RESEARCH ARTICLE

Determination of levofloxacin in human serum using liquid chromatography tandem mass spectrometry

Samiksha Ghimire[1], Kai van Hateren[1], Natasha Vrubleuskaya[1,2], Remco Koster[1], Daan Touw[1,3], Jan-Willem C. Alffenaar[1,*]

[1]University of Groningen, University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology, Groningen, The Netherlands.
[2]Department of Public Health TB Control, Metropolitan Public Health Service, The Hague, The Netherlands.
[3]University of Groningen, University Center of Pharmacy, Section of Pharmacokinetics, Toxicology and Targeting, Groningen, The Netherlands.

A rapid liquid chromatography tandem-mass spectrometry method was developed for the determination of levofloxacin and its metabolite (desmethyl-levofloxacin) in human serum. Sample preparation was done using protein precipitation technique. Our method had a run time of 2.5 min and retention times of 1.6 min for all analytes. The standard curves were linear within the concentration range of 0.10 to 5.00 mg/L for levofloxacin and 0.10 to 4.99 mg/L for desmethyl-levofloxacin; a correlation coefficient (R^2) of 0.999 and 0.998 respectively. The lower limit of quantification for both analytes was 0.10 mg/L. Within-day precision ranged from 1.4% and 2.4% for levofloxacin, 1.5% to 5% for desmethyl-levofloxacin and between-day precision ranged from 3.6% to 4.1% for levofloxacin and 0.0% to 3.3% for desmethyl-levofloxacin; whereas, accuracy ranged from 0.1% to 12.7% for levofloxacin and 0.2% to 15.6% for desmethyl-levofloxacin. This method could be a useful asset for routine therapeutic drug monitoring of levofloxacin in multi-drug resistant tuberculosis patients.

Introduction

The proportion of new cases with multi drug resistant tuberculosis (MDR-TB) was 4.1% in newly diagnosed cases and 19% in previously treated cases in year 2016 [1]. Higher levels of resistance (MDR-TB) and poor treatment outcomes are posing a threat to the control of TB globally. The world health organization (WHO) guidelines for the programmatic management of drug resistant TB, consist of at least four second line anti-TB drugs for effective treatment during the intensive phase [2]. This regimen includes pyrazinamide, a fluoroquinolone, a parenteral agent, ethionamide (or prothionamide) and cycloserine [2]. Levofloxacin, a fluoroquinolone antibiotic is the central drug in the treatment regimen of MDR-TB. Levofloxacin is started in a dose of 10-15 mg/kg once daily for 8-12 months intensive phase followed by continuation phase for 12 months [2].

Levofloxacin shows concentration dependent killing, and has moderate post antibiotic effect [3]. The greatest bactericidal effect of levofloxacin occurs at maximum

*Correspondence:
University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology. P.O. Box 30.001, 9700 RB Groningen, the Netherlands. Phone: +31 503614071; Fax: +31 503614087.
Email: j.w.c.alffenaar@umcg.nl

serum/plasma concentration to minimum inhibitory concentration (C_{max}: MIC) ratios of 8-10 and area under concentration time curve in 24 hrs to MIC (AUC_{0-24}: MIC) ratios of 125:1 [3-5]. Levofloxacin has a good in-vitro and in-vivo activity against strains of M.tuberculosis [6-8]. MIC values were low in laboratory and in clinical isolates [9] and in mouse models [10]. In addition, levofloxacin (1000 mg/day for 7 days in patients with pulmonary tuberculosis) showed extended early bactericidal activity (EBA) of 0.45log10 cfu/ml/day [11]. However, a too low serum concentration of levofloxacin contributes to the acquired drug resistance and treatment failure in MDR-TB patients especially with comorbidities and is of great concern. The need of therapeutic drug monitoring (TDM) came into light when patients failed directly observed and theoretically adequate therapy with treatment success rate of less than 50% for MDR-TB[1, 3, 12]. One of the reasons was a sub-optimal drug concentration. So, there is a need for a good and fast analytical procedure to accurately measure the concentration of levofloxacin in patients' blood. Different analytical methods such as capillary electro kinetic chromatography [13-16], solid phase spectrofluorimetry [17], and high performance liquid chromatography with fluorescent or UV detection [18-27] have been developed to measure the levofloxacin concentrations in plasma, urine, bile, faeces and other biological fluids [28]. To our knowledge, so far only five studies [29-33] have quantified levofloxacin concentrations by LC-MS/MS; however, among these studies some studies measured levofloxacin in human urine [27], other studies used plasma samples but did not provide description on run time and the ones that did had a relatively long run time and sample preparation time.

Therefore, to maximize the efficiency of existing LC-MS/MS methods and further compliment TDM, we aimed to develop and validate a rapid, selective, simple and robust method without extensive sample processing and short run times, to quantify levofloxacin and its metabolite desmethyl- levofloxacin in the serum of MDR-TB patients.

Materials and methods
## Chemicals and Reagents

Levofloxacin and levofloxacin-$^{13}C_2H_3$ (internal standard), desmethyl-levofloxacin and desmethyl-levofloxacin-$^2H_8$.HCl (internal standard) were purchased from Sigma-Aldrich (MO, USA) and Alsachim (Illkirch, France) (see **Figure 1** for chemical structures). Ultrapure water was obtained from a Milli-Q system (Millipore Corporation, MA, USA). Ammonium acetate and acetic acid were both obtained from Merck (NJ, USA), while trifluoroacetic acid and acetonitrile of ultra LC-MS grade were obtained from Biosolve (Valkenswaard, The Netherlands). As per the guidelines of the University Medical Center Groningen, pooled human serum samples were made available.

