

## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF SOFOSBUVIR BY PURE AND TABLET DOSAGE FORM”

**Jayesh A. Patil\*, S. T. Patil, B. R. Rane and S. P. Pawar**

Department of Quality Assurance P.S.G.V.P. Mandal's Cop Shahada Dist. Nandurbar,-  
425409 Maharashtra.

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### \*Corresponding Author

**Jayesh A. Patil**

Department of Quality  
Assurance P.S.G.V.P.  
Mandal's Cop Shahada Dist.  
Nandurbar,-425409  
Maharashtra.

### ABSTRACT

A simple, precise, rapid and reproducible RP-HPLC method was developed and validated for the determination of Sofosbuvir in Pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a C18 (PRIMESIL) column (250 X 4.6 mm i.d., particle size 5 $\mu$ m.). The mobile phase consisted of Acetonitrile:Water (pH 2.4 adjusted with 0.05% orthophosphoric acid) in ratio 80:20 (v/v) an gradient elution at a flow rate of 0.7 ml/min at ambient temperature. The detection was carried out at 260nm using UV-Visible detector. The retention time of Sofosbuvir is found to be 4.3 min and the calibration curve was linear in the concentration range of 20-100  $\mu$ g/ ml ( $r^2=0.997$ ). The limit of detection and the limit of

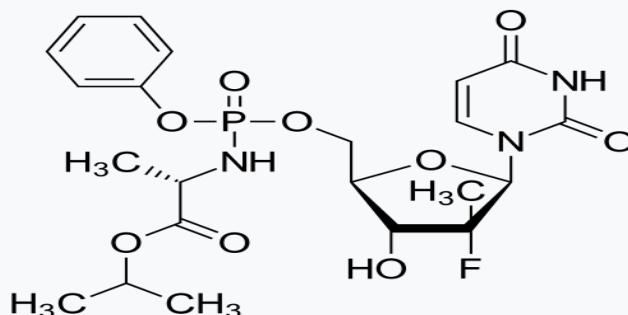
quantification were found to be 0.5355 and 1.6228  $\mu$ g/ml respectively. The amount of sofosbuvir present in the formulation was found to be 99%. The method was validated statistically using the SD, %RSD and values are found to be within the limits and the recovery studies were performed and the percentage recoveries was found to be 98% ( $\pm 1\%$ ) to 102% ( $\pm 1\%$ ). So, the proposed method was found to be simple, specific & linear. Hence it can be applied for routine analysis of sofosbuvir in the pharmaceutical formulations.

**KEYWORDS:** RP-HPLC, Sofosbuvir, UV detection, Acetonitrile:water.

### INTRODUCTION

IUPAC name of sofosbuvir is Isopropyl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxypyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate. with empirical formula of C<sub>22</sub>H<sub>29</sub>FN<sub>3</sub>O<sub>9</sub>P and molecular

weight 529.4525 g/mol. It is a White to Off-white non- hygroscopic Crystalline solid. slightly soluble in water (pH 1.2-7.7), freely soluble in ethanol and acetone, soluble in 2-propanol and insoluble in Heptane. The chemical structure of SOF was shown in Fig.1.<sup>[1,2,3]</sup>



**Fig. 1 chemical structure of sofosbuvir**

FDA has received the New Drug Application for sofosbuvir was submitted on 8 April 2013, granted priority review status and was approved by FDA for the treatment of chronic hepatitis C on 6<sup>th</sup> December 2013. The FDA approved sofosbuvir in combination with the other drug. Hepatitis caused by HCV has become a major emerging infectious disease, affecting the liver, caused by hepatitis C virus (HCV). The hepatitis c virus (HCV) is spread by blood-to-blood contact and major cause of chronic liver disease with ~170 million infected worldwide.(ref. seeff LB. natural history of chronic hepatitis C. hepatology 2002;36(suppl 1):S35-46). approximately 18 million people infected with hepatitis C virus (HCV).<sup>[4]</sup>

Sofosbuvir is a pro-drug using the Pro-tide biotechnology strategy. It is metabolized to the active anti-viral agent, 2'-deoxy-2'-  $\alpha$  -fluoro- $\beta$ -methyluridine-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.<sup>[5]</sup>

“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 SOF in pure and tablet dosage forms as per ICH guidelines.”<sup>[6]</sup>

## EXPERIMENTAL

### REAGENTS AND MATERIALS

**Chemical:** Pure standard of Sofosbuvir was obtained as gift sample from reliable's shree industrial training centre jalgoan.

**Reagents:** Acetonitrile (HPLC grade), Water (HPLC grade), Potassium dihydrogen phosphate (GR grade), Orthophosphoric acid (GR grade).

SOFOVIR Tablets (Hetero Drugs Limited) containing Sofosbuvir Marketed formulation was purchased from local market, High precision weighing balance (wensar instruments, hyderabad), micro pipette (in labs, 10100  $\mu$ l) 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°C. However, the Chemical structure and purity of the sample obtained were confirmed by melting point study.

