Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 637–640

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Determination of nifeviroc, a novel CCR5 antagonist: Application to a pharmacokinetic study

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ARTICLE INFO

Article history:
Received 6 March 2011
Received in revised form 26 June 2011
Accepted 28 June 2011
Available online 5 July 2011

Keywords:
Nifeviroc
CCR5 antagonist
LC-ESI-MS/MS
Pharmacokinetics

ABSTRACT

Nifeviroc is a novel CCR5 antagonist used for the treatment of HIV type-1 infection. A LC-ESI-MS/MS method for the determination of nifeviroc in human plasma was developed and validated. The calibration curve ($r^2 = 0.9993$) of nifeviroc was established at the range of 1.924–2935 $\mu g\,L^{-1}$. The intra- and inter-day precisions (RSD%) were all less than 7%, and the accuracies at three concentration levels were all within $100 \pm 5\%$. This validated method was then successfully applied to a pharmacokinetic study in health Chinese volunteers.

1. Introduction

AIDS is a potentially fatal condition resulting from the failure of the immune system and Human immunodeficiency virus (HIV) is the causative pathogen. The AIDS epidemic continues to be a significant global threat causing a death of 25 million. Although highly active antiretroviral therapy (HAART) was successful in reducing the AIDS-related mortality rates dramatically, the rapid emergence of viral strains resistant to current clinical treatments and the dynamic nature of the HIV-1 genome demand a continuous effort to identify alternative points of intervention in the viral life cycle. Targeting the viral entry process has become a new focus on research of the next generation of HIV antiretroviral therapies [1].

CCR5 is an essential co-receptor for HIV-1 recognition which entry into CD4+ macrophages and T-cells, but not essential for human functions [2–4]. Many pharmaceutical companies and academic institutions have been enthusiastically investigating potent antagonists against CCR5 as viral entry inhibitors [5], and indeed several small-molecule CCR5 antagonists, including nifeviroc [6], UK-427857 [7], GW-873140 [8] and TAK 220 [9], are now being evaluated in clinical trials. Among them, UK-427857 (maraviroc) has become the first CCR5 antagonist approved by FDA for the treatment of HIV infection [10], and nifeviroc was approved by SFDA to enter phase I clinical trial.

In this article, a method for the determination of the novel CCR5 antagonist by LC-ESI-MS/MS was developed and validated. The method has been successfully applied to a pharmacokinetic study involving twenty-four healthy Chinese volunteers. And the main pharmacokinetic parameters of nifeviroc are reported for the first time.

2. Material and methods

2.1. Apparatus

The chromatography was carried out using an Agilent 6410B triple quadrupole LC/MS system (Agilent Technologies, Waldbrom, Germany) equipped with an atmospheric pressure chemical ionization (APCI) source. The system was controlled by an Agilent MassHunter workstation software version B.01.04.

2.2. Chemicals and reagents

Pure nifeviroc and TD0591, as internal standard (IS), were both provided by Shanghai Targetdrug Co. Ltd (Shanghai, China). The molecular structure of nifeviroc and TD5091 are shown in Fig. 1. Acetonitrile and methanol were HPLC grade (Tedia, Fairfield, USA). Deionized water was purified through a Milli-Q water purification system (Millipore, Milford, USA). All other chemicals were of the analytical grade from commercial sources.

