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Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin and cinoxacin by high-performance liquid chromatography: application to levofloxacin determination in human plasma

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Abstract

A selective, sensitive and accurate liquid chromatographic method with UV and fluorescence detection was developed, validated and applied for the determination of fluoroquinolones in human plasma. The effects of mobile phase composition, ion-pair and competing-base reagents, buffers, pH, and acetonitrile concentrations were investigated on the separation of six quinolones (cinoxacin, levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin and trovafloxacin). Sample preparation was carried out by adding internal standard and displacing agent and processing by ultrafiltration. This method uses ultraviolet and fluorescence detection and separation using a C_{18} column. The recovery, selectivity, linearity, precision, and accuracy of the method were evaluated from spiked human plasma samples. The method was successfully applied to patient plasma samples in support of a levofloxacin pharmacokinetic study.

Keywords: Levofloxacin; Ciprofloxacin; Gatifloxacin; Moxifloxacin; Trovafloxacin; Cinoxacin

1. Introduction

Quinolones have evolved from antibacterial agents with a limited gram-negative spectrum to a class of antibiotics with a broad spectrum of activity and extensive indications for the treatment of infections. The 3-carboxylic quinolones investigated in this study are illustrated in Fig. 1. Except for cinoxacin, the others (levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin and trovafloxacin) are all fluorinated at the 6-position, and termed fluoroquinolones. There have been numerous publications describing the determination of fluoroquinolone concentrations by high-performance liquid chromatography (HPLC) with UV or fluorescence detection [1–14], most commonly for determination of ciprofloxacin or ofloxacin concentrations [1–6]. More recently, assays describing the determination of trovafloxacin [15,16] and moxifloxacin concentrations [17,18] in human body fluids by HPLC have been reported.

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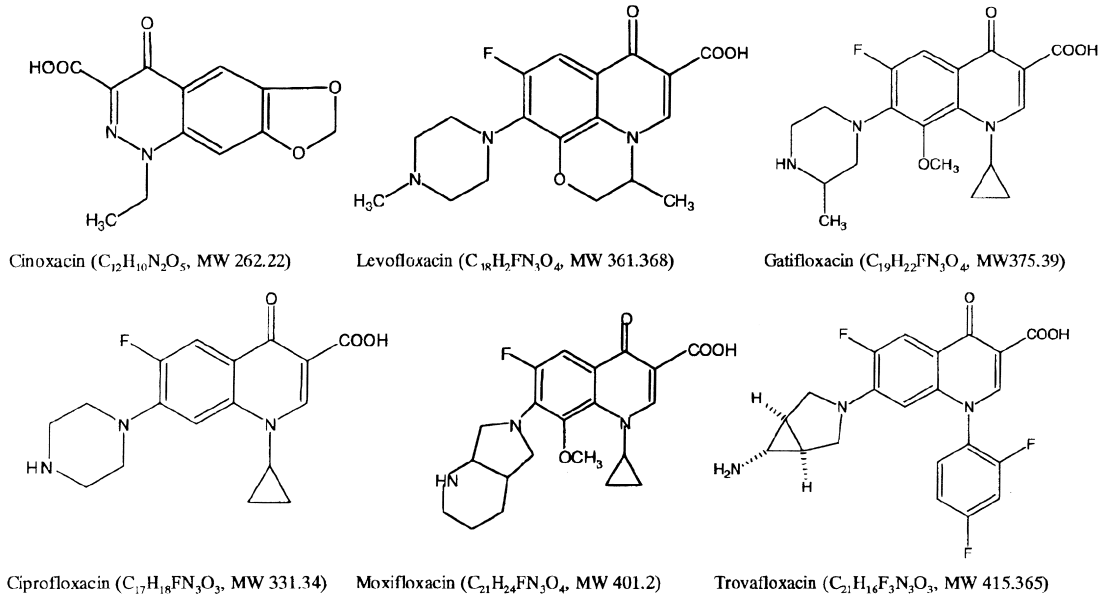

Fig. 1. Chemical structures of quinolones investigated in this study.

These assays have all described the quantitation of a single compound. In addition, several papers have reported the separation and/or simultaneous quantification of two or more fluoroquinolones [19–25], Two reviews [26,27] are available that describe the current status of analytical techniques for the quinolone antibiotics, the reader is directed to these for in depth discussion of these analytical techniques.

No currently available methods are available for the separation of the five fluoroquinolones described in this paper that are of interest in our laboratory. The aim of the present study was to establish a efficient, reliable, accurate and sensitive method for the separation of five different fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin, trovafloxacin) and quantitative determination of levofloxacin concentrations in human plasma. Specifically, our aim was to develop a method that allowed for the determination of each agent without the need for development of separate and distinct methods for each agent. In order to fulfill the aim, the method was first developed for the separation of and determination of quinolone concentrations by optimizing the experimental parameters and determining linearity for the five fluoroquinolones. Then we validated the method for the determination of levofloxacin concentrations by evaluating recovery, selectivity, linearity, precision, and accuracy. Finally, the method was applied to samples obtained from a levofloxacin pharmacokinetic study in patients with end-stage renal disease.

