Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 850 (2007) 68–73
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

High performance liquid chromatography–electrospray ionization mass spectrometric determination of balofloxacin in human plasma and its pharmacokinetics

Zuwei Bian [a,b], Yuan Tian [a,b], Zunjian Zhang [a,b,*], Fengguo Xu [a,b], Jinheng Li [c], Xiaomei Cao [c]

[a] *Key Laboratory of Drug Quality Control and Pharmacovigilance (China Pharmaceutical University), Ministry of Education, PR China*
[b] *Center for Instrumental Analysis, China Pharmaceutical University, Nanjing 210009, PR China*
[c] *Nanjing Command General Hospital, Nanjing 210002, PR China*

Received 15 February 2006; accepted 1 November 2006
Available online 11 December 2006

Abstract

A selective and sensitive high performance liquid chromatography–electrospray ionisation–mass spectrometry method has been developed for the determination of balofloxacin (BLFX) in human plasma. The sample preparation was a liquid–liquid extraction, and chromatographic separation was achieved with an Agilent ZORBAX 300SB C18 2.1 mm × 150 mm column using a mobile phase comprised of methanol–water (10 mM CH_3COONH_4, pH 3.0) = 40:60 (v/v). Standard curves were linear (r = 0.9992) over the concentration range of 0.03–3 μg/ml and had good accuracy and precision. The within- and between-batch precisions were within 10% relative standard deviation (R.S.D.). The limit of detection (LOD) was 0.02 μg/ml. The validated HPLC–electrospray ionization (ESI)–MS method has been used successfully to study balofloxacin pharmacokinetics in healthy volunteers.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Balofloxacin; HPLC–ESI–MS; Human plasma; Pharmacokinetics

1. Introduction

Balofloxacin (BLFX) is a new fluoroquinolone. In it, a methylaminopiperidine group substitutes the 7 position of the fluoroquinolone ring and the 8 position is modified with a methoxy group. The antibacterial spectrum of balofloxacin is broad, ranging from gram-positive bacteria to gram-negative bacteria. Balofloxacin exhibits excellent antibacterial activity against gram-positive bacteria such as multiple-drug-resistant staphylococci and pneumococci [1–3]. It is metabolized in the kidney. The balofloxacin glucuronide and *N*-desmethyl balofloxacin are the major metabolites [4,5].

Among the literatures available until now, a HPLC with spectrofluorimeter method has been described and applied to study the pharmacokinetics of balofloxacin in animals and human beings [4–7], but it is unstable because there are many factors, such as fluorescence quenching, which could affect fluorescence detection. Mi and Xiao reported a HPLC-UV method for determination of balofloxacin capsule in vitro [8]. Nakashima et al. reported the single and repeated oral administration study of balofloxacin [9,10], but did not refer to the food and gender effect. In consideration of getting full clarification of pharmacokinetic properties of balofloxacin in human, this paper presents a sensitive and selective HPLC–MS method suitable for the determination of balofloxacin in plasma for the safe application of balofloxacin in human.

2. Experimental

2.1. Chemicals and reagents

Balofloxacin tablets and balofloxacin reference standard (99.5% purity) were supplied by X.F Corporation (Heifei, PR China); naphazoline chloride (NAP) reference standard (internal standard, 98.5%) was supplied by the Institute for Drug

* Corresponding author. Tel.: +86 8327 1454; fax: +86 8327 1454.
E-mail address: zunjianzhangcpu@hotmail.com (Z. Zhang).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.11.001

Control of Jiangsu (Nanjing, PR China). HPLC grade methanol was purchased from Merck (Merck Company, Germany). Other chemicals were all of analytical grade and were used as received. Water was purified by redistillation before use.

2.2. Instrumentation and operating conditions

2.2.1. Liquid chromatography

The HPLC system is consisted of a Shimadzu LC-10AD vp pump, a Shimadzu DGU-14AM degasser, a Shimadzu SIL-HTc auto sampler and a Shimadzu CTO-10A vp column oven (Shimadzu, Kyoto, Japan). The column was an Agilent ZORBAX 300SB vp 2.1 mm × 150 mm and was operated at 40 °C. The mobile phase is consisted of methanol:water (10 mM CH_3COONH_4, pH 3.0) = 40:60 (v/v) which was set at a flow rate of 0.2 ml/min.

