Journal of Pharmaceutical and Biomedical Analysis 125 (2016) 369–375

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

A UHPLC–MS/MS method for the quantification of direct antiviral agents simeprevir, daclatasvir, ledipasvir, sofosbuvir/GS-331007, dasabuvir, ombitasvir and paritaprevir, together with ritonavir, in human plasma

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ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form 15 April 2016
Accepted 19 April 2016
Available online 20 April 2016

Keywords:
Liquid chromatography
Tandem mass spectrometry
HCV
DAAs
TDM
Multiplexed

ABSTRACT

To date, the new standard for treatment of chronic hepatitis C is based on the administration of novel direct acting antivirals. Among these, sofosbuvir, simeprevir, daclatasvir, ledipasvir, dasabuvir, ombitasvir and paritaprevir already entered the clinical use. Anyway, since few pharmacokinetic studies have been conducted on these drugs in a "real life" context poor knowledge is available about their optimal therapeutic range. Without this background, therapeutic drug monitoring is not applicable for treatment optimization. Up to now, a few methods are reported to quantify these drugs in human plasma, and none of them in a simultaneous way. The aim of this work was to develop and validate a simple, fast and cheap, but still reliable UHPLC–MS/MS method for the quantification of these drugs, feasible for a clinical routine use.

Solid phase extraction was performed using HLB C18 96-well plates. Chromatographic separation was performed on a BEH C18 1.7 μm, 2.1 mm × 50 mm column, settled at 50 °C, with a gradient run of two mobile phases: ammonium acetate 5 mM (pH 9.5) and acetonitrile, with a flow rate of 0.4 mL/min for 5 min. Tandem-mass detection was carried out in positive electrospray ionization mode.

Both inter and intraday imprecision and inaccuracy were below 15%, as required by FDA guidelines, while both recoveries and matrix effects resulted within the acceptance criteria. The method was tested on 80 patients samples with good performance.

Being robust, simple and fast and requiring a low plasma volume, this method resulted eligible for a clinical routine use.

1. Introduction

To date, HCV infection affects more than 130 million people worldwide, being an important cause of liver cirrhosis, hepatocellular carcinoma and liver transplantation [1].

HCV is a single strand RNA virus, belonging to the Flaviviridae family with a genome coding for 10 proteins, among which protease NS3-4A and polymerases NS5A/NS5B have been identified as druggable targets.

Up to 2011, the standard for treatment of HCV infection consisted in the combination of ribavirin (RBV) and pegylated-interferon α (Peg-IFNα). More recently, the better knowledge of viral life cycle and of its enzymes lead to the development of new direct acting antivirals (DAAs) [2].

The first generation of protease inhibitors, telaprevir (TVR) and boceprevir (BOC), has been approved in 2011 for use in combination with Peg-IFNα/RBV and, despite the enhanced response rate, these agents caused important side effects: anemia, neutropenia, and disguise for BOC and anemia, skin rash, and anorectal symptoms for TVR [2,3].

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http://dx.doi.org/10.1016/j.jpba.2016.04.031
0731-7085/

The goal of the research of new therapies is to develop drugs with pangenotypic activity, high genetic barrier and fewer side effects for patients with HCV.

These drugs include Nucleoside Inhibitors (NIs) and Non-Nucleoside Inhibitors (NNIs) of viral polymerase NS5A/5B and Protease Inhibitors (PIs) [4].

Among NIs, sofosbuvir (SOF) is currently the most used, because of its high pangenotypic effectiveness, alone or in combination with Peg-IFNα/RBV or with PIs (such as simeprevir, SMV) or NNIs (as ledipasvir, LDV, or daclatasvir, DAC), with or without RBV. An issue emerging in the evaluation of SOF kinetics is its fast metabolism to its main plasma metabolite (>90%), GS-331007, which can be considered a good marker for SOF plasma exposure [5].

Other than SOF-based regimens, therapeutic alternatives include the co-administration of SMV or DAC with Peg-IFNα/RBV and, more recently, the single tablet formulation of ritonavir-boosted ombitasvir and paritaprevir (OMV and PAR, a NNI and a PI, respectively), with or without dasabuvir (DBV, a NI) and/or RBV [4]. However, poor knowledge about these drugs pharmacokinetics in plasma and, going further, about the possible correlation between plasma concentrations and therapeutic response and/or toxicity is currently available.

Analyses on previous anti-HCV drugs, as RBV, BOC and TVR, already revealed in the past years the relationship between plasma concentration of these drugs and some of adverse effects, such as anemia, or therapeutic failure [6,7].