2. Experimental

2.1. Apparatus

Chromatography was performed on Beckman HPLC equipment consisting of a 118 solvent module, a 166 UV detector, a Jasco FP-920 fluorescence detector, and a 507e autosampler (Beckman Instrument, Fullerton, CA, USA), and on Shimadzu HPLC equipment (Shimadzu Scientific Instruments, Columbia, MD, USA), which comprised a liquid chromatograph (LC-10 ADVP), a system controller (SCL-10AVP), a diode array detection (DAD) system (SPD-M 10AVP), and an automatic injector (SIL-10ADVP). Data and chromatograms were collected using Gold Nouveau software from the Beckman system and Class-VP software from the Shimadzu system. Data were analyzed with Gold Nouveau and Class-VP software and Microsoft Excel 2000.

The pH of the solutions was adjusted with a Corning pH/ion meter 450 (Corning, NY, USA). Human plasma samples were prepared using an Amicon Centrifree micropartition device (Millipore, Bedford, MA, USA).

2.2. Chemicals

Cinoxacin was purchased from Sigma (St. Louis, MO, USA). The five fluoroquinolones were kindly provided: levofloxacin from R.W. Johnson Pharmaceutical Research Institute (Raritan, NJ, USA), ciprofloxacin and moxifloxacin from Bayer (West Haven, CT, USA); gatifloxacin from Bristol-Myers Squibb (Wallingford, CT, USA) and trovafloxacin from Pfizer (Groton, CT, USA).

All chemicals were analytical-reagent grade: sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$), sodium dodecyl sulfate (SDS), sodium 1-octane sulfonate (SOS), triethylamine (TEA) from J.T. Baker (Phillipsburg, NJ, USA); citric acid, acetonitrile (ACN), isopropanol and methanol from EM Science (Gibbstown, NJ, USA); sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) from VWR Scientific Products (West Chester, PA, USA); tetrabutylammonium acetate (TBAA) from Sigma and acetolhydroxamic acid (AHAA) from Aldrich (Milwaukee, WI, USA).

2.3. Chromatography

Analytical separation and guard columns were Adsorbosphere HS C_{18} 5 μm (Alltech Associates, Deerfield, IL, USA). The dimension of the separation column (part No. 28935, serial No. 00021067.1) was 250×4.6 mm I.D. with 5 μm particle size. The guard column cartridge (part No. 96079, serial No. 00061439.1) was 7.5×4.6 mm I.D. with 5 μm particles of identical chemistry to the separation column. Prefilter elements were 4.0 mm diameter with 2 μm particles (part No. 28640, Lot 072100). Between the sample injections, the injection needle was washed with 70% aqueous methanol. All experiments were carried out at ambient temperature, approximately 23 °C, and the flow-rate of the mobile phase was 1 ml/min.

2.4. Preparation of various solutions

2.4.1. Mobile phase

During the optimization of mobile phase composition, 50% aqueous acetonitrile was used first. Then the mobile phase containing 25 m*M* phosphate with 50% acetonitrile was prepared and adjusted pH to 3.0. Further, 10 m*M* SOS was added into the above mobile phase to run ion-pair chromatography. Instead of SOS, different amounts of SDS were added into the mobile phase to contain 0, 5, 7.5, 10, 12.5, 15 and 20 m*M* SDS, respectively. In addition, 10 m*M* TEA, TBAA or AHAA was, respectively, added to the mobile phase containing 10 m*M* SDS to study the effect of different competing bases on the separation of quinolones. Moreover, the mobile phase containing 10 m*M* SDS, 10 m*M* TBAA, 25 m*M* citric acid with 50% acetonitrile was prepared and adjusted pH to 3.4, 3.5, 4.0, 4.5, 5.0 and 6.0, respectively. The mobile phases containing 10 m*M* SDS, 10 m*M* TBAA, 25 m*M* citric acid and different concentrations of acetonitrile (40, 43, 45 and 50%) at pH 3.4 were prepared. The final optimized mobile phase consisted of 10 m*M* SDS, 10 m*M* TBAA, 25 m*M* citric acid with 43% acetonitrile at pH 3.5.

2.4.2. Displacing reagent

The stock solutions of 0.3 *M* $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 7H_2O$ were first prepared and then diluted to 75 m*M* with water and adjusted to pH 7.5 and 0.5% (mg/ml) SDS was added into the buffer. The displacing reagent consisted of the buffer containing 0.5% SDS–acetonitrile (4:1).