2.2.2. Mass spectrometry

Mass spectrometric detection was performed using a Shimadzu LCMS-2010A liquid chromatography mass spectrometer with an electrospray ionization (ESI) interface. All measurements were carried out under the positive ESI mode and all analytes were assayed by quantifying the $[M + H]^+$ ions with balofloxacin detected at m/z 390 and IS at m/z 211. The MS operating conditions were optimized as follows: nebulizer gas rate 1.5 l/min, CDL temperature 250 °C, block temperature 200 °C, probe voltage 1.5 kV. The quantification was performed via peak area. Data acquisition and processing were accomplished using Shimadzu LCMS solution Software for LCMS-2010A system.

2.3. Preparation of stock solutions, sample, quality control samples and standard curves

The stock solution of balofloxacin was prepared in 0.05 M HCl and the stock solution of naphazoline chloride was prepared in methanol. Both of them were 1.0 mg/ml and were stored at 4 °C. Working solutions of balofloxacin were prepared daily in redistilled water by appropriate dilution at 1.5, 2.5, 5, 10, 15, 25, 50, 100 and 150 μg/ml. And the stock solution of naphazoline chloride was further diluted with methanol to prepare the working internal standard solution containing 1 μg/ml of naphazoline chloride.

The preparation of plasma was by liquid–liquid extraction. The working internal standard solution (30 μl × 1 μg/ml) and 5 ml dichloromethane-ethyl acetate (20:80, v/v) were added into a 1 ml aliquot of the collected plasma sample from a human volunteer and then were vortexed for 2 min. After centrifuged at 2500 rpm for 10 min, the upper organic phase was transferred to another 10 ml centrifuge tube and evaporated to dryness under a gentle stream of nitrogen gas in water bath at 40 °C. The dried extract was redissolved in 100 μl mobile phase. Then an aliquot of 10 μl was injected into the LC–MS system.

Quality control samples were prepared by spiking different samples of 1 ml plasma, each of which were with proper volume of the corresponding standard solution, to produce a final concentration equivalent to low level (0.05 μg/ml), middle level (0.5 μg/ml) and high level (2 μg/ml) of balofloxacin with 30 ng/ml (30 μl × 1 μg/ml) IS each. The following procedures were the same as described above.

Proper volume of one of the above mentioned working solutions were added into 10 ml centrifuge tubes, with 1 ml aliquot blank plasma, to produce the standard curve points equivalent to 0.03, 0.05, 0.1, 0.2, 0.3, 0.5, 1.0, 2.0 and 3.0 μg/ml of balofloxacin. The following assay procedures were the same as describe above. In each run, a blank plasma sample (no IS) was also analyzed.

2.4. Method validation

2.4.1. Assay specificity

The healthy human blank plasma used for the preparation of the blank sample was obtained from six different people. Each blank sample was tested for the visible interference. When using LC–MS for analysis, a matrix effect by ionization competition between the analytes and co-eluents may possibly exist. To evaluate the matrix effect in the experiment, three different concentration levels of balofloxacin (0.05, 0.5 and 2 μg/ml) were added into the dried extracts of 1 ml blank sample, respectively, and then were dried and dissolved with 100 μl mobile phase. The neat standards at the same concentrations were dried directly and dissolved with the same volume of the mobile phase. The matrix effect of internal standard (30 ng/ml) was evaluated by the same method.

2.4.2. Linearity

The linearity of the standard curves of nine concentrations was evaluated through least squares linear regression analysis of peak area ratios of balofloxacin/IS versus balofloxacin concentrations in spiked plasma samples. Blank plasma samples were analyzed only to ensure the lack of interferences, but not to construct the calibration function. The lower limit of detection (LLOD) and the lower limit of quantification (LLOQ) were determined as the concentrations with a signal-to-noise ratio of 5 and 10, respectively, and the latter should be identifiable, discrete and reproducible with a precision correspondence to max 20% relative standard deviation (R.S.D.).

