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The development and validation of a method using high-resolution mass spectrometry (HRMS) for the qualitative detection of antiretroviral agents in human blood

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ABSTRACT

Background: Antiretroviral drugs are used for the treatment and prevention of HIV infection. Non-adherence to antiretroviral drug regimens can compromise their clinical efficacy and lead to emergence of drug-resistant HIV. Clinical trials evaluating antiretroviral regimens for HIV treatment and prevention can also be compromised by poor adherence and non-disclosed off-study antiretroviral drug use. This report describes the development and validation of a high throughput, qualitative method for the identification of antiretroviral drugs using high-resolution mass spectrometry (HRMS) for the retrospective assessment of off-study antiretroviral drug use and the determination of potential antiretroviral therapy (ART) non-compliance.
Methods: Serum standards were prepared that contained 15 antiretroviral drugs: 9 protease inhibitors (PIs), 4 nucleotide/nucleoside reverse transcriptase inhibitors (NRTIs), and 2 non-nucleoside/nucleotide reverse transcriptase inhibitors (NNRTIs). Analytical separation was achieved on a Hypersil Gold PFP (100 × 3 mm) column and the eluent was analyzed using the Thermo Exactive Orbitrap mass spectrometer (Exactive-MS) operated in full scan mode. Limit of identification, signal intensity precision, retention time analysis, selectivity, and carryover studies were conducted. Concordance with liquid chromatographic–tandem mass spectrometric (LC–MS/MS) methods was evaluated using remnant plasma samples from a clinical trial.
Results: The limit of identification ranged from 5 to 10 ng/ml for 14 drugs (9 PIs, 1 NNRTI, 4 NRTIs) and was 150 ng/ml for 1 NNRTI. Precision studies with high and low control mixtures revealed signal intensity coefficients of variation of 3.0–27.5%. The Exactive-MS method was selective for the compounds of interest. Overall, concordance ranged from 89.1% to 100% for the screening of antiretroviral drugs in clinical plasma specimens as compared to LC–MS/MS methods.
Conclusion: Using the Exactive-MS, we developed and validated a highly selective, robust method for the multiplexed detection of 15 antiretroviral drugs.

1. Introduction

An estimated 34 million people are living with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) worldwide, with approximately 2.5 million new infections occurring annually [1]. Numerous antiretroviral drugs are approved for HIV treatment by the United States Food and Drug Administration (FDA) in single drug and multi-drug formulations [2]. The most widely used classes of antiretroviral drugs are nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) [3,4]. Antiretroviral drugs were first introduced in the late-1980s; however, subsequent research demonstrated that potent, multi-drug regimens are needed for durable suppression of viral replication and clinical benefit [3]. Most antiretroviral treatment regimens include two NRTIs with an NNRTI or PI [4,5]. Newer classes of antiretroviral drugs include entry inhibitors, fusion inhibitors, and integrase inhibitors [6,7].

While antiretroviral drugs are effective for treatment of HIV infection, non-adherence to treatment regimens can lead to treatment failure and emergence of drug-resistant HIV strains [8,9]. Adherence counseling is an important component of HIV treatment. However, the complexity of treatment regimens, adverse events, and other

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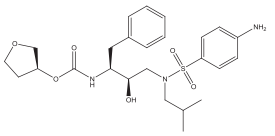
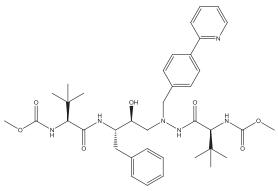
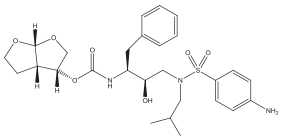
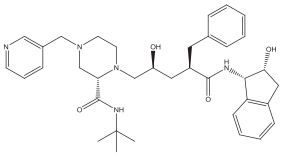
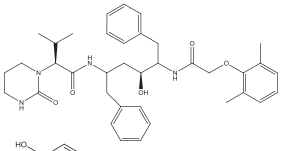
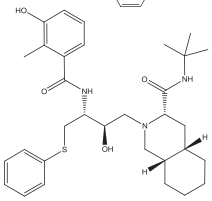
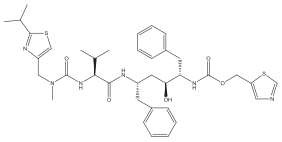
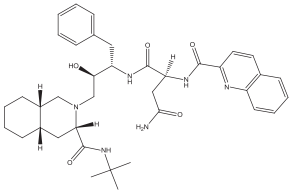
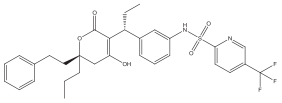
Table 1
Antiretroviral agents monitored in Exactive-MS method.

Antiretroviral agent	Structure	Molecular formula	$[M + H]^+$ (m/z)	Fragment Ions
Protease inhibitors				
Amprenavir (APV)		$C_{25}H_{35}N_3O_6S$	506.2319	245.1637
Atazanavir (ATV)		$C_{38}H_{52}N_6O_7$	705.3970	335.1948 168.0801
Darunavir (DRV)		$C_{27}H_{37}N_3O_7S$	548.2425	392.1989 69.0335
Indinavir (IDV)		$C_{36}H_{47}N_5O_4$	614.3701	513.2847 465.2845 421.2339
Lopinavir (LPV)		$C_{37}H_{48}N_4O_5$	629.3697	447.2621 183.1121 155.1172
Nelfinavir (NFV)		$C_{32}H_{45}N_3O_4S$	568.3204	467.2342 330.1142
Ritonavir (RTV)		$C_{37}H_{48}N_6O_5S_2$	721.3200	296.1413 268.1465
Saquinavir (SQV)		$C_{38}H_{50}N_6O_5$	671.3915	570.3052 433.1853
Tipranavir		$C_{31}H_{33}F_3N_2O_5S$	603.2135	585.1999 375.2175 333.1707