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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF SOFOSBUVIR IN BULK AND FORMULATION

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ABSTRACT
A Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the estimation of Sofosbuvir which is a new antiviral drug. The RP-HPLC was succeeded on a Phenomenex Luna® LC C18 column (150×4.6mm, 5µm). The mobile phase was effective according to the polarity of studied drug. The mobile phase was consisting of Acetonitrile: Methanol: Water in the ratio of 50:30:20 v/v/v using at flow rate of 1ml/min. with injection volume of 20 µL was selected for this present work. Detection was made by using UV detector at 260nm. Retention time was found to be 2.1min. The developed method was validated according to the ICH guidelines. The calibration curve was linear for Sofosbuvir in the concentration range of 10-50 µg/ml was good. The developed method was validated for Linearity, Precision, Accuracy and Robustness of Sofosbuvir drug and was accurate, precise and reliable for the analysis of Sofosbuvir in formulation. The Relative Standard Deviation for all the parameters were found to be less than 2 which shows the validated method and results obtained by this method is with fair agreement. Hence, this developed method can be easily effortlessly adopted for routine analysis for Sofosbuvir in bulk and formulation.

Keywords: Sofosbuvir, Method Development, Validation, RP-HPLC, ICH.

1. INTRODUCTION
The chemical name of Sofosbuvir is propane-2-yl(2S)-2-{[[2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy-phenoxy-phosphosphoryl]amino}propanoate. Its molecular formula is $C_{22}H_{29}FN_3O_9P$ and molecular weight is 529.458g/mol (Fig.1) [1, 2].

Sofosbuvir is an antiviral drug from the nucleotide polymerase inhibitors class, used to treat chronic hepatitis C virus (HCV) infection [3]. Sofosbuvir has approval to treat HCV infected patients with HCV genotype 1, 2, 3 or 4 and also experienced patients including those with HIV-coinfected and compensated cirrhosis. Limited data is available for treatment of chronic HCV infection caused by genotype 5 or 6. Metabolism of Sofosbuvir was cleaved by Cat and CES 1 and eventually activation steps included amino acid removal by histidine triad nucleotide-binding protein 1 (H1NT1) and phosphorylation by uridine monophosphate- cytidine monophosphate (ump-cmp) kinase and nucleoside diphosphate (NDP) kinase [4]. NS5B protein is a RNA dependent RNA polymerase so it is critical for the viral reproduction cycle [5].

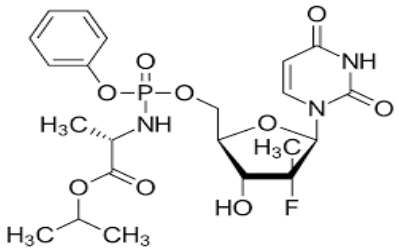

Fig. 1: Structure of Sofosbuvir

Sofosbuvir is a nucleotide prodrug and Hepatitis C Virus (HCV) NS5B polymerase inhibitor with the potential HCV inhibiting activity. Administration of orally dose, Sofosbuvir has metabolized to 2'-deoxy-2'-alpha-fluorobeta-C-methyluridine-5-monophosphate, then converted into the active triphosphate nucleotide that inhibits the NS5B polymerase thereby prevents viral replication [6].

The pharmacokinetics of Sofosbuvir and predominant circulating metabolite GS-331007 have been evaluated in healthy subject and subject with chronic hepatitis C.

Oral administration doses up to 400mg given to both subjects, Sofosbuvir was absorbed with peak plasma concentration observed at ~0.5-2 hours post dose and regardless of the dose level and GS-331007 peak was observed between 2-4 hours post dose [7].

From literature survey, it was found that few methods are available for determination of single drug and in combination containing Sofosbuvir and Ledipasvir by UV-VIS spectroscopes [8-11], RP-HPLC methods [11-18]. The aim of the study is to develop and validate a sample, precise and accurate RP-HPLC method for Sofosbuvir in bulk and formulation as per ICH guidelines.

2. MATERIAL AND METHODS

2.1. Chemicals and reagents

The pure standard drug of Sofosbuvir was obtained as a gift sample from MSN Pharmachem Pvt. Ltd. in Telangana, India and it was supplied by Zim Laboratories Ltd. Nagpur, India. Hepcinat 400 mg manufactured by NATCO Pharma Ltd. Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade) were obtained from Merck pharmaceuticals Private Ltd., Mumbai, India. Methanol 99.5% Extra pure was obtained from Loba Chemie Pvt. Ltd., Mumbai.

