Journal of Pharmaceutical and Biomedical Analysis 94 (2014) 65–70

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

A validated HPLC-MS method for quantification of the CCR5 inhibitor maraviroc in HIV+ human plasma

Marco Simiele, Lorena Baietto, Alessio Audino, Mauro Sciandra, Stefano Bonora, Giovanni Di Perri, Antonio D'Avolio*

Unit of Infectious Diseases, University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, Turin, Italy

ARTICLE INFO

Article history:
Received 3 August 2013
Received in revised form 20 January 2014
Accepted 22 January 2014
Available online 31 January 2014

Keywords:
Maraviroc
HPLC-MS
HIV
Therapeutic drug monitoring
Plasma

ABSTRACT

Maraviroc is a CCR5 inhibitor approved in 2007 for treatment of therapy experienced adult patients infected with CCR5-tropic HIV-1 virus. International guidelines for HIV therapy indicate a plasma concentration cutoff of maraviroc for response. We developed and validated a new HPLC-MS method to quantify maraviroc concentrations in human plasma. 6,7-Dimethyl-2,3-di(2-pyridyl)quinoxaline was used as internal standard and added to 100 μL of plasma. Samples were then treated with 500 μL of acetonitrile for the protein precipitation procedure. An analytical T3 Atlantis column (150 mm × 4.6 mm i.d.) with a particle size of 5 μm was used to separate the compounds and ions were detected at *m/z* 257.5 and 313.3 for maraviroc and quinoxaline respectively. The calibration curve was linear up to 2500 ng/mL. The mean recovery of maraviroc was 89.1%. All validation data results were in accordance to Food and Drug Administration and European Medicines Agency requirements. The HPLC-MS method reported here could be used routinely to monitor plasma concentrations of maraviroc in HIV-infected patients.

1. Introduction

Since Highly Active Antiretroviral Therapy (HAART) became widely available in 1996, the number of AIDS-related deaths deeply decreased, and it led to improvement of quality of life to the people living with HIV. Combination therapy includes at least one protease inhibitor (PI) or a non nucleoside transcriptase inhibitor (NNRTI) and/or one or more nucleoside or nucleotide transcriptase inhibitors (NRTIs-NtRTI) and/or a fusion inhibitor (FI). HIV virions might acquire mutations conferring cross resistance to different compounds of each class, especially when the patient does not intake the therapy correctly. Therefore, new therapeutic compounds are needed that able to overcome the extensive class-resistances observed in multi-drug treated patients [1].

New compounds as raltegravir (RGV), the first integrase inhibitor, and rilpivirine (TMC278) the last NNRTI approved, have shown to be active against multidrug-resistant viral strains [2–6]. In contrast to other classes of drugs maraviroc has a target direct of the cell host rather than viral components. Maraviroc (MVC; Celsentri®, Selzentry®) is a chemokine CCR5 co-receptor antagonist [7–15] that is used at present in experienced R5-tropic HIV-infected patients, for whom previous antiretroviral regimens have failed. MVC is administered orally at a usual dose of 300 mg twice daily, but can be taken at different doses (150 mg or 600 mg) mainly depending on other concomitant drugs [16]. MVC is a substrate for CYP3A4, so potentially can interact with several other antiretrovirals. Therefore, it is administered at 150 mg bid if co-administered with a boosted protease inhibitor, at 300 mg bid if co-administered with nevirapine (NVP) or tipranavir/ritonavir (TPV/r), and at 600 mg bid if co-administered with etravirine(ETV) or efavirenz (EFV) [16,17].