Preparation of stock solutions, calibration standard and QC samples

Stock solutions of various concentrations were prepared in methanol. Two batches of levofloxacin and desmethyl-levofloxacin stock solutions of 200 mg/L in methanol were made. One batch was used to prepare the calibration samples in blank human serum by spiking with an appropriate volume of serially diluted stock solutions to make eight different concentrations for the calibration curve. Likewise, the other batch was used to generate internal quality control (IQC) samples at lower limit of quantification (LLOQ), low, medium and high concentration and stored at -20°C to be used for the validation. The amount of stock solution added to the serum did not exceed 5% of the total volume. The internal standard solution was made by diluting a stock solution of 200 mg/L levofloxacin-$^{13}C_2H_3$ and desmethyl-levofloxacin-$^2H_8$ to a final concentration of 0.2 mg/L for both internal standards.

Sample preparation

Samples were prepared by adding 750 μL precipitating reagent containing internal standards to 100μL of serum in a polypropylene tubes. The mixture was then vortexed for 1 min and centrifuged for 5 min at 10.000 rpm after-
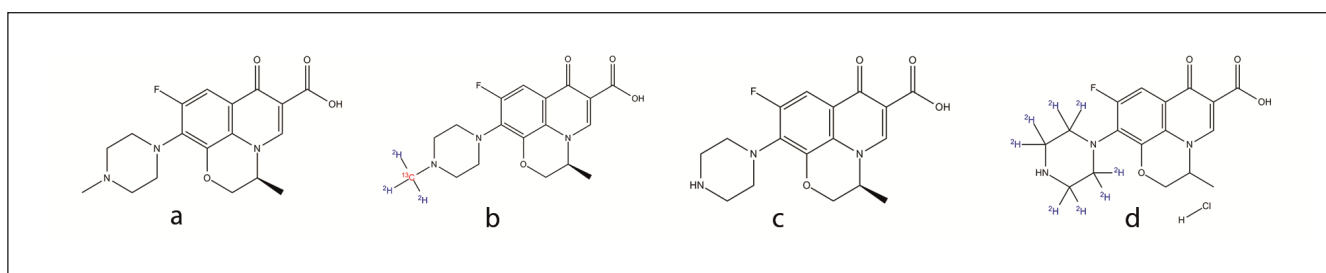
Figure 1. Chemical structures. (a) levofloxacin; (b) levofloxacin-($^{13}C_2H_3$); (c) desmethyl-levofloxacin and (d) desmethyl-levofloxacin-($^2H_8$).HCl

which 2 μL of supernatant was injected into the LC-MS/MS system. The analysis was performed on a triple quadrupole LC-MS/MS (Thermo Scientific, San Jose, CA, USA) with a surveyor MS pump Plus and auto sampler (Thermo Scientific, San Jose, CA USA). The mass spectrometer was a TSQ Quantum Access Max mass spectrometer. The auto sampler tray temperature was set at 10°C. The mobile phase had a flow of 300 μL/min and consisted of ultra-pure water, acetonitrile and an aqueous buffer (containing ammonium acetate 5.0 g/L, 100% acetic acid 35 mL/L and trifluoroacetic acid 2 mL/L in water. The elution gradient is shown in **Table 1**. The method had a run time of 2.5 min.

The high performance liquid chromatography (HPLC) system was coupled to a quadruple mass spectrometer. Quantifications were achieved in Selected Reaction Monitoring (SRM) mode and electrospray ionization was operated in positive mode with a with a spray voltage of 3500 V, a capillary temperature of 350°C and a sheath gas pressure and auxiliary pressure of 35 and 10 arbitrary units respectively. Mass transitions for levofloxacin were 362.1 $m/z \rightarrow$ 318.1 m/z and for desmethyl-levofloxacin 348.1 $m/z \rightarrow$ 310.1 m/z and for levofloxacin-$^{13}C_2H_3$ 366.1 $m/z \rightarrow$ 322.2 m/z, and for desmethyl-levofloxacin-$^2H_8$; 356.2 $m/z \rightarrow$ 318.2 m/z, using a scan width of 0.5 m/z.

Analytical method validation

The validation was performed based on US Food and Drug Administration (FDA) guidelines and European Medicines Agency (EMA) guidelines and parameters included were selectivity and sensitivity, linearity, accuracy and precision, matrix effects, recovery, process efficiency, dilution integrity and stability in human serum [34,35]. Linearity was tested by analyzing serum samples at 8 different concentrations of 0.100, 0.200, 0.500, 1.00, 2.00, 3.00, 4.00 and 5.00 mg/L for levofloxacin and of 0.100, 0.200, 0.500, 1.00, 2.00, 2.99, 3.99, 4.99 mg/L for desmethyl-levofloxacin (see **Table 2**). Four different concentrations of quality control samples were used, where LLOQ was 0.10 mg/L for levofloxacin and desmethyl-levofloxa-

