Journal of Chromatography B, 1011 (2016) 45–52

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Development and validation of an UFLC-MS/MS method for enantioselectivity determination of D,L-thero-methylphenidate, D,L-thero-ethylphenidate and D,L-thero-ritalinic acid in rat plasma and its application to pharmacokinetic study

Chenghao Zhang, Huafei Luo, Yubo Wu, Junyun Zhang, Furong Zhang, Guobei Lin, Hao Wang*

National Pharmaceutical Engineering Research Center, China State Institute of Pharmaceutical Industry, 1111 Ha Lei Road, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 1 November 2015
Received in revised form 8 December 2015
Accepted 19 December 2015
Available online 24 December 2015

Keywords:
Methyphenidate
Ethylphenidate
Chiral separation
UFLC-MS/MS
Pharmacokinetics
Drug interaction

ABSTRACT

A chiral UFLC-MS/MS method was established and validated for quantifying D-threo-methylphenidate (D-threo-MPH), L-threo-methylphenidate (L-threo-MPH), D-threo-ethylphenidate (D-threo-EPH), L-threo-ethylphenidate (L-threo-EPH) and D,L-threo-ritalinic acid (D,L-threo-RA) in rat plasma over the linearity range of 1–500 ng/mL. Chiral separation was performed on an Astec Chirobiotic V2 column (5 μm, 250 × 2.1 mm) with isocratic elution using methanol containing 0.003% ammonium acetate (w/v) and 0.003% trifluoroacetic acid (v/v) at a flow of 0.3 mL/min. All analytes and IS were extracted from rat plasma by a one-step liquid–liquid extraction (LLE) method. The intra- and inter-run accuracies were within 85–115%, and the intra- and inter-run precision were <10% for all analytes. Extraction recoveries were 55–62% for D-threo-MPH, 54–60% for L-threo-MPH, 55–60% for D-threo-EPH, 53–57% for L-threo-EPH and 25–30% for D,L-threo-RA. The validated UFLC-MS/MS method successfully applied to the pharmacokinetic interaction study of oral D-threo-MPH and L-threo-MPH (alone or in combination) in female Sprague Dawley rats. The EPH was not detected in rat plasma following oral administrated MPH without EtOH. As far as it is known to the authors, this study is the first one step liquid–liquid extraction method to extract and UFLC-MS/MS method to quantify D-threo-MPH, L-threo-MPH, D-threo-EPH, L-threo-EPH and D,L-threo-RA simultaneously.

1. Introduction

Methylphenidate (MPH) is widely prescribed in children and adolescents for the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy [1,2]. The drug is thought to block the uptake of norepinephrine and dopamine and to increase the release of these monoamines in the synaptic cleft [3,4]. Ethylphenidate (EPH) is a psychostimulant and analogue of methylphenidate. Interestingly it is also produced by the co-ingestion of methylphenidate and alcohol. The D-EPH selectively targets the dopamine transporter, whereas D-MPH exhibits equipotent actions at dopamine and norepinephrine transporters.

As show in Fig. 1, MPH and EPH have two stereogenic centers. However, only the threo enantiomers are used in the modern pharmaceutical formulations, because the erythro enantiomers exert only hypertensive and toxic effects [5,6]. Moreover, D-threo-enantiomer is more active than L-threo-enantiomer [7–9]. The threo enantiomers are subject to hydrolysis of the ester group via the enzyme carboxylesterase 1 (CES1) to the pharmacologically inactive D- or L-threo-ritalinic acid (RA), and it is well recognized that the CES1 exhibits enantioselectivity, preferring L-threo-enantiomer over D-threo-enantiomer [8,10]. Moreover, the CES1 enantioselectively transesterifies L-MPH with ethanol to yield L-EPH [11].

Appropriate drug therapy for older ADHD population requires a special consideration of lifestyle comorbidity [12]. For example, D,L-MPH/alcohol coabuse and dependence appear to be over-represented in older ADHD. As a novel psychoactive substances (NPS) psychostimulant drug, ethylphenidate is often sold under the street name “Nopaine” or “legal highs”. So justifiable societal concerns exist regarding the abuse of EPH as an “illicit drug”. For example, EPH abuse may have contributed to a recently documented post-mortem toxicological analysis [13,14]. This study can

* Corresponding author.
E-mail address: 13162596759@163.com (H. Wang).

