Development of a Novel LC-MS/MS Method for Simultaneous Detection of Anti-HIV Drugs Lopinavir, Ritonavir and Tenofovir in Plasma

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Purpose
In HIV treatment, anti-HIV drug combinations are typically used as highly active antiretroviral therapy (HAART), intended to maximize viral suppression and reduce harboring drug resistance. Three frequently used drugs in combination are the protease inhibitors Lopinavir and Ritonavir, and the nucleotide reverse transcriptase inhibitor Tenofovir. The goal of this study was to develop and validate a bio-analytical method for simultaneous extraction of the three drug combination in plasma and detection of these drugs by liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS).

Methods
The extractions were performed from 200µl plasma using a liquid-liquid extraction with 500µl methylene chloride followed by protein precipitation of the remaining aqueous layer using trifluoroacetic acid. The collected fractions were combined, dried in and reconstituted in 90/10 A/B of the mobile phase. Solvent A was composed of 0.1% acetic acid (HAc) in water and solvent B 0.1% HAc in acetonitrile. Samples were loaded on a Synergi Polar-RP column (100x2.0mm), using a gradient program on a Shimadzu LC system. The drugs were detected on ABSciex 3200Qtrap MS operating in positive MRM mode with the following m/z transitions: Lopinavir: 629.4/447.2, Ritonavir: 721.3/296.1 and Tenofovir: 288.1/176.1.

Results
The bio-analytical assay was optimized and validated to exhibit high extraction efficiency, recorded as 90%, 100% and 100% for Lopinavir, Ritonavir and Tenofovir respectively. The peak area for extracted drugs was linear between 1ng/ml to 1000ng/ml, R^2>0.99. The LOQ of amount on column for Tenofovir was 5pg and 250fg for Lopinavir and Ritonavir. The intra-day variation was ≤5% and inter-day variation was ≤10% for concentrations above 10ng/ml and ≤20% below 10ng/ml.

Conclusion
We have successfully developed a simple, efficient and sensitive method to simultaneously extract and determine the concentrations of the three drugs Lopinavir, Ritonavir and Tenofovir in plasma samples. This validated assay will be useful for evaluation of drug concentration in an efficient, selective and sensitive manner.

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