BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **21**: 1045–1051 (2007)
Published online 4 June 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/bmc.851

ORIGINAL RESEARCH

A rapid and simple high-performance liquid chromatography method for the determination of human plasma levofloxacin concentration and its application to bioequivalence studies

Zhi-Ling Zhou,[1] Min Yang,[1] Xi-Yong Yu,[1]* Huai-Yan Peng,[2] Zhi-Xin Shan,[1] Shu-Zhen Chen,[1] Qiu-Xiong Lin,[1] Xiao-Ying Liu,[1] Tie-Feng Chen,[1] Shu-Feng Zhou[3]* and Shu-Guang Lin[1]

[1]Research Center of Medical Sciences, Guangdong Provincial People's Hospital, Guangzhou 510080, People's Republic of China
[2]Department of Clinical Laboratory, The Third Xiangya Hospital, Central South University, Changsha 410013, People's Republic of China
[3]Division of Pharmacy, School of Life Sciences, Queensland University of Technology, 2 George Street, Brisbane, Queensland 4001, Australia

Received 13 October 2006; revised 9 March 2007; accepted 17 March 2007

ABSTRACT: A high-performance liquid chromatography method with fluorescence detection (HPLC-FLD) for the determination of levofloxacin in human plasma is described. Neutralized with phosphate buffer (pH 7.0), the sample (0.1 mL) was extracted with dichlormethane (1 mL). After voltex-mixing and centrifuged at 3000***g*** for 6 min at 4°C, the upper aqueous layer was aspirated using a micro vacuum pump and the organic layer was directly transferred to a clean test tube without pipetting. The organic solvent was evaporated and the residues were reconstituted with the mobile phase. Levofloxacin and terazosin (internal standard, IS) were chromatographically separated on a C_{18} column with a mobile phase containing phosphate buffer (pH 3.0, 10 mM), acetonitrile and triethylamine (76:24:0.076, v/v/v) at a flow rate of 1 mL/min. The analytes were detected using fluorescence detection at an excitation and emission wavelength of 295 and 440 nm, respectively. The linear range of the calibration curves was 0.0521–5.213 μg/mL for levofloxacin with a lower limit of quantitation (0.0521 μg/mL). The retention times of levofloxacin and terazosin were 2.5 and 3.1 min, respectively. Within- and between-run precision was less than 12 and 11%, respectively. Accuracy ranged from −6.3 to 4.5%. The recovery ranged from 86 to 89% at the concentrations of 0.0521, 0.5213 and 5.213 μg/mL. The present HPLC-FLD method is sensitive, efficient and reliable. The method described herein has been successfully used for the pharmacokinetic and bioequivalence studies of a levofloxacin formulation product after oral administration to healthy Chinese volunteers. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: levofloxacin; HPLC-FLD; bioequivalence; pharmacokinetics

INTRODUCTION

Levofloxacin (Fig. 1), a chiral fluorinated carboxyquinolone, is a synthetic broad spectrum antibacterial agent for oral and intravenous administration. Chemically, levofloxacin is the pure (–)-(*S*)-enantiomer of the racemic drug substance ofloxacin. Levofloxacin at 200 mg twice daily or 100 mg three times daily, the usual dose currently approved for antimicrobial treatment in China,

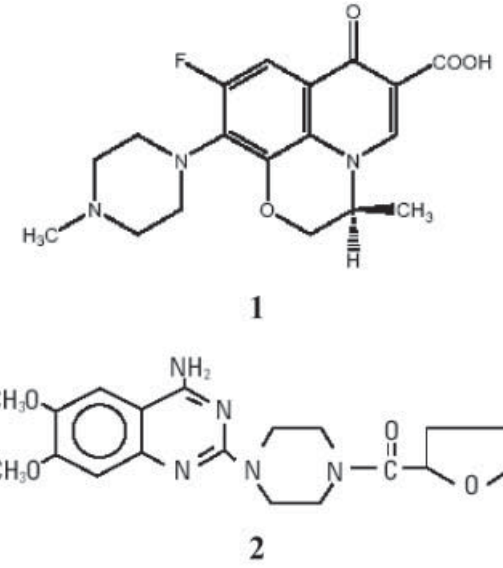

Figure 1. Chemical structures of levofloxacin (1) and terazosin (2).