2.2. Instruments

Analytical balance (Shimadzu AY 220), UV-Spectrophotometer (Systronic 2201), Sonicator (Oscar Microclean 103), High Performance Liquid Chromatography (Younglin Acme 9000) were used.

2.3. Chromatographic equipment and conditions

Chromatographic separation was achieved on a Phenomenex Luna® LC C18 Column (150 × 4.6mm, 5µm). The mobile phase used for the separation of Sofosbuvir was Acetonitrile: Methanol: Water in the ratio of 50:30:20 v/v/v. Flow rate was set at a 1ml/min at ambient temperature 30°C, an injection volume of 20µL. Using UV-Visible detector (UV730D) at wavelength of 260nm. The mobile phase was ultra-sonicated for degassing and filtered through 0.45µm membrane nylon filter using vacuum pump before pumping into HPLC system.

2.4. Preparation of Mobile Phase

HPLC grade Acetonitrile, Methanol and Water were used for the preparation of mobile phase in the ratio 50:30:20v/v/v. Each component of mobile phase was filtered twice using 0.45µm membrane filter and degassed for 15min by sonicating each component of mobile phase.

2.5. Preparation of Solution

2.5.1. Preparation of Standard stock solution

Standard stock solution of Sofosbuvir was prepared by transferring 10mg in 10 ml volumetric flask by using methanol as a diluent and volume was made up to mark and sonicated for 5min. (Conc.= 1000µg/ml). 3ml standard stock solution was pipette out and transferred in to10ml of volumetric flask and volume was made up to the mark with same diluent and further added 20 ml of same diluent to get concentration 100µg/ml.

2.5.2. Preparation of sample solution

Twenty Hepcinat 400mg of film coated tablets were weighed, crushed, finely powdered and mixed well. A portion of tablet powder equivalent to weight of 10 mg of Sofosbuvir was transferred to a 10 ml of volumetric flask and volume was made up to the mark with same diluent (Methanol) and sonicated for 15 minutes to complete dissolution of drug. The solution was filtered through Whatman filter paper no.1 to remove insoluble residue. The above prepared solution was further diluted to get required concentrations then analyzed the by proposed procedures.

2.6. Analytical Method Development [19]

The proposed RP-HPLC method of analysis was validated following ICH Q2 (R1) guidelines by studying the parameters like system linearity, accuracy, assay, precision, suitability, specificity, LOD, LOQ and robustness.

3. RESULTS AND DISCUSSION

3.1. Method Development

The proposed RP-HPLC method was developed and optimized for a series of trials in a terms of mobile phase selection, composition, wavelength, choice of stationary phase of column, flow rate and column temperature. Sofosbuvir showed the absorbance maxima at 260nm. Hence, the wavelength was selected as a working wavelength for the proposed RP-HPLC method.

3.2. Linearity and Range

The calibration curve was constructed between concentrations versus peak area by preparing in the

concentration range of 10-50µg/ml. The regression equation was found to be Y=15.33x+7.1 and correlation coefficient of Sofosbuvir was noted at 0.999 (Fig. 2). Overlay of chromatograms of concentration 10-50µg/ml of Sofosbuvir is shown in Fig.3. The ranged from 10-50µg/ml.

Table 1: linearity of Sofosbuvir

Conc.(µg/ml)	Peak Area
10	169
20	306
30	464
40	615
50	781

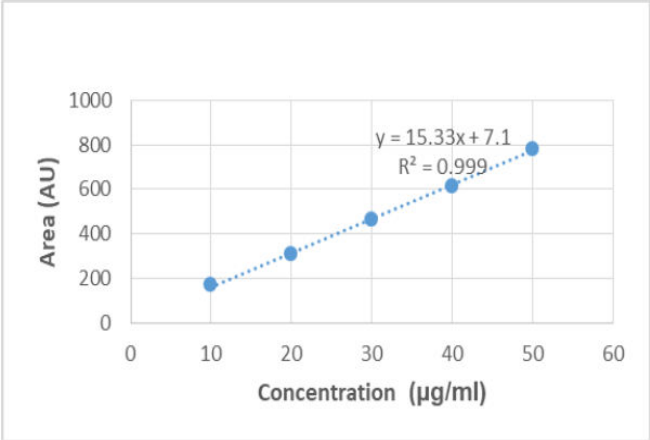

Fig. 2: Calibration curve of Sofosbuvir

3.3. Accuracy

The accuracy of the method was validated by analyzing three quality control samples of Sofosbuvir representing three concentration levels covering the specified linearity range and then calculating the percentage recovery. It was obtained from 98.8-100.5% for the method which confirms the accuracy of developed method (Table 2).