### HPLC APPARATUS AND CHROMATOGRAPHIC CONDITIONS

Chromatographic separation was achieved using a C-18 column (250mm  $\times$  4.6mm id., 5 $\mu$ m particle size) of Younglin (S.K) Gradient system that is equipped with UV Detector. Sonicator (Labindia Instruments).

**Table no:1 Chromatographic Condition**

Sr.no.	Parameter	Description
1	Stationary Phase	C <sub>18</sub> column with 250 mm $\times$ 4.6 mm id and 5 $\mu$ m particle size
2	Mobile Phase	Acetonitrile:Water(80:20) pH2.4 adjusted with 0.05%OPA
3	Flow Rate	0.7 ml/min
4	Detection wavelength	260nm
5	Detector	UV detector
6	Injector	Rheodyne Injector
7	Injection volume	20 $\mu$ l
8	Column Temperature	Ambient

### Preparation of standard stock solution

10mg accurately weighed drug (sofosbuvir) was put into 10ml volumetric flask containing 10 ml of methanol and sonicated for 10 mins. That makes 1000 $\mu$ gm/ml standard stock solution.

### Preparation of sample solutions

1) Take 0.2 ml from stock I and make vol. 10 ML with mobile phase

- = 20  $\mu\text{gm/ml}$  SOFOSBUVIR
- 2) Take 0.4 ml from stock I and make vol. 10 ML with mobile phase  
= 40  $\mu\text{gm/ml}$  SOFOSBUVIR
- 3) Take 0.6 ml from stock I and make vol. 10 ML with mobile phase  
= 60  $\mu\text{gm/ml}$  SOFOSBUVIR
- 4) Take 0.8 ml from stock I and make vol. 10 ML with mobile phase  
= 80  $\mu\text{gm/ml}$  SOFOSBUVIR
- 5) Take 1 ml from stock I and make vol. 10 ML with mobile phase  
= 100  $\mu\text{gm/ml}$  SOFOSBUVIR

## RESULT AND DISCUSSION

The development of the HPLC method for determination of drugs has received considerable attention in recent years because of its importance in routine quality control analysis. A HPLC method was proposed as a suitable method for the estimation of sofosbuvir in pure and tablet dosage form. A good separation was achieved using a C-18 (250 x 4.6 mm, 5  $\mu\text{m}$ ). The Chromatographic condition was adjusted in order to provide a good performance of assay. The method involved a mobile phase consisting of Acetonitrile and Water (pH 2.4 adjusted with 0.05% orthophosphoric acid) in ratio of 80:20 (v / v) accomplished at 260 nm. The retention time 4.35 at flow rate of 0.7 ml/min. and the injection volume was 20  $\mu\text{l}$  the total run time for an assay was approximately 4.5 min.

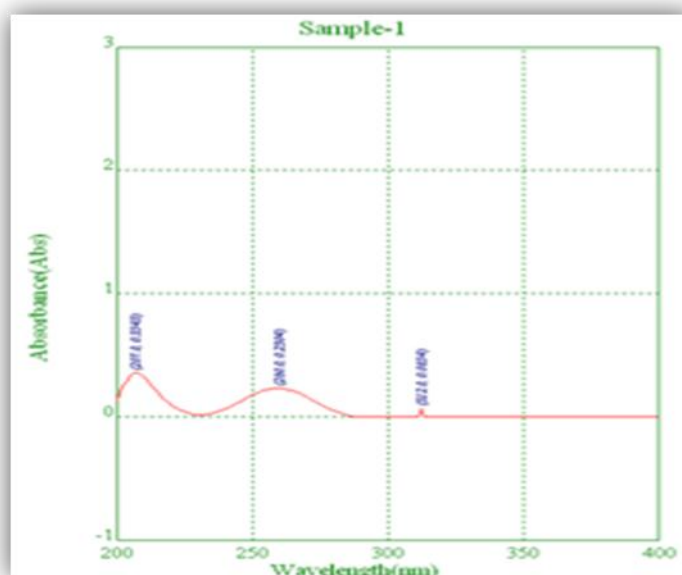


Fig. no. 2: Overlain spectra of sofosbuvir

- HPLC Method development and optimization: the finally chromatographic condition are,