*Correspondence to: Xi-Yong Yu, Research Center of Medical Sciences, Guangdong Provincial People's Hospital, 96 Dongchuan Road, Weilun Bldg, Guangzhou 510080, People's Republic of China.
E-mail: yuxyen@hotmail.com
Shu-Feng Zhou, E-mail: S4.zhou@qut.edu.au

Abbreviations used: HPLC-FLD, high-performance liquid chromatography method with fluorescence detection; IS, internal standard; QC, quality control.

Contract/grant sponsor: National Science Foundation of China; Contract/grant number: 30672077, 30571850.
Contract/grant sponsor: Guangdong Provincial Science Foundation; Contract/grant number: 015015, 06020831.

has been shown to be safe and efficacious for the treatment of several commonly encountered infections in adults, including acute bacterial exacerbations of chronic bronchitis, community-acquired pneumonia, uncomplicated skin infections and urinary tract infections (Bellmann *et al.*, 2004; Hu *et al.*, 2004; Jiang *et al.*, 2004; Liang *et al.*, 1999; Wang, 2003). In clinical settings, the plasma concentrations of levofloxacin need to be closely monitored. To develop novel formulations, bioequivalence studies of levofloxacin are also required. Therefore, it is important to develop a sensitive, rapid, simple and low-cost method for the determination of levofloxacin for routine therapeutic drug monitoring and bioequivalence studies. To date, determinations of levofloxacin in various biological matrixes have been established by the use of ultraviolet spectrometry (Liang *et al.*, 2002; Rimmele *et al.*, 2004; Santoro *et al.*, 2006), mass spectrometry (Bucci, 2004) or fluorescence detection (Bottcher *et al.*, 2001; Horstkotter and Blaschke, 2001; Liang *et al.*, 2002; Nguyen *et al.*, 2004; Oberdorfer *et al.*, 2004; Schulte *et al.*, 2006; Siewert, 2006; Swoboda *et al.*, 2003). While some of these methods are successful in the determination of levofloxacin, these methods have several limitations. These include a long chromatographic running time, and complicated and time-consuming sample pretreatment procedures (Bottcher *et al.*, 2001; Liang *et al.*, 2002; Siewert, 2006). The methods may have a low sensitivity (Nguyen *et al.*, 2004; Siewert, 2006) or require expensive instruments such as for mass spectrometry (Bucci, 2004; Guo *et al.*, 2006; Nguyen *et al.*, 2004) which are not commonly available in many cases. Thus, we have developed a simple high-performance liquid chromatography method with fluorescence detection (HPLC-FLD) method for the determination of levofloxacin in human plasma with a simplified sample pretreatment procedure and a short running time. The assay described here required a small sample volume only, and it was fully validated and successfully used for the pharmacokinetic and bioequivalence studies of a levofloxacin formulation product.

EXPERIMENTAL

Equipment and reagents

An HPLC system (Agilent Technologies 1050, USA) with a fluorescence detector (Agilent Technologies 1046A, USA) was used. Hewlett Packard Chemstation was utilized to process the raw data. Reference formulation (lot number 050468; 100 mg/tablet; Zhejiang Medicine Co. Ltd, Shanghai, China) and test formulation (lot number: 050501; 100 mg/tablet; Guangdong Yikang Pharmaceutical Co. Ltd, Guangdong, China) of levofloxacin, levofloxacin (purity > 98.8%), desmethyl-levofloxacin (purity > 90.5%) and levofloxacin-*N*-oxide (purity > 89.2%) were kindly provided by Guangdong Yikang Pharmaceutical Co. Ltd (Guangzhou, Guangdong, China). Terazosin (Fig. 1, IS, purity > 98.6%) was obtained from Guangzhou Pui's Pharmaceutical Factory (Guangzhou, Guangdong, China). Methanol and acetonitrile of HPLC grade were purchased from Tedia (Fairfield, OH, USA). Blank human plasma from healthy donors was obtained from the Blood Service Center of Guangzhou (Guangdong, China) or from the healthy volunteers.

