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Determination of gemifloxacin in different tissues of rat after oral dosing of gemifloxacin mesylate by LC–MS/MS and its application in drug tissue distribution study

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ABSTRACT

A simple, sensitive and specific liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated to evaluate the accumulation of gemifloxacin in different tissues of Wister albino rat. The analytical method consists of the homogenization of tissues followed by simple liquid–liquid extraction and determination of gemifloxacin by an LC–MS/MS. The analyte was separated on a Peerless basic C_{18} column (33 mm × 4.6 mm, 3 μm) with an isocratic mobile phase of methanol–water containing formic acid (1.0%, v/v) (9:1, v/v) at a flow rate of 0.6 ml/min. The MS/MS detection was carried out by monitoring the fragmentation of *m/z* 390.100 → 372.100 for gemifloxacin and *m/z* 332.100 → 314.200 for ciprofloxacin (internal standard; IS) on a triple quadrupole mass spectrometer. The validated method was accurate, precise and rugged with good linearity in all tissue homogenates. The accuracy and precision value obtained from six different sets of quality control samples of all tissues and serum analyzed in separate occasions within 91.833–102.283% and 0.897–5.291%, respectively. The method has been successfully applied to tissue distribution studies of gemifloxacin. The present study demonstrates that the highest tissue concentration of gemifloxacin was obtained in lung (11.891 ng/g), followed by liver (10.110 ng/g), kidney (10.095 ng/g), heart (4.251 ng/g), testis (3.750 ng/g), stomach (3.182 ng/g), adipose tissue (1.116 ng/g) and brain (0.982 ng/ml) in 3 h after multiple oral dosing of 200 mg gemifloxacin mesylate for 7 days. This method may also be used for gemifloxacin tissue distribution modeling study in rat tissues and antibiotic residue analyses in other animal tissues.

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] (SB-265805; LB-20304a) (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 [4–6]. 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) 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. [7]. 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 [8].

Gemifloxacin demonstrates concentration dependent killing of organisms. The ratio of the peak concentration (C_{max}) to the MIC and of the area under the curve (AUC) to the MIC appears to be the parameters that best correlate with clinical efficacy [9]. The pharmacokinetic profile of gemifloxacin is characterized by high concentration in plasma, tissues and body fluid and a long half-life permitting extended dosing intervals to be applied. If the free drug AUC/MIC ratio is >33.7, the probability of a favorable clinical outcome is quite high (>100%) for patients infected with gram-positive organisms [10]. Using this cutoff criterion (AUC/MIC ratio, >33.7), ciprofloxacin clearly fares poorly against gram-positive organisms, whereas gemifloxacin exceeds this threshold.

Tissue penetration of gemifloxacin is very well. Concentration in the respiratory tract exceeds serum levels, and cellular

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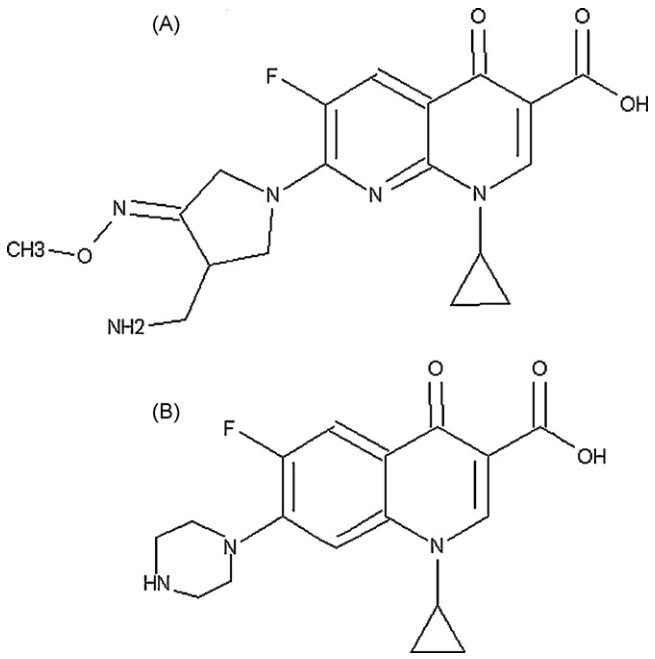

Fig. 1. The chemical structures of: (A) gemifloxacin and (B) ciprofloxacin (IS).

penetration in alveolar macrophages is 90-fold greater than serum levels for gemifloxacin [10,11]. In experimental meningitis models, gemifloxacin CSF levels were 22–33% of serum levels [12]. On the other side gemifloxacin eliminates by both renal (60%) and hepatic (40%) routes, and active tubular secretion is evident in the kidney. Therefore, clinical efficacy of gemifloxacin cannot just be predicted relating to the serum concentration to MIC and attention should be paid to tissue pharmacokinetic concept. Clinical effects can still observe due to the penetration of gemifloxacin into target tissues, even fallen below MIC.

Very few methods have been published for the quantification of gemifloxacin in human plasma. A method was developed by Doyle et al. [13] for the determination of gemifloxacin in human plasma using liquid chromatography–tandem mass spectrometry. Allen et al. [14] has quantified concentrations of gemifloxacin in serum and urine by a reversed-phase liquid chromatography (LC) method with fluorescence detection. But no such method has been published for quantification or to observed tissue distribution of gemifloxacin in animal model. Previous studies have shown that gemifloxacin displays a favorable plasma pharmacokinetic profile allowing a once-daily dosing regimen in human [14,15]. Hence, to accurately and reliably examine all tissue distributions after multiple dosing in Wistar albino rats, a simple and rapid LC–MS/MS method was developed and validated for quantification of gemifloxacin in different tissue homogenates.

2. Experimental

2.1. Materials and reagents

Gemifloxacin mesylate (purity ≥99%) bulk drug was obtained from Micro Lab. Ltd. (Bangalore, India). Ciprofloxacin (purity ≥99%) bulk drug was supplied by Nobel Healthcare (Haryana, India). Formic acid (98%) (analytical-reagent grade) and methanol (HPLC-grade) were purchased from Merck Pvt. Ltd. (Mumbai, India). HPLC grade water (resistivity of 18.2 MΩ cm) generated from Milli Q water purification system (Elix, Milli Q A10 Academic, Molsheim, France) was used throughout the analysis. The blank rat serum without any anticoagulant and different tissues were collected from Pharmacological & Toxicological Unit (PTU) of Bioequivalance Study Centre, Jadavpur University, Kolkata, India. All other reagents used were of analytical grade (Merck Pvt. Ltd., Mumbai, India).

2.2. Apparatus and chromatographic conditions

The liquid chromatographic (LC) system consists of LC-10AD pump, SIL-HTC autosampler and CTO-10ASvp column oven (Shimadzu, Kyoto, Japan). Chromatographic separation was achieved using a Peerless basic C_{18} column (i.d. 33 mm × 4.6 mm, 3 μm; Chromatopak, Mumbai, India), maintained at 35 °C. The samples were eluted using a mixture of methanol–water containing formic acid (1.0%, v/v) (9:1, v/v) at a flow rate of 0.6 ml/min with a total run time of 12.5 min.