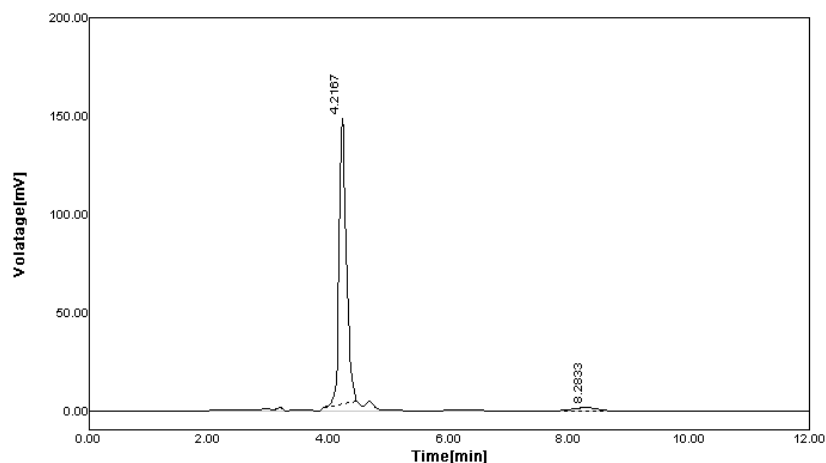


Fig. no. 3: Optimized chromatogram of sofosbuvir

### Parameters of Validation

#### 1. Linearity and Range

The linearity of an analytical method is its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. The range of an analytical method is the interval between upper and lower analyte concentration in sample including these concentrations for which it has been established that the analytical method has a suitable level of linearity, accuracy and precision. To establish the linearity and range of proposed methods, various aliquots of standard solution of drug were prepared from stock solution and analyzed. Sample solutions of drug with different conc. from 20 to 100 µg/ml were analyzed by HPLC at 260 nm respectively. Their area measured.

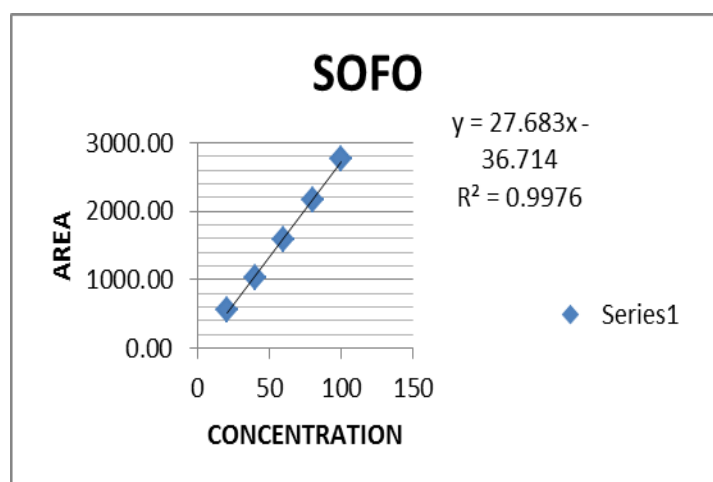
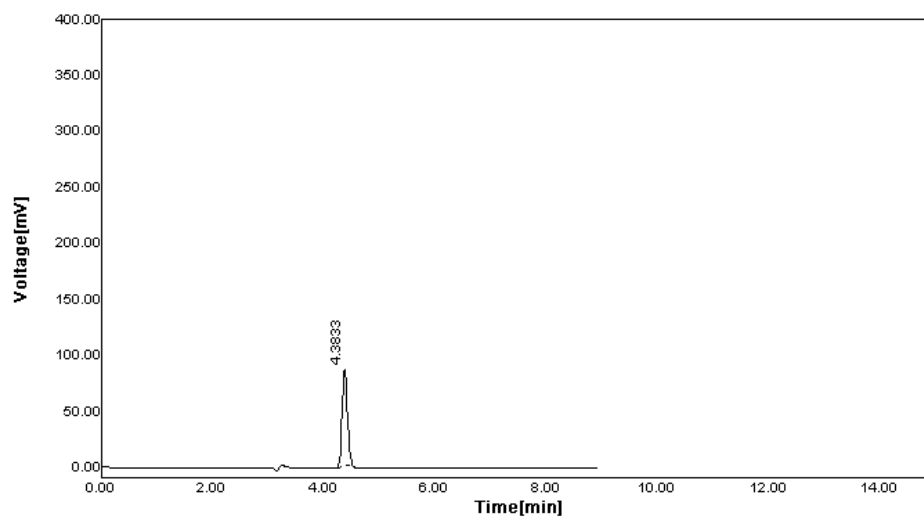
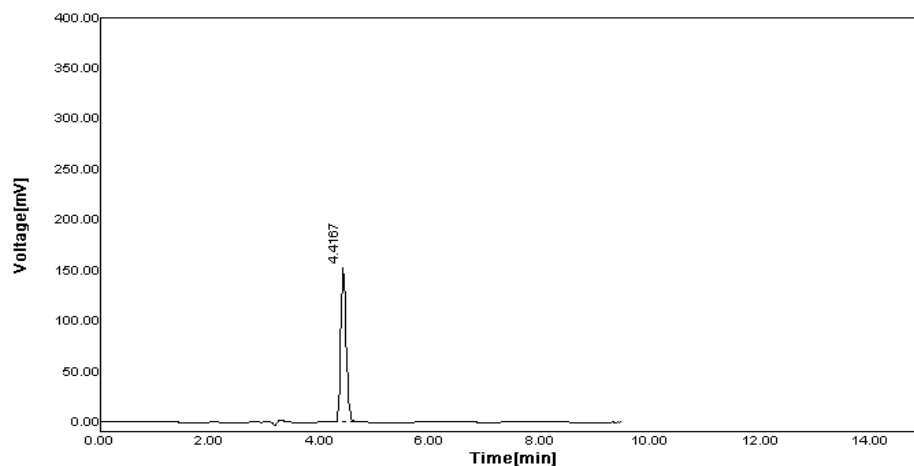
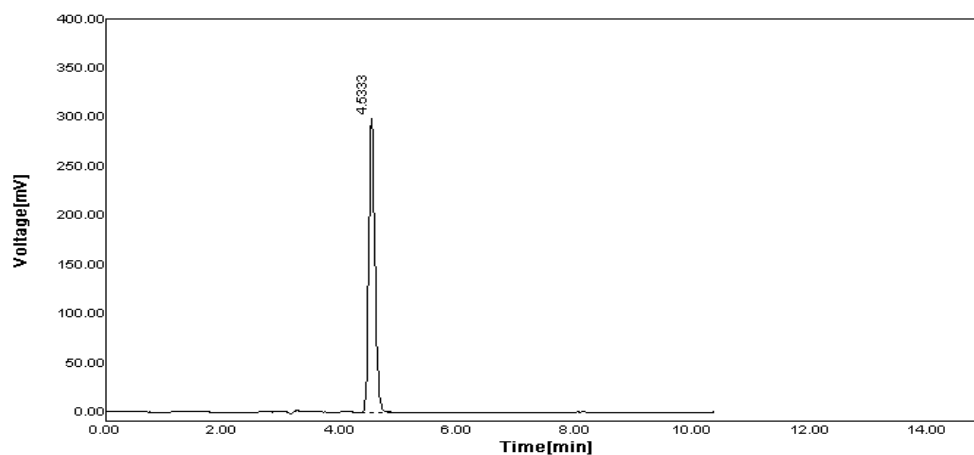


