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High Performance liquid chromatographic Method for the Determination of Sofosbuvir in pharmaceutical dosage forms

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ABSTRACT

A simple, rapid, precise and accurate reversed phase high performance liquid chromatographic method has been developed for the determination of Sofosbuvir. This method uses Agilent Eclipse XDB-C18 (5µm, 4.6 x 250mm) analytical column, a mobile phase of acetonitrile: potassium dihydrogen phosphate buffer pH 2.5 adjusted with orthophosphoric acid in ratio (55:45 v/v). The instrumental settings are a flow rate of 1.0 ml/min and Photon Diode Array detector wavelength at 260 nm. The retention times for Sofosbuvir were 3.16 min. The method was validated and shown to be linear. The linearity range for Sofosbuvir was 140-420 µg/ml. The Percentage recoveries for Sofosbuvir are ranged between 87.81 to 112.36. The correlation coefficient of Sofosbuvir was 0.999. The relative standard deviation for six replicates is always less than 2%. The Statistical analysis proves that the method is suitable for routine analysis of Sofosbuvir as a bulk drug and in pharmaceutical formulation.

Keywords: High Performance Liquid chromatographic method, Sofosbuvir, correlation coefficient.

INTRODUCTION

The chemical name of Sofosbuvir is (S)-Isopropyl 2-((S)-(((2R, 3R, 4R, 5R)-5-(2, 4-dioxo-3, 4-dihydro pyrimidin- 1(2H) - yl)-4-fluoro-3-hydroxy-4-methyl tetrahydro furanyl) methoxy)-(phenoxy) phosphoryl amino) propanoate [1].

Sofosbuvir (brandnames Sovaldi, Hepcinat, Resof, Hep cvir, and SoviHep) is a nucleotide analog used in combination with other drugs for the treatment of hepatitis C virus (HCV) infection. Sovaldi® 40 mg Film-coated tablet are manufactured by Gilead Sciences. They are used for oral administration and available in 40 mg, 30 mg, or 20 mg of Sofosbuvir (equivalent to 59.12 mg, 44.34 mg, or 29.56 mg Sofosbuvirdimaleate, respectively) [2]. There are no analytical methods that have been reported for the estimation of Sofosbuvir in bulk and in pharmaceutical formulations [3-15]. The present HPLC method deals with new simple, accurate and reliable estimation of Sofosbuvir in sterile pharmaceutical tablets. The other methods reported mainly on the determination of Sofosbuvir in plasma, blood samples and biological fluids including tissue homogenates. Such methods may not be suitable for regular/routine analysis for Sofosbuvir in pharmaceutical industry because of diversity and complexity in sample matrix.

MATERIALS AND METHODS

Sofosbuvir API is obtained as a gift sample. Acetonitrile (HPLC grade), Di-Potassium hydrogen Orthophosphate (AR grade) were obtained from Rankem Pvt. Ltd. Delhi, India. The 0.45 µm membrane filter was used throughout the experiment. The tablets of Sofosbuvir (Sovaldi®) were purchased from Local market. Double distilled water was used throughout the experiment. Other chemicals used in the experiment were of analytical or HPLC grade

Preparation of standard solution of Sofosbuvir

350 mg of Sofosbuvir was accurately weighed and transferred into a 100 ml clean dry volumetric flask,

about 70 ml of diluents was added, sonicated it completely and the volume was made up to the mark with the same solvent to give a concentration of 3500 µg/ml. (Stock solution) Further 5 ml was pipetted out from the above stock solution into a 50ml volumetric flask and diluted up to the mark with diluents to give a concentration of 350 µg/ml of Sofosbuvir. The stock solutions were filtered through a 0.45µ membrane filter paper.

Preparation of sample solution of Sofosbuvir

10 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 350 mg of active ingredient present in Sofosbuvir was transferred into a 100 ml clean dry volumetric flask, 70 ml of diluents was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluents to give a concentration of 3500 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 5 ml of upper clear solution was transferred to a 50 ml volumetric flask and diluted with diluents up to the mark to give the respective concentrations as per standard solution. The solution was filtered through a 0.45µ filter before injecting into HPLC system.

