Journal of Chromatography B, 957 (2014) 36–40

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short Communication

Determination of sitafloxacin in human plasma by liquid chromatography–tandem mass spectrometry method: Application to a pharmacokinetic study

Kai Huang, Jie Yang, Jing Zhang, Ying Ding, Lan Chen, Wen-Yan Xu, Xue-Jiao Xu, Ru Duan, Qing He*

Drug Clinical Trial Institution, Wuxi People' Hospital, Nanjing Medical University, Wuxi 214023, China

ARTICLE INFO

Article history:
Received 23 December 2013
Accepted 3 March 2014
Available online 12 March 2014

Keywords:
Sitafloxacin
Plasma concentration
LC–MS/MS
Pharmacokinetics

ABSTRACT

A high-performance liquid chromatographic–tandem mass spectrometric (HPLC–MS/MS) method was developed and validated to determine sitafloxacin in human plasma with dextrorphan as internal standard. Chromatographic separation was performed on a ZORBAX SB-C18 column (3.5 μm, 2.1 mm × 100 mm) with the mobile phase of methanol/water (containing 0.1% formic acid) (46:54, v/v) at a flow rate of 0.2 mL/min. Quantification was performed using multiple-reaction monitoring of the transitions at *m/z* 410.2 → 392.2 for sitafloxacin and *m/z* 258.1 → 157.1 for dextrorphan, respectively. The calibration curve was linear over the range of 5–2500 ng/mL with the lower limit of quantification of 5 ng/mL for sitafloxacin. The intra- and inter-day precisions were less than 8.3% and the deviations of assay accuracies were within ±4.1%. Sitafloxacin was sufficiently stable under all relevant analytical conditions. This method was successfully applied to the pharmacokinetic study of sitafloxacin in healthy Chinese volunteers.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Sitafloxacin {(2)-7-[(7S)-7-amino-5-azaspiro[2,4]heptan-5-yl]-8-chloro-6-fluoro -1-[(1R,2S)-2-fluoro-1-cyclopropyl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid sesquihydrate}, a new fluoroquinolone antimicrobial agent, is generally used for the treatment of systemic bacterial infections [1]. On the one hand, sitafloxacin exhibits effective inhibitory activities against aerobic and anaerobic Gram-positive and -negative bacteria, *Chlamydia* spp. and *Mycoplasma* spp. On the other hand, it also shows marked antibacterial effects on quinolone-resistant methicillin-resistant *Staphylococcus aureus*, *Pneumococcus* spp. and *Pseudomonas* spp. [2,3]. Furthermore, this antibiotic was proved to inhibit DNA gyrase and topoisomerase in bacteria much more than other quinolones [4,5]. Therefore, sitafloxacin possesses the widest spectrum and strongest antibacterial effects among newly available quinolones.

Sitafloxacin has excellent pharmacokinetics, as characterized by its high serum levels, good oral bioavailability and extensive distribution into many tissues [6]. Renal excretion is the major route of elimination of sitafloxacin and metabolism plays only a small role [7]. Thus, the overall systemic antibacterial property is primarily contributed from sitafloxacin itself. Since 2008, sitafloxacin has been approved for clinical use in Japan, a growing body of clinical evidence has indicated that this drug is extremely beneficial for treating pneumonia, cystitis and pyelonephritis [8].

Up to now, several analytical methods for measuring sitafloxacin in human plasma have been reported, such as, LC-fluorescence [9,10] and LC–UV [11]. However, these methods often suffer from disadvantages including long analysis time and complex analytical procedures, which make them unsuitable for analyzing large numbers of samples. To the best of our knowledge, no entirely validated LC–MS/MS method for the quantification of sitafloxacin in human plasma has been reported so far.

The purpose of present study was to establish a simple, rapid and sensitive LC–MS/MS method for the pharmacokinetic study of sitafloxacin in healthy Chinese volunteers.

2. Experimental

2.1. Materials and reagents

Sitafloxacin (purity: 99.7%) was provided by Haiyue Pharmaceutical Co., Ltd (Changchun, China). Dextrorphan (IS, purity: 98%) was purchased from Toronto Research Chemicals Inc. (Canada).

* Corresponding author. Tel.: +86 10 85350347; fax: +86 10 85350351.
E-mail address: najang@126.com (Q. He).

http://dx.doi.org/10.1016/j.jchromb.2014.03.004
1570-0232/© 2014 Elsevier B.V. All rights reserved.

HPLC grade of methanol was obtained from CNW Technologies GmbH (Dusseldorf, Germany). All other chemicals were of analytical reagent grade. Deionized water was produced using a Milli-Q system (Millipore, USA). Blank plasma used in this study was supplied by Wuxi People's Hospital Blood Bank.

2.2. Instruments and chromatographic-mass conditions

The samples were analyzed using a LC–MS/MS system that consisted of a Accela Surveyor auto-sampler, a Accela 1250 pump and a TSQ Qantum Access TM triple quadrupole mass spectrometer with an electrospray ionization (ESI) source. Xcalibur 1.4 software was used for data acquisition and processing (Thermo Finnigan, USA). The HPLC separation was performed with a ZORBAX SB-C_{18} analytical column (3.5 μm, 2.1 mm × 100 mm, Agilent), which was maintained at 35 °C. The mobile phase consisted of methanol/water (containing 0.1% formic acid) (46:54, v/v) was run at a flow rate of 0.2 mL/min. The total analytical runtime was 4.0 min.