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doi:10.1016/j.jpba.2011.06.027

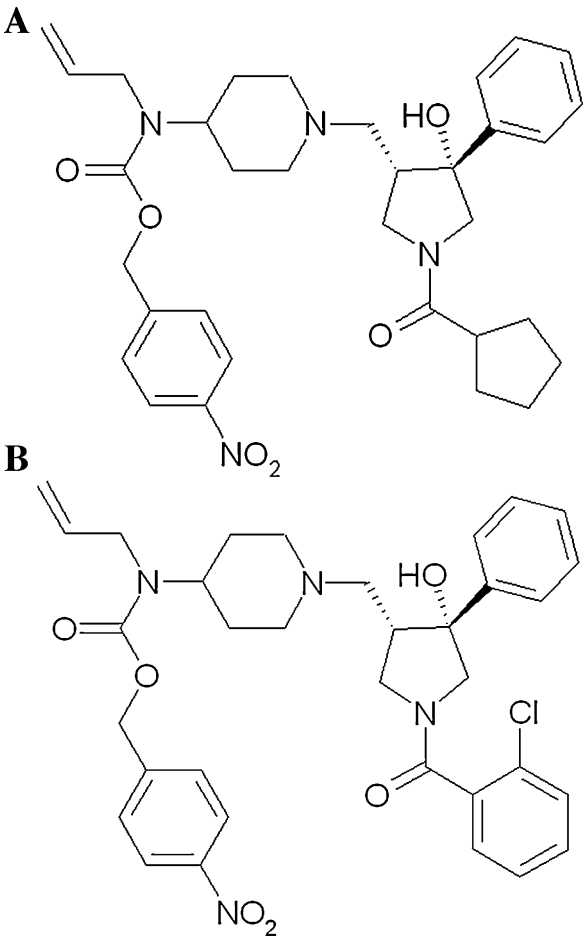

Fig. 1. The molecular structure: (A) nifeviroc; (B) TD0591.

2.3. Chromatographic conditions

The chromatographic separation was performed on a TC-C18 column (5 μm, 150 mm × 2.1 mm i.d., Agilent, Walbdbrom, Germany), operated at 30 °C. A Gemini C18 column (5 μm, 3.0 mm × 4.0 mm i.d., Phenomenex, Torrance, USA) was employed as guard column. The mobile phase, formed by acetonitrile-50 mM ammonium acetate buffer (pH 3.5) (85:15, v/v), was delivered with a flow rate of 0.2 mL min^{-1}, and the injection volume was 10 μL.

2.4. Mass spectrometric conditions

Mass spectrometric quantification was carried out in multiple reaction monitoring (MRM) mode, monitoring ion transitions of *m*/*z* 591.3–332.2 for nifeviroc, and *m*/*z* 633.2–332.2 for the internal standard in positive ion mode. The MS–MS operating parameters were set with a drying gas (N_2) flow of 9 L min^{-1}, nebulizer pressure of 40 psi, drying gas temperature of 325 °C, capillary voltage of 4 kV.

2.5. Preparation of standard and quality control (QC) samples

A stock solution of nifeviroc was prepared in methanol at the concentration of 117.4 mg L^{-1}. Nifeviroc stock solution was further serially diluted with methanol to prepare the working standard solutions at the concentrations of 29.35, 11.74, 4.696, 1.878, 0.7514, 0.3005, 0.1202, 0.04809 and 0.01924 mg L^{-1}. Then 50 μL of each working standard solution of nifeviroc was evaporated under gentle nitrogen flow at 40 °C, and 500 μL of blank human plasma was added to prepare the standard plasma sample (2935, 1174, 469.6, 187.8, 75.14, 30.05, 12.02, 4.809, 1.924 μg L^{-1}). And the quality control (QC) samples were set at three concentration levels of 4.809, 75.14 and 2935 μg L^{-1}. All standard samples and QC samples were freshly prepared daily.

The internal standard was dissolved in methanol to obtain a stock solution at the concentration of 828 mg L^{-1}, and the solution was then diluted in methanol to prepare internal standard working solution at 828 μg L^{-1}.

2.6. Sample preparations

After adding 50 μL of IS working standard solution (828 μg L^{-1} TD0591 in methanol) to 250 μL of plasma sample, 750 μL of methanol was added. After vortex mixing for 2 min and centrifuging at 15,400 × *g* for 10 min at 4 °C, the plasma protein was precipitated. 10 μL of the supernatant was injected into the LC-ESI-MS/MS system.

