile for cleaning up study samples. The use of trichloroacetic acid in acetonitrile yields sufficient recovery of moxifloxacin and levofloxacin (Fig. 1B). It also provides a sufficient cleaning up for lipemic and hemolyzed plasma samples. The analytical method was fully validated covering all aspects of required parameters listed in the international guidelines for bioanalytical method validation. It provides rapid sample preparation and time-saving procedures for analysis of moxifloxacin in therapeutic drug monitoring and pharmacokinetic studies, which is suitable for clinical routine. Finally, the validated method was successfully applied in a pharmacokinetic study of a single-dose oral administration of moxifloxacin 400-mg tablet in Thai healthy volunteers to demonstrate the applicability of the analytical method.

2. Investigations and results

2.1. Method validation

2.1.1. Specificity and sensitivity

Six different lots of blank human plasma were used to evaluate the specificity of the analytical method. No interference peak was observed at the retention times of moxifloxacin and levofloxacin. The representative chromatogram of ULOQ sample (3000 ng/mL) is presented in Fig. 2. Typical chromatograms of extracted blank plasma, blank plasma spiked with Na_2EDTA, levofloxacin and LLOQ sample (20 ng/mL) are shown in Fig. 3. The mean S/N of moxifloxacin was 28.57. The %deviation of six replicates of LLOQ ranged from 0.93 to 15.64% with %CV of 4.10%.

Table 1: Mean inter-day back-calculated standard and standard curve results (*n=3*)

Nominal conc. (ng/mL)	Back-calculated conc. (ng/mL)			Mean back-calculated conc. (ng/mL)	%Deviation	%CV
	Day 1	Day 2	Day 3			
20	19.899	19.925	19.871	19.898	-0.51	0.15
80	81.630	81.311	82.428	81.790	2.24	0.70
400	400.387	400.883	394.129	398.466	-0.38	0.94
1000	1020.504	976.168	976.641	991.438	-0.86	2.54
1600	1547.930	1586.411	1586.090	1573.477	-1.66	1.41
2200	2118.424	2212.314	2209.741	2180.160	-0.90	2.45
3000	3098.353	3032.682	3055.096	3062.044	2.07	1.09

2.1.2. Calibration curves and linearity

Calibration curves for moxifloxacin were constructed over the range of 20 – 3000 ng/mL. The calibration curve results are presented in Table 1. The weighted least square linear regression analysis showed that $1/x^2$ is the most appropriate weighting factor for constructing a calibration curve of moxifloxacin. The mean linear regression equation for moxifloxacin is y = 0.00060203x + 0.00078603, where y is the peak are ratio of moxifloxacin to IS and x is the concentration of moxifloxacin. The correlation coefficient (r^2) was found to be > 0.99. The %deviation of mean back-calculated concentrations ranged from -1.66 to 2.24 % with %CV of 0.15-2.54%.

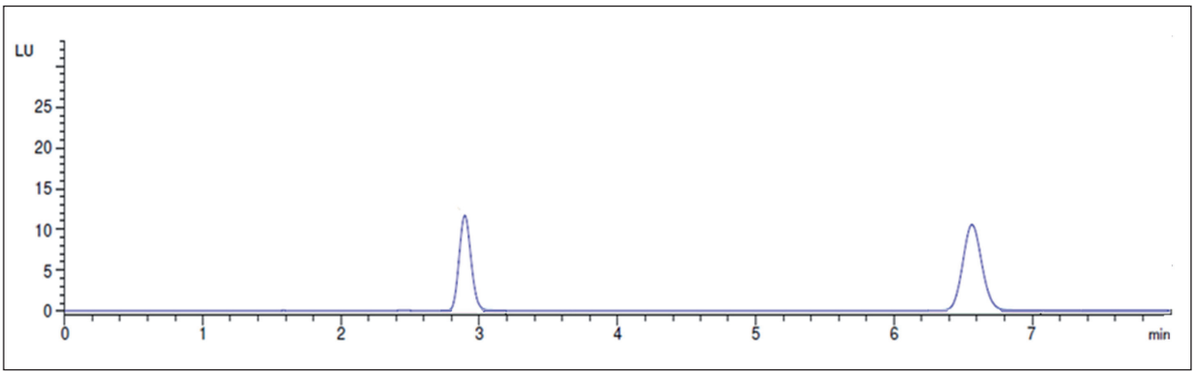

Fig. 2: A typical chromatogram of plasma spiked with 3000 ng/mL of moxifloxacin (ULOQ) and 570 ng/mL of levofloxacin (IS).