Method validation parameters

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH guidelines, the validation parameters were:

System suitability

Sample solution of Sofosbuvir was injected three times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard Chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from three replicate injections.

Linearity

Preparation of sample stock solution: About 350 mg of Sofosbuvir samples was weighed in to 100 ml volumetric flask, it was dissolved with diluents and the volume was made up to the mark with same diluents (3500µg/ml of Sofosbuvir as primary standard solution). Further 5 ml was pipetted out from the above stock solution into a 50ml volumetric flask and diluted up to the mark with diluents to give a concentration of 350 µg/ml of Sofosbuvir as secondary standard solution.

Preparation of Level – I (140µg/ml of Sofosbuvir) 4ml of secondary stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluents.

Preparation of Level–II (210 µg/ml of Sofosbuvir) 6ml of secondary stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluents.

Preparation of Level –IIII (280µg/ml of Sofosbuvir) 8ml of secondary stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluents.

Preparation of Level–IV (350 µg/ml of Sofosbuvir) 10ml of secondary stock solution had taken in 10ml of volumetric flask.

Preparation of Level –V (420µg/ml of Sofosbuvir) 1.2ml of primary stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluents.

Precision

The precision of the method was checked by repeated injected sample solution of Sofosbuvir 350 µg/ml

Accuracy

Assay was performed in triplicate for various concentrations of Sofosbuvir equivalent to 80, 100, and 120 % of the standard amount was injected into the HPLC system as per the test procedure.

Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate increase, flow rate decrease and different column which may differ but the responses were still within the specified limits of the assay.

Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. The flow rate was varied at 1.0 ml/min to 1.2 ml/min and to 0.8 ml/min. Standard solution 350 ppm (350 µg/ml of Sofosbuvir was prepared and analyzed using the varied flow rates along with method flow rate. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%. The method is robust only in less flow condition. The effect of variation of flow rate was evaluated.

Limit of detection The limit of detection was checked by signal to noise ratio. The prepared solution of 0.105µg/ml was checked by repeated injected sample solution.

Limit of quantification The limit of quantification was checked by signal to noise ratio. The prepared solution of 0.035µg/ml Sofosbuvir was checked by repeated injected sample solution.