Standard solutions and phosphate buffer (pH 7.0)

Phosphate buffer (pH 7.0) was prepared by dissolving 0.68 g KH_2PO_4 in 29.1 mL sodium hydroxide solution (0.1 mol/L) and 70.1 mL distilled water. The primary stock solutions of levofloxacin (1010 μg/mL) and terazosin (IS, 2180 μg/mL) were prepared by dissolving appropriate amounts of pure substance in methanol. The working solutions were prepared by diluting the stock solutions with methanol. Terazosin (IS) working solution (0.872 μg/mL) was acquired by diluting the stock solutions with phosphate buffer at pH 7.0. All the standard solutions were stored at –20°C until analysis. Plasma was spiked with stock solutions of levofloxacin to achieve the following calibration standard concentrations: 0.0521, 0.1478, 0.5213, 0.9901, 2.4390 and 5.213 μg/mL. Quality control (QC) samples that were run in each assay were prepared in the same way.

Chromatographic conditions

The analytes were separated on a Kromasil C_{18} (4.6 × 150 mm, 5 μm, Dikma Technologies, Dalian, China) column equipped with a guard column (Gemini C_{18} 4.0 × 3.0 mm, Phenomenex®, USA) with a column temperature of 25°C. The mobile phase was 10 mM phosphate buffer at pH 3.0 (containing 0.01% triethylamine)–acetonitrile (76:24, v/v) and was filtered using a 0.45 μm filter in a solvent filtration apparatus and not recirculated. The flow-rate was 1.0 mL/min. The wavelengths of the fluorescence detector were set to 295 (excitation) and 440 nm (emission), according to a HPLC method (Jiang *et al.*, 2004).

Sample pretreatment

The sample (0.1 mL) was neutralized by adding 0.2 mL phosphate buffer (terazosin working solution, pH 7.0, containing IS 0.872 μg/mL) and then shaken for 10 s. One milliliter of dichlormethane was added to the sample. After voltex-mixing for 1 min, the mixture was centrifuged at 3000***g*** for 6 min at a low temperature (4°C). The upper aqueous layer was aspirated using a micro vacuum pump and the remained organic layer was directly transferred to a clean test tube without pipetting. The organic solvent was evaporated under a stream of nitrogen gas in a water bath at 37°C for 5–6 min. The residues were reconstituted in 40 μL mobile phase. Twenty microliters of the solution was injected onto the HPLC for analysis with an injector (Valco Instruments Co. Inc., Switzerland).

Method validation

Specificity. Specificity of the method was established by measuring six independent sources of blank human plasma

DOI: 10.1002/bmc

or plasma samples spiked with levofloxacin and terazosin (IS). Additionally, desmethyl-levofloxacin and levofloxacin-*N*-oxide, the two metabolites identified in human urine (Almeida *et al.*, 2005; Fish and Chow, 1997), were investigated.

Extraction recoveries. The extraction recoveries were determined at three concentration levels (low: 0.0521 μg/mL; middle: 0.5213 μg/mL; and high: 5.213 μg/mL) for levofloxacin and at one concentration level for terazosin by comparing the analyte peak areas obtained from the quality control samples ($n = 5$) after extraction with those obtained from the corresponding unextracted reference standards prepared at the same concentrations, respectively.

Precision and accuracy. Precision and accuracy assays were carried out five times using three different concentrations (low: 0.0521 μg/mL; middle: 0.5213 μg/mL; and high: 5.213 μg/mL) on the same day and over five different days. The mean, standard deviation (SD), RSD and accuracy of the intra-day and inter-day experiments were calculated. The accuracy is expressed as the relative error of measurement (RE, %):

$$\mathrm{RE}\ (\%) = \left(\frac{\text{Mean calculated concentration} - \text{nominal concentration}}{\text{Nominal concentration}} \right) \times 100$$

Calibration curves. The peak area ratio of levofloxacin to terazosin (internal standard) was determined for each calibration sample using the calibration curve from 0.0521 to 5.213 μg/mL. Calibration was performed by a least-squares linear regression of the peak-area ratios of the analyte over the IS vs the respective standard concentration.

Stability. The stability of the stock solutions and working solutions of levofloxacin and terazosin, stored at –20°C for 3 months and at room temperature (25°C) for 24 h, was tested by comparing the instrument response with that of freshly prepared solutions. The stability experiments of levofloxacin in human plasma were carried out under three conditions: after three freeze–thaw cycles, after storage at room temperature for 24 h and at –70°C for 3 months. The stability of levofloxacin in human plasma was investigated using five replicates of each low, medium and high QC samples. Stability was determined by comparing the nominal concentration of levofloxacin and the calculated concentration of test samples.

