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A quantitative RP-HPLC method development and validation for sofosbuvir in bulk and tablet dosage form

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ABSTRACT

A new isocratic simple and rapid reverse phase high performance liquid chromatographic method was developed and successively validated for the estimation of Sofosbuvir. In this newly developed method, chromatographic separation of Sofosbuvir was achieved on a Phenomenex C18- column (250 × 4.6) mm within a short runtime of 6 min using mobile phase containing 0.1% Formic acid in water (pH at 2.3) and Acetonitrile in the ratio of 50:50% v/v. Sofosbuvir was estimated with UV detection at 262 nm and it was found to be eluted at 2.983 min. The above mentioned method was validated as per International Conference on Harmonization (ICH) guidelines with respect to accuracy, precision, linearity, limit of detection (LOD) and limit of quantitation (LOQ) and robustness. The method was found specific for Sofosbuvir and linear (r^2 =0.9994) over concentrations ranging from 20 to 100 µg/mL. The method was found statically accurate (mean recovery = 99.46%), precise with both intra-day and inter-day relative standard deviation (RSD) values < 1.0% and robust. The obtained results concluded that the proposed RP-HPLC method is convenient, reliable and useful in routine analysis for estimation of Sofosbuvir in its bulk form and dosage form.

Keywords: Sofosbuvir, RP-HPLC, Anti-HIV drugs, Development &Validation, ICH guidelines.

INTRODUCTION

Chromatography is defined as separation of mixture of compounds into individual components by using stationary and mobile phases. RP-HPLC technique is fast growing analytical technique for the estimation of chemicals and formulations. Sofosbuvir is a medication used for the treatment of hepatitis-C. It inhibits the hepatitis- C-NS5B protein. It is a prodrug and metabolized to the active antiviral agent GS-461203 (2'-deoxy-2'-α-fluoro-β-*C*-methyluridine-5'-triphosphate). GS-461203 serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis [1]. Although it has a 3' hydroxyl group to act as a nucleophile for an incoming NTP, a

similar nucleotide analogue, 2'-deoxy-2'-α-fluoro-β-C-methylcytidine, is proposed to act as a chain terminator because the 2' methyl group of the nucleotide analogue causes a steric clash with an incoming NTP [2]. It would act in a similar way. Chemically it is designated as Isopropyl (2*S*) - 2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl) - 4-fluoro– 3 – hydroxyl -4 - methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate [3 – 4].

Literature survey reveals RP-HPLC [5] and UPLC-MS/MS [6] plasma studies which are almost related to bioequivalence studies and few of are available on combination of drugs analysis or simultaneous estimations by HPLC.

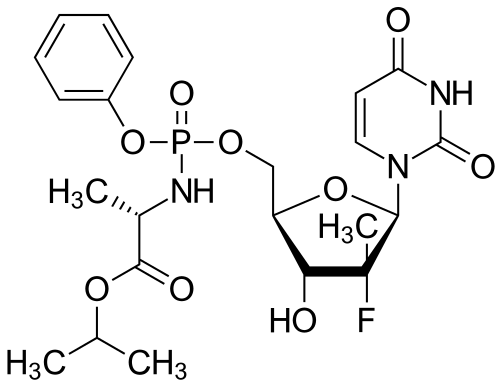

Fig 1: Structure of Sofosbuvir

The proposed method was optimized and validated in accordancewith International Conference on Harmonization (ICH) guidelines [7]. The aim of present work is to develop a simple, rapid, precise, accurate and selective reversed phase chromatographic method and to estimate the Sofosbuvir in bulk and its solid dosage forms.

MATERIALS AND METHODS

Materials and reagents

Sofosbuvir Standard drug sample was obtained as gift sample from by Aurobindo Pharma Ltd, Hyderabad. Sovaldi (Sofosbuvir 400 mg) Tablet dosage form procured from the local market. Other reagents used like HPLC grade Acetonitrile, formic acid were purchased from SD Fine Chemicals, Mumbai, India

Instrument

Quantitative HPLC was performed on Agilent 1200 series with Auto Sampler equipped with variable wavelength detector (UV detector). The chromatograms were recorded using EZChrom software.

