Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 857 (2007) 224–230
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Development and validation of an enantioselective HPLC–UV method using Chiralpak AD-H to quantify (+)- and (−)-torcetrapib enantiomers in hamster plasma—application to a pharmacokinetic study

Ravi Kumar Trivedi [a,b], P.K. Dubey [b], Ramesh Mullangi [a,*], Nuggehally R. Srinivas [a,c]

[a] *Drug Metabolism and Pharmacokinetics, Discovery Research, Dr. Reddy's Laboratories Ltd., Miyapur, Hyderabad-500 049, India*
[b] *Department of Chemistry, JNTU College of Engineering, Kukatpally, Hyderabad-500 085, India*
[c] *Drug Development, Discovery Research, Dr. Reddy's Laboratories Ltd., Miyapur, Hyderabad-500 049, India*

Received 9 May 2007; accepted 14 July 2007
Available online 6 August 2007

Abstract

A chiral selective, accurate and reproducible high-performance liquid chromatographic (HPLC) method was developed and validated for direct separation of individual enantiomers of torcetrapib (TTB) [(+)-TTB and (−)-TTB]. TTB enantiomers and IS were extracted from a small aliquot of plasma (100 μL) by simple liquid–liquid extraction using acetonitrile as extraction solvent. The enantiomers were resolved on Chiralpak AD-H® (250 mm × 4.6 mm, 5 μm) with the mobile phase consisting of *n*-hexane:isopropyl alcohol (IPA) in the ratio of 95:5 (v/v). The eluate was monitored using an UV detector set at 254 nm. Baseline separation of the TTB enantiomers and the internal standard (IS, DRL-17859), free from endogenous interferences was achieved. The resolution factor between the enantiomers was optimized and found to be not less than five. During method development, the IPA content in the mobile phase was optimized for separation of peaks of interest. Additionally, both flow rate and column temperature were optimized for an improved baseline separation of the enantiomers. Ratio of peak area of each enantiomer to IS was used for quantification of plasma samples. Nominal retention times of (+)-TTB, (−)-TTB and IS were 9.4, 13.8 and 17.5 min, respectively. The standard curves for TTB enantiomers were linear ($r^2 > 0.999$) in the concentration range 0.1–10 μg/mL for each enantiomer. Absolute recovery, when compared to neat standards, was 88.7–90.0% for TTB enantiomers and 100% for IS from the hamster plasma. The lower limit of quantification (LLOQ) for each enantiomer of TTB was 0.1 μg/mL. The inter-day precisions were in the range of 4.57–6.32 and 5.66–11.0% for (+)-TTB and (−)-TTB, respectively. The intra-day precisions were in the range of 1.60–7.36 and 2.76–13.6% for (+)-TTB and (−)-TTB, respectively. Accuracy in the measurement of quality control (QC) samples was in the range of 95.6–109% and 92.7–108% for (+)-TTB and (−)-TTB, respectively. Both enantiomers were stable in a series of stability studies, viz. bench-top (up to 12 h), auto-sampler (up to 24 h) and freeze/thaw cycles ($n = 3$). Stability of TTB enantiomers was established in hamster plasma for 15 days at −80 °C. The application of the assay to a pharmacokinetic study of (−)-TTB in hamsters is described.

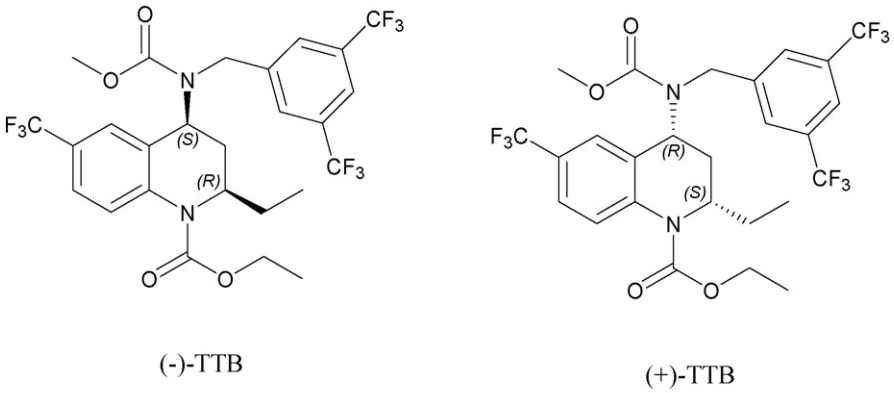
Keywords: Torcetrapib; Enantioselective; Quantitation; Chiralpak AD-H®; Chirality; HPLC–UV; Hamsters; Pharmacokinetics