Fig No: 4. linearity studies of sofosbuvir

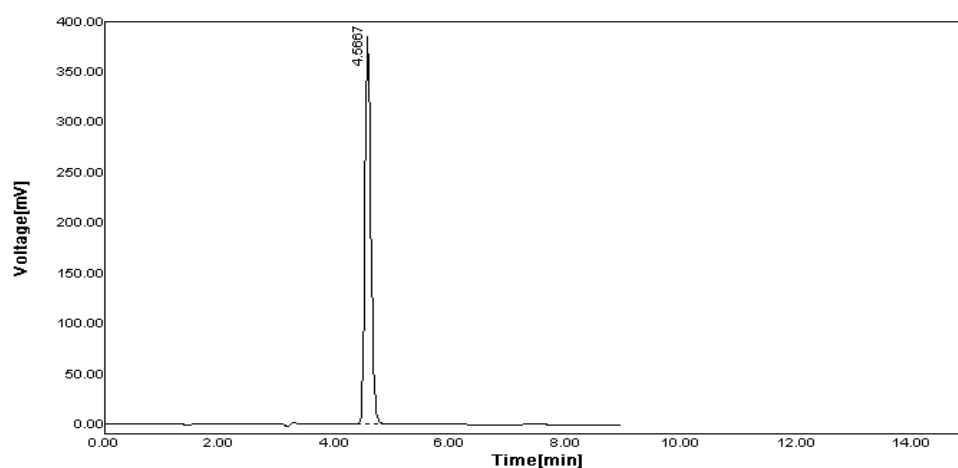
Table No: 2. linearity of sofosbuvir

Sr.No.	Concentration $\mu\text{g/ml}$	Area	%RSD
1	20	567.98	0.22
2	40	1034.08	0.56
3	60	1534.08	1.17
4	80	2164.20	0.32
5	100	2771.20	0.12

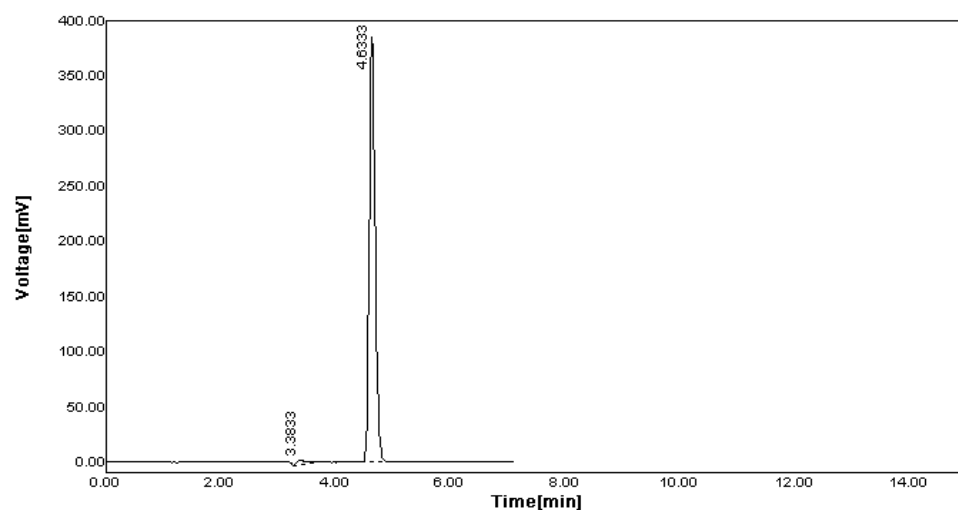
Fig No: 5. chromatogram of linearity of sofosbuvir (20 $\mu\text{g/ml}$ )Fig No: 6. Chromatogram of linearity for sofosbuvir (40 $\mu\text{g/ml}$ )