Stationary PHASE

Reverse phase Phenomenex C18- column (250 × 4.6) mm dimension was used as stationary phase.

Selection of wavelength detection

The multiple spectra scan of 50µg/mL of the Sofosbuvir were recorded on UV- visible spectrophotometer in the UV range of 200-800nm. From the UV spectrum, wavelength of 262 nm showing maximum absorbance and it was selected for detection of HPLC method.

Preparation of standard solution

20 mg of Sofosbuvir pure sample was weighed accurately and transferred into 100 mL clean and dry volumetric flask, and then added 50 mL of mobile phase. The solution was sonicated for 20 min and made up to the final volume with diluent. From the above stock solution, 2.5 mL was pipetted out in to a 10 ml clean dry Volumetric flask and then made up to the final volume with mobile phase.

Preparation of sample solution

60 mg sample of Sofosbuvir (equivalent weight of Sovaldi) was weighed accurately and transferred

into a 100 mL volumetric flask, 50 mL of mobile phase was added, sonicated for 45 min, and made up to the final volume with mobile phase and filtered. From the filtered solution 2.5 ml was pipetted out into a 10 ml clean dry volumetric flask and made up the volume up to 10 mL with mobile phase. Similar preparations were followed to achieve desired concentrations to perform for the validation of Sofosbuvir.

Analytical method validation

The objective of validation of an analytical method is to demonstrate that it is suitable for its intended purpose as stated in ICH guidelines Q2 (R1) on validation of analytical procedures. Parameters to be considered during validation of the developed method as per ICH guidelines are:

Specificity

Specificity is defined as ability to measure the analyte in the presence of other components which may present along with the analyte. To estimate specificity of the method Blank, placebo, standard and sample chromatograms were obtained for Sofosbuvir.

Linearity

The linearity of the method expresses the relation between the rest results and the concentration of the analyte. According to the linearity rule the test results should be directly proportional to the concentration of the analyte. A linear relationship was evaluated across the range of 20 - 100 µg/mL for Sofosbuvir. It was obtained by plotting peak area against concentration of standard and finding regression coefficient (r^2).

Accuracy

The accuracy of the method expresses the closeness between the obtained value and an accepted reference value or the conventional true value. Accuracy was evaluated using 3 replicates of 3 different concentrations within the range. In the present work recovery studies were calculated by selecting triplicates of three concentration levels viz. 80%, 100%, 120% by the addition of known amount of Sofosbuvir standard.

Precision

The precision of the method expresses the closeness between a series of determinations obtained from multiple sampling of the same sample under the same analytical conditions. Precision may be performed in three different levels: repeatability, reproducibility and intermediate precision. Precision is stated by mean, standard deviation and percentage relative standard deviation.

Limit of detection (lod) & limit of quantification (loq)

The detection limit defined as the lowest amount of analyte which can be identified but cannot be quantified exactly. The quantitation limit defined as the lowest amount of analyte which can be quantified with suitable accuracy and precision. LOD and LOQ can be estimated by following formulas:

- $LOD=3.3\times\sigma/S$
- $LOQ=10\times\sigma/S$
- σ = Standard deviation estimated based on the calibration curve.
- S = Slope of the calibration curve.

Robustness

The robustness of method expresses the resistance of chromatographic conditions by small change in the analytical conditions. To estimate robustness of analytical method chromatographic conditions like temperature, pH of mobile phase, flow rate and mobile phase composition were varied.

