Antiretroviral Testing: Development and Validation of LC-MS/MS Assays in Unique Specimen Sources to Support Clinical Trials

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Background: Infection with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) still remains a significant public health concern, with 2.5 million new infections occurring annually. One of the primary modalities in the management and treatment of HIV/AIDS is the administration of antiretroviral (ARV) agents. Further, ARV administration has proven effective in the prevention of sexual transmission of the virus in high-risk populations[1]. However, treatment success is impeded by lack of adherence as well as the potential development of multi-drug resistance. In order to better understand the pharmacokinetic-pharmacodynamic (PK-PD) relationships of current ARVs in disease prevention and management, compartmentalized PK studies are required to assess localized drug concentrations. Thus, the quantification of ARVs in unique specimen sources, including cervicovaginal secretions (CVS), rectal fluid (RF) and luminal tissue are critical in more fully characterizing drug efficacy. This work is focused on the development and validation of liquid chromatographic-tandem mass spectrometric (LC-MS/MS) methods for the quantification of several antiretroviral drugs, including emtricitabine (FTC) and tenofovir (TFV), in CVS and RF.

Methods: Blank CVS and RF were collected from healthy human subjects who consented to an institutional review board (IRB)-approved protocol for biological sample collection. Specimens were appropriately diluted (CVS) or centrifuged (RF) prior to spiking with ARVs. FTC and TFV were acquired from the AIDS Research and Reference Reagent Program and isotopically-labeled internal standards were acquired from Moravek Biochemicals, Inc. (Brea, CA). Following the generation of master and working stock solutions of FTC and TFV, drugs were spiked into CVS or RF. To mimic clinical studies, drug-containing CVS or RF samples were applied to polyester-tipped Dacron swabs (Puritan ®, Fisher Scientific). FTC and TFV were removed from the polyester-tipped collection device using a 50:50 methanol:water solution, evaporated to dryness and reconstituted in water. Reconstituted material was combined with an

isotopically-labeled internal standard mixture and analytes were extracted via the Waters Oasis MCX solid phase extraction (SPE) plate. Samples were evaporated to dryness and reconstituted in water containing 0.5% acetic acid. Samples were separated under a gradient elution at room temperature on a Zorbax Eclipse Plus XDB-C18, 2.1 x 50 mm, 35 micron column (Agilent Technologies, Santa Clara, CA). Analytes were detected on an API 4000 triple quadrupole mass spectrometer (AB SCIEX, CA, USA) with an ESI source operated in positive ionization mode. Analytes were monitored in selective reaction monitoring (SRM) mode. The assays were validated in accordance with the Food and Drug Administration (FDA) Guidance for Industry, Bioanalytical Method Validation guidelines[2].

Results: The analytical measuring ranges for FTC and TFV in both CVS and RF applied to polyester-based Dacron swabs were 2.5 – 640 ng/swab and 0.625 – 160 ng/swab, respectively. Analytical metrics assessed included both intra- and inter-assay precision and accuracy, dilutional analysis studies, stability challenges in response to freeze-thaw cycles, post-extraction and in sample matrix, as well as matrix effects analysis and selectivity studies. Quality control (QC) samples prepared at the lower limit of quantitation, as well as low, mid and high QC levels yielded intra- and inter-assay coefficients of variation (%CVs) ranging from 1.4 to 12.1% and 2.3 to 7.2% for FTC and 1.4 to 13.3% and 2.3 to 7.4% for TFV in RF, respectively. Intra- and inter-assay precision for FTC and TFV in CVS were 1.4 to 11.6% and 4.7 to 8.2%, and 0.9 to 16% and 3.0 to 11.0%, respectively. Dilutional analysis studies demonstrate that for drug concentrations above the limit of quantification, both CVS and RF samples can be diluted and quantified, and observed concentrations are within 15% of theoretical concentrations in both specimen sources for both analytes. Further, both FTC and TFV are stable in CVS and RF following three freeze thaw cycles on the polyester-tipped swab, for three days in sample matrix maintained at room temperature, and for 3 days post-extraction in water containing 0.5% acetic acid. Acceptability criteria include $\leq$ 15% deviation from QC samples immediately prepared and analyzed. Finally to assess the potential influence of endogenous compounds on analyte suppression or enhancement, matrix effects studies were performed following the guidelines of Matuszewski and colleagues[3]. In RF, ion suppression was observed for both FTC (71.3% of expected peak area signal) and TFV (63.9% of expected signal); however the isotopically labeled internal standards for FTC and TFV showed comparable suppression (72.2% and 61.7% of

expected signal, respectively), indicating that there are negligible relative matrix effects observed. Notably, minimal ion suppression was observed in CVS for both FTC and TFV.

Conclusions: The described work illustrates the workflow and considerations required for the development and validation of assays using alternate specimen sources, such as CVS and RF, including removal of the drug from collection devices used in clinical trials. The validated LC-MS/MS methods described herein have been used in completed and ongoing clinical trials aimed at better understanding the compartmentalized pharmacokinetics of FTC and TFV at the site of viral transmission. This approach can be modified and applied to other specimen sources, including tissue, amniotic fluid and breast milk.

References:

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