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Short communication

Development a validated highly sensitive LC–MS/MS method for simultaneous quantification of Ledipasvir, sofosbuvir and its major metabolite GS-331007 in human plasma: Application to a human pharmacokinetic study

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ABSTRACT

A highly sensitive and rapid LC–MS/MS method was developed, fully optimized and validated for the simultaneous determination of Ledipasvir (LED) and Sofosbuvir (SOF) in the presence of its major metabolite GS-331007 in human plasma using Daclatasvir as internal standard (IS). The extraction of analytes and IS from plasma was performed using liquid-liquid extraction with ethyl acetate. The chromatographic separation of these prepared samples was achieved on Xterra MS C_8 column (4.6 × 50 mm,5 μm) using gradient elution with a mobile phase of ammonium formate buffer (pH 3.5; 10 mM), acetonitrile and methanol pumped at a flow rate 0.7 mL min^{-1}.The detection was performed on API4000 triple quadrupole tandem mass spectrometer using multiple reaction monitoring (MRM) positive electrospray ionization interface. The method was validated according to FDA guidelines for bio-analytical methods with respect to linearity, accuracy, precision, selectivity, carry-over, stability and dilution integrity. Linearity was obtained over a concentration range of 0.1–1000, 0.3–3000 and 3.0–3000 ng mL^{-1} for LED, SOF and GS-331007; respectively by applying weighted least-squares linear regression method ($1/x^2$). The wider range of quantification in a shorter period of separation time less than 5.0 min allowed monitoring the serum concentration of analytes up to 144 h. The proposed method can be successfully applied for pharmacokinetic and bioequivalence studies in healthy human volunteers.

1. Introduction

Sofosbuvir [Isopropyl(2*S*)-2-[(2*R*,3*R*,4*R*,5*R*)-5-(2,4dioxopyrmidin1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran]methoxy-phenoxyphosp-horyl]amino]propanoate] Fig. 1 belongs to a new class of antivirals have been marketed lately in 2013. SOF is an oral nucleotide analog that inhibits the RNA polymerase of the HCV virus by being a defective substrate [1]. It provides a high cure rate with fewer side effects compared to previous treatments, approximately, four-fold reduced duration of therapy allows patients to be treated successfully without the use of interferon [2]. Ledipasvir, [Methyl *N*-[(2*S*)-1-[(6*S*)-6-[5-[9,9-Difluoro-7-[2-[(1*S*,2*S*,4*R*)-3-[(2*S*)-2-(methoxycarbonylamino)-3-methylbutane-yl]-3azabicyclo [2.2.1heptan-2-yl]- 3*H*-benzimidazol-5-yl] fluoren-2-yl]-1-*H*-imidazol-2-yl]- 5azaspiro [2,4] heptan-5-yl]-3-methyl-1-oxobutan-2-yl] carbamate Fig. 1.It is a novel HCV NS5A inhibitor that has proven potent antiviral activity against genotypes 1a and 1b HCV infection [3]. The latter combination provides high cure rates in people infected with genotype 1 without the use of interferon, regardless of the failure of the pre-treatment or the presence of liver cirrhosis [4,5]. To the best of author's knowledge, there was one reported LC–MS/MS method for simultaneous determination of LED, SOF and its metabolite GS-331007 in human plasma [6] and the other in rat plasma [7]. Other published LC–MS/MS and HPLC methods were reported for the simultaneous determination of SOF and/or LED in plasma [6,8–12]. However,