**Fig No: 7. Chromatogram of linearity for sofosbuvir (60µg/ml)**



**Fig No: 8. Chromatogram of linearity for sofosbuvir (80µg/ml)**



**Fig No: 9. chromatogram of linearity for sofosbuvir (100µg/ml)**

**Table No: 3. Linearity results of sofosbuvir**

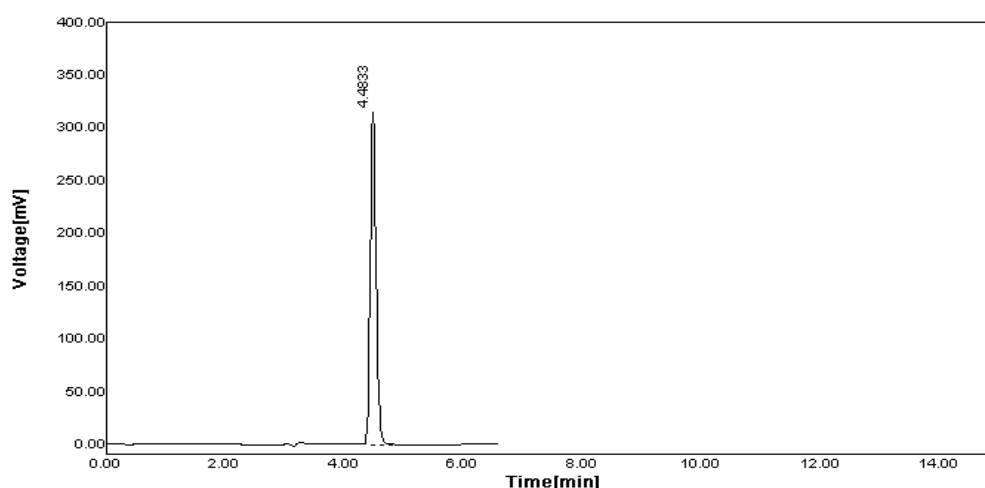
Conc. $\mu\text{g/ml}$	20	40	60	80	100
Peak Area	567.08	1038.14	1570.72	2159.37	2769.52
Correlation	0.997				

**Table no: 4. Calibration parameter of sofosbuvir**

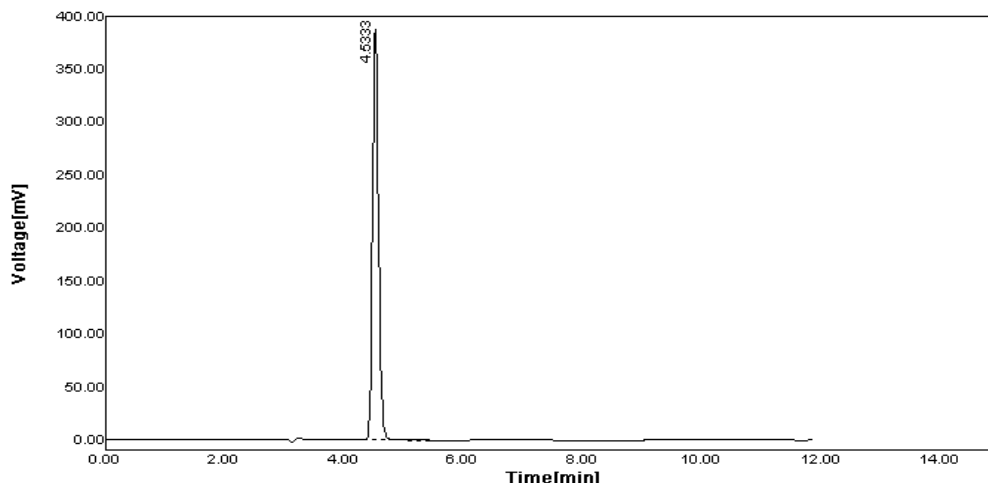
Parameter	Result
Slop	27.68
Intercept	36.71
Correlation co-efficient	0.997

## 2) PRECISION

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as standard deviation or relative standard deviation. A system precision was evaluated by measuring the peak response of the drug for three replicate injection of the standard solution prepared as per the proposed method. The chromatogram was shown in Fig. no. 10 & 11 % RSD for peak area obtained in three replicate injections are given in table no. 5 & 6.

