Journal of Chromatography B 1083 (2018) 209–221

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of antiretroviral drugs in human hair with liquid chromatography-electrospray ionization-tandem mass spectrometry

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ARTICLE INFO

Keywords:
Tenofovir
Lamivudine
Nevirapine
Hair
LC-ESI-MS/MS

ABSTRACT

The determination of the concentrations of antiretroviral drugs in hair is believed to be an important means for the assessment of the long-term adherence to highly active antiretroviral therapy. At present, the combination of tenofovir, lamivudine and nevirapine is widely used in China. However, there was no research reporting simultaneous determination of the three drugs in hair. The present study aimed to develop a sensitive method for simultaneous determination of the three drugs in 2-mg and 10-mg natural hair (Method 1 and Method 2). Hair samples were incubated in methanol at 37 °C for 16 h after being rinsed with methanol twice. The analysis was performed on high performance liquid chromatography tandem mass spectrometry with electronic spray ionization in positive mode and multiple reactions monitoring. Method 1 and Method 2 showed the limits of detection at 160 and 30 pg/mg for tenofovir, at 5 and 6 pg/mg for lamivudine and at 15 and 3 pg/mg for nevirapine. The two methods showed good linearity with the square of correlation coefficient > 0.99 at the ranges of 416–5000 and 77–5000 pg/mg for tenofovir, 12–5000 and 15–5000 pg/mg for lamivudine and 39–50,000 and 6–50,000 pg/mg for nevirapine. They gave intra-day and inter-day coefficient of variation < 15% and the recoveries ranging from 80.6 to 122.3% and from 83.1 to 114.4%. Method 2 showed LOD and LOQ better than Method 1 for tenofovir and nevirapine and matched Method 1 for lamivudine, but there was high consistency between them in the determination of the three drugs in hair. The population analysis with Method 2 revealed that the concentrations in hair were decreased with the distance of hair segment away from the scalp for the three antiretroviral drugs.

1. Introduction

Highly active antiretroviral therapy (HAART) consisting of multiple antiretroviral drugs is widely applied to maintain the virological suppression and reduce the morbidity and mortality of patients living with HIV. Strict adherence to HAART tends to favorable virological outcomes [1–3] and poor adherence to HAART seems the most common reason for the failure to achieve the optimal treatment benefits [4]. Therefore, the assessment of the adherence to HAART is important for predicting virological outcomes and avoiding drug resistance.

The adherence to HAART can be evaluated with various methods, such as self-reports, electronic monitoring with the medication event monitoring system, pill counts and pharmacy refill data, the visual analogue scale and the detection of the concentration of antiretroviral drugs in different biomatrices [5]. Among these methods, the detection of drug concentration is the most objective and direct method for the assessment of adherence to HAART [5].

In the previous, drug concentration was mostly performed in biofluids for the assessment of the adherence, such as plasma, peripheral blood mononuclear cell and saliva [6–11]. However, these indices have major limitations in time span and long-term stability when they are utilized to assess long-term adherence to HAARRT. For instance, drug level in plasma just reflects the adherence in a short-term period (e.g., several hours or days) and is easily affected by external conditions [12,13]. Alternatively, hair analysis overcomes the limitations in the previous analyses (e.g., blood analysis) [13,14]. The drug concentration in the 1-cm hair strands closest to the scalp can retrospectively reflect the drug usage over one month if the hair growth rate is 1 cm per

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https://doi.org/10.1016/j.jchromb.2018.03.021
Received 31 October 2017; Received in revised form 4 January 2018; Accepted 10 March 2018
Available online 11 March 2018
1570-0232/

month. It also shows high stability in storage and is non-invasive in the sampling [15–17]. Furthermore, it shows high consistency with other indices (e.g., the oral dosage and the drug concentration in plasma) [18,19]. Therefore, drug concentration in hair may be more superior to the other assessments in predicting long-term adherence [20–22].

High performance liquid chromatography mass spectrometry or tandem mass spectrometry (HPLC-MS or HPLC-MS/MS) were utilized as the primary method for detecting the concentrations of the antiretroviral drugs because of its high sensitivity, specificity and less analysis time [10,23–25]. Atmospheric pressure chemical ionization (APCI) and electro-spray ionization (ESI) are main ionization methods for HPLC-MS/MS determination. APCI is appropriate for low-polar and non-polar compounds while ESI can be applied to the wider range from low-polar to polar compounds [26]. Therefore ESI is more apposite for simultaneous analysis of different antiretroviral drugs with different polarity including strong polarity and low polarity.

Nowadays, lamivudine (3TC) and tenofovir (TFV) or zidovudine in combination with nevirapine (NVP) or efavirenz are adopted as the first-line antiretroviral therapeutic regimen of National Free Antiretroviral Treatment Program, China. Because 3TC, TFV and NVP are the important drugs in the first-line regimen, their simultaneous determination should be conducted. The simultaneous detection had been conducted in blood [27]. However, there was no study reporting simultaneous determination in hair although individual drug concentration was separately determined in several previous studies [17,22,28,29]. Additionally, incubation temperature, incubation duration, hair matrix effect and the position of hair segment in hair shaft are important factors influencing the determination of the concentrations of the three drugs in hair. Previous studies had proven that 37 °C is the optimum incubation temperature for the three drugs [22,28,29], and that 14–16 h is the optimum incubation duration for TFV and NVP [28,29]. However, there was no study reporting the optimization of the incubation duration for 3TC. The effects of hair matrix and the position of hair segment were also unclear.

