Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 873–877

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Determination of zabofloxacin in rat plasma by liquid chromatography with mass spectrometry and its application to pharmacokinetic study

Hyo-Eon Jin [a], Kyeong-Ryoon Lee [b], In-Hyul Kang [a], Suk-Jae Chung [b], Chang-Koo Shim [a,b,*]

[a] National Research Laboratory for Transporters Targeted Drug Design, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea
[b] Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea

ARTICLE INFO

Article history:
Received 27 July 2010
Received in revised form 30 October 2010
Accepted 2 November 2010
Available online 9 November 2010

Keywords:
Zabofloxacin
LC–MS
Rat
Pharmacokinetics
Plasma

ABSTRACT

The objective of the present study was to develop a rapid and sensitive method for the determination of zabofloxacin, a novel, broad-spectrum fluoroquinolone antibiotic, in rat plasma. Rat plasma samples were deproteinized with methanol, and then were injected into an LC–MS system for quantification. Zabofloxacin and enrofloxacin, which served as an internal standard, were analyzed by selected ion monitoring (SIM) at *m*/*z* transitions of 402 for zabofloxacin and 360 for the internal standard. The lower limit of quantification (LLOQ) was determined to be 10 ng/mL, with acceptable linearity ranging from 10 to 5000 ng/mL ($R > 0.999$). The validation parameters for zabofloxacin, such as absolute matrix effect (107.7–116.0%), accuracy (92.5–101.1% for intra-day and 90.3–103.8% for inter-day), precision (7.7–10.2% for intra-day and 4.2–8.9% for inter-day), and stability in rat plasma (96.0–101.8%), were found to be acceptable according to the assay validation guidelines of the FDA (2001). Following oral administration of zabofloxacin to rats at a dose of 20 mg/kg, the concentration of zabofloxacin in plasma was quantifiable in plasma samples collected up to 8 h following zabofloxacin administration. The method described in the present study is applicable to routine pharmacokinetic studies in rats.

1. Introduction

Fluoroquinolones are a class of antibiotics that are of great significance in the field of antibacterial chemotherapy due to their remarkably broad spectrum of activity [1]. However, as the use of fluoroquinolones has increased, a continual increase in bacterial resistance to these drugs has been widely recognized [2,3]. Therefore, the development of new antibacterial substances that are effective even in drug-resistant bacteria appears to be a matter of utmost importance. Zabofloxacin (DW-224a, Fig. 1) is a novel fluoroquinolone that was developed by Dong Wha Pharmaceuticals (Seoul, Korea). It is a potent and selective inhibitor of the essential bacterial type II topoisomerases (DNA gyrase) and topoisomerase IV, which are involved in DNA replication and metabolism [4]. Zabofloxacin has broad-spectrum antibacterial activity, with enhanced activity against gram-positive and gram-negative organisms, particularly against *Streptococcus pneumoniae*, including pneumococci strains that are resistant to other fluoroquinolones [5]. The overall antibacterial activity of zabofloxacin against gram-positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin resistant coagulase-negative staphylococci, *S. pneumoniae*, *S. pyogenes*, and *Enterococcus faecalis*, was more potent than the activity of reference compounds, such as ciprofloxacin, moxifloxacin, and gemifloxacin [6]. Therefore, zabofloxacin, with its expanded anti-pneumococcal activity, is expected to be very useful for the treatment of community-acquired respiratory tract infections, urinary tract infections, septicemia, systemic infections, skin and soft tissue infections, bacteremia, otitis media, and possibly endocarditis [7–9]. Although zabofloxacin has much clinical utility, a quantitative method for biological samples and pharmacokinetics is not well known. The objective of the present study, therefore, was to develop and validate a method to assay zabofloxacin in rat plasma that is applicable to routine pharmacokinetic studies in rats using LC–MS equipped with an electro-spray ionization (ESI) mode.

2. Experimental

2.1. Chemicals

Zabofloxacin hydrochloride (99.0% purity, Batch No. ZF-07-001) was provided by Dong Wha Pharmaceuticals Co. (Seoul, Korea). Enrofloxacin (purity ≥ 98%), which served as the internal standard (IS) in this assay, was purchased from Sigma–Aldrich (St.

