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Rapid and sensitive UHPLC-DAD method for simultaneous determination of sofosbuvir and ledipasvir in human serum

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Highlights

- Simultaneous analysis of Ledipasvir and Sofosbuvir in human serum performed using UPLC-DAD.
- This method is the first report for analysis of the drugs in human serum of healthy volunteers using UPLC-DAD.
- This method is sensitive for pharmacokinetic and clinical trial studies of the drugs when LC-MS MS system is not available.

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Abstract

Today, the direct-acting antiviral agents (DAAs) such as sofosbuvir (SOF) and ledipasvir (LED) are widely used to treat the hepatitis virus infection. The aim of this study was to develop a rapid, simple and valid method for simultaneous determination of SOF and LED in human plasma for bioavailability and pharmacokinetic studies. Chromatographic analysis was performed on the C_{18} column (Blue Orchid, 1.8 μm, 50 × 2 mm) using 0.1 % formic acid in water (pH 2.6) and acetonitrile (60:40; v/v) as mobile phase at a flow rate of 0.5 mL/min. The UV detector was set at 328 nm and 260 nm for analysis of SOF and LED, respectively. To 400 μL of plasma, 100 μL of clonazepam as the internal standard (I.S, 7 μg/mL) was added and the mixture subjected to liquid-liquid extraction using 1000 μL diethyl ether. The calibration curves were linear with coefficients of variation less than 8% for all analyses. The limit of quantification (LOQ) was 20 and 5 ng/mL for SOF and LED, respectively. The results of inter-day and intra-day precision showed good reproducibility and the total analysis time was 1.2 min. This method successfully applied for determination SOF and LED in four healthy volunteers.

Keywords

Hepatitis; Sofosbuvir; Ledipasvir; Ultra-high performance liquid chromatography; Diode array detector

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Introduction

Viral hepatitis is the seventh cause of death in the world. Recently, the use of direct-acting antiviral agents (DAAs) has been recognized as one of the most effective strategies for the treatment of hepatitis C virus (HCV) infection [1,2]. Sofosbuvir (SOF, Fig. 1 a) and Ledipasvir (LED, Fig. 1 b) are nucleotide NS5B and NS5A inhibitors respectively, which are classified in DDAs category. The combination of these two drugs has been approved by the FDA in 2014 for the treatment of, genotypes 1, 4, 5, and 6 chronic HCV infections [3]. Several analytical methods have been published for quantification of the drugs using tine layer chromatography (TLC) [4], high-performance liquid chromatography (HPLC-UV) [[5], [6], [7]], LC–MS/MS [8], UHPLC-MS/MS [9,10], and spectroscopic [[11], [12], [13], [14]] methods. The review of these papers shows that most of these studies have been conducted on non-human serum and pharmaceutical dosage form and/or are time-consuming with low sensitivity. [[5], [6], [7]] and [[11], [12], [13]]. Also, a high sensitive UPLC-MS/MS method was reported for determination SOF and LED and GS-331,007, a SOF metabolite, by Pan et al. at 2016. Their study however, has been performed on the rats with different pharmacokinetic parameters compared to human using the expensive UPLC MS/MS system [10].

The present method is the first report for the simultaneous determination of SOF and LED in human serum by the UHPLC-DAD method. In this study, we designed a rapid, sensitive and low-cost method for determination SOF and LED in human serum. The procedure was successfully applied for determination the drugs in four healthy volunteers.

