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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY FOR DETERMINATION OF MOXIFLOXACIN IN HUMAN PLASMA

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Abstract. A simple reversed phase HPLC method with UV detection has been successfully developed and validated for determination of moxifloxacin in human plasma. The sample pretreatment involves only single-step protein precipitation with trichloroacetic acid. Moxifloxacin was measured in plasma using a validated HPLC method with UV detector at 295 nm, C18 column (25 cm × 4.5 mm, 5 µm), a mixture of phosphate buffer pH 4.0 and acetonitrile (30:70, v/v) as mobile phase at a flow rate of 0.8 mL/min. Retention time of moxifloxacin was found to be 7.4 min. The mean recovery for the drug was obtained 97.30 %. The calibration curve was linear over the concentration range of 0.3 to 25.0 µg/mL with coefficient correlation of 0.9991. This method was successfully applied for therapeutic drug monitoring.

Keywords: moxifloxacin, protein precipitation, trichloroacetic acid, HPLC method, human plasma.

Classification numbers: 3.2.1.

1. INTRODUCTION

Moxifloxacin (1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4aS, 7aS) octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4 dihydroquinoline-3-carboxylic acid (Fig. 1)) [1] is a fourth-generation of flouroquinolone antibacterial agent. It possesses bacteriostatic activity against Gram-positive and Gram-negative bacteria [2, 3]. Since having an azabicyclo-substitution at C-7, its activity against Gram-positive bacterial as well as atypical pathogens is improved. It is available for oral and parenteral administration which is specially used for patients with respiratory or skin infection [3].

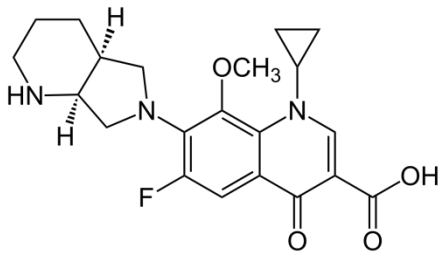


Figure 1. Molecular structure of moxifloxacin.

A variety of methods on high-performance liquid chromatography (HPLC) for measuring moxifloxacin concentration in plasma have been reported. HPLC with fluorescence detector was applied in several studies [4-7]. On the other side, HPLC with UV detector was employed for applications in routine therapeutic drug monitoring or clinical pharmacokinetic studies of moxifloxacin. Most reported HPLC-UV methods involve a sample pretreatment step in which methanol [8], acetonitrile [9, 10] or dichloromethane [11] was used as precipitating solvent. Alternatively, in this paper, we report a simple HPLC-UV method for determination of moxifloxacin in plasma using trichloroacetic acid within deproteinization step. Precipitation can be achieved by varying the pH of the medium. At low pH's, proteins have a net positive charge because the amide gains an extra proton. At high pH's, they have a net negative charge due to the carboxyl on the protein backbone losing its proton. At their pI value, a protein has no net charge. This leads to reduced solubility because the protein is unable to interact with the medium and will then fall out of solution.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals and reagents

Moxifloxacin hydrochloride standard (MOXI), yellow powder, was obtained from National Institute of Drug Quality Control with stated purity of 94.36 %. All solvents used were HPLC grade (Merck, Germany). Potassium phosphate and phosphoric acid were purity grade products from Merck. The used water is deionized. Other chemicals were at least of analytical grade and used as received.

2.1.2. Chromatographic system

Chromatographic analysis was performed on HPLC system (Shimadzu, Japan) with a PDA detector and using a MRC-ODS C18 column, 250 × 4.5 mm, 5 µm. The chromatographic conditions were achieved in this study according to the Liqin Zhu's method [10]. The mobile phase was a mixing of 20 mM phosphate buffer (pH 4.0 adjusted by phosphoric acid) and acetonitrile with a ratio 30:70 (v/v), delivered at 0.8 mL/min rate. The detection was observed at 295 nm. The column was maintained at ambient temperature with an injection volume of 20 µL.

