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Original Research Article

Potential of RP-UHPLC-DAD-MS for the qualitative and quantitative analysis of sofosbuvir in film coated tablets and profiling degradants

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ABSTRACT

Sofosbuvir is one of the new direct-acting antiviral drugs against hepatitis C virus (HCV) infection. This drug has recently been launched into the market, and generic versions of the medication are expected to be produced by local drug producers in some countries. Therefore, new methods are required to control sofosbuvir in pharmaceuticals. In the present study, a new method based on reversed phase (RP)-ultra-high performance liquid chromatography (UHPLC) coupled to diode array detection (DAD) and mass spectrometry (MS) was developed to facilitate the qualitative and quantitative analysis of sofosbuvir in film coated tablets. A wavelength of 260 nm was selected to perform a cost-effective quantification and the method showed adequate linearity, with an R^2 value of 0.9998, and acceptable values of accuracy (75%–102%) and precision (residual standard deviation < 5%). The detection and quantification limits were 0.07 μg/mL and 0.36 μg/mL, respectively. Furthermore, the use of high-resolution MS enabled us to ensure the specificity, check impurities and better sensitivity. Therefore, this methodology promises to be suitable not only for the routine analysis of sofosbuvir in pharmaceutical dosage forms, but also for potential degradants.

1. Introduction

Hepatitis C virus (HCV) can cause both acute and chronic hepatitis infection. It is a major public health concern since approximately 150 million people have chronic hepatitis C infection worldwide and 500,000 people die annually from hepatitis C-related liver diseases [1]. In this context, several nucleosides are investigated as potential inhibitors of HCV [2]. This class includes new direct acting antiviral drugs, such as sofosbuvir, that can cure hepatitis C infection with minimal side effects in comparison with interferon and ribavirin [3], but access to diagnosis and treatment is very limited and expensive [1,4] depending on the country. Sofosbuvir (CAS no 1190307-88-0; GS-7977) has recently been discovered [2] and proved to be effective against HCV 1, 2, 3, 4 and 6 genotypes [5]. This compound shares a similar structure to the nucleotide uridine monophospate and its mechanism of action is based on the inhibition of HCV NS5B RNA polymerase enzyme, which is essential for virus multiplication [2,5]. Moreover, sofosbuvir can be administered once daily, which is associated with better compliance by patients compared to other drugs [5].

Liquid chromatography (LC) coupled to UV detection or mass spectrometry (MS) has been applied to determine oral anti-HCV drugs in pharmaceutical preparations [6], biological samples [7–10] or even in wastewater [11]. In these studies, reversed phase (RP) liquid chromatography using silica-based C_{18} columns is the most common mode. However, modified ones or porous graphitic carbon stationary phase can be used to cover the analysis of hydrophilic anti-HCV drugs as ribavirin and highly lipophilic ones such as telaprevir [7,11]. The column particle size ranges between 1.7 and 5 μm, and isocratic or gradient modes are employed depending on the purpose and analyte. Moreover, in MS applications, electrospray ionization (ESI) is used in the positive ionization mode. In addition to these studies, pharmaceutical approaches are also addressed to identify impurities and degradation products [12], MS being the most versatile detector.

In this context, it is expected that generic drugs containing sofosbuvir will be produced and launched to the international market, reinforcing the necessity of valid and robust analytical methods for monitoring sofosbuvir, its impurities and degradants in final pharmaceutical products. Therefore, in the present study a fast and simple RP-

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UHPLC-UV methodology for the determination of sofosbuvir in film coated tablets was developed and validated. This study was also supported by the use of MS to perform the qualitative analysis of this compound in both positive and negative ionization modes, ensure the specificity and check impurities or degradants that may be helpful for further MS-based studies on sofosbuvir.

2. Material and methods

2.1. Chemicals

Acetonitrile (LC-MS grade) and water (LC-MS grade) and methanol (analytical grade) were provided by Fisher Chemicals (Loughborough, Leics, UK). Formic acid (purity > 98%) was purchased from Sigma-Aldrich (Steinheim, Germany). Sofosbuvir commercial pattern with a purity of 99% was obtained from Alsachim (Strasbourg, France). Film coated tablets (Sofolanork) were donated by Medical Cables (Málaga).

2.2. Tablets pretreatment

Tablets were weighed and ground to obtain a fine powder. Subsequently, a suspension of 0.5 mg/mL was prepared with a solution of methanol:water (80:20, v/v). The mixture was homogenized for 1 min, sonicated for 15 min, and filtered using syringe filters of regenerated cellulose (25 mm, 0.45 μm pore size). Prior to analysis, the samples were appropriately diluted in methanol:water (80:20, v/v) and centrifuged at 2400*g* for 2 min to avoid any solid particle that could cause problems during analysis. Finally, for determining the content of sofosbuvir, the method was applied to six tablets and two replicates were performed per tablet.

