Talanta 83 (2010) 110–116

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Validated liquid chromatographic-fluorescence method for the quantitation of gemifloxacin in human plasma

Badraddin M.H. Al-Hadiya [a,*], Adnan A. Khady [b], Gamal A.E. Mostafa [b]

[a] Clinical Pharmacy, College of Pharmacy, King Saud University, P.O.Box 2457, Riyadh 11451, Saudi Arabia
[b] Pharmaceutical Chemistry Department, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 23 June 2010
Received in revised form 26 August 2010
Accepted 26 August 2010
Available online 22 September 2010

Keywords:
Gemifloxacin
HPLC
Method of validation
Human blood plasma
Pharmacokinetic study

ABSTRACT

A highly selective, sensitive and rapid high performance liquid chromatographic method has been developed and validated to quantify gemifloxacin in human plasma. The gemifloxacin and internal standard (ciprofloxacin) were extracted by ultrafiltration technique followed by injection into chromatographic system. Chromatographic separation was achieved on a reversed phase C_{18} column with a mobile phase of acetonitrile:0.1% trifluoroacetic acid (20:80, v/v) using isocratic elution (at flow rate 1 mL min^{-1}). The analytes were detected at 269 and 393 nm for excitation and emission, respectively. The assay exhibited a linear range of 25–5000 ng mL^{-1} for gemifloxacin in human plasma. The lower limit of detection was 10 ng mL^{-1}. The method was statistically validated for linearity, accuracy, precision and selectivity following FDA guidelines. The intra- and inter-assay coefficients of variation did not exceed 7.6% deviation of the nominal concentration. The recovery of gemifloxacin from plasma was greater than 97.0%. Stability of gemifloxacin in plasma was excellent with no evidence of degradation during sample processing (auto-sampler) and at least 3 months storage in a freezer at −70 °C. This validation method is applied for clinical study of the gemifloxacin in human volunteers.

1. Introduction

Gemifloxacin [(R, S)-7-(3-aminomethyl-4-syn-methoxyimino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylic acid methanesulfonate] (CAS number 175463-14-6), is a recently developed fluoroquinolone antibacterial compound with a broad spectrum of activity (Fig. 1) [1–3]. It has shown potent antibacterial activity against clinical isolates and reference strains in both in vitro studies and experimental models of infection in animals [4,5]. It has particularly enhanced activity against gram-positive organisms, and displays fourfold higher activity than that of moxifloxacin against *Streptococcus pneumoniae* (minimum inhibitory concentration to inhibit 90% of isolates [MIC_{90}] is 0.03 μg mL^{-1}) in vitro [5]. Gemifloxacin has also shown potent activity against other major pathogens involved in respiratory tract infections, including *Haemophilus influenzae* and *Moraxella catarrhalis* and the atypical organisms, *Legionella pneumophila*, *Chlamydia* spp., and *Mycoplasma* spp. [6]. Furthermore, the compound has shown potent activity against many organisms that cause urinary tract infections. The adverse reaction profile is similar to that of older members of this class [7]. The pharmacokinetic properties of fluoroquinolone antibacterial agents have been well described [8]. Gemifloxacin is rapidly absorbed with a time to maximum plasma concentration (T_{max}) of 0.5–2 h in healthy subjects and displays linear pharmacokinetics over the dosage range studied (20–800 mg). The long terminal phase half-life ($t_{1/2}$) is 8 h after single or repeated administration. Approximately 20–30% of the administered dose is excreted unchanged in the urine and plasma protein binding of gemifloxacin is about 70% [9,10].

A few analytical methods have been published for quantification of gemifloxacin in human plasma using liquid chromatography–mass (LC/MS) [9–11] and –mass/mass (LC/MS/MS) [12]. However the LC/MS machine is quite expensive and not readily available in the most clinical, bioanalytical, educational research laboratories.

A method was described by Rote and Pingle [13] for the determination of gemifloxacin in spiked human plasma using liquid chromatography with UV detection. The calibration range was 30–600 ng mL^{-1} using liquid/liquid extraction.

Liquid chromatographic methods with fluorescence detection (HPLC-FL) were developed for the determination of gemifloxacin in human serum and urine using a reversed phase, and liquid–liquid extraction (unpublished data) [9,10].

