Journal of Chromatography B 1060 (2017) 200–206

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Development and validation of a highly sensitive LC-ESI-MS/MS method for estimation of IIIM-MCD-211, a novel nitrofuranyl methyl piperazine derivative with potential activity against tuberculosis: Application to drug development

Asmita Magotra[a,b], Anjna Sharma[a,b], Ajai Prakash Gupta[c], Priya Wazir[a], Shweta Sharma[d], Parvinder Pal Singh[d], Manoj Kumar Tikoo[a], Ram A. Vishwakarma[e], Gurdarshan Singh[a,*], Utpal Nandi[a,*]

[a] PK-PD, Toxicology and Formulation division, CSIR- Indian Institute of Integrative Medicine, Jammu 180 001, India
[b] Academy of Scientific and Innovative Research (AcSIR), CSIR- Indian Institute of Integrative Medicine, Jammu 180 001, India
[c] Quality Control and Quality Analysis Division, CSIR- Indian Institute of Integrative Medicine, Jammu, J & K, 180 001, India
[d] Medicinal Chemistry Division, CSIR- Indian Institute of Integrative Medicine, Jammu 180 001, India
[e] Director, CSIR-Indian Institute of Integrative Medicine, anal Road, Jammu 180001, J & K, India

ARTICLE INFO

Keywords:
Bioanalytical method
LC–MS/MS
IIIM-MCD-211
Pharmacokinetic study
Metabolic stability
Validation

ABSTRACT

In the present study, a simple, sensitive, specific and rapid liquid chromatography (LC) tandem mass spectrometry (MS/MS) method was developed and validated according to the Food and Drug Administration (FDA) guidelines for estimation of IIIM-MCD-211 (a potent oral candidate with promising action against tuberculosis) in mice plasma using carbamazepine as internal standard (IS). Bioanalytical method consisted of one step protein precipitation for sample preparation followed by quantitation in LC–MS/MS using positive electrospray ionization technique (ESI) operating in multiple reaction monitoring (MRM) mode. Elution was achieved in gradient mode on High Resolution Chromolith RP-18e column with mobile phase comprised of acetonitrile and 0.1% (v/v) formic acid in water at the flow rate of 0.4 mL/min. Precursor to product ion transitions (*m/z* 344.5/218.4 and *m/z* 237.3/194.2) were used to measure analyte and IS, respectively. All validation parameters were well within the limit of acceptance criteria. The method was successfully applied to assess the pharmacokinetics of the candidate in mice following oral (10 mg/kg) and intravenous (IV; 2.5 mg/kg) administration. It was also effectively used to quantitate metabolic stability of the compound in mouse liver microsomes (MLM) and human liver microsomes (HLM) followed by its *in-vitro-in-vivo* extrapolation.

1. Introduction

One of the world's life-threatening communicable diseases is tuberculosis (TB). As per the global tuberculosis report of World Health Organization (WHO) in the year of 2014, an estimated 9.0 million people developed TB which included 1.1 million people with HIV-positive. Worldwide death from this disease is unacceptably high with high levels of resistance *i.e.* multi drug resistance (MDR), extensively drug resistance (XDR) and poor treatment outcomes [1]. Therefore, Anti-TB drug discovery programme involve continuous search for novel rapid acting candidates that can be effective against both susceptible and resistant strains with shortening the prolonged TB treatments [2,3]. Extensive research is going on for anti-TB drugs having new chemotype through whole cell screening approach which is attractive because of

