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A new HPLC UV validated method for therapeutic monitoring of deferasirox in thalassaemic patients

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ABSTRACT

We describe a new high performance liquid chromatography coupled with ultraviolet detection method for the quantification of plasma concentration of oral iron chelating agent deferasirox. A simple protein precipitation extraction procedure was applied on 500 μl of plasma aliquots. Chromatographic separation was achieved on a C18 reverse phase column and eluate was monitored at 295 nm, with 8 min of analytical run. This method has been validated following Food and Drug Administration procedures: mean intra and inter day variability was 4.64 and 10.55%; mean accuracy was 6.27%; mean extraction recovery 91.66%. Calibration curves ranged from 0.078125 to 40 μg/ml. Limit of quantification was set at 0.15625 while limit of detection at 0.078125 μg/ml. We applied methodology developed on plasma samples of thalassaemic patients treated with deferasirox, finding correlation between deferasirox plasma concentrations and serum ferritin levels. This methodology allowed a specific, sensitive and reliable determination of deferasirox, that could be useful to perform its therapeutic monitoring and pharmacokinetic studies in patients plasma.

1. Introduction

In thalassaemic patients iron overload, caused by regular transfusions need and increased gastrointestinal absorption, can lead to different clinical consequences [1]. Iron accumulation in the body over the time can damage liver, myocardium, spleen, and endocrine organs, inducing heart failure, diabetes, hypothyroidism, hypogonadism, and hepatic disease as cirrhosis or liver cancer [2,3]. The major cause of death reported in transfusion iron overload is heart failure [4]. Achievement of safe tissue iron concentrations, by promoting a negative iron balance and iron detoxification, can be pursued with chelation treatment initiation [1]. Chelators such as deferipone and deferoxamine, standard cares for the past thirty years, have been widely used to remove excess iron in the body [5] although with evident drawbacks. Deferipone (Ferriprox©), formulated as solid tablets and administered 3 times a day, has a narrow therapeutic window, and its safety risks may include drug related agranulocytosis and arthropathy [6,7]. On the other hand, the uncomfortable way of administration of Deferoxamine (Desferal©), injected by slow subcutaneous or intravenous infusion over 8–12 h, due to the low oral drug bioavailability and to its short half life, results in a therapy compliance often poor with limited efficacy [8]. This situation has prompted to investigate for a more convenient iron chelating agent. Deferasirox (ICL670, Fig. 1), following indicated as DFX, is a tridentate orally administered iron chelator recently approved by Food and Drug Administration (FDA) and licensed by European Medicines Agency (EMA) to this purpose. Commercially known as Exjade©, DFX represents a new approach to the management of chronic iron overload in patients with chronic anemias who require blood transfusions [6,9,10]. Currently approved in many countries for the treatment of patients over 2 years of age, its once daily administration leads to high patient satisfaction and compliance [11]. DFX dose between 20 and 30 mg/kg/day generally produces a net negative iron balance [6], however, a recent retrospective study demonstrated that doses of DFX greater than 30 mg/kg/day are safe and more effective in reducing the iron burden [12]. The current maximum FDA approved dose of DFX has been recently increased to 40 mg/kg/day in the Unites States [13].

DFX elimination half life is between 8 and 16 h, allowing to a convenient once daily administration. Its metabolism and that of the iron chelate (Fe $[DFX]_2$) is primarily mediated by glucuronidation, whereas elimination of both occurs by hepatobiliary excretion into the faeces [6]. Although the mentioned half life is the most frequent reported, one study has shown that the half life of DFX may decrease to 7 h in some patients [8], and this may decrease the total effective

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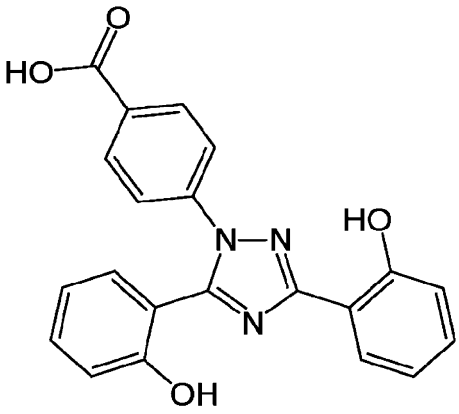

Fig. 1. Deferasirox structure.

time of drug coverage. Furthermore DFX is metabolized in hepatocytes by UDP glucuronyltransferase 1A1, with the formation of main metabolite, a glucuronide, that has no clinical use, up to now; this enzyme shows a variable expression, depending on common promoter polymorphism [14]. As previously described drug and its complex are excreted mainly in the bile: the complex is dissociated in intestinal lumen whereas DFX might enter an enterohepatic cycle [15]. In addition some patients, especially those heavily iron loaded, do not achieve adequate iron chelation and a negative iron balance, even when receiving DFX doses exceeding 30 mg/kg/day (poor responders). Others may experience DFX related adverse events (AEs) at the dose required to maintain the iron burden balance (intolerant patients). If AEs are managed by decreasing the dose of DFX or interrupting treatment, these patients will not be able to achieve adequate iron chelation and maintain a negative iron balance during their regular blood transfusions. Finally some DFX AEs may be dose dependent [6] and related to peak drug levels.