cin, LOW was 0.50 mg/L for both, medium (MED) was 2.02 mg/L and 2.01 mg/L, and HIGH was 4.03 mg/L and 4.02 mg/L for levofloxacin and desmethyl-levofloxacin respectively (see **Table 3**). Our LOW was two-three times LLOQ at 0.50 mg/L. HIGH was at 80% and MED at 40%. We used over-curve as a quality control (QC) sample in a range higher than the calibration curve. Ion suppression or enhancements were examined by injecting 6 blank serum samples into the liquid chromatography (LC) system and compared it to a response from high concentrations of stock solutions (each at 5 mg/L) for levofloxacin and desmethyl-levofloxacin and their internal standards, that were continuously post column infused in the ionization source. Ion suppression occurred in the early stages in between 0.4 to 0.8 min for all analytes, before the retention time of these analytes. During three days, each day, a single calibration curve was analyzed and accuracy was determined by using five determinations per QC sample for three consecutive days. Precision was expressed in terms of percentage of coefficient of variation (CV%) and within-day, between- day and overall CV were calculated. The acceptance limits were CV <15% for precision and within ±15% of the nominal value for accuracy for all QC levels, but for the LLOQ a CV <20% for precision and within ±20% of the nominal value for accuracy were accepted. One-way ANOVA was performed to calculate with-in day, between day and all the CV's. After linearity was proven during validation, the validation was also calculated using a 1-point calibration curve with the highest calibrator. This approach saves 7 calibrator injections and minimizes the sample turnaround time. Dilution integrity was determined by diluting the over curve (OC) ten times with blank serum in fivefold, for three consecutive days with all the analytes at concentrations above the HIGH.

Matrix effects, recovery and total process efficiency were assessed at three different concentrations (LOW, MED and HIGH) in quintuplicate in a single run [32]. Matrix effects were tested as the ratio of peak area substance/IS of the spiked extract of the blank matrix to the peak

Table 1. Gradient elution.			
Time (min)	**A (%)**	**B (%)**	**C (%)**
0.00	5	95	0
1.00	5	0	95
1.90	5	0	95
1.91	5	95	0
2.50	5	95	0
A= ammonium acetate 5.0 g/L, acetic acid 100% 35 mL/L, trifluoroacetic acid 2 mL/L, B= ultrapure water, C= acetonitrile.			

Table 2. Calibration lines.

Compound	Slope (± St. dev)	Intercept (± St. dev)	Corr. coefficent	Regr. coefficent
Levofloxacin	0.485 (± 0.00330)	0.00445 (± 0.00868)	0.99949	0.99898
Desmethyl-levofloxacin	0.834 (± 0.00946)	0.00165 (± 0.0248)	0.99859	0.99718
St.dev= standard deviation; corr.= correlation; regr.= regression.				

area substance/IS of spiked extraction solution. Recovery was calculated as the ratio of peak area substance/IS of spiked matrix to the peak area substance/IS of spiked extract of blank matrix whereas total process efficiency was calculated by dividing the peak area substance/IS of the extract of the spiked matrix by the peak area substance/IS of the spiked extraction solution.

For stability testing, LOW and HIGH QC samples for both levofloxacin and desmethyl- levofloxacin were taken. The testing conditions included storage stability and freeze thaw stability. Stability was defined as a loss of the initial drug concentration by ≤15%. Room temperature and auto-sampler stability were determined after 7 days of storage. Storage stability of levofloxacin and desmethyl-levofloxacin were examined by storing QC samples at room temperature (20°C-25°C), and after sample preparation in the auto sampler at 10°C. Stability was also tested using five freeze-thaw cycles at -20°C. All stability tests were done in quintuplicate per concentration level.

Clinical application of the method

This developed method has been used in our hospital both for clinical and research purpose. For a clinical trial (identifier number NCT 02169141) on the pharmacokinetics of levofloxacin, the method was used to assay samples from 20 multidrug-and extensively drug-resistant tuberculosis patients, treated with once daily 750-1000 mg of levofloxacin. The median levofloxacin dose was 15.8 mg/kg (IQR; 13.3-19.6). The median time of blood collection was 52.2 (IQR; 22-110) days after start of treatment with levofloxacin in (14/20 or 60%) of the included patients. The medical ethical review committee in Republic of Belarus, approved the study protocol. Written informed consent was obtained from all patients. Blood samples were collected before dosing and 1,2,3,4,7 and 12 hrs post-dosing at steady state. Serum samples were prepared by centrifuging collected blood samples for 5 min at 3000 rpm. Upper layer was decanted and transferred to a cryo vials. All the serum samples were frozen at -20°C until analysis [36]. During analysis, serum samples were allowed to thaw in the room temperature after which 100 µL of serum was pipetted out and added to 750 µL precipitating reagent containing internal

standards. The mixture was vortexed for 1 min and and centrifuged for 5 min at 11000 rpm. Finally, 2 µL of supernatant was injected into the LC-MS/MS system.

