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Determination of moxonidine in human plasma by liquid chromatography–electrospray ionisation–mass spectrometry

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Abstract

A sensitive and specific liquid chromatography–electrospray ionisation–mass spectrometry (LC–ESI–MS) method has been developed and validated for the identification and quantification of moxonidine in human plasma. After the addition of clonidine–HCl, the internal standard (IS) and sodium hydrogen carbonate, plasma samples were extracted using 5 mL ethyl acetate. The compounds were separated on a Lichrospher ODS (5 μm, 250 mm × 4.6 mm) column using an elution system of 10 mmol/L ammonium acetate buffer–methanol (20:80 v/v) as the mobile phase. Analytes were determined using electrospary ionization in a single quadrupole mass spectrometer. LC–ESI–MS was performed in the selected-ion monitoring (SIM) mode using target ions at *m*/*z*: 242.2 for moxonidine and *m*/*z*: 230.1 for the IS. The method has shown to be sensitive and specific by testing six different blank plasma batches. Linearity was established for the range of concentrations 0.01976–9.88 ng/mL with a coefficient of correlation (*r*) of 0.9999. The lower limit of quantification (LOQ) was identifiable and reproducible at 0.01976 ng/mL. The method has been successfully applied to study the pharmacokinetics of moxonidine in healthy male Chinese volunteers.

Keywords: Moxonidine; HPLC–ESI–MS; Human plasma; Pharmacokinetics

1. Introduction

Moxonidine–HCl (Fig. 1a) is a new centrally-acting antihypertension agent that reduces blood pressure by stimulating the central α_2-adrenoceptor. Determination of the pharmacokinetic profile of moxonidine is important for gaining a better understanding of its mechanism of action and for ensuring more efficient therapeutic application. Martin et al. [1,2] developed a gas chromatographic method with mass spectrometric detection for determination of moxonidine in human plasma. LOQ of moxonidine in plasma was 0.1 ng/mL. Qiang Zhang et al. [3] reported an RP-HPLC method for the quantification of moxonidine–HCl in material and preparations.

In this study, a sensitive liquid chromatography–electrospray ionisation–mass spectrometry (LC–ESI–MS) method has been novel developed for determination of moxonidine in human plasma. The assay is validated over the ranges of 0.01976–9.88 ng. The method has been successfully applied to study pharmacokinetics of moxonidine in healthy male Chinese volunteers.

2. Experimental

2.1. Chemicals and reagents

Moxonidine test patch, Moxonidine–HCl standard, and IS was supplied by Yabao Pharmaceutical Company (Shanxi Province, PR China); moxonidine reference patch was supplied by Tiantaishan Pharmaceutical Co. Ltd. (Chengdu Province, PR China). Methanol was chromatographic pure grade and purchased from Merck (Merck Company, Germany). Other chemicals were all of analytical grade. Deionized water was distilled before using.

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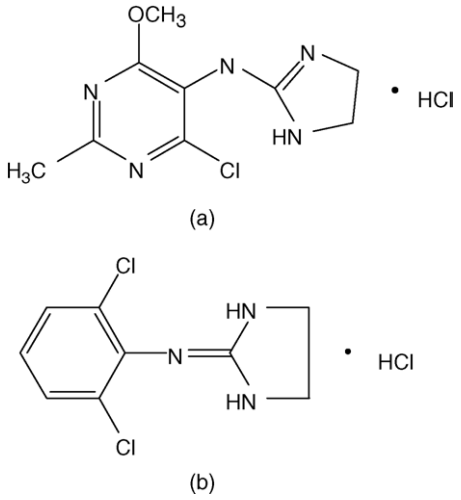

Fig. 1. Chemical structure of moxonidine hydrochloride (a) and clonidine hydrochloride (b).

