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Short communication

A HPLC-fluorescence method for the quantification of abiraterone in plasma from patients with metastatic castration-resistant prostate cancer

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ABSTRACT

Abiraterone acetate is an oral prodrug of abiraterone, a selective inhibitor of CYP17, used for patients with metastatic castration-resistant prostate cancer (mCRPC). To date, a single liquid chromatographic–tandem mass spectroscopy method has been reported to assay abiraterone concentration in plasma from mCRPC patients. The aim of this study was to develop a simple and sensitive high performance liquid chromatographic (HPLC) method with fluorescence detection for quantification of abiraterone in plasma from mCRPC patients. After protein precipitation with acetonitrile and a liquid–liquid extraction with diethyl ether, abiraterone, and hydroxy-itraconazole (internal standard) were separated on a C8 Xterra® MS column using a mobile phase of acetonitrile and glycine buffer 88.4 mM (pH 9.0) (60:40, v/v). Samples were eluted isocratically at a flow rate of 0.9 ml/min throughout an 11-min run. Fluorescence wavelengths' excitation and emission were 255 and 373 nm, respectively. The calibration was linear in the range 1.75–50 ng/ml. Inter- and intraday imprecision were less than 3.5 and 7%, respectively. This method is simple, sensitive, and selective. This analytical method was successfully applied to determine the steady-state plasma exposure to abiraterone in mCRPC patients. This method can be used in routine clinical practice to monitor plasma abiraterone concentrations in mCRPC patients.

1. Introduction

Prostate cancer, a hormonal-dependant cancer, is the second leading cause of cancer-related death in developed countries and affects one in six men [1]. Androgen deprivation therapy currently remains the first line therapy for metastatic prostate cancer. However, all patients eventually develop metastatic castration-resistant prostate cancer (mCRPC), which is invariably fatal [2]. Abiraterone acetate (AA) is a new oral prodrug of abiraterone, which is a selective inhibitor of cytochrome P450 17α-hydroxylase/17,20 lyase (CYP17). Abiraterone catalyzes critical reactions in the synthesis of androgens in testicular, adrenal, and prostatic tumor tissues [3]. AA (Zytiga®) in combination with prednisone was approved by the Food and Drug Administration (FDA) and European Medicines Agency for the treatment of patients with mCRPC. The recommended daily dose of AA is 1000 mg (once daily) in combination with prednisone 5 mg twice daily [4].

After oral administration, AA is hydrolyzed to abiraterone by esterases. Time (T_{max}) to reach maximum plasma abiraterone concentration (C_{max}) is approximately 2 h [5]. AA should be taken in a fasted state because bioavailability can increase up to 10-fold, when administered with a high-fat meal. Abiraterone has a large apparent distribution volume (5630 L) and is highly bound to human plasma

Abbreviations: AA, abiraterone acetate; C_{min}, steady-state plasma trough concentration; C_{max}, maximum plasma concentration; CYP17, cytochrome P450 17α-hydroxylase/17,20-lyase; DMSO, dimethylsulfoxide; FDA, Food and Drug Administration; HPLC, high performance liquid chromatography; IQC, in-house quality control; IS, internal standard; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LLE, liquid–liquid extraction; LOD, limit of detection; LLOQ, lower limit of quantification; mCRPC, metastatic castration-resistant prostate cancer; OH-itraconazole, hydroxy-itraconazole.

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proteins (99.8%) [6]. Abiraterone undergoes sulfation, hydroxylation, and N-oxidation, mainly in the liver. This metabolism leads to two main inactive metabolites: abiraterone sulfate, and N-oxide abiraterone sulfate via CYP3A4 and SULT2A1 [7]. Besides, 13 other metabolites have been identified in the plasma but their pharmacological activity currently remains unknown. Fecal excretion is the major route of elimination of abiraterone. The mean half-life of abiraterone in plasma is approximately 15 h. Finally, a reduced dose of AA is recommended in patients with moderate hepatic impairment while AA should not be used in patients with severe hepatic impairment [8].

Abiraterone has shown impressive efficacy in treatment of mCRPC. However, responses are inconsistently durable over the time. Additionally, some patients have a primary resistance to this therapy [2]. Different reasons may explain these treatment failures: the large interindividual variability in pharmacokinetics of abiraterone and the noncompliance. Furthermore, abiraterone is candidate to pharmacokinetic drug–drug interactions through CYP3A4 metabolism pathway. These different data suggest that plasma drug monitoring could be helpful in daily clinical practice for the management of outpatients treated with AA.

To our knowledge, a single high performance liquid chromatography with UV detection (HPLC–UV) method has been described to assay abiraterone concentrations in rat plasma [9]. However, this method is not enough sensitive for plasma drug monitoring in mCRPC patients. Two liquid chromatographic–tandem mass spectroscopy (LC–MS/MS) methods have been reported for the analysis of abiraterone exposure in human [10] and rat plasma [11]. But, such equipment is not available in most clinical laboratories.

In this intent, we have developed a simple and sensitive HPLC-fluorescence method to quantify abiraterone in plasma from mCRPC patients in daily clinical practice.