Application to bioequivalence studies

The ethics permit was obtained from Guangdong Provincial People's Hospital, Guangzhou, China. All participants gave written consent to this study after explanation of the aims and risks of the study according to Helsinki–Tokyo Declaration. The bioequiavailability of levofloxacin tablet at a single dose of 200 mg was assessed in an open, randomized, crossover trial, with a minimum washout period of 7 days. A total of 20 healthy Chinese male volunteers (age, mean ± SD, 24.4 ± 1.9 years; body mass index, 21.2 ± 1.5) were recruited into the study. All subjects received a complete medical history, physical examination and routine laboratory tests, and all volunteers had no hepatic, renal, cardiac, hematologic or other diseases. Smokers and persons with alcohol dependence were excluded.

Blood samples were obtained over 24 h (at baseline, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 9.0, 12 and 24 h post-dose) in test tubes containing heparin. Following centrifugation (3000***g*** × 10 min at 4°C), plasma was collected and stored immediately at –70°C until analysis. Levofloxacin plasma concentration levels were determined using the present HPLC method.

RESULTS AND DISCUSSION

Method development

The retention times of levofloxacin and terazosin were approximately 2.5 and 3.1 min, respectively. The chromatograms of blank human plasma, human plasma spiked with levofloxacin and volunteers' samples are shown in Fig. 2.

The effect of pH, buffer and composition of mobile phase on retention and resolution of levoflxacin and terazosin were investigated. After optimization, the mobile phase consisting of 10 mM phosphate buffer (containing 0.01% triethylamine, pH 3.0) and acetonitrile (76:24, v/v) was chosen as it gave a shortest retention time, better shapes of peaks, and good resolution of levofloxacin and terazosin (Fig. 2).

Various extraction solvents were evaluated to acquire the best analytical results. Dichlormethane and phosphate buffers (pH 7.0) were found to provide the highest recoveries (Table 1) and there were no any interfering peaks (Fig. 3) compared with other tested extraction solvents such as acetic ether or diethyl ether and HCl (0.15 M). The chosen extraction solvent also provided a higher safety of column protection than one-step protein precipitation. Furthermore, dichlormethane was evaporated quickly under a stream of nitrogen in water bath (37°C) for 5–6 min.

An improvement is the transferring technique of dichlormethane without pipetting in the present study. With the operation of pipetting, the tip might be contaminated and the volume of dichlormethane under the aqueous layer might change. The upper aqueous was aspirated with a micro vacuum pump and the

Table 1. Extraction recoveries for levofloxacin-spiked human plasma ($n = 5$)[a]

Amount of levofloxacin (μg/mL)	Mean (%)	RSD (%)
0.0521	86	9.3
0.5213	89	4.5
5.213	87	4.6

[a] The mean extraction recovery for each level was calculated from five levofloxacin spiked replicates.
RSD, relative standard deviation.