2.2. Instrumentation and operating conditions

HPLC analyses were performed using an Agilent 1100 LC–MSD system (Agilent company, USA) with an Agilent Chem Station (Agilent company, USA) with a Lichrospher ODS C18 column (4.6 mm × 250 mm, 5 μm, Huaiyin Hanbang science Co. Ltd., PR China). Using an elution system of 10 mmol/L ammonium acetate buffer–methanol (20:80 v/v) as the mobile phase, and the column temperature was maintained at 25 °C. A constant mobile phase flow-rate of 1.0 mL/min was employed throughout the analyses. LC–ESI–MS was carried out using nitrogen to assist nebulization. A quadrupole mass spectrometer equipped with an electrospray ionization source was set with a drying gas (N_2) flow of 10 L/min, nebulizer pressure of 40 psi (1 psi = 6894.76 Pa), drying gas temperature of 350 °C and the positive ion mode The fragmentor voltage was 90 V. LC–ESI–MS was performed in SIM mode using target ions at *m*/*z*: 242.2 for moxonidine and *m*/*z*: 230.1 for the IS. The MS data acquisition started at 2 min after sample injection, and the stream selection valve was set to waste until data acquisition started.

2.3. Preparation of stock solutions

Primary stock solutions of moxonidine–HCl for preparation of standards and quality controls (QC) were prepared from separate weighings. The primary stock solutions were prepared in methanol at a concentration of 0.988 mg/mL. Working solutions of moxonidine–HCl were prepared daily in methanol by appropriate dilution at 0.988, 9.88, 98.8 and 197.6 ng/mL.

The IS stock solution was prepared by dissolving 5.56 mg of clonidine–HCl in 10 ml of methanol producing a concentration of 0.96 mg/mL. A 0.048 μg/mL internal standard working solution was prepared by diluting the stock standard solution of clonidine–HCl with the methanol.

All the solutions were stored at 4 °C and were brought to room temperature before use.

2.4. Calibration curves

Calibration curves were prepared by spiking different samples of 1 mL blank plasma each with one of the above mentioned working solutions to produce the calibration curve points equivalent to 0.01976, 0.0494, 0.0988, 0.1976, 0.494, 0.988, 1.976, 4.94 and 9.88 ng/mL of moxonidine. Each sample was also spiked with 40 μl IS working solution (0.048 μg/mL) and extract as the 2.6 procedure. In each run, a plasma blank sample (no IS) was also analyzed. Calibration curves were prepared by determining the best-fit of peak area ratios (peak area of analyte/peak area of IS) versus concentration, and fitted to the equation $f = bC + a$ by unweighted least-squares regression.

2.5. Preparation of quality control samples

Quality control samples were prepared at four different concentration levels. QC samples were prepared daily by spiking different samples of 1 mL blank plasma each with the corresponding standard solution to produce a final concentration equivalent to 0.0494, 0.1976, 0.988 and 4.94 ng/mL of moxonidine and 1.92 ng of internal standard.

2.6. Extraction procedure

QC, calibration curve, and clinical plasma samples were extracted employing a liquid–liquid extraction technique. To each tube containing 1 ml plasma, 40 μl of internal standard solution (0.048 μg/mL) was added, 200 μl of saturated sodium hydrogen carbonate was added for alkalinizing and 5 ml ethyl acetate were added and vortex for 3 min. Afterwards, samples were centrifuged for 5 min at 4000 × *g*. The organic layer was evaporated under a stream of nitrogen at 45 °C. The residue was reconstituted in 100 μl mobile phase. An aliquot of 50 μl was injected into the LC–MS system.

2.7. Assay validation

The method validation assays were carried out according to the currently accepted US Food and Drug Administration (FDA) bioanalytical method validation guidance [4]. The following parameters were considered.

The method's specificity was tested by screening six different batches of healthy human blank plasma. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic/spectroscopic conditions and compared with those obtained with an aqueous solution of the analyte at a concentration near to the LLOQ.

The matrix effect on the ionization of analytes was evaluated by comparing the peak area of analytes resolved in blank sample (the final solution of blank plasma after extraction and reconstitution) with that resolved in mobile phase.