Journal of Pharmaceutical and Biomedical Analysis 131 (2016) 333–344

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Development and validation of a liquid chromatographic-tandem mass spectrometric method for the multiplexed quantification of etravirine, maraviroc, raltegravir, and rilpivirine in human plasma and tissue

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ARTICLE INFO

Article history:
Received 22 July 2016
Received in revised form 21 August 2016
Accepted 24 August 2016
Available online 4 September 2016

Keywords:
Antiretroviral
LC–MS/MS
Validation
Tissue
HIV

ABSTRACT

Background: Analytical methodologies for antiretroviral (ARV) quantification are important in determining both systemic and localized drug concentrations. The CCR5-antagonist maraviroc (MVC), the non-nucleoside reverse transcriptase inhibitors (NNRTIs) etravirine (ETV) and rilpivirine (RPV), as well as the integrase strand transfer inhibitor (INSTI) raltegravir (RAL), have all been evaluated using both oral and non-oral dosing regimens, demonstrating a need for dynamic and sensitive bioanalytical tools for drug quantification in plasma and tissue.
Methods: K_2EDTA plasma or blank luminal tissue lysate were spiked with ETV, MVC, RAL, and RPV. Following the addition of isotopically-labeled internal standards and sample extraction via protein precipitation or solid phase extraction, respectively, samples were subjected to liquid chromatographic-tandem mass spectrometric (LC–MS/MS) analysis. Chromatographic separation was performed using a Waters BEH C8, 50 × 2.1 mm, 1.7 μm particle size column, and detected on an API 5000 mass analyzer operated in selective reaction monitoring mode. The method was validated according to FDA Bioanalytical Method Validation guidelines.
Results: Analytical methods were optimized for the multiplexed monitoring of ETV, MVC, RAL, and RPV in plasma and homogenized tissue lysate. The lower limits of quantification (LLOQs) for ETV, RAL, and RPV were 1 ng/mL and the LLOQ for MVC was 0.1 ng/mL in plasma; the LLOQs for all ARVs in homogenized tissue lysate was 0.05 ng/sample. Standard curves were generated via weighted quadratic (plasma) or linear (tissue) regression of calibrators. Intra- and inter-assay precision and accuracy studies demonstrated %CVs ≤ 15.93% and %DEVs ≤ ± 13.52%, respectively. Stability and matrix effects studies, as well as external proficiency testing assessment, were also performed. All results were acceptable and in accordance with the guidelines recommended by the FDA, Guidance for Industry: Bioanalytical Method Validation document.
Conclusions: LC–MS/MS assays that are sensitive, specific, and dynamic have been developed and validated for the multiplexed quantification of ETV, MVC, RAL, and RPV in plasma and homogenized tissue lysate. The described methods meet sufficient throughput criteria to support large research trials.

1. Introduction

Antiretroviral therapy (ART) is the primary modality for the treatment and management of HIV/AIDS. Currently, there are more than 25 antiretroviral (ARV) agents approved by the United States Food and Drug Administration (FDA) for disease management in both single and combinatorial drug formulations [1]. Drug antiviral effects are achieved through inhibition of the various stages of the HIV life cycle; ARVs can target viral entry, RNA reverse transcription, integration of viral cDNA into the host cell genome, or the assembly and release of viral progeny. ARVs are stratified based on their mechanism of action, and are classified as viral entry inhibitors, nucleoside/nucleotide and non-nucleoside reverse transcriptase inhibitors (NRTI/NtRTI and NNRTI, respectively), integrase strand transfer inhibitors (INSTIs) and protease inhibitors (PIs). Therapeutic regimens typically include drugs from more than one

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http://dx.doi.org/10.1016/j.jpba.2016.08.016
0731-7085/

ARV class for the sustained suppression of viral replication and propagation [2,3]. In addition to their application in HIV management, ARVs have also shown success as pre-exposure prophylactic (PrEP) agents in high-risk populations [4–6].

