Journal of Chromatography B 1061–1062 (2017) 49–56

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous estimation of lisofylline and pentoxifylline in rat plasma by high performance liquid chromatography-photodiode array detector and its application to pharmacokinetics in rat

Kishan S. Italiya, Saurabh Sharma, Ishit Kothari, Deepak Chitkara, Anupama Mittal*

Industrial Research Laboratory (IRL), Department of Pharmacy, Birla Institute of Technology and Science (BITS-PILANI), Pilani, Rajasthan, 333031, India

ARTICLE INFO

Keywords:
Lisofylline
Pentoxifylline
Method validation
HPLC
Plasma
Pharmacokinetics

ABSTRACT

Lisofylline (LSF) is an anti-inflammatory and immunomodulatory agent with proven activity in serious infections associated with cancer chemotherapy, hyperoxia-induced acute lung injury, autoimmune disorders including type-1 diabetes (T1DM) and islet rejection after islet transplantation. It is also an active metabolite of another anti-inflammatory agent, Pentoxifylline (PTX). LSF bears immense therapeutic potential in multiple pharmacological activities and hence appropriate and accurate quantification of LSF is very important. Although a number of analytical methods for quantification of LSF and PTX have been reported for pharmacokinetics and metabolic studies, each of these have certain limitations in terms of large sample volume required, complex extraction procedure and/or use of highly sophisticated instruments like LC–MS/MS.

The aim of current study is to develop a simple reversed-phase HPLC method in rat plasma for simultaneous determination of LSF and PTX with the major objective of ensuring minimum sample volume, ease of extraction, economy of analysis, selectivity and avoiding use of instruments like LC–MS/MS to ensure a widespread application of the method.

A simple liquid-liquid extraction method using methylene chloride as extracting solvent was used for extracting LSF and PTX from rat plasma (200 µL). Samples were then evaporated, reconstituted with mobile phase and injected into HPLC coupled with photo-diode detector (PDA). LSF, PTX and 3-isobutyl 1-methyl xanthine (IBMX, internal standard) were separated on Inertsil® ODS (C18) column (250 × 4.6 mm, 5 µm) with mobile phase consisting of A-methanol B-water (50:50 v/v) run in isocratic mode at flow rate of 1 mL/min for 15 min and detection at 273 nm. The method showed linearity in the concentration range of 50–5000 ng/mL with LOD of 10 ng/mL and LLOQ of 50 ng/mL for both LSF and PTX. Weighted linear regression analysis was also performed on the calibration data. The mean absolute recoveries were found to be 80.47 ± 3.44 and 80.89 ± 3.73% for LSF and PTX respectively. The method was successfully applied for studying the pharmacokinetics of LSF and PTX after IV bolus administration at dose of 25 mg/kg in Wistar rat. In conclusion, a simple, sensitive, accurate and precise reversed-phase HPLC-UV method was established for simultaneous determination of LSF and PTX in rat plasma.

1. Introduction

Lisofylline (LSF, 1-(5-Hydroxyhexyl)-3,7-dimethylxanthine) is a modified methyl xanthine derivative (Fig. 1), having anti-inflammatory and immunomodulatory properties [1,2]. It was originally developed and tested to reduce cellular damage due to autoimmunity, hypoxia and ischemic reperfusion [3,4]. LSF has been used to overcome morbidity and mortality during serious infections associated with cancer chemotherapy and for treatment of acute lung injury after severe trauma [5,6]. LSF is also reported for its therapeutic potential in early treatment of diabetes, wherein it enhanced glucose-stimulated insulin secretion [7,8], caused reversal of insulin insensitivity by modulating oxidized free fatty acids (FFA) and glucose-induced phosphorylation of the insulin receptor [9]. The protective role of LSF in diabetes is mainly attributed to the promotion of mitochondrial metabolism in β-cells, normalizing the membrane potential of mitochondria and thus stimulating energy production [10,11]. This broad spectrum of activity suggests that LSF bears significant clinical utility in preventing both Type-1 diabetes mellitus (T1DM) and Type-2 diabetes mellitus (T2DM) [1,12]. Its therapeutic potential is further reflected by a clinical trial