MVC is also a substrate of P-glycoprotein [18]. Ritonavir, which is commonly administered as a booster for protease inhibitors, is a strong inhibitor of both CYP3A4 and P-glycoprotein, and can consequently cause an increase of MVC plasma concentrations. On the basis of the exposure-response analysis from the MOTIVATE studies, an approximate maximal efficacy is achieved at a maraviroc trough concentration (C_{trough}) above 50 ng/ml determined in a population predominantly receiving MVC (150 mg) with boosted protease inhibitors [19]. Although average plasma concentrations (C_{avg}) have a stronger correlation with virological efficacy, the measurement of average plasma concentrations in the clinical setting is difficult because of the requirement for multiple samples and the associated cost. Therefore, C_{trough} is more commonly used.

Accurate measurement of antiretroviral plasma concentrations is crucial for pharmacokinetic/pharmacodynamic analyses, drug–drug interaction studies, and therapeutic drug monitoring (TDM) [20]. The latter is currently considered a useful tool for the

* Corresponding author at: Laboratory of Clinical Pharmacology and Pharmacogenetics, Unit of Infectious Diseases, University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, CorsoSvizzera 164, 10149 Turin, Italy.
Tel.: +39 011 4393979; fax: +39 011 4393996.
E-mail address: antonio.davolio@unito.it (A. D'Avolio).

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http://dx.doi.org/10.1016/j.jpba.2014.01.031

Table 1
Chromatographic conditions for gradient HPLC analysis of maraviroc: Mobile phase: Solvent A (HPLC grade water + 0.05% formic acid) and Solvent B (HPLC grade acetonitrile + 0.05% formic acid). Temperature of the column was set at 35 °C.

Time (min)	Flow (ml/min)	Solvent A %	Solvent B %
0	1	80	20
4	1	60	40
7.5	1	30	70
8.1	1	5	95
15	1	5	95
15.1	1	80	20
20	1	80	20

optimization of antiretroviral therapy in international guidelines [20–23]. An understanding of the pharmacokinetics (PK) of MVC in the clinical setting, pharmacokinetic/pharmacodynamic properties and drug interaction profiles, is still limited due to the recent availability of this compound. Moreover, recent data suggest the involvement of pharmacogenetic factors in the PK of MVC [24]. Therefore, PK studies of MVC are requested in order to define the possible role of TDM in the clinical context. Few methods for MVC quantification in human plasma have been published to date [25–31].

The aim of our study was to develop and validate a cheap, fast and reliable HPLC-MS analytical method for the quantification of MVC in human plasma.

2. Experimental

2.1. Chemicals

Maraviroc was purchased from Pfizer Inc. (Groton, CT, USA). Acetonitrile HPLC grade and methanol HPLC grade were purchased from J.T. Baker (Deventer, Holland). HPLC grade water was produced with Milli-DI system coupled with a Synergy 185 system by Millipore (Milan, Italy). Quinoxaline [6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline] (QX) and formic acid were obtained from Sigma–Aldrich (Milan, Italy). Blank plasma from healthy donors was kindly supplied by the Blood Bank of Maria Vittoria Hospital (Turin, Italy).

2.2. Chromatographic and MS conditions

The HPLC-MS instrument used was a Waters system (Milan, Italy), with binary pump model 1525, AF degasser, 717-plus autosampler, and Micromass ZQ mass detector. LC–MS Empower 2 Pro software (version year 2007, Waters; Milan, Italy) was used. Chromatographic separation was performed at 35 °C using a column oven, and an Atlantis T3 5 μm column (150 mm × 4.6 mm i.d.) (Waters, Milan, Italy), protected by a SecurityGuard with C18 (4.0 mm × 3.0 mm i.d.) pre-column (Phenomenex, CA). Runs were performed with a gradient (Table 1), and the mobile phase was composed of Solvent A (HPLC grade water + 0.05% formic acid) and Solvent B (HPLC grade acetonitrile + 0.05% formic acid).