The present study aimed to develop a sensitive LC-MS/MS method for simultaneous determination of TFV, 3TC and NVP in hair under ESI in the positive mode and multiple reactions monitoring (MRM). In previous studies, different hair weights were used for the detection of the three drugs, such as 2-mg hair for 3TC and NVP [22,28] and 10-mg or 50-mg hair for TFV [17,29]. Therefore, 2-mg and 10-mg hair samples were utilized in the present analysis (i.e., Method 1 and Method 2). Then the validated method with good performances was applied to detect the concentrations of the three drugs in natural hair from HIV patients and then investigate the effects of the position of hair segment on the determination of the three drugs in hair.

2. Materials and methods

2.1. Participants and hair collection

Participants were 33 female HIV positive patients (P01–P33) who were randomly recruited from the Guangxi Zhuang Autonomous Region, China. Of these, 31 patients were using 3TC over the past 12 months, 27 patients were taking TFV and 10 patients were using NVP. All participants provided written informed consent prior to inclusion. The present study followed the Declaration of Helsinki and was approved by the Health Science Research Ethics Board of Southeast University. The research was also approved by IRB at University of South Carolina in the USA and Guangxi Center of Disease Control in China.

All participants provided hair strands longer than 12 cm in the posterior vertex region. Prior to use, the first 6-cm hair strands closest to the scalp were cut into six segments with 1 cm in length and the second 6-cm hair strands were cut into three segments with 2 cm in length. The nine hair segments were utilized to probe the change of the concentrations of the three drugs with the distance of hair segment away from the scalp.

2.2. Chemicals and reagents

Standards, lamivudine, tenofovir and nevirapine were purchased from TargetMol (Shanghai, P.R. China). Lamivudine-d3 (3TC-$^{15}N_2$, ^{13}C) and nevirapine-d3 used as internal standards (IS) for lamivudine and nevirapine were purchased from the Toronto Research Chemicals (Toronto, Canada). Adefovir as IS of tenofovir and methanol (HPLC grade) were obtained from Sigma Aldrich (St. Louis, MO, USA). Ammonium acetate was from Tedia (Fairfield, OH, USA).

Stock solutions of lamivudine, nevirapine and their ISs were prepared in methanol at 1 mg/mL and stored at −20 °C. Tenofovir and adefovir were dissolved in distilled water as stock solution at a concentration of 1 mg/mL and were stored at 4 °C. The working solutions of standards were obtained by diluting with methanol to the desired concentrations from 0.1 to 2000 ng/mL. IS working solutions were obtained by diluting with methanol at 20 ng/mL of 3TC-d3, NVP-d3 and 200 ng/mL of adefovir.

2.3. Hair treatment

Hair segments with 1 cm or 2 cm in length were rinsed with 2 mL methanol for 2 min and dried at 50 °C under a blow of pure nitrogen gas. The washing procedure was done twice. After being cut into pieces (1–2 mm) with surgical scissors, hair samples were weighed and transferred to a clean plastic centrifuge tube and then 950 μL methanol and 50 μL IS working solution were added. The mixture was vortex-mixed for 30 s and was incubated for the desired duration (e.g., 16 h) at 37 °C in thermostat water bath. After a 2-min vortex and 5-min centrifugation at 12,000 rpm, 800 μL supernatant was transferred into another clean centrifuge tube and evaporated using nitrogen air at 50 °C·Finally, the residue was reconstituted with 50 μL mobile phase for next LC-MS/MS analysis.

2.4. Simultaneous analysis of the three drugs

5 μL of the reconstituted solution was injected into a LC-MS/MS system consisting of an Agilent 1200 HPLC system (Agilent, Waldbronn, Germany) and 3200 QTRAP tandem mass spectrometer (ABI, Foster City, CA, USA). The Platisil ODS C18 (5 μm, 150 mm × 4.6 mm; Dikma) reverse phase column was used in the HPLC system for chromatographic separation. A mixture of methanol-water (80:20, v/v) containing ammonium acetate (2 mM) was prepared as mobile phase. It was filtered through micro porous membrane and conducted with over 10 min ultrasonic process after preparation. The column oven temperature was maintained at 40 ± 1 °C and the flow rate was set at 200 μL/min.

Mass spectrometric condition was also optimized for analysis of three drugs. Liquid nitrogen was gasified and used as nebulizing gas. The spectrometer was equipped with electrospray ionization source (ESI) operated in MRM positive mode. Its ion-spray voltage was set at 4500 V. The symmetric heaters were at 400 °C. Dwell time was set at 100 ms to monitor all analytes. For gas setting, curtain gas was maintained at 10 psig and collision gas at medium, and both gas 1 (ion source gas) and gas 2 were set at 40 psig. The precursor ion and product ion of individual drug in the optimum condition were shown in Fig. 1. The optimum condition of ionization and fragmentation for three analytes was listed in Table. 1.

2.5. Method validation

The validation was done under the solution of the standards spiked with blank hair matrices that were the hair strands 12 cm away from the scalp from a healthy female adult without the use of the three drugs.