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Short communication

High throughput LC–MS/MS method for simultaneous determination of zidovudine, lamivudine and nevirapine in human plasma

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ABSTRACT

A selective and sensitive high performance liquid chromatography–tandem mass spectrometry method has been developed and validated for simultaneous determination of zidovudine (ZDV), lamivudine (3TC) and nevirapine (NVP) in human plasma. After Solid phase extraction (SPE), analytes and ISTDs were run on Peerless Basic C18 column with an injection volume of 3 μL and run time of 3.0 min. An isocratic mobile phase of 0.1% formic acid in water:methanol (15:85, v/v) was used with positive mass spectrometric detection. The method was validated over a concentration range of 5–1500 ng/mL for ZDV and 3TC and over the concentration range of 10–3000 ng/mL for NVP. The intraday and interday precision and accuracy across four validation runs were ranged from 1.6 to 10.1% and 93.8 to 110.8% respectively.

1. Introduction

Multi-class combination treatment has become the most common regime in the management of acquired immunodeficiency syndrome (AIDS) [1]. Due to increased resistance for the causative human immunodeficiency virus (HIV) [2], US Department of Health and Human services has recommended single and multiple class combination regimens which are often referred to as highly active antiretroviral therapy (HAART) [3]. The most common combination at the beginning of the treatment consists of two nucleoside reverse transcriptase inhibitors (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI).

Zidovudine (ZDV) a nucleoside reverse transcriptase inhibitor acts as chain-terminator of viral DNA during reverse transcription [4]. Absorption of ZDV is rapid and nearly complete on oral administration [5], and because of first pass metabolism the systematic bioavailability of ZDV is approximately 65%. Lamivudine (3TC), another nucleoside reverse transcriptase inhibitor is active against HIV type-1 and hepatitis B (HBV) [6]. Phosphorylated active metabolites of 3TC competitively inhibit the HIV reverse transcriptase enzyme and act as a chain terminator of DNA synthesis. On oral administration 3TC absorption is rapid and absolute bioavailability is approximately 86% for both tablet and oral solution. Nevirapine (NVP) is a potent, non-nucleoside reverse transcriptase inhibitor used in combination with nucleoside analogs for HIV infection [7]. NVP binds directly to reverse transcriptase and blocks the RNA dependent and DNA dependent DNA polymerase activities by causing disruption in the enzymes catalytic site. On oral administration its systemic availability is about 90% with a relatively longer half life of 45 h.

Several analytical methods [8–16] have been developed for the determination ZDV, 3TC and NVP individually or in combination in pharmaceutical formulations, however very few methods are reported for simultaneous determination of ZDV, 3TC and NVP in biological matrices. Malm et al. [10] developed a combination method on blood samples using gradient HPLC with UV detection. The limit of quantification was 0.11, 0.13, and 1.3 μg/mL for 3TC, ZDV and NVP respectively. Vandana et al. [13] developed a gradient HPLC-UV method for the three drugs in plasma using 950 μL sample volume with a quantification limit of 51 ng/mL. Zhou et al. [14] developed an LC–MS/MS method for determination of ZDV, 3TC and NVP with a LOQ of 20 ng/mL using protein precipitation technique, which has a high potential for ion-suppression in LC–MS/MS analysis. Krishna et al. [15] developed a simultaneous LC–MS/MS method with a run time of 3.5 min and LOQ of 25 ng/mL for ZDV and 3TC and 81 ng/mL for NVP which is not sufficient for the current application.

Aim of the current study was to develop and validate [17,18] a more sensitive and selective high throughput LC–MS/MS method that can be efficiently used in pharmacokinetic studies, to evaluate bioavailability and bioequivalence for this potent combination