A "T" switch tube was applied post-column to introduce only 200 μL/min of total flow (1 mL/min) into the MS detector. Optimization of the MS conditions has been performed by direct infusion of reference standards (1 μg/mL) in mobile phase (solvent A:solvent B (50:50)) at 10 μL/min; at the same time a flow of 1 mL/min of mobile phase (solvent A:solvent B (50:50)) was introduced in the column to perform the infusion in combined mode. MS parameters were been optimized to maximize sensitivity as follows: ESI, positive polarity ionization; capillary voltage, 3.5 kV; source temperature, 110 °C; desolvation temperature, 350 °C; nitrogen desolvation flow, 400 l/h; nitrogen cone flow, 50 L/h. The ion transitions and cone voltages were: 513.3 → *m/z* 257.5 with a cone voltage of 35 V for MVC and 312.3 → *m/z* 313.3 with a cone voltage of 50 V for QX (IS).

2.3. Stock solutions, standards (STD) and quality controls (QC)

MVC and QX stock solutions were made in a solution of methanol and HPLC grade water (90:10, v/v) to obtain a final concentration of 1 mg/mL; all stock solutions were then refrigerated at 4 °C until use, within 1 month. A working solution of Internal Standard (IS) was made for every validation analyses with QX (3 μg/mL) in methanol and HPLC grade water (50:50, v/v). The highest calibration standard (STD 10) and four quality controls (QCs) were prepared adding a stock solution to blank plasma; the others STDs were prepared by serial dilution from STD 10 (2500 ng/mL) to STD 1 (4.9 ng/mL) with blank plasma, to obtain 10 different spiked concentrations plus a blank sample (STD 0). QC concentrations were 2000 ng/mL, 500 ng/mL, 50 ng/mL and 12.5 ng/mL for QC-High, QC-Medium, QC-Low and QC-Low-Low, respectively.

The range of MVC concentrations was established in accord with relevant clinical studies [8–12,14–16] in order to include all described concentrations. As for patient samples, STDs and QCs were inactivated for 35 min at 58 °C to provide HIV-free plasma, prior to storing them at −20 °C for no more than three months. They were thawed in the beginning of analysis avoiding more then two freeze–thaw cycles.

2.4. STD, QC and samples preparation

Patients receiving standard dosing of MVC (150 mg, 300 mg or 600 mg) underwent blood sampling for the measurement of plasma drug concentrations. The study was conducted in compliance with the Declaration of Helsinki and with the local Review Board regulations; all patients gave written informed consent according to the local ethic committee standards. Blood samples were collected in lithium heparin tubes (7 mL), and plasma was obtained after centrifugation at 1400 × *g* for 10 min at +4 °C (Jouan Centrifuge, Model BR4i; Saint-Herblain, France) and then underwent heat inactivation as described above.

A protein precipitation procedure was performed for extraction of maraviroc from plasma. Fifty μL of IS working solution was added to 100 μL of sample (STDs, QCs or patient samples) in a PTFE microfuge tube, then 500 μL of protein precipitation solution (acetonitrile 100%) was added. The tube was vortexed for 10 s and then centrifuged at 13,000 × *g* for 10 min at 4 °C. Supernatant was transferred into glass tubes and treated by vortex vacuum evaporation to dryness at 60 °C and then reconstituted with 125 μL of HPLC grade water and acetonitrile solution (70:30, v/v). Fifty μL were injected into the HPLC column. All QCs samples were performed in duplicate during validation sessions, and all procedure steps were carried out at room temperature.

During every routine analysis session, the patients samples have been processed together with STDs from 0 to 10 (STD0 was a blank plasma) and QCs (High, Medium, Low, Low-Low). Before the injection of these samples a series of blank (water and acetonitrile solution (70:30, v/v)) was injected for the conditioning of the HPLC-MS system. A fresh mix with the analytes (10 μg/mL in water:acetonitrile solution (70:30, v/v)) was also injected to verify the retention times. Concentrations data of patients samples were considered reliable if standard deviation of all QCs from nominal values were below 15%.

2.5. Specificity and selectivity

Interference from endogenous compounds was investigated by analysis of six different blank plasma samples. Potential interference by antiretroviral drugs concomitantly administered