RESULTS AND DISCUSSION

System suitability

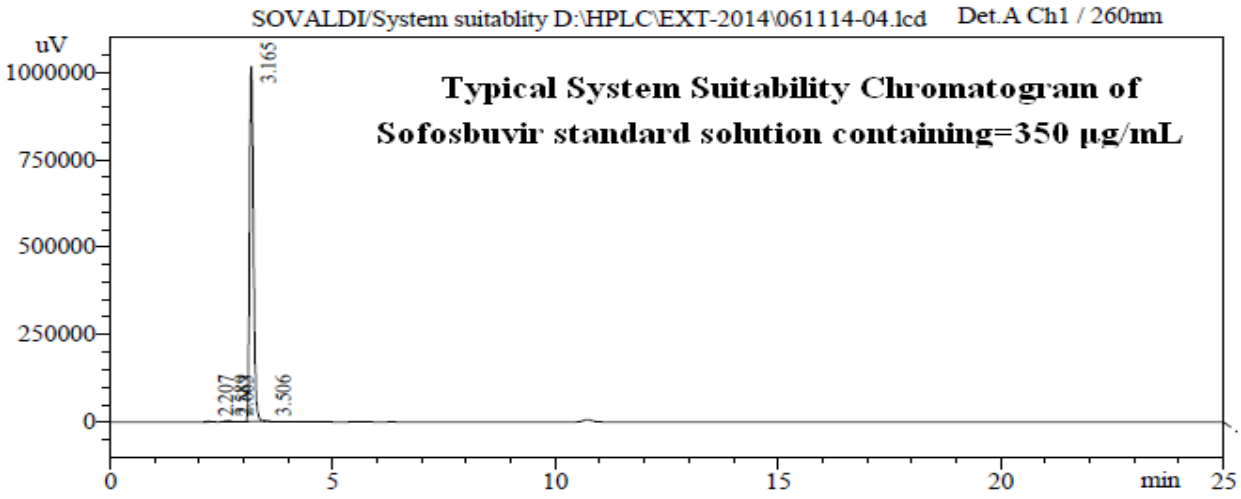

Fig 1: Typical System suitability Chromatogram of Sofosbuvir Working standard solution.

Table 1: Typical System suitability Chromatogram of Sofosbuvir Working standard solution

Peak#	Ret. Time	Name	Area	Area%	Relative retention time	Theoretical plate#	Tailing Factor	Resolution
1.	2.207		11615	0.184	0.697	2685.919	1.007	0.000
2.	2.589		9497	0.151	0.818	0.475	0.000	0.050
3.	2.663		22295	0.354	0.841	2968.165	0.000	0.010
4.	3.165	Sofosbuvir	6241811	99.105	1.000	5384.883	1.352	2.726
5.	3.506		12955	0.206	1.108	8803.624	1.401	2.119
Total			6298174	100.00				

Linearity

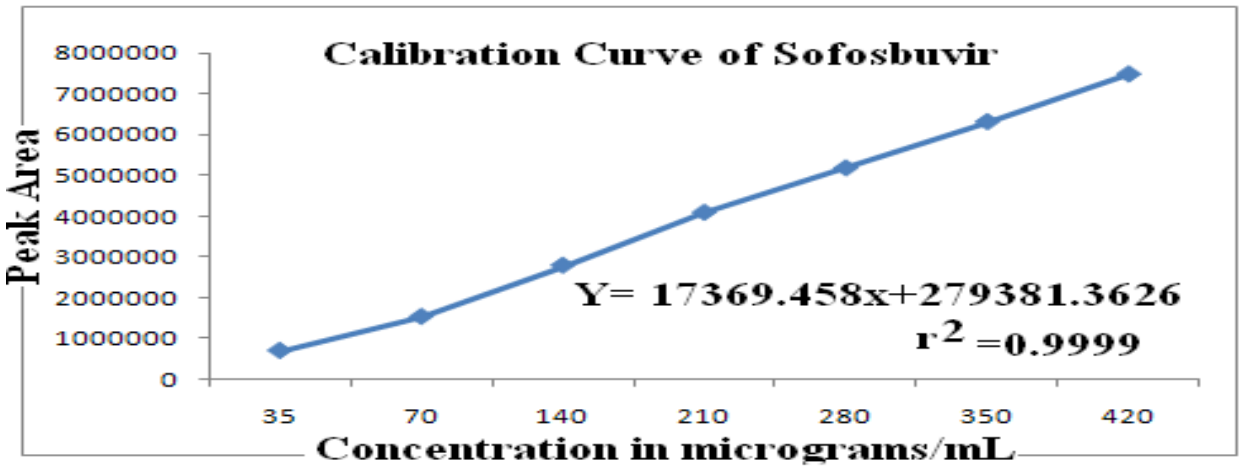

Fig 2: Linearity data

Table 2: Linearity data

Concentration of drug (µg/mL)	Retention time	Peak Area
35	3.167	698762
70	3.168	1534217
140	3.169	2791236
210	3.166	4089902
280	3.170	5180679
350	3.170	6315827
420	3.172	7486081