Recovery was estimated by spiking tablet samples with the commercial standard (300 μL of a methanolic solution 0.5 mg/mL) and following the procedure previously described. The amount of sofosbuvir estimated in the spiked samples was compared with the theoretical amount and the result was expressed as % [13]. This experiment was performed in triplicate.

2.3. Preparation of the calibration standards

An initial concentration of sofosbuvir (commercial standard) of 0.5 mg/mL was prepared in methanol:water (80:20, v/v) and proceeded analogously to what is described in Section 2.2. Afterwards, it was appropriately diluted using methanol: water (80:20, v/v) to obtain different calibration points in a concentration ranging from 0.003 to 250 μg/mL. Prior to analysis, these solutions were centrifuged at 2400*g* for 2 min. Moreover, quality control was prepared at three levels, namely, 4 μg/mL (low), 31 μg/mL (intermediate) and 250 μg/mL (high).

2.4. Instrumentation

Analyses were made with an Agilent 1200 series rapid resolution (Palo Alto, CA, USA) equipped with a binary pump, an autosampler and a diode array detector (DAD). The system was coupled to a 6540 Agilent Ultra-High-Definition (UHD) Accurate-Mass quadrupole-time-of-fligth (QTOF) mass spectrometer, which was equipped with an Agilent Dual Jet Stream (Dual AJS) electrospray ionization (ESI) interface. The mobile phases consisted of water with 0.2% formic acid (mobile phase A) and acetonitrile (mobile phase B). A multistep linear gradient was then applied: 0–1 min, 0%–47.5% B; 1–6 min, 47.5%–95% B; 6–6.5 min, 95% B; 6.5–7 min, 95%–0% B. The latter value (100% A and 0% B) was held for 3 min to equilibrate the column with the initial conditions prior to the next injection. The flow rate was set at 0.5 mL/min throughout the gradient. Separation was carried out with a Zorbax Eclipse XDB-C_{18} column (4.6 mm×50 mm, 1.8 μm of particle size) (Agilent) at 24 °C (column temperature). The injection volume was 2 μL. The absorbance was monitored between 190 and 600 nm, and a wavelength channel of 260 ± 4 nm was applied for quantitative purposes.

The operating conditions in negative and positive ionization modes were as follows: gas temperature, 325 °C; drying gas, nitrogen at 10 L/min; nebulizer pressure, 20 psig; sheath gas temperature, 400 °C; sheath gas flow, nitrogen at 12 L/min; capillary voltage, 4000 V; skimmer, 45 V; octapole radiofrequency voltage, 750 V; focusing voltage, 500 V, with the corresponding polarity automatically set. Spectra were acquired over a mass range from *m/z* 50 to 1700. Reference mass correction of each sample was performed with a continuous infusion of Agilent TOF mixture containing two mass references for each ionization mode. The detection window was set to 100 ppm. Data acquisition (2.5 Hz) in the profile mode was governed *via* the Agilent MassHunter Workstation B.05.01.

The acquisition program was B.05.01 Agilent MassHunter Workstation and spectral data were processed with B.06.00 Agilent MassHunter Qualitative Analysis program. For characterization, the isotope model selected was common organic molecules with a peak spacing tolerance of *m/z* 0.0025 and 7 ppm. The molecular formulae were obtained with an error of less than 5 ppm and with an MS score ≥90, which is related to mass accuracy, isotopic distribution spacing and isotopic abundances in MS mode.

2.5. Validation of the analytical method

The validation of the quantitative analysis was carried out according to the guidelines of the European Medicines Agency (EMEA) CPMP/ICH/381/95 [14]. The calibration curve was plotted by determining the linear relationship between the standards concentration (calibration standards) *vs.* the chromatographic peak area at 260 nm (RP-UHPLC-UV). Alternatively, another calibration curve was constructed using peak area of the extracted ion chromatogram (EIC) of sofosbuvir at *m/z* 530.1698 (RP-UHPLC-ESI-QTOF-MS). Three replicates at the aforementioned concentrations were studied to build the curves. The estimation of the regression parameters was done by the ordinary least squares method. The assumption of linear regression was checked by ANOVA using Statgraphics Plus version 5.0 (StatPoint Technologies, Warrenton, VA, USA).

Precision was estimated by the relative standard deviation (RSD) of sofosbuvir peak area for three consecutive analyses performed on the same day (intraday repeatability) and six analyses (interday repeatability) on six different days. For the RP-UHPLC-UV method, standard solutions used as quality control were injected at three levels: low (4 μg/mL), intermediate (31 μg/mL) and, high (250 μg/mL). In the case of the UHPLC-ESI-QTOF-MS method, the concentrations analyzed to estimate the precision were 0.003 (low), 0.5 (intermediate) and 4 μg/mL (high).

