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Quantification of the HIV-integrase inhibitor raltegravir and detection of its main metabolite in human plasma, dried blood spots and peripheral blood mononuclear cell lysate by means of high-performance liquid chromatography tandem mass spectrometry

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ABSTRACT

For the quantification of the HIV-integrase inhibitor raltegravir in human plasma, dried blood spots and peripheral blood mononuclear cell (PBMC) lysate, an assay was developed and validated, using liquid chromatography coupled with tandem mass spectrometry. The assay also allowed detection, but no quantification due to absence of reference substance, of the main metabolite, raltegravir–glucuronide.

Raltegravir was extracted from plasma by means of protein precipitation with a mixture of methanol and acetonitrile using only 50 μL plasma. Extraction from dried blood spots was performed with a simple one-step extraction with a mixture of methanol, acetonitrile and 0.2 M zincsulphate in water (1:1:2, v/v/v) and extraction from cell lysate was performed in 50% methanol in water. Chromatographic separation was performed on a reversed phase C18 column (150 mm × 2.0 mm, particle size 5 μm) with a quick stepwise gradient using an acetate buffer (pH 5) and methanol, at a flow rate of 0.25 mL/min. The analytical run time was 10 min. The triple quadrupole mass spectrometer was operated in the positive ion-mode and multiple reaction monitoring was used for drug quantification. The method was validated over a range of 50–10,000 ng/mL in plasma and dried blood spots and a range of 1–500 ng/mL in PBMC lysate. Dibenzepine was used as the internal standard. The method was proven to be specific, accurate, precise and robust. Accuracies ranged from 104% to 105% in plasma, from 93% to 105% in dried blood spots and from 82% to 113% in PBMC lysate. Precision over the complete concentration range was less than 6%, 11% and 13% in plasma, dried blood spots and PBMC lysate, respectively. The method is now applied for therapeutic drug monitoring and pharmacological research in HIV-infected patients treated with raltegravir.

1. Introduction

Until now antiretroviral drugs have targeted the viral enzymes reverse transcriptase, protease or interfered with viral entry. Raltegravir (Fig. 1A) is a drug from a new class of antiretroviral drugs called integrase inhibitors. These agents target the viral enzyme that catalyzes insertion of viral DNA into the host genome [1]. Raltegravir has shown potent antiretroviral effect in treatment-experienced human immunodeficiency virus 1 (HIV-1) infected patients [2,3]. Based on promising results, the FDA and EMEA granted approval of raltegravir for the use of HIV-1 treatment in treatment-experienced adults [4,5].

Currently, determination of drug concentrations in plasma is the gold standard for purposes of therapeutic drug monitoring (TDM) or pharmacokinetic studies. However, quantification of drug levels in dried blood spots obtained with a simple fingerprick provides a patient-friendly alternative for sample collection in patient populations where intensive venous sampling is unethical or impossible and it allows non-hospital based sampling. Moreover, when using dried blood spots for drug quantification, there is no need for the use of anticoagulant containing sampling tubes, plasma separation or the necessity of cold sample storage. Lastly, dried blood spots can be easily stored or transported without the requirements of special storage, allowing easy and cheap shipment.

The site of action of raltegravir is within the infected cell. Cell-associated drug levels of raltegravir provide information on drug disposition in a compartment where HIV replicates and may therefore be useful in understanding its clinical pharmacology.

We here present the development and validation of a sensitve and fast assay for the determination of raltegravir in plasma, dried blood spots and peripheral blood mononuclear cell (PBMC) lysate by means of liquid chromatography coupled with electrospray tandem mass spectrometry (LC–MS/MS). Previously, 2 different assays