2.5. Calibration standards

Eight calibrators of each quinolone for UV detection (10 000, 5000, 2000, 1000, 500, 200, 100 and 50 ng/ml) and for fluorescence detection (5000, 2500, 1000, 500, 200, 100, 50, and 20 ng/ml) were prepared by making serial dilutions from stock solutions and spiking them into the drug-free human plasma. The blank biological matrix was obtained from the local blood bank (Wishard Health Services, Indianapolis, IN, USA) of drug-free healthy volunteers. All samples were stored at −70 °C and protected from light until assayed. The peak area ratio of target to internal standard was determined for

each calibration sample. The linear equation describing the relationship between target (fluoroquinolone) concentration and the peak area ratio of target to internal standard was determined by least-squares weighted (1/concentration) for UV detection or unweighted (fluorescence detection) regression methods.

2.6. Sample preparation and extraction

The blank human plasma, calibrator, quality control and unknown samples were thawed and vortexed for 30 s. A 450-μl volume of displacing reagent and 50 μl of internal standard (10 μg/ml) or water for blank samples were added to 500 μl of sample and vortexed for 30 s. Each sample was transferred to an Amicon Centrifree (Millipore) micropartition device and centrifuged at 1500 *g* for 30 min. A 200-μl volume of each ultrafiltrate was transferred to a vial for HPLC analysis and the autosampler injected 10 μl of each sample.

2.7. Assay validation for levofloxacin

2.7.1. Linearity of standard curve

The peak area ratio of levofloxacin to ciprofloxacin (internal standard) was determined for each calibration sample using the calibration curve from 50 to 10 000 ng/ml. The linear equation describing the relationship between levofloxacin concentration and the peak area ratio of levofloxacin to ciprofloxacin was determined by least-squares weighted (1/concentration) regression methods. Six standard curves were prepared on 6 separate days and appropriate regression statistics were determined.

2.7.2. Precision and accuracy

For the intra-day assay precision and accuracy, six replicates of each low (120 ng/ml), middle (1500 ng/ml) and high (6000 ng/ml) quality control samples were analyzed. For the inter-day assay precision and accuracy, six replicates of each low, middle and high quality control samples were analyzed daily for 6 days. The mean, standard deviation, RSD and accuracy of the intra-day and inter-day experiments were calculated. Relative error (RE) of mean predicted concentration compared with nominal concentration was determined.

2.7.3. Stability evaluation

The stability experiments of levofloxacin were carried out under three conditions: during 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 three replicates of each lower limit of quantification (LLOQ), low, medium and high quality control (QC) samples. All test samples were analyzed at the same time as freshly thawed QC samples. Stability was determined by comparing the nominal concentration of levofloxacin in freshly thawed QC samples and the test samples.

2.8. Application of method to a levofloxacin pharmacokinetic study

Following completion of a scheduled hemodialysis treatment, each subject received 250 mg levofloxacin administered intravenously over 1 h through the venous limb of the hemodialysis access. Blood samples were obtained (5 ml each) at 0 (pre-infusion), and 0.5, 1, 1.5, 2, 3, 5, 25 and 48 h after the initiation of the infusion. All samples were obtained from the arterial port of the subject's hemodialysis access, in blood collection tubes, allowed to clot and centrifuged at 3000 rpm for 15 min. After separation, all samples were stored at −70 °C until analysis.

The concentrations of levofloxacin were determined based on the daily standard curve. The eight concentrations of calibration standards and three quality control samples were analyzed with each analytical set of samples. These calibrator and control batches were separated into aliquots in sample vials, stored at −70 °C and used in the sample analyses.

3. Results and discussion

3.1. Method development

3.1.1. Optimization of mobile phase composition

When 50% aqueous acetonitrile was used as the mobile phase, the fluoroquinolones were overlapped and the peak tailing was extremely serious due to the secondary interaction between silanol groups on column packing material and amino groups on

quinolones. Although Adsorbosphere HS C_{18} columns are endcapped, there is still 1% free silanol remaining. In order to overcome the secondary interaction, 25 m*M* phosphate buffer at pH 3 was added to the above mobile phase, which resulted in the peak shape becoming very sharp but the fluoroquinolones still overlapped. They were nonretained at this pH because they were completely ionized. The effect of SOS and SDS on separation of the six quinolones was studied to increase retention and selectivity of the quinolones (Fig. 2). Further, the effect of SDS concentrations from 5 to 20 m*M* in mobile phase was investigated on separation of the quinolones (Fig. 3). The retention and resolution of the 5 fluoroquinolones increased with SDS concentrations but the retention of cinoxacin did not. Their resolution did not improve very much at SDS concentrations higher than 10 m*M* and this concentration was chosen to minimize the equilibration time of column after each analytical batch of experiment. Improvement in the peak shape and resolution of quinolones was still needed with 10 m*M* SDS in mobile phase. Therefore competing base agents such as TEA, TBAA and AHAA were added. Based on our experiments, the best peak shape and resolution of quinolones were attained with 10 m*M* TBAA and