RESULTS AND DISCUSSION

To optimize the RP-HPLC method, mobile phases of different compositions were tried. A satisfactory separation and good peak symmetry for Sofosbuvir were obtained with a mobile phase 0.1% Formic acid in water (pH at 2.3) and Acetonitrile in the ratio of 50:50% v/v and at a flow rate of 1 mL/min. The retention time obtained for Sofosbuvir at 3.1 min at 262 nm wavelength. System suitability parameters was calculated and compared as per ICH guideslines

Linearity

The standard curve was observed by preparing five serial dilutions of Sofosbuvir using a standard stock solution and dilution were made with mobile phase. Responses were recorded as peak area. The peak areas were plotted against concentrations to

obtain the calibration curve. Linear relationship was observed across the range of 20-120 µg/mL. Linearity range and linear regression data of calibration plot for Sofosbuvir is given in table no.1 and calibration curve is represented in Figure 2.

Table-1: Linearity of Sofosbuvir

Parameters	Results Obtained
Linearity range (ppm)	20-100
Correlation coefficient (r^2)	0.9994
Slope	26178
Intercept	8885.6

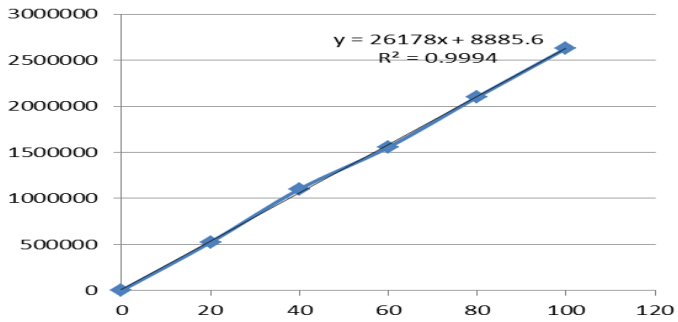

Figure-2: Linearity Graph for Sofosbuvir

SPECIFICITY

Specificity was tested by evaluating chromatogram of blank run and standard Sofosbuvir. The HPLC chromatograms recorded for the blank showed almost no peaks, interfering peak or baseline noise within a retention time of 2.983 min. Chromatogram of blank run and of 50 ppm of Sofosbuvir in mobile phase is given Figure 3 and 4.

