Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Journal of Chromatography B, 854 (2007) 26–34
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Separation and quantification of two diastereomers of a Drug Candidate in rat plasma by ultra-high pressure liquid chromatography/mass spectrometry

Haiping Wang, Richard W. Edom [1], Sanjeev Kumar, Stella Vincent, Zhongzhou Shen *

Department of Preclinical Drug Metabolism, Merck Research Laboratories, Rahway, NJ 07065, USA

Received 15 January 2007; accepted 26 March 2007
Available online 5 April 2007

Abstract

Ultra-high pressure liquid chromatography (UHPLC) is a relatively new technology which utilizes chromatographic media with a 1.7 μm particle size. This technology has the potential to offer significant advantages in resolution, speed, and sensitivity for analytical determinations, particularly when coupled with mass spectrometric detection. Drug Candidate A, under development at Merck Research Laboratories, contains two chiral centers which have the absolute configuration *R*, *S*. Under *in vivo* and *ex vivo* conditions, one of the chiral centers readily epimerizes to produce the *R*, *R* diastereomer. Initially, a traditional high performance liquid chromatography/tandem mass spectrometry (HPLC–MS/MS) method was developed to separate and quantify these two diastereomers in rat plasma. The lower limit of quantification (LOQ) of the two analytes was 2 ng/mL, and a chromatographic run time of approximately 11 min was needed to separate R, S-(A) and R, R-(A). In this study, we explored a simple and robust UHPLC–MS/MS method in order to increase sample throughput and productivity. We were able to achieve a two-fold reduction in the lower limit of quantification and a three-fold reduction in retention time utilizing the UHPLC method, while keeping the same sample extraction procedure and similar MS/MS methodology. The new method exhibited good intra- and inter-day accuracy and precision, and was linear over a dynamic range of 1–500 ng/mL for each diastereomer. The method was successfully applied for the determination of R, S-(A) and R, R-(A) concentrations for *in vitro* and *in vivo* studies of epimerization of A in Sprague-Dawley rats.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Diastereomers; UHPLC–MS/MS; Quantitation

1. Introduction

The recently commercialized technology, *ultra-high pressure liquid chromatography* (*UHPLC*), has been made possible by the availability of reversed-phase chromatographic media with a 1.7 μm particle size. Based on the van Deemter equation, the use of smaller particles can significantly reduce the height equivalent of a theoretical plate (HETP) generated in a separation by improved mass transfer [1]. The separation efficiency is three times greater with 1.7 μm versus 5 μm particles, and two times greater compared to 3.5 μm particles. Furthermore, the resolution is 70% higher than with 3.5 μm particles. Typically, shorter retention times can be achieved using 1.7 μm particles compared to 5 μm particles with the same efficiency, and the flow rate can be three times higher, again with little loss of efficiency. An additional benefit of UHPLC is the enhanced sharpness of chromatographic peaks, which should, in concept, lower the limit of detection by at least a factor of two-fold relative to separations conducted with HPLC [1–5]. Thus, this technology could offer significant advantages in resolution, speed, and sensitivity for analytical determinations, particularly when coupled with mass spectrometric (MS) detection. Applications of UHPLC coupled with MS detection have been reported recently, such as the use of UHPLC–MS/MS for priority pesticides in baby food [6] and ground water [7], forensic and toxicological analysis [8], quantitative bio-analysis of drugs [9–12], and investigating metabolites using UHPLC–qTOF [13]. Another interesting

* Corresponding author at: Mail Stop: RY 80-141, Merck Research Laboratories, P.O. Box 2000, Rahway, NJ 07065, USA. Tel.: +1 732 594 6367; fax: +1 732 594 2382.
E-mail address: zhongzhou_shen@merck.com (Z. Shen).
[1] Present address: Johnson & Johnson Pharmaceutical Research & Development, LLC, Raritan, NJ 08869, USA.

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.03.040

study on the diastereomers *ephedrine* and *pseudoephedrine* demonstrated that utilizing a UHPLC–MS method significantly improved the sensitivity and reduced the analysis time compared to a well-optimized HPLC–MS method [14].

Drug Candidate A, under development at Merck Research Laboratories, contains two chiral centers which have the absolute configuration *R*, *S*. Under *in vivo* and *ex vivo* conditions, one of the chiral centers can readily epimerize to produce the *R*, *R* diastereomer. Since chiral compounds can have distinct pharmacokinetic, pharmacological and toxicological properties, they must be characterized individually if racemates are administered or if inter-conversion of stereoisomers is possible [15–18]. In order to address stereo-chemical concerns for developing new drugs under current FDA guidelines [19], a rapid and sensitive assay for Drug A was required to quantify the two diastereomers for *in vitro* and *in vivo* studies. Initially, a traditional high performance liquid chromatography/tandem mass spectrometry (HPLC–MS/MS) method was developed. With this assay, the limit of quantification (LOQ) of the two analytes in rat plasma was 2 ng/mL, and a chromatographic run time of approximately 11 min was needed to separate R, S-(A) and R, R-(A).