2.4.3. Precision and accuracy

Precision and accuracy were assessed by determining QC samples at three concentration levels (0.05, 0.5 and 2 μg/ml). Within-batch precision and accuracy was determined by analyzing the group of samples on 1 day ($n = 5$ parallel samples per level), while between-batch precision and accuracy was on determined three consecutive days ($n = 5$ per day and total $n = 15$ per level). The concentration of each sample was determined using standard curve prepared and analyzed on the same day.

2.4.4. Extraction recovery and stability

Recovery experiments of balofloxacin were performed by comparing the analytical results of extracted samples (balofloxacin/IS peak area ratios) with unextracted standards (balofloxacin/IS peak area ratios) at the same three concentrations.

2.4.4.1. Freeze and thaw stability. The stability was assessed at three concentrations (0.05, 0.5 and 2 μg/ml), and these QC plasma samples were stored at the storage temperature (−20 °C) for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze–thaw cycles were repeated twice, then the samples were tested after three freeze (−20 °C)–thaw (room temperature) cycles.

2.4.4.2. Short-term stability. Three concentration levels of QC plasma samples were kept at room temperature for a period that exceeded the routine preparation time of samples (around 6 h).

2.4.4.3. Long-term stability. Three concentration levels of QC plasma samples kept at low temperature (−20 °C) were studied for a period of 2 weeks.

2.4.4.4. Post-preparative stability. The auto sampler stability was conducted reanalyzing extracted QC samples kept under the auto sampler conditions (4 °C) for 12 h.

2.4.5. Standard curve and quality control sample in each batch

A standard curve in each analytical run was used to calculate the concentration of balofloxacin in the unknown samples in each run. It was prepared at the same time as the unknown samples in the same batch and was analyzed in the middle of the run.

The QC samples in five duplicates at three concentrations (0.05, 0.5 and 2 μg/ml), were prepared and were analyzed with processed test samples at intervals per batch.

2.5. Pharmacokinetic study and statistical analysis

To demonstrate the reliability of this method for clinical studies, it was applied to determine balofloxacin concentrations in plasma samples of healthy volunteers. There were 12 volunteers participating in the single- and multiple-dose experiment and another 12 volunteers participating in the food effect experiment with six males and six females, respectively. The age of the first and the later 12 healthy volunteers ranged from 21 to 28 years (23 ± 2.0 years) and 21 to 27 years (23.5 ± 1.7 years), and the weight ranged from 55 to 78 kg (64 ± 7.3 kg) and 50–70 kg (59.4 ± 6.2 kg), respectively. All volunteers gave written informed consent to participate in the study. The volunteers were asked to stop taking any medicines at least 2 weeks before the study.

In the single-dose study, each volunteer received an oral administration of balofloxacin after overnight fasting. Then serial blood samples were collected from vein at 0, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, 6, 9, 12, 24 and 36 h post-dose. The multiple-dose study started after the single-dose study with a week washout. In the multiple-dose study, the same 12 volunteers received an oral administration of 100 mg of balofloxacin twice daily (at 7:00 and 19:00) until the sixth day's morning and received once on the sixth day's morning. Venous blood samples were obtained at 72, 96, 120, 120.17, 120.33, 120.5, 120.75, 121, 121.5, 122, 124, 126, 129, 132, 144 and 156 h post-dose. In the food effect experiment, after overnight fasting, another 12 volunteers randomized to be fed consumed a standard food in 0.5 h. Then doses of 100 mg of balofloxacin were administered and blood samples were collected at the same time points as the single-dose study. Each plasma sample was immediately separated by centrifugation at 2000 × g for 10 min to separate the plasma fractions and was stored frozen at −20 °C until analysis.

The pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}) of balofloxacin after single- and multiple-dose administration were assessed utilizing a two-tailed unpaired t-test on the ln-transformed data. Unpaired t-test was also used to compare the pharmacokinetic parameters based on the ln-transformed data between different genders after the single- and multiple-dose oral administration of balofloxacin and the study of food effect. A value of $p < 0.05$ was considered to be statistically significant.

