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A new RP-HPLC method development and validation of Sofosbuvir in bulk and pharmaceutical dosage forms

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ABSTRACT

A Simple, accurate and rapid RP-HPLC method has been developed for the estimation of Sofosbuvir (SOF) in bulk and pharmaceutical dosage forms using a Kromasil C18 (250mm × 4.6 mm, 5 µm) with mobile phase comprising 0.1% Ortho phosphoric acid buffer and acetonitrile in the ratio 55:45 (v/v). The flow rate was 1 ml/min and detection was carried out by photodiode array detector at 259nm. The retention time for SOF was found to be 2.069 min. the proposed method has permitted the quantification of SFB over linearity in the range of 0.10 – 0.60 mg/ml and its percentage recovery was found to be 100.39 %. The % RSD of intraday and inter day precision were found 0.34% and 0.60% according to International Conference on Harmonization (ICH) Q2B guidelines.

Keywords: Sofosbuvir, RP-HPLC, Validation and method development.

INTRODUCTION

SFB is a prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is phosphorylated intra cellularly to the active triphosphate form. Used for the treatment of chronic Hepatitis C. [1] Chemically, SOF is Propan-2-yl(2S)-2-{[(S)-{[(3R,4R,5R)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl] methoxy} (phenoxy) phosphoryl] amino} propanoate [2] with empirical formula of $C_{22}H_{29}FN_3O_9P$ and molecular weight 529.4525 g/mol. It is a White to Off-white non-hygroscopic crystalline solids. [3] Slightly soluble in water (pH 1.2-7.7), freely soluble in ethanol and acetone, soluble in 2-propanol and insoluble in heptanes.[4] The chemical structure of SOF was shown in Fig.1. SFB is a prodrug nucleotide analog used as part of combination therapy to treat hepatitis C virus (HCV) infection or to treat co-infection of HIV and HCV. After metabolism to the active antiviral agent 2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate (also known as GS-461203), the triphosphate serves as a defective substrate for the NS5B protein, an RNA-dependent RNA polymerase required for replication of viral

RNA. SOF is used in combination therapy to treat chronic hepatitis C virus (HCV) infected patients with HCV genotype 1,2,3, or 4, and to treat HCV and HIV co-infected patients. The combination therapy includes either ribavirin alone or ribavirin and peg-interferon alfa. [5] Sofosbuvir is nucleotide analog inhibitor, which specifically inhibits HCV NS5B polymerase. Sofosbuvir prevents HCV viral replication by binding to the two Mg2+ ions present in HCV NS5B polymerase's GDD active site motif. [6]

An extreme literature survey revealed that very few analytical methods have been reported such as HPLC for Sofosbuvir in individual and combination with other drugs. In order to minimize the batch –to- batch variation, it is very important to develop suitable analytical methods for day –to-day analysis of drugs. It was found that one attempt has been made to develop estimation of Sofosbuvir by RP-HPLC at the starting of my work. Therefore, it was thought of interest in development and validating an advanced new sensitive, specific, precise, accurate RP-HPLC method for estimation of Sofosbuvir in bulk drug and in pharmaceutical dosage form. We here in report a simple, rapid and reliable HPLC for the estimation of SFB in bulk and pharmaceutical dosage forms as per ICH guidelines. [7-10]

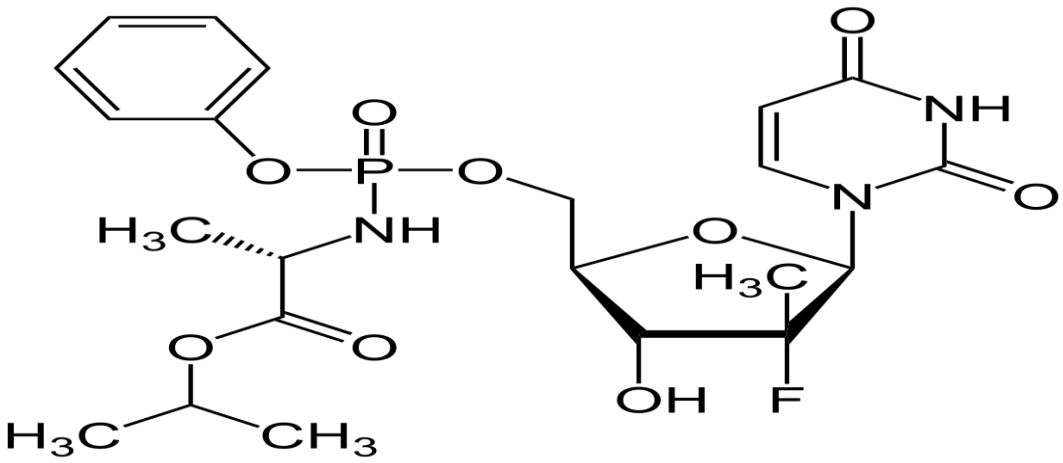

Fig 1: Structure of Sofosbuvir.