Results

Quantification was performed in SRM mode with a retention time of 1.6 min (see F**igure 2**). We analyzed the chromatograms of six different lots of human serum for interference. There was an absence of interfering peaks from endogenous substances at the time of retention of levofloxacin, desmethyl-levofloxacin, levofloxacin $-^{13}C_2H_3$ and desmethyl-levofloxacin-$^2H_8$. The calibration curves were linear over a range of 0.10 to 5.00 mg/L for levofloxacin and 0.10 to 4.99 mg/L for desmethyl-levofloxacin with a correlation coefficient (R^2) of 0.999 and 0.998 for levofloxacin and desmethyl-levofloxacin respectively (see **Table 2**). Accuracy calculated ranged from 0.1% to 12.7% for levofloxacin and 0.2% to 15.6% for desmethyl-levofloxacin. With-in day precision ranged between 1.4% and 2.4% for levofloxacin and 1.5% to 5.0% for desmethyl-levofloxacin and between-day precision ranged from 3.6% to 4.1% for levofloxacin and 0.0% to 3.3% for desmethyl-levofloxacin (see **Table 3**). Results of matrix effects, recovery and total process efficiency for levofloxacin, desmethyl-levofloxacin with their internal standards are shown in **Table 3**.

Table 4 showed the stability of levofloxacin and desmethyl-levofloxacin using different test conditions. The concentrations of QC samples (LOW and HIGH) measured for freeze-thaw stability biased between -1.2% and 2.7% for levofloxacin and 0.9% and 9.7% for desmethyl- levofloxacin. Levofloxacin and desmethyl-levofloxacin remained stable at room temperature (RT) for 7 days (168 hrs), with a bias between -1.7% and 3.8% for levofloxacin and -0.3% and 5.9% for desmethyl-levofloxacin. When stored in the auto sampler for 7 days (168 hrs), concentrations biased between 0.1% and 2.8% for levofloxacin and 5.2% and 7.3% for desmethyl-levofloxacin. We did not study stability at 4°C because levofloxacin and desmethyl-levofloxacin remained stable at 10°C and 25°C for 7 days so it was not needed. We did not perform long term stability testing at (-20°C ± 5°C for 1 year) either, because that has already been established.

Table 3. Validation results.

Criteria	QC concentration level				
	LLOQ	LOW	MED	HIGH	OC
Nominal concentration (mg/L)					
Levofloxacin	0.10	0.50	2.02	4.03	8.06
Desmethyl-levofloxacin	0.10	0.50	2.01	4.02	8.05
Accuracy (bias %)					
Levofloxacin	12.7	2.1	0.1	2.0	1.0
Desmethyl-levofloxacin	15.6	4.1	0.2	1.4	1.0
Within-day precision (CV%)					
Levofloxacin	2.4	2.4	1.6	1.4	2.6
Desmethyl-levofloxacin	5.0	3.5	2.9	1.5	3.4
Between-day precision (CV%)					
Levofloxacin	3.9	4.1	3.9	3.6	0.7
Desmethyl-levofloxacin	0.0	2.5	3.0	3.3	0.0
Matrix effects (bias%)					
Levofloxacin	n/a	100.7	96.6	96.3	n/a
Desmethyl-levofloxacin	n/a	104.2	95.7	90.4	n/a
Recovery (bias%)					
Levofloxacin	n/a	95.4	98.4	107.4	n/a
Desmethyl-levofloxacin	n/a	93.5	95.7	109.2	n/a
Total process efficiency (bias%)					
Levofloxacin	n/a	96.0	95.1	103.4	n/a
Desmethyl-levofloxacin	n/a	97.4	91.6	98.7	n/a

Table 4. Stability results

Concentration level	HIGH	LOW
Bench top stability (168 hrs) (bias %)		
Levofloxacin	3.8	-1.7
Desmethyl-levofloxacin	5.9	-0.3
Freeze-thaw stability (after five freeze-thaw cycles) (bias %)		
Levofloxacin	2.7	-1.2
Desmethyl-levofloxacin	9.7	0.9
Auto sampler stability (168hrs) (bias%)		
Levofloxacin	2.8	0.1
Desmethyl-levofloxacin	7.3	5.2

Clinical application

This method of analysis for levofloxacin quantification in serum/plasma samples has been used in the Clinical Toxicology and Drug Analysis Laboratory in our hospital. For a clinical trial (identifier number NCT 02169141) on the pharmacokinetics of levofloxacin in 20 MDR-TB patients, the median observed AUC_{0-24} and C_{max} were 98.8 (IQR; 84.4-159.6) mg*h/L and 10.05 (IQR; 8.4-16.2) mg/L respectively. More than 65% of the patient showed rapid absorption of levofloxacin, with a T_{max} of 1 hour. However, about 25% of the patients had Cmax below the expected minimum C_{max} of 8 mg/L [36]. The chromatogram of levofloxacin and internal standard at 1.5 mg/L and desmethyl-levolfoxacin and internal standard at 0.1mg/L of a patient at t_0 is shown in **Figure 3**.

Discussion

MDR-TB is a growing problem. Failure of the government to implement effective MDR-TB control programs due to cost constraints, lack of diagnostic infrastructure for the early case detection and its management, supply of low quality, poor stability and inappropriate drug combination regimens by counterfeit drug makers, lack of knowledge with doctors about the doses, duration of treatment, prescription errors with indiscriminate prescription of drugs in variety of other respiratory illnesses have exacerbated the multiplication of mutant strains of Mycobacterium tuberculosis. In addition, lack of proper information to the patient and their relatives about the drugs, its therapeutic benefit and risk associated with default, notorious side effects of the drugs itself and, social myths and misconceptions have contributed to poor adherence and treatment failure [37]. Among these factors,

presence of co-morbidities such as HIV, diabetes and presence of inter-patient pharmacokinetic variability in absorption, distribution, metabolism and excretion leading to low serum concentration of drug is crucial contributing to acquired drug resistance [38]. In this scenario, TDM could be a powerful technique to measure and adjust the dose and is especially important for antibiotics that exhibit concentration dependent killing such as levofloxacin. So, we developed a simple and rapid analysis method using LC-MS/MS to determine levofloxacin and its metabolite in serum of patients with MDR-TB.