3. Result and discussion

3.1. Selection of IS

It is necessary to use an internal standard to get high accuracy when HPLC is equipped with MS as the detector. Naphazoline chloride was adopted in the end because of its similar retention action, stable peak response and extraction efficiency. The structures of the ionized (protonated) forms of balofloxacin and naphazoline chloride are shown in Fig. 1.

3.2. Chromatography

3.2.1. Extract conditions

Ethyl acetate, dichloromethane-ethyl acetate (20:80, v/v) and n-hexane-isopropanol (95:5, v/v) were all attempted as the extract solutions, and dichloromethane-ethyl acetate (20:80, v/v) was finally adopted because of its high extraction efficiency and less interference. Extractions with 1 M HCl (100 μl), 1 M NaOH (100 μl), or none were tried. And it was found that there was less interference when extraction without the addition of HCl or NaOH.

3.2.2. Selection of mobile phase

NH_4Ac (10 mM, pH 6.65) and NH_4Ac (10 mM, pH 3.0) were both attempted as the water phase, and NH_4Ac (10 mM, pH 3.0) was chosen finally for the symmetry chromatographic peak shape and larger peak area of the analytes.

3.3. Mass spectra

Positive electrospray ionization mass scan spectra of balofloxacin and IS were shown in Fig. 1, respectively. According to the mass scan spectrum, m/z 390 produced by the quasimolecule ion $[M+H]^+$ of balofloxacin and m/z 211 produced by the quasimolecule ion $[M+H]^+$ of naphazoline were selected for monitoring.

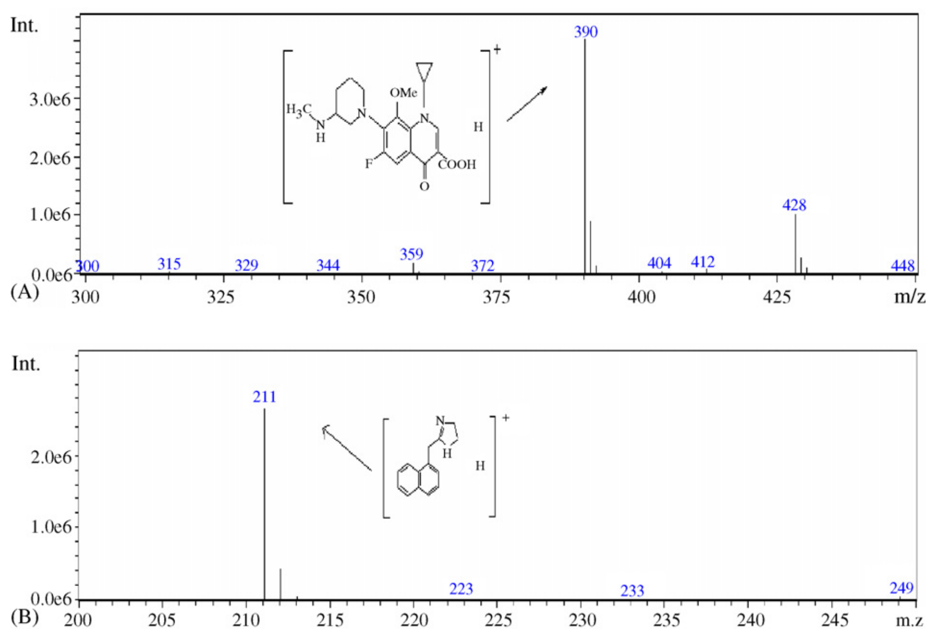

Fig. 1. Positive ion electrospray mass scan spectrum of: balofloxacin (A) and naphazoline chloride (B).

The SIM (+) chromatograms extracted from supplemented plasma are depicted in Fig. 2C. As shown, the retention times of balofloxacin and the IS were 3.6 and 2.8 min, respectively. The total HPLC–MS analysis time was 5 min per sample. A representative chromatogram of a plasma sample obtained at 1.5 h from a subject who received a single oral dose (100 mg) is shown in Fig. 3.