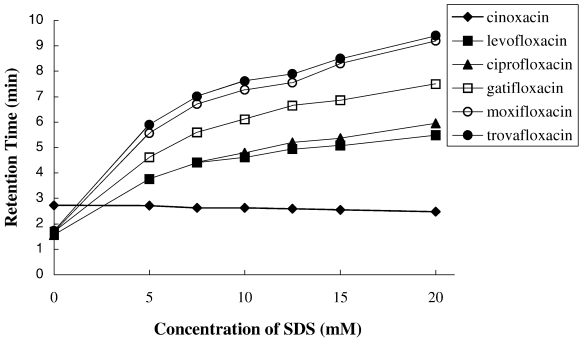

Fig. 3. Effect of SDS concentrations on separation of the quinolones. The mobile phase was composed of 25 m*M* phosphate and 50% acetonitrile with SDS varying from 0 to 20 m*M* at pH 3.0. Other experimental conditions as in Fig. 2.

thus 10 m*M* TBAA was chosen in the following mobile phase.

The effect of two types of buffers (phosphate and citric acid) were studied on the separation of quinolones. The resolution of quinolones was much better using citric acid than phosphate buffer (Fig. 4). In addition, the buffer capacity of citric acid is much wider than that of phosphate at low pH. Therefore, the citric acid was chosen as buffer in the mobile phase. At this point in our experiments, the optimized mobile phase consisted of 10 m*M* SDS, 10

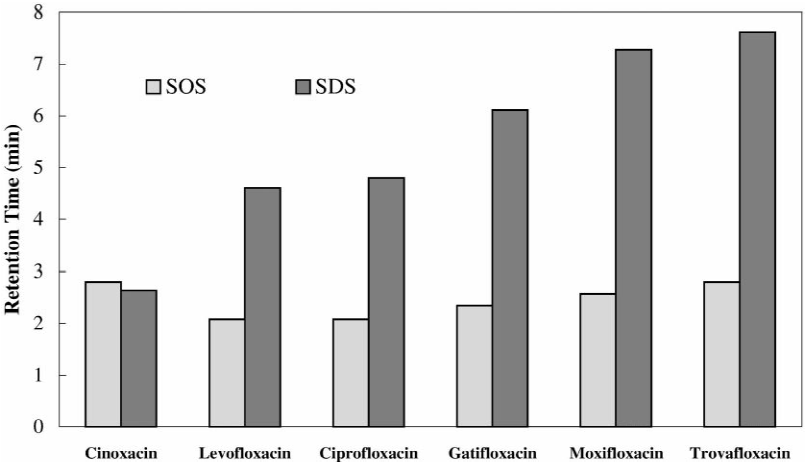

Fig. 2. Effect of ion-pair agents on separation of the quinolones. The mobile phase was composed of 25 m*M* phosphate and 50% acetonitrile with 10 m*M* sodium 1-octane sulfonate (SOS) or sodium dodecyl sulfate (SDS) at pH 3.0. Experimental conditions: the experiment was performed on Beckman HPLC equipment with UV detection. The mobile phase was composed of 50% acetonitrile; detection wavelength at 280 nm, injection volume: 10 μl, and flow-rate at 1 ml/min. The separation column was an Alltech Adsorbosphere HS C_{18} (250×4.6 mm I.D. with 5 mm particle size). Between consecutive analyses, a needle for the automated injector was washed with 70% aqueous methanol. All experiments were carried out at ambient temperature around 23 °C.

