Journal of Pharmaceutical and Biomedical Analysis 152 (2018) 155–164

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Ultrasensitive spectrofluorimetric method for rapid determination of daclatasvir and ledipasvir in human plasma and pharmaceutical formulations

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ARTICLE INFO

Article history:
Received 22 October 2017
Received in revised form 16 January 2018
Accepted 18 January 2018

Keywords:
Direct-acting antivirals
Daclatasvir
Ledipasvir
Native fluorescence
Real human plasma
Pharmaceutical formulations

ABSTRACT

Direct-acting antivirals (DAAs) represent a revolution in the treatment of chronic hepatitis C which have emerged at an extremely rapid pace over the past few years. DAAs act directly on the hepatitis C virus at various points in the viral life cycle to inhibit viral production. Among these novel DAAs, are daclatasvir (DCS) and ledipasvir (LDS). Herein, a novel, fast, simple, ultrasensitive and cost-effective spectrofluorimetric method was designed for determination of DCS and LDS in miscellaneous matrices. The method is based on investigation of the native fluorescence of the cited drugs. The relative fluorescence intensity (RFI) was measured at $\lambda_{ex}/\lambda_{em}$ equal to 315/381 nm for DCS and 332/387 nm for LDS. Under the optimum conditions, the linear ranges of calibration curves were 0.2–30 and 6–120 ng mL^{-1} for DCS and LDS, respectively with correlation coefficients ≥0.9998. The detection limits were 0.047 and 1.939 ng mL^{-1} for DCS and LDS, respectively indicating ultrasensitivity of the proposed method. Consequently, this permits in vitro and in vivo application of the proposed method in spiked and real human plasma with good percentage recovery (96.6–103.6%). The method was validated in compliance with ICH guidelines and US-FDA guidelines. Furthermore, the application was extended to analysis of DCS and LDS in its pharmaceutical formulations (either alone or in presence of other co-formulated drugs) and in synthetic mixture with sofosbuvir or ribavirin.

1. Introduction

Direct-acting antivirals (DAAs) represent the beginning of the new era of hepatitis C virus (HCV) treatment [1]. DAAs have emerged at an extremely rapid pace over the past few years [2]. They act directly on the hepatitis C virus at various points in its life cycle to inhibit the viral production [2]. HCV represents a global health problem that affects up to 185 million people worldwide [1]. Chronic hepatitis C infection (CHC) frequently progresses to cirrhosis, end-stage liver disease, hepatocellular carcinoma and death [3]. Over the past decade, the standard of care for treatment of CHC had been a combination of pegylated interferon and ribavirin [4]. DAAs replaced this combination due to its drawbacks including its long course duration, severe side effects, in addition to its high cost which makes it not affordable for many patients in limited-resource countries. DAAs showed high potency, favourable tolerability profile, higher barrier to resistance, pangenotypic coverage, fewer drug interactions, shortened treatment duration and reduced pill burden [5]. In addition, all DAA are orally administered [5]. Thus, there is great analytical significance for determination of these novel hepatitis C antivirals. Among these novel DAAs, are daclatasvir (DCS) and ledipasvir (LDS) [6]. Both DCS and LDS (Fig. 1) are HCV NS5A replication complex inhibitors [7,8]. DCS and LDS were co administered with other drugs such as sofosbuvir (SOF) and/or ribavirin (RBV) [6]. Literature survey reveals that bioanalysis of DCS or LDS with or without other drugs was achieved by chromatographic methods mainly LC–MS technique [9–14]. LC–MS technique is considered as a relatively expensive and requires technical experiences. Biological analysis requires analysis of large number of samples within short time. The target of this article is to develop simple, ultrasensitive and cost-effective method for analysis of the studied drugs in plasma within short time that avoids interference from any endogenous constituent of the plasma. Only one fluorimetric method was reported for determination of LDS [15] which has lower sensitivity than our method and applied on dosage forms only. To best of our knowledge, this is the first spectrofluorimetric method for determination of DCS or LDS in

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https://doi.org/10.1016/j.jpba.2018.01.038
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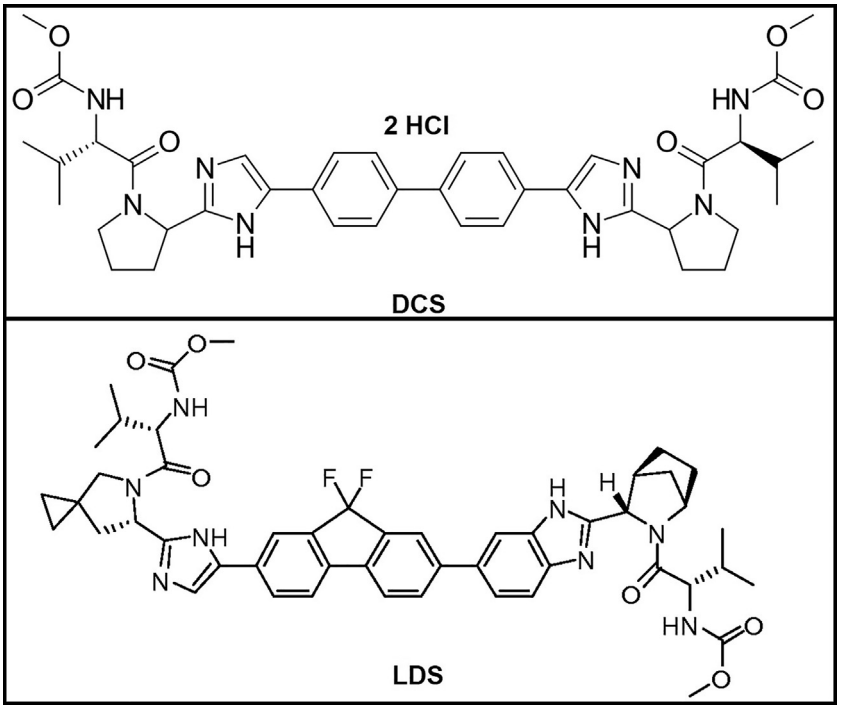