Accuracy was determined in the quality control as well as in the presence of tablet matrix. For this, given that control film coated tables with the same composition but without the analyte are not available, sofosbuvir standard was added to a sample prepared as described in Section 2.2 and at three levels: 25, 50 and 125 μg/mL. The accuracy (%) was expressed as the ratio of the calculated concentration over the known concentration of sofosbuvir. All samples were analyzed in triplicate.

The limits of detection (LOD) and quantification (LOQ) were based on the signal-to-noise ratio (S/N) of sofosbuvir determined with the program MassHunter Qualitative Analysis B.06.00, and subsequent extrapolation to S/N =2 and S/N =10, respectively. Finally, these limits were confirmed by an independent analysis of standard solutions with a concentration of sofosbuvir close to the LOD and LOQ.

Finally, the robustness of the quantitative method was checked by testing the influence of varying the wavelength at 245 and 275 nm and analyzing the quality control (n=3). The results are expressed as the value of RSD (%) for peak response.

Table 1
Recovery and validation parameters of the RP-UHPLC-UV and RP-UHPLC-ESI-QTOF-MS: linearity, limits of detection (LOD) and quantification (LOQ).

Parameters	RP-UHPLC-UV	RP-UHPLC-QTOF-MS
a[a]	39.47 ± 9.87	2,381,000 ± 44,210
b[a]	26.05 ± 0.09	−77070 ± 74,650
n[b]	7	11
R^2[b]	0.9998	0.995
P value[b]	< 0.0001	< 0.0001
$S_{y/x}$[b]	34.38	217,300
CV[b] (%)	1.8	8.1
Concentration range (μg/mL)	4–250	0.003–4
LOD (μg/mL)	0.07	0.0004
LOQ (μg/mL)	0.36	0.002
Intraday repeatibility (RSD,%)[c]	0.4	7.0
Interday repeatibility (RSD,%)[c]	2.2	12.4
Accuracy (%)[c]	92.6	90.9
Recovery (%)	101 ± 8	
Sofosbuvir content (mg per tablet)	372 ± 7	
Experimental mass of the tablets (mg)	1184 ± 31	

[a] All regression coefficients (a, b) presented are significantly different from zero ($P < 0.05$).
[b] n, number of points; $S_{y/x}$, residual standard deviation; CV (%)=($S_{y/x}/\bar{y}$)×100; R^2, determination coefficient; *P*-value of ANOVA test for the regression model ($y = a + bx$).
[c] Mean value of three concentration levels, i.e. low, intermediate and high concentration value of the linear calibration range for each method.

3. Results and discussion

3.1. Optimization of the analytical methodology

A simple solid-liquid extraction protocol was performed to determine sofosbuvir in film coated tablets. For this purpose, different water and methanol combinations as extractants were tested, as well as physical methods to facilitate the extraction (sonication, centrifugation, and filtering). Finally, methanol:water in a proportion of 80:20 (v/v) was chosen; the suspension was sonicated and filtered. The extraction efficiency was determined as percent recovery, obtaining a mean value of 101% (Table 1). Thereby, the pretreatment proved to be suitable since no loss of the compound was observed.

The analytical conditions were preliminarily checked using RP-UHPLC-DAD-MS (with a C_{18} column) and ESI in both positive and negative ionization modes. In general, an adequate ionization was achieved using water with 0.2% formic acid and acetonitrile as mobile phases. A gradient mode was optimized in order to separate putative matrix interfering compounds, impurities or degradants. The selected analytical conditions produced a final backpressure lower than 120 bars. Consequently, it can be reproduced in a conventional HPLC system, which is common in pharmaceutical laboratories due to the overall costs involved in the analysis [15].