**Fig No: 10 .Chromatogram of interday Precision for sofosbuvir**





**Fig No: 11. Chromatogram of intraday Precision for sofosbuvir**

- Precision results for sofosbuvir**

**Table no: 5. Interday precision study of sofosbuvir**

Conc. ( $\mu\text{g/ml}$ )	Peak area		Mean area	S.D	%RSD
	Trial 1	Trial 2			
40	1050.04	1070.65	1060.35	14.57	1.37
60	1624.72	1652.5	1638.61	19.64	1.20
80	2156.3	2126.29	2141.30	21.22	0.99

**Table no: 6. Intraday precision studies of sofosbuvir**

Conc. ( $\mu\text{g/ml}$ )	Peak area		Mean area	S.D	%RSD
	Trial 1	Trial 2			
40	539.37	548.69	544.03	6.59	1.21
60	1535.21	1555.39	1545.36	14.27	0.92
80	2553.11	2560.32	2556.72	5.10	0.20

### 3) Repeatability

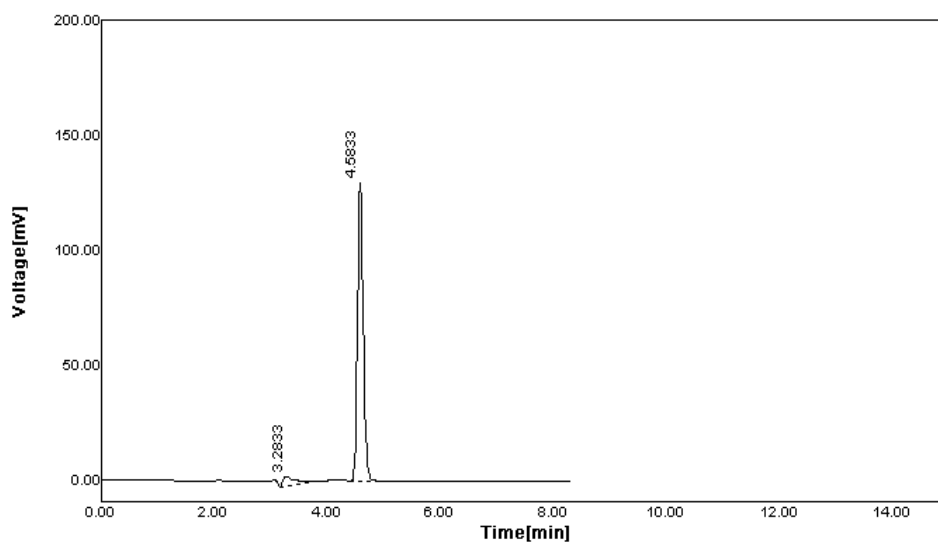
It was determined by preparing three replicates of same concentration (40  $\mu\text{g/ml}$  HPLC method) of sample and their area measured.

**Table No: 7. Repeatability study of sofosbuvir**

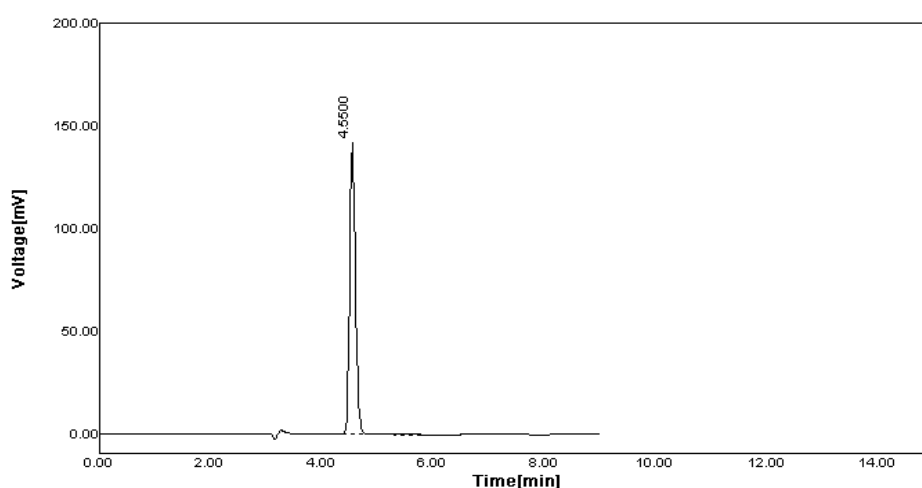
Sr. no.	Conc.	Peak area	Amt found	% amt found
1	40	1010.35	9.45	97.68
2	40	1032.13	6.65	24.13
3	40	1033.12	9.66	99.32
		Mean	9.59	73.71
		S.D.	0.12	42.95
		%RSD	1.24	58.27

#### 4) Accuracy

Accuracy of a method is the degree to which observed results correspond to true value of analyte in the sample. The determination was done at three different levels (80%, 100%, and 120% of sample concentration). Three samples of each level were prepared and total 6 determinations done as per ICH conditions. The samples were analyzed and their area measured and results indicated as % RSD.



