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A novel LC–ESI-MS method for the simultaneous determination of etravirine, darunavir and ritonavir in human blood plasma

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ABSTRACT

The new potent combination of antiretrovirals etravirine, darunavir, and ritonavir requires a new bioanalytical method for clinical pharmacology investigations and potential therapeutic drug monitoring. The development and validation of a novel LC–MS method for the simultaneous quantification of the most recently FDA-approved protease inhibitor and non-nucleoside reverse transcriptase inhibitor is described. This novel method was developed and validated using a sub-2 μm particle column, and provides excellent chromatographic separation and peak shape for all three analytes and internal standard. The method was validated over the range of 0.002–2.0 μg/mL. Intra- and inter-day accuracy of all analytes ranged from 88 to 106%, and intra- and inter-day precision was <7%. Dilution of samples 2-, 5-, and 10-fold maintained accuracy and precision, using a sample volume as low as 10 μL. Finally, the applicability of the method was investigated with clinical samples and external quality assurance proficiency testing samples.

1. Introduction

Etravirine (ETR; Intelence™) is a new non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus type 1 (HIV-1) [1,2]. The chemical name of etravirine is 4-[[6-amino-5-bromo-2-[(-4-cyanophynyl) amino]-4-pyrimidinyl]oxy]-3,5-dimethylbenzonitrile (Fig. 1). ETR is a highly potent inhibitor of HIV-1 replication, with activity in the nanomolar range comparable to that of the commonly prescribed NNRTI efavirenz [2].

Darunavir (DRV; Prezista™) is a new protease inhibitor of HIV-1 [3,4]. The chemical name of darunavir is [(1S,2R)-3-[[(4-aminophenyl)sulfonyl] (2-methylpropyl)amino]-2-hydroxy-1(phenylmethyl)propyl]-carbamic acid (3R,3aS,6aR)-hexahydo-furo[2,3-b]furan-3-yl ester monoethanolate (Fig. 1). DRV is also a potent inhibitor of HIV-1 replication, particularly active against strains with multiple protease inhibitor resistance mutations [5–10].

For maximum efficacy and durability, antiretrovirals must be used in combination [11–13]. However, treatment failure due to HIV drug resistance mutations is common [14–16]. New therapies, such as ETR and DRV, are aimed at treating patients harboring drug resistant HIV-1 variants. DRV must be given with ritonavir (RTV, Fig. 1) to enhance systemic drug exposure. Recently, the combination of ETR, DRV, and RTV has been demonstrated safe and effective in small numbers of highly drug resistant HIV-infected individuals [17,18].

Further pharmacologic study, including therapeutic drug monitoring (TDM), of these drugs require an accurate and sensitive bioanalytical method. To date, there has been no published LC–MS method for the simultaneous quantification of ETR, DRV, and RTV in human biological fluids. Consequently, this paper describes the development and validation of such a method in human blood plasma using HPLC with electrospray ionization and mass spectrometry detection and (ESI-MS) after a simple liquid–liquid extraction procedure.

2. Materials and methods

2.1. Chemicals

Etravirine, darunavir ethanolate and ritonavir (Fig. 1) were obtained from the NIH AIDS Research & Reference Reagent Program (McKesson HBOC BioServices, Rockville, MD, USA). Alprazolam (Fig. 1) was used as an internal standard and was purchased from Sigma Chemical Company (St. Louis, MO, USA). Tertiary butyl methyl ether and HPLC-grade chemicals and HPLC-grade water were purchased from Fisher Scientific (Norcross, GA, USA). Purified compressed nitrogen gas used was obtained from National Welders Supply (Charlotte, NC, USA). Sodium EDTA (Biological Specialty Corporation, Colmar, PA, USA).