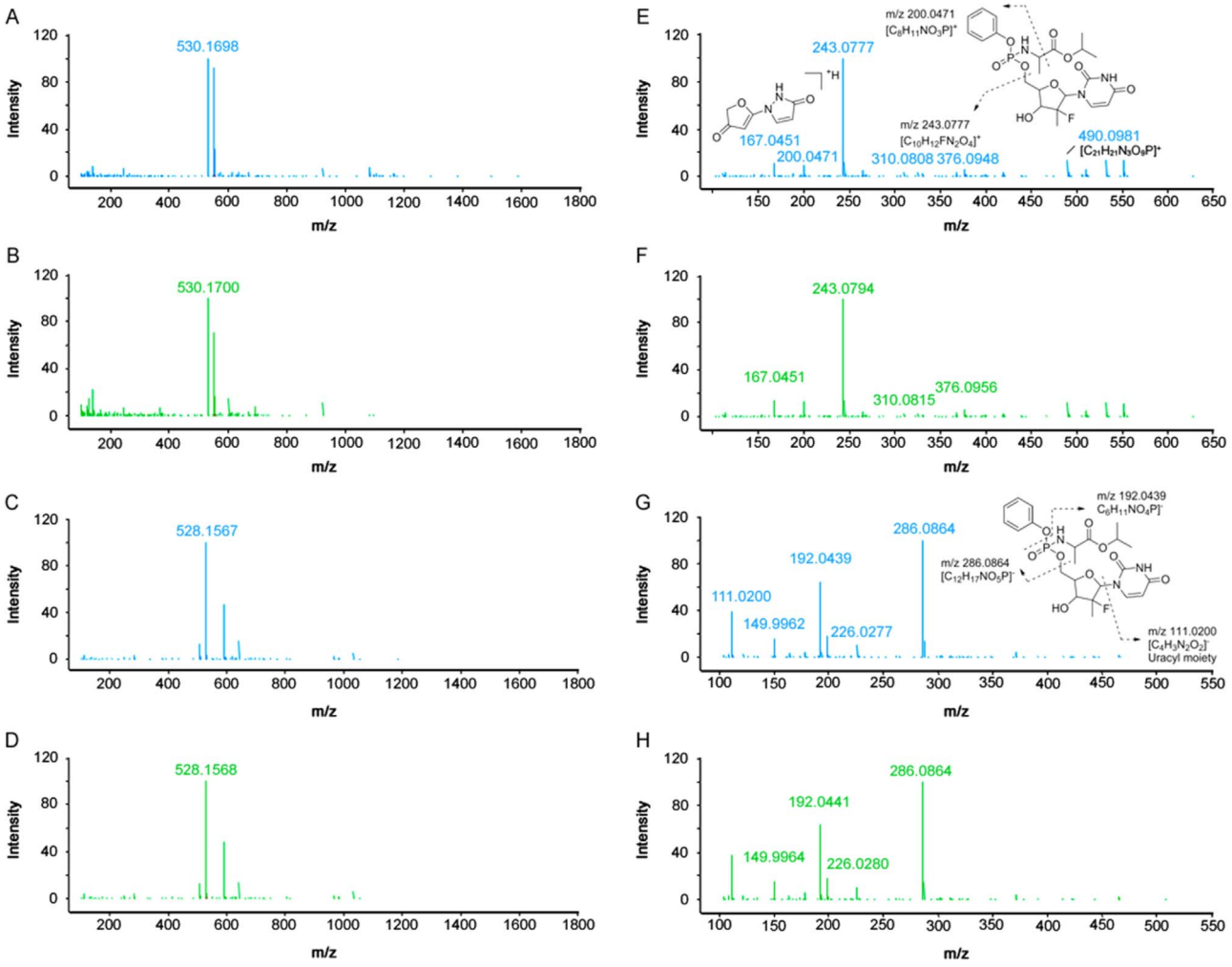

Fig. 1. Mass spectra and tandem mass spectra of sofosbuvir in a pharmaceutical tablet in the positive (A and E) and negative ionization modes (C and G) and commercial standard (125 mg/mL) in the positive (B and F) and negative ionization modes (D and H), respectively.

