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Determination of antiretroviral agents in human serum by capillary electrophoresis

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Abstract

In this work, a simple and rapid electrokinetic chromatography method for the simultaneous separation of different protease inhibitors (indinavir, ritonavir, saquinavir, nelfinavir), nucleoside reverse transcriptase inhibitors (stavudine, zidovudine, didanosine) and non-nucleoside reverse transcriptase inhibitors (nevirapine, efavirenz) was developed. The analyses were performed in a 75 μm i.d. uncoated fused-silica capillary with 48.5 cm length (effective length of 40 cm) using a running buffer consisting of 20 mmol L^{-1} sodium dodecyl sulfate, 10 mmol L^{-1} sodium tetraborate, 30% acetonitrile and 5% ethanol. Samples were injected hydrodynamically by applying 50 mbar pressure during 6 s. All analytes were separated within 10 min with a voltage of 20 kV. The proposed method was validated for zidovudine, didanosine and efavirenz in human serum. Serum samples were prepared using a solid-phase extraction procedure (Waters® Oasis HLB cartridges). For quantitative purposes, stavudine was chosen as the internal standard (IS). Method validation parameters were determined revealing good migration time repeatability (<0.7% RSD) and peak area repeatability (<1.2% RSD). Intra- and inter-day precisions were less than 1.7% and 4.4% RSD, respectively. Matrix matching analytical curves for each drug were linear in the 1.0–20.0 μg mL^{-1} interval ($r > 0.998$). Limits of detection (LOD) were in range of 0.3–0.5 μg mL^{-1}. The extraction recoveries were higher than 90% with exception of efavirenz, which was 77.4%. Based on the performance characteristics, the proposed method was found suitable for the determination of zidovudine, didanosine and efavirenz in serum samples.

Keywords: Micellar electrokinetic chromatography; Antiretroviral agents; Human immunodeficiency virus

1. Introduction

The acquired immunodeficiency syndrome (AIDS) was first recognized in 1981, and the human immunodeficiency virus (HIV), the causative agent of AIDS, was identified in 1983 [1]. Currently, HIV/AIDS is the fourth greatest cause of death worldwide. It is estimated that 40 million people are infected with HIV and 22 million have died of the disease [2].

For a decade, nucleoside analogue reverse transcriptase inhibitors (NRTIs), such as zidovudine, didanosine, zalcitabine, stavudine and abacavir, were the only drugs available to treat HIV-1 infection. This unsatisfactory situation was dramatically changed with the introduction of two additional drug classes: protease inhibitors (PIs) that include saquinavir, ritonavir, indinavir, nelfinavir and amprenavir and non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as efavirenz, nevirapine and delavirdine. Standard therapy consists of two nucleoside analogues in combination with either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor [3,4]; the triple combination therapy using these antiretroviral drugs was termed highly active antiretroviral therapy.

Therapeutic monitoring of these drugs is recommended in order to avoid or to delay the occurrence of viral resistance, to assess the usually underestimated non-adherence to treatment and to study drug interactions. Up to now, high-performance

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liquid chromatography (HPLC) is the most widely adopted technique for the quantitative determination of protease and reverse transcriptase inhibitors in biological fluids [5–9]. However, this method has presented some practical disadvantages: long analysis time (20–55 min), extensive sample preparation, large solvent volumes and complicated system operation and maintenance.