2.7. Method validation

The analytical method was validated to demonstrate the specificity, linearity, LLOQ, accuracy and precision, matrix effects and recovery of measurement. Specificity was verified by the lack of interference peaks to nifeviroc and internal standard chromatographic peaks in LLOQ samples. Linearity was tested at nine levels of concentration covering a range from 1.924 to 2935 μg L^{-1}, and the lowest concentration level was set as LLOQ. The intra-day accuracy and precision of the assay were determined by assaying quality control samples in five replicates at three concentration levels (4.809, 75.14 and 2935 μg L^{-1}) within the same day ($n = 5$). The inter-day accuracy and precisions were determined by analyzing of QC samples on three different days ($n = 15$). By comparing the response of sample prepared by spiking analyte to blank plasma processed solution with that prepared by spiking analyte to mobile phase, the matrix effects were studied in three nifeviroc concentration levels and one IS concentration level. Extraction recoveries were studied at the same concentrations of nifeviroc and IS by comparing the response of standard plasma sample processed with the above method with that of standard solution at the same concentrations.

All stability tests were performed with three replicates at the concentration levels of 4.809, 75.14 and 2935 μg L^{-1}. The short-term stability of plasma samples was evaluated at ambient temperature (~22 °C) over 4 h. The long-term stability at −20 °C was examined for 30 days. The freeze-thaw stability was determined after three freeze-thaw cycles (room temperature to −20 °C). The post preparation stability was evaluated at room temperature over 24 h.

3. Application

This method was applied to a pharmacokinetic study of nifeviroc after multiple doses of nifeviroc tablets at an escalation dosage range of 200–800 mg by oral administration in healthy volunteers.

The pharmacokinetic study was approval by the State Food and Drug Administration of China and the Clinical Research Ethics Committee of Tumor Hospital of Hunan Province. Written informed consent was obtained from each healthy volunteer prior to enrollment in the study. All subjects aged ≥18 years had to undergo a physical examination, ECG evaluation, hematological and blood chemistry test, urine chemistry test and a thorough medical history to determine all inclusion criteria were met. All subjects were non-smokers and free from any prescription and non-prescription medications two weeks before the study.

A randomized, placebo-controlled, dose escalation trial design was used. All subjects received oral nifeviroc three times per day

for 7 consecutive days, and there were two cases of the placebo in each group (1 case of men and 1 case of women). Trial was started from the low dose group. Dose escalation was not permitted in individual subjects. Blood samples were collected at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0 h after the last dose administration. Blood samples were centrifuged for 5 min at 12,000 × *g* at 4 °C. The plasma samples were collected and stored at −20 °C prior to bio-analysis.

The pharmacokinetic parameters were compared using analysis of variance with the software SPSS 13.0, *P* values <0.05 were considered statistically significant. Peak plasma concentrations and the times at which they occurred were determined by inspection of the individual patient's nifeviroc concentration–time curves. The elimination half-life ($t_{1/2}$) was determined from linear least squares regression of the day 5 plasma concentration–time points in the terminal log-linear region of the plasma concentration–time profiles. The area under the plasma concentration–time curve (AUC) from time 0 to 48 h after oral administration was calculated using the linear trapezoidal rule. Clearance (CL) was estimated by dividing the dose (in milligrams per meters squared) by the AUC on day 7.

4. Results and discussion

4.1. Chromatography

The selectivity of the assay was assessed by extracting samples of six different batches of blank human plasma, and then comparing the chromatogram of blank plasma with the corresponding plasma samples spiked with IS and nifeviroc (1.924 μg L^{-1}). The retention time of nifeviroc and IS were about 2.9 min and 3.0 min, respectively. Typical chromatograms are shown in Fig. 2, which were visually inspected for endogenous interferences.