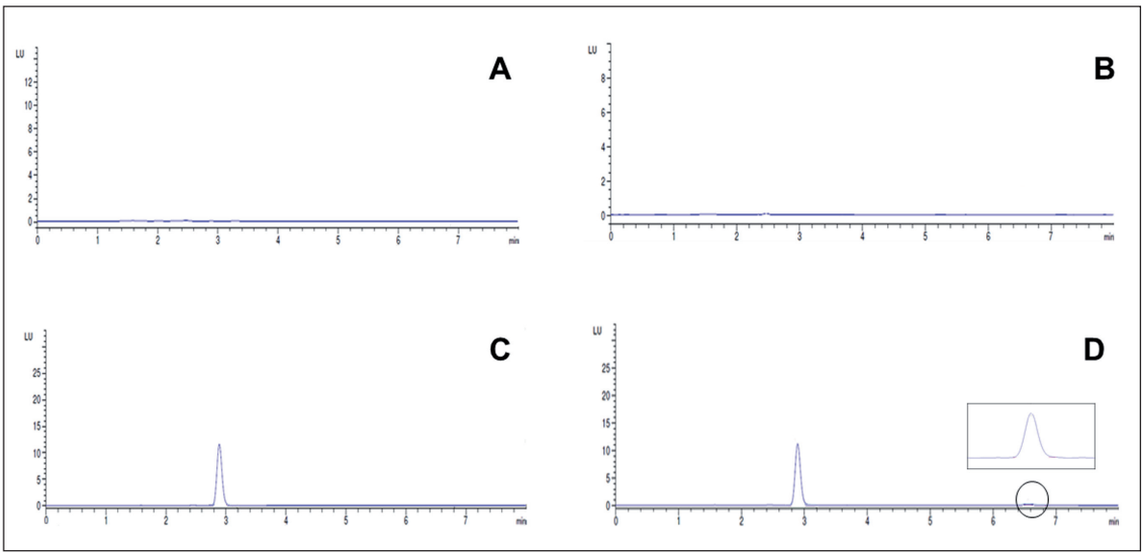

Fig. 3: Typical chromatograms of (A) blank human plasma, (B) blank human plasma spiked with Na_2EDTA, (C) spiked plasma with 570 ng/mL of levofloxacin (IS) and (D) spiked with 20 ng/mL of moxifloxacin (LLOQ) and 570 ng/mL of levofloxacin (IS).

Table 2: Intra-day and inter-day accuracy and precision of moxifloxacin determination

Nominal conc. (ng/mL)	Intra-day (*n=6*)			Inter-day (*n=18*)		
	Mean back-calculated conc. (ng/mL)	%Deviation (Accuracy)	%CV (Precision)	Mean back-calculated conc. (ng/mL)	%Deviation (Accuracy)	%CV (Precision)
20	21.386	6.93	4.40	21.905	9.53	4.11
60	59.397	-1.01	3.37	60.982	1.66	3.05
1200	1163.016	-3.08	3.58	1185.311	-1.22	3.11
2500	2474.369	-1.03	4.33	2466.187	-1.35	3.13
3000	2906.878	-3.10	3.94	2921.957	-2.60	3.84

2.1.3. Accuracy and precision

Accuracy and precision of the assay were determined at 20, 60, 1200, 2500 and 3000 ng/mL in six replicates. The intra-day accuracy showed %deviation between -3.10 and 6.93 % with the intra-day precision of 3.37 - 4.40 % CV. The inter-day accuracy showed %deviation from -2.60 - 9.53 % and the inter-day precision of 3.05 - 4.11%CV. The accuracy and precision results are summarized in Table 2.

2.1.4. Recovery

The recovery of moxifloxacin was evaluated at LQC, MQC and HQC levels. The mean recoveries at LQC, MQC and HQC levels were 58.56, 60.81 and 60.68 % with the %CV of 4.58, 5.28 and 2.14 %, respectively. The extraction recovery of levofloxacin determined at 570 ng/mL was 83.76 % with the %CV of 5.54%.

2.1.5. Hemolytic and lipemic effects

The effects of hemolyzed and lipemic plasma on the performance of the analytical method were assessed using LQC and HQC samples. For effect of hemolysis, the %deviation at LQC and HQC levels were -1.58 and -5.20% with the %CV of 1.12 and 2.72 %. For lipemic effect, the LQC and HQC samples shows the %deviation of 1.53 and 3.33 % with 1.84 and 2.55 %CV.