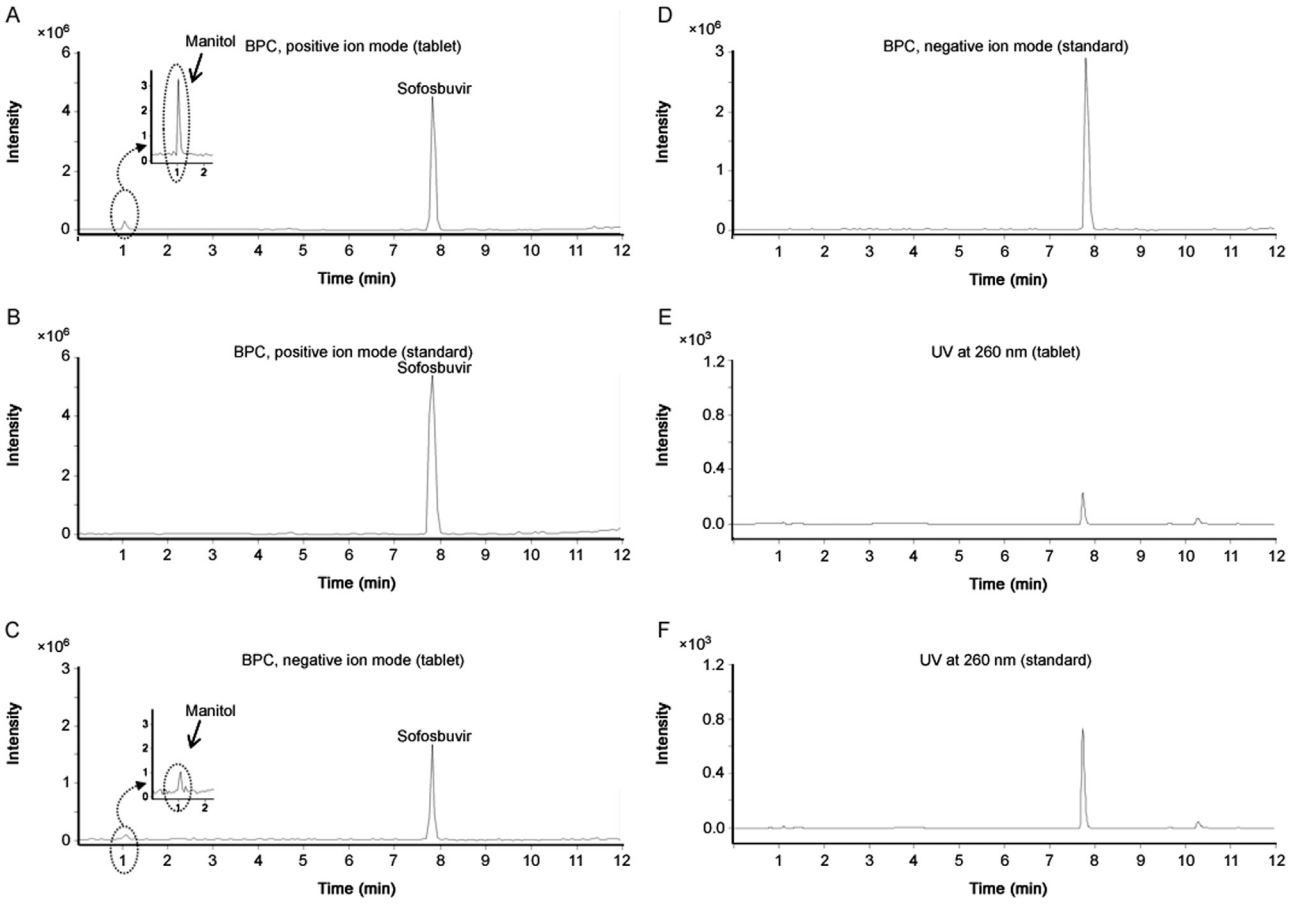

Fig. 2. Base peak chromatograms (BPC) obtained in positive and negative ionization modes and UV at 260 nm: sofosbuvir in a pharmaceutical tablet (A, C and E) and commercial standard (B, D and F).

Table 2
Precision and accuracy of the RP-UHPLC-UV method for the quantitative estimation of sofosbuvir.

Concentration (μg/mL)	Intraday repeatibility (RSD, %)		Interday repeatibility (RSD, %)		Accuracy (%)	
	Peak area	RT	Peak area	RT	Intraday	Interday
250	0.3	0.1	4.5	0.3	99.4	101
31	0.5	0.1	1.3	0.3	102	101
4	0.4	0.1	0.9	0.1	76	75

3.2. Qualitative analysis of sofosbuvir in film coated tablets and profiling degradants

On the one hand, the qualitative composition of pharmaceutical tablets was determined by RP-UHPLC-DAD-MS and -MS/MS obtaining the characteristic UV-Vis absorption (190–600 nm), MS and MS/MS spectra. As an example, Fig. S1 shows the characteristic absorption region of sofosbuvir, showing a maximum at 260 nm. Fig. 1 depicts the mass spectra with the characteristic molecular ions of sofosbuvir in a pharmaceutical tablet, and the commercial standard. Mass errors were less than 5 ppm, considering the molecular formula of sofosbuvir ($C_{22}H_{29}FN_3O_9P$) and the theoretical *m/z* values of the monoisotopic ion: 530.1698 ($[M+H]^+$) and 528.1553 ($[M-H]^-$). Additionally, MS/MS experiments (Fig. 1E-H) in both ionization modes enable us to obtain the fragmentation pattern of sofosbuvir. The product ions generated in MS/MS experiments depended on the ionization mode. For example, the main product ion was at *m/z* 243.0777 ($[C_{10}H_{12}FN_2O_4]^+$) in the positive ionization mode, whereas the counterpart was found at *m/z* 286.0864 ($[C_{12}H_{17}NO5P]^-$) in the negative ionization mode. These data could be useful for further MS-based analysis in order to enhance selectivity and sensitivity when more complex biological samples are analyzed. In this sense, sofosbuvir is typically analyzed in plasma using ESI in the positive ion mode [8–10] although the negative ionization mode also seems a potential alternative.

Overall, the aforementioned results indicate that the chemical structures of the compound in the pharmaceutical product and the commercial standard were the same and corresponded to the chemical structure of sofosbuvir. Besides sofosbuvir, mannitol (sweetener or diluent excipient) was also detected in the pharmaceutical samples under our analytical conditions: at *m/z* 205.0688 ($[M+Na]^+$) and 181.0727 ($[M-H]^-$) (Fig. 2).