EXPERIMENTAL

REAGENTS AND MATERIALS

Pure standard of Sofosbuvir was obtained as gift sample from Lupin pharmaceuticals, Mumbai Acetonitrile, Water HPLC grade (Merck Specialties Pvt Ltd, Mumbai, India)**,** Ortho phosphoric acid HPLC (merck specialities pvt ltd, mumbai), All solvents used in this work are HPLC grade. MyHep Tablets (Mylan Pharmaceuticals Private Limited) containing Sofosbuvir Marketed formulation was purchased from local market, High precision weighing balance (wensar instruments,hyderabad), micro pipette (in labs,10-100 µl) were employed in the study. All the glassware employed in the work cleaned with hot water followed acetic anhydride then acetone and dried in hot air oven whenever required. Working environment was maintained in 25^{o}C.

HPLC APPARATUS AND CHROMATOGRAPHIC CONDITIONS

The analysis was performed on A Waters 2695 RP-HPLC separation module (Waters Corporation, Milford, USA) equipped with PDA detector having back pressure 5000psi, automatic injector and Kromasil C18 (250mm × 4.6 mm, 5µ). Data acquisition was performed by using Empower 2 software. Single pan Balance (Shimadzu, AUX-220), Sonicator (poLabindia Instruments). Different mobile phases were tested in order of their polarity to find out the best conditions for the separation of sofosbuvir. An isocratic RP-HPLC system was used for analysis of samples at 25^{o}C column oven temperature. The chromatographic separation was achieved on Kromasil 250 mm x 4.6 mm, 5µ column using 0.1% Ortho phosphoric acid buffer

and acetonitrile 55:45 % v/v as mobile phase at a flow rate of 1 ml/min. The mobile phase was filtered through 0.45 µm nylon membrane filter and degassed before use. The injection volume was 10 µl and the total runtime was set as 5 minutes. The determination of analytes was carried out at 259 nm using PDA detector.

PROCEDURE RECOMMENDED

Preparation of mobile phase

1 ml of ortho phosphoric acid solution in a 1000ml of Volumetric flask add about 100ml of milli-Q water and final volume make up to 1000ml with milli-Q water and 100% acetonitrile taken in the ratio 55:45 (v/v) were employed as a mobile phase.

Preparation of stock solution

Accurately Weighed and transferred 40mg Sofosbuvir working Standard into a 10ml clean dry volumetric flask, add 5 ml of diluents (first dissolved in methanol and make up Acetonitrile: Water (50:50)), sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluents.

Table.01: Chromatographic Condition of Sofosbuvir in API

Parameters	Condition
Column	Kromasil 250 mm x 4.6 mm, 5m.
Column Temperature	25^0C
Wavelength	259nm
Diluent	First dissolved in methanol and make up quantity with Acetonitrile: Water in the ratio of (50:50)
Injector volume	10 µl
Flow rate	1 ml/min
Runtime	5 min
Retention time	2.06 min
Theoretical Plates	2613