2.1.6. Dilution integrity

The effect of dilution on the accuracy (%deviation) and precision (%CV) of spiked samples containing known amounts of moxifloxacin (4200 and 6000 ng/mL) are summarized in Table 3. The %deviation were -3.67 and -6.89 with the %CV of 5.63 and 3.17 at the concentration of 4200 and 6000 ng/mL of moxifloxacin, respectively.

Table 3: Dilution integrity test (1:1 and 1:3 dilutions of plasma samples which moxifloxacin concentrations at 140% and 200%ULOQ, respectively (n=5))

Nominal conc. (ng/mL)	Mean back-calculated conc. (ng/mL)	%Deviation (accuracy)	%CV (precision)
4200 (140% ULOQ)	4045.961	-3.67	5.63
6000 (200% ULOQ)	5586.776	-6.89	3.17

2.1.7. Stability

Stability results of moxifloxacin are summarized in Table 4. Moxifloxacin was found to be stable in human plasma for at least four freeze-thaw cycles (kept at the freezing temperature of -20 °C and -70 °C), for 6 h at room temperature (25±5 °C) under sodium lamp and day light exposure, for 8 weeks at -20 °C and -70 °C, and for 48 h in the autosampler (4 °C).

2.2. Pharmacokinetic study

The method was applied to the determination of moxifloxacin in human plasma after oral administration of 400 mg moxifloxacin tablets (Siam Bheasach, Thailand). The mean plasma concentration vs. time plot of moxifloxacin is shown in Fig. 4. The mean area under the plasma concentration-time curve ($AUC_{0\rightarrow t}$) and the area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0\rightarrow inf}$) of moxifloxacin were 36772.26±7653.74 ng·h/mL and 37599.88± 737.94 ng·h/mL, respectively. The ratio of $AUC_{0\rightarrow t}$ to $AUC_{0\rightarrow inf}$ was about 97.75%. The mean peak plasma concentration (C_{max}) was 2343.10±625.38 ng/mL and the mean time to peak plasma concentration (t_{max}) was 2.05±1.13 h. The elimination half-life ($t_{1/2}$) of moxifloxacin was 12.61±2.09 h.

Table 4: Stability of moxifloxacin in samples under different conditions (*n=3*)

Storage conditions	Nominal conc. (ng/mL)	Mean back-calculated conc. (ng/mL)	%Deviation	%CV
Freeze–thaw stability (4 cycles)				
Freeze at -20°C	60	65.449	9.08	0.68
	2500	2718.279	8.73	2.92
Freeze at -70°C	60	65.738	9.56	3.22
	2500	2665.112	6.60	1.32
Short-term stability (at 25°C, 6 h)				
Under sodium lamp	60	56.505	-5.82	3.67
	2500	2336.237	-6.55	1.96
Under day light exposure	60	56.234	-6.28	2.35
	2500	2312.972	-7.48	3.63
Long-term stability (8 weeks)				
At -20°C	60	63.581	5.97	0.36
	2500	2649.807	5.99	2.74
At -70°C	60	63.846	6.41	1.38
	2500	2666.592	6.66	0.35
Post-preparative stability				
Autosampler (4°C) for 48 h	60	61.927	3.21	1.83
	2500	2473.551	-1.06	1.22

3. Discussion

The specific aim of this study was to develop a LC method for determining moxifloxacin in human plasma with simple sample preparation and short analysis time. For HPLC analysis of moxifloxacin, fluorescence detection was chosen to give sufficient sensitivity and selectivity. The fluorescence detection theoretically shows the specificity on fluoroquinolone agents above other spectrophotometric techniques. Levofloxacin is an appropriate molecule to serve as an internal standard (IS) under the developed sample preparation and chromatographic condition because of the similarity of physical and chemical characteristics between moxifloxacin and levofloxacin. The chromatographic condition can unequivocally separate moxifloxacin from the IS and plasma matrix interference with appropriate time of analysis. In comparison with the previously reported method (Kumar and Ramachandran 2009), the method presented here has higher sensitivity (20 ng/mL vs. 125 ng/mL). The sensitivity at LLOQ of 20 ng/mL and fast analysis time are adequate for pharmacokinetic determination or therapeutic drug monitoring of moxifloxacin in patients. Additionally, the developed method is suitable for the routine analysis and easier affordable in clinical settings than sophisticated LC-MS methods.

