Journal of Chromatography B, 971 (2014) 52–57

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Quantitation of clevidipine in dog blood by liquid chromatography tandem mass spectrometry: Application to a pharmacokinetic study

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ARTICLE INFO

Article history:
Received 26 June 2014
Accepted 13 September 2014

Keywords:
Clevidipine
LC–MS/MS
Pharmacokinetics
Dog blood

ABSTRACT

Clevidipine, a vascular selective calcium channel antagonist of the dihydropyridine class, is rapidly metabolized by ester hydrolysis because of incorporation of an ester linkage into the drug molecule. To characterize its pharmacokinetic profiles in dogs, a simple, rapid and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for quantitation of clevidipine in dog blood. After one-step protein precipitation with methanol, the chromatographic separation was carried out on an Ecosil C_{18} column (150 mm × 4.6 mm, 5 μm) with a gradient mobile phase consisting of methanol and 5 mM ammonium formate at a flow rate of 0.5 mL/min. The quantitation analysis was performed using multiple reaction monitoring (MRM) at the specific ion transitions of m/z 454.1 $[M-H]^- \rightarrow m/z$ 234.1 for clevidipine and m/z 256.1 $[M-H]^- \rightarrow m/z$ 227.1 for elofesalamide (internal standard) in the negative ion mode with electrospray ionization (ESI) source. This validated LC–MS/MS method showed good linearity over the range 0.5–100 ng/mL with the lower limit of quantitation (LLOQ) of 0.5 ng/mL together with the satisfied intra- and inter-day precision, accuracy, extraction recovery and matrix effect. Stability testing indicated that clevidipine in dog blood with the addition of denaturant methanol was stable on workbench for 1 h, at −80 °C for up to 30 days, and after three freeze–thaw cycles. Extracted samples were also observed to be stable over 24 h in an auto-sampler at 4 °C. The validated method has been successfully applied to a pharmacokinetic study of clevidipine injection to 8 healthy Beagle dogs following intravenous infusion at a flow rate of 5 mg/h for 0.5 h.

1. Introduction

Acute arterial hypertension occurs in many clinical conditions, such as the operating room, intensive care and emergency care units [1,2]. Patients who suffer from acute arterial hypertension demand intravenous therapy to rapidly and effectively control the blood pressure [3,4]. Clevidipine, a vascular selective calcium channel antagonist of the dihydropyridine class, structurally related to felodipine, is used to effectively control the blood pressure through arterial-specific peripheral vasodilation in association with cardiac surgery. Clevidipine has the advantages of allowing precise titration to target blood pressure levels and maintenance of tight control over time because of its direct effect activity and ultra-short half-life [5–8].

The drug is pharmacologically active in the low nanomolar level, so a highly sensitive analytical method was required in order to characterize the pharmacokinetic profiles. Until now, only one article has been reported for the determination of clevidipine in dog blood using capillary gas chromatography–mass spectrometry with negative ion chemical ionization [9]. However, the GC–MS method is not prevalent any more for high-throughput analysis in pharmacokinetic studies, due to its time consuming and labor intensive sample pretreatment procedure. In current pharmacokinetic studies, LC–MS/MS is playing an important role for quantification of different kinds of drugs. Hence, it is necessary to develop a robust, sensitive, and simple LC–MS/MS method for the pharmacokinetic study of clevidipine. On the other hand, because of incorporation of an ester group into the drug molecule, clevidipine is rapidly metabolized to an inactive carboxylic acid metabolite by blood esterase [10]. Therefore, it is very crucial to avoid ester hydrolysis by enzymatic degradation when developing a method for the determination of clevidipine in biological matrix. In a previous study [9], sodium dodecyl sulfate (SDS) was used as an esterase

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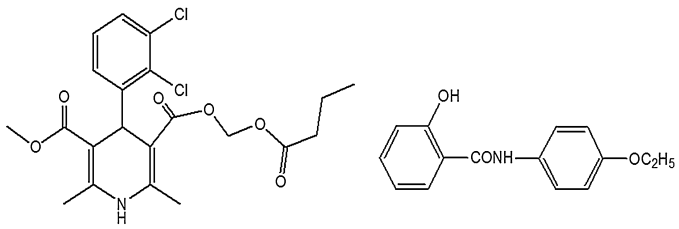
http://dx.doi.org/10.1016/j.jchromb.2014.09.019
1570-0232/



Clevidipine Etofesalamide

Fig. 1. Chemical structures of Clevidipine and Etofesalamide (IS).

inhibitor to prevent ester hydrolysis of clevidipine in blood, and liquid–liquid extraction was conducted prior to gas chromatographic determination. However, SDS, as a surfactant, is not an ideal esterase inhibitor for LC–MS/MS analysis, because it could contaminate the mass spectrometer.

In this study, a simple, robust and sensitive LC–MS/MS method was developed and validated for the quantification of clevidipine in dog blood. Methanol was used to inhibit ester hydrolysis of clevidipine and to simultaneously precipitate proteins, which was more time-saving and simpler than the mentioned method [9]. The validated assay successfully applied for the pharmacokinetic study of clevidipine injection following intravenous infusion at a flow rate of 5 mg/h for 0.5 h.