3.4. Assay

The proposed method was successfully adopted for the determination of Sofosbuvir in Hepcinat 400mg film coated tablet. Percentage recovery of Sofosbuvir was found to be 102.8% (Table 3). The method can be employed for routine analysis of estimation of Sofosbuvir in the formulation.

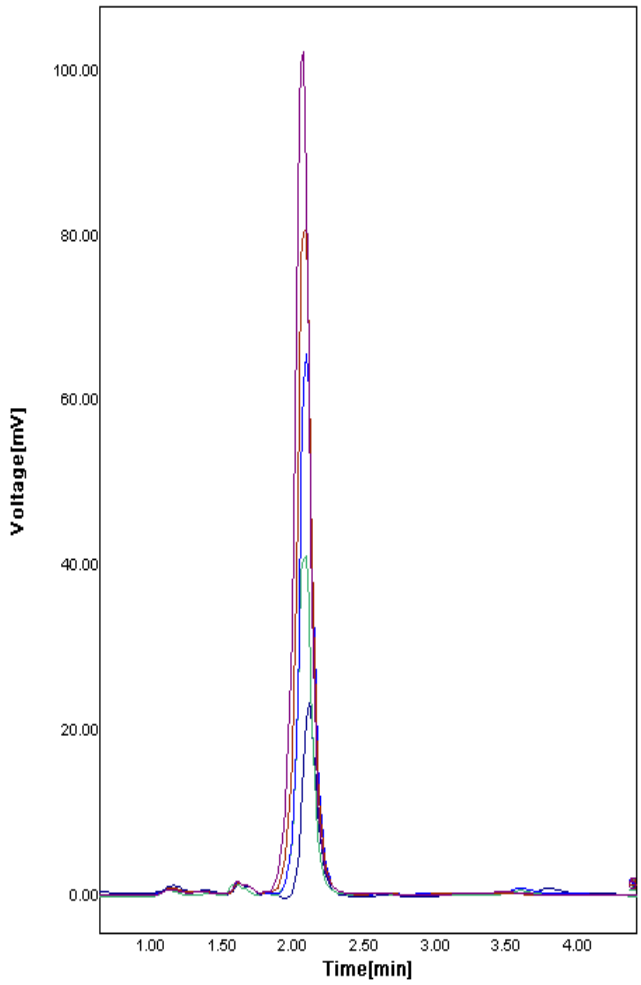

Fig. 3: Overlay of chromatograms of concentration 10-50µg/ml of Sofosbuvir

Table 2: Accuracy result of Sofosbuvir

Formulation	Spiked level (%)	Amount standard drug added (µg/ml)	Amount found (µg/ml)	% Recovery
Hepcinat	80	8	7.9	98.8
	100	10	10.05	100.5
	120	12	12.01	100.1

Table 3: Assay result of Sofosbuvir formulation

Brand name	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery
Hepcinat 400 mgtablet (NATCO Pharma Ltd. India)	30	30.85	102.8

3.5. Precision

The % RSD values for intra-day and inter-day precisions were found to be < 2% for the proposed method which confirm the good precision of the method (table 4).

Table 4: Intraday and Interday Precision results of Sofosbuvir

Sr. No.	Intraday Precision		Interday Precision	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
1	20	333	20	537
2		327		540
3		325		558
4		337		536
5		319		544
6		330		529
Average		328.5	**Average**	540.7
STDEV		6.32	**STDEV**	9.83
%RSD		1.923	**%RSD**	1.818

3.6. System suitability and Specificity

All the system suitability parameters such as retention time, tailing factor and theoretical plates were studied from standard chromatogram and to evaluate system suitability are given in Table 5.

The analytical parameter of specificity study is to determine whether the effect of excipients and other additives that are usually present in the pharmaceutical formulations of sofosbuvir are interfering with the peaks of the analytes or not in optimum chromatographic conditions. The specificity of the study was determined by comparing test results of standard solution and sample solution. Chromatograms were observed and compared for interference of excipients (Fig.4).

