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Simple chromatographic and spectrophotometric determination of sofosbuvir in pure and tablet forms

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ABSTRACT

Two methods, a reversed phase high-performance liquid chromatographic (RP-HPLC) method and a direct ultra-violet spectrophotometric method, were adopted and validated for the quantification of sofosbuvir, which is a new antiviral agent used for treatment of patients with hepatitis C virus (HCV). Validation parameters such as linearity, accuracy, precision, specificity, limits of detection and quantification were determined according to the guidelines of International Conference on Harmonization (ICH)-Q2B. The RP-HPLC method was applied on Hypersil™ ODS C18 column (150×4.6 mm, 5 µm) as a stationary phase. The mobile phase was optimized according to the polarity of the studied drug. It was methanol: acetonitrile (90:10, *v:v*), pumped using an isocratic mode with flow rate of 1 mL/min and UV detection at 260 nm. The UV spectrophotometric method was performed for the studied drug at 260 nm. The calibration curves were linear in the ranges of 2-60 µg/mL and 5-40 µg/mL for the RP-HPLC and UV spectrophotometric methods, respectively. The proposed methods are accurate, sensitive and precise, so they can be successfully adopted for the reliable determination of sofosbuvir content in its tablet form.

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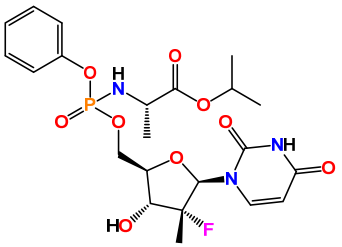
1. Introduction

Sofosbuvir (SFV) (isopropyl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl] methoxy-phenoxy-phosphoryl] amino] propanoate) is a nucleotide prodrug analog that can be used for treatment of patients with hepatitis C virus (HCV) (Scheme 1) [1]. It can be metabolized to the pharmacologically active uridine analog triphosphate. This metabolite can be incorporated into the ribonucleic acid (RNA) of the HCV and so acts as a chain terminator. The termination of RNA synthesis prevents replication of the virus and so leads to a rapid decrease of HCV viral count [2]. SFV can be used alone or in combination with other drugs like ribavarin and ledipasvir [3]. SFV has been included in the essential medicines list of World Health Organization (WHO) which shows the high potentiality of the drug in the treatment regimen of HCV [4].

There is no much reported literature concerning the quantification of SFV due to the recent launch of the drug in the market (2013). All the performed methods used the ultra-performance liquid chromatography - tandem mass spectroscopy (UPLC-MS/MS) technique for the studied drug determination with other antiviral drugs like ribavirin, ledipasvir or with its metabolite [5-7].



Scheme 1

From the previous literature review, there is an urgent need for a simple, accurate and economic analytical method for the rapid quantification of SFV either in pure form or in its tablet form as all the published methods used a complicated expensive technique (UPLC-MS/MS). The target of the present work is to apply and validate analytical methods characterized with accuracy and simplicity like RP-HPLC and UV spectrophotometry for the quantification of the studied drug either in pure or in dosage forms and can be applied for the rapid estimation of SFV in quality control laboratories.

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2. Experimental

2.1. Chemicals and reagents

Pure sofosbuvir (PSI-7977) was purchased from Cayman Chemical Company, Ann Arbor, United States of America (USA); its purity was certified to be 99.9%. Acetonitrile and methanol (HPLC grade) were supplied by Sigma Aldrich, St. Louis, USA.

2.2. Pharmaceutical formulation

Sovaldi® 400 mg film coated tablets with batch number PMPW. It was manufactured by Gilead Sciences, Inc. Foster City, USA.

2.3. Instrumentation

The liquid chromatographic method was performed on high performance liquid chromatograph consisted of a binary pump (Waters, 1525), a UV-visible wavelength detector (Waters, 2489) and an auto-sampler (Waters, 2707). The chromatograph is a product of Waters, Massachusetts, USA. The spectrophotometric analysis was done using double beam spectrophotometer (JASCO, Japan) with 1 cm path length matched quartz cuvettes. It is connected to IBM compatible computer with HP 680 inkjet printer (Hewlett Packard, USA).

