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A rapid and sensitive LC–MS/MS-ESI method for the determination of tolvaptan and its two main metabolites in human plasma

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ABSTRACT

A liquid chromatography–tandem mass spectrometry (LC–MS) method to quantify tolvaptan and its two main metabolites and applied to human study was first developed and validated as a measure of compliance in clinical research. Because of the structure similarity of tolvaptan and its multiple metabolites, the method was optimized to obtain a chromatographic and MS separation of the endogenous interference and isotope ions as well as high analysis throughput. Tolvaptan, its two main metabolites and the internal standard were extracted from human serum (0.1 mL) using solid-phase extraction, separated on a Waters nova-pak C_{18} column (150 × 3.9 mm, 5 μm) using isocratic elution with a mobile phase composed of acetonitrile, water and formic acid (65:35:0.25, v/v/v). The total run-time was shortened to 3.5 min. The mass transition ranges under positive electrospray ionisation that were monitored for quantitation included *m/z* 449–252 for tolvaptan, *m/z* 479–252 for metabolite DM-4103, *m/z* 481–252 for metabolite DM-4107 and *m/z* 463–266 for the internal standard (IS). The limit of quantification in plasma for all three analytes was 1 ng/mL. The method was validated over a linear range from 1 to 500 ng/mL for all three analytes with acceptable inter- and intra-assay precision and accuracy. The stability of the analytes was determined to be suitable for routine laboratory practices. The method was successfully applied to samples taken from research volunteers who ingested a 15 mg tolvaptan tablet.

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1. Introduction

Tolvaptan is an oral, non-peptide selective arginine vasopressin (AVP) V2-receptor antagonist prescribed in the United States for the treatment of hypervolemic and euvolemic hyponatremia associated with heart failure, cirrhosis, and Syndrome of Inappropriate Antidiuretic Hormone (SIADH) [1]. Tolvaptan is approved in Japan for the treatment of excess water retention in patients with cardiac failure and liver cirrhosis for patients who do not respond to treatment with other diuretics, including loop diuretics [2,3]. Tolvaptan takes approximately 2–4 h after oral administration to reach the maximum plasma concentration [4], with absolute bioavailability approximately 56% [5]. Tolvaptan is principally metabolized in the liver by CYP3A4 and is subsequently eliminated in feces [6]. Tolvaptan is also a P-glycoprotein substrate that had a drug–drug interaction with digoxin in vivo [7]. Tolvaptan shows linear pharmacokinetic characteristics over the range of dosages previously studied [8–10].

Tolvaptan can be degraded to form multiple metabolites which were structurally similar. Although the determination of tolvaptan in plasma from animals and humans has been reported in detail [4–8,11,12,], only one previous animal study has reported the quantitative analysis of tolvaptan metabolites [13]. According to this report, two metabolites were found at higher concentration than tolvaptan. Hence, quantification of the two metabolites may be an important tool in monitoring the pharmacokinetics in human clinical trials. It is worth noting that non-clinical animal studies are significantly different from clinical trials in humans. In the former, the dosages administered are typically considerably higher than dosages in a clinical trial in humans. The metabolic pathways of the different species may also be significantly different.

The present study describes a high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) method for the quantitation of tolvaptan and its two main metabolites, DM-4103/DM-4107 in human plasma. The method was fully validated and was successfully employed in a clinical