To the best of our knowledge, the following validation parameters: linearity, precision, accuracy, recovery and stability have not been reported combined in a single gemifloxacin HPLC-FL method

* Corresponding author. Fax: +966 4677480.
E-mail address: b_alhadiya@yahoo.com (B.M.H. Al-Hadiya).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.08.047

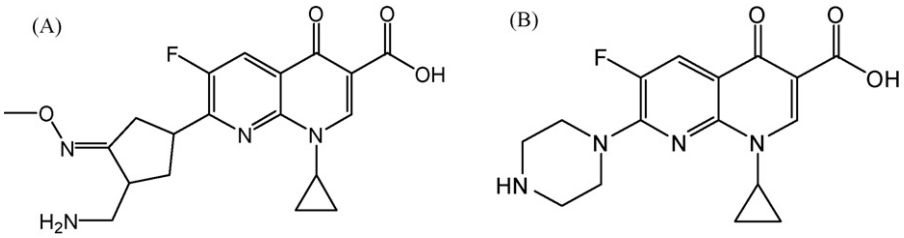

Fig. 1. The chemical structure of: (A) gemifloxacin and (B) ciprofloxacin (IS).

with its clinical application. So we developed a simple, rapid, sensitive and selective HPLC-FL method for the quantification of gemifloxacin in human plasma. The plasma sample containing the drug and IS was ultrafiltrated and the supernatant was injected into analytical column without any further clean up. The drug and internal standard were detected at 269 and 393 nm for excitation and emission, respectively. The lower limit of quantification is 25 ng mL^{-1} using 500 μL of human plasma, with lower limit of detection of 10 ng mL^{-1}. The total run time was ~8 min. The method proved very robust and was successfully applied for the analysis of clinical samples from male volunteers dosed with gemifloxacin.

2. Experimental

2.1. Chemicals and reagents

Gemifloxacin mesylate (purity ≥ 99.0%) and ciprofloxacin hydrochloride (purity ≥ 99.0%) were purchased from Sigma chemical (St. Louis, MO, USA). Deionized water was purified using cartridge system (Picotech water system, RTP, NC USA). Acetonitrile and ethanol were of HPLC grade (BDH, England). Trifluoro acetic acid was of analytical grade (Riedel-deHaen, Germany).

2.2. Apparatus

The LC system consisted of a water binary pump, model 1525 (Milford, MA, USA), equipped with a fluorescence detector model 2487, an autosampler model 717. Waters solvent delivery system was used to operate the isocratic flow. The data was collected with Millennium software (version 4.0) for data acquisition analysis. The chromatographic separations were performed using a symmetry LC-18 stainless steel column (150 mm × 3.9 mm, 5 μm) coupled with a symmetry C_{18}-sentry guard column (20 mm). Micron® (ultracell centrifugal filter paper Millipore cooperation MAO 1730, USA).

2.3. Chromatographic conditions

The mobile phase was a mixture of 0.1% trifluoroacetic acid: acetonitrile (80:20, v/v). The mobile phase was freshly prepared, then filtered through a Millipore filter (pore size 0.45 μm) and degassed continuously by an on-line degasser in the HPLC. Separation was performed at room temperature using a 1.0 mL min^{-1} flow-rate and 8 min run time. The injection volume was 75 μL and the detection wavelengths were set at 269 and 393 nm for excitation and emission respectively.

2.4. Standard solution

Standard solutions preparation was conducted at room temperature under subdued light (protected form direct light). The solution were protected from light with aluminum foil wrapping and stored at −70 °C. Gemifloxacin standard stock solution was prepared in 50% ethanol in water to produce a final concentration of 1 mg mL^{-1}. The working standard solution was prepared by diluting 1.0 mL of stock solution into 10 milliliters measuring flask with 50% ethanol in water to give a 100 μg mL^{-1} concentration. The internal standard (IS), ciprofloxacin stock solution was prepared in deionized water to produce a concentration of 1.0 mg mL^{-1}. Ten mL of this stock solution was diluted to 100 mL with sodium dihydrogen phosphate buffer to produce a working solution of 100 μg mL^{-1}.

2.5. Sample processing

Fifty microliters of ciprofloxacin (IS, 100 μg mL^{-1}), were added to 500 μL plasma sample in a 1.5 mL micro centrifuge tube (Eppendorf) thoroughly vortex-mixed for 30 s, then 450 μL sample was transferred to an ultrafiltration tube and centrifuged at 15,000 r.p.m. for 10 min. Aliquot of the ultrafiltrate were loaded in the autosampler tray and 75 μL of this sample was injected onto the analytical column.

2.6. Bioanalytical method validation

The described method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, specificity, stability, precision and accuracy according to international guidelines regarding bioanalytical method validation [14–16]. Limit of detection and LOQ were calculated from the residual standard deviation of the regression line (δ) of the calibration curve and its slope (S) in accordance to the following equations: LOD = 3.3 (δ/S) and LOQ = 10 (δ/S).

