Talanta 161 (2016) 278–287

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Online solid phase extraction and liquid chromatography-mass spectrometric determination of nucleoside drugs in plasma

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ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form
30 July 2016
Accepted 16 August 2016
Available online 18 August 2016

Keywords:
Nucleoside drugs
Online SPE-LC-MS/MS
Bioanalysis

ABSTRACT

The bioanalysis and especially the sample preparation of nucleoside drugs in complex media, such as human plasma, has been challenging due to the high polarity and high solubility of these drugs in water. Online solid phase extraction (SPE) offers significant advantages, such as automation and timesaving. Thus, several types of SPE columns have been developed for compounds with different polarities. In this study, SPE was applied to overcome the issue of sample pretreatment of nucleoside drugs in human plasma, with the final aim of establishing a robust analytical platform for drugs with similar structures. A simple, easy-to-use, and efficient method is described for the simultaneous determination of lamivudine, zidovudine, didanosine and emtricitabine in human plasma via online SPE and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Following a simple centrifugation step, a 10 μL plasma sample was injected directly onto the HPLC system. The Oasis MCX cartridge was washed, and the analytes were removed by back-flushing directly onto the analytical column. The analytes were quantified using a triple-quadrupole tandem mass spectrometer in multiple-reaction monitoring mode. Similarly, with the development and application of a Bond Elut phenylboronic acid (PBA) SPE cartridge, a fully automated online SPE-HPLC-MS/MS method was established for the simultaneous determination of ribavirin and taribavirin in human plasma. Linear calibration curves were obtained over the range of 0.5–2000 ng mL^{-1}, and the limit of quantification ranged from 0.5 ng mL^{-1} to 10 ng mL^{-1}, which is sensitive enough for clinical drug monitoring. The intra- and inter-day precisions were in the range of 0.2–8.9%, and the trueness ranged between 88.9% and 113.1%. Excellent recoveries from plasma were achieved with a range between 86.7% and 105.1%. This procedure is easier to perform and requires less sample handling compared to methods previously described in the literature. This high-throughput method involving the direct injection of plasma samples may provide a practical solution for the analysis of multiple nucleoside drugs in clinical research. The method was tested in plasma samples from some patients and showed good performance.

1. Introduction

At present, in addition to their use as cancer therapeutics and immunosuppressants [1], nucleoside drugs account for approximately 50% of antivirals already listed and used in clinics. These drugs play an important role in antiviral therapy for human immunodeficiency virus (HIV), hepatitis viruses B and C (HBV and HCV) and human herpes viruses (HSV) [2]. To improve the therapeutic effects in HIV-infected individuals, a combination of nucleoside reverse transcriptase inhibitors (NRTIs), such as lamivudine, zidovudine, didanosine and emtricitabine, are frequently used as a "nucleoside core"; other drugs are added to this core to achieve highly active anti-retroviral therapy (HAART) regimens [3].

The lack of compliance with antiviral treatments is one of the primary causes of therapeutic failure. The virus may multiply and develop resistance when exposed to subtherapeutic levels of antiviral drugs [4–6]. Therefore, high treatment compliance is

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http://dx.doi.org/10.1016/j.talanta.2016.08.050
0039-9140/

necessary for antiviral therapy. For instance, the required level of treatment appliance of HAART is above 95%. Poor compliance may also lead to a higher risk of mortality and a poor immunological response [7,8]. Therapeutic drug monitoring of nucleoside drugs is crucial to ensure treatment compliance, and the routine analysis of drug concentrations in the plasma could be a useful tool for achieving this goal. However, the bioanalysis of nucleoside drugs has been very challenging due to their high polarity and high solubility, which result in extreme difficulties during sample pretreatment.

The matrix clearance and the extraction of target nucleoside drugs from the matrix samples through in vivo pharmacokinetics (PK) studies have attracted great attention in bioanalytical research. Several methods relying on techniques such as high-performance liquid chromatography-ultra violet (HPLC-UV), HPLC-MS, capillary electrophoresis (CE), etc., have been developed for the determination of the nucleoside drugs in biological samples [9–16]. In most cases, sample pretreatment has been carried out using protein precipitation (PPT), liquid-liquid extraction (LLE), membrane filtration, off-line SPE, and other methods. In some cases, radioactive labelling and pre-column derivatization have been used to achieve the analytical goals [17]. Due to the high polarity and high solubility of nucleoside drugs, off-line manual operation processes such as PPT or LLE normally result in low recoveries and poor sensitivity [9–16]. In addition, these off-line manual operation processes are prone to human error and are time-consuming, which significantly affect the throughput, trueness, selectivity, reproducibility and recoveries during the downstream bioanalysis. The development of on-line sample pretreatment technologies can prevent not only low precision and poor recoveries but also the disadvantages of isotope labelling and pre-column derivatization [17]. Several methods using online SPE sample pretreatment for the simultaneous determination of a variety of nucleoside reverse transcriptase inhibitors (NRTIs) have been published. However, these previously reported pretreatment methods are rather complex and generally require large volumes of plasma samples (usually greater than 100 μL). In addition, it is difficult to achieve rapid turn-around times and high-throughputs for therapeutic drug monitoring (TDM) using these methods [18–20]. For instance, the bioanalysis of ribavirin has been reported using HPLC-MS/MS methods, but the sensitivity and recovery rate were both low [11,12,16,21]. In recent years, although several methods have been developed to quantify biological samples of ribavirin using reversible chemical adsorption, the sample pretreatment is still the major issue [22–24]. In the case of taribavirin, a prodrug of ribavirin, the only available bioanalysis method involves isotope labelling and SPE-based normal phase chromatography, which may indicate the challenges of its sample pretreatment [25,26].

