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Original Article

Development and validation of new RP-HPLC method for the determination of sofosbuvir in pure form

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ABSTRACT

The present work is concerned with application of simple, precise, accurate, reproducible and specific RP-HPLC method for estimation of Sofosbuvir in bulk. Separation of SFS was successfully achieved on a Hisil C18 (4.6 x 250mm, 5 µm) Waters or equivalent in an isocratic mode utilizing Phosphate Buffer (4.0 pH): Methanol (50:50%v/v) at a flow rate of 0.8 mL /min and eluate was monitored at 262 nm, with a retention time of 1.01 minutes. The method was validated and the response was found to be linear in the drug concentration range of 5 µg/mL to30µg/mL. The values of the slope, intercept and the correlation coefficient were found to be 0.07, -0.4 and 1.000 respectively. The RSD values for system precision and method precision were found to be 0.19 % (Intra-day), 0.21% (Inter-day) and 0.20 % (Intra-day), 0.23 % (Inter-day) respectively.

INTRODUCTION

Sofosbuvir 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]. The nucleoside triphosphate is a non-obligate chain-terminating analogue of UTP that competes for incorporation at the HCV NS5B polymerase active site. Viral RNA synthesis is inhibited secondary to incorporation of the phosphorylated metabolite into nascent viral RNA by the HCV RNA-dependent RNA polymerase. Chemically, It is (*S*)-isopropyl-2-((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyl tetrahydrofuran-2-yl) methoxy) - (phenoxy) phosphorylamino) propanoate (Fig.No.1).It is a White to off-white non-hygroscopic crystalline solid[2]. Slightly soluble in water (pH 1.2-7.7), freely soluble in ethanol and acetone, soluble in 2-propanol, and insoluble in heptanes [3]

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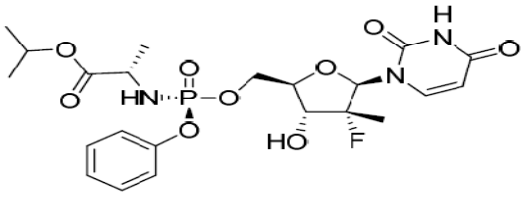

Figure 1. Structure of Sofosbuvir

Literature survey reveals a few HPLC methods, LC-MS method have been used. The objective of the present work was to develop simple, rapid, accurate, specific and economic RP-HPLC stability indicating method.

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP-HPLC C18 method with UV detection for the determination of Sofosbuvir in bulk form. The important features and novelty of the proposed method included simple sample treatment with sonicator of small amount of powder sample at ambient temperature, shot elution time(less than 5 min)SFS, good precision (R.S.D. less than 2%)

Conformation of the applicability of developed method validated according to the international conference on Harmonization (ICH)[6].

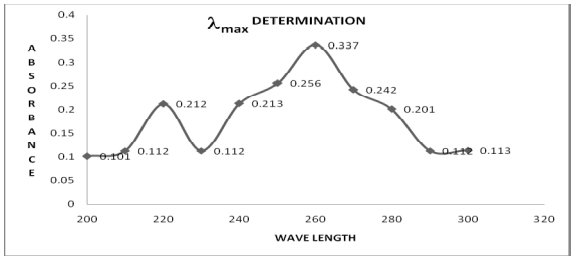

Figure 2. UV Spectrum of Sofosbuvir IN pH 4 Phosphate Buffer (10µg/ml)

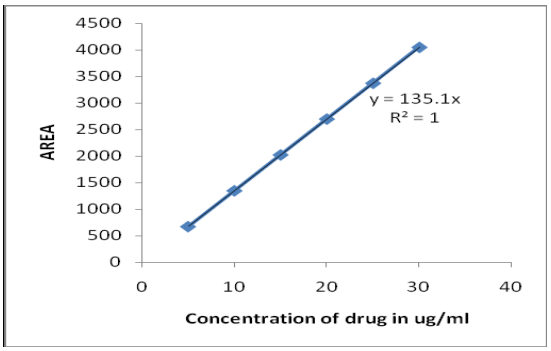

Figure 3.Calibration graph of Sofosbuvir

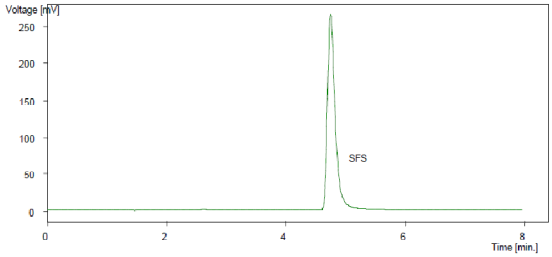

Figure 4. A model chromatogram for Sofsobuvir

MATERIALS AND METHODS

Chemicals

Sofosbuvir was obtained from HETERO Pharmaceuticals. And was used as such without further purification[7].