2.4. Standard solutions

Stock standard solution (1 mg/mL) was prepared by accurate weighing and transferring of 50 mg of pure SFV into 50 mL measuring flask. The drug was dissolved by aid of a vortex mixer in 20 mL methanol then the volume was completed to the mark using the same solvent. Working standard solution (100 µg/mL) was prepared by accurate dilution of 5 mL of the stock standard solution (1 mg/mL) into 50 mL measuring flask using methanol as a diluting solvent.

2.5. Method validation

The developed analytical methods were fully validated according to ICH-Q2B guidelines [8].

2.5.1. Linearity

2.5.1.1. HPLC-method

Aliquots (0.2-6.0 mL) of SFV working standard solution (100 µg/mL) were accurately and separately transferred into a series of 10 mL measuring flasks, the volume was then completed with methanol to obtain a concentration range of 2-60 µg/mL. The prepared samples were filtered through a 0.45 µm membrane filter then injection was carried out by the aid of a 50 µL Hamilton® syringe. Conditioning and pre-washing of the stationary phase was performed by passing about 60-70 mL of the mobile phase.

Samples were then chromatographed using Hypersil™ ODS C18 column (250×4.6 mm, 5 µm) as a stationary phase. The mobile phase was methanol: acetonitrile (90:10, *v*:*v*). It was pumped using isocratic mode with flow rate of 1 mL/min and UV detection at 260 nm. Peak area ratios were plotted against concentration to obtain the calibration graph then the regression equation was computed.

2.5.1.2. UV-spectrophotometric method

Aliquots (0.5-4.0 mL) of SFV working standard solution (100 µg/mL) were transferred, accurately and separately, into a series of 10 mL measuring flasks then the volume was completed with methanol to obtain a concentration range of 5-40 µg/mL. The absorption spectrum of each solution was scanned using methanol as a blank. Absorbance at λ_{max} = 260 nm was recorded for each concentration. The calibration graph was plotted between absorbance and concentration then the regression equation was computed.

2.5.2. Accuracy

Accuracy can be defined as the percent of the recovered analyte from a known added quantity [9]. Data from nine samples representing three concentration levels covering the obtained linearity range (10, 30 and 50 µg/mL for the HPLC method and 10, 20 and 30 µg/mL for the UV-spectrophotometric method) was used to assess accuracy of both methods, so the previously mentioned procedures under linearity were carried out then the concentration of SFV samples was calculated using the corresponding regression equation.

2.5.3. Precision

Precision can be defied as the degree of repeatability under normal operational conditions. The assay precision can be determined by repeatability (intra-day) and intermediate precision (inter-day) then reported as % relative standard deviation (%R.S.D.), for a number of experiments which are statistically significant [10] so three concentrations of SFV (10, 30 and 50 µg/mL for the HPLC method and 10, 20 and 30 µg/mL for the UV-spectrophotometric method) were analyzed three times within the same day (intra-day) or on three successive days (inter-day) then the results were documented as %R.S.D.

2.5.4. Specificity

Specificity can be determined by comparing the HPL chromatogram and the UV-scan obtained for a mixture of SFV and the commonly used excipients, with those obtained from the blank (excipients solution in methanol without drug) [10]. The chosen excipients were microcrystalline cellulose, colloidal silicon dioxide, mannitol, croscarmellose sodium, and magnesium stearate. These inactive ingredients were used in the manufacture of Sovaldi® 400 mg film coated tablets as mentioned in its monograph. The drug to excipient ratio was similar to that used in the market product.

2.5.5. Limits of detection and quantification

Limits of detection (LOD) can be defined as the lowest concentration of the analyte that the analytical method can reliably differentiate from the background. Limits of quantification (LOQ) can be defined as the lowest concentration that can be quantified with acceptable accuracy and precision [10]. The LOD and LOQ were calculated as

$$LOD = 3.3\ \sigma / S \quad (1)$$

$$LOQ = 10\ \sigma / S \quad (2)$$

where, σ is the standard deviation of the lowest standard level and S is the slope of the standard curve.

2.5.6. Robustness

Robustness was assessed by evaluating the effect of minute variations on the proposed methods. The conditions studied, for the HPLC-method, were the flow rate variation by 0.1 mL/min and mobile phase composition with respect to methanol ±5 mL per each 100 mL of the mobile phase. On the other hand, for the spectrophotometric method the solvent composition was slightly changed by adding 1% acetonitrile.