DOI: 10.1002/bmc

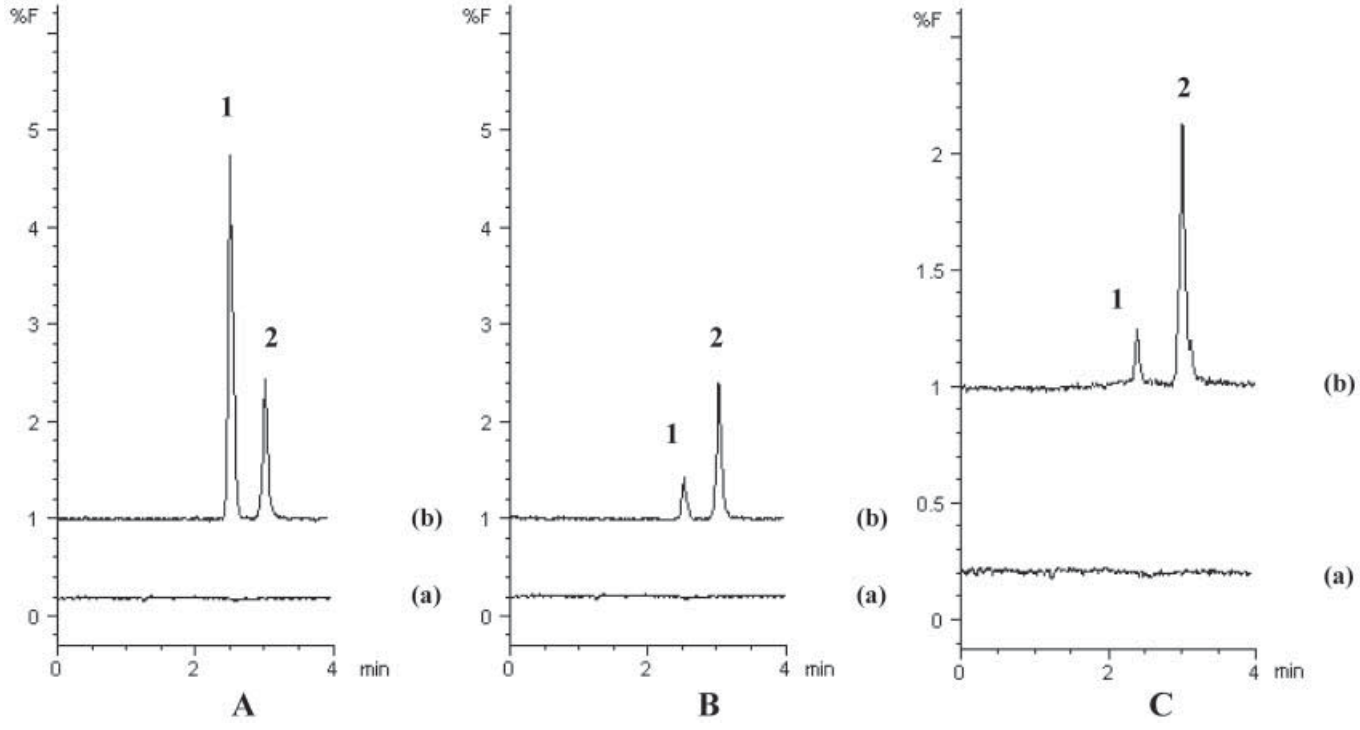

Figure 2. Representative chromatograms of: (A) blank human plasma (a) and a spiked plasma sample (b) containing 0.9901 μg/mL of levofloxacin and terazosin; (B) blank human plasma (a) and a plasma sample (b) at 24 h after dosing from a human volunteer containing 0.1102 μg/mL of levofloxacin; (C) blank human plasma (a) and a spiked plasma sample at the LOQ (b) with 0.0521 μg/mL of levofloxacin. Peaks: **1**, levofloxacin; **2**, terazosin (internal standard). For chromatographic conditions, see Experimental section.

