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Short communication

Determination of caderofloxacin lactate in rat plasma by high-performance liquid chromatography–mass spectrometry and its application in rat pharmacokinetic studies

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Abstract

A sensitive liquid chromatography–electrospray ionization mass spectrometric (LC–ESI-MS) method for the quantification of a newly active quinolone carboxylic acid caderofloxacin lactate in rat plasma was developed and validated after precipitation method with methanol. Chromatographic separation was achieved on a reversed-phase Shimadzu 2.0 μm C18 column (150 mm × 2.00 mm) with the mobile phase of methanol–0.02% formic acid and step gradient elution resulted in a total run time of about 10.0 min. The analytes were detected by using an electrospray positive ionization mass spectrometry in the selected ion monitoring (SIM) mode. A good linear relationship was obtained in the concentration range studied (5–2000 ng/mL) ($r = 0.9998$). The lowest limit of quantification (LLOQ) was 5 ng/mL and the lowest limit of detection (LLOD) was 2 ng/mL. Average recoveries ranged from 88.80 to 93.05% in plasma at the concentrations of 10, 100 and 1000 ng/mL. Intra- and inter-day relative standard deviations were 4.01–7.30 and 4.15–7.51%, respectively. This method was successfully applied in the pharmacokinetic studies in rats.

Keywords: Caderofloxacin lactate; LC–ESI-MS; Pharmacokinetics; Rat

1. Introduction

Quinolones are important antimicrobial agents for the effective treatment of patients afflicted with serious infections. These compounds have efficient orally absorption, long serum elimination half-lives, good tissue distributions and a broad range of activities against aerobic pathogens [1]. The compounds may be considered as the first-line agents for the treatment of complicated urinary tract infections, exacerbations of *Pseudomonas aeruginosa* respiratory tract infections in patients with cystic fibrosis and osteomyelitis infections caused by gram-positive or gram-negative bacteria [1]. In the past decade, a large number of quinolone derivatives have been synthesized, and most of them have been developed as orally administered antibacterial agents. Unfortunately, strains of quinolone-resistant *Staphylococcus aureus* and *P. aeruginosa* have increased in number, a large number of the existing quinolones have insufficient activity against these strains.

Caderofloxacin lactate (Fig. 1) (1-cyclopropyl-6-fluoro-8-difluoromethoxy-1,4-dihydro-7-[(3*S*)-methyl-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid lactate), derivatized from ciprofloxacin, is a newly developed active quinolone carboxylic acid. Initial susceptibility studies have documented that caderofloxacin shows effective against aerobic/anaerobic gram-positive and gram-negative bacteria, exhibits antibacterial activity markedly superior to ciprofloxacin, from which it derived, against *S. aureus*, *P. aeruginosa* [2,3].

The vast majority of published assays for quinolones in biofluids utilized HPLC with UV or fluorescence (FL) detection [4]. The previous methods required more biofluid and laborious sample pretreatment but provided lower recovery and sensitivity [5–7]. Employing a highly selective detector such as a mass spectrometer has many advantages, compared with using FL or UV detection which require more biofluid and longer runtimes to separate the target compound from interfering substances and to get the necessary selectivity and sensitivity.

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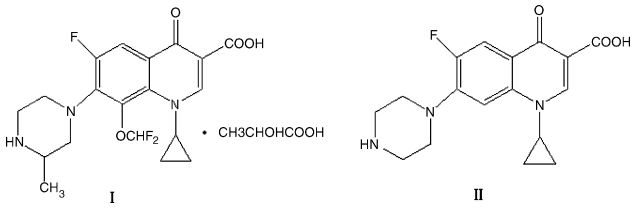

Fig. 1. Chemical construction of (I) caderofloxacin lactate and (II) ciprofloxacin.

At present it has been only reported that the analysis of impurity in caderofloxacin [8]. There was little pharmacokinetic information about this new quinolone. Developing and validating a satisfied bioanalytical method is a preliminary step for the further pharmacokinetics studies. We used an improved protein precipitation to prepare samples, developed and validated a simple, rapid, sensitive and reproducible quantification method for the followed pharmacokinetics evaluation of caderofloxacin lactate. The present method was successfully applied to monitoring the plasma concentration of caderofloxacin lactate after i.g. and i.v. administration to rats.

2. Experimental

2.1. Chemicals and reagents

Caderofloxacin lactate (purity > 99.0%) was kindly provided by Medicinal and Chemical Institute of China Pharmaceutical University. Ciprofloxacin (internal standard) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). Methanol (Merck, Germany) is of HPLC grade, formic acid is commercially available and is of analytical reagent.