Precision data

Table 3: Precision of Standard drug with statistics

Injection No.	**Name of the drug & conc. (350 µg/ml)**	**Retention time in min.**	**Peak Area**
1	Sofosbuvir injection-1	3.166	6241725
2	Sofosbuvir injection-2	3.164	6233791
3	Sofosbuvir injection-3	3.165	6232983
4	Sofosbuvir injection-4	3.167	6236755
5	Sofosbuvir injection-5	3.168	6235465
6	Sofosbuvir injection-6	3.168	6242724
Mean		3.166	6237240
% RSD.		0.043	0.066
Std. Deviation		0.001	4089

Table 4: Precision study of Sample Solution (Sovaldi® 40 mg, film coated tablets**) with statistics**

Injection No.	Name of the drug & conc. (350 µg/ml)	Retention time in min.	Peak Area
1	Sovaldi® injection-1	3.168	6232590
2	Sovaldi® injection-2	3.168	6233698
3	Sovaldi® injection-3	3.169	6234841
4	Sovaldi® injection-4	3.168	6234773
5	Sovaldi® injection-5	3.167	6228530
6	Sovaldi® injection-6	3.168	6229812
Mean		3.168	6232374
Std. Deviation		0.016	0.042
% RSD		0.001	2645

Accuracy data

Table 5: Recovery Peak areas of Sofosbuvir by Accuracy studies

S.No	Recovery at 80% dilution level Peak areas		Recovery at 100% dilution level Peak areas		Recovery at 120% dilution level Peak areas	
	Standard	Spiked	Standard	Spiked	Standard	Spiked
1	5127921	5677448	6224163	6717889	7012659	7724222
2	5131411	5680902	6231894	6709953	7012132	7770160
3	5128800	5686669	6233644	6717883	7018299	7786289
Avg	5129377.3	5681673.0	6229900.3	6715242	7014363.3	7760224
Std.Dev	1815.22	4659	5045.13	4580	3418.56	32204
%RSD	0.035	0.082	0.081	0.068	0.049	0.415
% Recovery	87.81%		89.36%		112.36%	

Robustness

Table 6: Robustness study of Sofosbuvir Standard solution at 100 % level (350 µg/mL)

Parameter	Peak areas of Sofosbuvir in Flow increase study		Peak areas of Sofosbuvir in Flow decrease study		Peak areas of Sofosbuvir in Variable column Study	
	Run time	**Peak Area**	**Run time**	**Peak Area**	**Run time**	**Peak Area**
Injection-1	2.882	5804467	3.509	7058410	3.168	6281207
Injection-2	2.884	5855502	3.509	7007582	3.166	6280946
Injection-3	2.881	5840748	3.511	7053305	3.167	6281445
Mean	2.882	5833572	3.510	7039766	3.167	6281199
% RSD	0.051	0.450	0.024	0.398	0.025	0.004
Std. Dev	0.001	26263	0.001	27989	0.001	250

Table 7: Robustness study of Sovaldi®-40 mg tablets solution at 100 % level (350 µg/mL)

Parameter	Peak areas of Sofosbuvir in Flow increase study		Peak areas of Sofosbuvir in Flow decrease study		Peak areas of Sofosbuvir in Variable column Study	
	Run time	**Peak Area**	**Run time**	**Peak Area**	**Run time**	**Peak Area**
Injection-1	2.883	5843702	3.511	7060432	3.168	6262054
Injection-2	2.884	5844134	3.509	7050012	3.167	6261908
Injection-3	2.885	5824122	3.506	7056954	3.169	6261804
Mean	2.884	5837320	3.509	7055800	3.168	6261922
% RSD	0.041	0.196	0.068	0.075	0.025	0.002
Std.Dev	0.001	11432	0.002	5305	0.001	126

LOD and LOQ Data

Table 8: LOD and LOQ Data

Dilution level	Concentration	Area
0%	70	1344217
10%	35	698762
5%	17.5	355925
2%	7	141164
1%	3.5	72926
0.50%	1.75	36773
0.20%	0.7	10847
0.10%	0.35	8130
0.05%	0.175	4356
0.02%	0.07	1850
0.01%	0.035	1196
0.005%	ND	ND
LOD:		**0.01%**
LOQ:		**0.03%**