4.2. Method validation

The regression equation for calibration curves in the range of 1.924–2935 μg L^{-1} was $Y = 1.4766X + 0.0032$ ($r^2 = 0.9993$), indicating a good linearity. The lower limit of quantification (LLOQ) for nifeviroc was 1.924 μg L^{-1} with the signal-to-noise ratio (S/N) of over 10. The precision at this concentration was acceptable with RSD of 6.07% ($n = 6$) and accuracy was 104.6%, indicating a good sensitivity of the established method. Intra- and inter-day accuracies were respectively 99.3–103.7% and 101–103.7%, while intra- and inter-day precision were respectively 1.09–2.66% and 2.99–6.96%. The data demonstrate that the precision and accuracy of this assay are within the acceptable range and the method is accurate and precise. The recoveries of nifeviroc were 103.2%, 84.3% and 100.7% ($n = 5$) at 4.809, 75.14 and 2935 μg L^{-1}, respectively, while the mean recovery of internal standard was 95.7%, showing a good extraction recovery. The matrix effect (%) for nifeviroc at 4.809, 75.14 and 2935 μg L^{-1} were 95.3%, 104.5% and 104.3% ($n = 5$) respectively, while for the internal standard it was 103.0%. Stability tests showed that nifeviroc in human plasma was stable at room temperature for 4 h or at −20 °C for 30 days and the analyte was stable in plasma for three cycles when stored at −20 °C and thawed to room temperature. The post-preparative stability of QC samples showed that no significant degradation in the sample extract at room temperature for 24 h.

4.3. Application

Typical plasma concentration–time profiles of nifeviroc after oral administrating were shown in Figs. 3 and 4. The data were fitted well by one-compartment model with a weighting factor of $1/c^2$. Main pharmacokinetic parameters are shown in Table 1, which shows that there is large variation among individuals. And

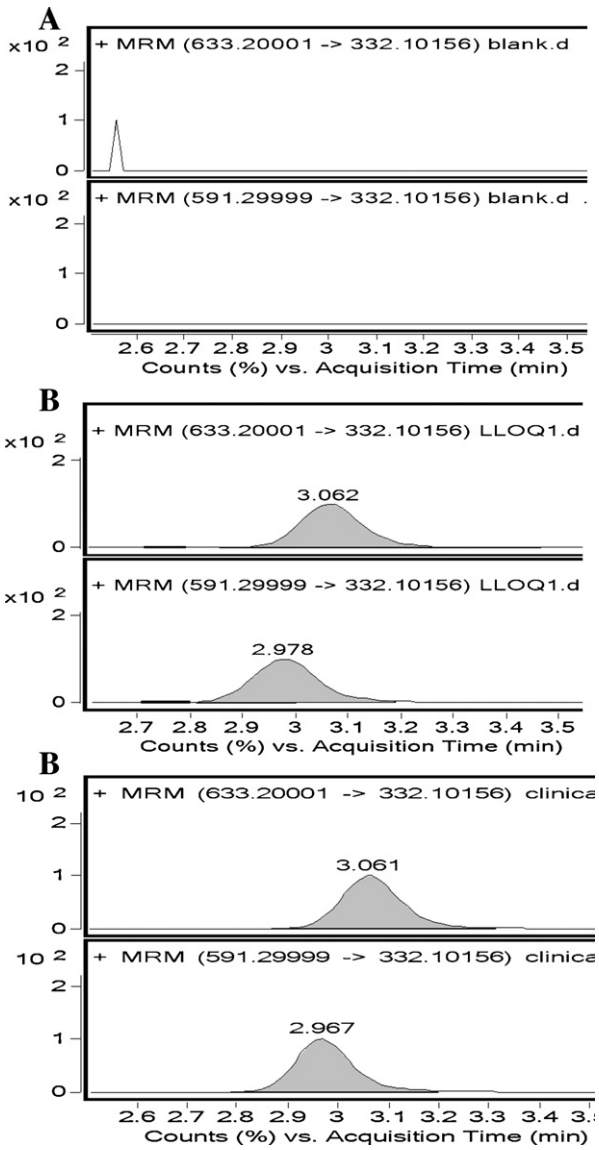

Fig. 2. Typical MRM chromatogram for nifeviroc and TD0591 (internal standard) in human plasma sample: (a) blank human plasma; (b) blank plasma spiked LLOQ level of nifeviroc (1.924 μg L^{-1}); (c) Plasma sample from a volunteer.