Fig. 1. Chemical structures of the studied compounds.

biological fluids and pharmaceutical formulations. The developed method can represent a good alternative to the already existing LC–MS methods. This method could be applied easily in developing countries where the cost is the main concern. The developed method was also successfully applied for determination of DCS or LDS in simulated synthetic mixture with sofosbuvir or ribavirin and in pharmaceutical formulations. The developed method involved nonextractive simple protein precipitation procedure coupled with ultrasound which characterized by good recovery with no need for sample preconcentration step. This greatly reduced the analysis time. Therefore, the relatively short analysis time as well as the ultrasensitivity and reliable results could contribute to the success of the method when it was applied for the therapeutic drug monitoring of the studied drugs in patients. The method was optimized and validated in accordance with ICH guidelines [16] and US-FDA guidelines [17–19]. This method was in vitro and in vivo applied for the determination of DCS and LDS in human plasma as well as analysis of pharmaceutical dosage forms.

2. Materials and methods

2.1. Apparatus

Spectrofluorophotometer RF-5301 PC (Shimadzu, Tokyo, Japan) was used with the excitation and emission slit control set at slit width 5 nm that equipped with 1 cm quartz cell. Ultrasonic cleaner (Branson ultrasonics corporation, Danbury, USA), pH meter model HI 4222 (Hanna Instruments Brazil, São Paulo, Brazil), sartorius handy balance H51 (Hannover, Germany), laboratory centrifuge Megafuge 11 (Thermo Electron Industries, France) were used. Whatman syringe filter with 0.45 μm pore size (Sigma Aldrich, USA) was used for filtration of plasma samples.

2.2. Chemicals and reagents

All chemicals and solvents were of analytical grade. Daclatasvir dihydrochloride (DCS), ledipasvir (LDS) and sofosbuvir (SOF) were kindly obtained from Mash Premiere for Pharmaceutical Industry (Badr city, Egypt). Ribavirin (RBV) was kindly obtained from Sigma (El-Menoufia, Egypt). Hydrochloric acid, acetic acid 96%, sulphuric acid, perchloric acid and nitric acid were obtained from El-Nasr Chemical Co. (Cairo, Egypt). Orthophosphoric acid 85% (H_3PO_4) was obtained from SDFCL-SD Fine-Chem Limited (Mumbai, India). 1.0 N of each acid solution was prepared in double distilled water. Methanol and acetonitrile were obtained from Fisher Scientific U.K. Limited (United Kingdom). Disodium hydrogen orthophosphate, citric acid, boric acid, sodium hydroxide, sodium dodecyl sulfate (SDS), tween-80, cetrimide, ethanol, isopropanol, acetone and dimethyl formamide (DMF) were obtained from El-Nasr Chemical Co. (Cairo, Egypt). Beta-cyclodextrin (B-CD), pluronic F-68 and pluronic F-127 were obtained from Sigma Aldrich (USA). Brij 58, brij 35 and dimethyl sulfoxide (DMSO) were obtained from Merck (Germany). Hydrochloric acid (0.1–2.0 M), phosphoric acid (0.05–2.0 M), disodium hydrogen orthophosphate (0.1 M), citric acid (0.1 M), sodium hydroxide (0.1 and 1 M) and Teorell and Stenhagen buffer (0.1 M, pH 2.0–12.0) [20] were prepared using double distilled water. Human plasma was kindly provided by Assiut University Hospital, Assiut, Egypt and stored at −20 °C until use after gentle thawing. Pharmaceutical formulations were purchased from the local market; Daklanork® tablets (Batch No. M166215) labeled to contain 60 mg DCS per tablet and Sofolanork Plus® tablets (Batch No. M147916) labeled to contain 400 mg sofosbuvir and 90 mg LDS per tablet (Mash Premiere for Pharmaceutical Industry, Badr city, Egypt).

2.3. Preparation of standard solutions

An accurately weighed amount (10 mg) of DCS or LDS was quantitatively transferred into 100 mL volumetric flasks, dissolved in 40 mL methanol with the aid of an ultrasonic bath and then completed to volume with the same solvent to obtain a stock standard solution of 100 μg mL^{-1} for each drug. The working standard solutions were prepared by further dilutions of suitable volumes of the stock solution with methanol to obtain the concentration ranges