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Development and validation of a new HPLC-DAD method for quantification of sofosbuvir in human serum and its comparison with LC–MS/MS technique: Application to a bioequivalence study

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Highlights

- The assay is first report for analysis of Sofosbuvir in human serum using HPLC and conventional UV detector.
- This study was aimed to evaluate applicability of HPLC to quantify sofosbuvir in human serum and its comparison with LC MS.
- The assay applied to quantify sofosbuvir in human serum after single dose oral administration of the drug in normal subjects.

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Abstract

Although for many analyses tandem mass spectrometry (LC–MS/MS) systems have significant advantage over the high-performance liquid chromatography with diode array detection (HPLC-DAD) however, the HPLC methods are easier, cheaper and more available to perform. As no published method is available for quantitative HPLC analysis of sofosbuvir (SOF), an orally administered anti-hepatitis drug in human serum, this study was aimed to evaluate applicability of the HPLC technique to quantify sofosbuvir and comparison of the two methods for analytical performance.

Following extraction of the drug and an internal standard (Hexobarbital), same chromatographic conditions were used for both the systems. After the chromatographic separation on a reverse phase C18 column using a mobile phase consisting of water (containing formic acid 0.5 mL/L) and acetonitrile (57:43; v/v) at a flow rate of 0.8 mL/min, the eluate was introduced into a DAD detector set at 261 nm, then passed through the mass spectrometry system in single ion monitoring mode (SIM).

For UV and mass spectrometry detections the calibration curves were linear over a concentration range of 25–3200 and 10–3200 ng/mL, respectively and the linearity was over 0.998 for both the systems. Lower limit of quantification (LLOQ) for mass spectrometry and DAD detections were 10 and 25 ng/mL, respectively. In conclusion sensitivity of DAD detection is sufficient enough to determine concentrations down to 0.5% of C_{max} which achieved in bioequivalence study of sofosbuvir and meet FDA requirements for these types of studies.

Keywords

Sofosbuvir; HPLC; UV detection; Bioequivalence study

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Introduction

Sofosbuvir (SOF 400 mg; Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate, MW 529.5. Fig. 1a) is a novel once-daily, orally administered anti- hepatitis C virus (HCV) agent [1]. SOF is widely used in combination with other drugs for treatment of the disease with higher cure rate and shorter duration of therapy [2], [3]. The drug is rapidly absorbed after oral administration and its maximum concentrations occur 0.5–2 h after ingestion. Metabolism and excretion of SOF is rapid with median half-life of 0.4 h and its concentrations fall to non- detectable levels in the blood of healthy volunteers by 4–5 h after single dose administration [4].

There are a few reports to quantify SOF in the literature. The current method of choice for analysis of SOF in biological matrix is UPLC–MS-MS [5], [6], [7], [8] and LC–MS-MS [9], [10] techniques. Using these methods, measurement of the drug with [5] or without [6], [7], [8], [9], [10] simultaneous analysis of its main inactive metabolite in human [5], [6], [9] or rat serum [7], [8] as well as its phosphorylated intracellular metabolites [10] have been reported. Performance data in the previously published methods have been compared in Table 1.

In spite of potential downsides of a non-MS based method, such as lower sample throughput and less assay selectivity, using of HPLC techniques in quantitative drug analysis are easier, cheaper and more available to perform. To our knowledge no published method is available for analysis of the drug in human serum using HPLC and conventional UV detector. This study was aimed to develop a reliable analytical method for determination of SOF in human serum using HPLC technique. Also applicability and efficiency of the new method was evaluated and compared with LC–MS-MS in a bioequivalence study following single dose oral administration of two different SOF preparations in twenty four healthy volunteers.

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Chemicals

SOF (purity 99.5%) was from Nontong chanyoo pharmatech co. (China) and kindly provided by Bakhtar Bioshimi pharmaceutical company (Kermanshah, Iran). Hexobarbital (Fig. 1b) was from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade), formic acid and diethyl ether were purchased from Merck (Darmstadt, Germany). Human drug-free serum was obtained from healthy volunteers in agreement with local ethics guidelines. Water was glass-double distilled and further purified with a Maxima purification …

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To compare UV and mass spectrometry detections, components in mobile phase, sample preparation technique and properties of I.S. should meet requirements suitability for both the system; namely resolution, UV absorbance and ionization. A large number of chemicals were tested and hexobarbital was selected as I.S. due to its appropriate retention time and ESI ionization properties. Different mobile phase systems including acetonitrile–water and methanol–water in various proportions were tested. …

Conclusion

In conclusion, a new, first report of specific method has been described for the determination of SOF in human serum by high performance liquid chromatography using UV detection. This method which has demonstrated to be suitable for its use in pharmacokinetic studies is sensitive adequate to measure SOF concentration up to 3.5h post dose and to provide a reliable estimate of the extent of exposure in single dose bioequivalence studies of the drug. …

Acknowledgments

This work was supported by Bakhtar Bioshimi Pharmaceutical Company and in part by Kermanshah University of Medical Sciences. …

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C. Pan *et al.*
Simultaneous determination of ledipasvir, sofosbuvir and its metabolite in rat plasma by UPLC–MS/MS and its application to a pharmacokinetic study
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Iranpanah A., ..., Farzaei M.H.

