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Liquid chromatography–tandem mass spectrometric assay for the light sensitive calcium channel antagonist lacidipine in human plasma

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ABSTRACT

A novel, rapid and sensitive liquid chromatography/tandem mass spectrometry method was developed and validated for the quantification of calcium channel antagonist lacidipine in human plasma. Carbamazepine was used as an internal standard. Analyte and the internal standard were extracted from human plasma by solid-phase extraction technique. The reconstituted samples were chromatographed on a C_{18} column by using a mixture of acetonitrile–ammonium acetate buffer (5 mM) (80:20, v/v) as the mobile phase at a flow rate of 1.0 mL/min. The calibration curve obtained was linear ($r^2 \geq 0.9990$) over the concentration range of 0.05–12.5 ng/mL. The multiple reaction-monitoring mode was used for quantification of ion transitions at m/z 456.2/354.2 and 237.1/194.1 for the drug and the internal standard, respectively. The results of the intra- and inter-day precision and accuracy studies were well within the acceptable limits. A run time of 2.2 min for each sample made it possible to analyze more than 300 plasma samples per day. The proposed method was found to be applicable to clinical studies.

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1. Introduction

Lacidipine is a calcium channel antagonist and developed for the treatment of hypertension [1–3]. In addition to its antihypertensive effect, lacidipine has also shown anti-atherosclerotic and antioxidant effects [4,5]. It has long duration of action because of its high degree of lipophilicity [6,7]. Lacidipine undergoes extensive first-pass hepatic metabolism and has a mean absolute bioavailability of about 10% (range 3–59%). It is completely metabolized in the liver by cytochrome P450 3A4 (CYP3A4) to pharmacologically inactive metabolites [2,3]. Therefore, the determination of the level of unchanged drug in plasma requires an analytical method with high sensitivity.

For the determination of lacidipine in biological fluids some high-performance liquid chromatographic methods [8–11] and liquid chromatography-mass spectrometric methods [12–14] were reported. Of all the above, only two methods are comparable with the present work. Ramakrishna et al. [13] reported a method with plasma concentration range of 0.1–25 ng/mL using 1.0 mL plasma sample volume. This method employs tedious liquid–liquid extraction; however, not sensitive enough for the determination of lacidipine concentrations for pharmacokinetic/bioequivalence studies. Another method proposed by Tang et al. [14] for quantification of lacidipine in human plasma (LLOQ of 0.025 ng/mL) also utilizes 1.0 mL plasma sample volume, which is very high compared to the present work.

In the present investigation, we have developed a method having a shorter run time with simple solid-phase extraction technique. The following are the advantages of the proposed method over those reported earlier: (1) because of the use of less plasma volume (250 μL), the volume of the sample to be collected per time point from an individual during the study is reduced significantly. This allows inclusion of additional points; (2) employing a simple solid-phase extraction procedure allows higher recoveries and the elimination of possible interference from endogenous and exogenous components; (3) greater sensitivity is achieved even with low plasma volumes and the method is well suited for pharmacokinetic analysis; (4) the rapid sample turnaround time of 2.2 min makes it an attractive procedure in high-throughput bioanalysis of lacidipine in human plasma.

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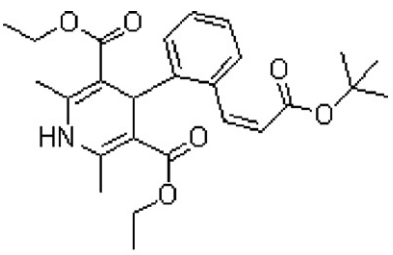
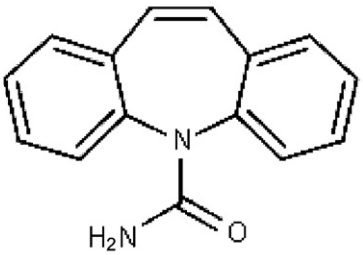


Lacidipine



Carbamazepine (IS)

Fig. 1. Chemical structures of lacidipine and carbamazepine (IS).

2. Experimental

2.1. Chemicals

Lacidipine reference standard (98.8% pure) was obtained from Clearsynth Labs Limited (Mumbai, India). Carbamazepine (99.2% pure) was employed as an internal standard (IS) and was obtained from Vivan Life Sciences Limited (Mumbai, India). Chemical structures are presented in Fig. 1. Drug free human plasma containing K2. EDTA as an anticoagulant was obtained from the Cauvery Diagnostics (Hyderabad, India). HPLC-grade LiChrosolv methanol, LiChrosolv acetonitrile were from Merck (Darmstadt, Germany). Ammonium acetate, formic acid and ethyl acetate were from Merck (Worli, Mumbai, India). HPLC Type I water from a Milli-Q system (Millipore, Bedford, MA, USA) was used. All other chemicals were of analytical grade.