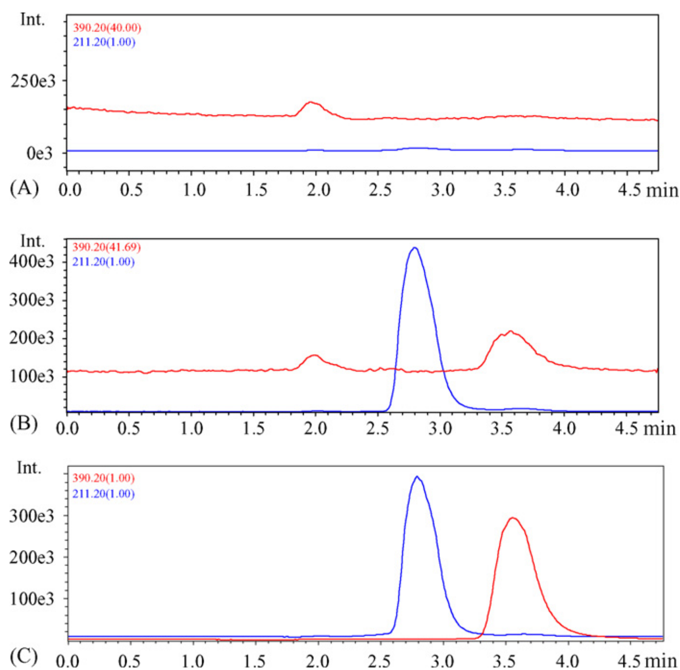

Fig. 2. The SIM (+) chromatograms of balofloxacin and naphazoline chloride. The retention times of balofloxacin and the IS were 3.6 and 2.8 min, respectively. (A) Blank plasma. (B) LLOQ (concentration of balofloxacin = 0.03 μg/ml). (C) Supplemented plasma (concentration of balofloxacin = 1 μg/ml). In (A) and (B), the baselines of sample are lifted up to make it more readable.

3.4. *Method validation*

3.4.1. *Assay specificity*

No interference of the analytes was observed. Fig. 2A shows a HPLC chromatogram for a blank plasma sample indicating no endogenous peaks at the retention positions of balofloxacin or IS. All the ratios of the peak area resolved in blank sample compared with that resolved in mobile phase are between 85 and 115%, which means no matrix effect for balofloxacin and IS in this method.

3.4.2. *Linearity and LLOQ*

The linear regressions of the peak area ratios of balofloxacin/IS versus concentrations were fitted over the concentration range of 0.03–3 μg/ml in human plasma. The mean standard curve was typically described by the least square equation: $C = 2.26 \times R + 0.00772$ ($r = 0.9992$), where R corresponds to the peak area ratio of balofloxacin to the IS and C refers to the concentration of balofloxacin added to plasma. The data shows the good linearity, and the precision and accuracy meet the requirement.

The LLOQ of balofloxacin was proved to be 0.03 μg/ml with an accuracy of 102.36 ± 2.14% and a precision of 10.27% in

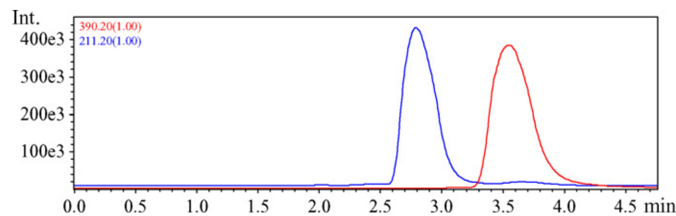

Fig. 3. The SIM (+) chromatogram of plasma sample of a healthy volunteer. The retention times of balofloxacin and IS were 3.6 and 2.8 min, respectively. The concentration of balofloxacin was 2.64 μg/ml.