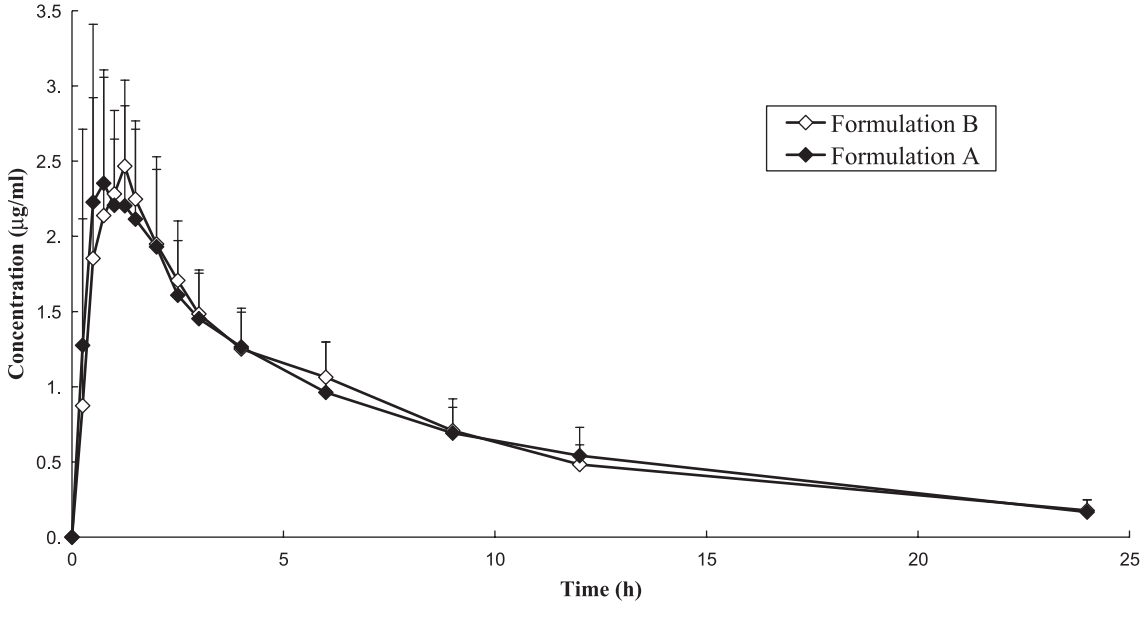

Figure 3. Mean plasma-levels of levofloxacin vs time profiles after administration of 200 mg levofloxacin in two oral formulations to 20 healthy volunteers. Data are the mean ± SD.

remaining organic layer was directly transferred into a clean test tube. This simple extraction procedure could avoid contamination, and it gave a constant sample volume. In this study, we found that there was nearly no emulsification when a small volume of sample was collected and centrifuged at a low temperature (4°C). In addition, only a small volume of plasma sample (0.1 mL) was required in this study, which resulted in a less consumption of extraction solvents and shorter sample mixing time.

DOI: 10.1002/bmc

Specificity

As shown in Fig. 2, no interfering peaks were observed in control (blank) human plasma.

Extraction recoveries

Under neutral conditions, the extraction recoveries of levofloxacin and terazosin were high by extraction with dichlormethane. For terazosin, the average extraction recovery was 90%, and the RSD was 5.1%. As shown in Table 1, the average extraction recoveries were at least above 86% for levofloxacin at all concentrations examined.

Calibration curves

The concentration range was 0.0521–5.213 μg/mL for levofloxacin. The peak area ratio (Y) of the analyte to IS was well correlated to the concentration (C). The regression equation was $Y = 2.0061C + 0.0317$, with a correlation coefficient (r) of 0.9999.

Accuracy and precision

The analysis of independent low, middle and high quality control samples was used to determine intra-day and inter-day precision and accuracy of the assay. The intra-day RSD for levofloxacin ranged from 8.5 to 12% and the accuracy from –3.1 to 2.1% (Table 2). The inter-day RSD for levofloxacin varied from 3.0 to 11% and the accuracy from –6.3 to 4.5% (Table 2). According to the FDA Guidance for Industry: Bioanalytical Method Validation for Human Studies (FDA, 2001), the above results indicated that the method was reliable, reproducible and accurate.

Sensitivity

Five quality control plasma samples were utilized to determine the sensitivity. The limit of quantitation (LOQ) was 0.0521 μg/mL for levofloxacin (signal-to-noise ratio, S/N = 9).

Stability

The stock solutions and working solutions of levofloxacin and terazosin (IS) stored at –20°C for 3 months and at room temperature (25°C) for 24 h, showed good stability with RE ranging from –4 to 3%. The stability data of levofloxacin in human plasma under three conditions are shown in Table 3. Five replicates of each low, middle and high quality control samples were analyzed. As shown in Table 3, no significant degradation of levofloxacin was observed under any of those conditions.

Table 2. Intra-day and inter-day precision and accuracy of levofloxacin in human plasma

Nominal concentration (μg/mL)	Mean ± SD calculated concentration (μg/mL)	RSD (%)	RE (%)
Intra-day (n = 30)			
0.0521	0.0505 ± 0.0063	12	–3.1
0.5213	0.5366 ± 0.0522	9.7	3.0
5.213	5.322 ± 0.453	8.5	2.1
Inter-day (n = 5 days)			
0.0521	0.0488 ± 0.0055	11	–6.3
0.5213	0.5384 ± 0.0160	3.0	3.3
5.213	5.446 ± 0.521	9.5	4.5

