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Determination of the antitubercular drug PA-824 in rat plasma, lung and brain tissues by liquid chromatography tandem mass spectrometry: Application to a pharmacokinetic study

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ABSTRACT

A selective, sensitive and high performance liquid chromatography-tandem mass spectrometry (LC–(ESI)MS/MS) method has been developed and validated for the quantification of the potent antitubercular drug candidate, PA-824, in rat plasma, lung and brain tissues. Sample clean-up involved protein precipitation and solid-phase extraction. Chromatographic separation was performed on YMC Triart C_{18} column (150 mm × 3.0 mm, 3.0 μm). The method was validated over the concentration range of 75–1500 ng/mL for plasma, 50–1200 ng/g for lungs and 100–1500 ng/g for brain tissue. Evaluation of the pharmacokinetic properties of PA-824 utilized Sprague Dawley rats with a dosage of 20 mg/kg at various time points. The new method was applied successfully for the determination of PA-824 with liquid desorption followed by liquid chromatography with ultra-high resolution quadrupole time-of-flight mass spectrometry in the different biological samples.

1. Introduction

The HIV pandemic has created circumstances where co-infection, the emergence of multidrug resistant and extensively drug resistant tuberculosis (MDR-TB and XDR-TB, respectively), and thus mortality attributed to tuberculosis worldwide is increasing [1–4]. Therefore, there is a need for novel drugs with activity against MDR, XDR and latent TB as well as shorter treatment periods. In particular these new agents should be safe and effective for use in HIV-infected TB patients. Nitroimidazoles belong to a class of antimycobacterial agents that are active against drug-susceptible and drug-resistant organisms [4–7]. Nitroimidazoles present similar activity against replicating and non-replicating organisms, which may indicate their potential to shorten therapy timelines [8]. PA-824 is a nitroimidazo-oxazine and a metronidazole derivative from the nitroimidazopyran class [9], developed by the TB Alliance. PA-824 is an antitubercular agent, whose mode of action affects protein and lipid synthesis of *M. tuberculosis*. In addition, it has demonstrated potential bactericidal activity comparable to that of isoniazid [10]. In the past several years, significant technological improvements in mass spectrometry have had a great impact on drug discovery and development [11–14]. At present, LC–MS is a well-established analytical method for the identification and quantification of analytes in sample mixtures and has been widely used in bioanalytical studies [15,16]. LC–MS/MS methods [17–21] frequently provide specific, selective and sensitive quantitative results, often with reduced sample preparation and analysis times compared with other commonly used techniques. Up to now, several methods had been developed for the pharmacokinetics and therapeutic drug monitoring of a number of antitubercular drugs [22–24]. Recently, Wang et al. [25] reported an analytical method for simultaneous determination of three antitubercular drugs, including PA-824 after an oral administration.

To examine the preclinical plasma pharmacokinetics and tissue distribution of PA-824 in a reproducible and precise manner, a validated assay is necessary. To the best of our knowledge, there is no published report literature that demonstrates validation of a sensitive assay for the determination of PA-824 in lungs and brain. The objective of the present study was to develop simple and sensitive method based on SPE/LC–(ESI)MS/MS for the determination of the PA-824 in plasma, lung and brain tissues to be applied in a pharmacokinetic study after oral and intraperitoneal administrations to rats.

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2. Materials and methods

2.1. Reagents and standards

PA-824 was purchased from DLD Scientific (Durban, South Africa). The chemical structure of the analyte is presented in Fig. 1. Acetonitrile (ACN), formic acid, methanol (MeOH) were of analytical grade all from Sigma Aldrich. Ultra-pure water was obtained using a Milli-Q purification system from Millipore Corporation (Bedford, MA, USA). Hybrid-SPE cartridges (30 mg, 1.0 mL) were supplied from Supelco-Sigma (St. Louis, MO). An internal standard (IS), carnidazole, was obtained from Sigma-Aldrich (Steinheim, Germany). Deuterated IS is recommended whenever possible; however, this was not commercially available for PA-824, therefore we decided to use a structurally similar nitroimidazole.

2.2. Instrumentation

The liquid chromatography tandem mass spectrometry (LC–MS) system consisted of a Shimadzu LC-20 AD series HPLC system (Shimadzu Corporation, Kyoto, Japan) coupled to a maXis 4G electrospray ionization (ESI) time-of-flight-mass spectrometry (TOF-MS) instrument (Bruker Daltonics, Bremen, Germany). All results were stored and analyzed with Data Analysis 4.0 SP 5 (Bruker Daltonics).