The developed method has fully been validated according to the U.S. FDA Guidance for Industry Bioanalytical Method Validation and EMA Guideline on bioanalytical method validation. The method is highly selective for moxifloxacin and levofloxacin even

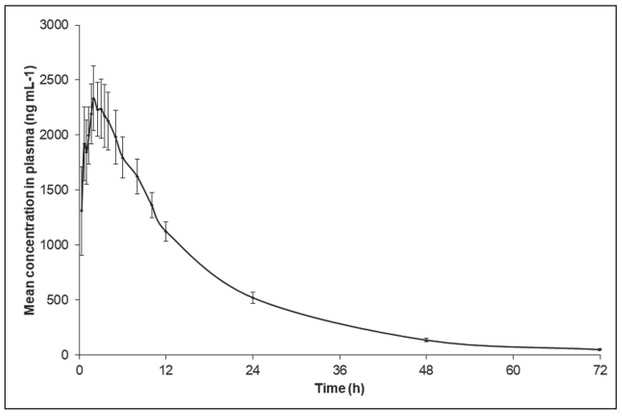

Fig. 4: Mean plasma concentration – time profile of six healthy volunteers after oral administration of a single dose of a moxifloxacin 400-mg tablet (*n*=6)

in the presence of matrix components. The linearity, accuracy, and precision data indicate that the method is linear, accurate and precise over the established concentration range. The recoveries of both moxifloxacin and levofloxacin were >50% with %CV < 15%, indicating that the recovery of the developed method was sufficient and reproducible. The hemolytic and lipemic effect studies suggest that the interference from red blood cells' components and plasma lipids had no negative impact on the accuracy and precision of the analytical method. Regarding sensitivity, the reported S/N, %deviation and %CV suggest that the analysis of moxifloxacin at 20 ng/mL is reliable in aspects of accuracy and precision which can be set as the LLOQ of the assay. The stability data indicates that moxifloxacin is stable along the recommended sample preparation and HPLC analysis periods.

The developed method has been applied to a pharmacokinetic study of moxifloxacin in human plasma after oral administration of 400 mg moxifloxacin tablets in six healthy volunteers. The ratio of ratio of $AUC_{0\rightarrow t}$ to $AUC_{0\rightarrow inf}$ was more than 80%, indicating that sampling time and LLOQ at 20 ng/mL are suitable for the study and the pharmacokinetic parameters i.e., *AUC, Cmax,* t_{max} and $t_{1/2}$ were successfully determined.

In conclusion, a simple, sensitive and rapid HPLC method was developed and validated for determination of moxifloxacin in human plasma. The fluorescence detector provides specificity and selectivity to the analytical method. The simple protein precipitation using 6% trichloroacetic acid in 20 % acetonitrile gave the sufficient recovery and minimized the sample preparation time. The developed method provides a sufficiently clean sample as well as an adequate sensitivity at LLOQ of 20 ng/mL.

4. Experimental

4.1. Chemicals and reagents

Moxifloxacin hydrochloride reference standard (lot number G0L202, purity 96.0%) was purchased from USP (USA). Levofloxacin hemihydrate (lot number 00000023220, purity 96.9%) was provided by Siam Bheasach Co., Ltd (Bangkok, Thailand). Moxifloxacin hydrochloride 400 mg tablets were kindly supplied by Siam Bheasach Co., Ltd. (Bangkok, Thailand). Analytical-grade trichloroacetic acid was bought from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate was obtained from Carlo Erba (Milano, Italy). HPLC-grade acetonitrile and methanol were purchased from Scharlau (Barcelona, Spain). K_3EDTA-blank human plasma was obtained from Bio Innova & Synchone Co., Ltd (Bangkok, Thailand). Hemolyzed and lipemic human plasma was obtained from National Blood Center (Bangkok, Thailand) and stored at -70 °C.

4.2. Instrumentation and chromatographic conditions

Liquid chromatographic system (Agilent Technologies, USA) consisted of an Agilent 1200 series with G1312A binary pump equipped with an Agilent G1379B degasser and Agilent G1367B thermostat autosampler was employed. The analytical separation was carried out using a Shishedo C18 column (4.6 x 250 mm, i.d. 5 µm) with fluorescence detection at the excitation wavelength of 296 nm and the emission wavelength of 504 nm. The column temperature was controlled at 35 °C. The mobile phase composed of 50 mM potassium dihydrogen phosphate buffer pH 2.4 and 100% acetonitrile (77:23, v/v) was pumped into the chromatographic system under an isocratic condition at a flow rate of 1.5 mL/min. The injection volume of the sample was 20 µL. All samples were kept in a thermostat autosampler (4 °C) for at least 60 min before analysis. The total analysis time was 8 min and the elution of moxifloxacin and levofloxacin were approximately observed at 6.5±0.5 min and 3.0±0.5 min, respectively. The data acquisition was processed using Chemstation software™ version B.02.01-SR2 [260] and the data report was generated using ChemStore C/S Revision B.03.02 Build (183)2SR3 and Chemstation software™ version B.02.01-SR2 [260].

