Journal of Chromatography B, 878 (2010) 456–460

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short communication

Quantification of raltegravir (MK0518) in human plasma by high-performance liquid chromatography with photodiode array detection

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ARTICLE INFO

Article history:
Received 7 August 2009
Accepted 27 October 2009
Available online 11 November 2009

Keywords:
Raltegravir
High-performance liquid chromatography
Photodiode array detection

ABSTRACT

A precise and accurate high-performance liquid chromatography (HPLC) method with photodiode array detection has been developed and validated for raltegravir, a human immunodeficiency virus integrase strand transfer inhibitor (HIV-1 INSTI). Plasma (300 μL) was extracted with dichloromethane/hexane 50:50 (v/v) after addition of the internal standard, 6,7-dimethyl-2,3-di(2-pyridyl) quinoxaline. The compounds were separated using a dC18 column and detected with ultraviolet detection at 320 nm. The limit of quantification was 10 ng/mL for raltegravir. The method was linear and validated over a concentration range of 0–10,000 ng/mL. The intra-day precision ranged from 3.1 to 12.3%, while the intra-day accuracy ranged from −15.0 to −0.5%, the inter-day precision and accuracy were less than 7%. The mean recovery was 76.8%. Application to clinical samples taken from patients treated with raltegravir indicated that the method is suitable for measuring plasma concentrations of raltegravir in pharmacokinetic studies of clinical trials.

1. Introduction

Raltegravir (MK0518, Isentress®) is a human immunodeficiency virus integrase strand transfer inhibitor (HIV-1 INSTI) indicated in treatment-experienced adult patients who have evidence of viral replication and HIV-1 strains resistant to multiple antiretroviral agents (400 mg bid). The efficacy of raltegravir after administration to HIV-infected patients whose virus is resistant to at least 3 classes of antiretroviral drugs has been clinically proven in recent Benchmark 1 and 2 clinical trials [1,2]. The number of patients who responded, the rapidity and durability of the antiretroviral response on exposure to raltegravir make this drug extremely powerful. No therapeutic range has yet been established for raltegravir, but the IC95 is 33 nM [3] (close to 15 ng/mL). Raltegravir is metabolized by glucuronidation involving UGT1A1 isoenzyme [4]. The pharmacological and clinical impact of a modification of exposure to raltegravir coadministered with other drugs (antiretroviral combination, prophylactic antimicrobial therapy) has to be assessed in HIV-infected patients, as the UGT1A1 activity may be increased by enzymatic inducers such as rifampicin [5], or decreased by specific enzymatic inhibitors such as atazanavir [6]. Consequently, a bioanalytical method to determine the concentration of raltegravir in human plasma was required to support clinical development studies. To date nine HPLC analytical methods for quantification of raltegravir have been published. Five of these use liquid chromatography–mass spectrometry [4,7–10], one uses fluorescence detection [11], and three others use ultraviolet (UV) detection after a liquid–solid phase [12–14]. Here we describe the validation of a simple, sensitive and cost effective HPLC method using photodiode array (PDA) detection and liquid–liquid extraction for quantification of raltegravir.

2. Experimental

2.1. Reagents

Acetic acid 99–100% was from Fisher Scientific (EC 200-580-7; Elancourt, France). Acetonitrile Chromasolv® for HPLC gradient grade (EC 200-835-2) and sodium acetate Sigma ultra min 99% (EC 204-823-8) were from Sigma–Aldrich (Steinheim, Germany). Raltegravir potassium salt was kindly supplied by Merck, and 6,7-dimethyl-2,3-di(2-pyridyl) quinoxaline was purchased from Sigma–Aldrich (EC 229-592-0, Steinheim, Germany).

2.2. Instrumentation

The HPLC system consisted of a LC 10 AD VP Shimadzu pump, a Waters 996 photodiode array detector, and a Waters 717 plus auto sampler used at ambient temperature. Empower 2.0 (Waters software) was used for data acquisition and processing.

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doi:10.1016/j.jchromb.2009.10.029