The aim of this study is to address and overcome the challenges of sample pretreatment for the detection of nucleoside drugs. We sought to find an efficient solution for identifying not just one specific drug but other drugs of the same class. A number of representative nucleoside drugs used in the clinic were selected for investigation. In addition, taribavirin was included in this study to make the investigation even more challenging. In recent years, online-SPE has been one solution for sample pretreatment in PK studies [27–32]. In this study, online SPE technology was applied to the sample preparation of nucleoside drugs in human plasma. A fully automated online SPE-HPLC-MS/MS method was established for the simultaneous determination of a variety of NRTIs (lamivudine, zidovudine, didanosine and emtricitabine) in human plasma. Furthermore, the method can be further developed for the simultaneous determination of ribavirin and taribavirin by replacing the SPE column with a Bond Elut PBA SPE cartridge. The procedure was found to be easier to carry out and required less sample handling than the methods previously described in the literature.

2. Experimental methods

2.1. Chemicals and reagents

HPLC grade acetonitrile (ACN) was purchased from Tedia (Tedia Company, USA). Acetic acid (CH_3COOH), ammonium hydroxide (NH_4OH), and ammonium acetate (CH_3COONH_4) were purchased from Shanghai Disino Pharma Co. Ltd. Ultrapure water was generated using a Milli-Q system (Millipore, MA, USA). Ribavirin, taribavirin, lamivudine, didanosine, emtricitabine and zidovudine (more than 98% pure) were obtained from Desano Pharma (Shanghai, China). Vigolibose and metronidazole were purchased from NIFDC (National Institutes for Food and Drug Control). Their chemical structures and physicochemical parameters are shown in Fig. 1. Blank human plasma samples from healthy volunteers were kindly donated by Tianjin AnDing Hospital. The plasma samples were collected under the approval of the ethical committee of Tianjin AnDing Hospital and informed consents were obtained from the volunteers. Patient samples were obtained from Tianjin second people's Hospital under the ethical approval number [2015] 01.

2.2. HPLC/MS/MS system

On-line SPE and HPLC analysis were carried out using a UltiMate 3000 × 2 Dual-Gradient HPLC system (Sunnyvale, CA, USA) equipped with two ternary pumps, a vacuum degasser, an autosampler and a thermostatted column compartment with a six-way valve. Data acquisition was performed with a triple quadrupole API4000$^+$ mass spectrometer from Applied Biosystems (Ontario, Canada). The data were processed using Analyst 1.6 software (Toronto, Canada).

2.3. Chromatography columns

Four online SPE columns were explored: Oasis HLB, 2.1 mm × 0 mm, 30 μm particle size; Oasis MCX, 2.1 mm × 20 mm, 30 μm particle size (Waters, Milford, MA, USA); LiChrospher RP-18 ADS, 3 mm × 20 mm, 25 μm particle size (Merck KGaA, Darmstadt, Germany); and Bond Elut PBA SPE cartridge, 4 mm × 10 mm, 40 μm particle size (packed in our lab using bondesil purchased from agilent technologies). The chromatographic separation was performed on an Acclaim 120 C18 column, 4.6 mm × 150 mm, 5 μm particle size (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Preparation of calibration work solutions and quality control standards

For didanosine, lamivudine, emtricitabine and zidovudine, the stock solutions were prepared at a concentration of 1.00 mg mL^{-1} with ACN/water (1:1, v/v). And for ribavirin and taribavirin, the stock solutions were prepared at the same concentration with water. All of the stock solutions were stored at 4 °C. The solutions of standards and quality controls were diluted with blank plasma (10/90, v/v) to prepare a series of samples with different concentrations. Two different internal standards were used respectively for analysis: metronidazole was the internal standard of didanosine, lamivudine, emtricitabine and zidovudine; viglibose was the internal stangdard for ribavirin and taribavirin. As internal standards, viglibose and metronidazole were dissolved and diluted with ACN/water (1:1, v/v) and water respectively to obtain the working solutions. The concentrations of metronidazole and