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Determination of efonidipine in human plasma by LC–MS/MS for pharmacokinetic applications

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ABSTRACT

Efonidipine hydrochloride is a new generation dihydropyridine calcium channel blocker designed to inhibit both T-type and L-type calcium channels. For the first time, a simple and robust LC–MS/MS method was developed for the determination of efonidipine in human plasma over the range of 0.100–20.0 ng/mL. Efonidipine was extracted from plasma by an LLE procedure, separated by LC and detected by MS/MS in positive mode ESI. The method was validated for selectivity, carryover, sensitivity, extraction recovery, matrix effects, linearity, accuracy and precision, dilution integrity and stability studies. The calibration curves were linear over 0.100–20.0 ng/mL ($r \geq 0.9980$). The lower limit of quantification (LLOQ) was established at 0.100 ng/mL. Intra- and inter-day precisions (LLOQ, low-QC, mid-QC, high-QC and ultra-high QC) were less than 12.5% in terms of relative standard deviation (RSD), and accuracies were between −5.0% and 5.0% in terms of relative error (RE). Matrix effect was acceptable (105.6–110.2%) and extraction recovery was reproducible (85.8–91.3%, RSD ≤ 10.0%). Efonidipine was stable in the investigated conditions. The method was applied to the pharmacokinetics of efonidipine in human subject.

1. Introduction

Efonidipine hydrochloride is a new generation dihydropyridine (DHP) calcium channel blocker designed to inhibit both T-type and L-type calcium channels with long lasting vasodilator actions and little reflex tachycardia [1,2]. The typical DHP calcium antagonists, such as nifedipine and amlodipine, selectively inhibit L-type calcium channels of vascular smooth muscle cells, resulting in vasodilation. In addition to exhibiting an antihypertensive effect through vasodilation by blocking L-type calcium channels, efonidipine has also been proved to regulate heart rate (HR) by inhibiting the T-type calcium channels, which are localized primarily in the sinoatrial node and involved in the pacemaker mechanism of the heart [3].

Furthermore, efonidipine has distinct properties when compared with other calcium channel blockers. The studies indicated that efonidipine therapy simultaneously improves blood pressure, endothelial function, and metabolic parameters without substantially altering insulin sensitivity in nondiabetic patients with hypertension [4,5].

Chronic kidney disease (CKD) includes conditions that damage the kidneys and decrease their ability to maintain normal health. High blood pressure was one of the main causes of CKD. Previous clinical studies have demonstrated that antihypertensive therapy with efonidipine-based regimens improves kidney function. Such improvement is observed even in the presence of impaired renal function and appears to be independent of systemic blood pressure (BP) [6,7].

Currently, efonidipine hydrochloride is only available in Japan and South Korea, and is developing in China. No pharmacokinetic information of the oral use of efonidipine in human could be found. To understand the pharmacokinetics of efonidipine hydrochloride, the development of a rapid and robust method for the quantitation of efonidipine concentrations in human plasma is required. To the best of our knowledge, there has been no method reported for the determination of efonidipine in human plasma to date. Therefore, the objective of our study was to develop a simple LC–MS/MS assay for the determination of efonidipine in human plasma and demonstrate its potential application

Abbreviations: DHP, dihydropyridine; HR, heart rate; CKD, chronic kidney disease; BP, blood pressure; IS, internal standard; LLOQ, lower limit of quantification; QC, quality control; ULOQ, upper limit of quantification; ESI, electrospray ionization; DP, declustering potential; CE, collision energy; RSD, relative standard deviation; RE, relative error; BMI, body mass index; ECG, electrocardiogram; ISR, incurred sample reanalysis.

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in the study of pharmacokinetics of efonidipine in human subject.

2. Experimental

2.1. Materials

Efonidipine hydrochloride monoethanolate (99.0%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Cilnidipine (99.6%, internal standard (IS)) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade methanol was purchased from Fisher Scientific (Waltham, USA). Ultra-pure grade water used throughout the study was prepared from deionized water. All other chemicals and solvents were commercially available analytical grade materials used without further purification. Blank (drug free) human plasma was obtained from Aerospace Center Hospital (Beijing, China). The tablets of efonidipine hydrochloride 20 mg were provided by Beijing JiaLin Pharmaceutical Co., Ltd. (Beijing, China).

2.2. Preparation of stock solution

Master stock solutions were prepared individually by dissolving efonidipine and cilnidipine in methanol at concentrations of 1.00 mg/mL. Each stock solution was stored in amber Eppendorf tubes at −20 °C, after a brief vortex. Working stock solutions in methanol–water (3:1, v/v) were prepared serially from the master stock solutions and stored at −20 °C. The lower limit of quantification (LLOQ) and quality control (QC) samples were spiked independently from the calibration standards, using separately prepared master stock solutions. The IS working solution was prepared at 250 ng/mL by diluting the stock solution with methanol–water (3:1, v/v).