Finally, to evaluate the potential of the methodology, a mass spectrometry-based targeted analysis was performed to look for sofosbuvir degradants in pharmaceutical samples at trace levels, which may be under LOD when using UV detection. Normally, these chemical structures are generated after forced degradation works, but not exhaustively studied in final commercial drugs. As an example, seven sofosbuvir degradants previously described by Swain and co-workers [16] after forced degradation of sofosbuvir *via* hydrolysis and oxidation were selected for a targeted study here in the positive and negative ionization modes: $C_{10}H_{14}FN_2O_8P$ (GS-606965) (at *m/z* 341.0545 and 339.0399), $C_{13}H_{19}FN_3O_9P$ (GS-566500) (at *m/z* 412.0916 and 410.0770), $C_{19}H_{23}FN_3O_9P$ (at *m/z* 488.1229 and 486.1083), $C_{16}H_{18}FN_2O_8P$ (at *m/z* 417.0858 and 415.0712), $C_{22}H_{28}N_3O_9P$ (at *m/z* 510.1636 and 508.1490), $C_{16}H_{25}FN_3O_9P$ (at *m/z* 454.1385 and 452.1240), and $C_{22}H_{29}FN_3O_{10}P$ (at *m/z* 546.1647 and 544.1502). Sofosbuvir is quite stable [17], but a potential oxidation product $C_{22}H_{29}FN_3O_{10}P$ could be detected using the positive (as an example, Fig. S2) and negative ionization modes.

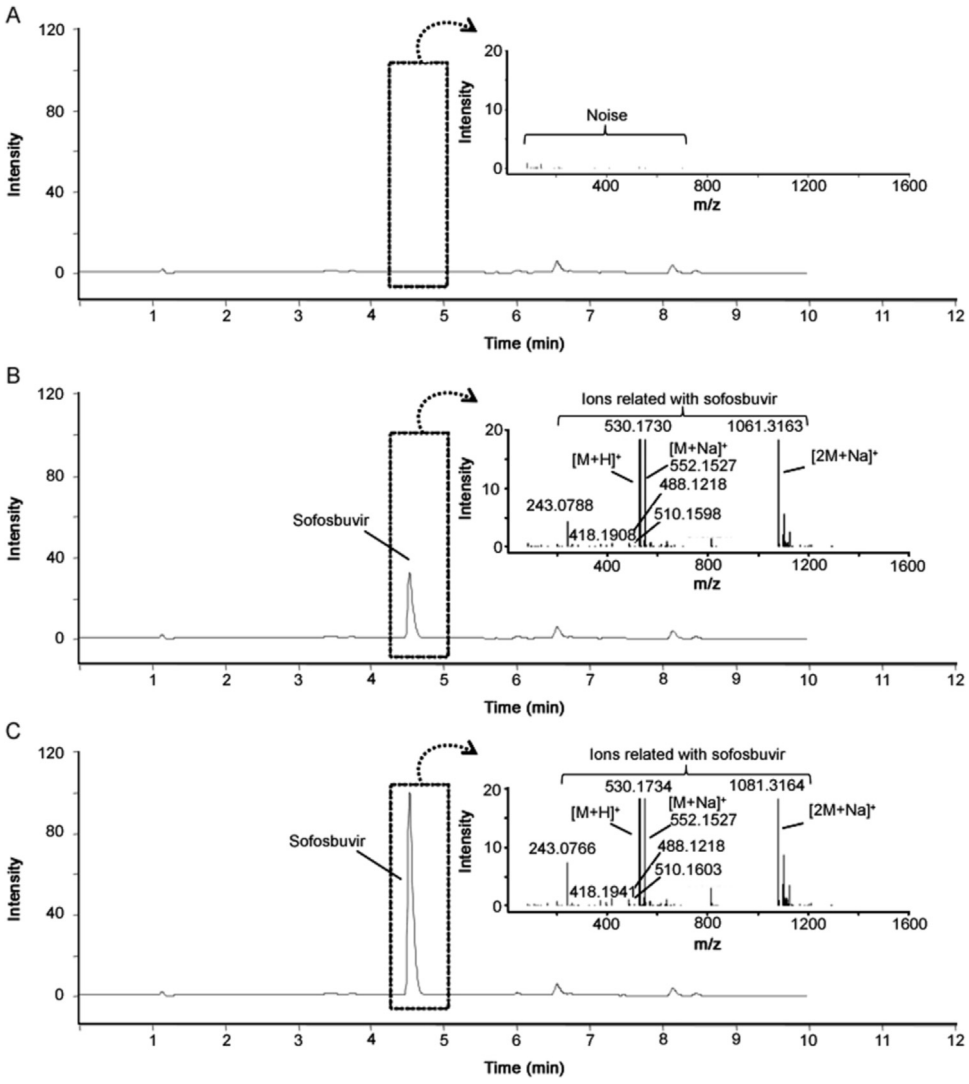

Fig. 3. Chromatograms at λ=260 nm of (A) blank, (B) pharmaceutical tablet, and (C) commercial standard (125 μg/mL) obtained with the optimized analytical method. Inset: the mass spectra at the elution time of sofosbuvir highlighting minor ions.