http://dx.doi.org/10.1016/j.jchromb.2015.12.043
1570-0232/

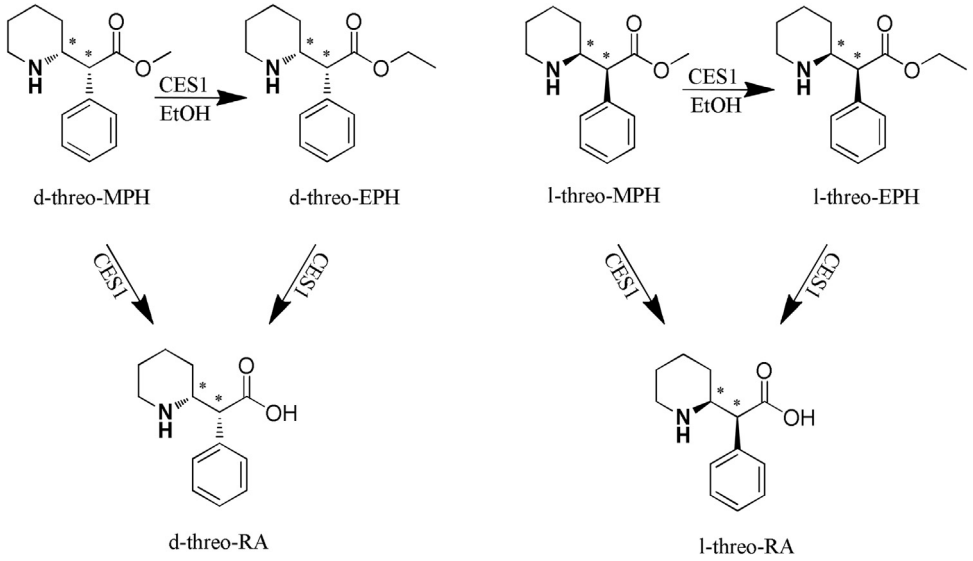

Fig. 1. The structure of threo-enantiomers and the CES1-mediated enantioselective transesterification and hydrolysis to EPH and RA, respectively.

be applied to provide the pharmacokinetic profiles and monitor drug abuse of D,L-threo-MPH and D,L-threo-EPH.

The D,L-threo-RA is an ampholytic substance, whereas D,L-threo-MPH and D,L-threo-EPH lack the acid group and are basic substances. These physicochemical differences make it difficult to extract these substances from biological matrices and chromatographically separate them by the same method. Only the solid-phase extraction (SPE) was used for the simultaneous extraction of these substances from plasma [5,15]. Several studies chirally analyzed D,L-threo-MPH, D,L-threo-EPH or D,L-threo-RA in various biological matrices [5,16–20]. However, in this study, we established a UFLC-MS/MS method for the first time to determine L-threo-MPH, D-threo-MPH, D-threo-EPH, L-threo-EPH and D,L-threo-RA in rat plasma using vancomycin as the chiral selector, and it was applied to study pharmacokinetic interaction between L-threo-MPH and D-threo-MPH following oral administration in rats. Moreover, a new liquid–liquid extraction (LLE) method was developed to replace the time-consuming SPE method.

2. Experimental

2.1. Reagents and chemicals

The L-threo-methylphenidate hydrochloride, D-threo-methylphenidate hydrochloride, D,L-threo-ethylphenidate hydrochloride, D,L-threo-ritalinic acid with purity of 99.0% were supplied by China State Institute of Pharmaceutical Industry (Shanghai, China). Methylphnidate-D_9 HCL, D,L-threo-ritalinic acid-D_{10} HCL, trifluoroacetic acid, ammonium formate (for HPLC, ≥99.0%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol and acetonitrile of high-performance liquid chromatography (HPLC) grade were obtained from Merck (Darmstadt, Germany) and Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), respectively. All other chemicals were of analytical grade and acquired from commercial sources.

2.2. Animals

Male Sprague-Dawley (SD) rats with body weight of 220 ± 10 g were supplied by the Shanghai Super-B&K Laboratory Animal Corporation Ltd. (Shanghai, China). The animals were allowed to acclimate for 2 weeks before the experiment was initiated. During the experimental period, Sprague-Dawley (SD) rats were housed under controlled conditions of 12:12 h light–dark cycle and 22 ± 2 °C, with food and water ad libitum. All studies concerning experimental animals were performed in accordance with the Ethical Guidelines for Investigations in Laboratory Animals and were approved by National Pharmaceutical Engineering and Research Center.

2.3. Standards and quality control samples

Each stock solution of D-threo-MPH, L-threo-MPH, D,L-threo-EPH, D,L-threo-RA and internal standard (IS) (D,L-MPH-D_9 and D,L-threo-RA-D_{10}) were prepared by dissolving accurately weighed samples in methanol to obtain concentrations of 1 mg/mL and stored at −20 °C until use. Mixed working stocks of D-threo-MPH, L-threo-MPH, D-threo-EPH, L-threo-EPH and D,L-threo-RA were serially diluted in methanol to obtain a series of standard solutions with different concentration levels and stored at −20 °C until use. Stock solution of IS (1 mg/mL) was diluted with methanol to obtain a concentration of 250 ng/mL. Calibration samples were prepared by spiking 100 μL of plasma with 10 μL of the working stocks and 10 μL of the IS solutions to obtain final concentrations of 1, 5, 10, 25, 50, 100, 250, 500 ng/mL for D-threo-MPH, L-threo-MPH, D-threo-EPH, L-threo-EPH and D,L-threo-RA; The quality control (QC) samples were separately prepared in the same way at the concentrations of 2, 20, and 400 ng/mL.

2.4. Sample preparation

All analytes were extracted using one step LLE method. Plasma samples were allowed to thaw thoroughly at room temperature. After vortex mixing, 100 μL aliquot of drug spiked plasma and 10 μL IS (250 ng/mL) as internal standard (IS) were pipetted into 1.5 mL centrifuge tubes. Samples were extracted using 1 mL 75:25(v/v) ethyl acetate-acetonitrile and the tubes were vortexed for 2 min prior to centrifugation at 17,800 × *g* for 3 min (Eppendorf, Hamburg, Germany). Then 800 μL supernatant was separated and evaporated to dryness at 40 °C with a vacuum centrifugal concentrator (miVac DUO, Genevac). The dried extract was dissolved with 200 μL mobile phase and vortexed for a further 1 min. The reconstituted solution was then centrifuged at 17,800 × *g* for 3 min. From these, 3 μL were injected in to the UFLC-MS/MS system for analysis.