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Short communication

A micellar liquid chromatography method for the quantification of abacavir, lamivudine and raltegravir in plasma

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ABSTRACT

An analytical methodology based on micellar liquid chromatography has been developed to quantify abacavir, lamivudine and raltegravir in plasma. These three antiretroviral drugs are prescribed as a set in highly active antiretroviral therapy to acquired immunodeficiency syndrome patients. The experimental procedure consists in the dilution of the sample in micellar media, followed by filtration and, without cleanup step. The analytes were resolved in less than 30 min using a mobile phase of 0.05 M sodium dodecyl sulphate at pH 7, running at 1 mL min^{-1} under isocratic mode at room temperature through a C18 column (125 × 4.6 mm, 5 μm particle size). The UV detection wavelength was set at 260 nm. The method was successfully validated following the requirements of ICH guidelines in terms of: linear range (0.25–2.5 μg mL^{-1}), linearity ($r^2 > 0.990$), intra- and interday precision (<6.8%) and accuracy (92.3–104.2%) and robustness (<7.1%). To the extent of our knowledge, this is the first published method to quantify these three drugs in plasma. Several blood samples from AIDS patients taking this HAART set provided by a local hospital were analyzed with satisfactory results.

1. Introduction

Since its discovery in 1981, acquired immunodeficiency syndrome (AIDS) has caused nearly 30 million deaths and approximately 34 million people are globally infected with human immunodeficiency virus 1 (HIV-1) [1–3]. Different attempts have been undertaken to find a cure for the disease, but with limited success. In 1996, highly active antiretroviral therapy (HAART) was introduced, with impressive clinical results in suppressing the activity of HIV. Generally, HAART regimen combines three or four different antiretroviral (ARV), which acts together against HIV. But this therapy is complex, has many unwanted effects, is difficult to adhere to and has to be lifelong because it does not get rid of HIV [4].

Recently, a new HAART combination (Table 1) [5], containing abacavir (Ziagen®), lamivudine (Epivir®) and raltegravir (Isentress®), has been introduced in the Spanish market, for patients in which fails the normal treatment with other mixtures [6]. Abacavir and lamivudine are reverse transcriptase inhibitors, which interacts with an HIV viral enzyme, used to generate new virus. Inhibition of this enzyme prevents the virus completes this reproductive cycle [7]. Raltegravir is an integrase inhibitor. It inhibits the integrating the viral genetic material into human chromosomes [8].

Therapeutic drug monitoring (TDM) consists in the quantification of drugs in physiologic matrices at several times after the consumption of the formulation. This information can be used to establish its pharmacokinetics and explain the therapeutic and adverse effects of the drug. This information can be used to determine the proper dose of a drug for a specific patient. This is important because the presence of sub-therapeutic levels of antiretroviral will result in the appearance of drug resistance mutations that can endanger drug treatment options [9]. On the other hand, the lack of compliance to HAART is the first cause of therapeutic failure and should be evaluated. A treatment compliance level above 95% is necessary [10]. In order to optimize the response of the patient to this new HAART regimen, clinicians need analytical methods to quantify abacavir, lamivudine and raltegravir in plasma.

Prominent among the techniques used for TDM, chromatographic methods such a thin layer chromatography (TLC), gas chromatography (GC) and HPLC are widely used to analyze antiretroviral [11]. Abacavir, lamivudine and raltegravir have been analyzed in several ways, such as HPLC-UV [12,13] and LC–MS/MS [14–16]. However, to the best of our knowledge, a method to

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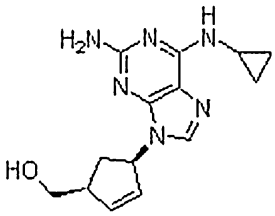
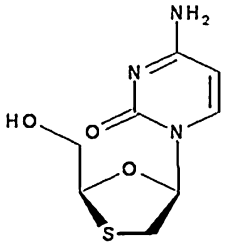
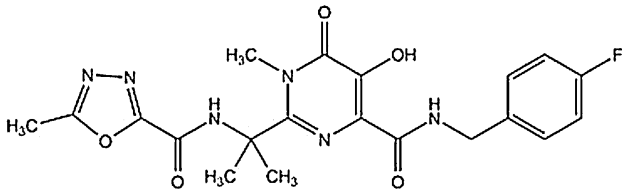
Table 1
Structure, pK_a and log $P_{o/w}$.