2. Materials and methods

2.1. Reagents

Abiraterone was purchased from LGC standards (Molsheim, France). Acetonitrile HPLC grade, glycine, dimethylsulfoxide (DMSO), and sodium chloride were purchased from Sigma (St. Louis, MO, USA). Diethyl ether was provided by Merck Millipore (Darmstadt, Germany). Drug-free plasma for calibration standards was purchased from UTAK laboratories (Valencia, Spain). Deionized purified water was prepared in the laboratory using an ELGA system (Veolia, Le Plessis Robinson, France).

2.2. Stock and working standard solution

Stock solutions containing 0.5 mg/ml of abiraterone (separate calibration and quality control stock solutions) and 0.1 mg/ml of hydroxy-itraconazole (internal standard) were prepared in DMSO and methanol respectively; then aliquoted and stored at −20 °C in the dark. Each day, three working solutions of abiraterone (50 μg/ml, 500 ng/ml, and 50 ng/ml) were freshly prepared with drug-free plasma for a set of seven calibrating standards ranging from 1.75 to 50 ng/ml. In-house quality controls (IQC) were prepared using different stock solutions of abiraterone. Concentrations of IQC were 2.5, 9, and 50 ng/ml. Finally, a working solution of internal standard (IS) (40 μg/ml) of hydroxy-itraconazole was freshly prepared with deionized purified water.

2.3. Chromatographic apparatus and conditions

The chromatography system consisted of Dionex Ultimate 3000 equipped with a gradient pump with degas option and gradient mixer, a fluorescence detector RF 2000, an autosampler, and a Chromeleon® chromatography workstation (Dionex Corporation, Sunnyvale, CA, USA). Chromatographic separation was achieved on C8 Xterra® MS (250 mm × 4.6 mm, 5 μm; Waters, Milford, USA) associated with a guard column packed with the same bonded phase. The mobile phase consisted of a mixture of glycine buffer (pH 9.0, 88.4 mM,) and acetonitrile (40:60; v/v), and was delivered at a flow rate of 0.9 ml/min throughout the 11-min run. Chromatography was performed at 50 °C. Fluorescence excitation and emission wavelengths were 255 and 373 nm, respectively.

2.4. Sample preparation

First, 100 μl of IS at 40 μg/ml was added to 400 μl of plasma (calibration standard, IQC, or plasma sample). After mixing, 400 μl of acetonitrile was added before a 10-min vortexing step with a VX-2500 Multi-Tube Vortexer® (VWR, Fontenay sous Bois, France). After centrifugation (10 min, 7970 × *g* at room temperature), 400 μl of the supernatant were transferred into a glass tube. Then 1 ml of water and 3 ml of diethyl ether were added. These solutions were mixed for 10 min with a mechanical rotator SB3 Stuart® (VWR), then centrifuged at 3082 × *g* for 5 min at room temperature. The organic phase was collected into a 5 ml glass tube and evaporated to dryness at 50 °C under nitrogen stream. The dry residue was reconstituted in 70 μl of mobile phase and 30 μl of each sample was injected into the chromatographic system.

2.5. Specificity and selectivity

Only blood samples from subjects not receiving any of the drugs of interest were used to test the selectivity and the specificity of the method. Interferences with endogenous compounds have been evaluated from plasma samples from patients experiencing severe hepatic ($n = 5$) or renal ($n = 5$) impairment. Drug interferences were investigated in plasma from 10 cancer patients.

2.6. Method validation

The method was validated according to the FDA guidelines for bioanalytical method validation [12]. Linearity of the method was determined by replicate analysis of six complete standard curves on six separate days. The three levels of IQC were assayed thrice with each standard curve. A linear regression was used to plot the peak area ratio (y) of analyte to IS vs. analyte concentration. Homoscedasticity of the model was assessed by the Levene test. Intra- and interday imprecision (coefficient of variation (CV%)) and accuracy expressed as bias were evaluated at the three levels of IQC. Six replicates of each level were assayed in one run for the intraday experiment. Three replicates of each level were assayed within six different days for the inter-day experiment. According to FDA guidelines, the accuracy and imprecision for all tested concentrations should be within ±15% except for the lower limit of quantification (LLOQ), in which case these parameters should not exceed 20%. The limit of detection (LOD) was determined from the smallest measurable peaks, which signal to noise ratio equals to three. Carry-over was evaluated by injecting a 2000 ng/ml concentration sample of abiraterone followed an injection of blank plasma sample ($n = 6$). The analyte concentration of blank was accepted whether it was found to be less than LOD. Dilution integrity exercise was carried out to ensure the integrity of analyte in samples, which are beyond upper limit of the standard curve and need to be diluted. A fresh stock solution of abiraterone was prepared and spiked in free-drug plasma to get a concentration level of two times of highest standard of the usual calibration standard. It was then diluted two and four times with the same free-drug plasma. Six aliquots of both dilutions were processed along with freshly spiked calibration standards and analyzed by back calculation using