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Chemicals and standard solutions

SOF and LED (purity 99.5 %) were from Nontongchanyoo pharmatech co. (China) and kindly provided by Bakhtarbioshimi pharmaceutical company (Kermanshah, Iran). The SOF 400 mg + LED 90 mg in tablets dosage forms were manufactured by Gilead Sciences International Ltd Cambridge. Acetonitrile (HPLC grade), formic acid, diethyl ether, and ethanol were obtained from Merck (Darmstadt, Germany). Human drug-

free serum was purchased from healthy volunteers in agreement with local ethics guidelines. Water …

The linearity of the calibration curve and LOQ

The calibration curves were linear over the concentration range of 20–1280 ng/mL for SOF and 5–1280 ng/mL for LED with respective regression equations of y = 0.1383x+3.8164 and y = 0.1145x+0.0074. The correlation coefficients of calibration curves for SOF and LED were obtained 0.9990. The calculated limits of quantification (LOQ) were 20 ng/mL and 5 ng/mL for SOF and LED, respectively. In the present study, the values of relative standard deviation (RSD %) were estimated <8% for SOF and <20 % …

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CRediT authorship contribution statement

Mohammad Bagher Majnooni: Writing - original draft, Data curation. **Seid-Shahram Miraghaee:** Project administration, Resources, Supervision, Data curation. **Samira Keshavarzi:** Formal analysis. **Bahareh Mohammadi:** Software, Formal analysis. **Soraya Sajadimajd:** Investigation, Methodology. **Razieh Hatami:** Visualization. **Gholamreza Bahrami:** Conceptualization, Funding acquisition, Validation, Writing - review & editing. …

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. …

References (16)

E.J. Gane *et al.*
Efficacy of ledipasvir plus sofosbuvir for 8 or 12 weeks in patients with hepatitis C virus genotype 2 infection
Gastroenterology (2017)

O.M. Abdallah *et al.*
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
J. Pharm. Biomed. Anal. (2017)

M.R. Rezk *et al.*
Quantification of sofosbuvir and ledipasvir in human plasma by UPLC–MS/MS method: Application to fasting and fed bioequivalence studies
J. Chromatogr. B (2016)

C. Pan *et al.*
Simultaneous determination of ledipasvir, sofosbuvir and its metabolite in rat plasma by UHPLC–MS/MS and its application to a pharmacokinetic study
J. Chromatogr. B (2016)

M.S. Eissa
Simultaneous determination of the brand new two-drug combination for the treatment of hepatitis C: Sofosbuvir/ledipasvir using smart spectrophotometric methods manipulating ratio spectra
Spectrochim. Acta A. Mol. Biomol. Spectrosc. (2017)

H.I. EL-Shorbagy *et al.*
Earth-friendly spectrophotometric methods for simultaneous determination of ledipasvir and sofosbuvir: application to average content and uniformity of dosage unit testing
Spectrochim. Acta A. Mol. Biomol. Spectrosc. (2018)

F.M. Salama *et al.*
Spectroflurimetric estimation of the new antiviral agent ledipasvir in presence of sofosbuvir
Spectrochim. Acta A. Mol. Biomol. Spectrosc. (2018)

M. Khalili *et al.*
Chemometric simultaneous determination of Sofosbuvir and Ledipasvir in pharmaceutical dosage form
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> *Citation Excerpt:*
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> …This combination was confirmed by the Food and Drug Administration (FDA) in 2014 for the therapy of HCV [10,11]. SOF was reported to be determined in combination with LED or other drugs using reverse phase-high performance liquid chromatography (RP-HPLC) [12,13], ultra-high-performance liquid chromatography (UHPLC) [14], ultra-performance liquid chromatography (UPLC) [15], and thin layer chromatography (TLC) [16]. The limitations of chromatographic techniques include the multi-step sample preparation, time-consuming analysis, expert operators, expensive instrumentation, the high maintenance cost of equipment, lack of a universal detector, and difficulty for novices [17,18].…

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> …Despite the ability of FLD, most drug molecules do not show fluorescence, so derivatization reagents are required for non-fluorescent drugs. In other studies, simultaneous analysis of sofosbuvir and ledipasvir has been performed using RP-HPLC-DAD [61] and UPLC-DAD [67] techniques in human plasma and serum, respectively. In the HPLC-DAD technique, the total analysis time was reported to be 9 min.…

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