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Short communication

Development and validation of a UPLC–MS/MS method for quantitation of droxidopa in human plasma: Application to a pharmacokinetic study

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ABSTRACT

In this study, a simple and sensitive ultra performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) method was developed and validated for quantitation of droxidopa in human plasma for the first time. A simple plasma protein precipitation method using methanol containing 3% formic acid was selected, and the separation was achieved by an Acquity UPLC™ BEH Amide column (2.1 mm × 50 mm, 1.7 μm) with a gradient elution using acetonitrile, ammonium formate buffer and formic acid as mobile phase. The detection of droxidopa and benserazide (internal standard, IS) was performed using positive-ion electrospray tandem mass spectrometry via multiple reaction monitoring (MRM). The precursor-to-product ion transitions m/z 214.2 → m/z 152.0 for droxidopa, and m/z 258.1 → m/z 139.1 for IS were used for quantification. A lower limit of quantification of 5.00 ng/mL was achieved and the linear curve range was 5.00–4000 ng/mL using a weighted ($1/x^2$) linear regression model. Intra-assay and inter-assay precision was less than 10.2%, and the accuracy ranged from 0.1% to 2.1%. Stability, recovery and matrix effects were within the acceptance criteria recommended by the regulatory bioanalytical guidelines. The method was successfully applied to a pharmacokinetic study of droxidopa in healthy Chinese volunteers.

1. Introduction

Droxidopa (L-threo-3,4-dihydroxyphenylserine) is an orally administered synthetic precursor amino acid converted both peripherally and centrally into norepinephrine [1–3]. Droxidopa is the ideal agent to treat orthostatic hypotension due to its direct conversion to norepinephrine, bypassing the rate-limiting hydroxylation step of the normal formation of norepinephrine from dopamine [4,5]. It is clinically used in Japan mainly for the treatment of neurogenic orthostatic hypotension (nOH), frozen gait, or dizziness associated with Parkinson disease (PD) since 1989 [6,7]. In February 2014, droxidopa received accelerated Food and Drug Administration (FDA) approval for the treatment of symptomatic nOH [8,9].

Although droxidopa is widely used in clinical practice, the report method concerning the quantitation of droxidopa in human plasma is scanty. The most widely used method is a high performance liquid chromatography (HPLC) with electrochemical detection [3,10,11], but this method needs a complex alumina extraction process and consumed large volume of biological samples. Jeon et al. [12] determinated droxidopa in rat serum by HPLC with fluorescence detection and this method was time-consuming for the fluorescence derivatization process. Gupta et al. [13] developed a novel electrochemical sensor based on ZnO nanoparticle and ionic liquid binder for square wave voltammetric to determine droxidopa in pharmaceutical and urine samples. Tajik et al. [14] determined droxidopa and carbidopa using a carbon nanotubes paste electrode. However, these two methods are not suitable for pharmacokinetic study for the cumbersome preparation of the sensor and electrode. In this paper, a new ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed and validated to quantify droxidopa in human plasma. This method consumed only 100 μL human plasma,

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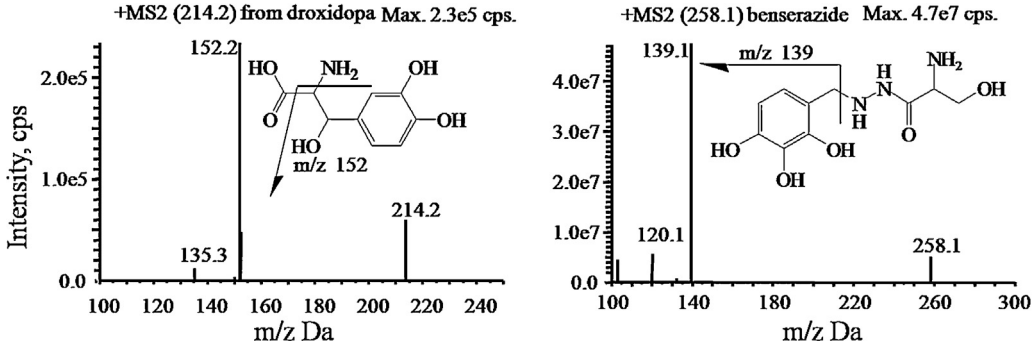

Fig. 1. Product ion mass spectra of (A) droxidopa and (B) benserazide and their proposed fragmentation patterns.

using a simple protein precipitation extraction method as a high-throughput tool for pharmacokinetic study.

