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Simultaneous determination of the HIV nucleoside analogue reverse transcriptase inhibitors lamivudine, didanosine, stavudine, zidovudine and abacavir in human plasma by reversed phase high performance liquid chromatography

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Abstract

A reversed phase high performance liquid chromatography method was developed for the simultaneous quantitative determination of the nucleoside reverse transcriptase inhibitors (NRTIs) lamivudine, didanosine, stavudine, zidovudine and abacavir in plasma. The method involved solid-phase extraction with Oasis MAX cartridges from plasma, followed by high performance liquid chromatography with a SymmetryShield RP 18 column and ultraviolet detection set at a wavelength of 260 nm. The assay was validated over the concentration range of 0.015–5 mg/l for all five NRTIs. The average accuracies for the assay were 92–102%, inter- and intra-day coefficients of variation (CV) were <2.5% and extraction recoveries were higher than 97%. This method proved to be simple, accurate and precise, and is currently in use in our laboratory for the quantitative analysis of NRTIs in plasma.

Keywords: Nucleoside reverse transcriptase inhibitors; Abacavir; Didanosine; Lamivudine; Stavudine; Zidovudine; HPLC; HIV

1. Introduction

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of drugs that were introduced as antiretroviral agents for the treatment of infection with human immunodeficiency virus (HIV). Four additional drug classes have since been developed: protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), and fusion inhibitors. The current standard of care, referred to as "highly active antiretroviral therapy" (HAART), is to combine at least three antiretroviral drugs. Two NRTIs should be combined with either one or two PIs, an NNRTI or a third NRTI [1]. The six NRTIs, approved for use in HIV infection are: abacavir (ABC), didanosine (ddI), lamivudine (3TC), stavudine (d4T), zalcitabine (ddC) and zidovudine (AZT). Zalcitabine is not recommended by HIV treatment guidelines and is therefore rarely used. NRTIs are prodrugs that require intracellular phosphorylation to their corresponding triphosphate derivates, which are the active inhibitors of HIV reverse transcriptase. There is no clear correlation between NRTI plasma concentrations and virological response or intracellular concentrations of the corresponding triphosphate derivates. Nevertheless, plasma concentration measurements may be important for research purposes and in patient care to check adherence to NRTIs, to guide dosing in patients with renal failure and to evaluate drug-drug interactions. Separation methods for NRTIs used for treatment of HIV-1 infection

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were reviewed in 2001 by Pereira et al. [2] Most of these reviewed methods allowed the quantification of only one drug. Only one method was capable of measuring abacavir, didanosine, lamivudine, stavudine and zidovudine simultaneously [3]. Since that time two other high performance liquid chromatography (HPLC) methods with UV detection for the simultaneous determination of NRTIs have been published [4,5]. Aymard et al. [3] showed chromatograms with a low signal-to-noise ratio possibly due to the use of an ion-pair reagent in their mobile phase. Ion-pair HPLC in a gradient mode is usually not recommended [6,7]. Simon et al. [4] had high standard deviations for their recovery and high quantification limits. Rezk et al. [5] used four different ultraviolet wavelengths for detection which can make their method less robust. They all used conventional silica-based solid-phase extraction sorbents and with these columns, care should be taken that the columns did not run dry during conditioning, in order to obtain reproducible results. This could complicate manual SPE extraction of large amounts of samples and compromise the accuracy of the assay. We considered none of these methods suitable for application in our laboratory.

Therefore, the aim of this study was to develop a simple and robust HPLC-UV method that could be used in our hospital for the simultaneous assay of abacavir, didanosine, lamivudine, stavudine and zidovudine in patient plasma samples.

2. Experimental

2.1. Chemicals

Abacavir sulphate was kindly supplied by Glaxo Smith Kline (London, UK), didanosine and stavudine were kindly provided by Bristol-Myers Squibb (Princeton, NJ, USA), lamivudine was purchased from Moravek Biochemicals (Brea, CA, USA) and zidovudine was obtained from Sigma (St. Louis, MO, USA). Chemical purity for all compounds was >99%. Super gradient acetonitrile and HPLC quality methanol were purchased from Labscan Analytical Sciences (Dublin, Ireland), and HPLC quality water from Baker (Deventer, The Netherlands). All other reagents were obtained from Merck (Darmstadt, Germany). The drugs that were investigated for possible interference with the assay were obtained from Sigma (St. Louis, MO, USA) or were extracted from commercial products.

2.2. Preparation of standards and quality control samples

Two series of stock solutions of each NRTI were prepared in methanol–HPLC-grade water (1:9) at a concentration of 0.5 mg/ml and kept at −20 °C. For the preparation of standard and quality control (QC) samples, both series of stock solutions were diluted with blank plasma to obtain a concentration that equalled the highest standard concentration (5 mg/l). To achieve 6 standard concentrations of 0.015, 0.050, 0.150, 0.50, 1.500 and 5 mg/l, appropriate amounts of one of the 5 mg/ml stocks in plasma were added to blank plasma. For the QC samples, concentrations of 0.10, 0.4 and 2.0 mg/l were prepared from the other 5 mg/ml plasma stock. The standard and QC samples were stored at −20 °C.

2.3. Equipment

The HPLC system consisted of a model P4000 solvent delivery pump, a model AS3000 autosampler, a model UV2000 programmable wavelength UV detector and a ChromJet integrator. All these instruments were from Thermo Electron (Breda, The Netherlands). The analytical column was a SymmetryShield RP 18 column (150 mm × 4.6 mm i.d./particle size 3.5 μm) protected by a SymmetryShield RP 18 guard column (20 mm × 3.9 mm i.d./particle size 3.5 μm), both from Waters (Etten-Leur, The Netherlands).

2.4. Solid-phase extraction method

Solid-phase extraction (SPE) columns (1 cc, 30 mg Oasis MAX Waters) were placed on a vacuum elution manifold (Baker spe 24G Column Processor) and rinsed with 0.5 ml of methanol, followed by 0.25 ml of distilled water. Next, 0.5 ml of distilled water and 0.5 ml of plasma sample were transferred onto the columns and were drawn into them by applying reduced pressure (flow rate <1 ml/min). The columns were then washed with two aliquots of 0.15 ml of distilled water, followed by vacuum suction for 5 min. Elution of the absorbed analytes was performed by using two volumes of 0.25 ml of methanol–HPLC quality water (80:20, v/v) and reduced pressure (flow rate <1 ml/min). The eluent was evaporated to dryness under a nitrogen stream at 40 °C. The residues were redissolved in 0.2 ml of acetonitrile–HPLC quality water (5:95, v/v), mixed on a vortex mixer for 20 s, and centrifuged for 5 min. The clear supernatants were placed in autosampler vials with inserts and aliquots of 25 μl were injected onto the column.

2.5. Chromatography and detection

The chromatographic separation was performed at 30 °C with a gradient elution. The mobile phase flow rate was set at 1.0 ml/min. The mobile phase components were an acetate buffer (20 mM potassium acetate adjusted to pH 4.60 with 20 mM acetic acid) and acetonitrile. Mobile phase (A) consisted of acetate buffer:acetonitrile (95:5, v/v) and mobile phase (B) consisted of acetate buffer:acetonitrile (76:24, v/v). After 10 min 100% mobile phase (A), the mobile phase (B) concentration was increased in 14 min linearly from 0 to 100%. After that, mobile phase (A) concentration returned to 100% in 2 min. The column was then re-equilibrated for 9 min. UV detection was performed at 260 nm. Peak height was used for integration.