The LC system was coupled with an atmospheric pressure ionization source (API-2000) triple quadruple mass spectrometer (AB Sciex Instruments, Foster, CA; Model: 029345-K) equipped with an electrospray ionization source, operating in positive mode. Analysis was performed in multiple reaction-monitoring (MRM) mode by monitoring the ion transitions from m/z 390.100 → 372.100 (gemifloxacin) and m/z 332.100 → 314.200 (IS). The MS/MS conditions were as follows: spray needle voltage, 4.5 kV; source temperature, 400 °C; auxiliary gas flow, 71 l/min; equalizer gas pressure, 80 psi. The instrument was controlled and the data integration was performed with analyst 1.4.1 software version (AB Sciex Instruments, Foster, CA).

2.3. Preparation of stock and standard solution

Stock solution of gemifloxacin was prepared by dissolving drug in water at a concentration of 1 mg/ml and stored in glass tubes at −20 °C. Serial (working) dilutions were prepared from this stock solution for the preparation of calibration and quality control (QC) samples. The internal standard (IS) master stock and working stock were prepared, respectively, at concentrations of 1 mg/ml and 250 ng/ml in water. Both the master and working internal standard were stored at −20 °C.

QC samples were prepared in batch, by addition of blank tissue homogenates and blank serum to the required amount of working solution in a volumetric flask, to obtain three different final concentrations in each matrix. The 20 μl aliquots of prepared QCs were stored at −20 °C.

2.4. Sample preparation

The calibration samples were prepared by spiking 20 μl of blank tissue homogenates and blank serum in polypropylene microcentrifuge tubes with 0.2 ml of the appropriate gemifloxacin working solution. The following method of sample preparation was used for all tissues including liver, lung, kidney, brain, heart, testis, stom-

Table 1
Tandem mass spectrometric parameters of gemifloxacin and ciprofloxacin (IS).

Compound	Molecular weight	Protonated ion	Fragment	CE (eV)	DP (V)	EP (V)	FP (V)	CXP (V)	Dwell time (ms)
Gemifloxacin	389.380	390.100	372.100	40.000	21.000	9.000	380.000	20.000	200.000
Ciprofloxacin	331.340	332.100	314.200	35.000	35.000	10.000	350.000	20.000	200.000

eV, electron volt; V, volt; CE, collision energy; DP, declustering potential; EP, entrance potential; FP, focusing potential; CXP, collision cell exit potential; ms, milliseconds.

Table 2
Linear regression data for the determination of gemifloxacin added to rat tissue homogenates and serum[a] ($n = 6$).

Tissue homogenate		Spiked concentration (ng/ml)							Equation	r^2
		0.250	0.500	1.000	2.500	5.000	10.000	20.000		
Lung	Mc	0.252 ± 0.004	0.493 ± 0.009	0.902 ± 0.033	2.272 ± 0.015	5.147 ± 0.149	10.222 ± 0.236	20.343 ± 0.610	$f = 0.2445C - 0.0022$	0.998
Liver	Mc	0.258 ± 0.011	0.545 ± 0.005	1.083 ± 0.042	2.233 ± 0.093	4.648 ± 0.080	10.260 ± 0.149	20.264 ± 0.543	$f = 0.2036C - 0.0505$	0.997
Kidney	Mc	0.252 ± 0.004	0.507 ± 0.018	1.036 ± 0.054	2.411 ± 0.092	5.066 ± 0.158	9.085 ± 0.106	20.490 ± 0.306	$f = 0.1352C - 0.0426$	0.994
Tissue homogenate		Spiked concentration (ng/ml)							Equation	r^2
		0.125	0.250	0.500	1.000	2.000	3.000	5.000		
Heart	Mc	0.122 ± 0.004	0.230 ± 0.007	0.492 ± 0.011	1.011 ± 0.022	1.959 ± 0.049	3.040 ± 0.126	4.936 ± 0.107	$f = 0.4837C + 0.0150$	0.996
Testis	Mc	0.117 ± 0.004	0.241 ± 0.010	0.513 ± 0.009	1.037 ± 0.032	1.865 ± 0.048	3.223 ± 0.049	4.938 ± 0.104	$f = 0.6254C + 0.0758$	0.989
Stomach	Mc	0.117 ± 0.003	0.233 ± 0.012	0.494 ± 0.013	1.068 ± 0.036	2.093 ± 0.064	3.104 ± 0.115	4.945 ± 0.102	$f = 0.4241C + 0.1194$	0.991
Tissue homogenate		Spiked concentration (ng/ml)							Equation	r^2
		0.075	0.150	0.250	0.500	1.000	2.500	5.000		
Adipose tissue	Mc	0.077 ± 0.004	0.147 ± 0.004	0.239 ± 0.010	0.540 ± 0.007	1.022 ± 0.041	2.588 ± 0.055	4.714 ± 0.102	$f = 0.5091C + 0.0413$	0.986
Brain	Mc	0.081 ± 0.002	0.164 ± 0.003	0.268 ± 0.010	0.470 ± 0.006	0.973 ± 0.034	2.392 ± 0.056	5.057 ± 0.100	$f = 0.5622C - 0.0467$	0.996
Serum	Mc	0.072 ± 0.003	0.136 ± 0.004	0.241 ± 0.011	0.490 ± 0.017	1.010 ± 0.019	2.480 ± 0.071	4.653 ± 0.117	$f = 0.5852C + 0.0344$	0.984

S.D., standard deviation; f, mean peak area ratios of gemifloxacin to IS; r^2 (correlation coefficient), linearity of the calibration curve; Mc, measured concentration (ng/ml).
[a] Measured concentration (Mc) presented by mean ± S.D.

Table 3
Accuracy and precision of the LC–MS/MS method for determination of gemifloxacin in representative tissue homogenates ($n = 6$).

Tissue homogenate	Spiked (ng/ml)	Intra-batch		Inter-batch	
		Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
Lung	0.750	97.777	1.243	98.059	2.248
	6.000	102.066	1.669	96.818	3.657
	16.000	95.902	2.468	96.279	1.883
Liver	0.750	102.283	2.975	98.651	1.880
	6.000	96.111	2.934	100.074	1.484
	16.000	98.854	0.897	98.750	1.867
Heart	0.400	91.833	2.347	96.697	3.022
	2.500	99.186	4.752	101.351	4.856
	4.000	93.533	4.203	95.730	3.342
Brain	0.300	95.222	2.382	97.407	4.237
	2.500	99.533	5.291	99.680	3.312
	4.000	101.683	1.635	98.861	2.181

Accuracy (%), [measured concentration/spiked concentration × 100]; RSD (%), relative standard deviation, [S.D./mean × 100].

ach and adipose tissue of Wistar albino rats. Tissues (0.3 g) were homogenized in 1 ml homogenate media [10× phosphate buffered saline (PBS) consists of 0.2 M potassium phosphate, 1.5 M sodium chloride, pH 7.2, prepared in Molecular Biology Grade water (DEPC treated)] twice for 30 s per time using an Ultra-Turrax T25 tissue disperser (IKA-Labortechnik, Germany) in an ice bath. The homogenized samples were sonicated for 5 min and centrifuged at 3500 × g for 10 min. Two hundred microliter aliquot from the resultant supernatants was spiked with 10 μl known concentration of internal standard (Ciprofloxacin, 25 ng/ml) in each polypropylene tube. Three milliliter of a mixture of chloroform–isoamyl alcohol (9:1, v/v) was added and the contents were hand mixed for 20 min, followed by centrifugation at 5000 rpm for 15 min. A 1.0 ml supernatant clear solution was separated and evaporated under nitrogen stream at 40 °C. The residue was reconstituted in 500 μl mobile phase and centrifuged at 5000 rpm for 5 min. The supernatant (150 μl) was transferred into a 1.0 ml glass vial, which was loaded into autosampler cabinet, and 5 μl aliquot was injected into the LC–MS/MS system. Serum samples were stored at −20 °C. Serum (200 μl) was used for extraction with the same steps as those for the tissue samples.