maximum plasma concentration of 400–200 mg was not doubled, furthermore, absorption half-life of 200 mg was not equals 400 mg, there is accumulation of nifeviroc in human. Accumulation of nifeviroc in the human body is related to not only inhibition of

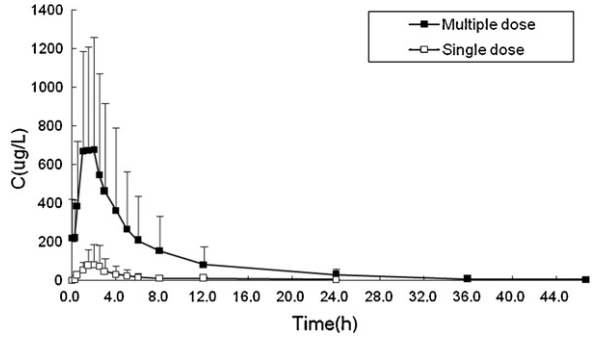

Fig. 3. Mean plasma concentration–time profile of nifeviroc in healthy volunteers after oral administration of single dose and multiple dose of 200 mg nifeviroc tablets ($n = 9$).

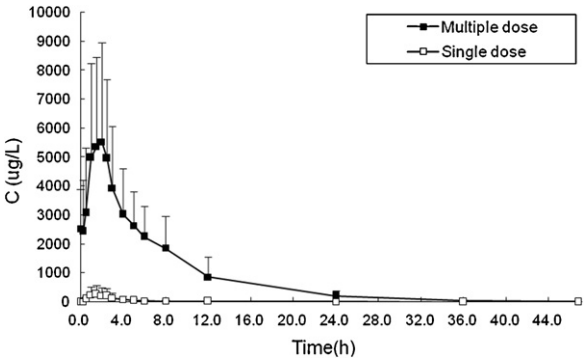

Fig. 4. Mean plasma concentration–time profile of nifeviroc in healthy volunteers after oral administration of single dose and multiple dose of 400 mg nifeviroc tablets ($n = 10$).

Table 1
Main pharmacokinetic parameters of nifeviroc.

	200 mg		400 mg	
	Mean	SD	Mean	SD
$t_{1/2}$ (h)	2.124	0.845	3.438	1.091
$AUC_{0\to t}$ ($mg\,L^{-1}\,h^{-1}$)	3594	3785	17,826	9076
$AUC_{0\to\infty}$ ($mg\,L^{-1}\,h^{-1}$)	3625	3806	17,983	9168
K_e (h^{-1})	0.355	0.123	1.574	0.600
CL ($L\,h^{-1}$)	0.126	0.123	0.027	0.011
T_{max} (h)	1.2	0.5	1.6	0.5
C_{max} ($mg\,L^{-1}$)	809.9	560.6	2946	1659

metabolism enzyme but also efflux transporter of absorption and inhibition of first pass effect. Nifeviroc is hardly excreted by the type of renal excretion. Further studies on nifeviroc absorption and metabolism in human are needed.

5. Conclusion

The pharmacokinetics of nifeviroc in human after oral administration has never been studied before. We developed and validated a sensitive and specific LC-ESI-MS/MS method for quantifying nifeviroc in human plasma. The method has been successfully applied to a pharmacokinetic study of nifeviroc involving nineteen healthy Chinese volunteers, and the main pharmacokinetic parameters of nifeviroc are obtained for the first time.

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