2.6.1. Calibration and control samples

Appropriate volumes of gemifloxacin working standard solution (100 μg mL^{-1}) were added to drug-free human plasma (20 mL) to prepare eight non-zero standard drug concentration (25, 50, 150, 300, 600, 1500, 3000 and 5000 ng mL^{-1}), and five quality control concentrations (25, 75, 750, 2500 and 4000 ng mL^{-1}). Standard drug concentrations used for the preparation of the calibration curves were different from those employed in the quality control studies. A calibration curve was constructed from blank plasma sample, a zero sample (a plasma spiked with IS) and eight non-zero samples covering the total range (25–5000 ng mL^{-1}), including lower limit of quantification (LLOQ).

Each validation run consisted of system suitability sample, blank sample, a zero sample (a plasma processed with IS) calibration curve consisting eight non-zero samples covering the total range (25–5000 ng mL^{-1}) and quality control samples at five concentration (n = 6, at each concentration). Such validation samples were generated on six consecutive days. Calibration samples were analyzed from low to high concentration at the beginning of each validation run and the other sample were distributed randomly through the run. The calibration curve had a correlation coefficient (r) of 0.9988.

2.6.2. Human plasma applications

Five healthy volunteers participated in this study to support the applicability of the developed method to quantify gemifloxacin for

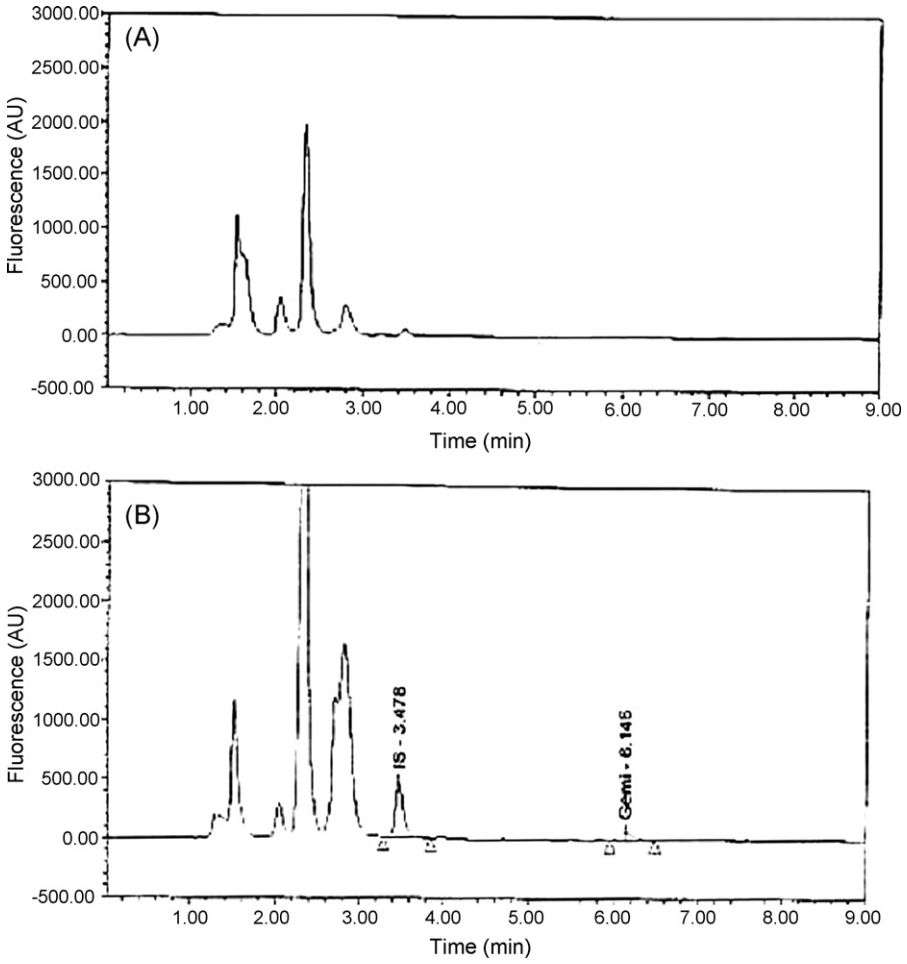

Fig. 2. Chromatogram resulting from (A) the analysis of blank human plasma, (B) human plasma spiked (B) with 25 ng mL^{-1} gemifloxacin and ciprofloxacin (IS).

pharmacokinetic purpose in bioequivalence and/or bioavailability studies. The volunteers were administered a single dose of Factive® tablets equivalent to 320 mg gemifloxacin/tablet (Oscient Pharmaceuticals, USA) under fasting conditions. Blood samples were drawn before drug administration and at 0.33, 0.66, 1.33, 1.66, 2.00, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 24.0, 36.0 and 48.0 h after drug administration. After centrifugation, plasma samples were separated and immediately stored in a freezer at a nominal temperature of −70 °C pending analysis.