* Corresponding author at: Department of Pharmacy, Birla Institute of Technology and Science (BITS), Pilani Campus, Pilani, Rajasthan, 333 031, India.
E-mail address: anupama.mittal@pilani.bits-pilani.ac.in (A. Mittal).

http://dx.doi.org/10.1016/j.jchromb.2017.06.043
Received 28 March 2017; Received in revised form 21 June 2017; Accepted 24 June 2017
Available online 29 June 2017
1570-0232/

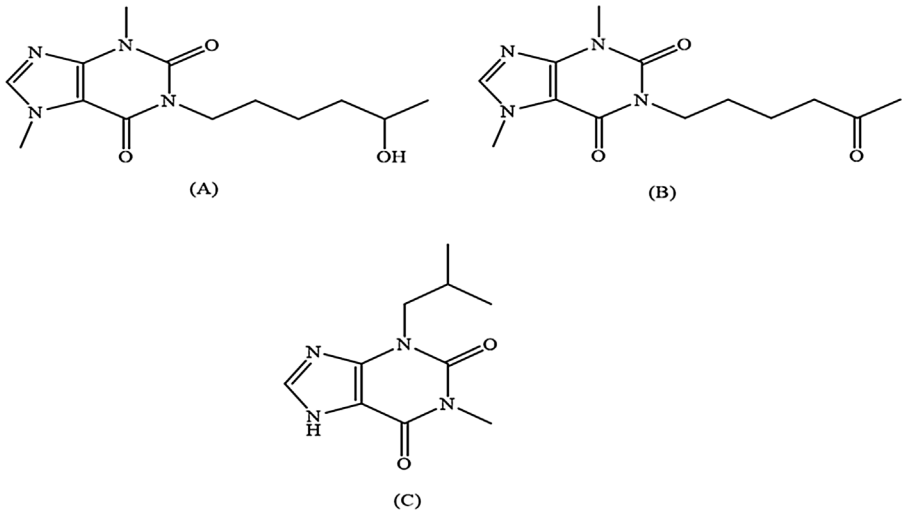

Fig. 1. Chemical structures of (A) Lisofylline, 1-(5-Hydroxyhexyl)-3,7-dimethylxanthine, (B) Pentoxifylline, 3,7-Dimethyl-1-(5-oxohexyl) xanthine and, (C) 3-Isobutyl 1-Methyl Xanthine, IBMX.

wherein, LSF is administered by continuous subcutaneous or intravenous route in subjects with T1DM to evaluate its safety, tolerability and bioavailability [13]. Apart from being administered as a drug itself, LSF is also reported to be an active metabolite of another drug, Pentoxifylline (PTX; 3,7-Dimethyl-1-(5-oxohexyl) xanthine, Fig. 1) which is clinically used as a haemorrheologic agent for the treatment of cerebrovascular and peripheral vascular disease [14]. It has also been reported that LSF and PTX undergo interconversion *in vivo* [15]. PTX has also demonstrated immunomodulatory effects in animal model of sepsis [16]. Considering the immense therapeutic potential and multiple pharmacological activities of LSF and PTX as stated above, appropriate and accurate quantification of LSF is of paramount significance.