2.5. Method validation

Validation was carried out on six different occasions, following the guidelines for Bioanalytical Method Validation published by Food and Drug Administration (FDA) [16]. On each occasion of analysis, calibration standards in duplicate at 0.250, 0.500, 1.000, 2.500, 5.000, 10.000 and 20.000 ng/ml for lung, liver and kidney; 0.125, 0.250, 0.500, 1.000, 2.000, 3.000 and 5.000 ng/ml for heart, testis and stomach; 0.075, 0.150, 0.250, 0.500, 1.000, 2.500 and 5.000 ng/ml for adipose, brain and serum, respectively. Quality control samples were prepared independently in batch before validation at required concentrations for different matrixes. Along with calibrators, six QC samples at each concentration were thawed and analyzed each occasion. Each validation run included two blank (zero concentration) samples and two samples containing only IS, along with the calibrators and QC samples. The results from QC samples in six runs were used to evaluate the accuracy and precision of the method. Accuracy was determined by the ratio of determined concentration and actual concentration multiplied by 100%, and precision was evaluated by the percentage relative standard deviations (RSD%).

In order to calculate recovery, the representative blank tissue homogenates (liver, lung, adipose and bladder) were mixed with

standard solutions of gemifloxacin at low, medium and high concentrations and extracted as described. Then, internal standard was added. In addition, homogenate of blank tissues was also extracted. The blank tissue extracts were mixed with standard solutions of gemifloxacin at low, medium and high concentrations and internal standard, respectively. The extraction recovery is calculated by the formula:

$$\text{recovery}\,(\%) = \frac{\text{the peak area ratio of extracted analyte to IS}}{\text{the mean peak area ratio of non-extracted analyte mixed with blank tissue extracts to IS}} \times W \times 100 \quad \left(W:\ \text{transferred fraction},\ \frac{5}{4}\right)$$

Matrix effect was determined at same concentration of analyte and IS as in recovery experiment. The matrix effect (ME) was calculated by using the following equation:

$$\text{ME} = \left[1 - \left(\frac{\text{response for post-extraction spiked drug}}{\text{response in pure solvent}}\right)\right] \times 100$$

Stability study was evaluated as part of the method validation. The autosampler stability was evaluated by comparing the extracted samples that were injected immediately (time 0) to the samples that were re-injected after 72 h. Stability of analyte in the tissue homogenates and serum after 8 h exposure in bench top was determined at all QC samples in six replicates. QC samples were kept at an ambient temperature for 24 h and analyzed against freshly spiked standard curve and QC samples for short-time stability study. For the long-term stability study, spiked tissue homogenates and serum stored for 1 and 3 months at −20 °C together with freshly spiked (for standard curve) and QC samples. The freeze–thaw stability was conducted by comparing the stability samples that had been frozen and thawed three times, with the samples thawed once. Six replicates of each QC samples were used from freeze–thaw stability evaluation.

2.6. Animal studies

Eighteen male Wistar albino rats weighing 250 ± 15 g, were used for the experiment. Animals were obtained from the animal house,

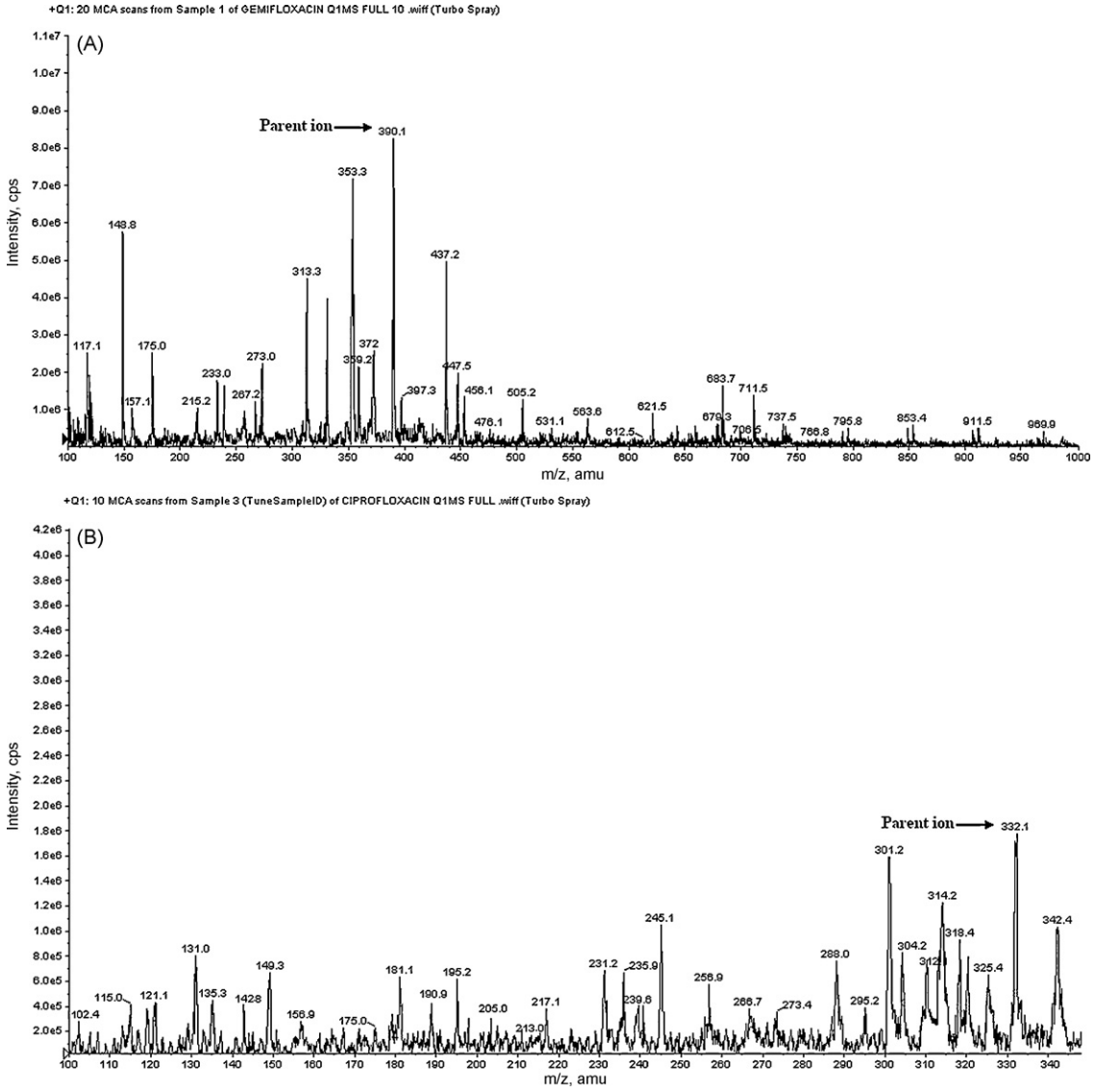

Fig. 2. Parent ion mass spectra of: (A) gemifloxacin (*m*/*z* 390.10) and (B) ciprofloxacin (*m*/*z* 332.10).

Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped and housed in wire cages with not more than six animals per cage, under good laboratory conditions (temperature $25 \pm 2\,^{\circ}C$; $50 \pm 20\%$ relative humidity) with dark and light cycle (12/12) for minimum of 8 days before the beginning of experiment to adjust the new environment and to overcome tress possibly incurred during transit. During this period they had free access to standard dry pellet diet (Hindustan Liver, Kolkata, India) and water *ad libitum*. The study was approved by Institutional Animal Ethics Committee of Jadavpur University (CPCSEA, Reg. No. 367), Kolkata, India.

Eighteen rats were divided into three groups, six animals in each group, corresponding to 1.5, 3 and 24 h for blood and tissue collection. Gemofloxacin mesylate bulk drug was orally administered at a dose of 200 mg/kg for once a day at 8.00 a.m. for 7 days. At the end of the experiment (on day 7) all animals were sacrificed at the predetermined sampling time and blood samples were collected. Serum was separated and stored frozen until analysis. Samples of a number of tissues (lung, liver, kidney, heart, testis, stomach, adipose tissue and brain) were quickly dissected. The tissues were rinsed with normal saline solution (0.9%, w/v), wiped dry and placed in plastic bags. The tissues were kept frozen at $-20\,^{\circ}C$ until analysis.

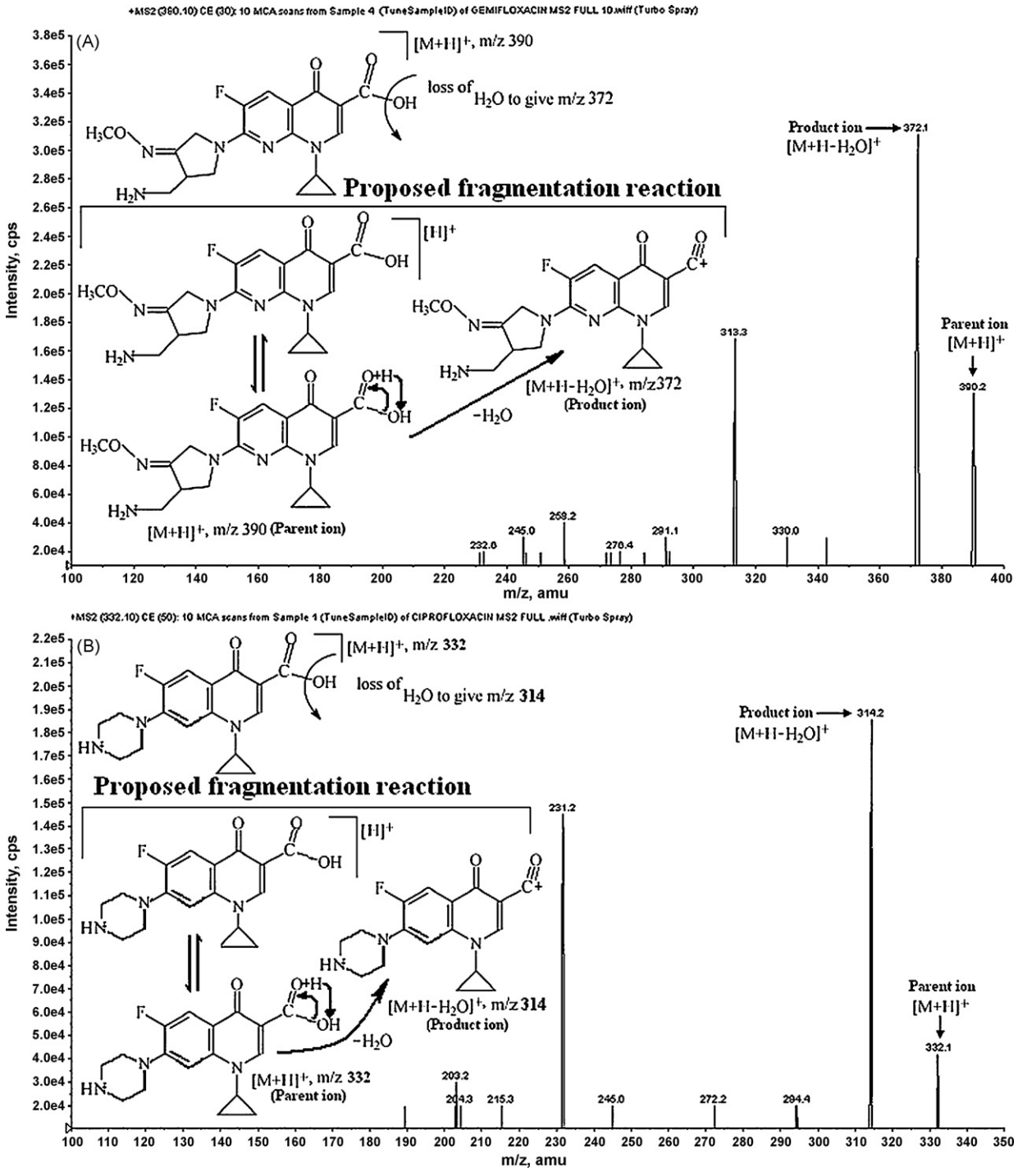

Fig. 3. Profile mass spectral data of: (A) gemifloxacin $(M+H)^+$ and (B) ciprofloxacin $(M+H)^+$ with their fragmentation interpretation.

