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EVALUATION AND VALIDATION OF BULK AND TABLET-BASED CHRONIC HEPATITIS DRUG: RP-HPLC

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ABSTRACT:

A simple, sensitive, precise, and accurate isocratic reverse phase high pressure liquid chromatographic method has been developed and validated for the estimation of sofosbuvir in bulk and tablet dosage form. To optimize, a column Phenomenex prodigy ODS-3V (150 mm x 4.6 mm, 5 µm), mobile phase mixture of methanol and (0.1%) tri-fluro acetic acid as buffer having pH of 3.2 in the ratio of (30:70 v/v) found to be an efficient system for elution of drug with good peak shape as well as retention time 2.990 min., flow rate 1.0 ml/min. at UV wavelength of 260nm. Quantitative linearity was obeyed in the concentration range of 100 to 600 µg/ml, the regression equations of concentration over their peak areas were found to be Y = 18864x + 58306 R^2 = 0.996, where Y is the peak area and X is the concentration of drug. The number of theoretical plates obtained was 2604.352 which indicate the efficient performance of the column. The limit of detection was 0.01 µg/ml and limit of quantification was 0.03 µg/ml, which indicates the sensitivity of the method the high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

Key words: Sofosbuvir, RP-HPLC, Methanol, Buffer as tri-fluro acetic acid.

INTRODUCTION

Sofosbuvir, IUPAC Name is 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,molecular formula and weight was $C_{22}H_{29}FN_3O_9P$ &529.453g/mol.

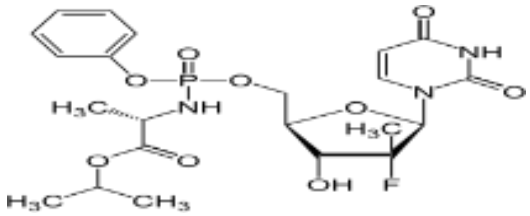

Fig. 1: Chemical structure of sofosbuvir.

Sofosbuvir[1] is a nucleotide analog used in combination with other drugs for the treatment of hepatitis C virus (HCV) infection. Compared to previous treatments, Sofosbuvir-based regimens provide a higher cure rate, fewer side effects, and a two- to four-fold reduced duration of therapy[2]. Sofosbuvir allows most patients to be treated successfully without the use of peginterferon[3], an inject able drug with severe side effects[4] that is a key component of older drug combinations for the treatment of HCV. Sofosbuvir inhibits the RNA polymerase that the hepatitis C virus uses to replicate its RNA. In 2013, the FDA approved sofosbuvir in combination with ribavirin (RBV) for oral dual therapy of HCV genotypes 2 & 3, and for triple therapy with injected pegylated interferon (peg.IFN) and RBV for treatment-naive patients with HCV genotypes 1 and 4. [5]In 2014, a combination of sofosbuvir with the viral NS5A inhibitor ledipasvir was approved.

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. [6]This latter combination provides high cure rates in people infected with genotype 1 (most common in US, Japan & much of Europe) without the use of interferon, irrespective of prior treatment failure or the presence of cirrhosis[7]. Sofosbuvir is also used in Hepatitis –C virus patients with an HIV confection. The treatment is based on a number of clinical trials, for example, the ELECTRON trial which showed that a dual interferon-free regimen of sofosbuvir plus ribavirin produced a 24-week post- treatment sustained virological response rate of 100% for previously untreated patients with Hepatitis-C virus genotype 2 or 3[8].

Sofosbuvir is a pro-drug using the Pro-tide biotechnology strategy. It is metabolized to the active anti-viral agent, 2'-deoxy-2'- α -fluoro-β-methyl-uridine-5'-tri-phosphate. The tri-phosphate serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis[9]. Prior to the discovery of sofosbuvir, a variety of nucleoside analogs had been examined as anti-hepatitis-C treatments, but these exhibited relatively low potency. This low potency arises in part because the enzymatic addition of the first of the three phosphate groups of the tri-phosphate is slow. The design of the sofosbuvir, based on the protide approach, avoids this slow step by building the first phosphate group into the structure of the drug during synthesis. Additional groups attached to the phosphorus temporarily mask the two negative charges of the phosphate group, thereby facilitating entry of the drug into the infected cell[10]. FDA approved sofosbuvir for the treatment of chronic Hepatitis-C[11]. In October 10th 2014, FDA approved the product combination of ledipasvir 90 mg / sofosbuvir 400 mg Brand –Harvoni[12.]