4.3. Preparation of standard solutions

Moxifloxacin was prepared in 100% methanol to obtain a primary stock solution at a concentration of 500 µg/mL. This solution was then diluted with the mobile phase to obtain a secondary stock solution at a concentration of 200 µg/mL. The working solutions of moxifloxacin at 400, 1600, 8000, 20000, 32000, 44000, and 60000 ng/mL for calibration curve standards were prepared from subsequently diluting the secondary stock solution with the mobile phase. Another secondary stock solution was prepared and diluted with the mobile phase to get working solutions at 400, 1200, 24000, 50000 and 60000 ng/mL for determination of lower limit of quantification (LLOQ), low quality control sample (LQC), medium quality control sample (MQC), high quality control sample (HQC), and upper limit of quantification (ULOQ). A stock solution of levofloxacin (internal standard, IS) at 300 µg/mL was prepared in methanol and further diluted with the mobile phase to obtain a working IS solution at a concentration of 12 µg/mL.

4.4. Preparation of calibration standards and quality control (QC) samples

Working solutions (15 µL) for calibration curve standards were individually spiked in a 285 µL of blank human plasma to yield calibration curve standards at concentrations of 20, 80, 400, 1000, 1600, 2200, and 3000 ng/mL. The lowest concentration standard (20 ng/mL) was used as a LLOQ sample. Five concentration levels of quality control (QC) samples were prepared by spiking another set of working standard solutions to blank plasma to yield QC samples at concentrations of 20, 60, 1200, 2500 and 3000 ng/mL, which correspond to LLOQ, LQC, MQC, HQC and ULOQ, respectively.

4.5. Sample preparation for HPLC analysis

Working IS solution (15 µL) was spiked into 300 µL of each plasma sample (i.e. calibration standards, QC samples and clinical plasma samples). The IS spiked samples were vortexed for 10 s. Then, the samples were deproteinized by the addition of 500 µL of freshly prepared 6 % trichloroacetic acid in 20 % acetonitrile. The samples were further vortexed for 30 s and subsequently centrifuged at 10000 rpm at 4 °C for 10 min. Finally, the supernatant was transferred to a HPLC vial for HPLC analysis.

4.6. Method validation

The method was validated for linearity, accuracy, precision, sensitivity, recovery and stability according to the U.S. FDA and EMA guidelines (U.S. FDA 2001; EMA 2011). The QC sample at 1200 ng/mL (MQC) was assigned as the system suitability sample for testing the equilibrium and readiness of the chromatographic system prior to HPLC analysis. The %CV calculated from five replicates of peak area ratio between moxifloxacin and levofloxacin should ≤ 2 %.

4.6.1. Specificity and sensitivity

Six different sources of blank plasma were used to investigate the specificity of the method in the presence of matrix interferences. The interference of K_3EDTA and Na_2EDTA was also evaluated in this experiment. The evaluation was conducted by comparing the chromatograms of blank plasma with and without K_3EDTA. The peak response of interfering components should be < 20% of the peak response of moxifloxacin in the LLOQ sample and < 5% of the peak response of levofloxacin. Sensitivity of the analytical method is the lowest concentration of moxifloxacin (LLOQ level) that can be accurately and precisely measured. The sensitivity is accepted if the signal-to-noise ratio (S/N) of moxifloxacin at LLOQ is at least five times to the baseline response. In addition, the analysis of moxifloxacin at six replicates of LLOQ samples from within day and between three different days must be within an accuracy of ±15 % deviation and a precision of ±20 %CV.

4.6.2. Calibration curves and linearity

Calibration curves were constructed by plotting concentrations of calibration curve standards of moxifloxacin against the peak area ratios between moxifloxacin and levofloxacin. The regression model was evaluated by weighted-least square linear regression. Linearity was assessed by constructing calibration curves on three consecutive days over the concentration range of 20-3000 ng/mL. The coefficient of determination (r^2) should be > 0.99. The accuracy of the calibration standards was expressed as a %deviation which should be within ±15 %, except for LLOQ which should be within ±20 %. The precision, which was expressed as a percentage coefficient of variation (%CV), of each calibration sample for three days fell within 15 %, except for LLOQ which should be ≤ 20%.

