

## Overview

A sensitive and specific liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method capable of quantifying moxifloxacin in Human Plasma is described.

In this method, moxifloxacin was extracted from plasma using a protein precipitation method. The LC separation was performed on a reverse phase C18 column with a special mobile phase, which greatly improved ion chromatography separation and peak shape.

The method has been successfully employed to quantify moxifloxacin in human plasma collected from a clinical study.

## Introduction

Moxifloxacin or AVELOX, as an antibacterial agent, is used to treat complicated intra-abdominal infections (cIAI) in adults. Two international studies are being carried out to study the effectiveness of this antibiotic as a new treatment for tuberculosis, the highly contagious bacterial disease that kills more than 2 million people worldwide each year. Several analytical methods have been developed for the determination of moxifloxacin in plasma. These HPLC/UV methods have low sensitivity and non specificity. To support clinical trials, a short run time and lower detection limit is needed. We report a rapid, specific, and highly sensitive LC/MS/MS method capable of quantifying moxifloxacin at levels as low as 1 ng/mL.

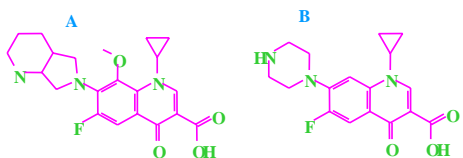


Figure 1. The structure of moxifloxacin (A), and ciprofloxacin as the internal standard (B).

## Experimental

### Sample Preparation

Moxifloxacin and the internal standard were extracted from human plasma by a protein precipitation method. The supernatant was evaporated to dryness under a nitrogen stream, and the residue was reconstituted with reconstitute solution.

### Liquid chromatography:

LC System: Pump Shimadzu LC-10AD  
 Autosampler Shimadzu SIL-HT  
 System Controller Shimadzu SCL-10A  
 Analytical Column: Luna C18 column, 2.0 x 30 mm, 5 μm  
 Gradient  
 Flow rate: 0.8 mL/min  
 Injection Volume: 5 μL

### Mass Spectrometry

MS System: PE Sciex API-4000  
 Condition: LC/(+)-ESI-MS/MS (MRM)

The mass spectrometer was set up for the following transition:

Moxifloxacin: 402 → 261  
 Internal Standard: 332 → 231

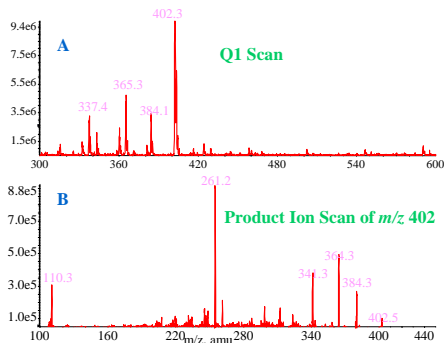


Figure 2. (A) Moxifloxacin LC/(+)-ESI-MS spectrum. (B) Moxifloxacin LC/(+)-ESI-MS/MS product ion spectrum.

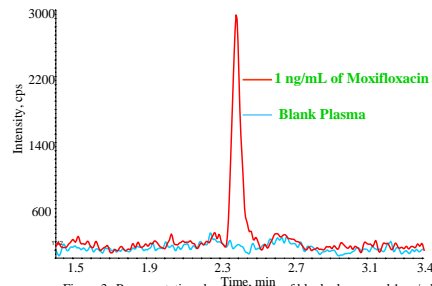


Figure 3. Representative chromatograms of blank plasma and 1 ng/mL moxifloxacin in plasma.



## Results and Discussion

In previous work, with UV used as the detector, the HPLC method not only gives low sensitivity, but its non-specificity also greatly increases the run time. In this study, a special mobile phase was used, which greatly improved the ion chromatography separation and peak shape.

Excellent linearity was obtained with a correlation coefficient greater than 0.995. The inter-day precision (CV%) and accuracy (RE%) for all QC samples, including LLOQ were <11% and <8%, respectively.

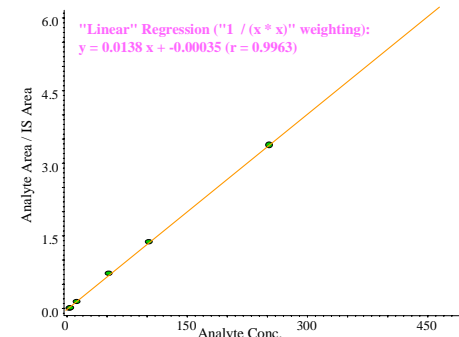


Figure 4. Representative calibration curve.

## Conclusion

A new LC/MS/MS method was developed and validated for quantifying moxifloxacin in human plasma, with a lower limit of quantitation (LLOQ) of 1 ng/mL in human plasma. The method has been used successfully for clinical sample analysis.