Table 1
The within- and between-batch precision, accuracy of the method for determination of balofloxacin (within-batch: $n = 5$; between-batch: $n = 5$ series per day)

Added concentration (μg/ml)	Within-batch			Between-batch		
	Detected concentration (mean ± S.D., μg/ml)	Mean accuracy (%)	R.S.D. (%)	Detected concentration (mean ± S.D., μg/ml)	Mean accuracy (%)	R.S.D. (%)
0.05	0.047 ± 0.004	94.31	7.78	0.048 ± 0.004	95.21	8.62
0.5	0.47 ± 0.02	94.69	4.67	0.49 ± 0.03	98.15	5.57
2	1.89 ± 0.06	94.26	3.15	1.95 ± 0.09	97.41	4.50

terms of R.S.D. ($n = 5$), and the LLOD was 0.02 μg/ml. Fig. 2B shows the chromatogram of an extracted sample that contained 0.03 μg/ml (LLOQ) of balofloxacin.

3.4.3. Precision and accuracy

Data for within- and between-batch precision and accuracy of the method for balofloxacin are presented in Table 1. The accuracy deviation is within 10% of the actual values. The precision determined at each concentration level does not exceed 10% of the relative standard deviation. The results revealed good precision and accuracy.

3.4.4. Extraction recovery and stability

The extraction recovery determined for balofloxacin was shown to be consistent, precise and reproducible. The recoveries of balofloxacin at three concentration levels ($n = 5$) were determined to be 78.49 ± 6.17, 80.19 ± 4.67 and 81.44 ± 2.58%, respectively.

All the results showed the stability behavior during these tests. The results of freeze and thaw stability indicated that the analytes were stable in human plasma for three cycles of freeze and thaw, when stored at −20 °C and thawed to room temperature. Short-term stability indicated reliable stability behavior under the experimental conditions of the regular runs. The findings from long-term test indicate that the storage of balofloxacin plasma samples at −20 °C is adequate when stored for 2 weeks and no stability-related problems would be expected during the samples routine analysis for the pharmacokinetic studies. The post-preparative stability of QC samples shows that balofloxacin was stable when kept at 4 °C in the auto sampler for 12 h.

3.5. Pharmacokinetic study

3.5.1. Single-dose study

Mean plasma concentration–time curves of balofloxacin following single oral doses of 100, 200 and 300 mg in 12 healthy volunteers are shown, respectively, in Fig. 4. Kinetic parameters could be calculated from Table 3 by the creation of the average from male and female data. The data indicated that C_{max} and AUC increased with dose. And other pharmacokinetic parameters, such as $t_{1/2}$, T_{max} and MRT, are of no significant difference between the doses of 100, 200 and 300 mg. Balofloxacin is absorbed and eliminated independently to the dose.

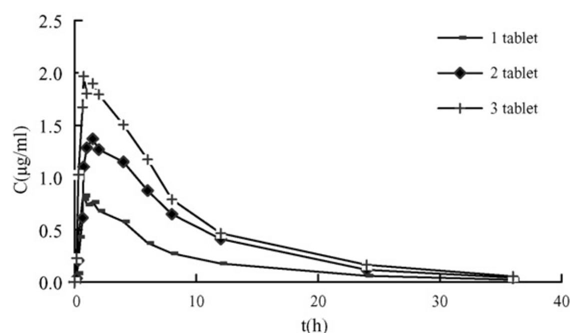

Fig. 4. Mean drug plasma concentration–time curves of balofloxacin in 12 volunteers after oral administration of balofloxacin in single-dose study.1 tablet: 100 mg; 2 tablet: 200 mg; 3 tablet: 300 mg.

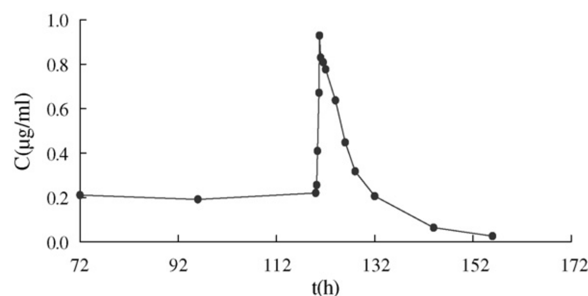

Fig. 5. Mean drug plasma concentration–time curve of balofloxacin in 12 volunteers after oral administration of balofloxacin in multiple-dose study.

