Journal of Pharmaceutical and Biomedical Analysis 117 (2016) 413–418

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Quantification of trantinterol, its two metabolites and their primary conjugated metabolites in human plasma by ultra-high-performance liquid chromatography- tandem mass spectrometry and its application to a pharmacokinetic study

Feng Qin[a], Bincan Yin[b], Lijuan Wang[a], Kunjie Li[a], Famei Li[a], Zhili Xiong[a,*]

[a] Department of Analytical Chemistry, School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China
[b] College of Medical Informatics, China Medical University, Shenyang 110013, PR China

ARTICLE INFO

Article history:
Received 31 May 2015
Received in revised form
16 September 2015
Accepted 16 September 2015
Available online 25 September 2015

Keywords:
Trantinterol
Metabolites
UHPLC-MS/MS
Human plasma
Pharmacokinetics

ABSTRACT

A highly rapid, selective and sensitive ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method was developed to simultaneously determine trantinterol, its major phase-I metabolites and their primary conjugated metabolites in human plasma. Waters Oasis HLB C18 solid phase extraction cartridges were used in the sample preparation. The separation was carried out on an ACQUITY UPLC™ BEH C_{18} column with methanol/0.2% formic acid (30:70, v/v) as the mobile phase at a flow rate of 0.25 mL/min. The detection was performed on a triple quadrupole tandem mass spectrometer in selective reaction monitoring (SRM) mode with the use of an electrospray ionization (ESI) source. The linear calibration curves for trantinterol, *tert*-butyl hydroxylated trantinterol (*tert*-OH-trantinterol) and 1-carbonyl trantinterol (trantinterol-COOH) were obtained in the concentration ranges of 0.200–250, 0.108–4.00 and 0.0840–5.02 ng/mL, respectively ($r^2 \geq 0.99$). The intra- and inter-day precision (relative standard deviation, RSD) values were less than 13%, and the accuracy (relative error, RE) was within ±9.9%, as determined from quality control (QC) samples for the analytes. The concentrations of conjugated forms of trantinterol and *tert*-OH- trantinterol in plasma were determined using selective enzyme hydrolysis. The method described herein was fully validated and successfully applied for the pharmacokinetic study of trantinterol in healthy volunteers after oral administration.

1. Introduction

Trantinterol, 2-(4-amino-3-chloro-5-trifluoromethyl-phenyl)-2-*tert*-butylaminoethanol, is a novel phenylethanolamine β_2 adrenoceptor agonist currently undergoing phaseIII clinical trials in China. It has exhibited both a potent trachea relaxing activity and high β_2 selectivity with low cardiac side effects [1]. The structures of 1-carbonyl trantinterol (trantinterol-COOH) and *tert*-butyl hydroxylated trantinterol (*tert*-OH-trantinterol), the major phase-I metabolites of trantinterol in vivo [2], are shown in Fig. 1. Trantinterol and *tert*-OH-trantinterol glucuronide conjugates are major phase- II metabolites in vivo [2]. To investigate the drug clearance mechanism, understand the role of metabolites in drug pharmacokinetic and pharmacodynamic assessment, evaluate drug interactions and assess drug safety, a simple, sensitive and rapid method for the simultaneous determination of trantinterol and its major metabolites in human plasma is necessary.

A number of methods have been reported for the quantification of β_2-adrenoceptor agonists in biological fluids [3–8]. However, few methods have been reported for the quantification of trantinterol in biological fluids [9–13], and only one published literature work reported a LC–MS/MS method [12] for the simultaneous determination of trantinterol and its major metabolites in rat urine and feces. The chromatographic run time needed in most of these methods was longer than 5 min. Herein, we present a fast, sensitive and selective UHPLC–MS/MS method using solid phase extraction for sample preparation for the determination of trantinterol and its major metabolites in human plasma. The total run time of the method was 2.6 min per sample. The present method has been proven to be more efficient in analyzing a large number of samples in biological fluids.

* Corresponding author. Fax: +86 24 2398 6289.
E-mail address: bearry200@126.com (Z. Xiong).