The present work reports the HPLC method for the analysis of sofosbuvir bulk, formulations and also in dissolutions, there was no HPLC method was reported. Some reported methods are [13]development of a sensitive UPLC-ESI MS/MS method for quantification of sofosbuvir and its metabolites in human plasma. [14]Simultaneous determination of lidipasvir, sofosbuvir and its metabolites in rate plasma by UPLC-MS/MS, and [15]Evaluation of a rapid method for the simultaneous quantification of ribavirin, sofobuvir and its metabolites in rat plasma by UPLC-MS/MS method.

EXPERIMENTAL METHODOLOGY

Instrumentation: Waters Alliance 2695 separation module (Waters Corporation, Milford, USA) equipped with 2998 UV detector with Empower-2 software was used for the analysis. The HPLC system was equipped with a column compartment with temperature control and an on-line degasser. Data acquisition, analysis, and reporting were performed by Empower-2 chromatography software. Phenomenex Prodigy ODS-3V (150 mm x 4.6 mm I.D; 5 µm) was used as stationary phase. Solubility of the compound was enhanced by sonication. All the weights in the experiments were done with Essea model: AJ220 Digital Electronic Balance.

Chemicals and Reagents: The reference samples of sofosbuvir were obtained from Natco Pharma Ltd., Hyderabad. Purified water was prepared by using Milli-Q water purification system. HPLC grade methanol (Merck, Mumbai), which was used for preparing dilutions and mobile phase. Analytical grade tri-fluro acetic acid (buffer) was obtained from Rankem Fine Chemicals Ltd., New-Delhi. Sovaldi, a formulation containing Sofosbuvir-500 mg was purchased from local market.

Preparation of standard solution: The standard solution was prepared by dissolving the suitable quantity of sofosbuvir of 500 mg in standard volumetric flask, and dilute up to the volume with diluent (water: methanol 60:40), from the above solution pipette out 5 ml into 50 ml standard volumetric flask and dissolve with diluent (500 µg/ml). This prepared standard (20 µl) was injected and chromate-graphed.

Preparation of standard solution: Twenty tablets were finely powdered and an accurately weighed sample equivalent to 500 mg (Avg. wt is 814mg) of sovaldi formulation into a 100 ml standard volumetric flask, dissolve and dilute to volume with diluent, Pipette out 5 ml into 50 ml standard volumetric flask dissolve with diluent (500 µg/ml), this prepared sample (20 µl) was injected and chromate-graphed and the results from assay are summarized.

METHOD DEVELOPMENT:

Binary mixture of (0.1%) trifluro acetic-acid in water adjust pH 3.2 (solvent-A) : methanol (solvent –B) in 30:70 v/v proportions in isocratic mode of elution was used as mobile phase. The resultant solution was thoroughly mixed and filtered (poly-tetra-fluoro ethanol (PTFE) filter of 0.45 µm pore size) using vacuum pump and degassed by sonication to expel the dissolved gases in solvent system. The flow rate of mobile phase was adjusted at 1.0 mL/min and 20 µL solution as injection volume were maintained. The eluted compounds were monitored at 260 nm by using UV detector. The column oven temperature was maintained at 30°C. Data acquisition, analysis, and reporting was performed by Empower-2 (Waters)

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chromatography software found to be an efficient system for elution of drug with good peak shape as well as retention time 2.990 min., flow rate 1.0 ml/min.