* Corresponding author at: National Research Laboratory for Transporters Targeted Drug Design, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, 599 Gwanangno, Gwanak-gu, Seoul 151-742, Republic of Korea. Tel.: +82 2 880 7873; fax: +82 2 888 5969.
E-mail address: shimck@snu.ac.kr (C.-K. Shim).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.11.001

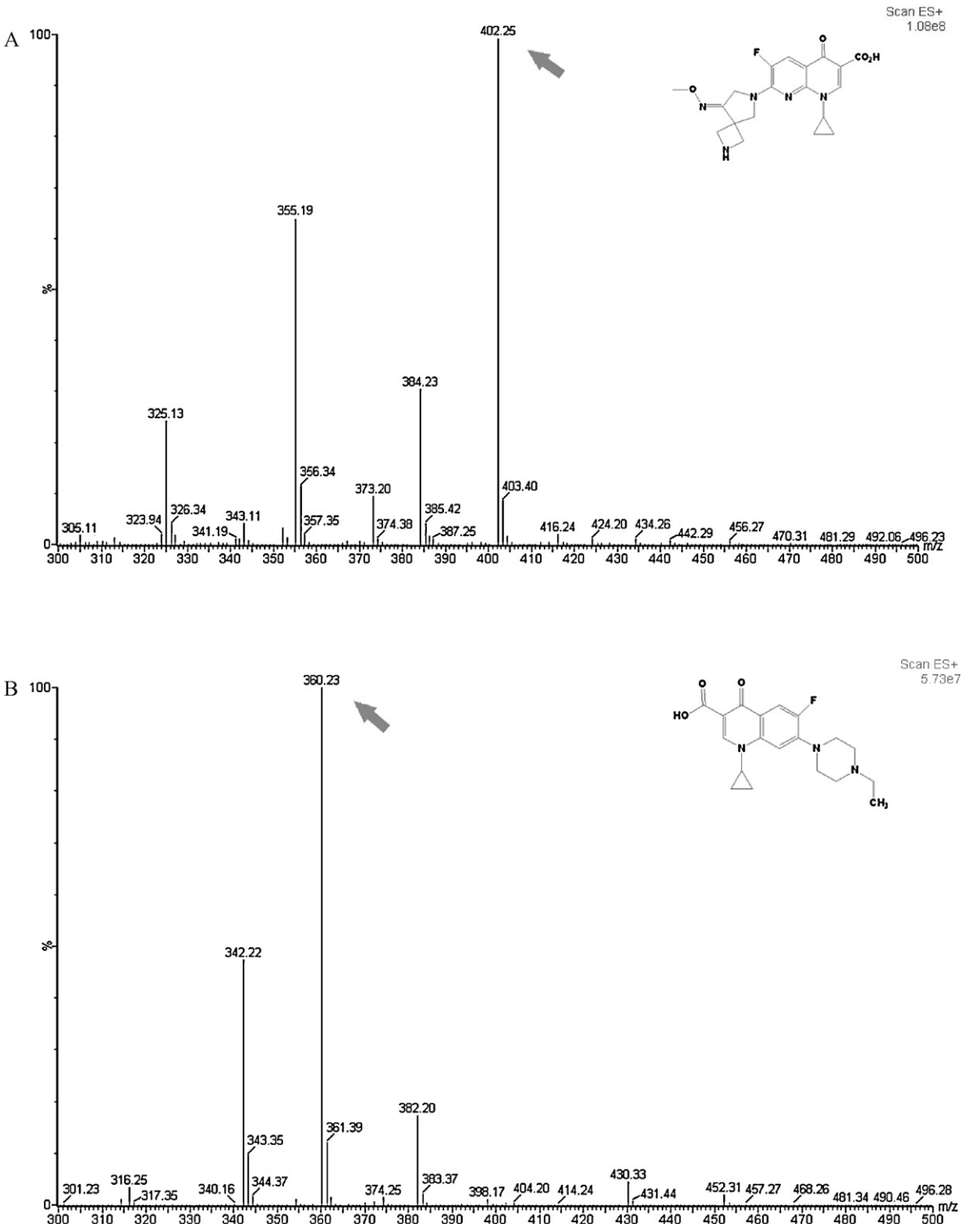

Fig. 1. Full scan mass spectra of (A) zabofloxacin (Mw 401.5) and (B) enrofloxacin (Mw 359.4).

Louis, MO, USA). Acetonitrile, methanol, and formic acid were purchased from Fischer Scientific (NJ, USA). All solvents were HPLC grade.