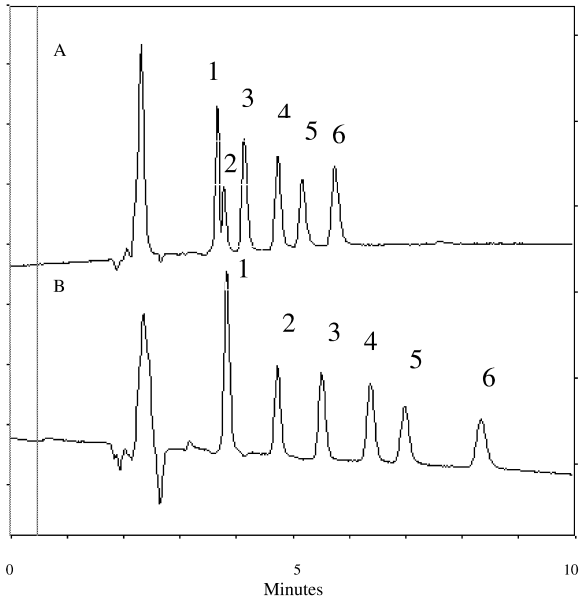

Fig. 4. Chromatograms of mixture of six quinolones showing the effect of buffers and competing base agents on retention time. The mobile phase was composed of 25 m*M* buffer (panel A: 25 m*M* phosphate; panel B: 25 m*M* citric acid) and 50% acetonitrile with 10 m*M* SDS and 10 m*M* TBAA at pH 3.5. Other experiment conditions are the same as in Fig. 2. 1: Cinoxacin, 2: levofloxacin, 3: ciprofloxacin, 4: gatifloxacin, 5: moxifloxacin, 6: trovafloxacin.

m*M* TBAA, 25 m*M* citric acid and 50% acetonitrile at pH 3.5.

3.1.2. Effect of pH and ionic strength of mobile phase

The effect of pH from 3.4 to 6.0 was investigated on the separation of the quinolones (data not shown). At pH 3.4, the largest degree of separation was obtained although the resolution and selectivity did not change appreciably from pH 3.4 to 6.0, indicating the method was quite robust. In order to investigate the robustness of the method further, the effect of acetonitrile concentrations from 40 to 50% in mobile phase was investigated. As seen in Fig. 5, the retention time and resolution of the quinolones increased with decreasing acetonitrile. As described earlier, the final optimized mobile phase for the remaining experiments consisted of 10 m*M* SDS, 10 m*M* TBAA, 25 m*M* citric acid and 43% acetonitrile at pH 3.5.

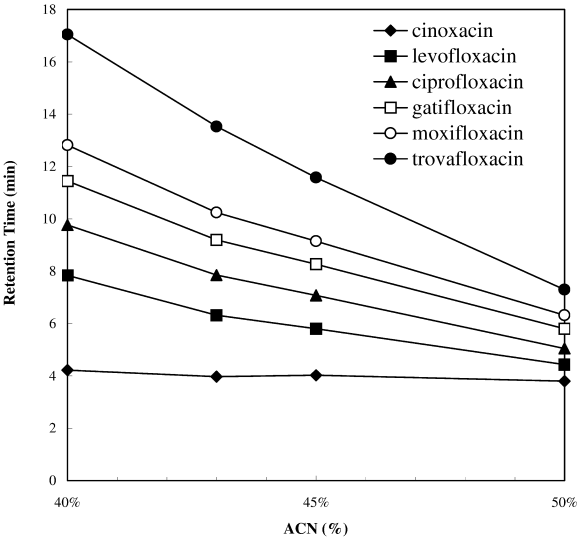

Fig. 5. Effect of acetonitrile concentration on retention of quinolones. The mobile phase was composed of 10 m*M* SDS, 10 m*M* TBAA, 25 m*M* citric acid with ACN, changing from 40 to 50% at pH 3.4. Other experimental conditions as in Fig. 2.

3.1.3. Selection of UV detection and fluorescence excitation/emission wavelengths

The most sensitive excitation/emission wavelengths were obtained at 280/450 nm for ciprofloxacin, at 293/500 nm for levofloxacin, gatifloxacin, and moxifloxacin, at 272/450 nm for trovafloxacin and at 263/450 nm for cinoxacin. Based on these experiments the optimal detection methods for our levofloxacin pharmacokinetic studies was determined to be UV detection at a wavelength of 293, using ciprofloxacin as the internal standard.

3.2. Extraction of fluoroquinolones from human plasma

Human plasma samples were processed by ultrafiltration and particulates and proteins in samples were removed. The protein binding of the quinolones varies from 20 to 76%. In order to displace the drug bound with proteins and to determine total drug concentrations, the displacing agent was added to samples before ultrafiltration. The extraction efficiency was determined by comparing peak areas of directly injected standards in the mobile phase and those from drug-free plasma spiked with standards and submitted to the sample preparation and ex-

traction procedures ($n=6$) at a concentration of 1000 ng/ml for each fluoroquinolone. The recovery of each of the five fluoroquinolones in human plasma processed by ultrafiltration was greater than 95%.

3.3. Method validation

3.3.1. Selectivity

The selectivity of the method was first assessed using independent sources of drug-free human plasma. No interfering peaks were observed (Fig. 6) in the blank human plasma processed by ultrafiltration at the retention times of the fluoroquinolones. Then, the selectivity was further assessed using blank patient samples collected immediately prior to administration of levofloxacin. The patients with end-stage renal disease were administered many other medications, which were different from patient to patient. However, no interfering peaks were found at the retention times of levofloxacin and ciprofloxacin as an internal standard (Fig. 7).