**Fig No: 12. Chromatogram of Accuracy (80%)**



**Fig No: 13. Chromatogram of Accuracy (100%)**

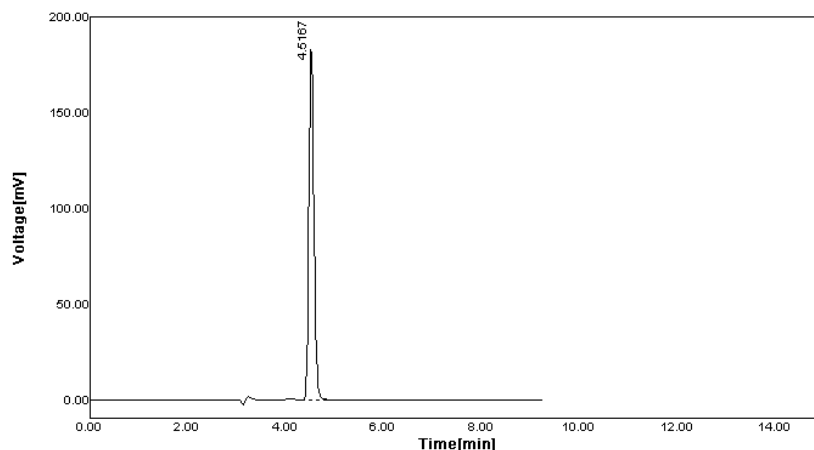


Fig No: 14. Chromatogram of Accuracy (120%)

Table No: 8. Accuracy study of sofosbuvir

Conc.	Amt added( $\mu\text{g/ml}$ )	Amt found ( $\mu\text{g/ml}$ )	%recovery	Mean recovery	Mean added ( $\mu\text{g/ml}$ )	Mean found	%RSD
80%	16	36.21	101.32	15.99	16	36.99	1.90
	16	37.78	98.63				
100%	20	39.74	98.74	19.64	20	74.28	0.72
	20	39.54	97.74				
120%	24	43.96	99.59	24.17	24	44.20	1.55
	24	44.42	101.80				

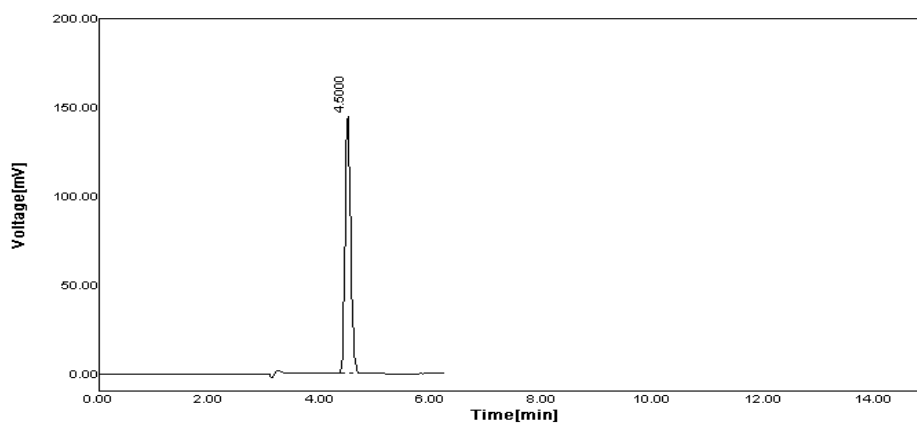
### 5) Robustness

Robustness of an analytical procedure are measure of its ability to remain unaffected by small changes in method parameters and provide an indication of its reliability. Analysis was carried out at three different wavelengths, flow rates and mobile phase respective area measured (HPLC method). The results were indicated as % RSD.

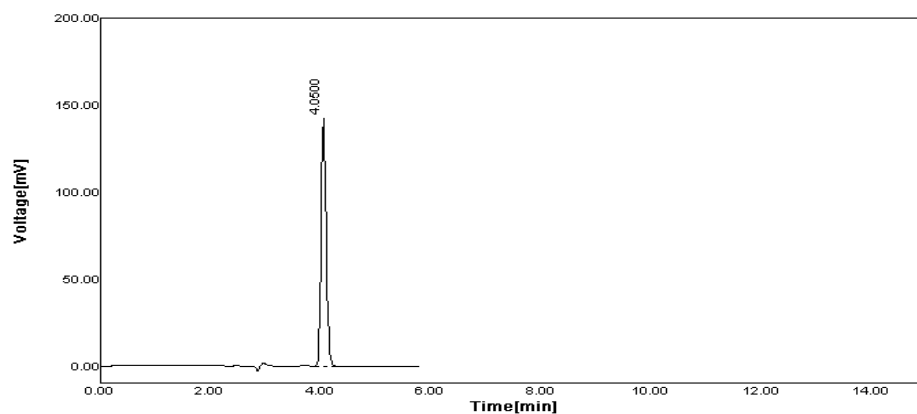
Table No: 9. Robustness study of sofosbuvir

A: flow rate=0.6			B: flow rate=0.8		
Sr. no.	Conc ( $\mu\text{g/ml}$ )	Peak area	Sr. no.	Conc ( $\mu\text{g/ml}$ )	Peak area
1	40	1038.71	1	40	1011.11
2	40	1040.1	2	40	1027.01
	Mean	1039.41		Mean	1019.06
	S.D.	0.98		S.D.	11.24
	%RSD	0.09		%RSD	1.10
Mobile phase Volume 79+21			Mobile phase Volume 81+19		