Table 3. Stability of levofloxacin in human plasma

		QC concentrations (μg/mL) (n = 5)		
Experimental conditions	Parameter	0.0521	0.5213	5.213
After three freeze–thaw cycles	Mean calculated concentration	0.0485	0.5504	4.633
	RSD (%)	13	5.2	8.0
	RE[c] (%)	–6.9	5.6	–11
At room temperature for 24 h	Mean calculated concentration	0.0508	0.5463	5.015
	RSD (%)	8.0	5.7	4.1
	RE (%)	–2.4	4.8	–3.8
At –70°C for 3 months	Mean calculated concentration	0.0486	0.5521	4.817
	RSD (%)	10	7.4	6.8
	RE (%)	–6.7	5.9	–7.6

DOI: 10.1002/bmc

Table 4. Pharmacokinetic parameters obtained after administration of 200 mg levofloxacin of two oral formulations to 20 healthy volunteers (mean A SD)

Parameter	Formulation A	Formulation B
T_{max} (h)	0.9 ± 0.5	1.1 ± 0.6
$T_{1/2}$ (h)	7.1 ± 1.8	6.5 ± 1.7
C_{max} (μg/mL)	3.1 ± 0.8	3.0 ± 0.8
AUC_{0-24} (μg h/mL)	17.6 ± 3.7	17.5 ± 2.8
AUC_{0-inf} (μg h/mL)	19.4 ± 4.2	19.1 ± 3.5

Application to bioequivalence studies

The mean plasma concentration–time curves and the pharmacokinetic parameters (T_{max} = time to reach peak or maximum concentration following a single administration of levofloxacin; $T_{1/2}$ = elimination half-life associated with the terminal slope of a semi logarithmic concentration–time curve; C_{max} = maximum drug concentration in plasma; AUC_{0-24} = area under the concentration–time curve during an administration interval (24 h); AUC_{0-inf} = area under the concentration–time curve from 0 to infinity) after administration of both formulations of levofloxacin are shown in Fig. 3 and Table 4, respectively. It can be seen that the plasma levofloxacin levels increased, reaching a maximum of about 3.0 μg/mL in about 1 h following dosing (Table 4). Then, the concentrations decreased with a half-life of about 6–7 h. No statistically significant difference was observed in any parameter between the two formulations ($p < 0.05$). In order to establish whether the formulations tested were bioequivalent, the ratios of C_{max}, AUC_{0-24} and of AUC_{0-inf} as well as the 90% confidence limits of these ratios were calculated and two one-sided *t*-tests were performed for each parameter comparison. The 90% confidence intervals (90% CIs; obtained by ANOVA using ln-transformed data) for overall bioequivalence analysis were 90.8–111.4% for C_{max}, 96.1–104.5% for AUC_{0-24} and 95.5–106.6% for AUC_{0-inf}; all were within the predefined ranges. The data clearly indicate that the formulations tested are bioequivalent.

CONCLUSIONS

In conclusion, a simple, rapid and reliable HPLC–FLD method has been developed for the determination of levofloxacin in human plasma samples. The method reported here requires a small sample volume, with a short chromatographic run. It is sensitive, specific, reliable and accurate. Finally, the method has been successfully applied to bioequivalence studies of a levofloxacin formulation product after oral administration to healthy volunteers.

Acknowledgments

The authors would like to thank Wei-si Zheng for her valuable technical skills, and are grateful to the nurses in the fourth ward of Internal Medicine of Cardiology, Guangdong Cardiovascular Institute for their enthusiastic clinical assistance. This work was supported by the National Science Foundation of China (30672077, 30571850) and Guangdong Provincial Science Foundation (015015, 06020831).

REFERENCES

Almeida S, Filipe A, Almeida A, Wong H, Caparros N and Tanguay M. Comparative bioavailability of two formulations of levofloxacin and effect of sex on bioequivalence analysis. Data from a randomised, 2 × 2 crossover trial in healthy volunteers. *Arzneimittelforschung* 2005; **55**: 414–419.

Bellmann R, Kuchling G, Dehghanyar P, Zeitlinger M, Minar E, Mayer BX, Muller M and Joukhadar C. Tissue pharmacokinetics of levofloxacin in human soft tissue infections. *British Journal of Clinical Pharmacology* 2004; **57**: 563–568.

Bottcher S, von Baum H, Hoppe-Tichy T, Benz C and Sonntag HG. An HPLC assay and a microbiological assay to determine levofloxacin in soft tissue, bone, bile and serum. *Journal of Pharmaceutical and Biomedical Analysis* 2001; **25**: 197–203.