3.3.2. Linearity

Linearity of the five fluoroquinolones in human plasma by HPLC with UV and fluorescence detection was evaluated (Table 1). The suitable excitation/emission wavelengths were selected to obtain a wider dynamic range (e.g., for levofloxacin at 280/450 nm) or a better LLOQ (293/500 nm). Based on these linearity experiments the optimal detection methods for our levofloxacin pharmacokinetic studies was determined to be UV detection at a wavelength of 293, with ciprofloxacin as the internal standard.

3.3.3. Precision and accuracy

The precision and accuracy experiments for levofloxacin were carried out for the application of this method to a pharmacokinetic study. The 6-day standard curve validation of levofloxacin is shown in Table 2. Overall mean precision (RSD) ranged from 2.68 to 5.32%. The accuracy of these calculations

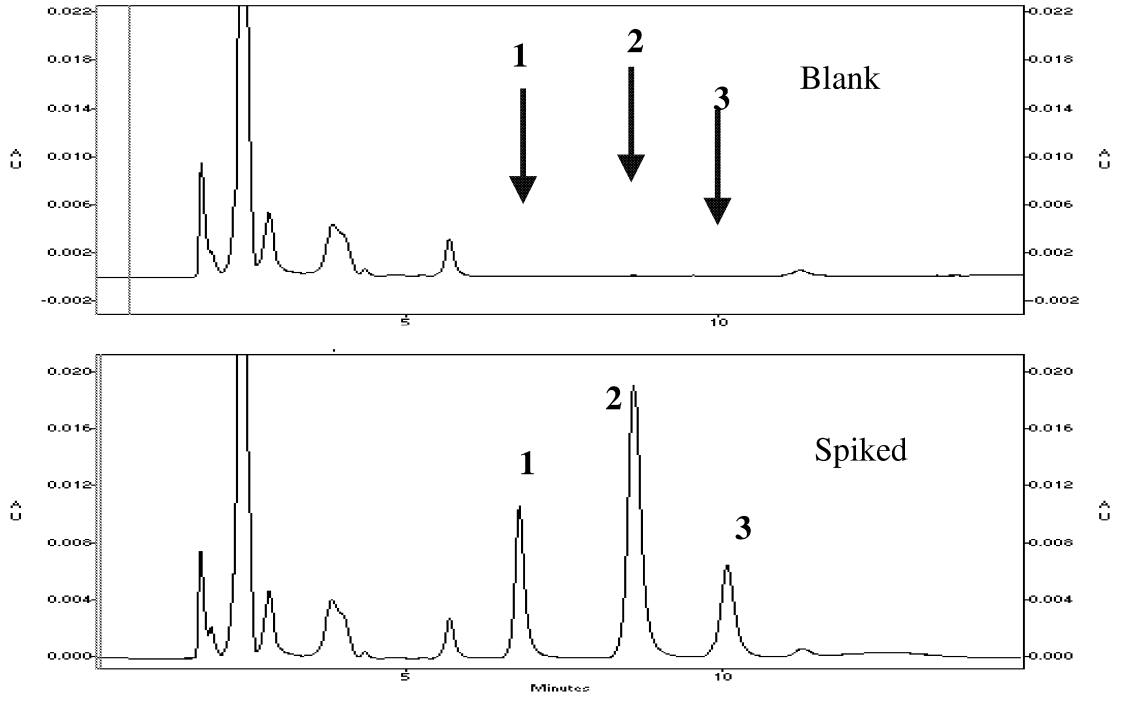

Fig. 6. Chromatograms of blank and spiked human plasma processed by ultrafiltration. The mobile phase was composed of 10 m*M* SDS, 10 m*M* TBAA, 25 m*M* citric acid containing 43% ACN at pH 3.5 with UV detection at 293 nm. Other experimental conditions as in Fig. 2. 1: Levofloxacin, 2: ciprofloxacin, 3: gatifloxacin.