2.6.3. Specificity

To evaluate the specificity of the method, drug free plasma samples were carried through the assay procedure and the retention time of the endogenous compounds in plasma were compared with those of gemifloxacin (25 ng mL^{-1}) or internal standard. Specificity of the method was assessed to test the matrix influence between different plasma samples.

2.6.4. Recovery

The absolute recovery of gemifloxacin was evaluated by comparing drug peak area of the spiked analyte samples to unrestricted analyte of stock solution that has been injected directly into an HPLC system. The assay absolute recovery for each compound, at each concentration, was computed using the following equation: absolute recovery = (peak area of extract/mean peak area of direct injection) ×100, and relative recovery = (conc. of extract/theoretical conc.) ×100.

2.6.5. Accuracy and precision

Within-batch accuracy and precision evaluations were performed by repeated analysis of gemifloxacin in human plasma. The run consisted of a calibration curve plus six replicates of each LLOQ, low, medium and high quality control samples. Between-batch accuracy and precision were assessed by analysis of samples consisting of a calibration curve and six replicates of LLOQ, low, medium and high quality control samples for gemifloxacin on three separate days. The overall precision of the method expressed as relative standard deviation and accuracy of the method expressed in term of bias (percentage deviation from true value).

2.6.6. Stability

The bench-top stability was examined by keeping replicates of the low and high plasma quality control samples at room temperature for approximately 6 h. Freeze–thaw stability of the samples was obtained over four freeze–thaw cycles, by thawing at room temperature for 6 h and refrozen for 12–24 h. Auto-sampler stability of gemifloxacin was tested by analysis of processed and reconstituted low and high plasma quality control samples, which were stored in the auto-sampler tray for 24 h at 8 °C. Long-term stability of gemifloxacin in human plasma was tested after storge at approximately −70 °C for 90 days. For each concentration and each storage condition, six replicates were analyzed in one analytical batch. The concentration of gemifloxacin after each storage period was related to the initial concentration as determined for the samples.