The HPLC system was coupled to a triple quadrupole mass spectrometer and quantification was performed in SRM mode with a run time of 2.5 min and retention times of 1.6 min for all the compounds. Our method has advantages over the five studies that have quantified levofloxacin by LC-MS/MS [29-33]. The first study quantified levofloxacin in human urine. The second study by Conte et al. used plasma samples, but lacked a detailed description of the procedure, information on different parameters such as run time and used ciprofloxacin hydrochloride monohydrate instead of stable isotopically labeled levofloxacin as an internal standard [30]. Ciprofloxacin is available without prescription in many underdeveloped countries and is used for a variety of bacterial infections; therefore, validation of the method using ciprofloxacin as an internal standard could result in the wrong interpretation of analytical results. The third study [29] quantified 20 anti-TB drugs (16 second line including levofloxacin and four first line drugs) in human plasma using LC-MS/MS but had a long run time of 13 min and used moxifloxacin-d4 as an internal standard for levofloxacin. The fourth study [31] simultaneously quantified azole antifun-
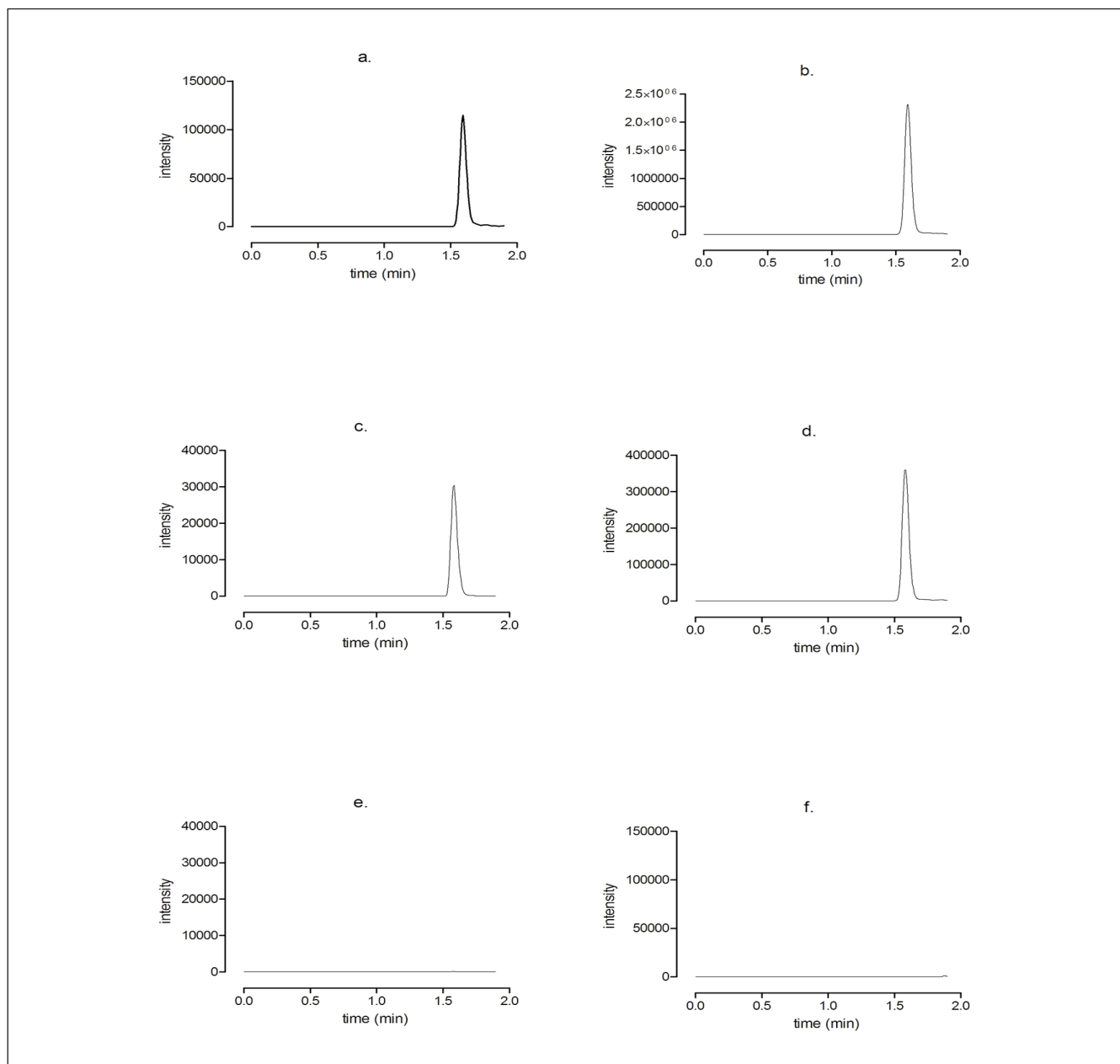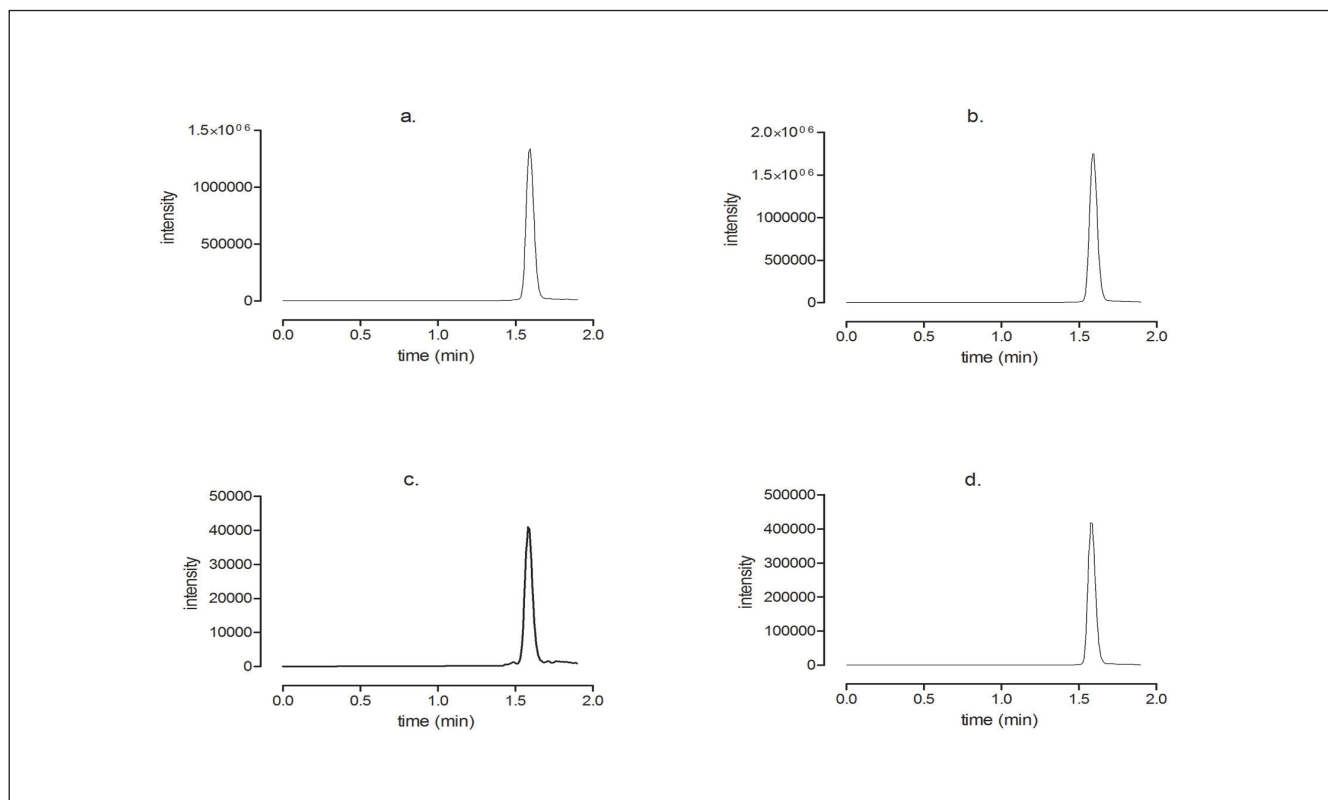
Figure 2. Chromatograms of four different analytes at LLOQ of 0.10 mg/L along with blank. (a) levofloxacin; (b) levofloxacin-($^{13}C_2H_3$); (c) desmethyl-levofloxacin and (d) desmethyl-levofloxacin-($^2H_8$); (e) desmethyl-levofloxacin blank with internal standard; (f) levofloxacin blank with internal standard.