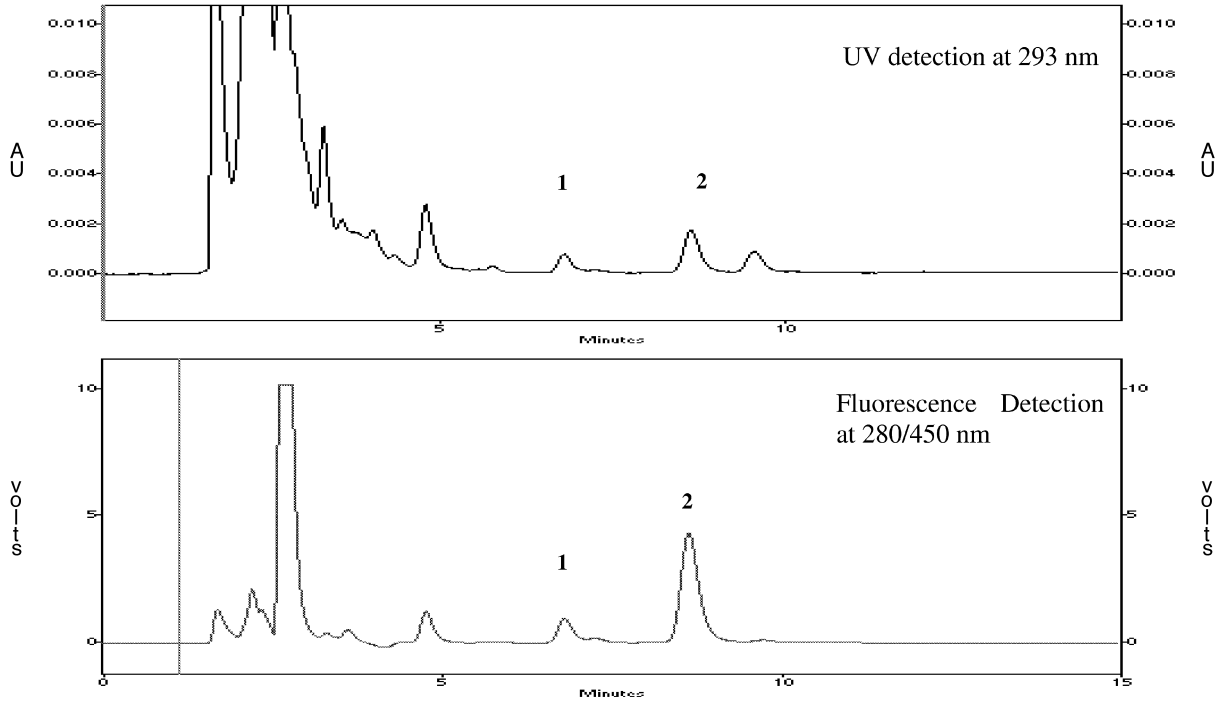

Fig. 7. Chromatograms of patient plasma sample spiked with ciprofloxacin processed by ultrafiltration. The mobile phase was composed of 10 m*M* SDS, 10 m*M* TBAA, 25 m*M* citric acid with 43% ACN at pH 3.5 with UV detection at 293 nm and fluorescence detection at 280/450 nm. Other experimental conditions as in Fig. 2. 1: Levofloxacin: 2: ciprofloxacin (internal standard).

ranged from −5.56 to 8.55%. Furthermore, 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 1.49 to 5.09% and the accuracy from −3.50 to 2.11% (Table 3). The inter-day RSD for levofloxacin varied from 3.30 to 7.55% and the accuracy from −5.67 to 3.63% (Table 3). The above results indicated that the method was reliable, reproducible and accurate.

Table 1
Linear range of five fluoroquinolones in human plasma by HPLC with UV and fluorescence detection

Target	Internal standard	Linear range (ng/ml) (R^{2a}≥0.999)	
		UV	FD[b]
Levofloxacin	Ciprofloxacin	50–10 000 (293 nm)	20–5000 (280/450 nm)
Ciprofloxacin	Levofloxacin	200–10 000 (280 nm)	20–5000 (293/500 nm)
Gatifloxacin	Ciprofloxacin	100–10 000 (293 nm)	20–5000 (280/450 nm)
Moxifloxacin	Ciprofloxacin	100–10 000 (293 nm)	20–5000 (280/450 nm)
Trovafloxacin	Gatifloxacin	200–10 000 (293 nm)	20–5000 (280/450 nm)

Experimental conditions: the experiment was performed on Beckman HPLC equipment with UV and fluorescence detection. The mobile phase was composed of 10 m*M* SDS, 10 m*M* TBAA, 25 m*M* citric acid containing 43% acetonitrile at pH 3.5; injection volume: 10 μl, and flow-rate at 1 ml/min. The separation column was an Adsorbosphere HS C_{18} (250×4.6 mm I.D. with 5 μm particle size). The calibrators spiked in human plasma were processed by ultrafiltration. Between consecutive analyses, a needle for the automated injector was washed with 70% aqueous methanol. All experiments were carried out at ambient temperature around 23 °C.

[a] Coefficient of determination.

[b] Fluorescence detection (excitation/emission wavelengths).