2.2. Animals

Twenty male Sprague–Dawley rats, weighed 240–260 g, were supplied by Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). The rats were housed under controlled environmental conditions (temperature, $23 \pm 1\,^{\circ}C$; humidity, $55 \pm 5\%$) with a commercial food diet and water free. Animal experiments were carried out according to institutional guidelines for the care and the use of laboratory animals, and approved by the Animal Ethics Committee of China Pharmaceutical University.

2.3. Preparation of stock solutions, standards and quality control samples

The stock solution of caderofloxacin lactate was prepared in methanol at the concentration level of 1 mg/mL and ciprofloxacin was prepared in methanol at the concentration level of 1 mg/mL, both of which stored at 4 °C until being used. "Individual working solutions" were prepared by diluting stock solutions in methanol for optimization of chromatographic and MS conditions. The working solution for internal standard (5 μg/mL) was prepared by diluting the stock solution with methanol. All caderofloxacin lactate and ciprofloxacin solutions were stored at 4 °C in polypropylene bottles.

Rat plasma calibration standards of caderofloxacin lactate were prepared by adding 10 μL working solution within the concentration range of 0.05–20.0 μg/mL into 90 μL of drug-free rat plasma and well mixed. For caderofloxacin lactate, concentration points were 5, 10, 20, 50, 100, 200, 500, 1000, 2000 ng/mL. These concentration ranges covered the plasma concentrations expected in our experimental studies. Quality control (QC) samples were prepared in the same way as calibration.

Standards with blank and QC sample concentrations were 10, 100 and 1000 ng/mL. QC samples were stored in polypropylene tubes at −20 °C until analysis.

2.4. HPLC–ESI-MS analysis

The assay was performed using Shimadzu (Japan) LC/MS 2010A system. Liquid chromatographic separations were achieved using a Shimadzu 2.0 μm C18 column (150 mm × 2.00 mm) that was preceded by a guard column (C18, 30 mm × 2.00 mm, Phenomenex, Torrance, CA, USA). The column and autosampler tray temperatures were kept constant at 40 and 4 °C, respectively. The mobile phase consisted of a mixture of 0.02% formic acid in water (A) and methanol (B), and was delivered at a flow rate of 0.2 mL/min. The gradient cycle consisted of an initial 1 min isocratic segment (80% A and 20% B). Then, the step gradient was started, increasing solvent B to 70% within 1.0 min and maintained from 2.0 to 5.0 min. After changing back to 20% solvent B at 7.0 min, the mobile phase gradient was maintained at this composition from 7.0 to 10.0 min for column equilibration. The flow rate was 0.2 mL/min during the whole gradient cycle.

The sample injection volume was 5 μL. Samples were ionized by positive-ion electrospray ionization (ESI) probe in the positive-ion mode under the following source conditions: gas flow: 4.5 L/min; curve dissolution line (CDL) voltage was fixed as in tuning, CDL temperature: 250 °C; block temperature: 200 °C. Mass spectra was obtained at a dwell time of 0.2 and 1 s for SIM and scan mode accordingly. Analysis was carried out using selected ion monitoring (SIM) for specific *m/z* 412.00 for caderofloxacin $[M+H]^+$ and 332.00 for ciprofloxacin $[M+H]^+$. Peak areas for all components were automatically integrated using LC/MS solutionVersion 2.04 (© 1997–2002 Shimadzu Corp.).

2.5. Sample preparation

Each collected blood sample was immediately centrifuged at 8000 rpm for 5 min and then transferred into a clean Eppendorf tube. The plasma samples were stored at −20 °C until analysis. A 100 μL volume of blank plasma, calibration standards, QC samples and plasma samples, spiked with internal standard working solution (10 μL), were vortex mixed for 30 s and then extracted with methanol (1 mL) for 3 min using a vortex mixer (Scientific Industries, Inc., USA). After centrifugation at 10,000 rpm for 5 min, the upper organic phase (800 μL) was transferred into clean tubes and evaporated to dryness in the Thermo Savant SPD 2010 SpeedVac System (Thermo Electron Corporation, USA). The residue was then reconstituted in 100 μL methanol

immediately before LC–MS analysis. After centrifugation at 20,000 rpm for 10 min at 4 °C twice, the supernatant was transferred to 1.5 mL auto sampler vial. A 5 μL of the supernatant was used.

2.6. Assay validation

2.6.1. Sensitivity and specificity

The lowest limit of quantitation (LLOQ) was determined as the minimum concentration that could be accurately and precisely quantified (lowest data point of the standard curve). The lowest limit of detection (LLOD on column) was defined as the amount that could be detected with a signal-to-noise ratio of 3. The specificity of the assay for the analytes versus endogenous substances in the matrix was assessed comparing the lowest concentration in the calibration curves with reconstitutions prepared with drug-free plasma from five different rats.

