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Validation of an LC-MS/MS Assay to Simultaneously Monitor the Intracellular Active Metabolites of Tenofovir, Emtricitabine, and Lamivudine in Dried Blood Spots

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Abbreviations: TFV, tenofovir; FTC, emtricitabine; 3TC, lamivudine; NRTI, nucleos(t)ide reverse transcriptase inhibitor; TFVdp, tenofovir diphosphate; FTCtp, emtricitabine triphosphate; 3TCtp, lamivudine triphosphate

Highlights:

- First assay to simultaneously quantify TFVdp, FTCtp, and 3TCtp in DBS.
- Assay precision and accuracy within ±15%.
- Calibration range utilized clinically relevant concentrations.
- Measure of adherence for MOST HIV treatment and prevention regimens.

Abstract

The ability to monitor adherence to antiretroviral therapy is critical for the interpretation of outcomes from clinical studies of HIV, and for optimizing patient care. The antiretrovirals tenofovir (TFV), emtricitabine (FTC), and lamivudine (3TC) are commonly included in drug regimens for HIV prevention and treatment. The active form of the drugs tenofovir diphosphate (TFVdp), emtricitabine triphosphate (FTCtp), and lamivudine triphosphate (3TCtp) are found intracellularly in erythrocytes and peripheral blood mononuclear cells (PBMCs). The ability to collect and analyze dried blood spot (DBS) samples is an attractive alternative to PBMC sampling in many resource limited settings. We developed and validated an assay to quantify all three intracellular metabolites over the range of 100-25000 fmol/sample. This assay utilizes a simple protein precipitation/liquid-liquid extraction of a single 3-mm DBS punch (from a Whatman 903 Protein Saver card) with isotopically labeled $^{13}C_5$-TFVdp included as the internal standard. Following extraction, samples are analyzed by anion exchange chromatography on a Thermo Biobasic AX 5micron column with detection by electrospray ionization in the positive mode on a AB Sciex API-5000 triple quadrupole mass spectrometer with a total run time of 8 minutes. The assay was linear over the entire range (R^2>0.996). The assay was accurate (inter-assay %bias within ±3.0%) and precise (inter-assay %CV ≤9.8%). The assay was also reproducible from multiple punches within a spot as well as punches from separate blood spots. Stability was established at room temperature for 3 days, and at -80°C for up to 63 days. Clinical samples were analyzed from subjects on Truvada®, Stribild®, Descovy®, and Triumeq® regimens and intracellular metabolites were detected in all samples as expected, indicating the assay performs well for all current formulations of TFV, FTC, and 3TC.

Keywords:

Dried blood spot; validation; HIV; adherence monitoring; antiretroviral; intracellular metabolite