3.5.2. Multiple-dose study

Mean plasma concentration–time data obtained at steady state after multiple oral doses of balofloxacin in the same 12 healthy volunteers are depicted in Fig. 5. Kinetic parameters could be calculated from Table 3 by creation of the average from male and female data. The mean steady state C_{max} and AUC_{ss} were smaller than the C_{max} and AUC_{0-t} after a

Table 2
Pharmacokinetic parameters of 12 healthy volunteers after oral administration of balofloxacin in food effect study

Parameters	Food intake condition	Fasting condition
$T_{1/2}$ (h)	7.72 ± 1.20	7.89 ± 1.68
T_{max} (h)	3.04 ± 3.04	1.23 ± 0.99
C_{max} (μg/ml)	0.64 ± 0.21	1.07 ± 0.38
AUC_{0-36} (μg h/ml)	5.32 ± 0.90	6.31 ± 1.38
$AUC_{0-\infty}$ (μg h/ml)	5.53 ± 0.94	6.56 ± 1.43
MRT (h)	9.11 ± 0.97	8.28 ± 0.80
Ka (1/h)	3.38 ± 3.98	7.26 ± 6.60
Ke (1/h)	0.18 ± 0.05	0.22 ± 0.07

Table 3
Kinetic parameters about single and multiple-dose study in male and female volunteers

Parameters	Single-dose study						Multiple-dose study	
	100 mg		200 mg		300 mg		Male	Female
	Male	Female	Male	Female	Male	Female		
$T_{1/2}$ (h)	7.57 ± 0.55	6.94 ± 1.28	9.24 ± 3.45	6.65 ± 0.75	6.66 ± 1.82	7.16 ± 2.76	7.67 ± 1.04	7.02 ± 0.85
T_{max} (h)	0.90 ± 0.49	2.04 ± 1.08	1.58 ± 1.23	1.92 ± 1.11	1.83 ± 1.20	1.46 ± 1.28	0.92 ± 0.30	1.63 ± 0.59
C_{max} (μg/ml)	1.39 ± 0.89	0.89 ± 0.22	2.09 ± 0.57	1.46 ± 0.57	2.31 ± 0.46	2.73 ± 0.34	1.05 ± 0.42	0.99 ± 0.27
AUC_{0-36} (μg h/ml)	7.17 ± 2.01	6.69 ± 1.17	12.57 ± 3.00	15.58 ± 6.91	18.08 ± 5.02	19.12 ± 4.92	–	–
$AUC_{0-\infty}$ (μg h/ml)	7.41 ± 2.04	6.86 ± 12.4	13.21 ± 3.11	15.91 ± 7.01	18.66 ± 5.28	19.67 ± 5.00	–	–
MRT (h)	8.24 ± 0.99	8.18 ± 0.99	8.40 ± 1.50	8.78 ± 0.49	8.33 ± 1.56	8.06 ± 1.54	8.60 ± 1.09	8.01 ± 0.55
Ka (1/h)	12.3 ± 11.22	2.35 ± 1.34	4.90 ± 3.03	5.53 ± 9.78	4.87 ± 6.08	8.99 ± 9.18	–	–
Ke (1/h)	0.34 ± 0.32	0.18 ± 0.05	0.23 ± 0.09	0.12 ± 0.03	0.17 ± 0.04	0.19 ± 0.09	0.16 ± 0.06	0.16 ± 0.02
C_{min} (μg/ml)	–	–	–	–	–	–	0.23 ± 0.11	0.22 ± 0.10
C_{av} (μg/ml)	–	–	–	–	–	–	0.41 ± 0.14	0.53 ± 0.20
AUC_{ss} (μg × h/ml)	–	–	–	–	–	–	4.98 ± 1.69	6.40 ± 2.43
DF	–	–	–	–	–	–	2.05 ± 0.72	1.50 ± 0.29

single-dose of 100 mg, but there is no significant difference between them ($p > 0.05$). And the pharmacokinetic parameters, such as T_{max} and MRT, obtained at steady state, were similar to those obtained after a single-dose of 100 mg.