Abbreviations: LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; FDA, Food and Drug Administration; IS, internal standard; ESI, electrospray ionization; MRM, multiple reaction monitoring; IV, intravenous; TB, tuberculosis; MTB, *Mycobacterium tuberculosis*; MDR, multi drug resistance; XDR, extensively drug resistance; WHO, World Health Organization; MIC, minimum inhibitory concentration; QC, quality control; LLOQ, lower limit of quantitation; LQC, low quality control; MQC, middle quality control; HQC, high quality control; MLM, mouse liver microsomes; HLM, human liver microsomes; RSD, relative standard deviation; SD, standard deviation; AUC_{0-t}, area under the curve for plasma concentration from zero to the last measurable plasma sample time; $AUC_{0-\infty}$, area under the curve for plasma concentration from zero to time infinity; C_{max}, maximum plasma concentration; T_{max}, time to reach maximum plasma concentration; Cl, clearance; V_d, volume of distribution; $T_{1/2}$, elimination half-life; CYP, cytochrome P450; $t_{1/2}$, *in-vitro* half-life; k_{el}, *in-vitro* elimination rate constant; CL_{int}, intrinsic clearance; CL_{int},H, hepatic intrinsic clearance; CL.H, hepatic clearance; BW, body weight

* Corresponding authors at: PK-PD, Toxicology and Formulation division, CSIR- Indian Institute of Integrative Medicine, Jammu, J & K 180 001, India.
E-mail addresses: singh_gd@iiim.ac.in (G. Singh), unandi@iiim.ac.in, utpalju@gmail.com (U. Nandi).

http://dx.doi.org/10.1016/j.jchromb.2017.06.015
Received 22 March 2017; Received in revised form 21 May 2017; Accepted 6 June 2017
Available online 08 June 2017
1570-0232/

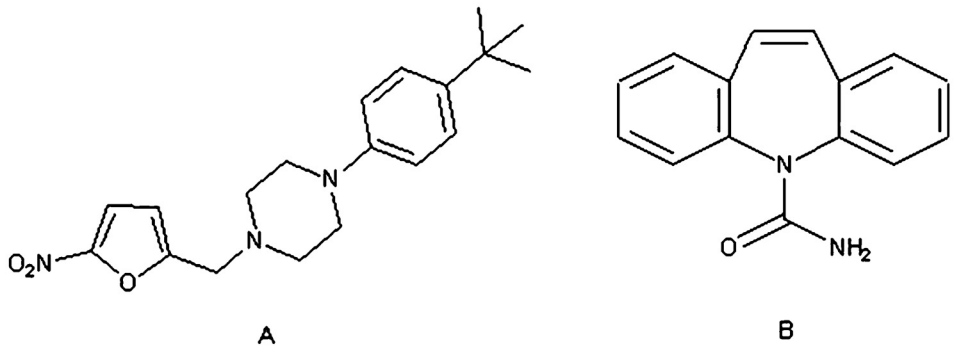

Fig. 1. Chemical structure of (A) IIIM-MCD-211 and (B) Carbamazepine (IS).

Table 1
Gradient program for elution of IIIM-MCD-211 and IS.

Time point (min)	Mobile phase ratio (%v/v)	
	0.1% Formic acid in water	Acetonitrile
0	70	30
5	70	30
8	20	80
10	20	80
11	70	30
12	70	30

discovery of several compounds such as bedaquiline (TMC207; diarylquinoline derivative), TCA-17 (benzothiazoles), Q-203 (imidazopyridine amides) *etc* [4–8]. However, the major hurdle is the undesirable pharmacokinetics during the development of a new chemical candidate into a drug candidate [9–12]. Thus, early evaluations of absorption, distribution, metabolism, and excretion (ADME) properties have become mandatory to speed up the discovery process. Moreover, detailed preclinical *in-vitro* and *in-vivo* metabolic stability and pharmacokinetic evaluation of new therapeutic candidate is a regulatory requirement before plunge into clinical studies for better perception of efficacy and safety [13–15].