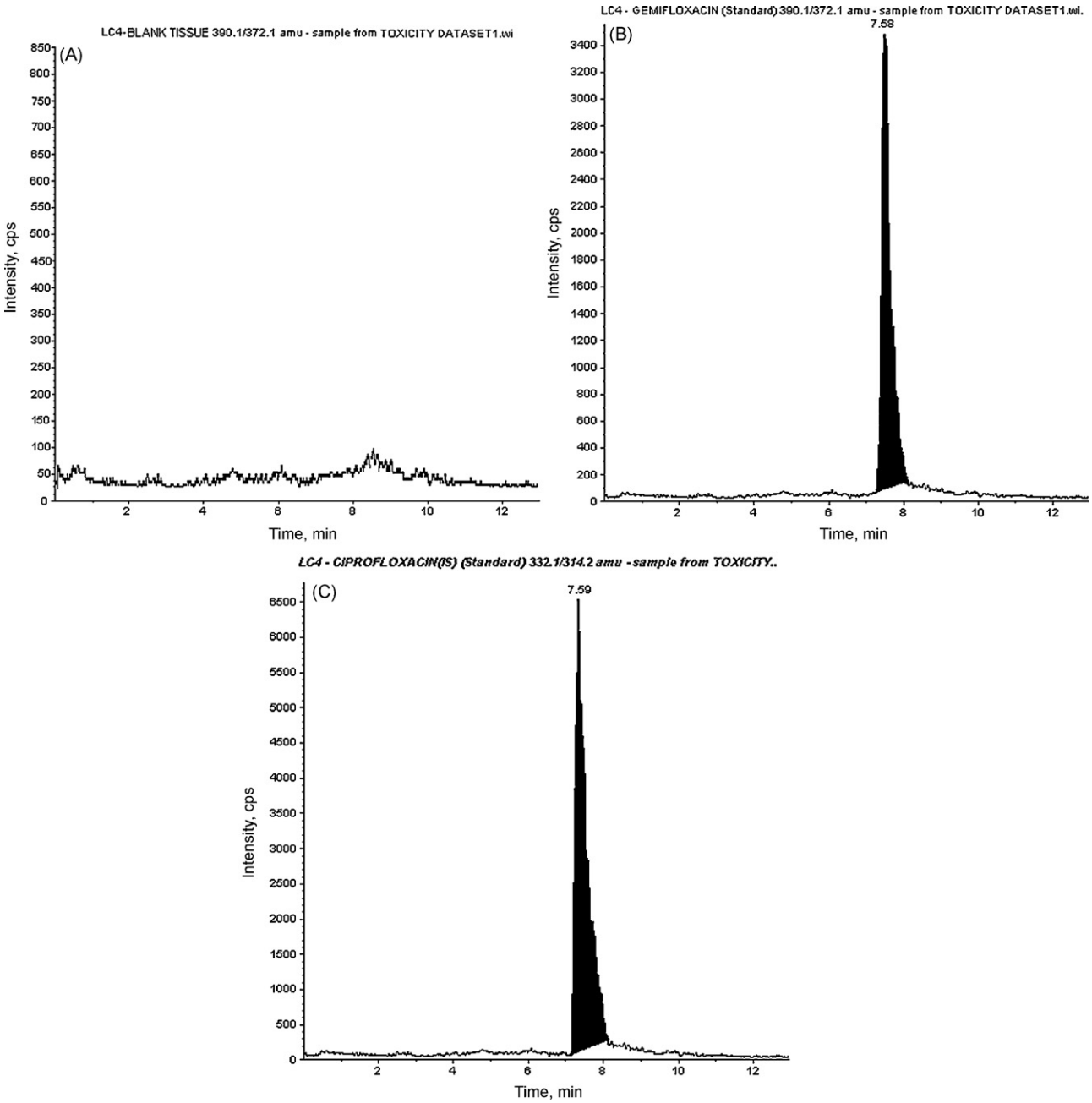

Fig. 4. Representative MRM chromatograms of: (A) blank rat lung tissue homogenate, (B) blank rat lung tissue homogenate spiked with gemifloxacin and (C) blank rat lung tissue homogenate spiked with ciprofloxacin (IS).

3. Result and discussion

3.1. Mass spectrometry and assay specificity

LC–MS/MS was used to determine the distribution and accumulation of gemifloxacin in different tissues of male Wistar albino rats. Positive electrospray mass spectra of gemifloxacin shows an intense $[M+H]^+$ ion at m/z 390.100 (Fig. 2A) and another intense $[M+H]^+$ ion at m/z 332.100 (Fig. 2B) as shown for ciprofloxacin. No interfering peaks of endogenous components were observed from any of the tissues studied. When these molecular ions undergo fragmentation in the collision cell, the product ions mass spectra (Fig. 3) were generated. With the experimental conditions used in these experiments gemifloxacin and ciprofloxacin show an intense product ion at m/z 372.100 and 314.200, respectively. Chromatographic conditions were optimized for peak shape. The collision energies and other optimized parameters used for analyte and IS are presented in Table 1. Typical representative chromatograms of blank lung tissues and tissues containing gemifloxacin and IS at 25 ng/ml level are presented in Fig. 4. Representative chromatograms of blank brain tissue and tissues containing gemifloxacin and IS at 0.500 ng/ml level are presented in Fig. 5. The retention times of gemifloxacin and internal standard were at 7.5 min.

3.2. Limit of quantification and linearity

Initially, validation was performed to evaluate the calibration, accuracy and precision of gemifloxacin in different tissues along

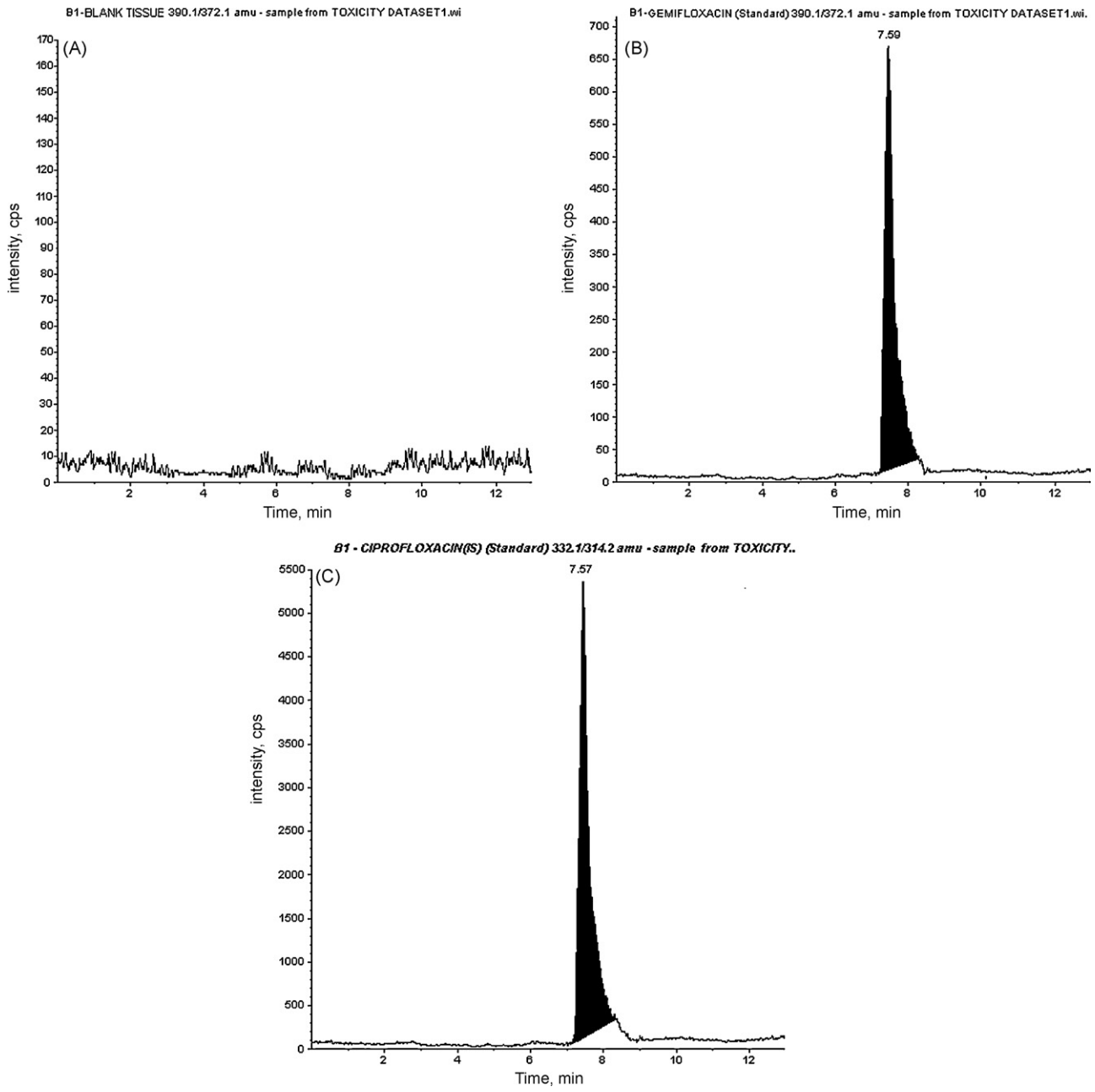

Fig. 5. Representative MRM chromatograms of: (A) blank rat brain tissue homogenate, (B) blank rat brain tissue homogenate spiked with gemifloxacin and (C) blank rat brain tissue homogenate spiked with ciprofloxacin (IS).