2.2. Methods

2.2.1. Standard preparation

Standard stock solution of moxifloxacin was prepared by direct weighing of standard substance with subsequent dissolution in deionized water. The concentration of the standard stock solution was 500 µg/mL. Working standard solution of MOXI was prepared by withdrawing an aliquot portion of the stock solution and diluting appropriately with methanol to get the concentration of 10 µg/mL. The solution was filtered through a 0.45 µm nylon filter and degassed by ultrasonic agitation before analysis.

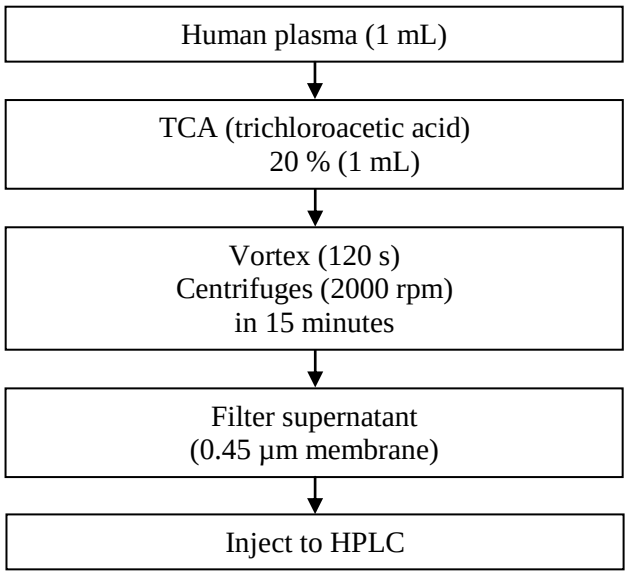
2.2.2. Sample preparation

Blank plasma samples were collected from healthy volunteers, who do not use moxifloxacin.

The spiked sample was prepared by taking a known volume of moxifloxacin stock solution and evaporating the solvent to collect the residue (temperature 40 °C, N_2 gas). The residue was diluted in blank plasma and vortex mixed for 2 minutes to get a spiked sample with known moxifloxacin concentration.

2.2.3. Optimization of sample pretreatment

For the analysis of biological samples, protein precipitation is the most common sample preparation procedure, which is the simplest approach that removes the majority of the protein from the sample. In our method development, trichloroacetic acid (TCA) was used as an acidic reagent for deproteinization. To achieve good extraction efficiency and protect the HPLC column, the human plasma pretreatment was optimized with respect to parameters including TCA/plasma ratio; concentration of TCA; vortex time; centrifugation time and rate. Peak area response was counted as changing one single variable. Optimal pretreatment procedure was obtained as in Scheme 1.



Scheme 1. Optimal pretreatment procedure.

2.2.4. Calibration curve in plasma matrix

Aliquot portions of standard working solution were added to 0.50 mL of blank plasma. The final moxifloxacin concentration was in a range of 0.3 - 25.0 µg/mL (10 solutions). These solutions were further treated to the same procedure as optimized conditions in section 2.2.3.

2.2.5. Linearity

The linearity of the standard curves was assessed with the intercept, slope and correlation coefficient (R^2) and their variations in the range of 0.3 - 25.0 µg/ml. The calibration samples of moxifloxacin (0.3, 0.5, 1.0, 2.0, 4.0, 8.0, 10.0, 14.0, 18.0, 25.0 µg/mL) were prepared by separately spiking of prepared working standard solution of moxifloxacin into 0.5 mL of blank plasma. The standard calibration curve for moxifloxacin was constructed by least square linear regression using peak areas.

2.2.6. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined by gradually adding moxifloxacin to blank plasma sample. LOD was obtained at the concentration in which height of peak signal to noise (S/N) equals to 3. LOQ was accepted as LOQ = 3.3 × LOD.