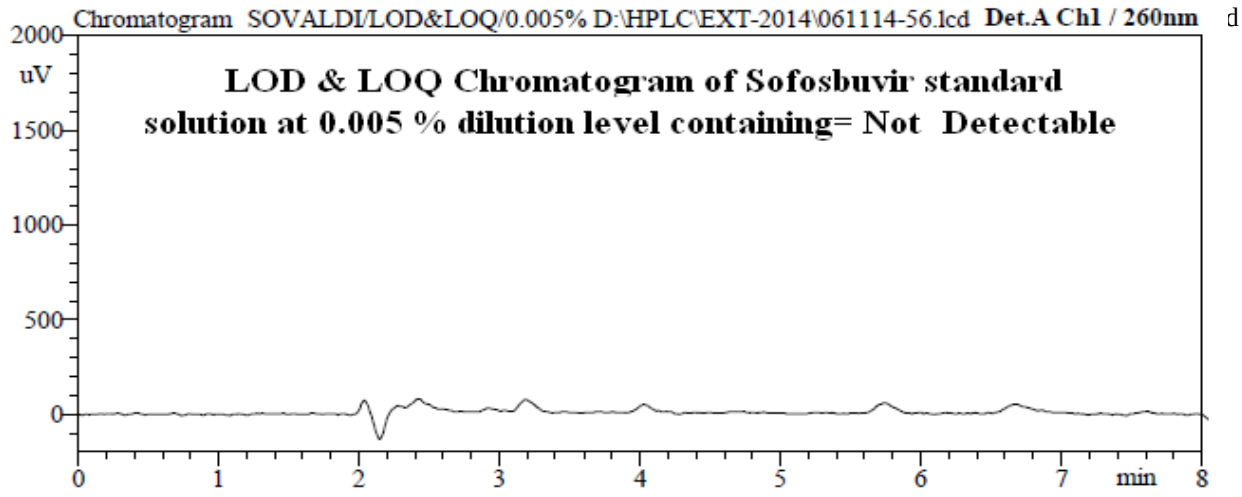

Fig 3: LOD &LOQ chromatogram of Sofosbuvir standard solution at 0.005%.

Table 9: Performance & Detection Characteristics of HPLC method

Parameter	Results of the proposed HPLC method	
	Sofosbuvir Standard solution	Sofosbuvir Sample (Sovaldi®-40 mg tablets) Solution
Retention time (min)	3.166	3.172
Theoretical plates (n)	5414.269	5391.792
Plates per meter (N)	21657.076	21567.168
HETP	$4.6175x10^{-5}$	$4.6366x10^{-5}$
Peak asymmetry (T)	1.353	1.358
Linearity range (µg/mL)	35-420	
Limit of Detection (µg/mL)	0.07	
Limit of Quantification (µg/mL)	0.21	

SUMMARY AND CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of sofobuvir was done by RP-HPLC. A sensitive, accurate and precise HPLC for the estimation of Sofosbuvir in bulk drug and in tablet dosage form is done. From the typical chromatogram of Sofosbuvir, it was it found that the retention time was 3.166 min. The contents of the mobile phase were Buffer: Acetonitrile 45: 55 (v/v). Solvent-A (Buffer) is 3.48 gms of Di Potassium hydrogen *ortho*-phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute orthophosphoric acid and Solvent-B is Acetonitrile in a isocratic mode of separation was used to resolute the Sofosbuvir at a flow rate of 1.0 ml/min and eluents were monitored at 260 nm, was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the A good linear relationship (r^2=0.9998) was observed between the concentration range of 35-420µg/mL. The assay of Sofosbuvir in bulk was found to be 99.34%. From the recovery studies it was found that about 111.12 % on average of Sofosbuvir was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the film coated tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and pharmaceutical tablet dosage form of Sofosbuvir within a short analysis time. The results obtained on the validation parameters met ICH and USP requirements. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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