METHOD VALIDATION:

As per (ICH) International Conference on Harmonization guidelines, the method validation parameters such as specificity, linearity, precision, accuracy, LOD, LOQ and robustness were optimized[16].

a) Specificity: Specificity is the extension to which the procedure applies to analyte of interest and is checked by examining the formulation sample for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in the formulation did not interfere with the drug peak and thus the method is specific. The HPLC chromatogram recorded for the drug matrix (mixture of the drug and excipients) showed almost no interfering peaks within retention time ranges. Fig. 2 & 3 showed the representative chromatograms for standard and the dosage form. The figure describes that the selected drug was clearly separated and the proposed HPLC method is selective.

b) Linearity: To establish linearity, the stock solutions were prepared as 1000 µg/ml using mobile phase, from the stock solution further dilutions were prepared in the concentration range of 600 - 100 µg/ml, elution's are made on HPLC by injecting 20 µg/ml of each concentration repeat it for two times. The coefficient of determination and regression coefficient (R^2) was obtained and shown in the table 2 and fig. 4.

c) Precision: The intraday and inter-day precision was determined by analyzing sofosbuvir standard (500 µg/ml) for six times on same day (intra-day) and repeated on the second day (inter-day) studies were given the table 3 & 4.

d) Accuracy: The accuracy of the method shall be demonstrated through determination on samples in three concentrations from 120% (600 µg/ml), 100% (500 µg/ml) and 80% (400 µg/ml), three replicates of each of the theoretical concentrations employed as per the usual procedure and the results are summarized in Table 5.

e) Limit of detection (LOD) and limit of quantification (LOQ):

A series of 12 replicate concentrations were analyzed and quantified. Set up the described chromatographic conditions and allow the system to equilibrate. Starting with concentration 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02%, 0.01% and 0.005% peak area values were given in the Table 6. From this LOD is 0.01% and LOQ was found to be 0.03%.

f) Robustness: The robustness of the method was determined as per USP guidelines, under different conditions including change in flow rate, different column, pH of buffer, and buffer concentration. The results obtained by deliberately variation in method parameters and data are summarized in table-7.

g) System suitability: For system suitability, six replicates of the working standard samples were injected and the parameters like – plate number (N), retention time (Rt), and peak asymmetry of samples were calculated and given in table-8.

RESULTS AND DISCUSSION

The aim of the present study was to develop a simple, sensitive, precise, and accurate RP-HPLC method for the analysis of sofosbuvir in bulk, formulation and disolution. To optimize the mobile phase, various combinations of buffer, methanol and acetonitrile solvents were studied, on a column Phenomenex prodigy ODS-3V (150 mm x 4.6 mm, 5 µ). Finally by using mixture of methanol and (0.1%) tri-fluro acetic acid with pH 3.2 as buffer in the ratio of (30:70 v/v) found to be an efficient system for elution of drug with good peak shape as well as retention time 2.990 min., flow rate 1.0 mL/min. at UV wavelength of 260 nm. Quantitative linearity was obeyed in the concentration range of 100 to 600 µg/ml, the regression equations of concentration over their peak areas were found to be Y= 18864 X + 58306, R^2=0.996, where Y is the peak area and X is the concentration of drug. The number of theoretical plates obtained was 2504.970 which indicate the efficient performance of the column. The Limit of detection was 0.01 µg/mL and limit of quantification was 0.03 µg/mL, which indicates the sensitivity of the method the high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by the proposed HPLC method.

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CONCLUSION

In conclusion a new isocratic RP-HPLC method was developed and validated for the estimation of sofosbuvir in bulk and tablet dosage form. The developed method is simple, precise and accurate and satisfactory results were obtained through the method validation data. The present method can be easily applicable for routine drug analysis in laboratories and pharmaceutical industry.

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Table 1: Results of assay from tablet dosage form.