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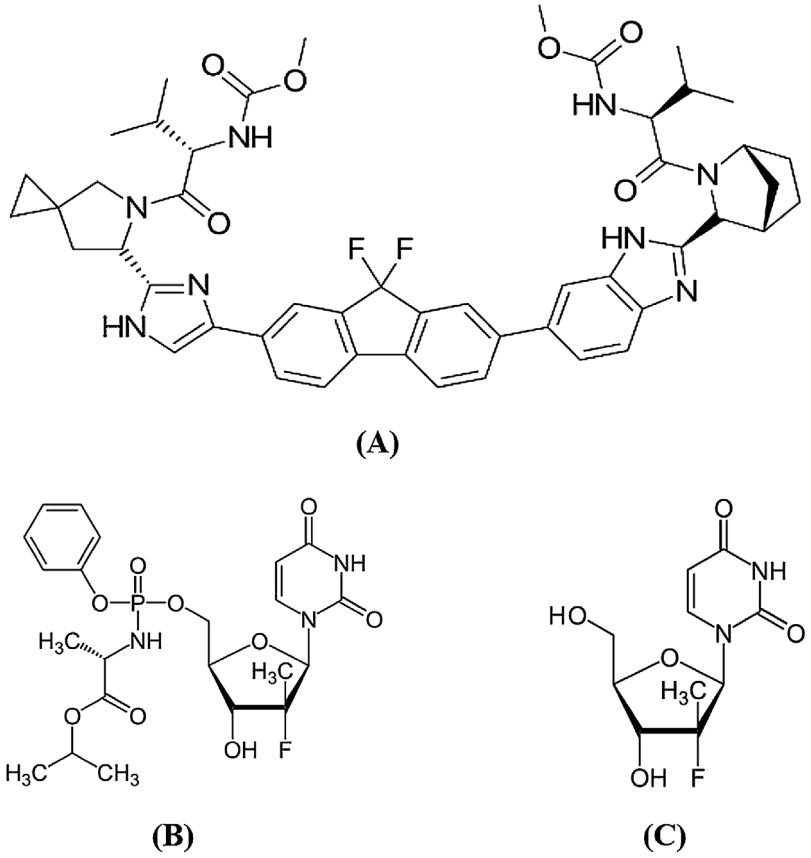

Fig. 1. Chemical structures for (A) Ledipasvir (B) Sofosbuvir (C) GS-331007.

simultaneous determination of LED, SOF and its major metabolite GS-331007 in human volunteer plasma would help to establish a pharmacokinetic correlation that requires administration of both drugs to achieve maximum efficacy.

The present work was aimed to develop a highly sensitive and selective LC–MS/MS method with good precision and accuracy. Moreover, quantification of LED and SOF in presence of its metabolite GS-331007 which has the greatest value of interest in clinical pharmacological studies, and accounts for more than 90% of total exposure in human plasma. The proposed method could reach a lower detection limit in human plasma and thus allow quantification of analytes up to 144 h as required by the new WHO bioanalytical guidelines [13]. The sensitivity of the method was found to be sufficient for accurately measuring the main pharmacokinetic parameters for the investigated analytes. The values obtained were in close agreement with the earlier reported values for healthy subjects.

2. Experimental

2.1. Chemicals and reagents

SOF was purchased from Optimus Ltd (Telangana, India), LED was purchased from BDR Pharmaceuticals Internationals Pvt. Ltd. (Mumbai, India). Daclatasvir (IS) was purchased from Virupaksha Organics Ltd. (Hyderabad, India). GS-331007 was purchased from Toronto Research Chemicals (North York, Canada). Methanol, acetonitrile and ammonium formate (HPLC grade) were obtained from Sigma-Aldrich (Milwaukee, USA). Ethyl acetate (HPLC grade) was purchased from Scharlab S.L.chemicals (Barcelona, Spain). Ultrapure water; resistivity > 18 MΩ cm^{-1} at 25 °C and TOC < 5 ppb was obtained from MilliQ UF-Plus system (Millipore, Bedford, MA, USA). Blank plasma samples were obtained from Holding Company for Biological Products and Vaccines (Giza, Egypt).

2.2. Pharmaceutical formulations

Harvoni® 400/90 mg tablets, was purchased from local market, manufactured by Gilead Sciences, limited IDA business and technology (Co.Cork, Ireland) for Gilead Sciences international (Cambridge, UK). Each tablet is claimed to contain 400/90 mg of SOF and LED, respectively.

2.3. Instruments

Quantitative analysis was performed using Agilent 1260 HPLC chromatographic system (Agilent Technologies, CA, USA) coupled to API4000 mass spectrometer (AB Sciex, Ontario, Canada) operated in positive electrospray ionization (ESI) through multiple reaction monitoring (MRM) mode. Data acquisitions were acquired by Analyst 1.6.2 software Hotfixes® (AB Sciex, Ontario, Canada).

2.4. Liquid chromatographic and mass spectrometric conditions

Chromatographic separations were achieved using waters Xterra MS C8 column (4.6 mm × 50 mm,5 μm; Waters, MA, USA) maintained at 30 °C through the column oven with an injection volume of 5 μL. The mobile phase was pumped with a gradient elution program consists of (A): ammonium formate buffer (pH 3.5; 10 mM), (B): 40% acetonitrile, 60% methanol, was delivered at flow rate 0.7 mL min^{-1} according to the following programs: 0.0–0.5 min (20% B), 0.5–0.7 min (90% B), 0.7–4.0 min (90% B), 4.0–4.2 min (20% B), 4.2–5.0 min (20% B). Each component of the mobile phase was degassed before use in an ultrasonic bath for 10 min. The mode