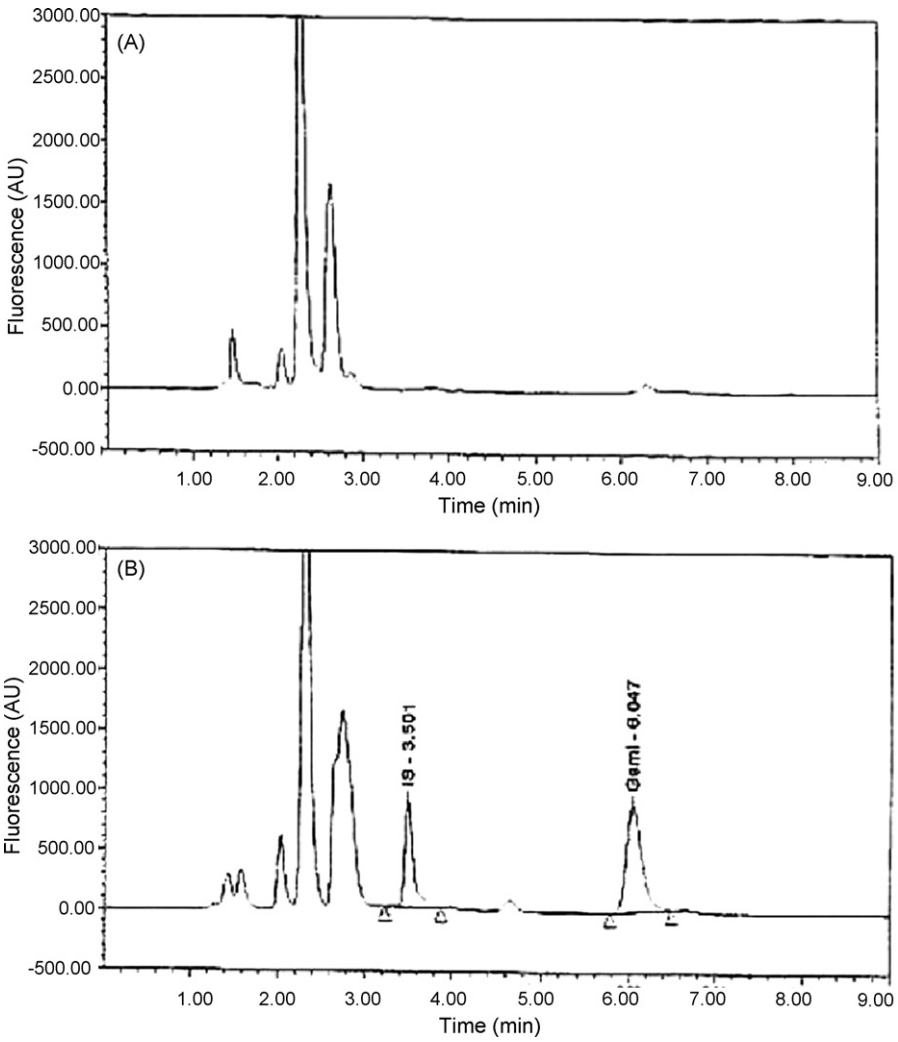

Fig. 3. Chromatogram resulting from (A) the analysis of volunteer sample at zero time, (B) volunteer sample obtained at 1.33 h after oral administration of 320 gemifloxacin tablet.

2.6.7. Ruggedness and robustness

The ruggedness of the HPLC method was evaluated by carrying out the analysis using two different analyst (operator) and different instruments on different days. The robustness of the method is demonstrated by the variation of different condition affecting on chromatographic resolution in the HPLC method were also studied.

3. Results and discussion

3.1. Separation

Fig. 2 shows the representative chromatograms of blank plasma (A), and blank plasma with internal standard and gemifloxacin (B). Fig. 3 shows the chromatograms of volunteer sample after oral administration of 200 mg dose of gemifloxacin Factive® after zero time (A) and after 1.33 h (B). The analytes were well separated from plasma samples after ultrafiltrated under the present chromatographic conditions at retention times of ~3.3 min and ~6.5 min for IS and gemifloxacin respectively. The total run time was ~8 min. The peaks were of good shape, completely resolved one from another at therapeutic concentration of gemifloxacin. Ultra filtration process of plasma sample was sufficient to isolate the gemifloxacin and ciprofloxacin from plasma without any interfering endogenous peaks at 269 nm and 390 nm for excitation and emission, respectively.

3.2. Method validation

The peak area ratio of gemifloxacin to IS in human plasma was linear with respect to the analyte concentration over the range 25–5000 ng mL^{-1}. The mean linear regression equation of calibration curve for the analyte was $y = 0.0013\ (\pm 0.0004)\ x + 0.0491\ (\pm 0.0033)$, where y was the peak area ratio of the analyte to the IS and x was the concentration of the analyte. The correlation coefficient (r) for the gemifloxacin was 0.9988 (±0.003) over the concentration range used. The lower limit of quantification, the lowest concentration of the standard curve which can be measured with acceptable accuracy and precision for the analyte from the normal human plasma was 25 ng mL^{-1}. The lower limit of detection was 10 ng mL^{-1}. The limit of detection and LOQ were determined at 3 and 10 times the base-line noise, respectively following the United States of pharmacopoeia procedure [17]. Table 1 summarizes the back-calculation of gemifloxacin concentration of the calibration standards in human plasma. The precision for the analyte covering the concentration range of 25–5000 ng mL^{-1} ranged from 1.59 to 7.29 % and the relative error was between 1 and 8.0 %.

3.2.1. The precision and accuracy

The precision and accuracy at the LLOQ and at low, medium and high concentration of gemifloxacin in plasma were within the acceptable limits (Table 2). Within- and between days rel-

Table 1
Data of back-calculated gemifloxacin concentration of the calibration standards in human plasma.

Nominal concentration ($ng\,mL^{-1}$)	Mean[a]	Standard deviation (SD)	Precision (%)	Accuracy (%)
25	25.27	1.71	6.76	101.09
50	51.29	3.74	7.29	102.58
150	162.69	5.60	3.44	108.46
300	311.13	7.46	2.00	103.71
600	645.93	12.83	1.99	107.66
1500	1546.86	41.99	2.66	103.12
3000	3173.40	50.44	1.59	105.78
5000	5107.01	168.63	3.30	102.14

[a] Average of six determinations.

ative standard deviation (precision, % CV) were less than 1.59 and 7.29%. Within- and between days relative errors were less than 4.52 and 7.22% respectively. Accuracy was expected as percent error (relative error) [(measured concentration−spiked concentration)/spiked concentration] × 100, while the precision was quantitated by calculating within and between days % CV values (Table 3).