2.2. Standards and quality control (QC) samples

Stock solutions (0.5 mg/mL for zabofloxacin and 1 mg/mL for enrofloxacin) were prepared by dissolving zabofloxacin and enrofloxacin (IS) in methanol. Standard solutions were then prepared by serial dilution of the stock solution with methanol to obtain the desired concentration range. Plasma standards of zabofloxacin were prepared by mixing 50 μL of each standard solution with 50 μL of blank rat plasma in Eppendorf tubes to give final zabofloxacin concentrations of 10, 20, 50, 100, 500, 1000, and 5000 ng/mL in plasma. An aliquot (50 μL) of the IS standard solution (5 μg/mL) was then added to each plasma standard and mixed by vortexing. Zabofloxaxcin concentrations of 50, 500, and 5000 ng/mL and enrofloxacin

concentrations of 500 and 5000 ng/mL served as QC samples.

2.3. Chromatographic and mass spectrometer operating conditions

The LC/MS system consisted of a Waters Alliance 2695 high-performance liquid chromatographic system (Waters, USA) and a Waters micromass ZQ selective detector. The separation of zabofloxacin and the IS from endogenous substances was performed on a Gemini LC/MS column [150 mm × 3 mm, 5 μm (Phenomenex, USA)] using an automatic injector (Waters Alliance 2695) and a SecurityGuard™ [4.0 mm × 3.0 mm, Phenomenex, USA].

The mobile phase, which was comprised of 0.1% formic acid and acetonitrile (80:20, v/v), was delivered at a flow rate of 0.2 mL/min. The MS was operated using an electro-spray ionization (ESI) source in the positive-ion mode. The voltages of the capillary, cone, extractor, and radio frequency (RF) lens were set at 3.80 kV, 43.00 V, 2.00 V, and 0.0 V, respectively. The temperatures of the source and desolvation were 120 and 150 °C, respectively. Gas flow rates were 50 and 250 L/h for the cone and desolvation, respectively.

2.4. Assay validation

2.4.1. Selectivity, sensitivity and linearity

Interference by endogenous compounds was assessed by comparison of the chromatograms for blank plasma, plasma spiked with the IS only and plasma spiked with zabofloxacin.

The limit of quantification (LOQ) was defined as the concentration with a precision of less than 20% of the relative standard deviation (RSD) and an accuracy between 80 and 120% of the theoretical value. The signal-to-noise ratio for the LOQ sample was confirmed to be not less than 10 [10].

The linearity of the assay was assessed using the zabofloxacin plasma standards over a concentration range of 10–5000 ng/mL. Calibration curves were constructed using the ratios of the zabofloxacin peak area to that of the IS in the plasma standards. The linearity of the calibration curve was determined using linear regression analysis [10].

2.4.2. Precision and accuracy

The precision and accuracy of the assay were evaluated using the RSD of the intra- and inter-variation (i.e., precision) and the deviation from the theoretical concentration (i.e., accuracy) following the assay of multiple batches of QC samples.

2.4.3. Matrix effect

The absolute and relative matrix effects of zabofloxacin and IS were assessed by analyzing 2 sets of standards. To determine the absolute matrix effect for zabofloxacin and IS, blank plasma obtained from three different rats were deproteinized, and zabofloxacin and IS were added to the post-deproteinization sample to reach each of the concentration levels (set 2). The mean peak areas of the analyte were compared with the mean peak areas from the neat solutions of the analyte in methanol (set 1). The relative matrix effect was determined by variability and was expressed as precision (CV, %) (set 2) [11].

2.4.4. Stability

Triplicate sets of zabofloxacin and IS QC samples in methanol were prepared and assayed, as described above, following storage at room temperature for 6 h or in a refrigerator at 4 °C for 1 week. The results obtained for the stored samples were compared with those of freshly prepared samples. The effects of various storage/handling conditions on zabofloxacin were also examined. QC samples (i.e., 50, 500, and 5000 ng/mL of zabofloxacin in rat plasma) were assayed after three freeze–thaw cycles. The short- and long-term stability of the QC samples were also examined.

