Fitoterapia 97 (2014) 34–42

Contents lists available at ScienceDirect

Fitoterapia

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

Pharmacokinetic and metabolism studies of rohitukine in rats by high performance liquid-chromatography with tandem mass spectrometry

Yashpal S. Chhonker [a,b], Hardik Chandasana [a,b], Deepak Kumar [a], Sunil K. Mishra [c], Shishir Srivastava [c], Vishal M. Balaramnavar [c], Anil Nilkanth Gaikwad [d], Sanjeev Kanojiya [e], Anil K. Saxena [c], Rabi S. Bhatta [a,b,*]

[a] Pharmacokinetics & Metabolism Div., CSIR — Central Drug Research Institute, Lucknow 226031, India
[b] Academy of Scientific and Innovative Research (AcSIR), Anusandhan Bhawan, Rafi Marg, New Delhi 110 001, India
[c] Medicinal & Process Chemistry Div., CSIR — Central Drug Research Institute, Lucknow 226031, India
[d] Pharmacology Div., CSIR — Central Drug Research Institute, Lucknow 226031, India
[e] Sophisticated Analytical Facility, CSIR — Central Drug Research Institute, Lucknow 226031, India

ARTICLE INFO

Article history:
Received 26 March 2014
Accepted in revised form 9 May 2014
Available online 16 May 2014

Keywords:
Rohitukine
Dysoxylum binectariferum
Pharmacokinetics
LC–MS/MS
Bioanalytical method
Bioavailability

ABSTRACT

A sensitive, selective, and rapid high performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed for the quantification of rohitukine in rat plasma. HPLC was performed using a Symmetry-Shield C_{18} (5 μ, 4.6 × 150 mm) column, and isocratic elution with ammonium acetate buffer (pH 4; 10 mM):methanol (08:92, v/v) at a flow rate of 0.6 mL/min. Sample clean-up involved solid phase extraction (SPE) of analyte and internal standard (phenacetin) from 100 μL plasma. The parent → product ion transitions (MRM) for analyte and IS were 306.1 → 245.1 *m/z* and 180.1 → 138.1 *m/z* respectively, and were monitored on a triple quadrupole mass spectrometer, operating in positive ion mode. The method was validated across the dynamic concentration range of 5–500 ng/mL for rohitukine, with a fast run time of 4.5 min. The analytical method measured concentrations of rohitukine with accuracy (% bias) of $<\pm 10\%$ and precision (% RSD) of $<\pm 12\%$. Rohitukine was stable during the battery of stability studies viz., bench-top, auto-sampler, freeze/thaw cycles and 30 days of storage in a freezer at -70 ± 10 °C. Finally, the applicability of this assay has been successfully demonstrated in vivo pharmacokinetic and in vitro metabolism studies in *Sprague–Dawley* rat. This method will therefore be highly useful for future preclinical and clinical pharmacokinetic studies of rohitukine.

1. Introduction

The Chromone alkaloids are important pharmacophore exhibiting promising medicinal properties. Rohitukine (Fig. 1a), a chromane alkaloid (5, 7-dihydroxy-8-((3S, 4R)-3-hydroxy-1-methylpiperidin-4-yl)-2-methyl-4H-chromen-4-one) was first isolated from *Amoora rohituka* and later from *Dysoxylum binectariferum*, both plants belonging to Meliaceae family [1–3]. The rohitukine is a major bioactive chemical constituent of ethanolic extract of *D. binectariferum*. The rohitukine has been reported to exhibit various biological activities, such as anticancer [4,5], anti-inflammatory and immune-modulators [1], anti-fertility and anti-implantation [6], anti-leishmania activity [7] and anti-ulcer activity [8]. Rohitukine also inhibits adipogenic differentiation in vitro as well as inhibit development of dyslipidemia in HFD fed hamsters in vivo [9]. Despite several pharmacodynamic properties of rohitukine, a detailed pharmacokinetic study has yet not been reported.

* Corresponding author at: Pharmacokinetics & Metabolism Div., CSIR — Central Drug Research Institute, 226031, India. Tel.: +91 522 2772974x4853.
E-mail address: rabi.cdri@gmail.com (R.S. Bhatta).

http://dx.doi.org/10.1016/j.fitote.2014.05.004
0367-326X/

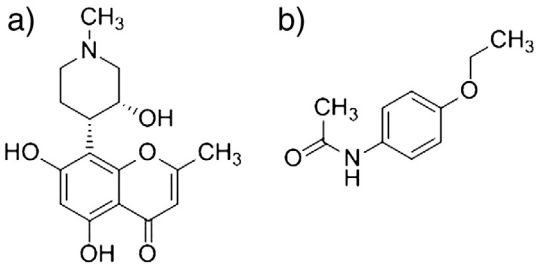

Fig. 1. The chemical structure of (a) rohitukine and (b) phenacetin (IS).