S.No.	Drug	Prepared concentration	Amount found	% Recovery
1	Std. Sofosbuvir	500 µg/ml	499.25	99.85
2	Sample Sovaldi (tablet)	500 µg/ml (equivalent wt.)	499.05	99.81

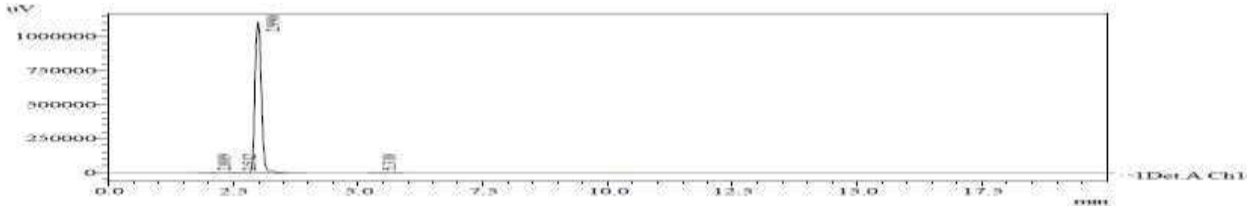

Fig-2 : Chromatogram of standard solution of Sofosbuvir.

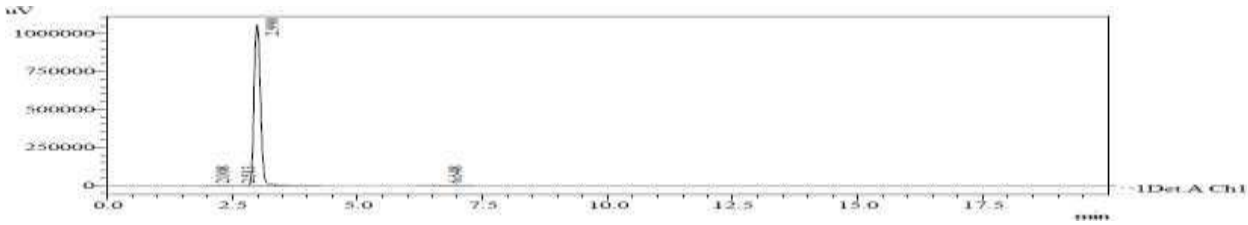

Fig-3: Chromatogram of Sovaldi-500 mg tablet dosage form.

Table – 2: Linearity results for sofosbuvir.

Concentration of drug (µg/mL)	Retention time (min.)	Peak Area
100	2.963	2205366
200	2.985	4450760
300	2.987	6584010
400	3.044	8065447
500	2.968	10058134
600	3.034	11749660

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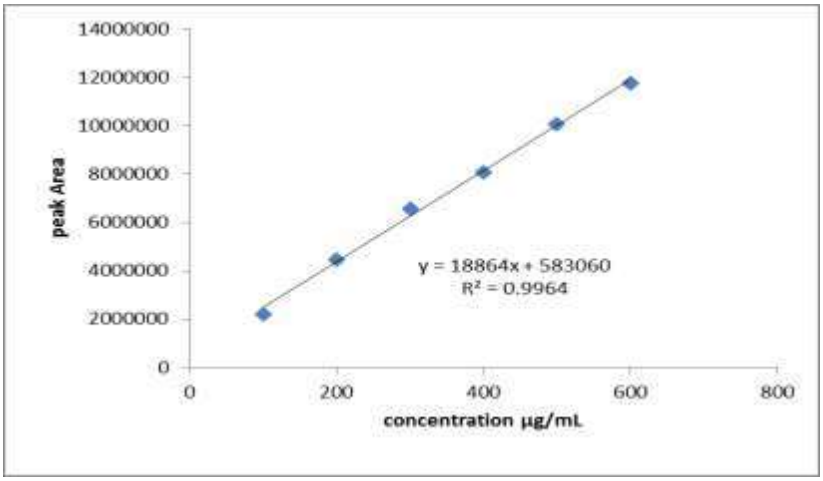

Fig.- 4: Calibration curve of Sofosbuvir.

Table – 3: Inter-day precision

Inj.	Retention time (min)	Peak Area
1	2.982	9931517
2	2.978	9927713
3	2.980	9929188
4	2.974	9926435
5	2.972	9925327
6	2.974	9906283
Mean	**2.977**	**9924410.5**
Std.Dev	0.131	9140.88
%RSD	0.004	0.092

Table – 4: Intra-day precision

Inj.	Retention time (min)	Peak Area
1	2.991	9982586
2	2.978	9933753
3	3.074	9962273
4	2.971	9954863
5	2.971	9952183
6	2.988	9913721
Mean	**2.996**	**9949896.5**
Std.Dev	1.313	23753
%RSD	0.039	0.239

Table- 5:

Accuracy results for Sofosbuvir.