3.2.2. Specificity

There were no interfering peaks present in six different randomly selected samples of drug free human plasma used for analysis at the retention times of either analyte or internal standard (Figs. 2(a) and 3(a)).

3.2.3. Stability

Stock solution of the gemifloxacin and the internal standard were prepared in 20% aqueous methanol. The solution was stable for at least 1 month when stored at −70 °C. Stability of plasma samples were performed as described earlier in the text (Tables 4 and 5 show short and long stability). Four freeze–thaw cycle (Table 6) and 6 h room temperature storage for low and high quality control samples indicated that gemifloxacin was stable in the human plasma under the experimental condition. QC sample were stable

Table 2
Data of intra- and inter-day accuracy and precision of gemifloxacin QC samples.

Day of analysis	Gemifloxacin measured concentration in human plasma				
	LLOQ 25 ($ng\,mL^{-1}$)	QC-1 75 ($ng\,mL^{-1}$)	QC-2 750 ($ng\,mL^{-1}$)	QC-3 2500 ($ng\,mL^{-1}$)	QC-4 4000 ($ng\,mL^{-1}$)
Day 1	28.69	77.82	662.47	2514.04	3864.58
	27.98	75.48	728.81	2281.58	3649.00
	24.32	78.59	718.26	2276.29	4431.40
	30.00	68.93	697.83	2661.21	4368.64
	24.43	74.30	789.04	2520.82	4337.95
	26.30	65.20	729.71	2604.00	4237.96
Day 2	26.83	73.06	720.36	2521.67	4414.21
	22.43	68.16	672.66	2419.56	4475.12
	24.00	78.15	778.56	2661.09	4209.90
	26.51	79.14	764.96	2676.75	4088.15
	26.01	65.32	788.42	2651.59	4289.07
	24.21	86.25	750.17	2373.83	4409.06
Day 3	26.09	76.83	745.15	2491.56	4263.64
	24.86	69.04	744.25	2554.21	3497.33
	27.41	72.12	772.09	2484.39	4168.59
	25.89	71.69	767.80	2626.63	3607.11
	24.42	74.70	743.10	2435.73	3877.38
	24.34	72.52	764.98	2514.35	3706.96
Mean	25.86	73.94	734.55	2514.64	4072.08
Standard deviation (SD)	1.87	7.91	40.78	113.66	293.82
Precision (CV) (%)	7.22	6.64	5.55	4.52	7.22
Accuracy	103.42	98.58	97.94	100.59	101.80

Table 3
Data of relative recovery of gemifloxacin.

Sample no.	QC-1 75 ($ng\,mL^{-1}$)		QC-2 750 ($ng\,mL^{-1}$)		QC-3 2500 ($ng\,mL^{-1}$)		QC-4 4000 ($ng\,mL^{-1}$)	
	Found	R[a] (%)	Found	R (%)	Found	R (%)	Found	R (%)
Mean[b]	77.83	103.78	774.1	103.2	2491.91	99.68	4004.90	100.12
Standard deviation (SD)	2.57	3.43	15.04	2.00	126.82	5.07	154.79	3.87
Precision (CV) (%)	3.3	3.3	1.94	1.94	5.09	5.09	3.86	3.86

[a] Relative recovery.
[b] Average of six determinations.

Table 4
Data of short-term stability for gemifloxacin plasma sample at four QC levels.

Sample no.	QC-1 75 ($ng\,mL^{-1}$)		QC-2 750 ($ng\,mL^{-1}$)		QC-3 2500 ($ng\,mL^{-1}$)		QC-4 4000 ($ng\,mL^{-1}$)	
	Initial	6 h at 25 °C	Initial	6 h at 25 °C	Initial	6 h at 25 °C	Initial	6 h at 25 °C
Mean[a]	74.53	73.67	715.08	721.25	2513.70	2516.85	3972.47	4255.28
Standard deviation (SD)	3.82	4.04	48.38	36.60	93.30	98.65	205.00	92.30
Precision (CV) (%)	5.13	5.48	6.77	4.94	3.71	3.92	5.16	2.17
Accuracy (%)	99.38	98.23	95.34	96.17	100.55	100.67	99.31	106.38
Stability (%)		98.85		100.87		100.13		107.12

[a] Average of six determinations.

Table 5
Data of long-term stability for gemifloxacin plasma sample at four QC levels.