2.2. LC–MS/MS instrument and conditions

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a Zorbax XDB-Phenyl column (75 mm × 4.6 mm, 3.5 μm; Agilent Technologies, Santa Clara, CA, USA), a binary LC-20AD prominence pump, an auto sampler (SIL-HTc) and a solvent degasser (DGU-20A_3) was used for the study. Aliquot of 10 μL of the processed samples were injected into the column, which was kept at room temperature (25 °C). An isocratic mobile phase consisting of a mixture of acetonitrile–ammonium acetate buffer (5 mM) (80:20, v/v) was used to separate the analyte and delivered at a flow rate of 1.0 mL/min into the electrospray ionization chamber of the mass spectrometer. Quantification was achieved with MS–MS detection in positive ion mode for the analyte and the internal standard using an MDS Sciex API-4000 mass spectrometer (Foster City, CA, USA) equipped with a Turboionspray™ interface at 500 °C. The ion spray voltage was set at 5000 V. The source parameters viz. the nebulizer gas (GS1), auxiliary gas (GS2), curtain gas and collision gas were set at 54, 35, 45, and 10 psi, respectively. Dwell time was set at 200 ms. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) were 72, 16, 10, 11 V for lacidipine and 75, 27, 10, 4 V for carbamazepine. Detection of the ions was carried out in the multiple-reaction monitoring mode (MRM), by monitoring the transition pairs of m/z 456.2 precursor ion to the m/z 354.2 for lacidipine and m/z 237.1 precursor ion to the m/z 194.1 product ion for the IS. Quadrupoles Q_1 and Q_3 were set on unit resolution. The analysis data obtained were processed by Analyst Software™ (version 1.4.2).

2.3. Preparation of plasma standards and quality controls

Standard stock solutions of lacidipine (1 mg/mL) was prepared in 1% formic acid in methanol, where as the IS (1 mg/mL) was prepared in methanol. Working solutions for calibration and controls were prepared by appropriate dilution in water–methanol (40:60, v/v; diluent). All the solutions were prepared under yellow monochromatic light and stored in dark to protect form photodegradation. The IS working solution (4 ng/mL) was prepared by diluting its stock solution with water–methanol (40:60, v/v; diluent).

Calibration samples were prepared by spiking 950 μL of control human plasma with the 50 μL working standard solution of the analyte as a bulk, to obtain lacidipine concentration levels of 0.05, 0.1, 0.41, 1.28, 2.57, 5.13, 7.6, 10 and 12.5 ng/mL. Similarly, quality control (QC) samples were also prepared as a bulk based on an independent weighing of standard drug, at concentrations of 0.05 (LLOQ), 0.15 (low), 1.91 (middle 1), 6.35 (middle 2) and 10.77 ng/mL (high) as a single batch at each concentration. The calibration and control bulk samples were divided into aliquots in micro centrifuge tubes (Tarson, 2 mL) and stored in the freezer at −70 °C until analyses.

2.4. Sample processing

A 250 μL aliquot of human plasma sample was mixed with 25 μL of the internal standard working solution (4 ng/mL of carbamazepine). To this, 250 μL of extraction buffer (0.5% formic acid in 100 mM ammonium acetate) was added after vortex mixing for 10 s. The sample mixture was loaded onto a Oasis HLB cartridge (30 mg/1 mL, Make: Waters Corporation) that was pre-conditioned with 1.0 mL of methanol followed by 1.0 mL water. The extraction cartridge was washed with 1.0 mL extraction buffer followed by 1.0 mL of water. Analyte and internal standard were eluted with 2.0 mL of dichloromethane and evaporated to dryness at 40 °C under a stream of nitrogen. The dried extract was reconstituted in 500 μL of mobile phase and transferred into amber-colored injector vials. From these, a 10 μL aliquot was injected into the chromatographic system. The entire sample processing and further LC–MS/MS analysis were carried out as soon as possible, under yellow monochromatic light in order to prevent the degradation of the lacidipine by light.

2.5. Bioanalytical method validation

The validation of the above method was carried out as per US FDA guidelines [17]. The parameters determined were selectivity, matrix effect, linearity, precision, accuracy, recovery, stability and dilution integrity. Selectivity was assessed by comparing the chromatograms of six different batches of blank plasma obtained from six different sources including one lipemic and hemolyzed plasma. Sensitivity was determined by analyzing six replicates of plasma samples spiked with the lowest level of the calibration curve concentrations. Matrix effect was checked with six different lots of K2. EDTA plasma. Three replicate samples each of LQC and HQC were prepared from different lots of plasma (36 QC samples in total). For checking the linearity standard calibration curves containing at least 9 points (non-zero standards) were plotted. In

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