New UPLC-MS/MS method using 96-well extraction plate for the simultaneous quantification of 16 antiretroviral drugsin human plasma and evaluation of drugs pharmacokinetic interaction

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Background: AIDS still affects millions of people in the world and no drugs is available for the eradication of the HIV infection. Anyway, since the advent of the HAART, a lots has been done for the improvement of the duration and quality of life of HIV positive patients. HAART consist in the combination of different classes of antiretroviral drugs, including integrase inhibitors (IIs).
Rilpivirine (RPV), dolutegravir (DTG) and elvitegravir (EVG) are the latest antiretroviral drugs approved for treatment of HIV infection. Since these drugs recently entered clinical use, poor informations are currently available concerning their pharmacokinetic properties, drug-drug interaction profile and their therapeutic ranges: these informations are crucial in order to make possible the use of therapeutic drug monitoring as a tool for treatment optimization. The low number of pharmacokinetic studies about these drugs can be partially due to the absence of an high-throughput method for their simultaneous quantification, together with other antiretroviral drugs.
Therapeutic drug monitoring (TDM) is an important tool for maintaining the concentration of the drugs in the range of efficacy, in order to prevent the development of resistance and to avoid adverse effects.
Pharmacokinetic studies are required to define the role of TDM and the pharmacokinetic and pharmacodynamic parameters of these new drugs.
Since the highly active antiretroviral therapy (HAART) is a combination of three or more antiretroviral drugs, in which at least two different antiretroviral drug classes are present, in particular protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs), there could be several drugs interaction. Some of these are note in literature, but more others are not, above all after the introduction of new drugs.
In this work, we describe the development and validation of a new UPLC-MS/MS method to quantify sixteen antiretroviral agents plus two boosters (cobicistat and ritonavir), in human plasma. We also want to evaluate possible durg-drug interaction.
Method: 100 ul of sample are transferred into OSTRO protein precipitation plate's well. Then 50 ul of internal standard solution (quinoxalina) are added into the well. Finally the sample is precipitated by the addition of 600 ul of Acetonitrile with 1% of formic acid. Then a positive pressure is applied by a manifold for 5 minutes and sample is collected in a collection plate. Then it is diluted with 700 ul of water.Chromatographic separation was performed on a Acquity® UPLC HSS T3 column (150 mm x 2.1 mm I.D) with a particle size of 1.8 μm and compounds were detected with a tandem mass detector, monitoring two ion transitions for each drugs.
SPSS is used for statistical analysis of drugs pharmacokinetic and interactions.
Results: The method is fully validate following the Food and Drug Administration guidelines. The mean recovery for all drugs is above the 50%. Accuracy and precision inter/intra-day were below 15% for all drugs. The use of the OSTRO plate allow us to process an high number of samples in around one hour. Indeed the extraction plate lead to a cleaner sample decreasing noise in the chromatographic run and avoiding damage to column when a lot of samples routinely run in the same analysis section.
All concentration data from real patients samples have been analysis by SPSS searching possible drug-drug interaction. The evaluation of interactions is carried on considering the therapeutic dosage in order to avoid bias do to different dose for the same drug.

Conclusion: Accurate measurement of drugs plasma concentrations is crucial for pharmacokinetic/pharmacodynamic analyses, drug–drug interaction studies, and therapeutic drug monitoring. The UPLC-MS/MS method reported here could be used routinely to monitor plasma concentrations of antiretroviral drugs in HIV-infected patients and then to evaluate and monitor drug-drug interactions.