The mass spectrometer was operated in positive ESI mode and the transitions monitored in the multiple-reaction monitoring (MRM) mode were m/z 410.2 → 392.2 with a collision energy (CE) of 22 eV for sitafloxacin and m/z 258.1 → 157.1 with CE of 37 eV for dextrorphan. The optimized parameters for monitoring the analytes were as follows: spray voltage, sheath gas, auxiliary gas, collision gas (argon) pressure and capillary temperature were 3500 V, 20 psi, 10 L/min, 1.5 mTorr and 350 °C, respectively.

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

A stock solution of sitafloxacin was prepared in methanol/water (containing 0.1% formic acid) (50:50, v/v) at a concentration of 1.0 mg/mL. Standard solutions (25, 50, 250, 500, 2500, 5000, 10,000 and 12,500 ng/mL) and quality control (QC) solutions (50, 2500 and 10,000 ng/mL) were prepared by serial dilution of the sitafloxacin stock solution with methanol/water (containing 0.1% formic acid) (50:50, v/v). The working solution of IS (250 ng/mL) was obtained by diluting a stock solution of dextrorphan (1.0 mg/mL) with methanol/water (50:50, v/v). All the solutions were stored at −20 °C prior to use.

Calibration curves were prepared by spiking 10 μL the appropriate standard solution into 50 μL of blank human plasma. QC samples were prepared by adding the stock solution of sitafloxacin into blank plasma to obtain the final concentrations of 10, 500 and 2000 ng/mL, which represented low, medium and high concentration of QC samples, respectively.

2.4. Sample preparation

A 50 μL of plasma sample was mixed with 10 μL of IS working solution (250 ng/mL). Then 1 mL of isopropanol was added and the mixture was vortexed for 5 min. After centrifugation at 13,000 rpm for 5 min, the supernatant was separated and evaporated to dryness at 40 °C in a vacuum concentration system (Concentration plus, Eppendorf AG, Germany). The dry residue was reconstituted with 400 μL of mobile phase and vortex-mixed for 1 min, 5 μL of supernatant was injected for LC–MS/MS system analysis.

2.5. Method validation

2.5.1. Selectivity

Selectivity was investigated by comparing chromatograms of six individual blank human plasma samples with a plasma sample spiked with sitafloxican and IS. Chromatographic peaks of analytes were identified on the basis of their MRM responses and retention times.

2.5.2. Linearity

The linearity of this method for the determination of sitafloxacin was evaluated by a calibration curve in the range of 5–2500 ng/mL. Calibration curves were created by plotting the peak area ratio of sitafloxacin to internal standard versus concentrations of sitafloxacin in plasma with $1/x^2$ weighted regression. The coefficient of correlation (r^2) should be more than 0.99. The LLOQ was defined as the lowest concentration of analyte determined with acceptable precision and accuracy (R.S.D.% did not exceed 20% and R.E.% was within ±20%). Moreover, signal-to-noise ratio (S/N) of analyte at this concentration level was at least 10.

2.5.3. Accuracy and precision

The intra- and inter-day accuracy and precision were determined by assaying five replicates of QC samples at three different concentrations (10, 500 and 2000 ng/mL for sitafloxacin) on five consecutive days. The variability of determination was depicted as the relative error (R.E.%) and relative standard deviation (R.S.D.%), respectively. The R.E.% must be within ±15% and R.S.D. % should not exceed 15%.

2.5.4. Matrix effect and recovery

The matrix effect was evaluated by comparing the peak areas of analytes spiked in pretreated blank samples from six individuals (A) with those of standard solutions in the mobile phase (B). The ratio (A/B) is defined as the matrix factor, the variability in matrix factors should be less than 15%.

Recoveries of sitafloxacin were evaluated by analyzing five replicates at each QC levels of 10, 500 and 2000 ng/mL. The recovery was determined by comparing the peak areas of processed QC samples with those of standard solutions.

2.5.5. Stability

The stability of sitafloxacin was assessed under various conditions using three levels of QC samples. Short-term stability was determined by analyzing samples kept at room temperature for 6 h. Long-term stability was examined by assaying samples stored at −80 °C for 66d. Freeze–thaw stability was investigated by testing samples after three freeze/thaw cycles (−80–24 °C). Additionally, Post-preparative stability was studied by analyzing samples left in auto-sampler vials at 24 °C for 24 h. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15% R.E.) and precision (15% R.S.D.).

2.5.6. Pharmacokinetic study

Eleven healthy Chinese volunteers, 6 males and 5 females, with a mean age of 22.8 ± 1.8 years, were included and all obtained written informed consents. The protocol was approved by the Ethics Committee of Wuxi People's Hospital. After an overnight fasting, volunteers received a single oral dose of 100 mg sitafloxacin tablets. Serial blood samples were collected prior dosing and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 24, 36 and 48 h post-dosing. The blood samples were placed in heparinized tubes and centrifuged at 3500 rpm for 10 min at 4 °C to obtain the plasma. All samples were stored at −80 °C until analyzed.

The pharmacokinetic parameters of sitafloxacin were calculated using DAS 3.0 pharmacokinetic program based on non-compartmental analysis (Chinese Mathematical Pharmacological Society).

3. Results and discussion

3.1. Method development

The mass spectrometric conditions were optimized for sitafloxacin to obtain the maximal sensitivity. Both positive and

Download English Version:

https://daneshyari.com/en/article/1212473

Download Persian Version:

https://daneshyari.com/article/1212473

Daneshyari.com