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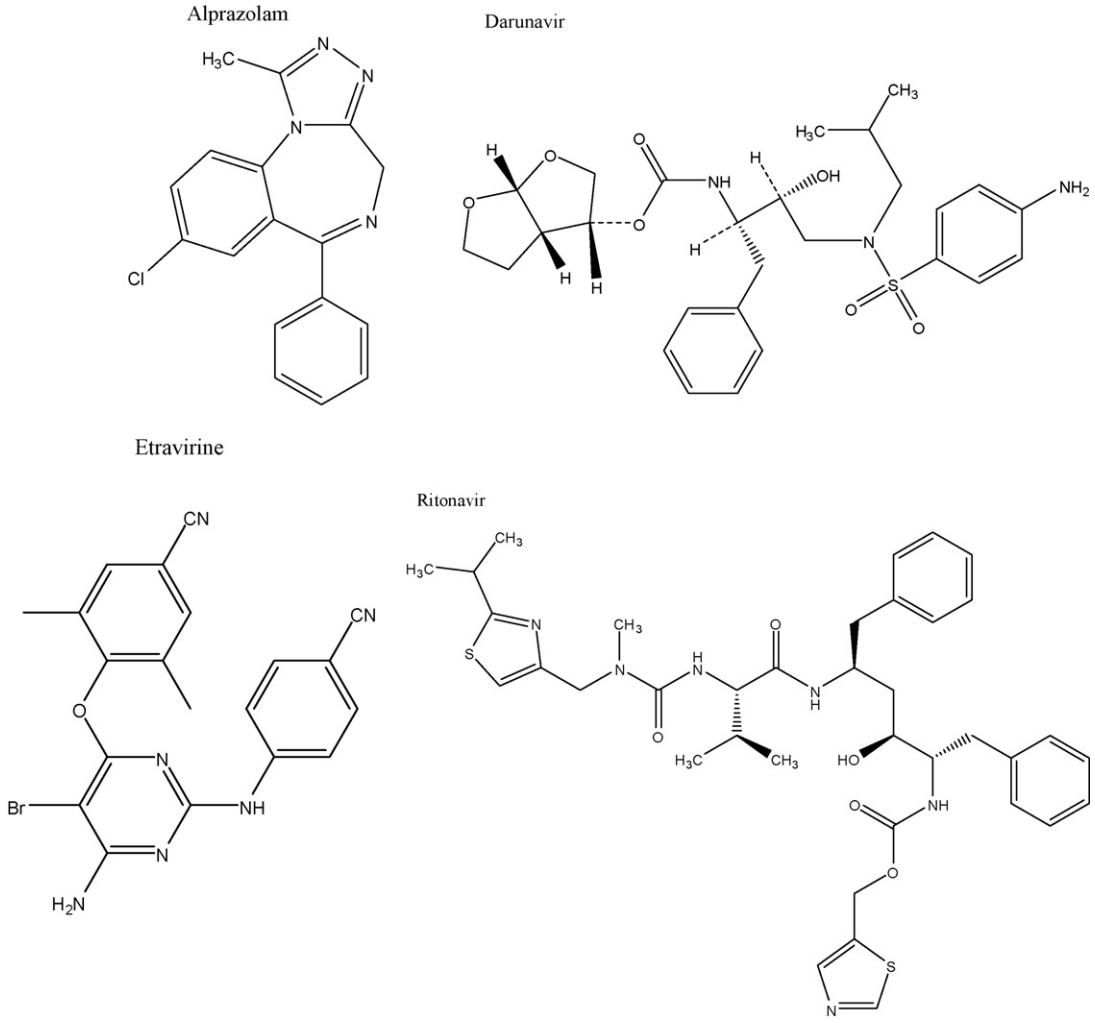

Fig. 1. Chemical structure of internal standard, darunavir, etravirine and ritonavir.

2.2. Equipments

An Eppendorf 5415D centrifuge (Eppendorf AG, Hamburg, Germany) was used for centrifugation during sample preparation. A high-performance liquid chromatography (HPLC) system consisting of an Agilent Technologies (Wilmington, DE, USA) HP1100 binary pump, degasser, thermostatic auto sampler was connected to an 1100 Series mass spectrometer using positive electrospray ionization. Chromatographic data analysis was performed by HP ChemStation software (Version A.09.03) run on a Dell computer (operated by Windows 2000 professional).

2.3. Preparation of standards

Two individual 1 mg/mL stock solutions of each compound were prepared. To prepare the DRV solution, 5.4 mg of darunavir ethanolate (593.73 g/mol) powder, with 99.5% purity, was accurately weighed and dissolved in a 5 mL volumetric flask using a solution of 1:1 solution of methanol:HPLC-grade water. To prepare the ETR solution which is less water soluble, 5.0 mg of ETR (435.29 g/mol) powder, with 100% purity, was dissolved in 5 mL of a 4:1 solution of methanol:HPLC-grade water. To prepare the RTV solution, 5.0 mg of RTV (720.96 g/mol) powder, with 100% purity, was dissolved in 5 mL of a 1:1 solution of methanol:HPLC-grade water.

A 100 μg/mL composite master stock solution was prepared by combining 1 mL of each separate analyte stock solution in a 10 mL volumetric flask, and adjusting the volume with a 4:1 solution of methanol:HPLC-grade water. This composite master stock solution was used to prepare seven intermediate calibrators (in a 4:1 solution of methanol:HPLC-grade water) in concentrations of 0.02, 0.1, 0.2, 1, 2, 10, 20 and 2 μg/mL. Seven calibrators working solutions in concentrations of 0.002, 0.01, 0.02, 0.1, 0.2, 1.0 and 2.0 μg/mL were prepared in plasma by diluting the seven intermediate calibrators 1:9 in drug free plasma. This plasma matrix was obtained from whole blood anticoagulated with sodium EDTA.

From another 100 mg/mL composite master stock solution, four intermediate solutions in concentrations of 0.06, 0.6, 6 and 18 μg/mL were prepared in a 4:1 solution of methanol:HPLC-grade water. Four quality control (QC) working solutions in concentrations of 0.006, 0.06, 0.6 and 1.8 μg/mL were prepared in plasma by diluting the four intermediate solutions 1:9 in drug free plasma.

A composite 50 μg/mL master stock solution containing sixteen potential drugs of interference (primarily other protease inhibitors, non-nucleoside reverse transcriptase inhibitors, nucleoside reverse transcriptase inhibitors, raltegravir and maraviroc) was prepared from individual 1 mg/mL primary stock solutions of each drug. This solution was prepared using a 3:1 solution of methanol:HPLC-grade water, and diluted in the final reconstitution solution (as described below) to 0.1 μg/mL before injection onto the HPLC system.