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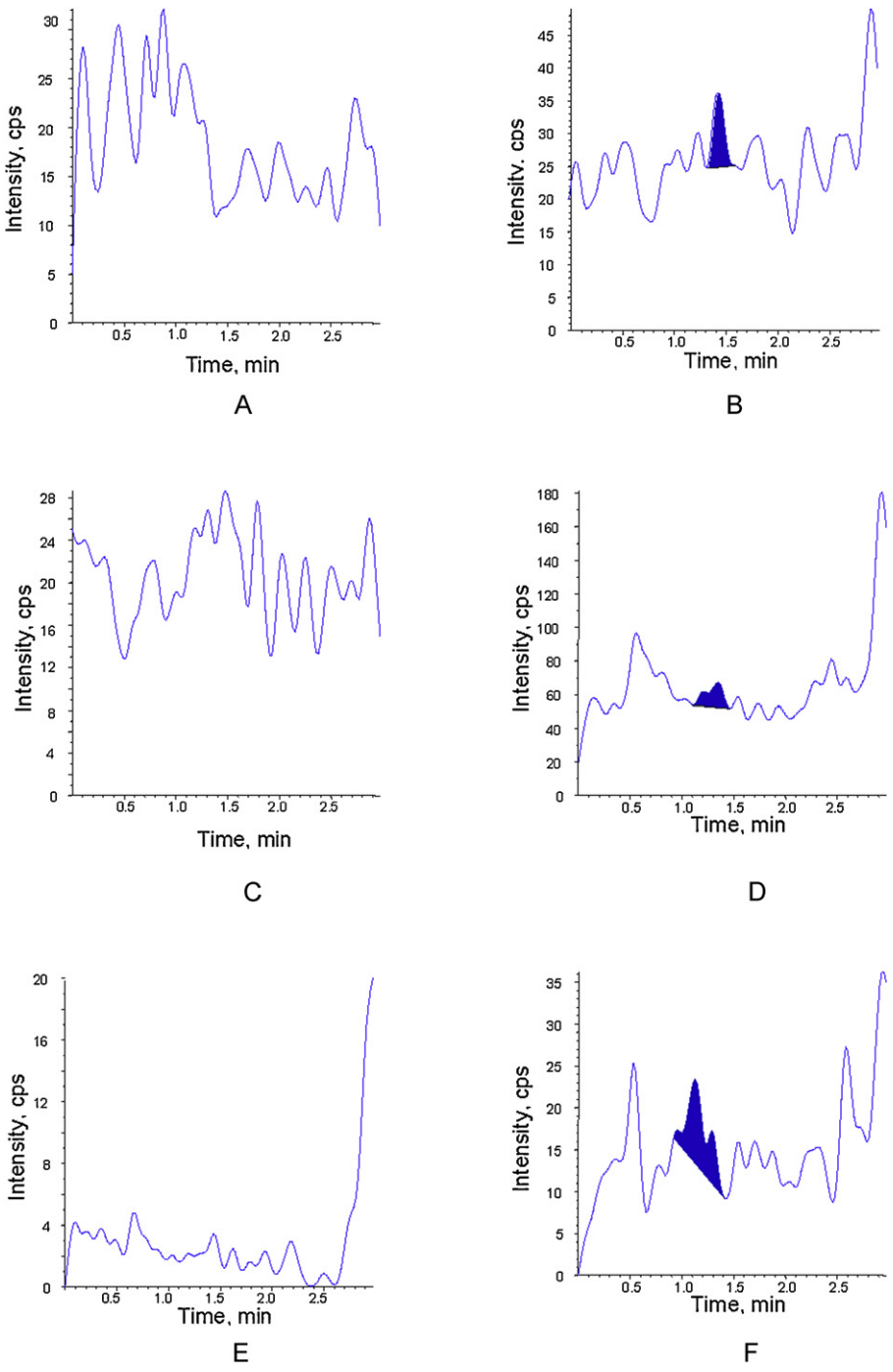

Fig. 1. Representative chromatograms of ZDV (A), DDI (B), 3TC (C), FTC (D), NVP (E) and ABC (F) in blank plasma.

of ZDV, 3TC and NVP. The new LC–MS/MS method was developed in human plasma containing K_2 EDTA as anticoagulant, and was completely validated as per FDA guidelines. A sensitive quantification limit of 5 ng/mL for ZDV and 3TC and 10 ng/mL for NVP was achieved with a sample processing volume of 300 μL.

2. Materials and methods

2.1. Chemicals and reagents

Working standards of zidovudine (ZDV), lamivudine (3TC), nevirapine (NVP) and ISTDs having purity more than 99% were obtained from Aurobindo Pharma Limited (Hyderabad, India). Didanosine (DDI), emtricitabine (FTC) and abacavir sulfate (ABC) were used as ISTDs for ZDV, 3TC and NVP respectively. HPLC grade methanol and acetonitrile were procured from Thermo Fisher Scientific India Private Limited (Mumbai, India). HPLC Type 1 water obtained from a Milli-Q gradient system (Millipore, Bedford, USA) was used in analysis. Oasis HLB 30 mg; 1 CC SPE cartridges were purchased from Waters Corporation (Milford, USA).

2.2. Instrumentation

HPLC system (Shimadzu, Japan), equipped with LC-20AD pumps for solvent delivery, DGU-20 A3 degasser, CTO-AS vp Column oven and a high throughput a SIL HTc autosampler was used for the