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Development and validation of LC-ESI-MS/MS method for analysis of moxifloxacin and levofloxacin in serum of multidrug-resistant tuberculosis patients: Potential application as therapeutic drug monitoring tool in medical diagnosis

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ABSTRACT

Moxifloxacin (MFX) and levofloxacin (LFX), class of fluoroquinolone antibiotics, are the two most prescribed drugs to multidrug resistant tuberculosis (MDR-TB) patients. A single, sensitive and reliable LC-ESI-MS/MS method was developed and validated to simultaneously quantitate the levels of these drugs in human serum where enrofloxacin (EFX) was used as internal standard (IS). Quantification was achieved by multiple reaction monitoring of selected mass transitions from precursor ions to product ions m/z 402.2 → 384.2 for MFX, 362.2 → 318.2 for LFX, and 362.1 → 318.3 for EFX. Calibration curves were plotted using concentrations ranging between 0.23–1000 ng/mL for MFX and 0.13–1000 ng/mL for LFX, and the correlation coefficients (r^2) were in excess of 0.999. Intra- and inert-day accuracy was ranged between 92.1–104% with mean recoveries of 96% and 95.5% for MFX and LFX, respectively and precision was <9% at all quality control concentration levels. Matrix effect analysis showed extraction efficiency of 93.0–94.6% for MFX and 90.9–99.5% for LFX. Application of the developed method to real sample analysis resulted in efficient quantification of MFX and LFX in serum samples obtained from ten MDR-TB patients. The result indicated that the method could be applied as a potential drug monitoring tool to accurately analyze MFX and LFX within a short run time.

1. Introduction

Multidrug-resistant tuberculosis (MDR-TB), caused by *Mycobacterium tuberculosis* (MBT), is one of the major growing health concerns because of the difficulty to treat it than drug susceptible TB. According to the world health organization (WHO), the number of people being infected with MBT is 1 in 3 worldwide and the number of new cases detected every year is reaching up to 9 million out of which 20% pretreatment cases being MDR-TB. In 2013 alone, 480,000 people developed MDR-TB globally [1–3]. Asian countries are among the regions with the highest growing risk of MDR-TB, and the prevalence of the disease reaches up to 2.8% among diagnosed TB patients in South Korea [4].

From the few known effective anti-MDR-TB drugs, moxifloxacin (MFX, 1-cyclopropyl-7-[(1*S*, 6*S*)-2,8-diazabicyclo[4.3.0]nonan-8-yl]-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid) and levofloxacin (LFX, (S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid) are the two most recommended and prescribed later-generation fluoroquinolone antibiotics (FQN) to patients (Fig. 1) [5–8].

Because of the growing concern of MTB infection, studies on MFX and LFX in combating MDR-TB have driven researchers' attention in various fields. Numbers of comparative and individ-

Abbreviations: EFX, enrofloxacin; ESI, electrospray ionization; HPLC, high performance liquid chromatography; LFX, levofloxacin; MDR-TB, multidrug resistant tuberculosis; MFX, moxifloxacin; MRM, multiple reaction monitoring; MS, mass spectrometry; QC, quality control.

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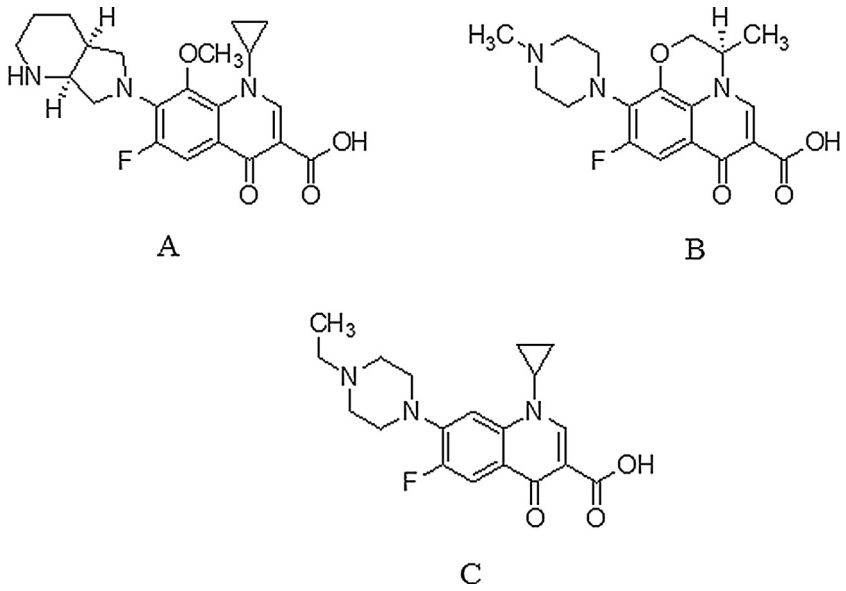