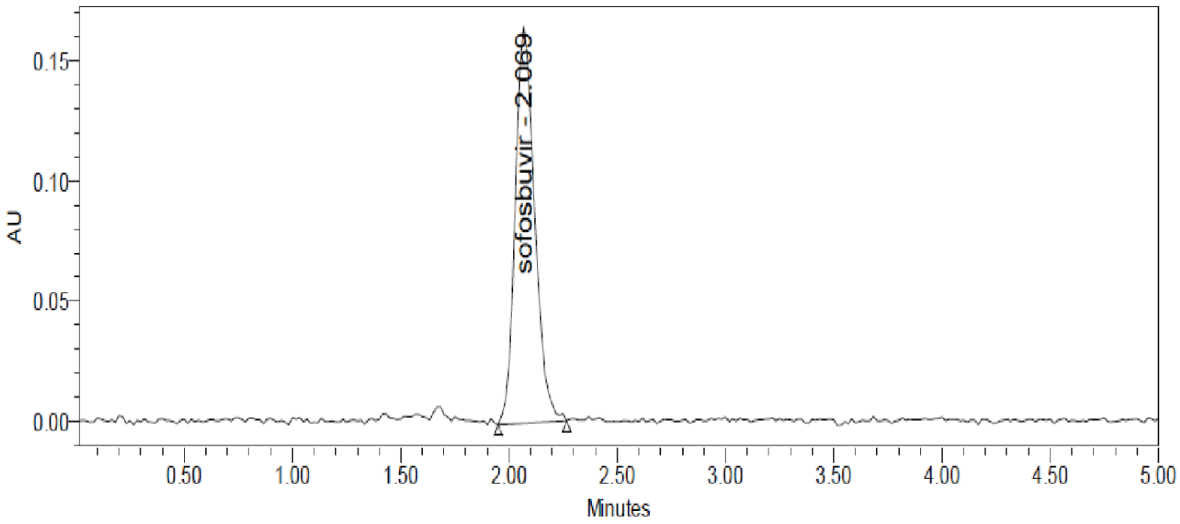

Fig.2; A typical chromatogram of SOF

CONSTRUCTION OF LINEARITY

The concentrations of analyte were prepared from the stock solution by taking suitable volume (0.25 – 1.5 ml) and diluted up to 10 ml to get the desired concentrations for linearity in the range of 100 – 600µg/ml. the prepared solutions were filtered through 0.45µm nylon membrane filter and each of the dilutions was injected three times into the column. The calibration curve for SFB was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

ESTIMATION OF SFB

Analysis of marketed formulation was purchased from local market. 10 tablets were weighed and average weight was calculated. Then from the transferred the equivalent to one tablet to 100ml volumetric flask, 75ml of diluent was added and the mixture was allowed to stand with intermittent sonication for 5 mins to ensure complete solubility of drug. Further the volume made up with diluent and the resulting solution was passed through 0.45µm membrane filtered. From the filtered solution, 1ml was pipette out into 10ml volumetric flask and made upto 10ml with diluent. From the solution, 10µl was injected into HPLC system and peak area was recorded (Fig.3) with detector at 259nm. The % assay was calculated with obtained peak area of detector response. The % assay was found to be 100.39% for Sofosbuvir. This indicates that developed method can be used for routine analysis.

Table.02: % Assay results of Sofosbuvir in formulation

Tablet	Drug	Dosage (mg)	Sample concentration	Amount found (µg/ml)	% Assay
1	**SFB**	**400**	**400**	**402**	**100.39**

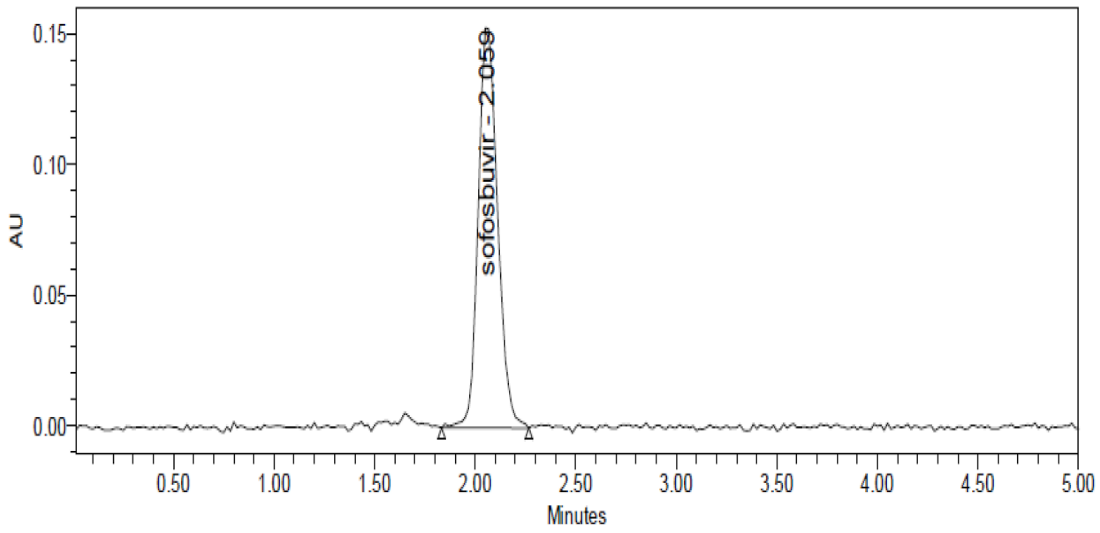

Fig 3: Chromatogram showing assay of Sofosbuvir marketed dosage form (MyHep tablets)

METHOD VALIDATION

As per the International Conference on Harmonization (ICH) guidelines, the method validation parameters such as Specificity, Linearity, Precision, Accuracy, Limit of Detection/ Quantification and Robustness were optimized.

