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Development and validation of a highly sensitive LC–MS/MS-ESI method for quantification of IIIM-019—A novel nitroimidazole derivative with promising action against Tuberculosis: Application to drug development

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ABSTRACT

The study aims to illustrate an analytical validation of a rapid and sensitive liquid chromatography (LC) coupled to tandem mass spectrometry (MS-MS) and electrospray ionization (ESI) method for quantification of IIIM-019 (a novel nitroimidazole derivative with potential activity against Tuberculosis) in mice plasma. The extraction of the analyte and the internal standard (Tolbutamide) from the plasma samples involves protein precipitation using acetonitrile. The chromatographic separation was accomplished using a gradient mode and the mobile phase comprised of acetonitrile and 0.1% formic acid in water. The flow rate used was 0.7 ml/min on a C_{18}e high performance Chromolith column. IIIM-019 and Tolbutamide (IS) were analyzed by combined reversed-phase LC/MS–MS with positive ion electrospray ionization. The MS–MS ion transitions used were 533 > 170.1, 533 > 198 for IIIM-019 and 271 > 74, 271 > 155 for internal standard (IS) respectively. The method was linear over a concentration range of 0.5–1000 ng/ml and the lower limit of quantification was 0.50 ng/ml. The entire study was validated for accuracy, precision, linearity, range, selectivity, lower limit of quantification (LLOQ), recovery, and matrix effect in accordance with the FDA guidelines of method validation. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. The intra and inter-day precisions were in the range of 0.51–11.18% and 0.51–7.55%. The pharmacokinetics was performed on male Balb/c mice by oral (2.5 mg/kg), intraperitoneal (2.5 mg/kg) and intravenous (1 mg/kg) routes. The oral bioavailability of IIIM-019 was 51.6%. The method was also applied successfully in determining microsomal stability wherein the compound was found to be very slightly metabolized by rat liver microsomes.

1. Introduction

Tuberculosis is the second leading cause of death transmitted by inhaling infected droplets containing Mycobacterium tuberculosis (Mtb). A survey in 2012, gives an account of 8.6 million people struggling with the disease amongst which 1.3 million die every year (out of which 13% deaths were of HIV infected patients) [1]. Approximately one third of the world's population is infected with latent tuberculosis wherein the patient is infected with Mtb but the disease is not contagious. Such individuals have a 10% risk of developing active Tuberculosis. The number gives an impression that tuberculosis appears to be a major global health issue. The growing pervasiveness of multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant TB (XDR) has led to the increasing efforts in the development of new drugs for the chemotherapy of tuberculosis [2–4].

It has been estimated by WHO that if the condition remains the same, 30 million people will be infected by Tuberculosis between

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the years 2000 and 2020 [5,6]. Thus, discovery of novel anti-tuberculosis molecules with good potency and less toxicity holds prime importance in minimizing the duration of therapy.

Nitroimidazoles have attained great importance among the researchers in the last decade for their promising action against tuberculosis. They are found to be active against both replicating and non-replicating bacteria [7,8]. 2-Nitroimidazoles were first in this class of compounds to show anti-mycobacterial activity [9]. The anti-tuberculosis activity of bicyclic nitroimidazoles was first identified by Hindustan Ciba-Geigy in 1989 [10]. This led to the generation of a lead compound CGI-17341 effective against both drug susceptible and MDR tuberculosis but its development was freezed due to its mutagenicity [11].

Further SAR studies led to the development of PA-824 (a nitroimidazopyran derivative, currently in Phase-II clinical trial) found to be active against both aerobically replicating as well as anaerobic non-replicating Mtb [12]. Otsuka pharmaceuticals Co. Ltd., developed OPC-67683 (delamanid), a 6-nitro-2,3-dihydroimidazooxazole derivative and has been recently approved by European Union for the treatment of MDR-TB [13,14]. Both the drugs are non-mutagenic and lipophilic in nature [15,16].

IIIM-019 is a nitrodihydroimidazooxazole (structure represented in Fig. 1) with potential activity against *Mycobacterium tuberculosis* H37Rv, non-replicating and resistant strains. The compound has a Minimum inhibitory concentration (MIC) against H37Rv MTB of 0.23 μM and also showed good safety index and *in vivo* efficacy [17]. This makes it an attractive target for preclinical and clinical studies for which a specific quantification method is essential.

In the present study, a simple and rapid HPLC method with MS–MS detection with a short run time and an adequate concentration range has been developed for quantification of IIIM-019 in mice plasma. The method was successfully applied to quantify levels of IIIM-019 in mice plasma in pharmacokinetic study. Extraction of IIIM-019 from the biological fluid i.e. plasma was achieved using liquid-liquid extraction to remove proteins and other interferants. The method was also applied in determining the metabolic stability of the molecule by using rat liver microsomes.