Table 2
Six-day standard curve validation of levofloxacin

Concentration (ng/ml) (n=2[a])		RSD	Relative error[c]
Nominal	Mean predicted[b] ± SD	(%)	(%)
50	54±2.8	5.2	8.6
100	104±2.9	2.8	3.6
200	198±8.8	4.5	−1.2
500	494±14.5	2.9	−1.3
1000	944±43.6	4.6	−5.6
2000	2006±53.8	2.7	0.3
5000	4971±264.7	5.3	−0.6
10 000	10 054±296.7	3.0	0.5

The experimental conditions are described in Table 1.
[a] n=Number of daily replicates.
[b] Mean predicted concentration of levofloxacin was calculated by daily linear regression equation.
[c] Relative error %=100·[(predicted concentration−nominal concentration)/nominal concentration].

3.3.4. Stability

The stability experiment was carried out under three conditions (Table 4). Triplicates of each LLOQ, low, middle and high quality control samples were analyzed. As shown in Table 4, no significant degradation of levofloxacin was observed for processed samples under any of those conditions.

3.4. Method application

The validated method was applied to patient samples in support of pharmacokinetic study of levofloxacin in patients with end-stage renal disease. Fig. 7 shows a chromatogram of unknown samples from a representative patient with end-stage renal disease after receiving levofloxacin, spiked with the internal standard, ciprofloxacin. A representative concentration–time curve after the administration of levofloxacin is shown in Fig. 8.

4. Conclusions

For the first time, levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovofloxacin and cinoxacin, have been successfully separated. The method has been developed and validated for the quantitative determination of levofloxacin in human plasma. The method reported here is selective, sensitive, reliable and accurate. Finally, the method has been success-

Table 3
Intra-day and inter-day precision and accuracy of levofloxacin in human plasma

Nominal concentration (ng/ml)	Mean±SD predicted concentrations (ng/ml)[b]	RSD (%)	Relative error[c] (%)
Intra-day precision and accuracy (n=36)[a]			
QC low 120	116±4.6	3.9	−3.5
QC middle 1500	1467±21.8	1.5	−2.2
QC high 6000	6126±311.6	5.1	2.1
Inter-day precision and accuracy (n=6 days of replicate samples)			
QC low 120	113±7.0	6.2	−5.7
QC middle 1500	1467±48.4	3.3	−2.2
QC high 6000	6218±469.5	7.6	3.6

The experimental conditions are described in Table 1.
[a] n=Number of replicates.
[b] Mean predicted concentrations of levofloxacin.
[c] Relative error %=100·[(predicted concentration−nominal concentration)/nominal concentration].

Table 4
Stability of levofloxacin in human plasma processed by ultrafiltration

Experimental conditions	Parameter	Concentration (ng/ml) (n=3)[a]			
		LLOQ[b] 50	Low QC 120	Middle QC 1500	High QC 6000
After three freeze–thaw cycles	Mean[c]	474	117	1463	6230
	RSD (%)	11.2	6.9	8.5	7.5
	RE[d] (%)	−5.2	−2.5	−2.5	3.8
At room temperature for 24 h	Mean	49	116	1466	6402
	RSD (%)	6.8	4.5	7.2	5.5
	RE (%)	−1.8	−3.4	−2.2	6.7
At −70 °C for 3 months	Mean	44	118	1491	6238
	RSD (%)	12.5	8.5	8.6	7.8
	RE (%)	−11.2	−6.0	−0.6	4.0

The experimental conditions are described in Table 1.
[a] n=Number of replicates.
[b] Lower limit of quantification.
[c] Mean predicted concentrations of levofloxacin.
[d] Relative error (RE) %=100·[(predicted concentration−nominal concentration)/nominal concentration].

fully implemented to clinical samples in support of pharmacokinetic studies of levofloxacin. The method will be further developed, validated and applied to pharmacokinetic studies of the other fluoroquinolone agents.

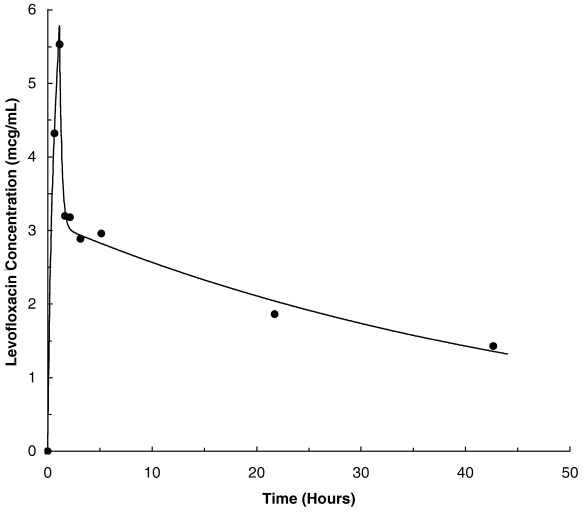

Fig. 8. Individual pharmacokinetic model fit of levofloxacin concentrations vs. time in a representative subject. The closed circles (·) represent the observed data points following a 250-mg levofloxacin intravenous dose. The solid line represent the fitted line to the data set.

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