1. Introduction

Despite on-going advancement in understanding and treatment, cardiovascular diseases continue to remain the leading cause of morbidity and mortality. Although significant reductions in cardiovascular risk can be achieved by effectively lowering low-density lipoprotein cholesterol (LDL-C), treated patients remain at substantial risk from recurring cardiovascular events. Epidemiological studies have established that higher levels of high-density lipoprotein cholesterol (HDL-C) are strongly associated with reduced cardiovascular risk, and therefore raising levels of HDL-C may be beneficial in addition to the traditionally well-accepted modality of lowering LDL-C [1]. In this regard the activity of cholesteryl ester transfer protein (CETP) appears to be inversely correlated with HDL-C levels [2]. Therefore, CETP has been projected as an attractive target for intervention to raise levels of HDL-C and potentially reduce residual cardiovascular risk [3,4].

* Corresponding author. Tel.: +91 40 23045439; fax: +91 40 23045438.
E-mail address: mullangiramesh@drreddys.com (R. Mullangi).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.07.045



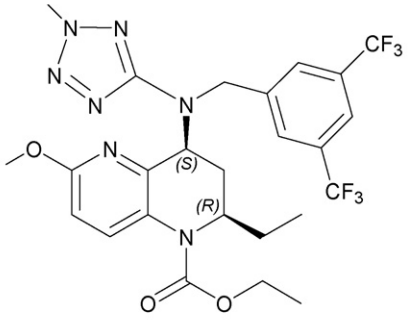


Fig. 1. Structural representation of (−)-TTB, (+)-TTB and (−)-DRL-17859.

CETP is a glycoprotein secreted mainly from the liver and circulated in plasma, bound mainly to high-density lipoprotein (HDL). CETP is endogenously expressed in hamsters, rabbits and humans [5]. Torcetrapib (TTB; CP-529,414; Fig. 1), chemically (−)-(2*R*,4*S*)-4-[(3,5-bis-trifluoromethyl-benzyl)-methoxycarbonylamino]-2-ethyl-6-tri-fluoromethyl-3,4-dihydro-2H-quinoline-1-carboxylic acid ethyl ester is a CETP inhibitor. In clinical studies, TTB lowered CETP activity, decreased apo-B (apolipoprotein-B) and LDL-C, elevated HDL-C and apo-E with no effect on triglycerides [4]. The development of TTB was recently suspended due to higher risk of cardiovascular event in patients who received TTB along with standard of care [6].

Lee et al. have described the distribution of (−)-TTB in human plasma and different lipoprotein components of human plasma by GC–MS/MS using a structural analog of (−)-TTB (CP-456,643) as an IS [7]. In this method Lee et al. did not furnish the details of the bioanalytical method. Hitherto there is no bioanalytical method reported for the estimation of TTB enantiomers on a chiral column. In this manuscript, we are presenting the HPLC method development and validation parameters for TTB enantiomers on a Chiralpak AD-H® column in hamster plasma and application of this method to derive the pharmacokinetic parameters for (−)-TTB. In order to optimize the enantiomeric separation of TTB enantiomers, different chromatographic conditions, viz. effect of mobile-phase composition, temperature and flow rate, were also studied and are described in this paper.

2. Experimental

2.1. Chemicals and reagents

(±)-TTB, (−)-TTB and (−)-DRL-17859 (IS, Fig. 1) were synthesized by Discovery Chemistry Group, Dr. Reddy's Laboratories Ltd. (DRL), Hyderabad, India, using the synthetic process reported by Damon et al. [8], and were characterized using chromatographic (HPLC, LC–MS/MS) and spectral techniques (IR, UV, Mass, ^{1}H and ^{13}C-NMR) by the Analytical Research Group, Discovery Research, DRL, Hyderabad. Purity was found to be more than 98.7% for all three compounds. HPLC-grade *n*-hexane was purchased from S.D. Fine Chemicals, Mumbai, India. HPLC-grade acetonitrile and isopropanol (IPA) were purchased from Ranbaxy Fine Chemicals Limited, New Delhi, India. All other reagents purchased from Qualigens (Mumbai, India) were of analytical reagent grade. Control hamster plasma was obtained from Department of Pre-clinical and Safety Evaluation, Discovery Research, DRL, Hyderabad.

2.2. HPLC operating conditions

The HPLC system consisted of a Shimadzu Prominence (Koyoto, Japan) system equipped with degasser (DGU-20A5), pump (LC-20AT), column oven (CTO-20A), auto-injector with sample cooler (SIL-20AC) and ultraviolet detector (SPD-20A). A Chiralpak AD-H® (250 mm × 4.6 mm, 5 μm) column (Daicel Chemical Industries Ltd., Japan) coupled with guard column