2.6.2. Linearity

Linearity of calibration was tested and assayed in consecutive 5 days. Calibration curves in the concentration range of 5–2000 ng/mL for caderofloxacin lactate were constructed by plotting the peak-area ratios of analyte/internal standard against the spiked concentrations. The linearity was determined from linear regression analysis on the calibration curves.

2.6.3. Accuracy and precision

The accuracy and precision (presented as relative standard deviation, R.S.D.) of the assay were determined using QC samples (at 10, 100 and 1000 ng/mL). Accuracy (%) was determined from the percentage ratio of measured over spiked QC concentration (mean of measured/spiked × 100%). Intra-day precision was determined by analyzing replicate aliquots of QCs ($n = 5$ per each concentration) on the same day. Inter-day precision was determined by repetitive analysis of QC samples (each concentration) on 5 consecutive days.

2.6.4. Recovery and ionization

To determine the recovery of caderofloxacin lactate by the precipitation method, plasma samples were spiked with caderofloxacin lactate at concentrations of 10, 100 and 1000 ng/mL. The recovery was assessed by comparing the peak-area ratios (analyte:internal standard) obtained from spiked plasma samples of different analyte concentrations to the peak-area ratios for the samples containing the equivalent amounts of the analyte and internal standard directly dissolved in the mobile phase.

Ion suppression of ionization was evaluated by comparing the absolute peak areas of control plasma extracted and then spiked with a known amount of caderofloxacin lactate, to neat standards injected directly in the same reconstitution solvent.

2.6.5. Stability

The effects of three freeze–thaw cycles and at room temperature in plasma for 4 h on the compound stability were evaluated by repeated analysis ($n = 3$) of QC samples. Long-term stability in plasma was also tested by assaying frozen QC samples after storage at −20 °C for 6 months. The amount of caderofloxacin lactate in these plasma samples was determined using a newly prepared calibration curve. Stability was expressed as a percentage of nominal concentration.

2.6.6. Application of the assay

The developed LC–ESI-MS assay method was used in the pharmacokinetic study after intravenous (i.v.) (6 mg/kg) and intragastic (i.g.) (6 mg/kg) administration of caderofloxacin lactate to rats. Animals were fasted for 12 h before dosing and 4 h afterwards, with free access to water. For intravenous route, caderofloxacin lactate powder dissolved in isotonic saline was delivered using a 1 mL syringe into a rat's femoral vein. For oral route, the same dosing solution was directly delivered by intragastric administration. The preparations were made immediately before drug administration. About 0.3 mL blood samples via the post-orbital venous plexus veins were collected in heparinized tubes at 3, 5, 10, 15, 30, 45, 60, 90, 120, 240, 360 and 480 min after intravenous (i.v.) (6 mg/kg) administration and at 5, 10, 15, 30, 45, 60, 90, 120, 240, 360, 480 and 600 min after intragastic (i.g.) (6 mg/kg) administration. The blood sample was transferred into a heparinized eppendoff tube and mixed gently, and then centrifuged (8000 rpm, 5 min) to obtain 100 μL plasma, which was kept at −20 °C until analysis.

Pharmacokinetic parameters were calculated from the plasma concentration–time data. The elimination half-life ($T1/2k_e$) was determined by linear regression of the terminal portion of the plasma concentration–time data. The area under the plasma concentration–time curve from zero to the last measurable plasma concentration point ($AUC_{0-\tau}$) was calculated by the linear trapezoidal method.

3. Results and discussion

3.1. Liquid chromatography and mass spectrometry

It was clear that the analyte and IS both of formed predominantly protonated molecules $[M+H]^+$ in the scan spectra, with m/z at 412.00 for caderofloxacin and 332.00 for IS ciprofloxacin. Representative chromatograms for caderofloxacin and ciprofloxacin in actual plasma samples were presented in Fig. 2.

As shown in Fig. 2, there were no endogenous compounds or other impurities interfered with the assay. The retention time of caderofloxacin and ciprofloxacin (IS) were approximately 5.7 and 5.6 min, respectively. The overall chromatographic run time was finished within 10 min.