2.3. Preparation of standards and calibration curves

Separate stock solutions of PA-824 and IS were prepared by dissolving 10 mg of each substance in 10 mL of methanol, and the solutions were stored at refrigerated temperature (0–4 °C). A series of PA-824 working standard solutions and an IS solution, were prepared by appropriate dilutions of their stock solutions with ACN:deionized water (1:1, v/v). Calibration standards were prepared by spiking working standard solutions and IS into 100 μL of blank rat plasma or different tissue homogenates of untreated rats to yield PA-824 concentrations of 75, 150, 250, 500, 750, 1000 and 1500 ng/mL (in plasma), and IS concentration of 250 ng/mL. Quality control (QC) samples at lower limit of quantification (LLQC), low (LQC), middle (MQC) and high (HQC) concentrations (75; 100, 800 and 1400 ng/mL) were prepared separately in the same fashion. More detailed information can be found in Section 2.8.

2.4. Chromatographic conditions

For HPLC separation, a YMC Triart C_{18} column (YMC Europe Gmbh, Dislanken, Germany), with spherical hybrid silica particles (150 mm × 3.0 mm; particle size 3 μm) equipped with the corresponding guard column (4 mm × 3.0 mm) was used. The mobile phase was Milli-Q water (0.1% v/v formic acid) and ACN (0.1% v/v formic acid). The flow rate was 0.2 mL min^{-1} and the temperature of the column oven was set at 25 °C. The gradient profile was initially from 30 to 90% ACN in 10 min (held 2 min), after which time the mobile phase was returned to the initial conditions (30% ACN) in 3 min. The sample injection volume was 5 μL. Column re-equilibration was set at 5 min.

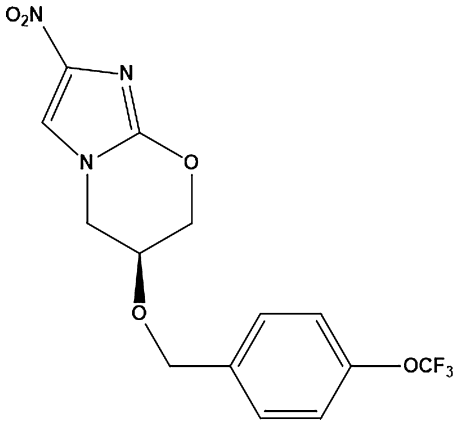

Fig. 1. Chemical structure of the target analyte.

2.5. Mass spectrometric analysis

To obtain accurate mass spectra of target analyte a maXis 4G ESI time-of-flight-mass spectrometry (TOF-MS) instrument (Bruker Daltonics) was used. The MS conditions were optimized. Acquisition parameters were: source type, ESI; ion polarity, positive; nebulizer, 1.5 bar; capillary, 3500 V; dry heater, 200 °C; scan range, *m/z* 100–1500; end plate offset, 500 V; dry gas, 8.0 L/min; collision cell radiofrequency, 600 Vpp; collision energy, 21 eV.

2.6. Plasma and tissue samples

Drug free plasma samples were purchased from Life Technologies (Burlington, ON, Canada) and stored at −20 °C prior to analysis. Plasma samples were thawed at room temperature before analysis. Rat brains and other tissues were separated and weighed, then placed into liquid nitrogen immediately. The organs were weighed, cut it into small pieces using scissors and mixed with ultra-pure water (3 μL/mg tissue). The tissues were then homogenized. All homogenate was stored at −80 °C till analysis.

2.7. Sample preparation

To an aliquot of 100 μL of spiked plasma sample, IS was added at a concentration level of 250 ng/mL and vortexed briefly. Subsequently, 800 μL of acetonitrile or methanol was added to induce the precipitation of plasma proteins. The mixture was vigorously mixed for 1 min, followed by centrifugation at 13,000 rpm for 15 min. The supernatants were filtered through a Hybrid-SPE-Phospholipid cartridge (30 mg/1 mL). The filtered samples (500 μL) were evaporated until dryness under a gentle stream of nitrogen, and reconstituted in 500 μL of mobile phase. All the vials containing samples were vortexed briefly and transferred into autosampler vials for injection into the chromatographic system.

All thawing of frozen plasma samples and tissues were completed at room temperature. For the brain and lung tissue samples, the IS was added to 100 μL brain homogenates samples and followed by addition of ultra-pure water. The IS was added to the homogenized tissue sample and thoroughly mixed. Subsequent extraction procedure of the drug from the tissue samples was the same as described for the plasma.

2.8. Method validation

During the process of method validation, specificity, linearity, lower limit of quantification (LLOQ), limit of detection, precision, accuracy, extraction recovery, and stability were evaluated. The present method was also validated using total error approach [26–28]. For each level, the limit of the β-expectation tolerance interval (β-TI) was calculated. β-TI is the interval within which the average proportion of future β results will fall. Calculations are detailed in the work of Rozet et al. [28]. β-TI should not exceed the threshold of 30% selected for the acceptance limits, in agreement with the recommendations [29].