http://dx.doi.org/10.1016/j.jpba.2015.09.020
0731-7085/

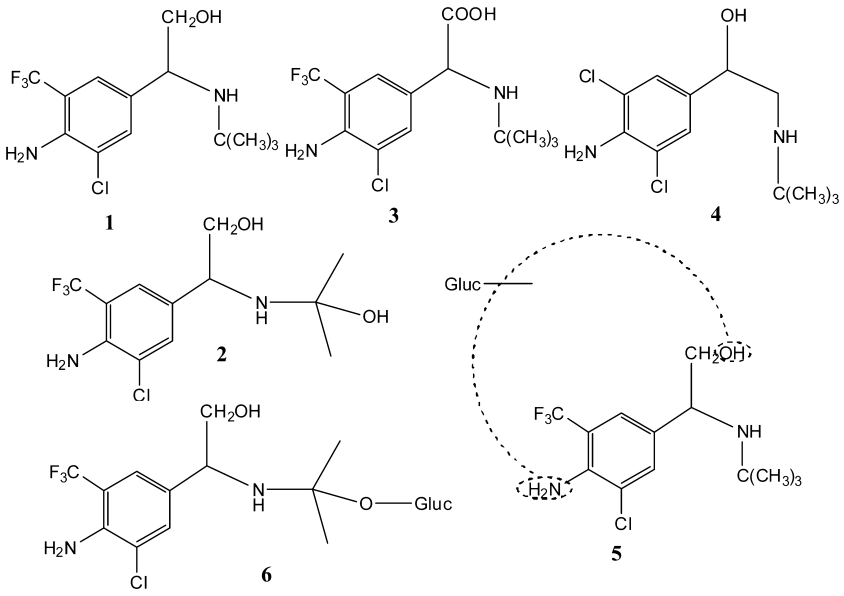

Fig. 1. Chemical structures of trantinterol (1), *tert*-OH-trantinterol (2), trantinterol-COOH (3), I.S. (4), trantinterol glucuronide (5) and *tert*-OH-trantinterol glucuronide (6).

2. Experimental

2.1. Chemicals and reagents

Trantinterol hydrochloride (99.3% purity) was generously supplied by the Department of Pharmaceutical Chemistry, Shenyang Pharmaceutical University (Shenyang, China). *Tert*-OH-trantinterol and trantinterol-COOH were isolated and purified in our laboratory. The purities of these metabolites were above 99.0% and were verified using HPLC. Clenbuterol (internal standard, I.S., 99.4% purity) was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China). β-Glucuronidase was a product of Sigma–Aldrich (St. Louis, MO, USA).

2.2. Apparatus and operating conditions

2.2.1. Liquid chromatography

The separation was carried out using an ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm; Waters Corp, Milford, MA, USA). The column temperature was maintained at 40 °C. The isocratic mobile phase consisted of methanol/0.2% formic acid (30:70, v/v) at a flow rate of 0.25 mL/min. The autosampler temperature was maintained at 4 °C, and 20 μL of sample solution was injected.

2.2.2. Mass spectrometric conditions

The mass spectrometer was operated in positive ionization mode with the capillary voltage set at 1.0 kV. The source and desolvation temperatures were set to 120 and 500 °C, respectively. Nitrogen was used as the desolvation and cone gas with flow rates of 600 and 30 L/h, respectively. Argon was used as the collision gas at a pressure of approximately 0.260 Pa. Quantification was performed using SRM of the following transitions: *m/z* 311 → *m/z* 238 for trantinterol, m/z 327 → *m/z* 238 for *tert*-OH-trantinterol, *m/z* 325 → *m/z* 252 for trantinterol-COOH and *m/z* 277 → *m/z* 203 for the I.S., with a scan time of 0.10 s per transition. The optimized cone voltages and collision energies for trantinterol, *tert*-OH-trantinterol, trantinterol-COOH and the I.S. were 15, 12, 15, 12 V and 15, 12, 18, 12 V, respectively.

2.3. Preparation of the standards and quality control samples

Stock standard solutions of trantinterol, *tert*-OH-trantinterol and trantinterol-COOH were prepared by dissolving appropriate amounts of reference standards of trantinterol, *tert*-OH-trantinterol and trantinterol-COOH in methanol. The solutions were then serially diluted with a mixture of methanol and water (50:50, v/v) to provide working standard solutions of the desired concentrations.

Calibration standards were prepared daily by evaporating 75 μL of working standard solutions (25 μL of every analyte) to dryness and then fully mixing them with 500 μL of blank plasma. The QC samples used in the validation and during the pharmacokinetic study were prepared with blank plasma at low, middle and high concentrations at the beginning of the experiment by independent dilution, and the aliquots were stored at −80 °C after preparation.

2.4. Sample preparation

To analyze trantinterol-COOH, free (unconjugated) trantinterol and *tert*-OH-trantinterol, 50-μL internal standard solutions were pipetted into a 10-mL glass tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was vortex-mixed with 500 μL of plasma for 30 s, and the mixture was loaded onto a Waters Oasis 10 mg HLB solid-phase extraction (SPE) cartridge with a flow rate of approximately 0.5–1 mL/min. All processing was performed on a SPE vacuum manifold. The SPE cartridge was successively preconditioned with 2 mL of methanol and 2 mL of water. Care was taken that the cartridge did not run dry. The SPE cartridge was rinsed with 1 mL of methanol/water (5:95, v/v), and the analytes were eluted with 2 mL of methanol. The eluate was evaporated under a nitrogen stream at 40 °C. The residue was reconstituted in 100 μL of mobile phase and vortexed for 30 s. An aliquot of 20 μL was injected into the UHPLC–MS/MS system for analysis.

To determine the total trantinterol and *tert*-OH-trantinterol (free plus conjugated trantinterol and *tert*-OH-trantinterol), 100 μL of β-glucuronidase enzyme solution (24 000 units/mL in 0.05 mol/L KH_2PO_4 buffer, pH 5.0) was added to a 500 μL aliquot of human plasma. The mixture was incubated in a water bath at 37 °C for 16 h. After enzymatic hydrolysis, the mixture was treated as described above. Those plasma samples whose concentrations were higher than the highest calibration point were diluted appropriately with