3.2. Method validation

3.2.1. Linearity

The linear regression analysis of caderofloxacin lactate was constructed by plotting the peak-area ratio of caderofloxacin against the internal standard (y) versus analyte concentration

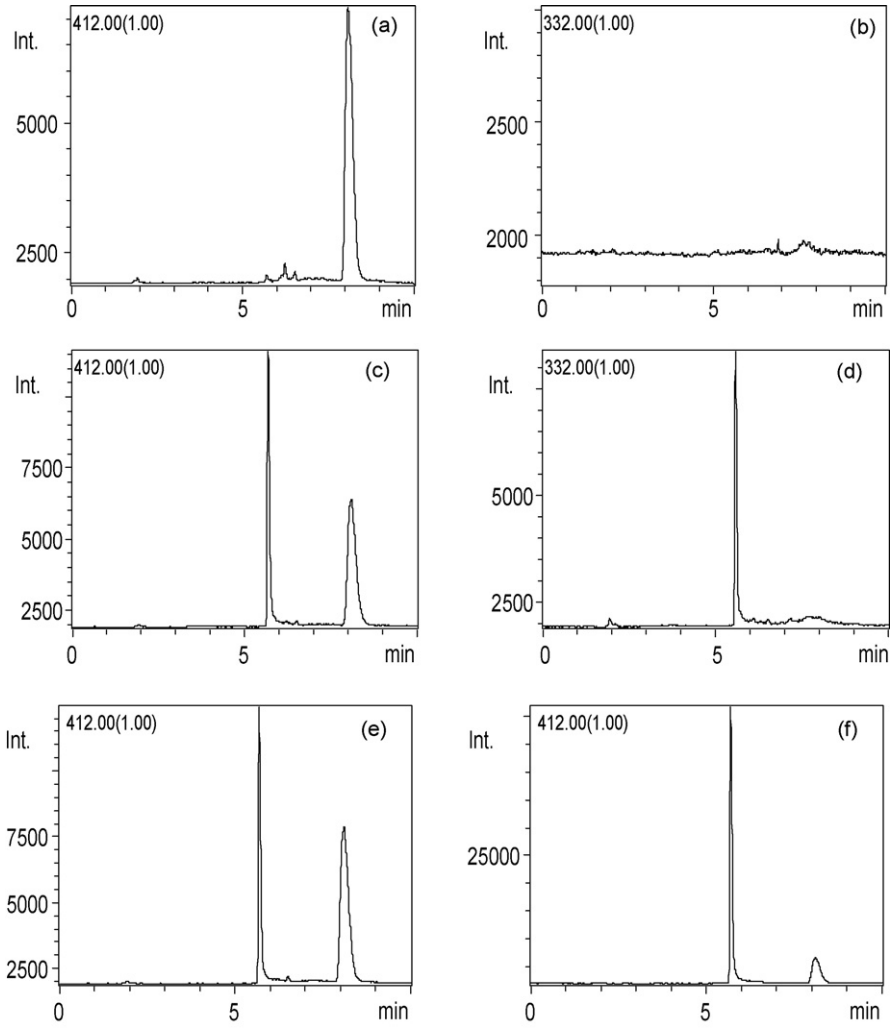

Fig. 2. Representative chromatograms obtained following extraction of (a and b) blank rat plasma; (c) blank plasma spiked with caderofloxacin lactate (500 ng/mL); (d) blank plasma spiked with ciprofloxacin (IS, 5.0 μg/mL); (e) a rat plasma sample 15 min after oral administration of caderofloxacin lactate (6 mg/kg); (f) a rat plasma sample 15 min after intraveanous administration of caderofloxacin lactate (6 mg/kg).

(ng/mL) in spiked plasma samples (x). The calibration curves were constructed in the range of 5–2000 ng/mL. The average regression equation of these curves and their correlation coefficients (r) were calculated as follows: $y = 0.0033x - 0.0022$ ($r = 0.9998$, $n = 5$). It showed good linear relationships between the peak areas and the concentrations. For caderofloxacin lactate, the lowest limit of quantification (LLOQ) was 5 ng/mL and the lowest limit of detection (LLOD) was 2 ng/mL.

3.2.2. Accuracy and precision

The intra-day precision (presented as relative standard deviation, R.S.D.) was shown in Table 1. The precision for concentrations of 10, 100 and 1000 ng/mL caderofloxacin lactate were 6.39, 7.30and 4.01%, respectively, and the accuracy, defined as (measured concentration/spiked concentration) × 100%, reached from 92.30 to 107.22% throughout the three concentrations examined. The inter-day precision was studied over 5 days, and the results were also given in Table 1. The precision ranged from 5.20 to 7.51%, and the accuracy reached from 95.91 to 102.46% throughout the three concentrations examined.