Table 3
Robustness of the RP-UHPLC-UV method tested at different wavelengths.

Concentration (μg/mL)	245 nm (RSD, %)	275 nm (RSD, %)
250	0.2	0.3
31	0.1	1.6
4	1.6	0.6

The use of a QTOF mass spectrometer enables to perform pseudo-selected ion monitoring, selected reaction monitoring or multiple reaction monitoring using the EIC mode, with higher selectivity and sensitivity than other mass analyzers [18]. It also provides a high confidence level for structure confirmation by generating the molecular formula and obtaining fragmentation patterns when possible. In our case, the potential detection of degradants and its differentiation from positive false responses was possible: the narrower the mass window is, the higher the selectivity you obtain. At this point, the oxidation product of sofosbuvir was tentatively confirmed by generating the molecule formulae with mass error lower than ± 1 mDa, but the fragmentation pattern could not be measured due to its low abundance. These results are preliminary, but could be useful for quality control assays and further toxicocological studies.

3.3. Assessment of quantitative methods for the determination of sofosbuvir

In order to facilitate a routine and cost-effective analysis of the active pharmaceutical ingredient sofosbuvir in film coated tablets, a wavelength of 260 nm (absorption maximum, Fig. S1) was selected to perform the quantification of sofosbuvir. In these analytical conditions, the peak shape of sofosbuvir was adequate (Fig. 2), and the tailing factor was always lower than 2. The validation parameters are shown in Tables 1 and 2. The value for the coefficient of determination (R^2) was 0.9998 and the regression was significant ($P < 0.05$), indicating that the fit was adequate in the proposed concentration range. In addition, the residual standard deviation expressed as CV was 1.8%. The LOD and the LOQ values were 0.07 and 0.36 μg/mL, respectively. Regarding precision, the RSD values for the peak area were lower than 1.5%, with the exception of the intermediate precision at the highest level (4.5%). In the case of the retention time, the RSD was lower than 0.5%. The accuracy in the quality control and in the presence of the tablet matrix was generally close to 100% (Tables 2 and S1), except for the lowest concentration. The selectivity of the methodology was also appropriate without interfering matrix substances or impurities co-eluted with sofosbuvir (Fig. 3).

Moreover, the robustness of this method was performed by testing the influence of varying the wavelength used for detection and covering

the range described in previous studies [16,19,20]. The value of RSD (%) for peak response was lower than 2% (Table 3).

The RP-UHPLC-UV method was compared with RP-UHPLC-QTOF-MS in terms of precision, accuracy and sensitivity since both detection techniques can be coupled online. Using the proposed analytical conditions, the LOD and LOQ could be diminished using MS spectrometry in the positive ionization mode (200 times lower, approximately). Alternatively, the worst precision values were achieved (7%–12.4%) (Table 1). Thus, since concentration of sofosbuvir is not a critical problem for our purpose, we selected RP-UHPLC-UV for quantification of sofosbuvir since this method achieved better precision and accuracy results, besides presenting lower purchasing and maintenance costs for routine determinations. However, the alternative method proposed here, RP-UHPLC-QTOF-MS, can be useful for other applications, in which a higher sensitivity is desired.

In comparison with other LC methods based on UV absorption to quantify HCV anti-viral drugs, this methodology showed lower LOD and LOQ values [6,16,19,20] and a similar precision to that in bulk or plasma [6,16,19–21]. In these studies, C_{18} columns preferably used are 150–250 mm in length, 4.6 mm of inner diameter and 3.5–5 μm of particle size [6,16,20], whereas a C_8 column (250 mm×4.6 mm, 5 μm) has also recently been applied [20] to separate sofosbuvir. UV wavelengths for quantification were 245, 254, and 260 nm. When comparing with MS detection, the sensitivity in terms of LOQ obtained using RP-UHPLC-UV is lower than that reported by other authors (0.5–10 ng/mL) [8–10], but this method is more precise. Thus, MS-based methods are useful for the determination of sofosbuvir in biological samples due to their higher sensitivity, which agreed with our results.

3.4. Quantification of sofosbuvir in film coated tablets

The methodology was applied to the assay of a pharmaceutical formulation containing 400 mg of sofosbuvir in each tablet. The experimental mass of the tablets was 1184 ± 31 mg (error −1.4%) (Table 1). The estimated amount of sofosbuvir with the selected method was 372 mg (93% ± 4%), which is in agreement with the declared content. This value was also comparable to that certified by the official Egyptian Organization for Drug Control and Research (95%).

4. Conclusions

The methodology based on RP-UHPLC-UV showed adequate recovery, precision, accuracy, specificity and selectivity for the determination of sofosbuvir in tablet dosage form. Its chromatographic runtime allows the analysis of a large number of samples in short period of time and the possibility to analyze sofosbuvir in combination with other HCV anti-virals. The use of a high resolution mass analyzer widens the range of possibilities to detect degradants at trace levels with a high degree of reliability and lower detection limits than UV. Therefore, the methodology proposed here is useful for a routine quality control of sofosbuvir in pharmaceutical preparations, and it promises to be suitable for clinical and toxicocological applications.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpha.2017.04.003.