2.5. Application to pharmacokinetic studies of zabofloxacin

The applicability of the developed assay to pharmacokinetic studies of zabofloxacin in rats was examined. All animal experiments were performed in accordance with the Guidelines for Animal Care and Use, Seoul National University. Male Sprague–Dawley rats (240–270 g, Orient Bio Inc., Seong-Nam, Korea) were anesthetized with ketamine (45 mg/kg) and acepromazine (5 mg/kg), and the femoral artery and vein were cannulated with a polyethylene tube (PE-50; Clay Adams, Parsippany, NJ, USA) filled with heparinized saline (20 IU/mL). Rats ($n = 4$) were orally administered zabofloxacin at a dose of 20 mg/kg. Blood samples (120 μL) were taken from the femoral artery cannula at 0, 2, 5, 10, 15, 30, 60, 90, 120, 240, 360, and 480 min after zabofloxacin administration. The plasma was separated by centrifugation of whole blood at 12,000 rpm for 5 min.

The plasma samples were processed as described above in Section 2.2. The peak concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from individual zabofloxacin plasma concentration–time profiles. The area under the plasma concentration–time curve (AUC_{0-t}) was calculated using the linear trapezoidal method from 0 to 8 h. The area under the plasma concentration–time curve from zero to time infinity ($AUC_{0-\infty}$) was calculated using the trapezoidal extrapolation method [12]. In the extrapolation, the area from the last datum point to time infinity was estimated by dividing the last measured plasma by the terminal-phase rate constant. Standard methods [12] were used to calculate the terminal half-life ($T_{1/2}$) and the mean residence time (MRT) using a non-compartmental analysis program (WinNonlin® 3.1, Pharsight Co., Mountain View, CA, USA). All data are presented as mean ± S.D.

3. Results and discussion

3.1. Chromatography

To determine the primary ion species for zabofloxacin and enrofloxacin (IS), full mass spectra were obtained (Fig. 1) using the corresponding standards. The spectra indicated that the primary ion for zabofloxacin was m/z 402. By comparison, the primary ion for the IS was m/z 360. Therefore, these primary ions for the compounds were used in the subsequent analysis.

3.2. Specificity, sensitivity and linearity

After optimizing the chromatographic separation conditions, the chromatogram for a blank rat plasma sample showed no interfering peaks for the retention times of the analyte and the IS. The retention times for zabofloxacin and the IS were determined to be 3.18 and 4.59 min, respectively, indicating that the analysis can be completed within 8 min. The peak shapes for the drug and IS were symmetrical.

The limit of quantification (LOQ) was determined as the analyte concentrations with an accuracy between 80 and 120% and a percent RSD (i.e., precision) of less than 20% [10]. Based on the estimated precision/accuracy, the limit of quantification for this assay was set at 10 ng/mL (Table 1). The signal-to-noise ratio was not less than 10 for the LOQ sample [10].

The calibration curves for zabofloxacin in rat plasma were found to be linear over a concentration range of 10–5000 ng/mL. The calibration equation for zabofloxacin was ($n = 3$, the slopes and intercepts in the mean ± SD) $y = (0.097 \pm 0.012)x + (0.00037 \pm 0.00051)$

Table 1
Precision and accuracy for zabofloxacin in rat plasma ($n = 5$).

Batch	Theoretical concentration (ng/mL)			
	LLOQ	LQC	MQC	HQC
	10	50	500	5000
(A) Intra-day accuracy and precision				
Mean estimated concentration (ng/mL)	9.5	46.2	505.7	4943.4
Precision (CV, %)[a]	7.9	7.7	10.2	8.9
Accuracy (%)[b]	95.0	92.5	101.1	98.9
(B) Inter-day accuracy and precision				
Mean estimated concentration (ng/mL)	9.7	46.1	518.9	5221.6
Precision (CV, %)[a]	8.9	8.8	4.2	4.4
Accuracy (%)[b]	90.3	92.2	103.8	102.8

[a] Precision (CV, %) = standard deviation of the concentration/mean concentration × 100.
[b] Accuracy (%) = (calculated concentration − theoretical concentration)/theoretical concentration × 100.

with $r^2 = 0.9997 \pm 0.0006$, where y, x, r represent the peak area ratio, the concentration of zabofloxacin in the plasma, and the correlation coefficient, respectively.

3.3. Precision and accuracy

QC samples at three different concentrations (50, 500, and 5000 ng/mL) were analyzed in five replicates to determine the intra- and inter-day accuracy/precision (Table 1). The intra-day accuracies for zabofloxacin ranged from 92.5 to 101.1%, while the intra-day precision in RSD was less than 7.7%. The inter-day accuracies for the analyte ranged from 92.2 to 103.8%, and the precision was less than 8.8%. These results indicate that the accuracy and precision of the current assay are within the recommendations for assay validation as stipulated in "Guidance for Industry: Bioanalytical Method Validation (FDA 2001) [10]."