Few methods have been reported in literature, which are based on either a complex sample preparation procedure or use of sophisticated instruments like LC–MS/MS that hinders its use in routine analysis for various applications. Chivers, et al. developed a HPLC-UV method for simultaneous quantification of LSF and PTX in plasma at a high flow rate (2 mL/min) using a sample volume of 1000 µL that required 10 mL of extracting solvent (methylene chloride) [17]. Grasela, et al. reported a HPLC-UV method requiring a multistep liquid–liquid extraction (LLE) process and high injection volume (250 µL) that may not be applicable for routine analysis [18]. A LC–MS/MS method has been reported to LSF and PTX up to 1 ng/mL however it requires a highly specific sample preparation method (lithium precipitation using Seraprep reagent) [19]. Although, use of LC–MS/MS method is a recommended procedure and significantly enhances the sensitivity of the method; it is not available in every research laboratory, requires highly trained personnel and is associated with a high running and maintenance cost. Moreover, LSF has been reported to be administered at a high dose ranging from 25 to 50 mg/kg in animals [1,20] and 1–3 mg/kg in humans [14]. Thus, the need of detecting a concentration below 50 ng/mL may not arise at all and hence HPLC based methods might also be equally useful.

The present work describes method development and its detailed validation for quantification of LSF and PTX in rat plasma within the range of 50–5000 ng/mL. The proposed method uses a simple liquid–liquid extraction (LLE) method using methylene chloride (2 mL) as an extracting solvent, and small sample volume (200 µL). 3-isobutyl 1-methyl xanthine (IBMX) was selected as internal standard (I.S) (Fig. 1). Full validation was carried out including selectivity, lower limit of quantification (LLOQ), limit of detection (LOD), precision, accuracy, carry over effect, dilution integrity and stability, using internationally accepted guidelines for bioanalytical method validation [21,22]. Stability studies were also performed to determine the stability of stock solutions and of plasma samples that were exposed to different storage conditions including repeated freeze-thaw cycles, autosampler, long term, and bench top storage. The developed method was applied to the pharmacokinetics (PK) studies of LSF and PTX (25 mg/kg, i.v.) in wistar rat.

2. Material and methods

2.1. Chemicals, reagents and experimental animals

(±) LSF (purity ≥99%, HPLC) was purchased from Cayman Chemicals Inc. (Michigan, USA). PTX and IBMX (purity ≥99%, HPLC) were obtained from Sigma Aldrich (St. Louis, MO, USA). HPLC grade solvents, acetonitrile (ACN), methanol and methylene chloride were obtained from Merck Limited (Mumbai, India). Purified water was used in our studies which refers to the Mili-Q Reference ultrapure water (Type 1, as described by ASTM®, ISO® 3696 and CLSI® norms) prepared using Milli-Q® Reference water purification system. Wistar rats (male; 8–10 weeks, 200–220 g) were procured from Central Animal Facility, BITS-PILANI (Pilani, India). Animal experiment protocol was approved by Institutional Animal Ethics Committee (IAEC), BITS-PILANI, Pilani and experiments were conducted as per CPCSEA guidelines. Rats were housed in well ventilated cages at standard laboratory conditions with regular light/dark cycles for 12 h and fed with standard normal diet *ab libitum*. All other chemicals and reagents were of analytical grade and used as obtained.

2.2. Liquid chromatographic conditions

A Shimadzu HPLC system (Kyoto, Japan) equipped with a binary pump (LC-20AD), Photo Diode Array (PDA) detector (SPD-M20A) and auto sampler (SIL-HTC, Shimadzu, Japan) were used to develop the analytical method. The HPLC system was equilibrated for approximately 40 min before beginning the sample analysis. LSF, PTX and IBMX were separated on Inertsil® ODS (C18) column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of A-methanol B-water (50:50 v/v) run in isocratic mode at a flow rate of 1 mL/min and injection volume of 80 µL. Eluents were monitored at a wavelength of 273 nm. Control of hardware and data handling was performed using LCsolution software version 1.22 SP1.

2.3. Preparation of stock solutions, calibration curve standards (CS) and quality control (QC) samples

Stock solutions of LSF (1 mg/mL) and PTX (1 mg/mL) were prepared by dissolving the accurately weighed amount of each of these analytes in Milli-Q water. The stock solution (1 mg/mL) of IBMX was