2.2.7. Precision

Precision of analytical method was expressed as SD (Eq. 1) and %RSD (Eq. 2) of series of replicate measurements.

$$SD = \sqrt{\frac{\sum (S_i - S_{tb})^2}{n-1}} \qquad (1)$$

$$RSD\ (\%) = \frac{SD}{S_{tb}} \times 100 \qquad (2)$$

where: S_i is the i^{th} recovery efficiency; S_{tb} is the average recovery of n analyses; n is the number of repetitions.

2.2.8. Recovery

Recovery (H) was calculated through the equation 3:

$$\%\ H = C_{tt}/C_{lt} \times 100 \qquad (3)$$

where C_{tt} is actual concentration of moxifloxacin obtained after analysis while C_{lt} denotes added amount of moxifloxacin.

3. RESULTS AND DISCUSSION

3.1. Method evaluation

3.1.1. Specificity

To evaluate the specificity of moxifloxacin determination in plasma, we performed two analysis for comparison: (a) blank plasma sample; (b) spiked sample with moxifloxacin of 3.0 µg/mL. The obtained chromatograms were shown in Figure 2. Under the optimized chromatographic conditions and sample processing procedure, the retention time of moxifloxacin was approximately 7.4 min. The chromatograms showed a clear and excellent separation between moxifloxacin and endogenous interferences from plasma. No interfering peaks were observed at the retention time of moxifloxacin.

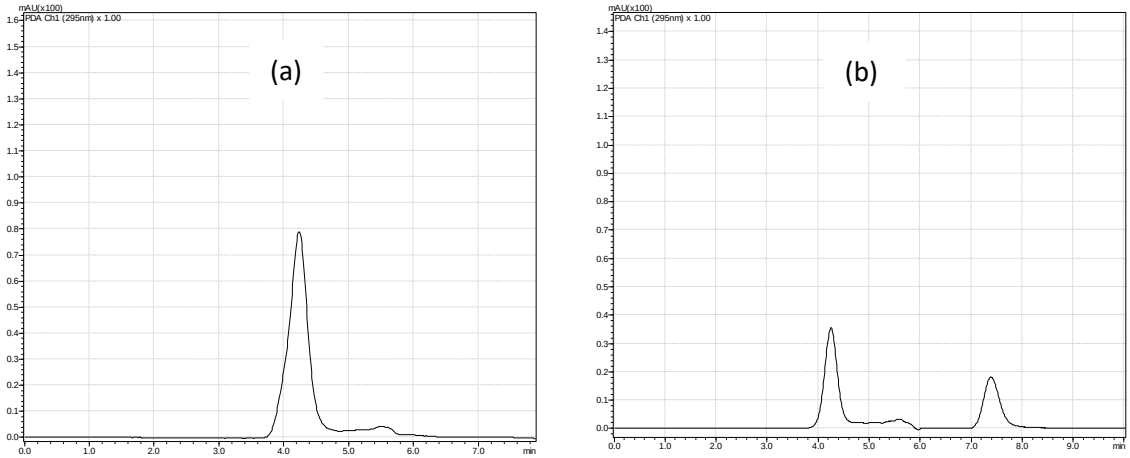


Figure 2. Chromatograms of (a) blank plasma sample and (b) spiked sample with moxifloxacin of 3.0 µg/mL

3.1.2. Linearity and regression analysis

The calibration line for human plasma analysis was evaluated. The linearity is in the range of 0.03-25 µg/mL with the regression coefficient (R^2) is of 0.9991.

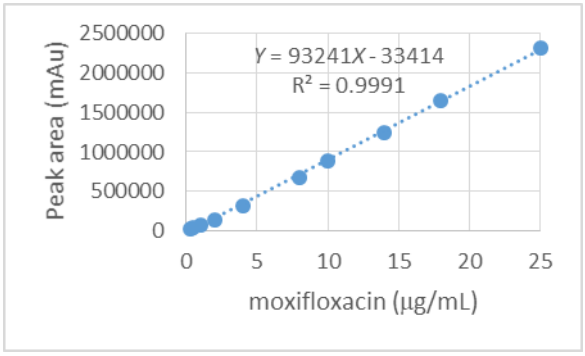


Figure 3. Calibration curve for determination of moxifloxacin in plasma.