3.4. Matrix effect

The absolute matrix effect ranged from 107.7 to 116.0% and from 145.3 to 147.6% for zabofloxacin and enrofloxacin, respectively (Table 2). The precision of set 2 (i.e., relative matrix effect) ranged from 2.1 to 11.8% for zabofloxacin and from 3.6 to 3.7% for enrofloxacin. The variability appeared comparable to the variability data obtained with the standard solution in which the analyte was dissolved in methanol (i.e., set 1, 2.6–12.0% for zabofloxacin and 1.8–4.5% for enrofloxacin). These data confirm the absence of the relative matrix effect for zabofloxacin and enrofloxacin.

Table 2
Matrix effect and precision (CV, %) for zabofloxacin and enrofloxacin (IS) in three different rat plasma.

Concentration (ng/mL)	Absolute matrix effect[a] (%)	Precision[b] (CV, %)	
		Set 1	Set 2
Zabofloxacin			
50	116.0	12.0	11.8
500	108.5	2.6	2.1
5000	107.7	2.8	10.5
Enrofloxacin (IS)			
500	147.6	4.5	3.6
5000	145.3	1.8	3.7

[a] Absolute matrix effect expressed as the ratio of the mean peak area of an analyte added post-deproteinization (set 2) to the mean peak area of the same analyte standards (set 1) multiplied by 100.
[b] Precision of determination of peak areas of zabofloxacin and enrofloxacin (IS) in set 1 and 2 as the measure of relative matrix effect.

Table 3
Stability of zabofloxacin in rat plasma ($n = 3$).

Concentration (ng/mL)	Stability (%)
Zabofloxacin	
Freeze–thaw stability (3 cycles)	
50	98.8 ± 5.6
500	101.8 ± 2.0
5000	99.1 ± 6.3
Short-term stability (24 h at room temperature)	
50	96.5 ± 10.4
500	96.4 ± 1.0
5000	100.0 ± 1.0
Long-term stability (1 week at −80 °C)	
50	96.0 ± 5.1
500	97.6 ± 4.6
5000	98.6 ± 2.3

3.5. Stability

The peak areas of zabofloxacin and the IS were 93.8–98.2% and 91.3–94.2%, respectively, following storage of the compounds in methanol at room temperature for 6 h, indicating no significant decrease in the peaks for these compounds under the given conditions. The stability of the IS during storage at 4 °C for 1 week was 83.0–95.4%.

The stability of zabofloxacin in the rat plasma was assessed for typical storage/handling conditions. The results are reported in Table 3.

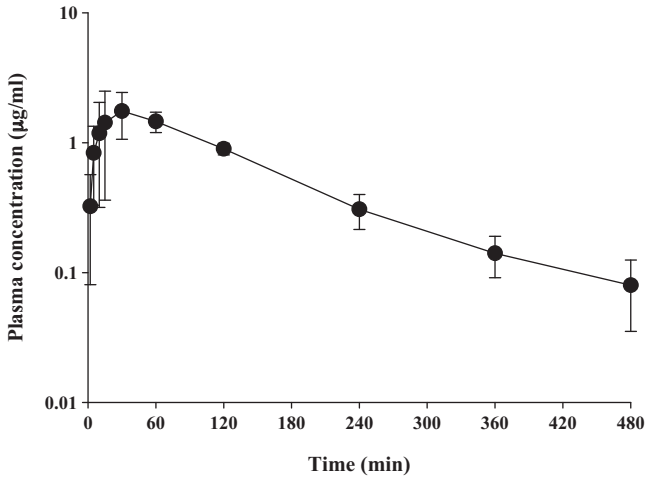

Fig. 2. Mean plasma concentration–time profiles of zabofloxacin following oral administration of 20 mg/kg zabofloxacin to rats (mean ± SD, $n = 4$ rats).

Table 4
Pharmacokinetic parameters of zabofloxacin following an oral administration of zabofloxacin at a dose of 20 mg/kg in rats ($n = 4$).