Sample no.	QC-1 75 (ng mL^{-1})			QC-2 750 (ng mL^{-1})		
	Initial	28-days at −70 °C	90-days at −70 °C	Initial	28-days at −70 °C	90-days at −70 °C
Mean[a]	74.53	76.82	76.41	715.08	774.82	752.21
Standard deviation (SD)	3.82	2.13	1.11	48.38	23.65	16.07
Precision (CV) (%)	5.13	2.78	1.45	6.77	3.05	2.14
Accuracy (%)	99.38	102.43	101.88	95.34	103.31	100.29
Stability (%)		103.07	102.52		108.35	105.19
Sample no.	QC-3 2500 (ng mL^{-1})			QC-4 4000 (ng mL^{-1})		
	Initial	28-days at −70 °C	90-days at −70 °C	Initial	28-days at −70 °C	90-days at −70 °C
Mean[a]	2513.70	2522.42	2571.36	3972.47	4053.47	4035.09
Standard deviation (SD)	93.30	59.98	62.50	205.00	192.42	89.03
Precision (CV) (%)	3.71	2.38	2.43	5.16	4.75	2.21
Accuracy (%)	100.55	100.90	102.85	99.31	101.34	100.88
Stability (%)		100.35	102.29		102.04	101.58

[a] Average of six determinations.

Table 6
Data of freeze and thaw stability of gemifloxacin plasma sample at four QC levels.

Sample No.	QC-1 75 (ng mL^{-1})		QC-2 750 (ng mL^{-1})		QC-3 2500 (ng mL^{-1})		QC-4 4000 (ng mL^{-1})	
	Initial	4th cycle	Initial	4th cycle	Initial	4th cycle	Initial	4th cycle
Mean[a]	74.53	73.67	715.08	721.25	2513.70	2516.85	3972.47	4255.28
Standard deviation (SD)	3.82	4.04	48.38	36.60	93.30	98.65	205.00	92.30
Precision (CV) (%)	5.13	5.48	6.77	4.94	3.71	3.92	5.16	2.17
Accuracy (%)	99.38	98.23	95.34	96.17	100.55	100.67	99.31	106.38
Stability (%)		98.85		100.87		100.13		107.12

[a] Average of six determinations

for at least 90 days if stored at approximately −70 °C (Table 5). Auto-injector stability of the samples is also mentioned in Table 4. Results indicate that the samples were stable when kept in the auto-injector for up to 6 h at room temperature (25 °C).

3.2.4. Clinical application

The developed HPLC method has been successfully used to quantification of the gemifloxacin concentration in human plasma samples after oral administration of gemifloxacin. Clinical plasma samples of volunteers were analyzed with own calibration curve and QC samples as one batch in a single analytical run. The standard calibration curve, including blank sample and standard zero samples, was used to determine the sample concentrations in the unknown clinical samples. QC samples (at each of low, medium and high concentrations) were analyzed together with the unknown clinical samples and were allocated judiciously taking into consideration the estimated drug level through the batch, in order to detect any analytical drift.

Fig. 4 shows the plasma concentration time profile of gemifloxacin (ng mL^{-1}) after administrating oral single dose of

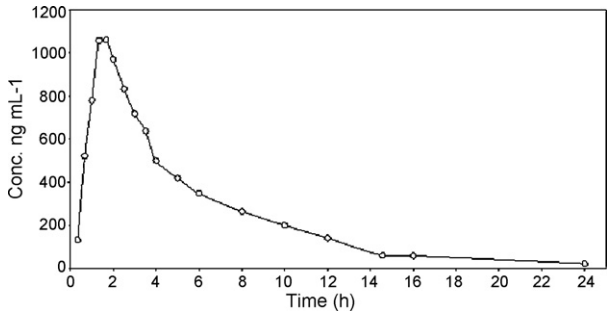

Fig. 4. Mean concentrations–time profile of gemifloxacin following oral administration one Factive® tablet (320 mg) to five health male volunteers.

Table 7
Pharmacokinetic parameters of gemifloxacin in five healthy male adults after oral administration of a single dose (320 mg) of Factive® Tablets.

Parameter[a]	Mean ± SD
$AUC_{0\to t}$ (ng h mL^{-1})	9564 ± 2914
$AUC_{0\to\infty}$ (ng h mL^{-1})	9874 ± 2951
C_{max} (ng mL^{-1})	1746 ± 302
T_{max} (h)	1.396 ± 0.247
K_{el} (h^{-1})	0.129 ± 0.047
$t_{1/2}$ (h^{-1})	6.169 ± 2.220
$AUC_{0\to t}/AUC_{0\to\infty}$	0.967 ± 0.011

[a] $AUC_{0\to t}$, area under the plasma concentration-time curve from time 0 to time t; $AUC_{0\to\infty}$, area under the plasma concentration-time curve from time 0 to infinity; C_{max}, peak plasma concentration; T_{max}, time to peak plasma concentration; K_{el}, elimination rate constant; $t_{1/2}$, elimination half life.

