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Simultaneous determination of tedizolid and linezolid in rat plasma by ultra performance liquid chromatography tandem mass spectrometry and its application to a pharmacokinetic study

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ABSTRACT

A sensitive and rapid ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was developed to determine tedizolid and linezolid in rat plasma simultaneously. Chromatographic separation was carried out on an Acquity UPLC BEH C18 column and mass spectrometric analysis was performed using a XEVO TQD triple quadruple mass spectrometer coupled with an electrospray ionization (ESI) source in the positive ion mode. Multiple reaction monitoring (MRM) mode was used for quantification using target fragment ions *m/z* 371.4 → 343.2 for tedizolid, and *m/z* 338.3 → 56.1 for linezolid. This assay method has been fully validated in terms of selectivity, linearity, recovery and matrix effect, accuracy, precision and stability. The linearity of this method was found to be within the concentration range of 5–5000 ng/mL for tedizolid, and 10–10,000 ng/mL for linezolid in rat plasma, respectively. Only 3.0 min was needed for an analytical run. This assay was used to support a pre-clinical study where multiple oral doses were administered to rats to investigate the pharmacokinetics of tedizolid and linezolid.

1. Introduction

Linezolid (Fig. 1A) is the first member of a new class of antibiotics, the oxazolidinones [1]. Linezolid exhibits a broad spectrum of activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci (CoNS), glycopeptide-resistant enterococci and penicillin-resistant *Streptococcus pneumonia* [2,3]. It is also active against mycobacterial species, including *Mycobacterium tuberculosis* and *Nocardia* spp [2]. An advantage of linezolid in the clinical practice is its availability as intravenous (iv) and oral formulations which allow the switch to an oral treatment after induction iv treatment. In the United States, linezolid was licensed by the FDA in adults and children in 2002.

Tedizolid phosphate is a novel oxazolidinone prodrug antibacterial that is rapidly converted by endogenous phosphatases to the active moiety tedizolid (Fig. 1B). Tedizolid has potent activity against a wide range of Gram-positive pathogens, including MRSA, vancomycin-resistant enterococci (VRE) and *cfr*-positive linezolid-resistant strains [4–6]. The efficacy of tedizolid phosphate 200 mg once daily for 6 days was shown not to be sub-standard to linezolid 600 mg twice daily for 10 days in each of 2 phase 3 clinical trials in patients with acute bacterial skin and skin structure infection (ABSSSI) [7,8]. In June 2014, the US Food and Drug Administration (FDA) approved tedizolid for treatment of patients with ABSSSI caused by certain susceptible Gram-positive pathogens [9].

To best of our knowledge, no published LC-MS/MS and HPLC based methods are reported for the quantification of tedizolid in plasma. Many methods for the determination of linezolid in biological fluids by LC [10–16] or LC-MS/MS [17–22] have been reported. However, reports describing a LC-MS/MS-based method for simultaneous determination of tedizolid and linezolid in plasma are not available. Simultaneous detection of tedizolid and linezolid in plasma would help establishing a pharmacokinetic and pharmacodynamic co-relation in animal models that require administration of both drugs to achieve maximal efficacy.

In the current article, we describe a highly sensitive, selective, and rapid ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method that was developed and fully validated for simultaneous estimation of tedizolid and linezolid in

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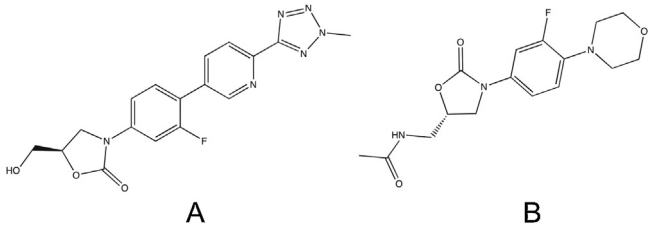

Fig. 1. The chemical structures of the analytes in the present study: (A) tedizolid; (B) linezolid.

rat plasma. This method offers a small turnaround time for analysis and utilizes only 100 μL rat plasma for sample processing using protein precipitation with acetonitrile. Translation of this methodology to pharmacokinetic studies is also demonstrated.

2. Materials and methods

2.1. Chemicals and reagents

Tedizolid (purity 98.0%), linezolid (purity 98.0%), and diazepam (internal standard, IS, purity 98.0%) were obtained from Sigma (St. Louis, MO, USA). Formic acid was analytical grade and purchased from the Beijing Chemical Reagents Company (Beijing, China). Acetonitrile was of LC grade and were purchased from Merck Company (Darmstadt, Hesse, Germany). LC grade water was obtained using a Milli Q system (Millipore, Bedford, MA, USA).

2.2. UPLC-MS/MS conditions

Liquid chromatography was performed on an Acquity ultra performance liquid chromatography (UPLC) unit (Waters Corp., Milford, MA, USA) with an Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 μm particle size) and inline 0.2 μm stainless steel frit filter. A gradient program was employed with the mobile phase, combining solvent A (0.1% formic acid in water) and solvent B (acetonitrile) as follows: 20–95% B (0–0.3 min), 95–95% B (0.3–1.5 min), 95–20% B (1.5–1.6 min), 20–20% B (1.6–3.0 min). The flow rate was 0.40 mL/min and the injection volume was 2 μL. The column and sample temperature were maintained at 40 °C and 4 °C, respectively.