Four ARVs that are used in HIV management include the CCR5-antagonist maraviroc (MVC), the NNRTIs etravirine (ETV) and rilpivirine (RPV), and the INSTI raltegravir (RAL). Although not administered combinatorially, these drugs are approved by the FDA for HIV treatment in conjunctions with other ARVs [3]. MVC acts as a CCR5-antagonist, preventing interaction between the viral gp120 protein and the host cell CD4 receptor, thus inhibiting downstream viral entry [7]. MVC was initially approved by the FDA in 2007 as a treatment for ART-experienced patients, but is now also approved in conjunction with other drugs for treatment in ART-naïve HIV positive patients [3,8,9]. ETV and RPV are diarylpyrmidine (DAPY) derivatives that act as NNRTIs; they were approved for use in treatment-experienced patients in 2008 and 2011, respectively [10,11]. Notably, both NNRTIs show significant antiviral activity against wild-type HIV and viral strains resistant to first-generation NNRTIs [11–13]. RAL was the first INSTI approved for HIV management in 2007, and while initially approved for use in treatment-resistant patients, RAL, in combination with tenofovir and emtricitabine, is currently a recommended therapeutic regimen for treatment-naïve patients [3]

Characterization of both systemic and localized drug concentrations is important to assess ARV pharmacokinetics (PK) and pharmacodynamics (PD). A number of studies have focused on alternative delivery mechanisms of ARVs to maximize both adherence as well as localized drug concentrations at the site of transmission to prevent viral acquisition [14–16]. MVC has recently been evaluated in a matrix-based ring formulation for vaginal application, and RPV has demonstrated promise in several clinical trials as a long-acting injectable [17–19]. Nanoformulation studies involving MVC, ETV, and RAL have also been conducted *in vitro,* showing improved neutralization of HIV-1 when compared to free drug [20]. Understanding matrix-specific drug concentrations can be informative in determining both systemic and compartmentalized drug PK.

Currently, there are several liquid chromatographic-tandem mass spectrometric (LC–MS/MS) methods published describing the development and validation of the ETV, MVC, RAL, or RPV in plasma, amongst others [21–24]. More recently, a report demonstrating ETV, MVC, and RAL quantification in several pigtail macaque matrices has also been published [24]. Additionally, our group has previously described an assay for MVC quantification with a LLOQ of 0.5 ng/mL [25]. Yet, several of the previously published assays are associated with substantial sample preparation, long analytical run times, or a lack of isotopically labeled internal standards [21–24]. Thus, in order to better characterize the systemic and localized PK of the aforementioned structurally disparate ARVs, we have developed and validated a dynamic, high-throughput, LC–MS/MS method for quantification of MVC, ETV, RAL, and RPV in both plasma and luminal tissue. The described method is currently being implemented in a number of forthcoming clinical trials.

2. Experimental

2.1. Chemicals

ETV ($C_{20}H_{15}BrN_6O$), the deuterated internal standard $^2H_8$-ETV (ETV-IS; $C_{20}H_7D_8BrN_6O$), MVC ($C_{29}H_{41}F_2N_5O$), the deuterated internal standard $^2H_6$-MVC (MVC-IS; $C_{29}H_{35}D_6F_2N_5O$), RPV ($C_{22}H_{18}N_6$), the deuterated internal standard $^2H_6$-RPV (RPV-IS; $C_{22}H_{12}D_6N_6$), and RAL ($C_{20}H_{21}FN_6O_5$) were all acquired from Toronto Research Chemicals (TRC, North York, ON). The carbon-13 labeled RAL internal standard, $^{13}C_6$-RAL (RAL-IS; $C_{14}{}^{13}C_6H_{21}FN_6O_5$), was a gift from Merck & Co., Inc. (Rahway, NJ). Chemical structures for ETV, MVC, RPV, and RAL are depicted in Fig. 1. Drug-free human K_2EDTA plasma was purchased from Biological Specialty Corporation (Colmar, PA). Whole blood collected in K_2EDTA tubes as well as luminal (vaginal and colorectal) tissue from post-surgical remnants were acquired under an institutional review board (IRB)-approved protocol for biological sample collection and maintained at −80° C for subsequent processing. HPLC-grade water, methanol, isopropanol, dimethyl sulfoxide and LC–MS-grade water with 0.1% formic acid were acquired from Fisher Scientific (Fair Lawn, NJ). Proteomics-grade formic acid and reagent-grade formic acid was purchased from ProteoChem (CO, USA) and Sigma-Aldrich (St. Louis, MO), respectively.