Table 5: System suitability results of Sofosbuvir

Parameters	Values
Theoretical plates (N)	3183
Retention time (min)	2.1
Tailing factor	1.18

3.7. Limit of Detection & Limit of Quantification

The developed method was highly sensitive with LOD of 1.87µg/ml and LOQ of 5.65µg/ml.

3.8. Robustness

The robustness of the developed method was accessed by small changes in method parameters such as flow rate (±0.1ml/min), mobile phase composition (±1%) and detection wavelength (±1nm). The %RSD of robustness was found to be less than 2% of the proposed method (Table 6).

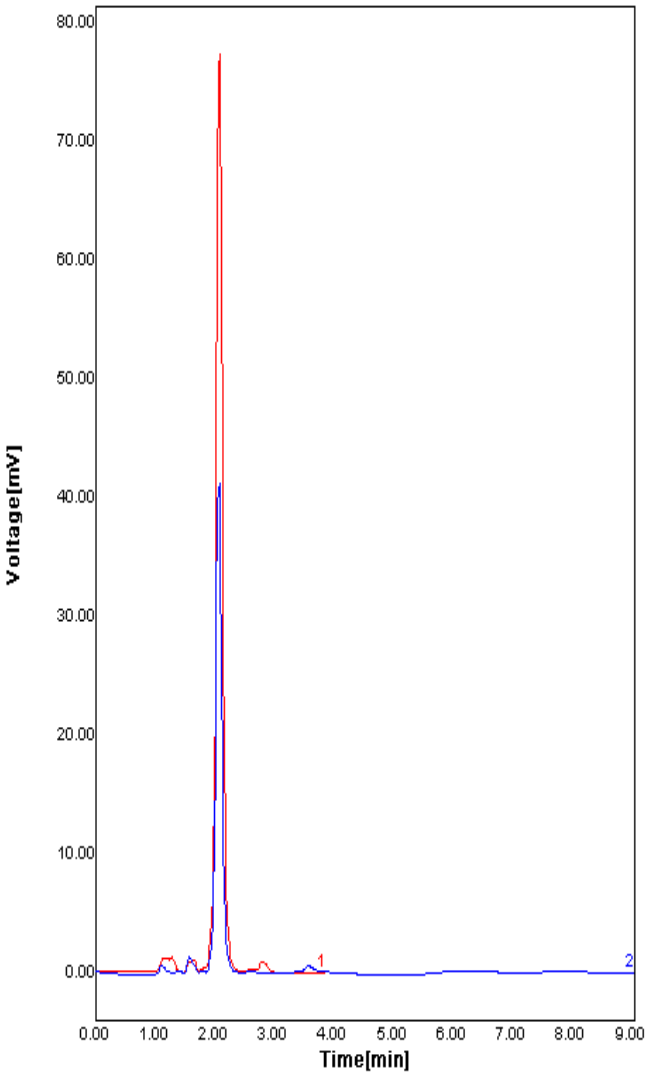

Fig. 4: Overlay of 20µg/ml standard solution chromatogram (Blue) and 30µg/ml of sample solution chromatogram (Red)

Table 6: Robustness result of Sofosbuvir

Parameters	Optimized	used	Area	Average	STDEV	%RSD
Flow rate (±0.1ml/min)	1.0	0.9	339	337	6.25	1.85
		1.0	330			
		1.1	342			
Detection wavelength (±1nm)	260	259	341	334.3	5.86	1.75
		260	330			
		261	332			
Mobile phase composition (Acetonitrile: Methanol:Water) [v/v/v]	50:30:20	49:31:20	560	571.3	10.26	1.796
		50:30:20	580			
		51:29:20	574			

Table 7: Summary of sofosbuvir

Parameter	Sofosbuvir
Linearity range (µg/ml)	10-50
Regression equation	Y=15.33x+7.1
Slope	15.33
Intercept	7.1
Regression coefficient	0.999
Repeatability(n=6) %RSD	Intraday=1.92
	Interday=1.82
% Recovery (Accuracy)	98.8-100.5%
LOD(µg/ml)	1.87
LOQ(µg/ml)	5.65

4. CONCLUSION

A novel RP-HPLC method was developed and successfully validated as per ICH guidelines for the estimation of sofosbuvir. All the results of various parameters were found to be within the acceptance limits. Hence the proposed RP-HPLC method for the analysis of sofosbuvir in bulk and its formulation was found to be specific, precise, accurate and fast.

5. ACKNOWLEDGEMENT

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