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 (10%)	Standard	Spiked (10%)	Standard	Spiked (10%)
1	8191294	8922484	9948703	10784935	11742762	12321031
2	8273988	8863628	9963923	10719931	11765161	12269195
3	8061684	8545883	9966516	10798878	11735631	12299054
Mean	**8175655**	**8777332**	**9959714**	**10767915**	**11747851**	**122964427**
Std.Dev	107012	202589	9624	42136	15409	26018
%RSD	1.309	2.308	0.097	0.391	0.131	0.212
% Recovery	**98.4**		**100.29**		**99.38**	
Average % Recovery	**99.35**					

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Table – 6: LOD & LOQ values for sofosbuvir.

S.No.	% concentration	Concentration(µg/ml)	Peak Area
1	10%	50	1109818
2	5%	25	562792
3	2%	10	225205
4	1%	5	111317
5	0.50%	2.5	56943
6	0.20%	1.0	23622
7	0.10%	0.5	12317
8	0.05%	0.25	7546
9	0.02%	0.1	3626
10	0.01%	0.05	1892
11	0.005%	0.025	ND
LOD:		**0.05 µg/ml**	
LOQ:		**0.15 µg/ml**	

Table – 7: Robustness results for sofosbuvir.

Parameter	Peak areas for flow rate		Peak areas for Variable Column		Peak areas for pH change	
	Flow rate 1.2 ml	Flow rate 0.8 ml	Zobrax eclipse XBD-C18	Inertsil ODS - C18	pH - 3.3	pH - 3.1
Injection-1	8977551	11249640	9918274	9915857	8973715	11011114
Injection-2	8931754	11094626	9924921	9890454	8979611	11019735
Injection-3	8999892	10956161	9927796	9977939	8983432	11318097
Mean	**8969732**	**11100146**	**9923664**	**9928083**	**8978919**	**11116315**
Std. dev	34735.36	146822.34	4884	45006	4895	174801
% RSD	0.387	1.323	0.049	0.453	0.055	1.572

Table – 8: System suitability parameters.

Parameter	Results of the proposed HPLC method
Retention time (min)	2.989
Theoretical plates (n)	2504.970
Plates per meter (N)	16699.80
HETP (L/n)	$5.759x10^{-5}$
Peak asymmetry (Tailing)	1.178
Linearity range (µg/mL)	100-600
Regression coefficient (R^2)	0.996
Limit of Detection (µg/mL)	0.05
Limit of Quantification (µg/mL)	0.15

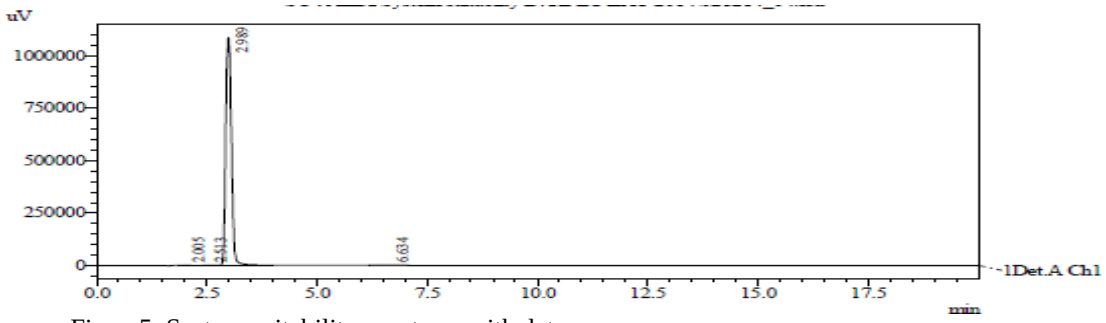

Fig. – 5: System suitability spectrum with data.

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