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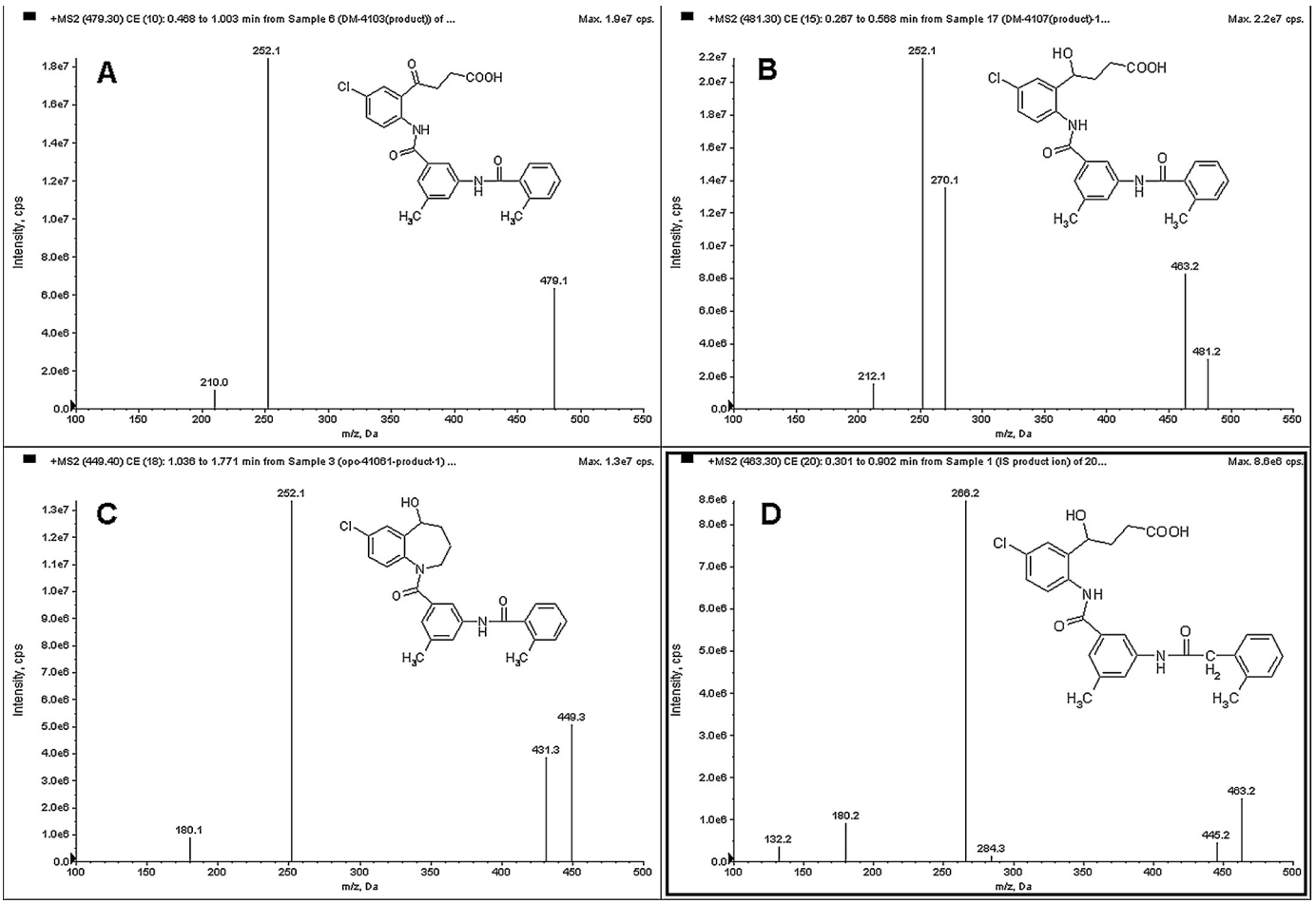

Fig. 1. Positive product ion mass spectra of DM-4103 (A), DM-4107 (B), tolvaptan (C) and the internal standard (D).

pharmacokinetic study in healthy male volunteers following oral administration of tolvaptan (15 mg).

2. Experimental

2.1. Chemicals and materials

Tolvaptan, the two main metabolites of tolvaptan (DM-4103, DM-4107), and the internal standard (IS; structure presented in Fig. 1) were sourced from BaiLi Medicine Co., Ltd. (Sichuan Province, China; purity >99.5%). Formic acid, methanol and acetonitrile (HPLC-grade) were purchased from Fisher Scientific Co., Ltd. Distilled water was prepared using a Milli-Q water purification system. The control plasma used for standards and quality controls (QCs) was obtained from healthy volunteers with heparin used as an anti-coagulant.

2.2. Instrumentation for liquid chromatography–mass spectrometry

An Agilent 1100 system (Wilmington, DE, USA) consisting of a vacuum degasser, binary pump and auto-sampler, was used for solvent and sample delivery. Chromatographic separation was achieved using a Waters nova-pak C_{18} column (150 × 3.9 mm, 5 μm). The column temperature was maintained at 35 °C. The mobile phase consisted of acetonitrile-water-formic acid (65:35:0.025, v/v), and the flow rate was 0.8 mL/min. An AB MDS Sciex API4000 mass spectrometer (Concord, Ontario, Canada) equipped with a TurboIonSpray ionisation (ESI) source was used for mass analysis and detection. Instrument control and data processing were performed using Analyst® version 1.4.1 software (AB Sciex, Concord, Ontario, Canada). The mass spectrometer was operated in positive-ion mode. The nebuliser and TurboIonSpray nitrogen gases were set at 45 and 40 instrument units, respectively. The TurboIonSpray voltage and temperature were optimized at 5000 V and 450 °C, respectively. For collision-induced dissociation, nitrogen was used as the collision gas, and the flow rate was set at medium. The curtain gas was set at 25 instrument units. Quantification was performed via multiple-reaction-monitoring (MRM) of the following transitions: *m/z* 449 to *m/z* 252 for tolvaptan, *m/z* 479 to *m/z* 252 for metabolite DM-4103, *m/z* 481 to *m/z* 252 for metabolite DM-4107, and *m/z* 463 to *m/z* 266 for IS. The optimized collision energies were 25, 18, 27 and 20 V for tolvaptan, DM-4103, DM-4107 and IS, respectively. The declustering potential was set at 67, 45, 55, 90 V for tolvaptan, DM-4103, DM-4107 and IS, respectively. The dwell time was 200 ms per transition. The mass spectrometer was operated at unit mass resolution for Q1 and Q3 (the peak width at half-height was set at 0.7 Da).

2.3. Preparation of standard solutions

2.3.1. Calibration standards

Stock solutions (1 mg/mL) of tolvaptan, DM-4103, DM-4107 and IS were prepared in methanol. Tolvaptan, DM-4103 and DM-4107 stock solutions were mixed (1/1/1, v/v/v) and serially diluted with acetonitrile to achieve standard working solutions at concentra-

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