gals, antibiotics (levofloxacin and others using levofloxacin-^{13}C-d3 as an internal standard for levofloxacin), imatinib, and raltegavir in human plasma with a fast run time of 5 min. The last study [33] developed and validated a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method for the analysis of levofloxacin in serum of MDR-TB patients using enrofloxacin as an internal standard, with a run time and retention time of 4 min and 2.58 min respectively. Two studies [29,33] did not use structural analogue

as an internal standard but rather moxifloxacin-d4 and enrofloxacin, which does not have the same retention time and comparable ionization characteristics as levofloxacin[39]. The study by Jourdil et al. [31] has advantage over two studies [29,33] in terms of using stable isotopically labeled levofloxacin internal standard.

We have minimized the run time for levofloxacin and desmethyl-levofloxacin compared to the procedure described in the previous studies by Kim et al. [29], Jourdil et al. [31] and Lee et al. [33] For sample preparation, all of

Figure 3. Chromatogram of a patient sample at t0. (a) levofloxacin; (b) levofloxacin-($^{13}C_2H_3$); (c) desmethyl-levofloxacin and (d) desmethyl-levofloxacin-($^2H_8$);

these studies [29,31,33] used protein precipitation which favors pharmacokinetic monitoring but took approx. 11 min for protein precipitation. In this study, we optimized the sample preparation time to 7 min. This is of great advantage because it allows to process more samples per hour thereby optimizing the throughput of the LC-MS/ MS in clinical laboratories. Hence, our method was rapid with a short sample preparation time and run times. Furthermore, our method allows to measure levofloxacin and its less active desmethyl-levofloxacin separately, in a single assay. This could be useful in patients with genetic polymorphisms in drug metabolizing cytochrome P450 iso-enzymes, facilitating major metabolic reactions which will allow to exactly quantify the increased/decreased metabolite and serum drug concentrations.