2. Experimental

2.1. Chemicals and reagents

IIIM-019 was obtained from Indian Institute of Integrative Medicine (IIIM) – Medicinal Chemistry Division. HPLC grade acetonitrile and formic acid were purchased from Rankem, Ranbaxy Fine Chemicals Limited (New Delhi, India). DMSO was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Ultra-pure water for the mobile phase was obtained using a Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals were of research grade.

2.2. Animals

All experiments were performed after the approval of the Institutional Animal Ethical Committee of Indian Institute of Integrative Medicine. Pharmacokinetics was conducted on male Balb/c mice (24–26 g) obtained from the Animal House of IIIM. Wistar rats (180–200 g) were used to make the liver microsomes. The animals were housed in a temperature and humidity controlled environment with a 12:12 light-dark cycle and were given free access to food (Lipton India) and water. After acclimatization under these conditions for at least 1 week before the start of experiment, mice were randomly divided into various groups with five in each group. The mice were fasted for over 4–6 h and the rats were fasted for 18 h wherein they were given free access to water.

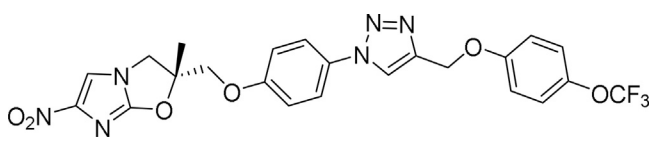

Fig. 1. Chemical Structure of IIIM-019.

2.3. Chromatographic conditions

The analyses were performed using an Agilent 1260 Infinity (Palo Alto, CA, USA) HPLC system equipped with 1260VL infinity quaternary pumps, autosampler, a thermostat compartment. The samples were separated on a Chromolith high resolution RP18e column (100 × 4.6 mm, Merck, Darmstadt, Germany) guarded by a Chromolith RP18e 10 × 4.6 mm analytical guard column. The mobile phase consisted of acetonitrile and 0.1% (*v/v*) formic acid in water. The isocratic elution was used with 7.3% (0.1%) formic acid in water and 2.7% acetonitrile. The flow rate was adjusted to 0.7 ml min^{-1} and column temperature was maintained at 60 °C. Triple-quadrupole tandem mass spectrometry (MS/MS) was carried out on a Agilent 6410 tandem triple quadrupole mass spectrometer (TQD-MS) equipped with an ESI ion source operating in both positive ion mode and negative mode. ESI source was operated in positive ionization mode. Quantification was performed in MRM mode. The MS parameters were optimized at capillary voltage 4.0 kV, chamber current 0.43 μA, capillary current 5679 nA and gas temperature 300 °C. Nitrogen was used as desolvation gas at the rate of 15 l min^{-1} and nebulizer pressure was maintained at 50 psi. Nitrogen was also used as the collision gas. Collision energies of 15 eV and 10 eV were used for IIIM-019 and the IS, respectively. The mass resolution was set at 0.7 μFWHM (unit mass resolution) for both quadrupoles. The fragmentor voltage values were 170 V for IIIM-019 and 90 V for IS. Quantification was performed using multiple reaction monitoring (MRM). The transitions were *m/z* 533> 170.1, 533 > 198 for IIIM-019 and 271 > 74, 271 > 155 for IS, respectively. The injection volume was 10 μl and the total run time was 10.0 min. All the data was collected in the centroid mode and acquired and processed using Agilent Mass Hunter work station software.

2.4. Standard solution preparation

The primary stock solution of IIIM-019 was prepared in DMSO (1 mg/ml). The intermediate stock solution of 100 μg/ml and the working standard of 1 μg/ml were prepared in acetonitrile. The primary stock solution in DMSO was stored in −20 °C and was found to be stable. The standards of IIIM-019 were prepared by dilution of the stock solution with acetonitrile to obtain the working stock solutions in plasma of the following concentrations: 0.5, 1.0, 5.0, 10, 50, 100, 500, 1000 ng/ml for calibration curve (CC); 0.50, 1.0, 500 and 1000 ng/ml for quality control (QC). The stock solution of Tolbutamide (1 μg/ml) was prepared in acetonitrile to obtain a final concentration of 10 ng/ml in each sample. Tolbutamide has high recovery from the plasma (minimum matrix interference) and its ability to give precise results on precision accuracy studies makes it a suitable compound to be used as an internal standard [18,19]. Calibration standards were prepared by spiking 100 μl of mice plasma with suitable working solution of IIIM-019 (10 μl) and IS (10 μl) to obtain a final concentration of 10 ng/ml of the IS in each sample. Samples for the determination of precision and accuracy were prepared by spiking control mice plasma with IIIM-019 at appropriate concentrations (0.50, 1.0, 500 and 1000 ng/ml). All the stocks and the standard solutions were stored under −80 °C until analysis.