RESULTS

The present RP-HPLC method for the quantification of SFB in bulk and pharmaceutical dosage forms, revealed as simple, rapid, accurate and precise method with significant shorter retention time of 2.069.min.

Accuracy

The accuracy of the proposed method was determined by standard addition method. It is the closeness of the analytical results obtained by the analysis of the true value. A known amount of standard drug was added to the fixed amount of injection solution. Accuracy was expressed as percentage recovery. Recovery test was performed with three different concentrations i.e. 200 µg/ml, 400µg/ml and 600 µg/ml for Sofosbuvir. The % recovery results were calculated and given in Table.03.

Table.03: % Recovery results of Sofosbuvir

Conc.	Sofosbuvir		
	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery
50%	200	198.21	99.10
	200	199.77	99.88
	200	198.47	99.24
100%	400	406.98	101.74
	400	406.49	101.62
	400	406.57	101.64
150%	600	599.28	99.88
	600	603.15	100.53
	600	598.95	99.82

Linearity

A series of six concentrations in the range of 100 to 600µg/ml of Sofosbuvir has been prepared and peak areas were recorded at 259nm. A calibration curve was plotted between peak area versus concentration of respective Sofosbuvir and the response of the drug was found to be linear. The linear regression equation (y = mx + c) was found to be y = 2452.2x + 22728. (Fig.4) for Sofosbuvir. The linearity results were given in Table.04 & 05.

Table.04: Linearity results of Sofosbuvir

Concentration (µg/ml)	Area	Average area	% RSD
100	291315	291440	0.09
	291748		
	291256		
200	514225	516267	1.10
	512112		
	522464		
300	763943	767713	0.43
	769903		
	769294		
400	983383	988458	0.44
	990845		
	991145		
500	1262003	1257597	0.84
	1245602		
	1265185		
600	1492919	1487202	0.36

Concentration (µg/ml)	Area	Average area	% RSD
	1482395		
	1486292		

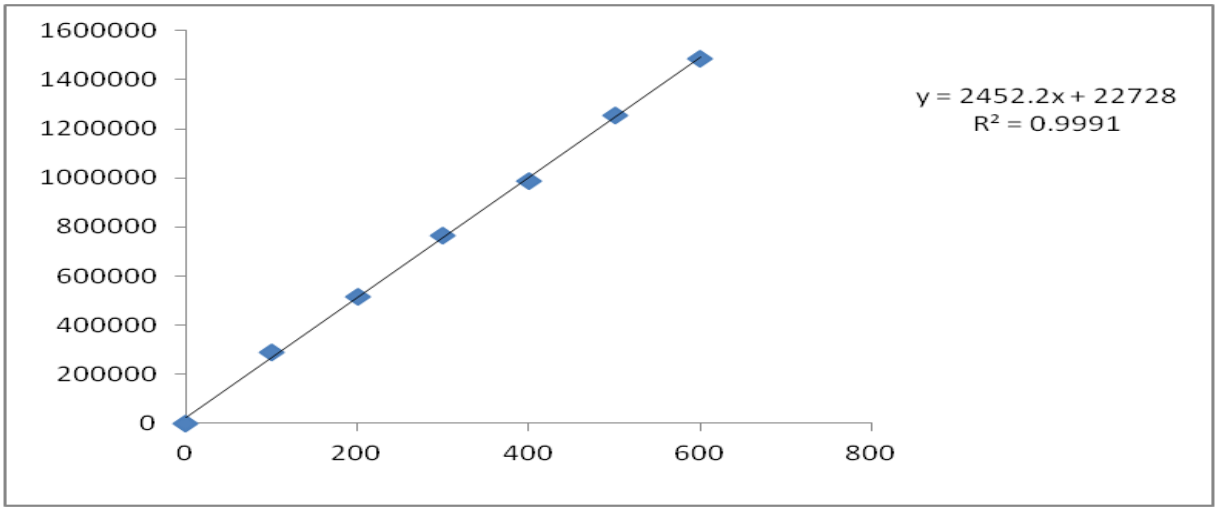

Fig .04: Calibration curve of Sofosbuvir

Table.05: Slope and intercept value of Sofosbuvir

Linearity curve	Sofosbuvir	
	Slope	**Intercept**
Value	2452.2	22728
Correlation coefficient (r^2)	0.9991	

Precision

Repeatability or Precision of the method was determined by injecting six replicates of standard solution at 400µg/ml of Sofosbuvir into HPLC system. From the results obtained it was found that the proposed method was precise.