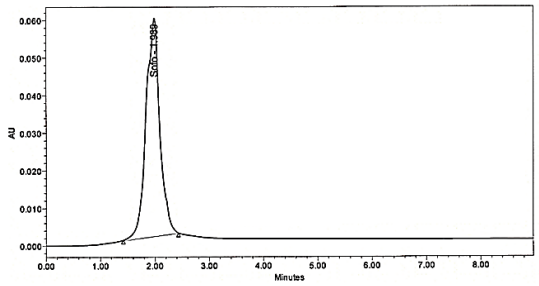

Figure 1. An HPL chromatogram of 20 µg/mL sofosbuvir.

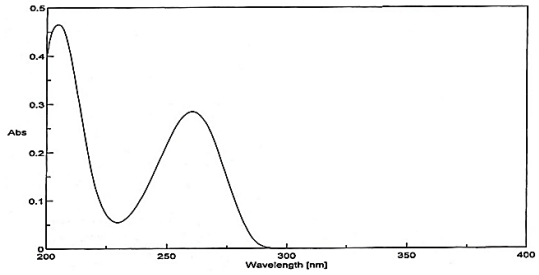

Figure 2. Absorption spectrum of 10 µg/mL sofosbuvir using methanol as blank.

2.5.7. System suitability

The system suitability for the chromatographic method was evaluated according to tailing factor, number of theoretical plates (N) and height equivalent to theoretical plates (HETP).

2.6. Analysis of pharmaceutical formulation

Ten Sovaldi® 400 mg film coated tablets were weighed to find the average weight of a tablet then crushed, finely powdered and mixed well. Tablet powder equivalent to 10 mg of SFV was transferred to a beaker of 250 mL capacity then; a suitable volume of methanol (40 mL) was added and stirred for about 20 minutes. Filtration was carried out into 100 mL measuring flask. The residue was washed with about 20 mL methanol (twice), and then the volume was completed to the mark with methanol and mixed well. The general procedures were followed as mentioned under linearity for both techniques then the concentration of SFV was obtained from the corresponding regression equation.

Moreover, standard addition procedure was applied by spiking different known quantities of pure SFV to the tablet formulation, and then the procedures were followed as mentioned before.

3. Results and discussion

Two simple analytical methods were developed for the quantification of SFV either in pure or tablet forms. These methods can be conveniently applied for the routine quality control analysis of pharmaceutical dosage forms. Both methods were developed and validated according to the guidelines of ICH-Q2B [8].

The first one is an isocratic reversed phase high-performance liquid chromatographic (RP-HPLC) method. The parameters were optimized to get an optimum performance of the method. The mobile phase selection was done depending on the polarity of the studied drug. Different stationary and mobile phases were tried to reach the best match and so get the best separation pattern of SFV. The best Gaussian peak with ideal peak symmetry was obtained on using Hypersil™ ODS C18 column (150×4.6 mm, 5 µm) as a stationary phase and methanol:acetonitrile (90:10, *v*:*v*) as a mobile phase. The mobile phase flow was operated using the isocratic mode with a rate of 1 mL/min and UV detection at 260 nm. By adopting the described chromatographic conditions, SFV was well separated with average retention time of 1.99±0.05 min as shown in Figure 1.

On the other hand, the second method was based on the absorption power of SFV in the ultra violet (UV) region. SFV has specific structural arrangement that absorb UV rays at a specific wavelength (260 nm) and this information was successfully used for its determination, Figure 2.

3.1. Method validation

3.1.1. Linearity

3.1.1.1. HPLC-method

Calibration standards at seven levels were prepared by suitable dilution of the working standard solution of SFV (100 µg/mL) to reach concentration range of 2-60 µg/mL. Each concentration was injected in triplicate, and peak area ratios were plotted against the corresponding concentrations to obtain the calibration curve then the regression equation was computed to be:

$$P = 0.0337 \times C + 0.0091 \ (r^2 = 0.9996) \quad (3)$$

where, P is the peak area ratio and C is the concentration in µg/mL.

3.1.1.2. Spectrophotometric method

Five concentration levels were prepared from the working standard solution of SFV (100 µg/mL) in the range 5-40 µg/mL. A linear correlation was obtained between absorbance at 260 nm and the corresponding concentration of the studied drug.

The following regression equation was computed to be:

$$A = 0.029 \times C - 0.0073 \ (r^2 = 0.9997) \quad (4)$$

Table 1. Method validation parameters for determination of sofosbuvir by the adopted methods.