In this context nitrofurans have attained great attention due to their promising action against *Mycobacterium tuberculosis* (MTB) including resistant strains [16–18]. Our lab Council of Scientific and Industrial Research-Indian Institute of Integrative Medicine (CSIR-IIIM) has identified a novel and potent anti-TB agent IIIM-MCD-211, chemically 1-(4-*tert*-butyl) phenyl)-4-(5-nitrofuran-2yl) methyl piperazine (Fig. 1). Some of the attributes which make this molecule attractive are good water solubility (40 μg/mL); excellent minimum inhibitory concentration like 0.0072 μM against H37Rv strain, 1.4 μM against non-replicating phase strain, 0.072 μM against resistant Rif^R strain, 0.029 μM against MDR strain of MTB; acceptable safety indices [19]. All these above characteristics have prompted further drug development process for which a specific quantitation method is required. Therefore, the aim of the present research work was to develop a simple, sensitive and rapid LC-ESI-MS/MS method for quantitation of IIIM-MCD-211 in mice plasma. Method was to be validated according to the FDA guidelines. This new method was applied to estimate the pharmacokinetics of IIIM-MCD-211 in mice following oral (10 mg/kg) and IV (2.5 mg/kg) administrations. Further, metabolic stability of the compound was to be evaluated in mouse liver microsomes (MLM) and human liver microsomes (HLM) followed by its *in-vitro-in-vivo* extrapolation.

2. Experimental

2.1. Chemicals and reagents

IIIM-MCD-211 (purity ≥ 96%) was synthesized in Medicinal Chemistry Division, Indian Institute of Integrative Medicine, Jammu. Carbamazepine (IS) (purity ≥ 99%) (Fig. 1) and verapamil (purity ≥ 98%) were purchased from Sigma-Aldrich (St. Louis, USA). Formic acid and acetonitrile (LC–MS grade) were procured from Rankem (New Delhi, India). Other chemicals or reagents used were of analytical grade. Water having resistivity of 18.2 MΩ-cm was used throughout the analysis (Millipore water purification system, Millipore, Bedford, USA).

2.2. Instrumentation and LC–MS/MS conditions

LC system (Make: Agilent Technologies; Model: 1260 series) equipped with a degasser, quaternary pump, autosampler, column compartment and detector was used to inject (10 μL) of the processed samples on a Chromolith High Resolution RP-18e column (50 × 4.6 mm) (Merck, India) which kept at room temperature. Elution was achieved at gradient mode (Table 1) using 0.1% (v/v) formic acid in water and acetonitrile that was delivered at 0.4 mL/min into mass spectrometer's electrospray ionization chamber. Autosampler was set at 4 °C while column was maintained at ambient temperature.

Quantitation was accomplished in + Ve ion mode for IIIM-MCD-211 and IS using a triple quad mass spectrometer (Make: Agilent Technologies; Model: 6410B) equipped with ESI source. Common MS parameters *viz*. capillary voltage, nebulizer and drying gas flow were set at 3000 V, 50 psi, 12 L/min, respectively. Compound dependent parameters like fragmentor voltage and collision energy were 121 V and 12 eV for IIIM-MCD-211 whereas 100 V and 10 eV for IS, respectively. Monitoring of the transition from the parent ion (344.5 for IIIM-MCD-211; 237.3 for IS) to product ions (218.4 & 176.3 for IIIM-MCD-211; 194.2 for IS) was carried out in MRM mode for analysis. Data were acquired by Mass Hunter software (ver. B06.00).

2.3. Preparation of stock, standard and quality control samples

Stock solution (1.0 mg/mL) was prepared in methanol for analyte and IS by weighing separately. Then, these were diluted further with methanol to prepare standard solutions/calibration standards/quality control (QC) samples. A nine point calibration curve for analyte was prepared by spiking serially diluted working solution into blank plasma to obtain final concentrations in the range of 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 ng/mL for the analyte. In the same way, QC samples were also prepared to attain the final concentrations of 1 (low QC ;LQC), 40 (middle QC; MQC) and 90 (high QC; HQC) ng/mL. All solutions were stored at −20 °C for further use.

2.4. Sample preparation

Protein precipitation procedure was employed to recover analyte from mice plasma. Calibration standards (10 μL of working solution of analyte was spiked into 90 μL of blank plasma) and pharmacokinetic study samples (100 μL of plasma) were processed by adding IS (10 μL of