with serum. The calibration curve was constructed by plotting the peak area ratio (f) of gemifloxacin to internal standard versus the nominal concentration (C) of the analyte. The linearity was determined by weighted ($1/C$) linear regression analysis. The regression equation of the calibration curve was then used to calculate the concentration of gemifloxacin in different tissues and serum. The back-calculated values of the concentrations were statistically evaluated. The back-calculated concentrations (mean ± S.D.) from the representative calibration standards by LC–MS/MS determination for gemifloxacin and the corresponding regression equations are given in Table 2. The method also showed good linear responses over the selected concentration range in tested matrixes of rat. Additional evaluation of the lower limit of quantitation (LLOQ) confirmed that those concentrations fitted with the proposed criteria. It is clear that (from Table 2) our method exhibited good sensitivity. Representative chromatograms of gemifloxacin in rat lung sample (tissue homogenate) with IS and rat brain sample (tissue homogenate) with IS, 3 h after oral administration of 200 mg/kg are shown in Figs. 6 and 7, respectively.

3.3. Accuracy and precision

The back-calculated concentration values for QC samples of different matrixes, run in sextuplicate at each concentration level on six different occasions, were used to assess the accuracy and precision of the assay. The accuracy and precision of gemifloxacin measurements were assessed with lung, liver, kidney, heart, testis, stomach, adipose tissue and brain tissue homogenates and serum (results of four representative tissue homogenates are shown in Table 3).

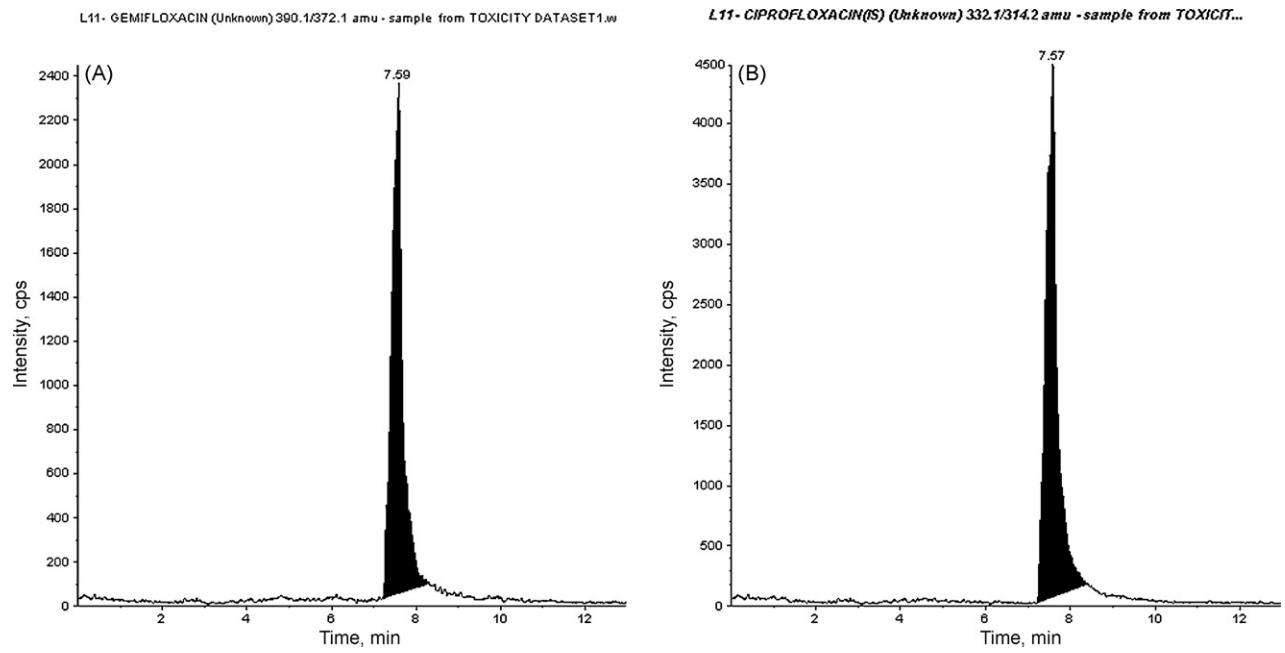

Fig. 6. Representative chromatograms of gemifloxacin in rat lung sample (tissue homogenate) with IS after oral administration of 200 mg/kg body weight.

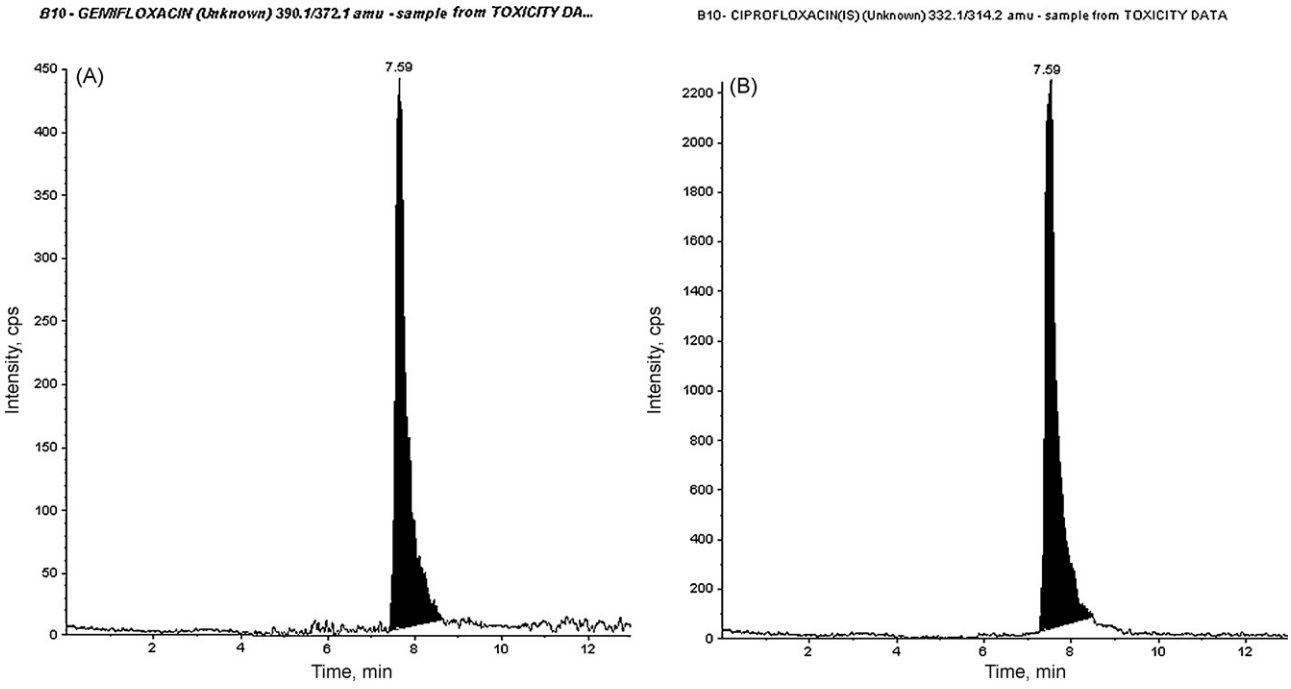

Fig. 7. Representative chromatograms of gemifloxacin in rat brain sample (tissue homogenate) with IS after oral administration of 200 mg/kg body weight.