Therefore it is clear that, due to all these mentioned parameters, an high inter individual variability of DFX exposure may occur, leading to inadequate chelation treatment or to a toxicity increase.

Therapeutic drug monitoring (TDM) has become recently an essential tool for the management of patients with different pathologies and may be useful also for thalassaemic patients. Measurement of DFX plasma concentrations in treated patients, in fact, could be useful to evaluate patient adherence to daily oral therapy, potential drug–drug interactions, and pharmacokinetic (PK)/pharmacodynamic (PD) relationship studies [16,17]. Furthermore recent data [18] show an inverse correlation between preadministration labile plasma iron, target of chelators, and DFX trough concentration (i.e. 24 h after last intake), following indicated as C_{trough}, sustaining the hypothesis that DFX C_{trough} could be related to treatment response.

In recent years, numerous papers have reported the use of high throughput bioanalytical procedures for the quantification of iron chelating drugs [8,10,13,19–23]. Those reporting the use of high performance liquid chromatography coupled with ultraviolet determination (HPLC UV) methods [8,10,13,20,23], all applied methodology developed by Rouan in 2001 [19]. More recently liquid chromatographic methods based on mass spectrometry (LC MS MS) detection have been developed to this purpose [21,22], although MS facilities are not always available in standard hospital laboratories. Chauzit et al. [22] reported also the analysis of DFX metabolite, with the evidence that glucuronide not interferes with DFX at its retention time and that it do not convert usually into the parent drug, increasing the DFX concentration *in vitro*. Method developed by Rouan [19] permitted separation and simultaneous plasma determination of DFX and its iron complex in a range of concentrations from 0.25 to 20 μg/ml. In order to preserve the ratio between complex and total form, method required plasma samples storage at 4 °C immediately after collection, and all samples processing maintained at low temperature, procedures often difficult both for hospital setting and laboratory analytical routine.

Therefore aim of the present study was to develop and validate an easier HPLC UV method for DFX plasma quantification, broadly applicable and defined by a wider range of concentrations (0.078125–40 μg/ml).

2. Materials and methods

2.1. Chemicals

DFX (ICL 670) and Imatinib (STI 571), used as internal standard (IS), were kindly provided by Novartis Pharma AG (Basel, Switzerland). Acetonitrile HPLC grade, methanol HPLC grade and triethylamine were purchased from VWR International (Milan, Italy). HPLC grade water was produced with Milli DI system coupled with a Synergy 185 system by Millipore (Milan, Italy). Blank plasma from healthy donors was kindly supplied by the Blood Bank of San Luigi Hospital (Orbassano, Italy).

2.2. Stock solutions, calibration standards (STD_s) and quality controls (QC_s)

Stock solution of DFX was prepared by dissolving an accurately weighed amount of drug in ethanol to obtain a final concentration of 1 mg/ml, then stored at −20 °C till analysis [22]. Stock solution of IS was prepared by dissolving an accurately weighed amount of drug in methanol to obtain a final concentration of 1 mg/ml, then stored at −20 °C till analysis, stable up to 3 months [24]. The highest calibration standard (STD_{10}: 40 μg/ml) and 3 QC_s, QC_{high} (20 μg/ml), QC_{medium} (5 μg/ml) and QC_{low} (0.3125 μg/ml) were prepared adding a determined volume of stock solution to blank plasma. Others STD_s were prepared by serial dilution from STD_{10} to the lowest calibration standard (STD_1: 0.078125 μg/ml) with blank plasma, to obtain 10 different spiked concentrations. A blank sample plus IS (STD_0) was also included. Calibration range, from STD_{10} to STD_1, and QC_s concentrations are listed in Table 1. STD_s and QC_s were stored at −20 °C until analyses.

2.3. STD_s and QC_s extraction

The extraction procedure was based on protein precipitation: 100 μl of IS working solution, made at the final concentration of 100 μg/ml in methanol and used immediately, was added to 500 μl of plasma sample. Then 750 μl of protein precipitation solution (methanol:acetonitrile 50:50, v/v) was added to each sample. After brief mixing (30 s), samples were centrifuged at 12,000 rpm for 15 min and 800 μl of the obtained supernatant were transferred to vials, for injection in column (20 μl).

All procedures (stock solutions, STD_s and QC_s preparation and extraction steps) were carried out at room temperature.

2.4. Chromatographic system and conditions

HPLC was performed with a VWR Hitachi system (LaChrom Elite) equipped with autosampler, spectrophotometer, and heated column compartment. System management and data acquisition were performed with the EzChrom Elite software. Separation was achieved with GraceSmart© RP18 column, 5 μ, 250 mm × 4.6 mm (Grace, Milan, Italy), preceded by a Security Guard Cartridge C18 4 mm × 3 mm (Phenomenex, Milan, Italy). Mobile phase consisted of 40% solvent A, 20% methanol, 40% acetonitrile. Solvent A consisted of water (72.5%) methanol (25%) and triethylamine (2.5%), adjusted for pH 9.3 by orthophosphoric acid. Analysis was carried