Reagents

Methanol (HPLC grade), Water (HPLC grade), Potassium dihydrogen phosphate (GR grade),Orthophosphoric acid(GR grade)

Instruments and Equipments

High Performance Liquid Chromatography (Shimadzu HPLC, Class VP series) with LC-10AT VP pumps, manual injector with loop volume of 10 µl (Rheodyne), programmable variable wavelength UV detector[8].

Preparation of buffer

Weigh accurately 1.75 g of potassium dihydrogen phosphate and dissolve it in 1000 ml of HPLC Grade water. And adjust the pH to 4 with dilute orthophosphoric acid, filter through 0.45µm nylon membrane filter and degas.

Preparation of mobile phase

Mix a mixture of above buffer 500mL (50%) and 500mL of Methanol HPLC (50%) and degas in ultrasonic water bath for 5minutes.Filter through 0.45µ filter under vacuum filtration.

Preparation of standard and sample solutions

Stock solution of SFS (1mg/mL) was prepared by weighing 10mg and dissolving in the mobile phase Phosphate buffer (pH4.0): Methanol (50:50%v/v). Standard solutions of SFS were prepared in the range of 5µg/mL to 30µg/mL by diluting the stock solution with mobile phase. The eluate was monitored at 260nm.Each solution was then injected into the column and chromatograms were recorded.

Table 1: Optimized method of parameters

PARAMETERS	METHOD
COLUMN	C_{18}(250×4.6MM,5µm)
MOBILE PHASE	Phosphate buffer : methanol(50 : 50)
FLOW RATE	0.8ml/min
RUN TIME	8min
COLUMN TEMPERATURE	Ambient
VOLUM OF INJECTION LOOP	10µl
DETECTOR WAVE LENGTH	262nm
DRUG RT	1.01min
LINEARITY RANGE	5-30 µg/ml

Table 2: Calibration curve in pH 4 phosphate buffer

Concentration ppm	Average Area	Statistical Analysis	
5	675	Slope	0.07
10	1351	y-Intercept	-0.4
15	2027	% of y- Intercept	-0.00006
20	2703	Correlation Coefficient	1.000
25	3378	r2	1.000
30	4054		

Table 3: System precision (Intra day)

INJECTION	Peak area	% Assay	
1	2701	Mean	2694.8
2	2696		
3	2690	SD	5.16
4	2689		
5	2698	%RSD	0.19

RESULTS AND DISCUSSION

In this paper we developing the reverse phased column procedure for a suitable method for the pharmaceutical analysis of Sofsobuvir drug. Atypical Chromatogram obtained by using the mobile phase (Figure No2),. The precision and Accuracy of the method was determined.

Table 4: System precision (Inter day)

INJECTION	Peak area	% Assay	
1	2704	Mean	2695
2	2690		
3	2696	SD	5.74
4	2690		
5	2695	%RSD	0.21

Table 5. System precision (Intra day)

INJECTION	Peak area	% Assay	
1	2703	Mean	2695.4
2	2696		
3	2689	SD	5.596
4	2691		
5	2698	%RSD	0.20

Table 6. System precision (Inter day)

INJECTION	Peak area	% Assay	
1	2701	Mean	2696.8
2	2696		
3	2690	SD	5.24
4	2689		
5	2698	%RSD	0.23

Sofosbuvir dosage forms inter and intraday studies were performed in two consecutive days. The method was validated for linearity, precision and accuracy parameters[9]. Linearity of the method was studied by injecting six concentrations of drug prepared in the mobile phase in the range 5-30 microgram/millilitre and solutions are analyzed through the high pressure liquid chromatographic technique (Figure No. 4). The peak area were plotted against concentration was subjected to linear plot and the results present in table (Table no.2). Precision of this method was studied in inter day and intra-day variation[12]. The precision of intraday studies was repeated on two consecutive days (Table No.3-6). The developed method was found to be precise as the percentage of RSD values for inter-day and intra –day precision studies were found to be less than 2% (Table no.7).

CONCLUSION

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of Sofosbuvir from pure and its dosage forms. The mobile phase is simple to prepare and economical. The developed method is accurate, precise and reliable for the analysis of Sofosbuvir in Pharmaceutical formulations.

This method was validated for linearity, accuracy and precision of sofosbuvir drug. The RSD values for all parameters were found to be <2, which indicates the validity of method and results obtained by this method is with fair agreement. Hence, this method can be easily and conveniently adopted for routine analysis of Sofosbuvir in pure form and also can be used for dissolution or similar studies

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Table 7. Robustness

Parameters	Optimum range	Conditions in procedure	Remarks
Flow rate (ml/min)	0.8-1.0	1.0	At lower flow rates the asymmetry factor was increased and at higher flow rates the retention time was decreased
pH OF MOBILE PHASE	3-7	Ambient	Beyond the optimum range of pH of the mobile phase ,change in RT and Peak shape was observed

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