3.5.3. Food effect

Mean plasma concentration–time curves of balofloxacin of the food effect experiment are shown in Fig. 6. Kinetic parameters are listed in Table 2. From the data, we could find that, C_{max} obtained after a standard food intake are significantly lower ($p < 0.05$); than obtained under fasting conditions. Although AUC_{0-t} and $AUC_{0-\infty}$ under food intake conditions are lower than they are under fasting conditions, there is no significant difference between them ($p > 0.05$), maybe the small sample size cause this result. Otherwise, T_{max} is delayed for about 2 h after food intake compared with fasting conditions. But there is little difference about $t_{1/2}$, MRT and Ke between the two conditions. This means that, taking food will affect the rate and the degree of absorption of balofloxacin significantly, but will not affect the rate of elimination of it.

3.5.4. Gender effect

Kinetic parameters of the single- and multiple-dose study in male and female volunteers are listed in Table 3, respectively.

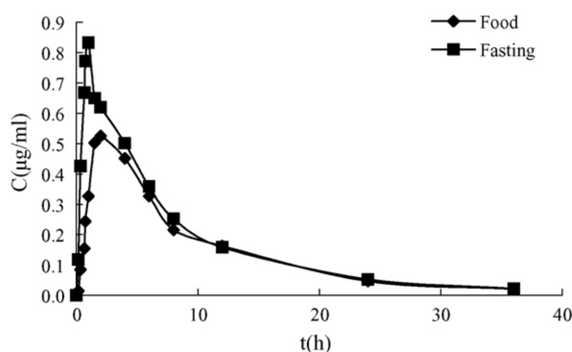

Fig. 6. Mean plasma concentration–time curves of balofloxacin of the food effect experiment.

Although C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ are a little higher or lower in men than those in women, these parameters have no statistically significant difference ($p > 0.05$) between men and women.

4. Conclusion

A sensitive, selective, accurate and precise HPLC method with selected ion monitoring by single quadrupole mass spectrometer with ESI interface was developed and validated for determination of balofloxacin in human plasma. The method has significant advantage over the other techniques used for the plasma sample analysis. The major advantages of this method are the rapidity of separation and the efficiency of analyzing the plasma sample. In these studies, more than 200 samples were analyzed per day, which makes the method suitable for the analysis of large sample batches. This method may be fully recommended for pharmacokinetic studies as well as for therapeutic drug monitoring.

References

[1] K. Marutani, M. Matsumoto, Y. Otabe, et al., Antimicrob. Agents Chemother. 37 (1993) 2217.
[2] Y. Gohara, S. Arai, A. Akashi, et al., Antimicrob. Agents Chemother. 37 (1993) 1826.
[3] M. Matsumoto, K. Kojima, H. Nagano, et al., Antimicrob. Agents Chemother. 36 (1992) 1715.
[4] T. Nakagawa, M. Ishigai, Y. Hiramatsu, et al., Arzneimittelforschung 45 (6) (1995) 716.
[5] T. Nakagawa, M. Ishigai, Y. Hiramatsu, et al., Arzneimittelforschung 45 (6) (1995) 719.
[6] D.W. Wang, L. Xie, J. Wang, X.D. Liu, J. China Pharm. Univ. 35 (2004) 160.
[7] O. Kozawa, T. Uematsu, H. Matsuno, M. Niwa, S. Nagashima, M. Kanamaru, Antimicrob. Agents Chemother. 40 (1996) 2824.
[8] Z.Y. Mi, W.H. Xiao, Chin. J. Spectrosc. Lab. 22 (2005) 186.
[9] M. Nakashima, T. Uematsu, M. Fukuchi, et al., Jpn. J. Chemother. 43 (s-5) (1995) 115.
[10] M. Nakashima, T. Uematsu, M. Fukuchi, et al., Jpn. J. Chemother. 43 (s-5) (1995) 141.