3.4. Recovery and matrix effect

Recovery and matrix effect were calculated for all six batches of QC samples (QC-low, QC-medium and QC-high) of all matrixes and the results are shown in Table 4. The gemifloxacin stock was added either pre-extraction or post-extraction, and the IS was added post-extraction in all the samples. Recovery was calculated as the response ratio (gemifloxacin peak area/IS peak area) measured in pre-extraction gemifloxacin spiked samples ($n = 6$), as a percentage of that measured from post-extraction gemifloxacin spiked samples ($n = 6$). The matrix effect was determined by comparison of response ratio in post-extraction gemifloxacin spiked samples ($n = 6$) with that of response ratio observed in pure solvent, i.e., mixture of methanol–water containing formic acid (1.0%, v/v) (9:1, v/v). Average matrix values obtained were <5% for analyte and IS among the all matrix. No significant peak area differences were observed.

3.5. Stability study

Each stability test included three replicates of three levels of QC samples [17]. All stability results, as well as the linear regression correlation coefficients of calibration curves generated from each stability test run for the analyte are presented in Tables 5–7.

Table 4
Recovery and matrix effect evaluation of gemifloxacin in different matrixes ($n = 6$)[a].

Matrix	Spiked concentration (ng/ml)					
	0.750		6.000		16.000	
	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)
Lung	92.060 ± 5.163	4.319 ± 0.303	91.284 ± 5.87	3.713 ± 0.204	93.480 ± 4.621	4.548 ± 0.188
Liver	89.361 ± 4.525	3.265 ± 0.162	84.962 ± 5.062	4.945 ± 0.110	87.661 ± 6.882	4.985 ± 0.116
Kidney	90.581 ± 3.444	1.908 ± 0.153	92.442 ± 4.350	0.995 ± 0.085	89.610 ± 3.812	2.926 ± 0.130
Matrix	Spiked concentration (ng/ml)					
	0.400		2.500		4.000	
	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)
Heart	81.962 ± 3.610	4.970 ± 0.298	82.922 ± 4.90	4.654 ± 0.241	81.941 ± 3.81	4.979 ± 0.341
Testis	94.202 ± 4.331	1.350 ± 0.045	90.948 ± 6.861	3.670 ± 0.164	88.351 ± 2.132	4.153 ± 0.104
Stomach	79.627 ± 2.980	4.948 ± 0.146	82.554 ± 5.142	2.954 ± 0.116	80.918 ± 4.142	4.991 ± 0.115
Matrix	Spiked concentration (ng/ml)					
	0.300		2.500		4.000	
	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)
Adipose tissue	80.312 ± 2.644	3.978 ± 0.089	84.016 ± 4.931	2.853 ± 0.118	83.433 ± 3.321	4.531 ± 0.184
Brain	78.658 ± 3.641	4.953 ± 0.135	80.111 ± 6.240	3.851 ± 0.122	82.181 ± 1.130	4.191 ± 0.155
Serum	79.318 ± 4.410	4.89 ± 0.218	88.213 ± 2.752	4.094 ± 0.256	86.832 ± 2.810	2.121 ± 0.305

S.D., standard deviation; recovery (%), [mean extracted response ratio/mean unextracted response ratio × W × 100]; W, transferred fraction (5/4); matrix effect (%), [mean unextracted response ratio/mean response ratio in pure solvent) × 100].

[a] The data presented in this table as mean ± S.D.%.

Table 5
Short-term and long-term stability data of liver, lung and kidney[a].

Organs	Storage condition	Low-QC (0.750 ng/ml)	Medium-QC (6.000 ng/ml)	High-QC (16.000 ng/ml)	r^2
Liver	3 freeze/thaw cycle	94.254 (4.350)	96.251 (2.353)	97.260 (2.121)	0.993
	24 h ambient	96.741 (5.221)	98.220 (3.880)	98.542 (2.867)	0.996
	72 h autosampler	94.313(3.681)	95.845 (3.412)	96.524 (3.095)	0.988
	8 h bench top	93.245 (4.822)	97.226 (2.986)	98.561 (2.270)	0.999
	1 month frozen (−20 °C)	94.241 (3.991)	93.221 (3.410)	94.452 (2.861)	0.997
	3 month frozen (−20 °C)	92.980 (4.241)	94.181 (3.552)	93.440 (3.141)	0.989
Lung	3 freeze/thaw cycle	95.212 (3.332)	95.965 (3.013)	96.212 (4.330)	0.984
	24 h ambient	93.541 (4.120)	94.781 (3.721)	95.611(2.193)	0.996
	72 h autosampler	95.311(2.511)	97.210 (2.415)	98.915 (2.221)	0.992
	8 h bench top	94.246 (3.640)	95.322 (4.416)	97.234 (3.843)	0.981
	1 month frozen (−20 °C)	93.643 (2.854)	94.351 (2.541)	96.252 (2.051)	0.996
	3 month frozen (−20 °C)	93.380 (5.243)	95.275 (3.274)	98.540 (2.961)	0.999
Kidney	3 freeze/thaw cycle	93.115(5.422)	95.414(3.651)	95.422 (2.651)	0.995
	24 h ambient	92.820 (4.752)	93.748 (2.982)	94.985 (2.241)	0.983
	72 h autosampler	96.321 (5.021)	96.483 (4.321)	98.744 (3.686)	0.996
	8 h bench top	93.454 (3.814)	95.440 (4.101)	99.212 (3.411)	0.982
	1 month frozen (−20 °C)	93.812 (3.142)	93.412 (3.782)	95.455 (2.583)	0.998
	3 month frozen (−20 °C)	92.315(3.055)	92.185 (3.422)	94.412 (2.083)	0.994

[a] The data presented in this table is the percentage of measured value vs. theoretical value with RSD in parentheses ($n = 6$); r^2 (correlation coefficient), linearity of the calibration.

In tissue homogenates gemifloxacin was stable for 3 freeze/thaw cycle to 3 month frozen condition at −20 °C. No detectable loss of gemifloxacin in the stored samples was detected.