Compound	Structure	pK_a	log $P_{o/w}$
Abacavir		5.01	1.2
Lamivudine		Not found	−0.952
Raltegravir		6.7	−1.055

simultaneously analyze these three drugs in plasma has not been previously issued. Mass spectrometry is an easy-to-contaminate instrumentation and quite expensive. Besides, chromatographic methods usually require tedious and time-consuming extraction and cleanup steps prior to the chromatographic resolution.

Authors have proven that plasma samples can be studied avoiding these problems using micellar liquid chromatography (MLC) [17]. Previously published papers have detailed the development of MLC-based methodologies, using sodium dodecyl sulphate (SDS) as surfactant, for the screening of thirteen ARV [4] and the quantification of 3 HAART mixtures by the use of only three mobile phases [3]. These methods can be adapted to the analysis of abacavir, lamivudine and raltegravir, to maintain the benefits of micellar liquid chromatography, as direct injection (after dilution and filtration), use of isocratic mobile phases, versatility and the use of low amount of pollutant chemicals, reducing the analysis time and cost [18].

The aim of this work was to develop a sensitive and reliable MLC-based method to determine three ARV included in a new HAART combination: abacavir, lamivudine and raltegravir, in plasma samples of AIDS patients. The method must be simple, inexpensive, environmentally-friendly and with a reduced experimental protocol. The developed method was validated according the ICH Guidelines [19] in terms of linear interval, calibration parameters, sensitivity expressed as limits of detection (LOD) and quantification (LOQ), precision, accuracy and robustness. Finally, the developed analytical method was used to quantify the ARV in human plasma from AIDS patients. Samples were supplied by the Hospital La Plana (HLP) of Vila-real.

2. Materials and methods

2.1. Reagents and instrumentation

The following ARV standards (purity > 99.5%) were taken for the analysis: abacavir, lamivudine (GlaxoSmithKline, Brentford, UK) and raltegravir (Merck, MSD, Darmstadt, Germany).

Selected surfactant was sodium dodecyl sulphate (purity > 99%, Merck, Darmstadt, Germany). Hydrochloride acid, sodium hydroxide and sodium dihydrogenphosphate (reagent quality) were purchased from Scharlab (Barcelona, Spain). Ultrapure water was prepared by purification of deionized water using an ultrapure water generator device (Millipore S.A.S., Molsheim, France) and used for preparing all mobile phases and solutions.

The instrumentation used in the study and the treatment of the data are described in [3].

2.2. Solutions and mobile phases

Mobile phases and solution of ARV were prepared and stored as reported in [3]. The concentrations of the stock solutions 100 μg mL^{-1} for abacavir and lamivudine, and 250 mg mL^{-1} for raltegravir.

2.3. Sample treatment

The blood collection and extraction of plasma was performed as reported in [3]. Afterwards, plasma samples were frozen and stored at −20 °C until its analysis, it was then thawed just before use. The samples were obtained after consent from the patients.

For validation and analysis purpose, 1 mL volume of plasma samples was introduced into a vial and the volume was adjusted to 5 mL with 0.05 M SDS at pH 7 (1/5 dilution). In the case of spiked samples, the appropriate amount of the ARV standard solution was added before filling. The diluted sample was vigorously shaken to favor homogenization and stored for one day in the fridge at 5 °C to favor the contact between antiretroviral and the sample [20]. These samples were filtered using 0.45-μm nylon membranes (Micron Separations, Westboro, MA, USA) and injected in the chromatographic system.