A XEVO TQD triple quadruple mass spectrometer equipped with an electrospray ionization (ESI) source (Waters Corp., Milford, MA, USA) was used for mass spectrometric detection. Multiple reaction monitoring (MRM) modes of *m/z* 371.4 → 343.2 for tedizolid, *m/z* 338.3 → 56.1 for linezolid, and *m/z* 285.2 → 193.1 for IS were utilized to conduct quantitative analysis. The Masslynx 4.1 software (Waters Corp., Milford, MA, USA) was used for data acquisition and instrument control.

2.3. Standard solutions, calibration standards and quality control (QC) sample

The stock solutions of tedizolid and linezolid used to make the calibration standards and quality control (QC) samples were prepared by dissolving 10 mg each compound in 10 mL methanol to obtain a concentration of 1.00 mg/mL of each compound. The stock solutions were further diluted with methanol to obtain working solutions at several concentration levels. Calibration standards and QC samples in plasma were prepared by diluting the corresponding working solutions with blank rat plasma. Final concentrations of the calibration standards were 5, 10, 20, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL for tedizolid, and 10, 20, 50, 100, 200, 500, 1000, 2000, 5000 and 10,000 ng/mL for linezolid in rat plasma, respectively. The concentrations of QC samples in plasma were 10, 200, and 4000 ng/mL for tedizolid, and 20, 400, and 8000 ng/mL for linezolid, respectively. IS stock solution was made at an initial concentration of 1 mg/mL. The IS working solution (200 ng/mL) was made from the stock solution using acetonitrile for dilution. All stock solutions, working solutions, calibration standards and QCs were immediately stored at −80 °C.

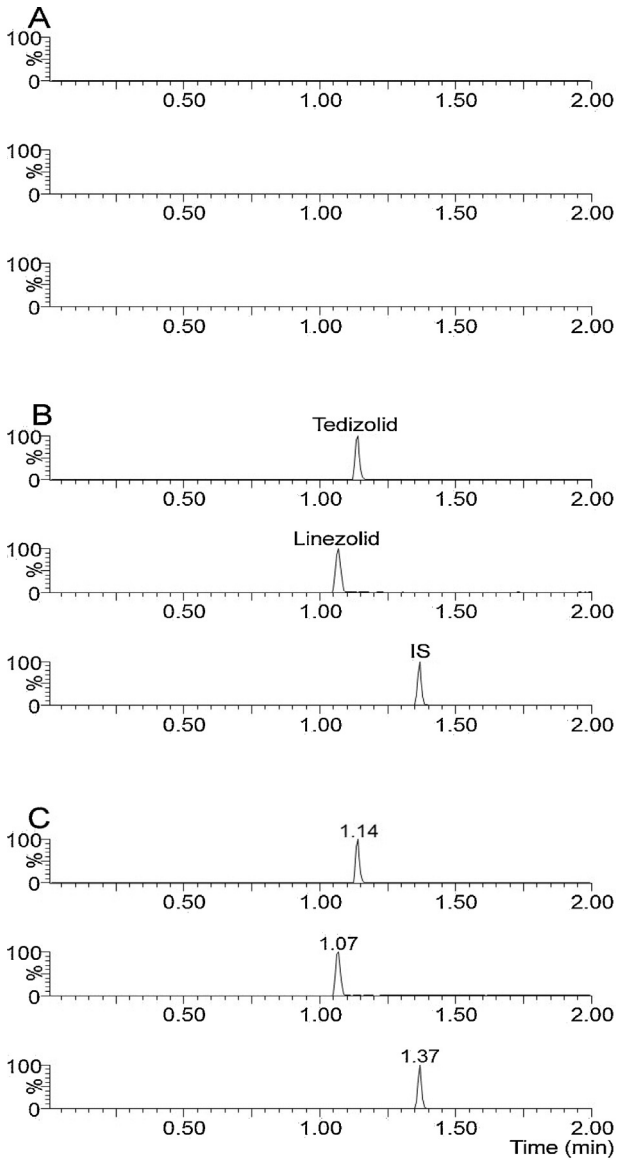

Fig. 2. Representative chromatograms of tedizolid, linezolid and IS in rat plasma samples. (A) a blank plasma sample; (B) a blank plasma sample spiked with tedizolid, linezolid and IS; (C) a plasma sample from a rat 1.0 h after an oral co-administration of tedizolid and linezolid.

2.4. Sample preparation

Before analysis, the plasma sample was thawed to room temperature. In a 1.5 mL centrifuge tube, an aliquot of 200 μL of the IS working solution (200 ng/mL in acetonitrile) was added to 100 μL of collected plasma sample. The tubes were vortex mixed for 1.0 min and spun in a centrifuge at 15,000 × *g* for 10 min. The supernatant (2 μL) was injected into the UPLC-MS/MS system for analysis.