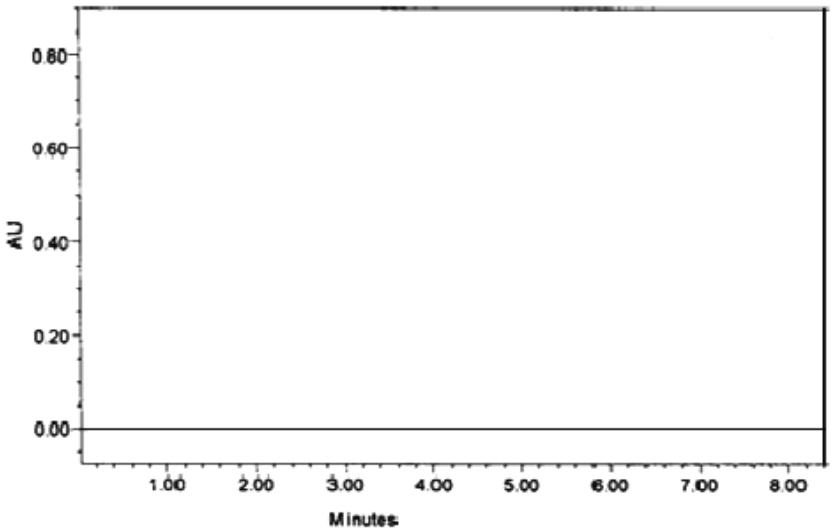

Fig-3: chromatogram of blank

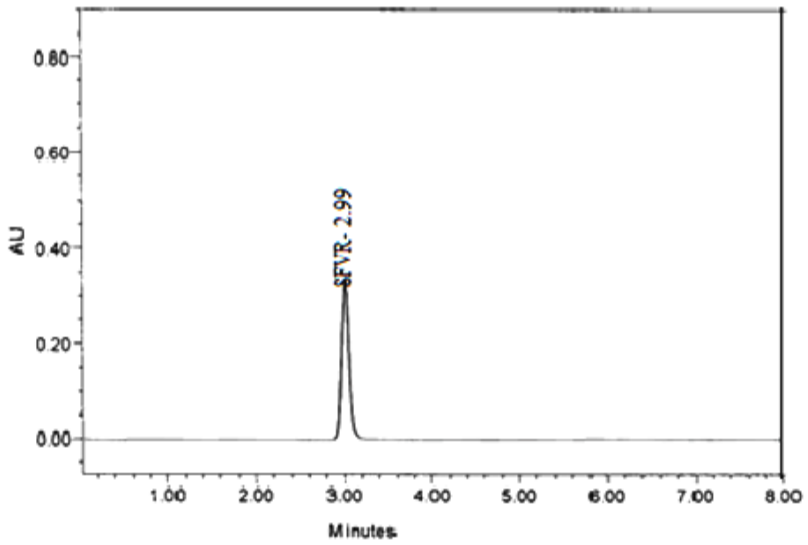

Fig-4: Chromatogram of Sofosbuvir

PRECISION

Intra-day precision

It was performed at three different concentration levels low (20µg/mL), mid (30µg/mL) and high (40µg/mL) within the same day at three different times (session 1, 2, 3).

Inter-day precision

It was carried out at same concentration levels on three consecutive days, using same homogeneous sample. The % RSD values for both intra-day and inter-day precision were found within acceptable limit. Results are presented in Tables 2 and 3 respectively.

Table-2: Intra-day precision results

Level		Low	Mid	High
Concentration (ppm)		20	30	40
Peak area	session 1	523565	785347	1147136
	session 2	530021	791431	1149627
	session 3	525645	779217	1146239
Average peak area		526410	785331	1163759
Standard Deviation		2690	4986	28757
%RSD		0.5	0.63	0.12

Table-3: Inter-day precision results

Level		Low	Mid	High
Concentation (ppm)		20	30	40
Peak area	session 1	523465	785447	1146936
	session 2	530121	792431	1149597
	session 3	525545	779317	1146239
Average peak area		526377	785731	1147591

Standard Deviation	2780	5357	1446
%RSD	0.51	0.68	0.12

LOD AND LOQ

Values of LOD and LOQ were calculated using slope of calibration curve. LOD and LOQ values of Sofosbuvir for HPLC method are tabulated in Table 4. Determined based on the standard deviation of the response and slope of the calibration curve.

Table 4: Results of LOD and LOQ

Parameters	Result
LOD	0.03 ppm
LOQ	0.063 ppm

ACCURACY

The accuracy of the method was determined on three concentration levels by recovery experiments. Accuracy of the method is reported as present recovery of known added amount of analyte in the placebo. The accuracy of the method was established by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% by adding known amount of Sofosbuvir. Results obtained were found to be within acceptable limits as shown in Table 5.

Table 5: Accuracy of Sofosbuvir

S.No	Spiked level	% Recovery of Sofosbuvir	% RSD
1	80%	100.5	
2	100%	99.85	0.906
3	120%	98.75	

ROBUSTNESS

Robustness of method was studied by making slight but deliberate changes in chromatographic conditions such as proportion of organic phase in mobile phase composition and flow rate. Effects of these changes on both the retention time (RT) and peak area were evaluated by calculating the relative standard deviations (%RSD). The results obtained are tabulated in Table 6.

Table 6: Robustness of Sofosbuvir

Parameter	Variation	% RSD
Robustness	i. Change in flow rate (± 0.1 mL/min)	0.92
	ii. Change in mobile phase (± 1mL)	0.45

CONCLUSION

A simple and precise stability indicating RP-HPLC method has been developed for determination of Sofosbuvir in tablet dosage form. The %RSD values in precision, recovery studies and robustness studies were found less than 2.0%, which indicates that the method is precise, accurate and robust. Limit of detection (LOD) and limit of quantification (LOQ) values for Sofosbuvir was 0.03 and 0.063 µg/mL. The % assay was found to be well within the acceptable limit. The % recovery is 98 -101% for Sofosbuvir. The proposed method can be used as an alternative method for the analysis of Sofosbuvir in its dosage forms.

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