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CONFERENCE

57th ASMS Conference on Mass Spectrometry

TITLE

LC-MS/MS Determination of Emtricitabine and Tenofovir in Human Plasma

AUTHORS

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ABSTRACT

Novel Aspect: Simple, efficient and inexpensive method offering excellent reproducibility and reliability which was demonstrated by both excellent validation and ISR results.

Introduction

Emtricitabine (FTC) and tenofovir (TFV) are in a combination medicine and used alone or combined with other antiviral drug for the treatment of HIV-infected adults. To support pharmacokinetic or bioequivalent studies of the combined medicine, FTC and TFV were determined in human plasma by HPLC and LC/MS/MS methods. To have the plasma samples better cleaned and subjected to less matrix effect (therefore achieve better reproducibility and reliability), solid-phase extraction was exclusively used in previously reported methods. In this work, we report a very reliable LC/MS/MS method for FTC and TFV using a simple and efficient protein precipitation approach achieving excellent reproducibility with little or no matrix effects. The reliability of the method was demonstrated by excellent incurred sample reanalysis results.

Methods

FTC and TFV were extracted along with stable isotope labeled internal standards from 100µL of human plasma by protein precipitation using methanol. Following vortexing and centrifugation, the supernatant was transferred to another 96-plate, solvent evaporated to dryness, reconstituted and injected for LC/MS/MS analysis. The whole extraction was performed on a TOMTEC Quatra-96 unit. The analytes and internal standards were chromatographed on an Aquasil C18 column (100x2 mm, 5µ) with gradient elution on Shimadru LC-10A pumps and CTC autosampler, and detected on a Sciex API4000 tandem mass spectrometer under positive TurboIonSpray mode. The retention times for the analytes were ~1.7 and ~2.6 min for FTC and TFV, respectively. The run time was ~7.5 min including column re-equilibration time.

Preliminary results

This automated protein precipitation-LC/MS/MS method was validated for a linear calibration range of 5-3000 ng/mL for both FTC and TFV. The validation data showed excellent precision and accuracy through 3 validation runs on 5 quality control concentrations: for FTC and TFV, intra-day precision of ≤8.4 and ≤8.0 %CV for LLOQ and ≤3.1 and ≤6.2 %CV for other concentrations; intra-day accuracy ranged from -9.8 to 3.3% and -9.2 to 2.7% RE; inter-day precision of ≤2.1 and ≤4.2 %CV, and inter-day accuracy ranged from -7.8 to 2.4% and -4.2 to 2.0 %RE, respectively. The method was selective, no interference peaks were found at the retention times of the analytes and the internal standards. Matrix effect evaluation showed a ~5% ion enhancement for FTC and a ~0.5% ion suppression for TFV – very little or no matrix effect. Extraction recovery was measured to be 72% for FTC and ~77% for TFV. Stability of the analytes was evaluated in human plasma under conditions of room temperature bench-top storage, freeze/thaw cycles, and freezer (-20°C and -70°C) storage. Both analytes showed to be stable under all conditions evaluated. The method was applied to support clinical study and ~1000 samples were analyzed. Incurred sample reanalysis (ISR) was performed and the excellent ISR data for both analytes

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substantiated the reproducibility and reliability of the method.