Journal of Ethnopharmacology • Volume 323 • 2024 • Article 117708

> *Citation Excerpt:*
>
> …The drying gas was heated to 300 °C and supplied at a 10 L/min flow rate. Fragmentation voltage and collision energy were set to 135 V and 25, respectively, with a dwell time of 200 ms for all analytes (Miraghaei et al., 2017). The m/z of the parent ions and their correlation with the m/z of the obtained fragments (their daughter ions), were used to investigate the mass spectrum results.…

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Development of a deep eutectic solvent-based ultrasound-assisted homogenous liquid-liquid microextraction method for simultaneous extraction of daclatasvir and sofosbuvir from urine samples

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Journal of Pharmaceutical and Biomedical Analysis • Volume 204 • 2021 • Article 114254

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Improved spectral resolution for the rapid simultaneous spectrophotometric determination of sofosbuvir and daclatasvir as anti hepatitis C virus drugs in pharmaceutical formulation and biological fluid using continuous wavelet and derivative transform

Keyvan K., ..., Motiee F.

Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy • Volume 251 • 2021 • Article 119429

> *Citation Excerpt:*
>
> …Good tolerance and safety, as well as greater effect, are their advantages compared with the older treatments [1,4]. SOF, Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl] methoxy-phenoxy phosphoryl] amino] propanoate (Fig. 1a) with molecular weight of 529.5 g/moL is an anti HCV agent and novel drug, which administered orally [5]. It is recommended in combination with other drugs such as peginterferon-alfa, DAC, LED, and SMV [6].…

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Computational design of molecularly imprinted polymer for electrochemical sensing and stability indicating study of sofosbuvir

Abdel-Hamied Abdel-Tawab M.A.H.A., ..., Nashar R.M.

Microchemical Journal • Volume 158 • 2020 • Article 105180

> *Citation Excerpt:*
>
> …Moreover, scientists have merged between RGO and MWCNTs for a synergistic effect on sensors' performance [16]. A variety of methods have been reported in literature for analysis of SOFO including high-performance liquid chromatography (HPLC) [12,18–21], thin layer chromatography (TLC) [22], ultra-performance liquid chromatography/mass spectrometry (UPLC-MS/MS) [23] and spectrophotometry [24]. Though these methods are of high accuracy, yet most of them require many complicated extraction procedures besides having a high rate of solvent consumption and requiring highly expensive instrumentation.…

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Optimization and modeling of a green dual detected RP-HPLC method by UV and fluorescence detectors using two level full factorial design for simultaneous determination of sofosbuvir and ledipasvir: Application to average content and uniformity of dosage unit testing

EL-Shorbagy H.I., ..., El-Brashy A.M.

Microchemical Journal • Volume 147 • 2019

> *Citation Excerpt:*
>
> …SOF in combination with compounds other than LED was determined by UPLC [7,8], RP-HPLC [9–14], and spectrophotometric methods [15,16]. Determination of SOF individually was reported using UPLC [17], RP-HPLC [18–23], TLC [20,24], capillary zone electrophoresis [20], and spectrophotometric methods [19,20,25]. Methods for determination of LED individually include RP-HPLC [26] and spectrofluorimetric methods [27].…

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Efficient HPTLC-dual wavelength spectrodensitometric method for simultaneous determination of sofosbuvir and daclatasvir: Biological and pharmaceutical analysis

Abo-Zeid M.N., ..., El-Shaboury S.R.

Journal of Pharmaceutical and Biomedical Analysis • Volume 156 • 2018

> *Citation Excerpt:*
>
> …This is the first HPTLC method for determination of DCS either in pure form or in binary mixture with SOF in miscellaneous matrices. The reported methods for real biological analysis of SOF and/or DCS were spectrofluorimetry [8] and chromatographic methods [9–16] especially LC–MS/MS methods. Only two methods were reported for simultaneous determination of SOF and DCS in human plasma which were LC–MS methods [12,16]….

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