References

[1] World Health Organization (WHO), Hepatitis C - Fact sheet No 164, 2015.
[2] M.J. Sofia, D. Bao, W. Chang, et al., Discovery of a β-D-2'-deoxy-2'-α-fluoro-2'-β-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus, J. Med. Chem. 53 (2010) 7202–7218.
[3] N. Afdhal, S. Zeuzem, P. Kwo, et al., Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection, N. Engl. J. Med. 370 (2014) 1889–1898.
[4] S. Tome, E. Otero, Access to new direct-acting antiviral agents against HCV infection: a view from Spain, Clin. Liver Dis. 5 (2015) 22–23.
[5] I. Gentile, F. Borgia, A.R. Buonomo, et al., A novel promising therapeutic option against hepatitis C virus: an oral nucleotide NS5B polymerase inhibitor sofosbuvir, Curr. Med. Chem. 20 (2013) 3733–3742.
[6] B. Lakshmi, K. Saraswathi, T. Reddy, RP-HPLC method development and validation for the analyisis of telaprevir in pharmaceutical dosage forms, Int. J. Res. Pharm. Biomed. Sci. 3 (2012) 1483–1487.
[7] M. Aouri, D. Moradpour, M. Cavassini, et al., Multiplex liquid chromatography-tandem mass spectrometry assay for simultaneous therapeutic drug monitoring of ribavirin, boceprevir, and telaprevir, Antimicrob. Agents Chemother. 57 (2013) 3147–3158.
[8] M.R. Rezk, E.B. Basalious, I.A. Karim, Development of a sensitive UPLC-ESI-MS/MS method for quantification of sofosbuvir and its metabolite, GS-331007, in human plasma: application to a bioequivalence study, J. Pharm. Biomed. Anal. 114 (2015) 97–104.
[9] X. Shi, D. Zhu, J. Lou, et al., Evaluation of a rapid method for the simultaneous quantification of ribavirin, sofosbuvir and its metabolite in rat plasma by UPLC–MS/MS, J. Chromatogr. B 1002 (2015) 353–357.
[10] L. Qu, W. Wang, D. Zeng, et al., Quantitative performance of online SPE-LC coupled to Q-Exactive for the analysis of sofosbuvir in human plasma, RSC Adv. 5 (2015) 98269–98277.
[11] C. Prasse, M.P. Schlüsener, R. Schulz, et al., Antiviral drugs in wastewater and surface waters: a new pharmaceutical class of environmental relevance?, Environ. Sci. Technol. 44 (2010) 1728–1735.
[12] R. Ravali, M. Phaneendra, J.K. Bhanu, et al., Recent trends in analytical techniques for the development of pharmaceutical drugs, J. Bioanal. Biomed. S11 (2011) 1–7.
[13] M. Gupta, H.N. Bhargava, Development and validation of a high-performance liquid chromatographic method for the analysis of budesonide, J. Pharm. Biomed. Anal. 40 (2006) 423–428.
[14] European Medicines Agency (EMEA), Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. CPMP/ICH/381/95 - ICH Q2 (R1), EMEA, 2006: 1–15.
[15] Z. Li, High-throughput analysis in support of process chemistry and formulation research and development in the pharmaceutical industry, P.G. Wang (Ed.), High-Throughput Analysis in the Pharmaceutical Industry, CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, 2009: 247–278.
[16] D. Swain, G. Samanthula, S. Bhagat, et al., Characterization of forced degradation products and in silico toxicity prediction of Sofosbuvir: a novel HCV NS5B polymerase inhibitor, J. Pharm. Biomed. Anal. 120 (2016) 352–363.
[17] European Medicines Agency (EMA), Assessment report. EMA/CHMP/688774/2013, 2013.
[18] L. Wilson, A. Arabshahi, B. Simons, et al., Improved high sensitivity analysis of polyphenols and their metabolites by nano-liquid chromatography-mass spectrometry, Arch. Biochem. Biophys. 559 (2014) 3–11.
[19] M. Nebsen, E.S. Elzanfaly, Stability-indicating method and LC–MS-MS characterization of forced degradation products of sofosbuvir, J. Chromatogr. Sci. 54 (2016) 1631–1640.
[20] B. Zaman, F. Siddique, W. Hassan, RP-HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form and its application to in vitro dissolution studies, Chromatographia 79 (2016) 1–9.
[21] G. Nannetti, S. Pagni, S.G. Parisi, et al., Development of a simple HPLC-UV method for the determination of the hepatitis C virus inhibitor simeprevir in human plasma, J. Pharm. Biomed. Anal. 121 (2016) 197–203.