320 mg/tablet to five health adult male subjects under fasting condition. Table 7 shows the calculated pharmacokinetic parameters ($AUC_{0\to t}$, $AUC_{0\to\infty}$, C_{max}, T_{max}, K_{el}, $t_{1/2}$ and AUC $_{0\to t}$/AUC $_{0\to\infty}$) of gemifloxacin in these subjects. Therefore, the present developed HPLC assay method could be successfully applied to the determination of gemifloxacin in several pharmacokinetic studies conducted in any institution. The terminal phase of gemifloxacin in the study was well characterized and the analytical assay was able to detect low concentrations at the end of the plasma concentration- time profile.

4. Conclusion

The developed HPLC method of analysis provided a reliable, reproducible and specific assay for gemifloxacin in human plasma. The method described here is sensitive enough to detect as low as 10 ng mL^{-1}. The validation method allows quantification of gemifloxacin in biological plasma samples for the purpose of bioequivalence study was linear from 25 to 5000 ng mL^{-1}. Compared to previously reported methods, the present assay method assessed

extensive validation parameters as per FDA guideline. The method has shown acceptable precision, accuracy and adequate sensitivity for use in the clinical pharmacokinetic studies and deems to be suitable for use in all laboratories equipped with sophisticated or unsophisticated instruments.

Acknowledgment

The volunteers supported by King Khaled hospital, King Saud University, are greatly appreciated.

References

[1] M.G. Cormican, R.N. Jones, Antimicrob. Agents Chemother. 41 (1997) 204.
[2] A.F. Hohl, R. Frei, V. Pünter, A. Von Graevenitz, C. Knapp, J. Washington, D. Johnson, R.N. Jones, Clin. Microbiol. Infect. 4 (1998) 280.
[3] J.-I. Oh, K.-S. Paek, M.-J. Ahn, M.-Y. Kim, C.Y. Hong, I.-C. Kim, J.-H. Kwak, Antimicrob. Agents Chemother. 40 (1996) 1564.
[4] M.E. Erwin, R.N. Jones, J. Clin. Microbiol. 37 (1999) 279.
[5] D.M. Johnson, R.N. Jones, M.E. Erwin, Diagn. Microbiol. Infect. Dis. 33 (1999) 87.
[6] P. Hannan, G. Woodnutt, Antimicrob. Agents Chemother. 45 (2000) 367.
[7] L.D. Saravolatz, J. Leggett, Clin. Infect. Dis. 37 (2003) 1210.
[8] R.C. Owens Jr., P.G. Ambrose, Med. Clin. North Am. 84 (2000) 1447.
[9] A. Allen, E. Bygate, S. Oliver, M. Johnson, C. Ward, A. Cheon, Y.S. Choo, I.C. Kim, Antimicrob. Agents Chemother. 44 (2000) 1604.
[10] A. Allen, E. Bygate, M. Vousden, S. Oliver, M. Johnson, C. Ward, A.-J. Cheon, Y.S. Choo, I.-C. Kim., Antimicrob. Agents Chemother. 45 (2001) 540.
[11] E. Doyle, S.E. Fowles, D.F. McDonnel, R. McCarthy, S.A. White, J. Chromatogr. B 746 (2000) 191.
[12] R. Bikash, D. Ayan, B. Uttam, K.S. Amlan, B. Anirbandeep, M. Jayanti, S.C. Uday, K.D. Anjan, T.K. Pal, J. Pharm. Biomed. Anal. 52 (2010) 216.
[13] A.R. Rote, S.P. Pingle, J. Chromatogr. B 877 (2009) 3719.
[14] Guidance for Industry: FDA Bioanalytical Method Validation (Guidelines US Dept. of Health and Human Services, Food and Drug Administration (FDA), Centre for Drug Evaluation and Research (CDER) 2001.
[15] Guidelines for Validation of Analytical Procedures: Methodology- step 4 International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 1996.
[16] Notes for Guidance on the Investigation of Bioavailability and Bioequivalence. The European Agency for the Evaluation of Medicinal Products (EMEA) 2001.
[17] The United State Pharmacopoeia, 245th ed., United State pharmacopoeia. Convention, Rockville, MD, 2000, 2150 pp.