3.2.3. Recovery and ionization

The recoveries of caderofloxacin lactate added to rat plasma were from 88.80 to 93.05%, which were given in Table 1. The results showed that there was no significant difference in the signals of analytes extracted from rat plasma and from the mobile phase, indicating that there were no matrix effects.

3.2.4. Stability

Stability of caderofloxacin lactate during sample handling (freeze–thaw, short-term temperature and stored at −20 °C for 6 months) were shown in Table 1. Caderofloxacin lactate was stable for at least 4 h at room temperature in plasma samples, and mean recoveries from the nominal concentration were more than 94%, respectively, at 10, 100 and 1000 ng/mL. Caderofloxacin was stable in plasma samples when stored at −20 °C for a 6-

Table 1
Accuracy, precision, recovery and stability of caderofloxacin lactate from spiked rat plasma

	Spikedconcentration(ng/mL)		
	10	100	1000
Accuracy and precision			
Intra-day precision ($n=5$)			
Measured concentration (ng/mL)	9.23 ± 0.59	96.53 ± 7.05	1072.24 ± 42.96
Accuracy (%)	92.30 ± 5.90	96.53 ± 7.05	107.22 ± 4.30
R.S.D. (%)	6.39	7.30	4.01
Inter-day precision ($n=5$)			
Measured concentration (ng/mL)	9.59 ± 0.72	101.98 ± 4.23	1024.56 ± 53.28
Accuracy (%)	95.90 ± 7.20	101.98 ± 4.23	102.46 ± 5.33
R.S.D. (%)	7.51	4.15	5.20
Recovery			
Measured concentration (ng/mL)	8.88 ± 0.72	93.05 ± 2.88	925.98 ± 33.37
Recovery (%)	88.80 ± 7.20	93.05 ± 2.88	92.60 ± 3.34
Stability of samples ($n=5$)			
Freeze–thaw (three cycles) (%)	90.81	97.12	102.63
Room temperature (for 4 h) (%)	94.14	99.41	103.72
Stored at $-20\,^{\circ}C$ (for 6 months) (%)	92.00	93.12	91.29

month period and following three freeze–thaw cycles. Mean recoveries from nominal concentration were all more than 90%.

3.2.5. *Pharmacokinetic study of caderofloxacin in rats*

The assay was used to obtain pharmacokinetic data for caderofloxacin lactate in rat plasma after i.v. administration (6 mg/kg) and i.g. administration (6 mg/kg). Fig. 3 shows application of the LC–ESI-MS method developed here to in vivo pharmacokinetic studies in rats. The pharmacokinetic parameters of caderofloxacin lactate were estimated as Section 2.6.6. The area under the plasma concentration ($AUC_{0-\tau}$) of caderofloxacin lactate after i.v. and i.g. administrations were3076.10 ± 832.88 ng/(mL h) and 2112.10 ± 550.18 ng/(mL h), respectively. The $T1/2k_e$ were 90.66 ± 24.89 min (i.v.) and 126.24 ± 41.26 min (i.g.). The absolutely bioavailability of caderofloxacin lactate was found to be 68.6%. The LLOQ of 5 ng/mL was sensitive enough for the pharmacokinetics research of caderofloxacin lactate.

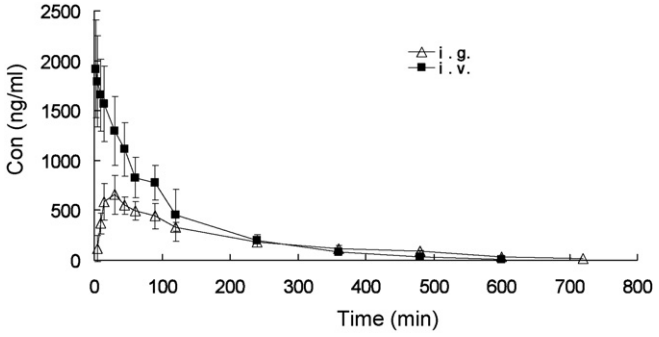

Fig. 3. Mean plasma concentration–time profile of caderofloxacin lactate after i.g. and i.v. administration of 6 mg/kg caderofloxacin lactate to six rats.

4. Conclusion

A reliable and sensitive LC–ESI-MS method for the analysis of caderofloxacin lactate in rat plasma had been successfully developed and validated. To obtain caderofloxacin from the plasma, an improved precipitation with methanol was used. This method demonstrated a relatively short analysis time and the acceptable sensitivity, precision, accuracy, selectivity, recovery and stability. The method was successfully applied to a pharmacokinetic study of caderofloxacin lactate in rats. And to our knowledge, it is the first report of LC–ESI-MS method on the determination of caderofloxacin lactate concentration in vivo.

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