Table.06: Precision data

Injection	Sofosbuvir concentration	Area
1	400µg/ml	988616
2		991620
3		983591
4		984602
5		989132
6		980856
Mean		986403
STDV		**4036.9**
%RSD		**0.4**

Robustness

Robustness study of the method was determined by changing the parameters such as flow rate, mobile phase ratio and temperature. Drug samples were analyzed under small changed conditions and chromatogram was recorded. It was found that these deliberate changes were not affected the chromatograms of both drug samples.

Table.07: Actual conditions and proposed conditions of the method

Parameters	Actual conditions	Proposed variations
Flow rate	1ml/min	0.9, and 1.1ml/min
Mobile phase ration	55:45 % v/v	±5%
Temperature	25 ^{0}C	20 ^{0}C, 30 ^{0}C

Effect of Flow rate

By changing the flow rate (1ml/min ±0.1ml) no drastic changes was seen in chromatographic parameters

Table.8: Robustness data at flow rate 0.9ml/min of Sofosbuvir

Parameters	RT	Area	Average area	% RSD
Sofosbuvir	2.260	971995	975116	0.3
	2.264	978519		
	2.266	974440		
	2.268	976791		
	2.275	978479		
	2.281	970471		

Table.09: Robustness data at 1.1ml/min of Sofosbuvir

Parameters	RT	Area	Average area	% RSD
Sofosbuvir	1.869	965678	955559	1.0
	1.875	943208		
	1.879	944178		
	1.881	962880		
	1.881	960063		
	1.883	957347		

Effect of Mobile phase

In mobile phase, organic phase was changed to ±10%. It was found that change in mobile phase was not affected the chromatogram parameters.

Table.10: Robustness data at mobile phase ration 45:55% v/v

Parameters	RT	Area	Average area	% RSD
Sofosbuvir	1.887	923741	931686	0.6
	1.890	929021		
	1.892	937085		
	1.892	927732		
	1.897	936415		
	1.903	936119		

Table.11: Robustness data at mobile phase ration 65:35% v/v

Parameters	RT	Area	Average area	% RSD
Sofosbuvir	2.228	961352	964502	0.64
	2.228	960588		
	2.229	955685		
	2.230	968539		
	2.232	967730		
	2.242	973119		

Effect of Temperature

Temperature of the column was changed to $\pm 5^0$C and chromatogram was recorded. From the results, it was found that change in temperature also not affected the chromatogram parameters.

Table.12: Robustness data at temperature 20 ^{0}C

Parameters	RT	Area	Average area	% RSD
Sofosbuvir	2.021	928242	925434	0.44
	2.025	919000		
	2.025	921372		
	2.036	923694		
	2.036	931974		
	2.043	928319		

Table.13: Robustness data at temperature 35 ^{0}C

Parameters	RT	Area	Average area	% RSD
Sofosbuvir	2.031	937150	939030	0.51
	2.037	940122		
	2.037	941376		
	2.037	932061		
	2.038	946158		
	2.045	937314		

Limit of Detection (LOD)

Limit of detection is the known concentration of Sofosbuvir and establishing minimum concentration at which the Sofosbuvir can be reliably detected. It was calculated based on the standard deviation of the response and the slope of the standard calibration curve. The LOD was found to be 0.762 µg/ml of Sofosbuvir.

Limit of Quantification (LOQ)

Limit of quantification is the known concentration of Sofosbuvir and establishing minimum level at which the Sofosbuvir can be quantified with acceptable accuracy and precision. The LOQ was found to be 2.308 µg/ml of Sofosbuvir. The LOD and LOQ results were given in Table.14.

Table.14: LOD and LOQ results of Sofosbuvir

Sample	LOD	LOQ
Sofosbuvir	0.762 µg/ml	2.308 µg/ml

CONCLUSION

In the present study, a newly RP-HPLC method was developed and successfully validated according to ICH guidelines for the estimation of Sofosbuvir. The method was validated for various parameters like specificity, linearity, accuracy, precision, robustness, LOD and LOQ. The validated method was applied for the assay of commercial tablets of Sofosbuvir in formulation. All the results obtained of various parameters were found to be within the acceptance limits. Thus the developed method in the present work is simple, sensitive, accurate, rapid, precise and robust. Hence the above method can be successfully applied for estimation of Sofosbuvir in both bulk and tablet dosage form.

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