4.6.3. Accuracy and precision

Intra-day accuracy and precision were evaluated from six determinations of five different concentration levels at 20 (LLOQ), 60 (LQC), 1200 (MQC), 2500 (HQC) and 3000 (ULOQ) ng/mL on the same day. The inter-day accuracy and precision

were evaluated based on accuracy and precision batches from three consecutive days. The acceptance limit of accuracy was within ±15%, except for LLOQ which should be within ±20%. The acceptance limit of precision was ≤ 15%CV, except for LLOQ, which should be ≤ 20%CV.

4.6.4. Recovery

Recovery was evaluated by comparing the peak areas of the moxifloxacin in extracted QC samples (LQC, MQC and HQC) with those obtained from the extracted blank plasma spiked with the working solutions of moxifloxacin at the same concentrations. The recovery of levofloxacin was determined at a concentration of 570 ng/mL. The experiment was performed in five replicates. The recovery of moxifloxacin and levofloxacin should be > 50%. Additionally, the precision of recovery of moxifloxacin and levofloxacin at each concentration level should be ≤ 15%CV.

4.6.5. Hemolytic effect

Hemolysis may possibly occur during blood sampling. To ensure the reliability of the bioanalytical method from the impact of hemolyzed plasma, hemolytic effect on the accuracy and precision of the method was evaluated on six replicates of LQC and HQC samples prepared in the hemolyzed blank plasma. The concentrations of moxifloxacin in the hemolyzed plasma samples were determined against a calibration curve prepared in human normal blank plasma. The acceptance limit of accuracy was within ±15 %. The acceptance of precision was ≤ 15%CV.

4.6.6. Lipemic effect

High fat plasma may impact of the accuracy and precision of bioanalytical method. Lipemic effect on the accuracy and precision was therefore determined to ensure the reliability of the method. Six replicates of LQC and HQC samples prepared in lipemic human plasma were used to evaluate the lipemic effect. The concentrations of moxifloxacin in lipemic plasma samples were determined against a calibration curve prepared in human normal blank plasma. The acceptance limit of accuracy was within ±15%. The acceptance limit of precision was ≤ 15%.

4.6.7. Dilution integrity

Dilution integrity was conducted to demonstrate that sample dilution with the same matrix did not affect the reliability of the results. Five replicates of samples containing 4200 and 6000 ng/mL of moxifloxacin were diluted at 1:1 and 1:3 dilution with blank human plasma, respectively. The acceptance limits were ±15% deviation for accuracy with the precision of ≤ 15%CV, respectively.

4.6.8. Stability

All stability studies of moxifloxacin in human plasma were performed in triplicate using the LQC and HQC samples. Freeze-thaw stability was examined by determining moxifloxacin in the QC samples after four freeze (in freezers, -20 °C and -70 °C) – thaw (at room temperature, 25 °C) cycles. Short-term stability was tested by storage of the QC samples under sodium lamp and day light exposure over 6 h at room temperature (25 °C). Long-term stability was tested by storing the QC samples in the freezers (-20 °C and -70 °C) for 8 weeks. For the stability in an autosampler, the extracted samples were kept in a controlled temperature autosampler (4 °C) for 48 h. The amount of moxifloxacin in the all stability samples was analyzed against freshly prepared calibration curve and QC samples. The assay values should be within ±15% deviation for accuracy and ≤ 15%CV for precision.

4.7. Pharmacokinetic study

A single 400 mg moxifloxacin tablet was orally administered to six Thai healthy volunteers (two males and four females). Blood samples were collected at pre-dose and at 0.33, 0.66, 1.00, 1.33, 1.67, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 24.00, 48.00 and 72.00 h after dosing using an indwelling cannula placed in a forearm vein and kept the volunteers with normal saline. The collected blood samples (kept in K_3EDTA blood collection tubes) were centrifuged at 3200 x *g* for 10 min. The obtained plasma was transferred into polypropylene tubes and stored at -70 °C under light protection before analysis. All the subjects were informed for the aims and risks of the study and written consent was obtained. The study protocol was reviewed and approved by the Ethical Review Committee for Research in Human Subjects, Institute of Development of Human Research Protection (IHRP). The study was conducted in accordance with the International guidelines for human research protection as Declaration of Helsinki, the Belmont Report, CIOMS Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Conflicts of interest: The authors report no conflicts of interest.

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