2.2. Preparation of standards and quality controls

Stock solutions of ETV, MVC, RAL, RPV, as well as isotopically labeled internal standards were independently prepared in methanol at final concentrations of 1 mg/mL. Stock solutions of ETV, MVC, RAL, and RPV were diluted with methanol to generate working stock solutions of 100, 10, 1, and 0.1 μg/mL; internal standards were diluted with methanol to generate a working solution containing 50 ng/mL of MVC-IS, RAL-IS, and RPV-IS, and 100 ng/mL of ETV-IS. For drug quantification in plasma, calibration standards were prepared by spiking human K_2EDTA plasma with appropriate volumes of working stock solutions. Final plasma calibration standards were as follows: ETV and RPV: 1, 2, 5, 20, 150, 500, 1000, 2500, and 5000 ng/mL; MVC: 0.1, 0.2, 1, 20, 75, 250, 500, 750, and 1000 ng/mL; RAL: 1, 2, 5, 20, 150, 500, 1000, 2000, 3000 ng/mL. Quality control (QC) master and working stocks were independently weighed and prepared in methanol as described above. QC materials were also generated by spiking human K_2EDTA plasma with working stock solutions. Concentrations at the plasma lower limit of quantification (LLOQ), low, mid, and high QCs were as follows: ETV and RPV: 1 (LLOQ), 3 (low), 300 (mid), and 4250 (high) ng/mL.; MVC: 0.1 (LLOQ), 0.3 (low), 150 (mid), 850 (high) ng/mL; RAL: 1 (LLOQ), 3 (low), 300 (mid), 2550 (high) ng/mL.

For drug quantification in homogenized tissue lysate, calibrators were prepared in a 70:30 methanol:water solution, which was the solvent system used for tissue homogenization. Calibrators were prepared by spiking 70:30 methanol:water solution with appropriate volumes of working stock solutions, and 0.1 mL of each calibrator was used to generate the tissue standard curve. Tissue calibrators were assigned as ng of drug per volume of sample analyzed; standards were as follows: ETV and RPV: 0.05, 0.1, 0.5, 2.0, 15, 50, 100, and 200 ng/sample; MVC: 0.05, 0.1, 0.5, 2.0, 7.5, 15, 30, and 40 ng/sample; RAL: 0.05, 0.1, 0.5, 2.0, 15, 50, 100, and 150 ng/sample.

Tissue QC samples were prepared in tissue (vaginal or colorectal) lysate. Upon equilibration to room temperature, tissue was partitioned into smaller pieces to facilitate homogenization, weighed, and combined with 70% methanol in water. Homogenization was achieved using a bead-based approach in the Bullet Blender Gold® (Next Advance, Averill Park, NY) homogenizer. During homogenization, temperature was maintained at 4 °C via dry ice. Tissue was homogenized for 5 min at speed setting 6. Homogenized lysates were pooled and diluted to a final concentration of 20 mg tissue/mL solvent. Following the preparation of tissue lysate, independently weighed and prepared master and working stock solutions were used to generate QCs at the following concentrations: ETV and RPV: 0.05 (LLOQ), 0.15 (low), 7.5 (mid), and 170 (high) ng/sample; MVC: 0.05 (LLOQ), 0.15 (low), 5.0 (mid), and 35 (high) ng/sample; RAL: 0.05 (LLOQ), 0.15 (low), 7.5 (mid), and 130 (high) ng/sample.