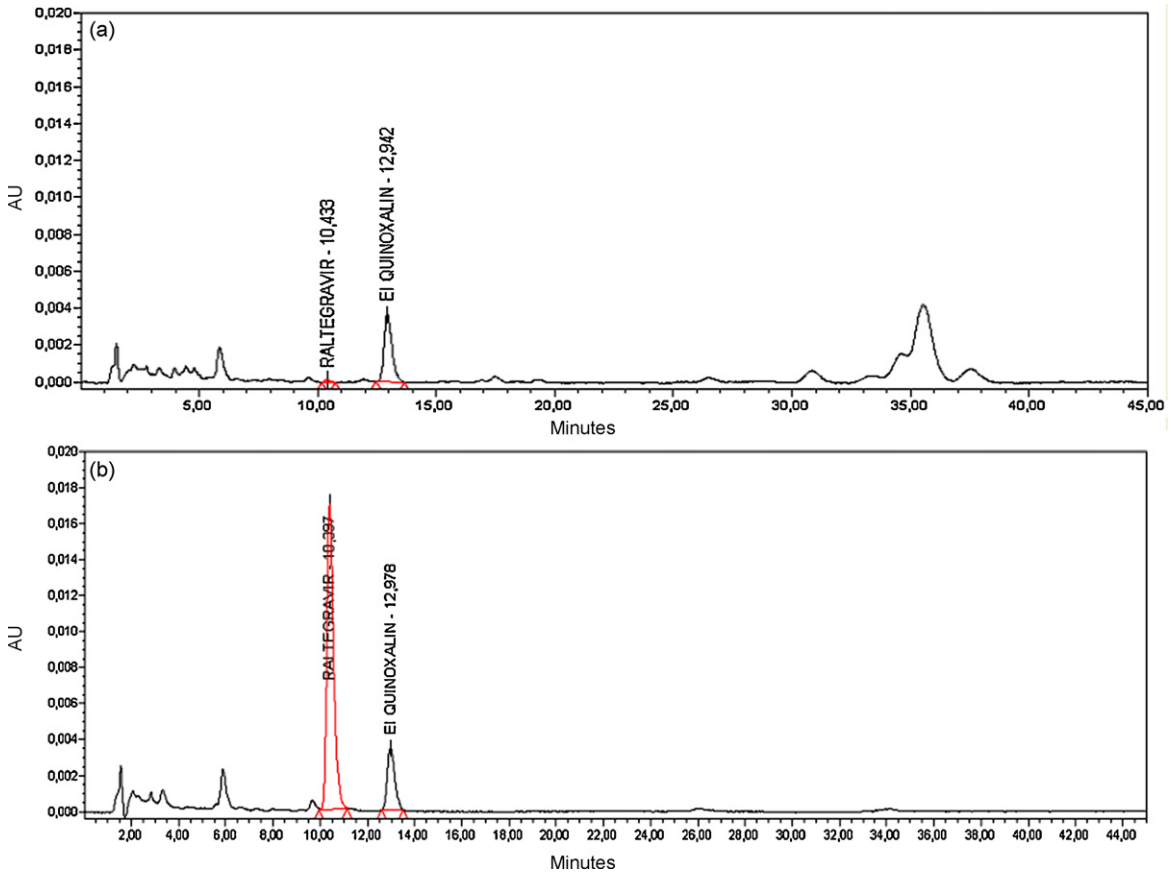

Fig. 1. Representatives chromatograms of the 10 ng/mL LOQ (a) and a patient treated by raltegravir (1254 ng/mL) (b).

2.3. Chromatographic conditions

Chromatography was performed on a 2.1 mm × 150 mm Atlantis® dC18 (3 μm) column with mobile phase consisting of 710/290 (v/v, %) acetate buffer (0.1 M, pH 4)/acetonitrile with a 0.4 mL/min flow rate. The PDA detector was set at 320 nm. A 80 μL full loop injection was used with a total run time of 45 min.

2.4. Preparation of calibration standards and quality control samples

2.4.1. Stock and diluted standard solutions

Two different 500 μg/mL stock solutions of raltegravir were prepared in water. After aqueous dilution of these raltegravir stock solutions, working standard solutions (50 and 5 μg/mL) aliquoted in polypropylene tubes and stored at −20 °C, were used to prepare calibration standards and quality controls. Internal standard solution of quinoxaline was prepared at 0.5 μg/mL in methanol.

2.4.2. Preparation of calibration standards and quality controls

Calibration standards of raltegravir ranged from 10 to 10,000 ng/mL and were prepared by adding an appropriate volume of stock or aqueous diluted solution (50 and 5 μg/mL) to aliquots of blank human plasma. Low, middle and high quality controls at 75, 1500 and 7500 ng/mL were prepared by diluting a separate 500 μg/mL raltegravir solution or aqueous diluted solutions (50 and 5 μg/mL) in aliquots of blank human plasma. Aliquots of calibration standard and quality control were transferred into polypropylene tubes and stored at −20 °C.

2.5. Extraction procedure

Plasma samples (300 μL) were pipetted into a glass tube and spiked with 100 μL of a 0.5 μg/mL working internal standard solution. Two milliliters of a dichloromethane/hexane (50/50, v:v) mixture and 200 μL of acetate buffer (0.1 M, pH 4) were added. After vortex-mixing for 4 min, tubes were centrifuged at 4000 rpm for 10 min. The organic layer was transferred into a glass tube and evaporated to dryness under a nitrogen stream at ambient temperature for 15 min. The dry residue was reconstituted in 100 μL of the mobile phase, mixed, and 80 μL were injected into the chromatographic system for analysis.

2.6. Data evaluation and calculations

Chromatograms were integrated and raltegravir concentrations were calculated with Empower 2.0 (Waters Software). Standard curve was constructed from linear least-squares regression was performed on the analyte to internal standard area ratio versus analyte concentration.

3. Validation of conditions

The method was validated according to guidelines for bioanalytical method validation [15,16]. Linearity of the method was assessed by analyzing seven complete standard curves (9 concentrations ranging from 10 to 10,000 ng/mL) on 7 separate days by linear regression with a weighting factor of $1/C$ where C is the analyte concentration. Intra- and inter-precision (coefficient of variation (CV%)) and accuracy (means, standard error) were evaluated using the 3 quality control samples. According to US