Assay performance was assessed based on different parameters such as linearity, precision and accuracy. The calibration curve was linear over a range of 0.10 to 5.00 mg/L for levofloxacin. The estimated mean Ctrough and C_{max} of levofloxacin in patients receiving an MDR-TB treatment regimen varies between 3.24-13.00 mg/L [40]. For this method, the upper limit is above the linearity range set for the assay. Although the linear range does not cover the concentration gradient, by having validated

diluted steps, this method can be used to accurately measure concentrations levels up to 40.0 mg/L.

The validation parameters were in the ranges recommended by FDA i.e. ± 20% for precision and accuracy for LLOQ and ±15% for LOW, MED, and HIGH- quality control samples, making this method reliable. Levofloxacin and desmethyl-levofloxacin were stable for up to 7 days in serum at a temperature between 20-25°C and 10°C in the auto sampler. This is beneficial in rural areas where the well-equipped central laboratory is far from the communities holding MDR-TB patients. The serum samples could be transported without a risk of significant loss of the concentration of levofloxacin and desmethyl-levofloxacin to the central laboratory and without refrigeration.

Conclusions

We developed and validated a method using LC-MS/MS for the quantitative analysis of levofloxacin and its metabolite (desmethyl-levofloxacin) in human serum. The method is simple, fast, accurate and precise. This method can be applied in clinical settings to measure pharmacokinetic parameters and in specialized laboratories for routine TDM of levofloxacin in the treatment of TB.

References

1. World Health Organization. Global Tuberculosis Report 2017. http://apps.who.int/iris/bitstream/10665/259366/1/9789241565516-eng.pdf?ua=1

2. World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug- resistant tuberculosis (2014). http://apps.who.int/iris/bitstream/10665/130918/1/9789241548809_eng.pdf

3. Berning SE. The role of fluoroquinolones in tuberculosis today. Drugs 61(1), 9-18 (2001).

4. Turnidge J. Pharmacokinetics and pharmacodynamics of fluoroquinolones. Drugs 58(2), 29-36 (1999).

5. Stein GE. Pharmacokinetics and pharmacodynamics of newer fluoroquinolones. Clin Infect Dis 23 Suppl 1, S19-24 (1996).

6. Schluger NW. Fluoroquinolones in the treatment of tuberculosis: which is best? Am J Respir Crit Care Med 188(7), 768-769 (2013).

7. JI B, Lounis N, Truffot-Pernot C, Grosset J. In vitro and in vivo activities of levofloxacin against Mycobacterium tuberculosis. Antimicrob Agents Chemother 39(6), 1341-1344 (1995).

8. Mor N, Vanderkolk J, Heifets L. Inhibitory and bactericidal activities of levofloxacin against Mycobacterium tuberculosis in vitro and in human macrophages. Antimicrob. Agents Chemother 38(5), 1161-1164 (1994).

9. Angeby KA, Jureen P, Giske CG et al. Wild-type MIC distributions of four fluoroquinolones active against Mycobacterium tuberculosis in relation to current critical concentrations and available pharmacokinetic and pharmacodynamic data. J Antimicrob Chemother 65(5), 946-952 (2010).

10. Rodrıguez J, Ruiz M, Climent A, Royo G. In vitro activity of four fluoroquinolones against Mycobacterium tuberculosis. Int J Antimicrob Agents 17(3), 229-231 (2001).

11. Johnson J, Hadad D, Boom WH et al. Early and extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. Int J Tuberc Lung Dis 10(6), 605-612 (2006).

12. Alsultan A, An G, Peloquin CA. Limited sampling strategy and target attainment analysis for levofloxacin in patients with tuberculosis. Antimicrob Agents Chemother 59(7), 3800-3807 (2015).

13. de Boer T, Mol R, de Zeeuw RA, de Jong GJ, Ensing K. Enantioseparation of ofloxacin in urine by capillary electrokinetic chromatography using charged cyclodextrins as chiral selectors and assessment of enantioconversion. Electrophoresis 22(7), 1413-1418 (2001).

14. Hernandez M, Borrull F, Calull M. Determination of quinolones in plasma samples by capillary electrophoresis using solid-phase extraction. J Chromatogr B Biomed Sci Appl 742(2), 255-265 (2000).

15. McCourt J, Bordin G. Development of a capillary zone electrophoresis–electrospray ionisation tandem mass spectrometry method for the analysis of fluoroquinolone antibiotics. J Chromatogr A 990(1), 259-269 (2003).

16. Ferdig M, Kaleta A, Vo TDT, Buchberger W. Improved capillary electrophoretic separation of nine (fluoro) quinolones with fluorescence detection for biological and environmental samples. J. Chromatogr A 1047(2), 305-311 (2004).

17. Ballesteros O, Vılchez JL, Navalón A. Determination of the antibacterial ofloxacin in human urine and serum samples by solid-phase spectrofluorimetry. J Pharm Biomed Anal 30(4), 1103-1110 (2002).