2. Experimental

2.1. Chemicals and materials

Clevidipine reference standard (Fig. 1, Purity: 99.6%) was kindly provided by the Centre for medicinal chemistry, Tianjin Institute of Pharmaceutical Research (Tianjin, China). Etofesalamide reference standard (internal standard, Fig. 1, Purity: 100%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The clevidipine injection (25 mg/mL, Batch No. 1312122131) was provided by the Centre for Pharmaceutical Formulation Design and Engineering, Tianjin Institute of Pharmaceutical Research (Tianjin, China). Methanol (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Analytical grade ammonium formate was purchased from Tianjin Guang Fu Fine Chemical Research Institute (Tianjin, China). All other reagents were of analytical grade. Deionized water was produced with a BM-40 quartz pure water distiller (Beijing Zhong Sheng Mao Yuan Tech Co. Ltd., Beijing, China). Freshly obtained drug-free heparinized dog blood was collected from the Beagle Dogs in our laboratory and stored at 4 °C before use.

2.2. Instrumentation and analytical conditions

Liquid chromatography tandem mass spectrometry (LC–MS/MS) analyses were performed using a Shimadzu high performance liquid chromatograph coupled with an AB SCIEX Triple Quad™ 5500 mass spectrometer. Analyst Ver. 1.5.2 software was used for data collection and processing (Applied Biosystems, Toronto, Canada).

Chromatographic separation was achieved on an Ecosil C_{18} column (150 mm × 4.6 mm, 5 μm) at 30 °C with a gradient mobile phase consisting of methanol and 5 mM ammonium formate at a flow rate of 0.5 mL/min. The gradient proportion of methanol was increased in a stepwise manner from 75% to 100% over 3.0 min, maintained at 100% for 6 min, and then returned to 75% within 0.1 s to re-equilibrate for 4 min prior to the next injection. The auto-sampler was maintained at 4 °C.

The mass spectrometer was operated in negative ion mode using electrospray ionization (ESI) source. The multiple reaction monitoring (MRM) modes were used to detect a specific transition of the precursor ion to the product ion at m/z 454.1 → 234.1 for clevidipine and m/z 256.1 → 227.1 for etofesalamide (IS). The optimal ESI-MS/MS parameters were as follows: the ion spray voltage and source temperature were −4500 V and 500 °C. The gas rates of nitrogen for nebulizing gas, turbo gas, curtain gas and collision gas were set to 40, 50, 16 and 5 psi, respectively. The declustering potential, collision energy, entrance potential and collision cell exit potential were set to −120, −20, −14 and −20 V for clevidipine, respectively, and −120, −16, −14 and −20 V for the IS, respectively.

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

Two stock solutions of clevidipine for standard solution and quality control (QC) were prepared from independent preparations by dissolving clevidipine in methanol to yield a concentration of 0.2 mg/mL. The working solutions of clevidipine were prepared by the serial dilution of stock solution with methanol to obtain the following clevidipine concentrations: 0.5, 1, 2, 5, 20, 50, and 100 ng/mL. Working solutions for QC samples with concentrations of 1, 10 and 80 ng/mL were prepared in the same manner. IS working solution was also prepared by diluting the IS stock solution (0.1 mg/mL) to a final concentration of 5 ng/mL with methanol. All the stock solutions were kept at −20 °C, and the working solutions were kept at 4 °C before use.

Calibration standards of clevidipine were prepared at the concentration ranged from 0.5 to 100 ng/mL by spiking 100 μL working solutions into 100 μL blank dog blood. QC samples were prepared in the same manner at three levels of 1, 10 and 80 ng/mL. All of the spiked blood samples were then treated according to sample preparation procedure. Both the calibration standard samples and the QC samples were applied in the method validation and the pharmacokinetic study.

2.4. Sample preparation

All blood samples were protected from light exposure during sample preparation. An aliquot of 100 μL working solutions, 100 μL IS working solution, 10 μL 5% ascorbic acid, and 700 μL methanol were added to 100 μL blood samples, followed by vortexing for 1 min, and then centrifuged at 12,000 rpm for 10 min. 10 μL of the supernatant was injected into the LC–MS/MS system for analysis.

2.5. Method validation

Method validation was performed according to guidelines set by the United States Food and Drug Administration (FDA) for bioanalytical method validation [11]. This method was validated in terms of specificity, linearity, lower limit of quantification (LLOQ), intra- and inter-day precision and accuracy, extraction recovery, matrix effect, and stability of the analyte during the sample storage and processing procedures.

2.5.1. Specificity

The specificity of the LC–MS/MS method was assessed by comparing the chromatograms of six different batches of the dog blank blood with the corresponding spiked blood samples, as well as the study samples. Chromatographic peaks of analyte and IS were identified on the basis of their retention times and MRM responses. Baseline noise should be <20% of analyte response at the LLOQ level [12].