3.1.3. LOD and LOQ

By gradually increasing amount of moxifloxacin in human plasma sample, the determined LOD and LOQ values were 0.15 and 0.50 µg/mL, respectively (Table 1). There are also lower levels of quantification for HPLC-UV method reported [8, 9, 11], however in clinical practice

this level is satisfactory for effective determination of the concentration of the drug because reported the mean trough concentration was 0.59 μg/mL [12].

Table 1. Regression analysis of the calibration lines.

Parameter	Value
Regression equation	$Y = (93241 \pm 1834.2)\ X - (33414 \pm 21155.6)$
Regression coefficient	0.9991
LOD (μg/mL)	0.15
LOQ (μg/mL)	0.50

Y = peak area, X = concentration of MOXI.

3.1.4. Accuracy and precision

The mean peak concentration of moxifloxacin in human plasma was 4.81 ± 1.03 μg/mL as previously reported by Ioannis Kioumis *et al*. [12] In our study, precision and accuracy were evaluated using blank plasma spiked with three different concentration levels of moxifloxacin: low (1.0 μg/mL), medium (5.0 μg/mL) and high (10.0 μg/mL). Each concentration level was analyzed repeatedly five times.

Table 2. Precision and accuracy evaluation (n = 5).

Actual conc. (μg/mL)	Found conc. ± SD (μg/mL)	Recovery (%)	RSD (%)
1.0	0.89 ± 0.09	89.20	10.22
5.0	4.27 ± 0.10	85.39	2.34
10.0	9.73 ± 0.11	97.32	1.18

The results (Table 2) show that the RSD is higher at lower concentration of MOXI. However, all RSD-values are smaller than 11 %, which is acceptable with the AOAC [13] requirement. On the other hand, mean value of the method recovery at three concentration levels ranged from 89.20 % to 97.32 %. According to AOAC, the analytical method for an analyte concentration between 1 - 10 ppm, its recovery should be within 80 - 110 %. Hence, it can be concluded that the proposed method has both high accuracy and precision and meets the requirement for analysis.

3.2. Application

The developed procedure was applied to determine moxifloxacin in plasma of patients who was infused Avelox drug with dose of 450 mg / 250 ml. These samples were collected from patients after 1-3 h infusing at Blood Bank of Vietnam 108-Military Hospital.

In the previous study, the concentration-time profiles of moxiflocaxin in plasma were demonstrated and the mean peak concentration was 4.81 μg/mL at 1 h after infusion 400 mg. Furthermore, Jian Lu *et al.* [11] found that peak concentrations ranged from 2.60 to 3.79 g/ml were able to be reached within 1–3 h (mean 1.75 h) after oral dose 400 mg. The analytical

results for individual patients in this study were shown in Table 3. Although the weight, age, healthy, liver function and renal function were the important factors which caused the changes of moxifloxacin concentration in human plasma, these results were consistent with the previous studies. It is suggested that the proposed method could be applied not only for therapeutic drug monitoring but also for pharmacokinetic studies.

Table 3. Analysis of moxifloxacin by proposed method.

No.	Name of patient	Time after infusing (h)	MOXI (µg/mL)
1	Tran Van A	1	1.52±0.05
2	Nguyen Van B	2	2.61±0.20
3	Cao Thi C	3	2.07±0.14

4. CONCLUSIONS

An HPLC-UV method for the determination of moxifloxacin in human plasma sample was developed and validated. The proposed method involves the following advantages: simple and effective sample preparation, precise and accurate assay for moxifloxacin analysis. The sensitivity and simplicity of the method makes it suitable for routine therapeutic drug monitoring or clinical pharmacokinetic studies of moxifloxacin.

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