In this study, we developed a simple and robust UHPLC–MS/MS method in order to increase sample throughput and productivity using a reversed-phase UHPLC column. We were able to achieve a two-fold reduction in the lower limit of quantification and a three-fold reduction in retention time utilizing the UHPLC method while keeping the same extraction procedure and similar MS/MS methodology. This method was successfully validated by evaluating the extraction recovery, stability, sensitivity, linearity, precision and accuracy for these two diastereomers. Sample handling conditions were optimized in order to minimize the *ex vivo* inter-conversion of these two diastereomers. This new method was successfully used to determine the R, S-(A)/R, R-(A) concentration ratios in plasma from Sprague-Dawley rats both *in vitro* as well as *in vivo* following oral administration of A.

2. Experimental

2.1. Chemicals, reagents and materials

Diastereomers (R, S-(A) and R, R-(A)) and the internal standard (IS) were synthesized at Merck Research Laboratories. HPLC-grade water and all analytical organic solvents were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Control rat plasma with sodium-EDTA anticoagulant was purchased from BioReclamation Inc. (Hicksville, NY, USA). Ammonium bicarbonate buffer was prepared by titrating 100 mM ammonium bicarbonate with ammonium hydroxide to pH 9.3 ± 0.05. Formate buffer was prepared by titrating 500 mM formic acid with sodium hydroxide to pH 3.0 ± 0.05. Deep 96-well collection plates were purchased from VWR Scientific Products (West Chester, PA, USA). Disposable glass centrifuge tubes (5 mL) were purchased from Kimble Corp. (Vineland, NJ, USA), and disposable culture tubes (13×100 mm) were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Animal studies and sample collection

All studies were reviewed and approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. Sprague-Dawley rats, 14–16 weeks old and weighing 300–400 g, were obtained from Charles River Laboratories (Wilmington, MA). The animals were fasted overnight before dosing and for 4 h post-dose, with water provided ad libitum. Pure R, S-(A) was administered orally by gastric gavage using a dosing vehicle composed of 0.5% aqueous methylcellulose containing 0.02% sodium lauryl sulfate at 5 mL/kg. Blood (~0.3 mL) was collected into sodium-EDTA-containing tubes at predetermined intervals (pre-dose, 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, and 72 h) from a previously implanted catheter in the femoral artery. Plasma samples were obtained after immediate centrifugation of blood at 4 °C and were stored at −70 °C until analyzed.

2.3. In vitro incubations

Pure R, S-(A) was dissolved in acetonitrile and added to fresh, EDTA-treated rat blood to produce a 10 μM final concentration. The total content of acetonitrile in blood was 0.5% (v/v). Incubations were carried out at 37 °C in a shaking water bath for predetermined intervals (0, 0.25, 0.5, 1, 2, 4 h). The incubations were terminated by placing the samples on ice. Plasma samples were obtained by centrifugation at 4 °C and then immediately extracted and analyzed by UHPLC–MS/MS.

2.4. Sample preparation for analysis

All sample preparations were performed in an ice–water bath, except as noted. The stock solutions of R, S-(A) and R, R-(A) (1 mg/mL) were prepared by dissolving the compounds separately in DMSO. An internal standard (IS) stock solution was prepared similarly. Two separate weighings for each diastereomer were used to prepare individual stock solutions for standard curve and quality control (QC) samples. All stock solutions were stored at −70 °C. Dilutions of the diastereomer and IS stock solutions were prepared in acetonitrile. Standard curve samples were prepared by spiking appropriate amounts of R, S-(A) and R, R-(A) separately into 100 μL of control rat plasma. The standard curves consisted of eight concentrations at 1.0, 2.5, 5.0, 12.5, 25.0, 125, 250, and 500 ng/mL in duplicate. The QC samples were prepared at four concentrations (1.0, 2.5, 100 and 400 ng/mL) by spiking appropriate amounts of R, S-(A) and R, R-(A) separately into 100 μL control rat plasma. The internal standard solution (20.0 μg/mL) was prepared in acetonitrile. A 20 μL aliquot of this solution was added to each sample in 5 mL glass centrifuge tubes. Then, 1 mL of an ethyl acetate/MTBE (methyl tert-butyl ether) (50/50, v/v) mixture was added, the tubes were capped and vortex-mixed for 5 min. Following centrifugation at 4 °C ($3000 \times g$ for 10 min), the aqueous layer was frozen in a dry-ice/acetone bath and the organic layer was transferred to clean disposable culture tubes. The culture tubes containing the organic layer were placed in a Zymark Turbo Vap LV (Hopkinton, MA, USA) and evaporated to dryness at

Download English Version:

https://daneshyari.com/en/article/1217785

Download Persian Version:

https://daneshyari.com/article/1217785

Daneshyari.com