Capillary electrophoresis (CE) has emerged in recent years as a powerful analytical technique for the separation of a large variety of substances, including pharmaceutical compounds [10,11]. CE possesses many unique advantages, such as small sample volumes, high separation efficiency, low operating and consumable costs, low consumption of reagents, short analysis time and easy conditioning of column. In the present literature, only a few publications describe the separation and determination of antiretroviral drugs using CE. Chelyapov et al. [12] developed a method for the quantitative determination of 4 PIs in deproteinized serum samples, using formic acid containing acetonitrile as electrolyte. Analysis time of 15 min and adequate sensitivity (62.5 ng mL^{-1}) are a few characteristics of their proposed method. Zeemann et al. [13] studied the separation of five PIs using an electrolyte containing phosphoric acid and hexadimethrin bromide (HDB), an electroosmotic flow (EOF) modifier, which allowed the establishment of a strong cathodic EOF. All five PIs were separated within 5 min. In latter study, the separation of 11 antiretroviral drugs in 8 min was achieved when a mixture containing phosphoric acid, acetonitrile and sodium polyanethol sulfonate (SPAS), an EOF modifier, was used as electrolyte [14]. More recently, the authors proposed a method where 15 drugs were separated in approximately 10 min using as electrolyte phosphoric acid containing SPAS, acetonitrile, ethanol and sodium dodecyl sulfate, SDS [15]. The separation of Zeeman et al., although impressive, does not seem useful for validation purposes and routine use in clinical laboratories or quality control of pharmaceutical formulations. System peaks and baseline perturbations in the vicinity of the solvent peak would compromise the quantitative determination of at least four drugs: didanosine, estavudine, zidovudine, ritonavir and, possibly, amprenavir. Moreover, as the authors showed the use of low pH electrolytes may be detrimental due to the fact that some analytes exhibit limited pH stability. Up to now, only Fan and Stewart [16] developed and validated method to determine serum concentration of anti-HIV drug mixtures using alkaline conditions (borate/phosphate buffer and SDS). Despite the method's excellent performance, only four drugs were evaluated.

In this work, a reliable, simple and rapid electrokinetic chromatographic (EKC) method was developed. This method employs an alkaline buffer, allowing simultaneous separation of nine anti-HIV drugs with baseline resolution. Since for most therapeutic studies only three drugs have to be monitored simultaneously, the proposed method was validated for zidovudine, didanosine and efavirenz in human serum (three routinely prescribed drugs of the highly active antiretroviral therapy).

2. Experimental

2.1. Instrumentation

All experiments were conducted in a capillary electrophoresis system (Agilent Technologies, model HP 3D CE, Palo Alto, CA, USA), equipped with a diode array detector set at 200 nm and a temperature control device maintained at 25 °C. Data acquisition and treatment software was supplied by the manufacturer (HP ChemStation, rev A.06.01). Fused-silica capillaries (Polymicro Tecnologies, Phoenix, AZ, USA) with dimensions 48.5 cm total length, 40 cm effective length and 75 μm i.d. × 375 μm o.d. were used. Samples were injected hydrodynamically, 50 mbar pressure (1 mbar = 100 Pa) during 6 s. The instrument was operated under positive polarity (injection end of the capillary). A constant voltage of 20 kV was used for all experiments.

2.2. Chemical and reagents

All reagents and solvents were of analytical grade and used with no further purification. Indinavir sulfate (IDV), nelfinavir mesylate (NFV), saquinavir (SQV), ritonavir (RTV), zidovudine (AZT), stavudine (D4T), didanosine (DDI) and nevirapine (NVP) were kindly donated by Cristália Produtos Químicos e Farmacêuticos LTDA (São Paulo, Brazil) and efavirenz (EFV) was kindly supplied by Instituto de Tecnologia de Fármacos–Fundação Oswaldo Cruz (Rio de Janeiro, Brazil). Sodium dodecyl sulfate (SDS) was purchased from Riedel-de Haën (Seelze, Germany). Sodium tetraborate was obtained from Aldrich (Milwaukee, WI, USA). Water was purified by deionization (Milli-Q, Millipore Corp., Bedford, MA, USA). Water® Oasis HLB 3cc cartridges were purchased from Waters Corporation (Milford, MA, USA). Drug-free human serum was obtained from the Faculdade de Ciências Farmacêuticas–USP (São Paulo, Brazil).

2.3. Analytical procedure

The electrolyte solution was prepared fresh daily. At the beginning of each day, the fused-silica capillary was conditioned by flushing with a 1 mol L^{-1} NaOH solution (5 min), followed by a 5 min flush of deionized water and electrolyte solution (40 min). In between runs, the capillary was rinsed with 0.1 mol L^{-1} NaOH for 3 min, followed by fresh electrolyte solution (3 min).

2.4. Preparation of standards

Individual stock solutions of all nine antiretroviral agents were prepared by dissolving appropriate amounts in methanol to a final concentration of 1000 μg mL^{-1}. All stock solutions were stored at −20 °C. Working solutions were prepared fresh daily by diluting appropriately the stock solutions with 9 mmol L^{-1} SDS solution.