18. Carlucci G. Analysis of fluoroquinolones in biological fluids by high-performance liquid chromatography. J Chromatogr A 812(1), 343-367 (1998).

19. Belal F, Al-Majed A, Al-Obaid A. Methods of analysis of 4-quinolone antibacterials. Talanta 50(4), 765-786 (1999).

20. Hernández-Arteseros J, Barbosa J, Compano R, Prat M. Analysis of quinolone residues in edible animal products. J Chromatogr A 945(1), 1-24 (2002).

21. Immanuel C, Kumar AH. Simple and rapid high-performance liquid chromatography method for the determination of ofloxacin concentrations in plasma and urine. J Chromatogr B Biomed Sci Appl 760(1), 91-95 (2001).

22. Cheng F, Tsai T, Chen Y, Hung L, Tsai T. Pharmacokinetic study of levofloxacin in rat blood and bile by microdialysis and high-performance liquid chromatography. J Chromatogr A 961(1), 131-136 (2002).

23. Espinosa-Mansilla A, de la Pena, A Munoz, Gómez DG, Salinas F. HPLC determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin with photoinduced fluorimetric (PIF) detection and multiemission scanning: application to urine and serum. J Chromatogr B 822(1), 185-193 (2005).

24. Liang H, Kays MB, Sowinski KM. Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin and cinoxacin by high-performance liquid chromatography: application to levofloxacin determination in human plasma. J Chromatogr B 772(1), 53-63 (2002).

25. Djabarouti S, Boselli E, Allaouchiche B et al. Determination of levofloxacin in plasma, bronchoalveolar

lavage and bone tissues by high-performance liquid chromatography with ultraviolet detection using a fully automated extraction method. J Chromatogr B 799(1), 165-172 (2004).

26. Wong F, Juzwin S, Flor S. Rapid stereospecific high-performance liquid chromatographic determination of levofloxacin in human plasma and urine. J Pharm Biomed Anal 15(6), 765-771 (1997).

27. Ballesteros O, Toro I, Sanz-Nebot V, Navalón A, Vılchez J, Barbosa J. Determination of fluoroquinolones in human urine by liquid chromatography coupled to pneumatically assisted electrospray ionization mass spectrometry. J Chromatogr B 798(1), 137-144 (2003).

28. Fish DN, Chow AT. The clinical pharmacokinetics of levofloxacin. Clin Pharmacokinet 32(2), 101-119 (1997).

29. Kim H, Seo K, Kim H et al. Simple and accurate quantitative analysis of 20 anti-tuberculosis drugs in human plasma using liquid chromatography–electrospray ionization–tandem mass spectrometry. J Pharm Biomed Anal 102, 9-16 (2015).

30. Conte JE, Golden JA, McIver M, Zurlinden E. Intrapulmonary pharmacokinetics and pharmacodynamics of high-dose levofloxacin in healthy volunteer subjects. Int J Antimicrob Agents 28(2), 114-121 (2006).

31. Jourdil J, Tonini J, Stanke-Labesque F. Simultaneous quantitation of azole antifungals, antibiotics, imatinib, and raltegravir in human plasma by two-dimensional high-performance liquid chromatography–tandem mass spectrometry. J Chromatogr B 919, 1-9 (2013).

32. Matuszewski B, Constanzer M, Chavez-Eng C. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 75(13), 3019-3030 (2003).

33. Lee SJ, Desta KT, Eum SY et al. 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. J Chromatogr B 1009, 138-143 (2016).

34. Food and Drug Administration US Department of Health, Guidance for industry, bioanalytical method validation, 2013. https://www.fda.gov/downloads/drugs/guidances/ucm368107.pdf

35. European Medicines Agency Committee for Medicinal Products for Human Use, Guideline on bioanalytical validation (EMEA/CHMP/EWP/192217/2009), European Medicines Agency, London, 2011. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf

36. van't Boveneind-Vrubleuskaya N, Seuruk T, van Hateren K et al. Pharmacokinetics of Levofloxacin in Multidrug-and Extensively Drug-Resistant Tuberculosis patients. Antimicrob Agents Chemother 00343-17 (2017).

37. Prasad R, Gupta N, Singh M. Multidrug resistant tuberculosis: trends and control. Indian J Chest Dis Allied Sci 56(4), 237-246. (2014).

38. Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 62(15), 2169-2183 (2002).

39. Veringa A, Sturkenboom MG, Dekkers BG et al. LC-MS/MS for therapeutic drug monitoring of anti-infective drugs. Trends Anal Chem 84, 34-40 (2016).

40. Baietto L, D'Avolio A, De Rosa FG et al. Simultaneous quantification of linezolid, rifampicin, levofloxacin, and moxifloxacin in human plasma using high-performance liquid chromatography with UV. Ther Drug Monit 31(1), 104-109 (2009).

Citation:

Ghimire S, van Hateren K, Vrubleuskaya N, Koster R, Touw D, Alffenaar JWC. Determination of levofloxacin in human serum using liquid chromatography tandem mass spectrometry. J Appl Bioanal 4(1), 16-25 (2018).

Open Access and Copyright:

Funding/Manuscript writing assistance:

Competing interest:

The authors have declared that no competing interest exist.