Pharmacokinetic parameters	Mean ± SD
T_{max} (min)	33.8 ± 18.9
C_{max} (μg/mL)	1.8 ± 0.8
$T_{1/2}$ (min)	107.0 ± 13.3
MRT (min)	145.2 ± 36.0
$AUC_{0-8\,h}$ (μg min/mL)	268.7 ± 40.6
AUC_{∞} (μg min/mL)	281.5 ± 41.7

Standard methods [12] were used in the calculation of $AUC_{0-8\,h}$, AUC_{∞}, and MRT (AUMC/AUC), using a non-compartmental analysis program (WinNonlin® 3.1, Pharsight Co., Mountain View, CA, USA), where AUMC indicate fraction absorbed and the first moment of AUC.

3.6. Applicability of the assay

The applicability of the current assay to the study of the pharmacokinetics of zabofloxacin in rats was also examined. Fig. 2 shows the profiles of zabofloxacin concentrations in plasma after a single oral administration of zabofloxacin at a dose of 20 mg/kg. The concentration of the drug was readily measurable in plasma samples collected up to 8 h following zabofloxacin administration. The standard pharmacokinetic parameters are listed in Table 4. These collective observations suggest that the current assay is applicable to pharmacokinetic studies of zabofloxacin in rats.

4. Conclusions

An LC/MS assay method was developed and validated for the determination of zabofloxacin in rat plasma. Advantages of this method are simple sample preparation and a short LC run time. In addition, the sensitivity, selectivity, linearity, accuracy, and precision of the assay were adequate, as was the stability of the compounds during the assay. This method is applicable to routine pharmacokinetic studies of zabofloxacin in rats.

Acknowledgements

This work was supported by the Korea Science and Engineering Foundation (KOSEF) through the National Research Laboratory. The program is funded by the Ministry of Science and Technology (No. R0A-2006-000-10190-0).

References

[1] P.C. Appelbaum, P.A. Hunter, The fluoroquinolone antibacterials: past, present and future perspectives, Int. J. Antimicrob. Agents 16 (2000) 5–15.
[2] M.E. Klepser, E.J. Ernst, C.R. Petzold, P. Rhomberg, G.V. Doern, Comparative bactericidal activities of ciprofloxacin, clinafloxacin, grepafloxacin, levofloxacin, moxifloxacin, and trovafloxacin against *Streptococcus pneumoniae* in a dynamic in vitro model, Antimicrob. Agents Chemother. 45 (2001) 673–678.
[3] F. Garcia-Garrote, E. Cercenado, J. Martin-Pedroviejo, O. Cuevas, E. Bouza, Comparative in vitro activity of the new quinolone gemifloxacin (SB-265805) with other fluoroquinolones against respiratory tract pathogens, J. Antimicrob. Chemother. 47 (2001) 681–684.
[4] H.S. Park, S.J. Jung, J.H. Kwak, D.R. Choi, E.C. Choi, DNA gyrase and topoisomerase IV are dual targets of zabofloxacin in *Streptococcus pneumoniae*, Int. J. Antimicrob. Agents (2010).
[5] H.J. Kim, M.J. Seol, H.S. Park, D.R. Choi, S.K. Seong, H.K. Shin, J.H. Kwak, Antimicrobial activity of DW-224a, a new fluoroquinolone, against *Streptococcus pneumoniae*, J. Antimicrob. Chemother. 57 (2006) 1256–1258.
[6] H.S. Park, H.J. Kim, M.J. Seol, D.R. Choi, E.C. Choi, J.H. Kwak, In vitro and in vivo antibacterial activities of DW-224a, a new fluoronaphthyridone, Antimicrob. Agents Chemother. 50 (2006) 2261–2264.
[7] R.N. Jones, D.J. Biedenbach, P.G. Ambrose, M.A. Wikler, Zabofloxacin (DW-224a) activity against *Neisseria gonorrhoeae* including quinolone-resistant strains, Diagn. Microbiol. Infect. Dis. 62 (2008) 110–112.
[8] U. Theuretzbacher, Future antibiotics scenarios: is the tide starting to turn? Int. J. Antimicrob. Agents 34 (2009) 15–20.
[9] E.F. Hendershot, Fluoroquinolones, Infect. Dis. Clin. North Am. 9 (1995) 715–730.
[10] U.S. Department of Health & Human Services, Food and Drug Administration, Guidance for Industry, Bioanalytical Method Validation, 2001.
[11] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS, Anal. Chem. 75 (2003) 3019–3030.
[12] M. Gibaldi, D. Perrier, Pharmacokinetics, 2nd revised and expanded, Informa HealthCare, New York, 1982, pp. 409–416.