Fig. 1. Chemical structures of moxifloxacin (A), levofloxacin (B) and enrofloxacin (C).

ual studies on the efficiency and role of the two drugs against MDR-TB have been reported [1,9,10]. On the other hand, various qualitative and quantitative techniques such as HPLC-UV detector [11–13], HPLC-fluorescence detector [14–17] and HPLC-MS/MS [18–20] have been reported for determination of MFX and LFX in human plasma. However, majority of these techniques were either focused on separate analysis of the two commonly prescribed drugs or the developed methods were not applied to real sample analysis and validated. In addition, most of the methods had limitations including the need of large amount of sample (plasma or serum), low sensitivity, time-consuming sample preparation and a long run time. These make them unsuitable for the rapid and accurate determination of MFX and LFX simultaneously.

Liquid chromatography-tandem mass spectrometry (LC–MS/MS) method operating the multiple reaction monitoring (MRM) has become one of the top choices for selective and sensitive analysis of compounds including drugs at a very low concentration with high speed [21,22]. The aim of this study was to develop and validate a single, rapid, sensitive, and reproducible LC-ESI-MS/MS utilizing MRM method to determine both MFX and LFX levels in human serum. The developed method could be used as a novel therapeutic drug monitoring tool for detection of MFX and LFX in human serum equally applied to real sample analysis obtained from MDR-TB patients. In addition, the method possibly be used for analysis of these drugs in patients infected by other diseases such as community acquired pneumonia or urinary tract infections, and prescribed to MFX or LFX.

2. Materials and methods

2.1. Chemicals and reagents

Moxifloxacin was obtained from Bayer AG (Leverkusen, Germany). Levofloxacin and enrofloxacin (EFX, internal standard) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The purity of all the standard drugs was ≥99%. HPLC grade methanol, acetonitrile, and water were purchased from Fisher Scientific (Pittsburgh, USA), and formic acid from Sigma–Aldrich. Blood samples from 10 MDR-TB patients prescribed to LFX or MFX (five for each analyte) were obtained from the National Masan TB Hospital, South Korea (Protocol: NCT00425113).

Table 1
Mass spectrometry parameters for the LC-ESI-MS/MS determination of levofloxacin and moxifloxacin.

Analyte	Q1 $[M+H]^+$ (m/z)	Q3 (m/z)	Time	DP	EP	CE	CXP
MFX	402.243	384.2	150	106	10	31	36
		358.2	150	106	10	25	10
LFX	362.157	318.2	150	81	10	25	32
		261.1	150	81	10	37	26
EFX	362.141	318.3	150	101	10	27	32

DP: Declustering potential; EP: Entrance potential; CE: Collision energy; CXP: Collision cell exit potential.

2.2. Instrumentation

2.2.1. High performance liquid chromatography

LC analysis was performed using Agilent 1200 series HPLC system equipped with a degasser, a quaternary pump, an autosampler, and column oven. Chromatographic separations were achieved by Atlantis dC18 column (4.6 mm × 150 mm, 5 μm; Waters, USA) which was maintained at 30 °C. A binary solvent system composed of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was used in isocratic elusion (60:40 v/v of A:B). The flow rate and injection volume were 500 μL/min and 5 μL, respectively throughout the run time.

2.2.2. Mass spectrometry

Ionization and detection of analyte and IS were carried out on an API 4000 MS/MS system (AB Sciex, USA) operating in a positive electrospray ionization (ESI) mode. MRM was used for quantification and monitoring of mass transitions from precursor ion to product ion (m/z). The precursor ions were filtered in the first quadrupole (Q1), and submitted to collision induced fragmentation in the second quadrupole (Q2) to afford the corresponding product ions, which were monitored via the third quadrupole (Q3) under the optimized identification and quantification parameters indicated in Table 1. The measurements were made under conditions of a source temperature: 450 °C, ion spray voltage: 5.5 kV, 3 collision gas (CAD), 15 curtain gas (CUR), 55 ion source gas 1 and 2, and dwell time: 150 ms. Softwares of Bio-analyst TM, version 1.4.2, and Analyst, version 1.4.2, were used for instrument control and data acquisition, respectively.