3.6. In vivo tissue distribution study

The LC–MS/MS assay described in this report was successfully used for the investigation of tissue distributions of gemifloxacin in rat. By applying this assay, the concentrations of gemifloxacin was determined in many tissues including liver, lung, kidney, stomach, testis, heart, brain, adipose tissue and serum after multiple oral dosing of 200 mg gemifloxacin mesylate for 7 days. Fig. 8 shows the tissue distribution results after oral administration at 200 mg/kg to Wistar albino rat. The highest tissue concentration of gemifloxacin was obtained in the lung (11.891 ng/g), followed by liver (10.110 ng/g), kidney (10.095 ng/g), heart (4.251 ng/g), testis

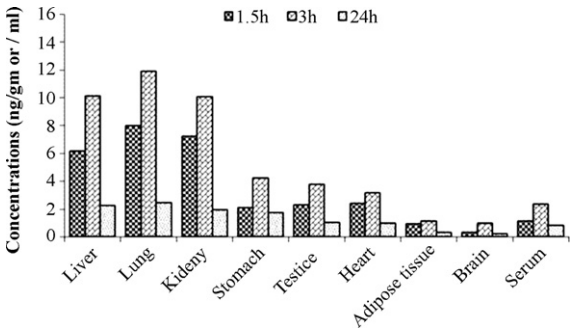

Fig. 8. Gemifloxacin concentrations (ng/g) in rat tissues at 1.5, 3 and 24 h after oral administration of gemifloxacin at 200 mg/kg, compared with the corresponding drug serum concentrations (ng/ml) ($n = 6$).

Table 6
Short-term and long-term stability data of stomach, testis and heart[a].

Organs	Storage condition	Low-QC (0.400 ng/ml)	Medium-QC (2.500 ng/ml)	High-QC (4.000 ng/ml)	r^2
Stomach	3 freeze/thaw cycle	92.680 (4.321)	94.356 (3.641)	96.243 (2.580)	0.991
	24 h ambient	94.512 (3.850)	93.651 (3.514)	94.472 (3.151)	0.983
	72 h autosampler	93.654 (5.151)	95.541 (4.120)	96.120 (3.411)	0.995
	8 h bench top	95.241 (4.742)	94.285 (2.681)	93.572 (1.983)	0.994
	1 month frozen (−20 °C)	94.855 (4.184)	94.814 (4.400)	94.755 (2.520)	0.998
	3 month frozen (−20 °C)	92.351 (3.541)	93.459 (2.891)	94.121 (2.184)	0.988
Testis	3 freeze/thaw cycle	96.322 (4.423)	95.861 (3.116)	98.560 (3.715)	0.990
	24 h ambient	94.157 (5.040)	95.740 (5.121)	97.453 (4.321)	0.992
	72 h autosampler	94.510 (6.101)	94.624 (4.523)	95.420 (3.220)	0.987
	8 h bench top	93.653 (6.246)	95.417 (5.010)	96.225 (4.151)	0.993
	1 month frozen (−20 °C)	92.875 (5.084)	96.422 (4.575)	95.480 (3.414)	0.988
	3 month frozen (−20 °C)	90.818 (4.131)	94.850 (3.880)	94.116 (2.951)	0.998
Heart	3 freeze/thaw cycle	93.653 (6.255)	94.563 (5.128)	96.485 (4.233)	0.991
	24 h ambient	92.470 (4.554)	95.620 (4.481)	95.750 (3.220)	0.988
	72 h autosampler	94.517 (4.290)	93.856 (5.481)	98.261 (2.986)	0.996
	8 h bench top	94.851 (5.115)	96.540 (3.813)	96.313 (4.611)	0.988
	1 month frozen (−20 °C)	93.642 (4.061)	94.513 (4.462)	98.547 (3.781)	0.997
	3 month frozen (−20 °C)	93.241 (3.657)	94.020 (3.880)	97.244 (3.552)	0.992

[a] The data presented in this table is the percentage of measured value vs. theoretical value with RSD in parentheses (n = 6); r^2 (correlation coefficient), linearity of the calibration.

Table 7
Short-term and long-term stability data of adipose tissue, brain and serum[a].

Organs	Storage condition	Low-QC (0.300 ng/ml)	Medium-QC (2.500 ng/ml)	High-QC (4.000 ng/ml)	r^2
Adipose tissue	3 freeze/thaw cycle	94.560 (5.465)	95.350 (3.884)	97.842 (3.554)	0.984
	24 h ambient	93.681 (6.280)	94.251 (4.483)	97.651 (4.306)	0.993
	72 h autosampler	94.543 (5.512)	95.452 (5.091)	98.411 (3.240)	0.997
	8 h bench top	95.241 (3.392)	97.411 (3.425)	99.283 (2.951)	0.988
	1 month frozen (−20 °C)	92.540 (4.270)	94.348 (3.742)	94.570 (3.221)	0.983
	3 month frozen (−20 °C)	89.452 (4.126)	92.434 (3.814)	93.422 (2.453)	0.994
Brain	3 freeze/thaw cycle	92.863 (6.425)	94.560 (4.595)	96.859 (3.423)	0.991
	24 h ambient	94.521 (5.571)	95.472 (4.461)	94.561 (4.201)	0.994
	72 h autosampler	92.780 (5.242)	97.255 (2.281)	97.263 (2.368)	0.967
	8 h bench top	95.237 (4.166)	94.323 (3.911)	95.441 (3.160)	0.989
	1 month frozen (−20 °C)	94.251 (3.758)	94.512 (3.243)	95.453 (3.563)	0.988
	3 month frozen (−20 °C)	91.383 (3.050)	93.246 (2.688)	94.120 (2.911)	0.991
Serum	3 freeze/thaw cycle	89.841 (3.682)	92.310 (4.230)	95.582 (2.681)	0.989
	24 h ambient	92.544 (4.128)	94.722 (5.108)	91.577 (4.505)	0.990
	72 h autosampler	93.450 (5.321)	88.321 (2.983)	98.322 (3.273)	0.979
	8 h bench top	94.684 (4.408)	91.475 (4.150)	94.351 (2.672)	0.9954
	1 month frozen (−20 °C)	91.688 (4.091)	95.551 (3.662)	92.601 (4.813)	0.990
	3 month frozen (−20 °C)	92.851 (4.283)	93.613 (2.710)	95.716 (2.570)	0.984

[a] The data presented in this table is the percentage of measured value vs. theoretical value with RSD in parentheses ($n = 6$); r^2 (correlation coefficient), linearity of the calibration.

(3.750 ng/g), stomach (3.182 ng/g), adipose tissue (1.116 ng/g) and brain (0.982 ng/ml) in 3 h. In our study, it was found that the concentrations of gemifloxacin in visceral tissues were significantly higher than those in serum (2.341 ng/ml in 3 h). The adipose tissue and brain tissue concentrations were lower than or nearly equal to the corresponding serum concentrations.

4. Conclusions

The LC–MS/MS method described here is a simple, economical and reliable method for the determination of gemifloxacin in rat tissues. In addition, the injection volume (5 μl) used in this method was very less. This would also help to protect the chromatographic column and minimize contamination. All these advantages make the method suitable for large sample analysis.

It has been successfully applied for the investigation of tissue distributions of gemifloxacin in rat after oral administration. The results showed the highest tissue concentration was observed in the lung. This method may also be used for gemifloxacin tissue distribution modeling study of rat tissues and antibiotic residue analyses in other animal tissues. Moreover the new method can be applied for serum sample analysis of gemifloxacin in human pharmacokinetic study.

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