Indeed, the therapeutic drug monitoring (TDM) of anti-HCV drugs plasma concentration could represent a useful tool for the clinicians to evaluate drug efficacy and to prevent adverse events, in order to optimize the therapy. Treatment optimization through TDM is already reported to improve the quality of life and the efficacy of the therapy itself, but also it could lead to a cost saving, reducing side effects and consequent clinical cost for patient's care, in many different contexts.

At the moment only few methods have been developed for the quantification of some of the new DAAs, and not altogether [8–12]. For these reasons, a robust quantification method for all the currently used drugs is currently needed. Therefore, the aim of this work was to develop and validate a new high-throughput UHPLC–MS/MS method for the simultaneous quantification in human plasma of SOF/GS-331007, SMV, DAC, LDV, OMV, PAR and DBV, togheter with RTV, eligible for a wide routine use following FDA guidelines [13].

2. Experimental

2.1. Chemicals

DAC and [$^{13}C_2$, $^{2}H_6$]-DAC (d-DAC), SMV, SOF and its metabolite GS-331007, DBV, OMV and PAR were all purchased from Alsachim (Illkirch Graffenstaden, France); LDV was purchased from Selleckchem (Munich, Germany). Acetonitrile (ACN) 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). 6,7-Dimethyl-2,3-di(2-pyridyl) quinoxaline (QX), RTV 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). Ammonium acetate and DMSO were purchased from Sigma Aldrich.

2.2. Chromatographic conditions

Chromatographic analysis was performed on a Shimadzu Nexera X2® LC system coupled with a LC-8050® tandem mass spectrometer (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on an Acquity UPLC BEH C18 column, (2.1 mm × 50 mm, 1.7 μm; Waters, Milan, Italy) maintained at 50 °C through the column oven.

Table 1
Chromatographic gradient of mobile phases A (ammonium acetate 5 mM pH 9.5) and B (acetonitrile).

Time (min)	Solvent B %	Flow (mL/min)
0.00	3	0.40
0.50	8	0.40
0.60	55	0.40
1.80	70	0.40
2.00	95	0.40
3.50	95	0.40
3.60	3	0.40
5.00	3	0.40

Compounds separation was obtained through a gradient (Table 1) of mobile phases A (Ammonium acetate 5 mM buffer, pH 9.5) and B (ACN) at flow rate of 0.4 mL/min and a time run of 5 min. Auto-sampler was settled at 4 °C and the injection volume was 0.3 μL, with a sampling rate of 1 μL/sec. Data processing and system control was managed through the LabSolution® software (Shimadzu, Kyoto, Japan) version 1.0.

2.3. Stock solutions, standards and quality controls

Stock solutions of DAC, SMV, LDV, SOF/GS-331007, DBV, OMV and PAR were prepared in DMSO at a concentration of 1 mg/mL and stored at −80 °C. QX, RTV and d-DAC stock solutions (1 mg/mL) were prepared in pure methanol and stored at 4 °C until analysis. Internal standard working solution (IS) was made with QX and d-DAC (both at [0.625 μg/mL]) in water:methanol (50:50 v:v) at the time of the analysis.

The highest standard sample (STD 9) and the three quality controls, high (H), medium (M) and low (L), were prepared by spiking blank plasma with stock solutions; Lower STDs were prepared by serial 1:1 dilution from STD 9 to STD 1 with blank plasma, in order to obtain 9 different spiked concentrations plus a blank sample (STD 0). STDs and QC were stored at −80 °C.

Calibration ranges and QCs concentrations for all drugs are listed in Table 2.

2.4. Sample extraction

HLB C18 96 wells plates were chosen for the samples extraction. Each well (cartridge) was activated with 1 mL of pure methanol and equilibrated with 1 mL of water, in a positive pressure-96 manifold® (Waters, Milan, Italy).

Two hundred microliters of plasma were diluted 1:2 with H2O 1% phosphoric acid, added with 40 μL of IS working solution and centrifuged at 21,000 *g* for 10 min: then, the supernatants were loaded into the corresponding wells. After a washing step with 200 μL of pure water, the samples were eluted in a 96 well 2 mL collection plate with 500 μL of methanol:ACN 90:10 (vol:vol): 0.5 μL of the resulting extracts have been injected in the chromatographic system.

2.5. Mass conditions

Tandem mass spectrometric detection was carried-out through electrospray ionization source set in positive ionization mode (ESI+) for all the considered analytes.

Ionization conditions were optimized by directly injecting solutions containing each single drug, prepared in a mixture of the two mobile phases (A and B) 50:50 (vol:vol), bypassing the column (Fast