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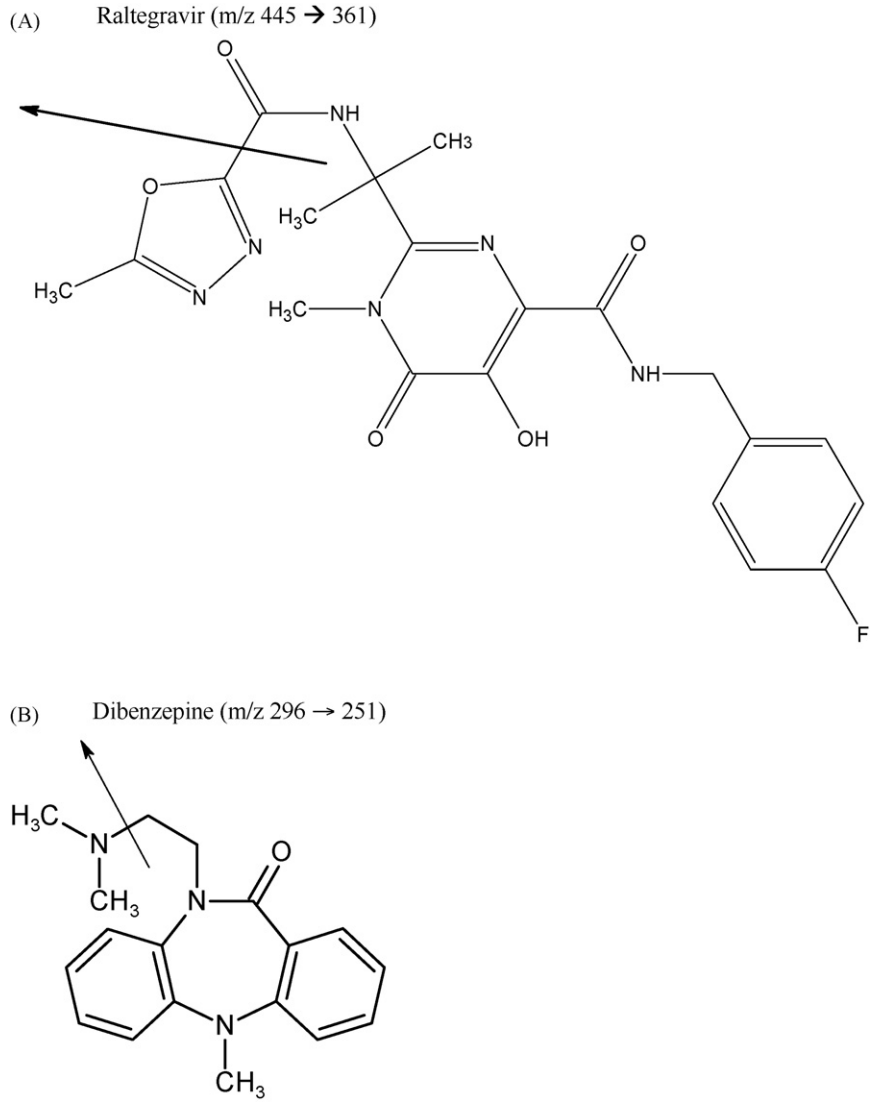

Fig. 1. Proposed fragmentation pathways of raltegravir (A) and the internal standard dibenzepine (B). (A) Raltegravir (m/z 445 → 361) and (B) dibenzepine (m/z 296 → 251).

for the determination of raltegravir in human plasma by means of LC–MS/MS have been described [6,7]. To the best of our knowledge, no assay for raltegravir has been previously published for the quantification of raltegravir in PBMC lysate or dried blood spots. Furthermore, contrary to the previous developed methods, our developed method uses the same sample pretreatment, chromatographic setup and detection as in methods previously described by us for the quantification of protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) from dried blood spots and plasma [8,9]. This allowed simultaneous quantification of raltegravir, PIs and NNRTIs from a single plasma or dried blood spot sample using the same setup, thereby reducing analysis time and costs when concentrations of multiple antiretroviral drugs have to be quantificated.

2. Experimental

2.1. Chemicals

Raltegravir potassium salt originated from Merck Sharp & Dohme (Haarlem, The Netherlands), dibenzepine hydrochloride was obtained from TEBU-BIO (Heerhugowaard, The Netherlands). Acetonitrile and methanol were HPLC-grade and obtained from Biosolve (Valkenswaard, The Netherlands). Ammonium acetate, dimethylsulfoxide (DMSO), glacial acetic acid and zinc sulphate heptahydrate were obtained from Merck (Amsterdam, The Netherlands). Distilled water originated from Aqua B. Braun (Melsungen, Germany). Drug-free plasma, blood and buffy coat was obtained from healthy human volunteers. Whatman 903 protein saver cards® for sample collection, 2 mL Eppendorf reaction vials, 1.5 mL autosampler vials, autosampler vial inserts, BD Vacutainer® CPT™ cell preparation tubes, BD Vacutainer® EDTA anticoagulated blood collection tubes, phosphate buffered saline (PBS), 14 mL sterile polypropylene tubes and Ficoll® were obtained from VWR international B.V. (Amsterdam, The Netherlands). A 0.25-in. diameter punch was obtained from Fiskars (Madison, WI, USA). Haemolance plus® high flow lancets for single use for patient sampling were obtained from HaeMedic AB (Munka Ljungby, Sweden).

2.2. Chromatographic and mass spectrometric conditions

An Agilent (Agilent technologies, Palo Alto, CA, USA) HPLC system was used consisting of an 1100 series pump and cooled autosampler (4 °C). Separation was carried out on a Phenomenex Gemini C18 column (150 mm × 2.0 mm) with a Phenomenex Securityguard Gemini C18 precolumn (4.0 mm × 2.0 mm) (Torrence, Ca,