2. Experimental

2.1. Reagents and chemicals

Droxidopa (purity > 98.9%) and benserazide hydrochloride (internal standard, IS) were purchased from Tokyo Chemical Industry Co., LTD (Tokyo, Japan) and Sigma-Aldrich (St. Louis, MO, USA), respectively. The structures of droxidopa and IS are shown in Fig. 1. Chromatographic grade methanol and acetonitrile were bought from Tedia Company (Fairfield, OH, USA). Formic acid and ammonium formate of analytical grade was purchased from ANPEL Laboratory Technologies Inc. (Shanghai, China). Ultrapure water up to a resistivity of 18.2 MΩ prepared by a Milli-Q water purification system (Millipore, Mississauga, Canada) was used throughout the study. All other chemicals were of HPLC grade.

2.2. Liquid chromatography and mass spectrometry

The LC–MS/MS analysis was carried out on an ekspert™ ultra LC 110-XL system (SCIEX, Concord, Ontario, Canada), composed of a quaternary pump, an autosampler, a column oven, and an AB QTRP 4500 mass spectrometer equipped with an electrospray ion source. Analyst Version 1.6.2 software was used for data acquisition and analysis. Chromatographic separation was performed on an Acquity UPLC™ BEH Amide column (2.1 mm × 50 mm, 1.7 μm; Waters, Wexford, Ireland) protected by a VanGuard Pre-column (5 mm × 2.1 mm, Waters, Wexford, Ireland), and the column oven was set at 40 °C. Gradient elution was achieved using the mobile phase consisting of (A) water-1 mol/L ammonium formate-formic acid (1000:10:1, v/v/v) and (B) water-acetonitrile-1 mol/L ammonium formate-formic acid (950:50:10:1, v/v/v) at a flow rate of 0.3 mL/min. The gradient elution program started by 100%B for 1.5 min, followed by a linear ramp to 30%A: 70%B at 2.5 min, and held this mix solvent until 5 min before re-equilibration to initial conditions for one min. The injection volume was 20 μL.

The mass spectrometer with electrospray ionization (ESI) source was operated in positive mode. Detection was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 214.2 → 152.0 for droxidopa, and *m/z* 258.1 → 139.1 for IS with a dwell time of 200 ms per transition. Optimal MS parameters were as follows: curtain gas 40 psi; collision gas medium; source temperature 600 °C; nebulizer gas (GS1) and turbo gas (GS2) 60 psi; ion spray voltage 5500 V; collision energies 13 and 21 eV for droxidopa and IS, respectively; declustering potential 34 V and 17 V for droxidopa and IS, respectively.

2.3. Preparation of calibration standards and quality control (QC) samples

Stock solution of droxidopa was prepared by dissolving droxidopa in hydrochloric acid (0.1 M) and diluting with methanol to yield 1.0 mg/mL solution and stored at 4 °C. Stock solution was further diluted in 50% aqueous methanol to provide working standard solutions of desired concentrations of 0.25, 0.50, 1.50, 5.00, 15.0, 50.0 and 200 μg/mL. Calibration standards for droxidopa were prepared by spiking 490 μL blank human plasma with 10 μL of the respective working standard solutions to yield final concentrations of 5.00, 10.0, 30.0, 100, 300, 1000 and 4000 ng/mL. All solutions were stored at −20 °C until analysis. LLOQ (lower limit of quantitation), low, medium and high QC samples were similarly prepared at concentrations of 5.00, 8.00, 500 and 2500 ng/mL respectively and stored at −20 °C. The IS stock solution (1 mg/mL) was prepared in 20% aqueous methanol. It was diluted with methanol containing 3% formic acid to obtain a 500 ng/mL working IS solution and stored at 4 °C.

2.4. Sample preparation

The frozen plasma samples were thawed in room temperature and vortex-mixed thoroughly. An aliquot of 50 μL of the IS solution (500 ng/mL) was added to 100 μL of plasma sample in a 1.5 mL centrifuge tube. Subsequently, 300 μL methanol containing 3% formic acid was added and vortex-mixed for 5 min immediately. After centrifugation at 13600*g* for 10 min at 4 °C, the supernatant was separated and transferred to the auto-sampler vials. 20 μL of the aliquot was injected into the UPLC–MS/MS system for analysis.

2.5. Method validation [15]

2.5.1. Selectivity

Selectivity was investigated by analyzing the chromatograms of six batches of human blank plasma and the corresponding spiked plasma samples at LLOQ level. The assay was considered to exclude the potential interference of endogenous substances at the retention times of droxidopa and IS if the response was <20% of that of the LLOQ for droxidopa and <5% of that of the IS.

2.5.2. Linearity

The linearity of calibration curves was evaluated by plotting the peak area ratios of analyte to IS (y) against the nominal concentrations, using the least-square linear regression with weighing factor $1/x^2$ (x, concentration). The acceptable linearity was achieved when the correlation coefficients (r) were >0.995 and back-calculated concentrations were <20% at the LLOQ and <15% at other concentrations.