Therefore, in order to probe more efficiently the potential benefits, pharmacokinetics, and drug interaction potential of rohitukine consumption, it is necessary to develop analytical methodologies which are capable of the sensitive and accurate quantification of rohitukine in low volumes of biometrics. To date, only HPLC-UV analytical methods have been reported to quantify rohitukine in hamster plasma, however they lack sensitivity and require larger plasma volume [9]. Therefore, it was necessary to develop a sensitive and selective LC–MS/MS method to the quantification of rohitukine in low volume of plasma. The application of HPLC and LC–MS/MS is the technique that is currently considered the method of choice for supporting preclinical and clinical pharmacokinetic studies [10–13]. In the present study, therefore, we developed and validated a highly sensitive, simple and rapid LC–MS/MS method for the quantification of rohitukine in rat plasma. This research is the first to report the successful application of such a method in the pharmacokinetic study of rohitukine after oral and *intra-venous* (*I.V.*) administration of rohitukine to *Sprague–Dawley* (SD)-rats.

2. Experimental

2.1. Chemical and reagents

Pure reference standards of rohitukine (>98%) were procured from Medicinal and Process Chemistry Division, CSIR — Central Drug Research Institute (CDRI) India. The phenacetin used as internal standard (IS) was purchased from Sigma-Aldrich (India) Pvt. Ltd. (Fig. 1b). Methanol, HPLC grade, was purchased from Sigma-Aldrich (India) Pvt. Ltd. DSC-18 cartridges (Discovery Supelco Cat. no. 52602-U) were purchased from Sigma-Aldrich (India) Pvt. Ltd. Ultra pure water of 18.2 MΩ·cm was obtained from a Milli-Q PLUS PF system. Heparin sodium injection I.P. (1000 IU/mL) was purchased from Biologicals E. Ltd. (Hyderabad, India). Rat plasma was collected from healthy adult; drug free male SD rats at Laboratory Animal Division of CDRI (Lucknow, India). Plasma was obtained by centrifuging the heparinized blood at 2000 ×*g* for 5 min. Pooled plasma samples were stored at −70 ± 10 °C till use. All the other reagents were of analytical grade. All the animal experiments were conducted in accordance with the current legislation on animal experiments as per Institutional Animal Ethical Committee at CSIR — Central Drug Research Institute (IAEC approval no IAEC/2012/91Ns).

2.2. LC–MS/MS conditions

A Shimadzu (Shimadzu, Japan) SIL series LC system equipped with a degasser (DGU-20A3), isopump (LC-20 AD) and column oven (CTO-10AS) along with an auto-sampler (SIL-HTc) was used to inject 20 μL aliquots of the processed samples onto a Symmetry-Shield C_{18} (5 μ, 4.6 × 150 mm) column, preceded with a guard column. The system was calibrated in isocratic mode with a mobile phase consisting of 10 mM ammonium acetate (pH 4): methanol (08:92, v/v) at a flow rate of 0.6 mL/min. The column oven temperature was maintained at 30 °C and the total LC run time was 4.5 min. Other chromatographic parameters like rinsing volume, rinsing speed, needle stroke, sampling speed, purge time and rinse dip time were kept at 300 μL, 25 μL/s, 52 mm, 3.0 μL/s, 1.0 min and 10 s respectively. Rinsing solution was kept at methanol:water (80:20). Rinsing mode was set before and after aspiration to ensure negligible carryover effect.

Mass spectrometric detection was performed on an API 4000 Q trap mass spectrometer (Applied Biosystems, Canada) equipped with an electro-spray ionization (ESI) source. The MS/MS system was operated at unit resolution in the multiple reaction monitoring (MRM) mode, using ion precursor → product ion combinations of 306.1 → 245.1 *m/z* for rohitukine and 180.1 → 138.1 *m/z* for IS. The instrument response was optimized for rohitukine and IS by infusing a constant flow of a solution of the drug dissolved in mobile phase. Electro-spray ionization (ESI) was performed in the positive ion. The source temperature was 500 °C. Ion spray voltage of 5500 V was applied in positive mode. Nitrogen was used as the collision gas. The curtain gas was kept at 12. The optimized GS1 and GS2 were 45 and 50, respectively. Compound dependant parameters set for rohitukine and IS, were declustering potential (DP), 76 and 46 V; entrance potential (EP), 10 V for both; collision energy (CE), 35 and 50 eV; and collision exit potential (CXP), 10 and 12 V, respectively.

Q1 and Q3 were maintained at unit resolution and the dwell time was kept at 200 ms. Zero air was used as source gas while nitrogen was used as both curtain and collision gas. All raw data were processed with PE SCIEX Analyst Software (Version 1.4.2) from Applied Biosystems.

2.3. Preparation of standard stock, calibration curve and quality control sample

Stock solution, calibration curve and quality control sample preparation. Stock solutions (1 mg/mL) of rohitukine and phenacetin were prepared by dissolving in methanol and stored in a refrigerator (1 to 6 °C). The working solutions of analyte were obtained by step-wise dilution of the stock solution. Calibration curve of rohitukine was prepared in blank normal rat plasma over a concentration range of 5–500 ng/mL. The calibration curve (CC) consisted of one replicate of eight non-zero standards, with two standards below the low quality control (QC) and one standards above the high QC. Different stocks were used to prepare the calibration standards and QC samples.

Calibration standards and QC samples of appropriate concentration were prepared by spiking (5% of total volume) blank plasma with an appropriate analyte stock. The lower limit of quantitation (LLOQ) was 5.0 ng/mL. Quality control samples (five sets) at concentrations of low, 15 ng/mL (LQC), medium, 200 ng/mL (MQC), and high, 400 ng/mL (HQC), concentrations were prepared in rat plasma and analyzed with each assay validation run to ensure acceptable assay