Calibration standards in blank human heparinized plasma were prepared fresh daily in duplicate at final plasma concentrations of 0.100, 0.300, 1.00, 3.00, 10.0, 15.0, and 20.0 ng/mL for every analytical run. QC samples were prepared at final concentrations of 0.100 (LLOQ), 0.250 (low-QC), 3.00 (mid-QC), 16.0 (high-QC) and 80.0 ng/mL (5-fold dilution QC, ultra-high QC) by adding the required amount of working stock solution to plasma. QC samples were vortexed, aliquotted and stored at −80 °C until analysis.

2.3. Sample preparation

Frozen plasma samples were thawed at 25 °C and vortex-mixed briefly. To a 500 μL aliquot of human plasma in 10 mL glass tube, 100 μL of IS working solution (250 ng/mL), 50 μL of methanol–water (3:1, v/v) and 200 μL of 1 M sodium hydroxide were added. The sample was vortexed for 30 s and 3 mL of diethyl ether–dichloromethane (3:2, v/v) was added. The mixture was vortex-mixed for 60 s, then shaken on mechanical shaker for 10 min. After centrifugation at 3500 rpm for 10 min, the upper organic layer was then transferred into another set of glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 150 μL of methanol–water (3:1, v/v). A 10 μL aliquot of the solution was injected into the LC–MS/MS system for analysis. Samples at the concentration higher than the upper limit of quantification (ULOQ), including the ultra-high QC samples, should be diluted with the dilution factor of 5 and re-assayed (100 μL of sample was added to 400 μL pooled blank human plasma).

2.4. Instrument conditions

The LC system was a Shimadzu Prominence-20A series (Kyoto, Japan) consisting of two LC-20AD solvent delivery modules, a DGU-20A3 on-line degasser, an SIL-20AHT autosampler, a CTO-20A column oven and a CBM-20A system controller. Chromatography was carried out on a C18 analytical column (Synergi Hydro-RP 80A, 150 mm × 2.0 mm i.d., 4 μm, Phenomenex, USA) fitted with a C18 guard column (AQ, 4.0 mm × 2.0 mm i.d., 5 μm, Phenomenex, USA). The mobile phase consisted of methanol and 5 mM ammonium acetate (0.1% formic acid) (90:10, v/v). The flow rate was 0.3 mL/min with column temperature maintained at 35 °C. Total run time for each injection was 3.5 min. An API 3200 triple-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Concord, Ontario, Canada) equipped with an electrospray ionization (ESI) source was used for mass analysis and detection. Analyst software (Applied Biosystems/MDS SCIEX, version 1.4.2) was used for data acquisition and processing. The mass spectrometer was operated in the positive ion mode with the MRM transitions and correlative optimized declustering potential (DP) and collision energy (CE) listed in Table 1. Other parameters were as follows: collision gas, curtain gas, gas 1 and gas 2 (all nitrogen) 4, 20, 30 and 60 psi, respectively; dwell time 300 ms; IonSpray voltage 5500 V; source temperature 550 °C. Unit resolution was used for Q1 and Q3 mass detection.

2.5. Validation

This study was conducted in accordance with the EMA and the FDA guidance on bioanalytical method validation [8,9]. So far, there are no published studies regarding the determination of efonidipine in human plasma.

2.5.1. Selectivity

Selectivity was determined in human plasma samples spiked at the LLOQ level ($n = 3$). Six blank human plasma samples without efonidipine and IS were prepared. The blank samples were visually compared to the LLOQ samples for any significant interference at the retention times of efonidipine and IS. The acceptance criteria for selectivity were that the mean response, i.e., signal-to-noise ratio (S/N) of efonidipine in the LLOQ samples was at least five times the response compared to the blank samples, and there was no significant matrix interference at the retention times of efonidipine and IS in the blank samples compared with the LLOQ samples.

2.5.2. Cross-talk

The cross-talk phenomena among MS/MS channels were assessed injecting efonidipine and IS, separately, at the highest concentrations for calibration curve and monitoring the response in the other MS/MS channels.

2.5.3. Carryover

Carryover was evaluated by three injections of a ULOQ sample of the calibration curve, immediately followed by three injections of a blank human plasma sample. Carryover was considered acceptable if the mean peak area counts of efonidipine and IS was not more than 20% for efonidipine, and 5% for IS, compared to the area counts in the LLOQ sample.

2.5.4. Matrix effects and extraction recovery

Matrix effect factors were determined at three QC concentrations (0.250, 3.00 and 16.0 ng/mL) of efonidipine and at the working concentration of IS by comparing the peak areas of post-extraction blank plasma (from six different individuals) spiked with efonidipine and IS with mean peak areas of solutions containing efonidipine and IS at corresponding concentrations. The relative standard deviation (RSD) should be within 15%.

Extraction recoveries were determined at three QC concentrations (0.250, 3.00 and 16.0 ng/mL) of efonidipine and at the working concentration of IS by comparing the peak areas of extracted QC