Bucci FA, Jr. An *in vivo* study comparing the ocular absorption of levofloxacin and ciprofloxacin prior to phacoemulsification. *American Journal of Ophthalmology* 2004; **137**: 308–312.

FDA. Guidance for Industry: Bioanalytical Method Validation for Human Studies; www.fda.gov/cder/giudance/4252fnl.pdf, 2001.

Fish DN and Chow AT. The clinical pharmacokinetics of levofloxacin. *Clinical Pharmacokinetics* 1997; **32**: 101–119.

Guo L, Qi M, Jin X, Wang P and Zhao H. Determination of the active metabolite of prulifloxacin in human plasma by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B, Analytical Technology in the Biomedical and Life Sciences* 2006; **832**: 280–285.

Horstkotter C and Blaschke G. Stereoselective determination of ofloxacin and its metabolites in human urine by capillary electrophoresis using laser-induced fluorescence detection. *Journal of Chromatography B, Biomedical Sciences and Applications* 2001; **754**: 169–178.

Hu Q, Yan GL, Wang LQ, Liu XF and Wang XC. Clinical evaluation of levofloxacin. *Journal of Chinese Hospital Pharmacy* 2004; **24**: 37–38.

Jiang XL, Sun JG, Wang GJ, Li H, Li P and He H. Determination of levofloxacin in dog plasma using RP-HPLC and its pharmacokinetics. *Chinese Journal of Clinical Pharmacology and Therapeutics* 2004; **9**: 669–672.

Liang DR, Xu L, Miao J, Xiu QY, Fang Z, Chen DY and Wang RL. Multicentre clinical research of levofloxacin in treatment of acute bacterial infection. *Chinese Journal of Antibiotics* 1999; **24**: 1–11.

Liang H, Kays MB and Sowinski KM. Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin and cinoxacin by high-performance liquid chromatography: application to levofloxacin determination in human plasma. *Journal of Chromatography B, Analytical Technology in the Biomedical and Life Sciences* 2002; **772**: 53–63.

Nguyen HA, Grellet J, Ba BB, Quentin C and Saux MC. Simultaneous determination of levofloxacin, gatifloxacin and moxifloxacin in serum by liquid chromatography with column switching. *Journal of Chromatography B, Analytical Technology in the Biomedical and Life Sciences* 2004; **810**: 77–83.

Oberdorfer K, Swoboda S, Hamann A, Baertsch U, Kusterer K, Born B, Hoppe-Tichy T, Geiss HK and von Baum H. Tissue and serum levofloxacin concentrations in diabetic foot infection patients. *The Journal of Antimicrobial Chemotherapy* 2004; **54**: 836–839.

Rimmele T, Boselli E, Breilh D, Djabarouti S, Bel JC, Guyot R, Saux MC and Allaouchiche B. Diffusion of levofloxacin into bone

and synovial tissues. *The Journal of Antimicrobial Chemotherapy* 2004; **53**: 533–535.

Santoro MI, Kassab NM, Singh AK and Kedor-Hackmam ER. Quantitative determination of gatifloxacin, levofloxacin, lomefloxacin and pefloxacin fluoroquinolonic antibiotics in pharmaceutical preparations by high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis* 2006; **40**: 179–184.

Schulte S, Ackermann T, Bertram N, Sauerbruch T and Paar WD. Determination of the newer quinolones levofloxacin and moxifloxacin in plasma by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatographic Science* 2006; **44**: 205–208.

Siewert S. Validation of a levofloxacin HPLC assay in plasma and dialysate for pharmacokinetic studies. *Journal of Pharmaceutical and Biomedical Analysis* 2006; **41**: 1360–1362.

Swoboda S, Oberdorfer K, Klee F, Hoppe-Tichy T, von Baum H and Geiss HK. Tissue and serum concentrations of levofloxacin 500 mg administered intravenously or orally for antibiotic prophylaxis in biliary surgery. *The Journal of Antimicrobial Chemotherapy* 2003; **51**: 459–462.

Wang Y. Comparison of efficacy and safety between gatifloxacin and levofloxacin in treatment of patients with light to moderate acute lower respiratory tract infections. *Chinese Journal of Clinical Pharmacology and therapeutics* 2003; **8**: 434–437.

DOI: 10.1002/bmc