Parameter	Method	
	HPLC	UV Spectrophotometry
Linearity		
Range, µg/mL	2-60	5-40
Slope	0.0337	0.029
Intercept	0.0091	-0.0073
r^2	0.9996	0.9997
Accuracy		
Mean±S.D. [a]	98.94±0.899	100.96±0.745
Variance	0.808	0.555
%R.S.D.	0.909	0.738
Precision		
Intraday precision, %	99.81±1.121	100.94±0.874
Interday precision, %	98.59±1.412	101.84±1.028
Robustness	0.657 [b] 0.574 [c]	0.691
LOD, µg/mL	0.25	1.50
LOQ, µg/mL	1.70	4.50

[a] Standard deviation, average of three determinations.
[b] Variation of flow rate.
[c] Variation of mobile phase composition.

Table 2. Established system suitability parameters for the HPLC method.

Parameter	SFV peak	Significance
t_R (min) *	1.99±0.05	-
Tailing factor	1.1	T = 1 for a typical symmetric peak
Number of theoretical plates (N)	253	Increase with the increase in column efficiency
Height equivalent to theoretical plate (HETP)	0.099	Decrease with the increase in column efficiency

* Average of triplicate runs.

Table 3. Determination of sofosbuvir in Sovaldi® 400 mg film coated tablets and application of standard addition procedure by the proposed methods.

Method	Content uniformity		Standard addition	
	Mean±S.D. *	% R.S.D.	Mean±S.D. *	% R.S.D.
HPLC method	102.08±0.974	0.954	101.74±0.786	0.773
UV spectrophotometric method	98.96±0.842	0.851	99.74±0.652	0.654

* Average of three determinations.

where, A is the absorbance at 260 nm and C is the concentration in µg/mL.

3.1.2. Accuracy

The accuracy of the proposed methods was validated by analyzing nine quality control samples of SFV representing three concentration levels covering the specified linearity range then calculating the recovery and percent of relative standard deviation (% R.S.D.) which is considered satisfactory as it was less than 1% for both methods which confirm the accuracy of the developed methods (Table 1).

3.1.3. Precision

The intraday and interday precisions were checked by analyzing three different concentrations of SFV by adopting the proposed methods, either in the same day or during three successive days. The % R.S.D. values for intraday and interday precisions were less than 2% for the proposed methods, which confirm the good precision of both methods (Table 1).

3.1.4. Specificity

The proposed methods were specific as none of the tried excipients interfered with the drug of interest, so, the methods were suitably applied for assaying the commercial product.

3.1.5. Limits of detection and quantification

The obtained values of LOD and LOQ confirmed the sufficient sensitivity of the proposed methods (Table 1).

3.1.6. Robustness

It is a measure of the method capability to maintain unaffected by slight changes in its parameters. The studied parameters were the variation in flow rate or mobile phase composition (HPLC method) and solvent composition (spectrophotometric method). The proposed methods were not affected by the slight changes in their conditions where the % R.S.D. values were less than 1% and so this confirms the robustness of the methods (Table 1).

3.1.7. System suitability

System suitability parameters for the chromatographic method were studied, to evaluate the peak symmetry and column efficiency. The studied parameters were tailing factor, number of theoretical plates (N) and height equivalent to theoretical plates (HETP). The values of the studied parameters confirmed the excellent peak symmetry and high column efficiency, Table 2.

3.2. Analysis of pharmaceutical formulation

The proposed methods were successfully adopted for the quantification of SFV in Sovaldi® 400 mg film coated tablets to assess content uniformity (Table 3). The commercial dosage form showed acceptable recoveries by applying the proposed methods which showed acceptable limits of content uniformmity. Also, the standard addition procedure was applied by spiking different known quantities of pure SFV to the tablet formulation to ensure the applicability and reliability of the proposed methods. The results showed satisfactory recoveries of the pure added drug by the proposed methods (Table 3).

4. Conclusion

The proposed RP-HPLC and UV spectrophotometric methods are simple, sensitive and selective having acceptable accuracy and precision. Also, the short analysis time for both methods makes them suitable for the assay of SFV in its commercial product during the routine analysis in quality control laboratories. Moreover, the UV-spectrophotometric

method offers a cost effective alternative to the RP-HPLC method of analysis.

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