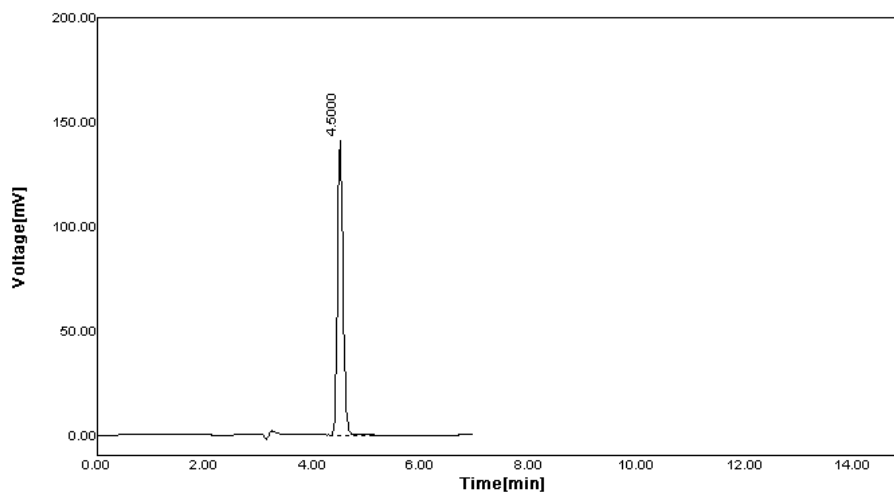
Sr. no.	Conc.( $\mu\text{g/ml}$ )	Peak area		Sr. no.	Conc ( $\mu\text{g/ml}$ )	Peak area
1	40	1006.7		1	40	1014.39
2	40	1011.22		2	40	1024.35
	Mean	1008.96			Mean	1019.37
	S.D.	3.20			SD	7.04
	%RSD	0.32			%RSD	0.69
<b>Wavelength Change=259</b>				<b>Wavelength change =261</b>		
Sr No.	CONCE $\mu\text{g/ml}$	Peak Area		Sr. No.	Conc( $\mu\text{g/ml}$ )	Peak Area
1	40	1030.75		1	40	1069.4
2	40	1023.65		2	40	1089.34
	Mean	1027.20			Mean	1079.37
	SD	5.02			SD	14.10
	%RSD	0.49			%RSD	1.31



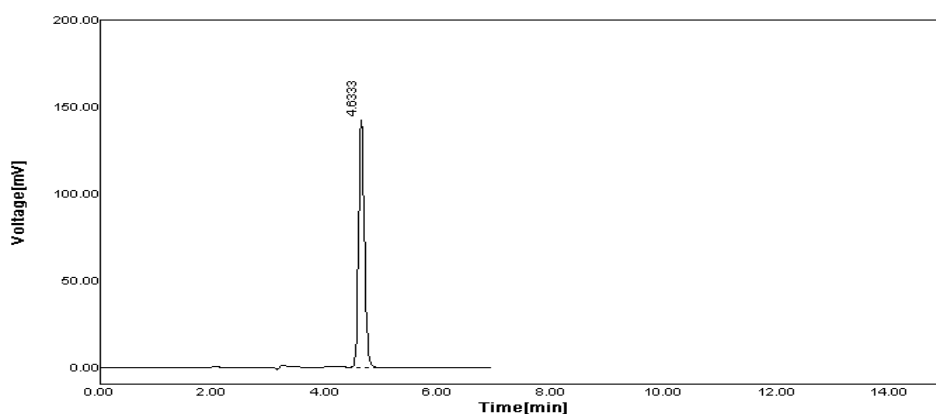
**Fig No: 15. Chromatogram of Robustness (Flow Rate 0.6 ml/min)**



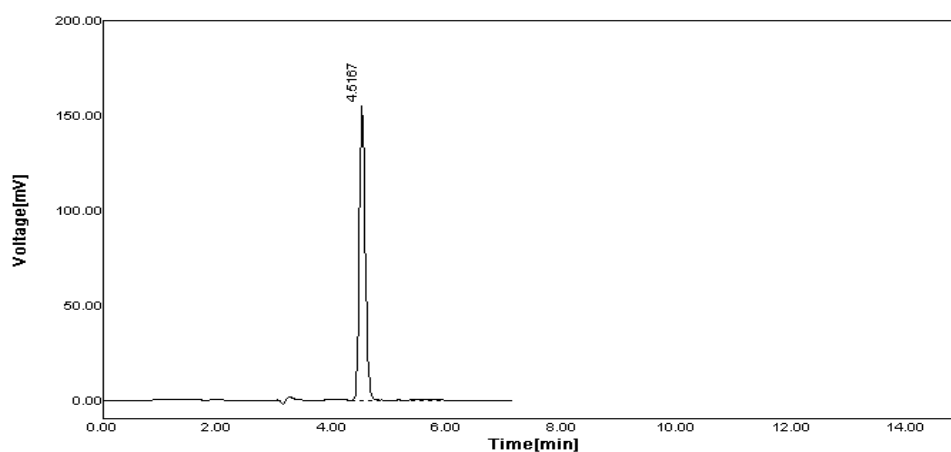
**Fig No: 16. Chromatogram of Robustness (Flow Rate 0.8 ml/min)**



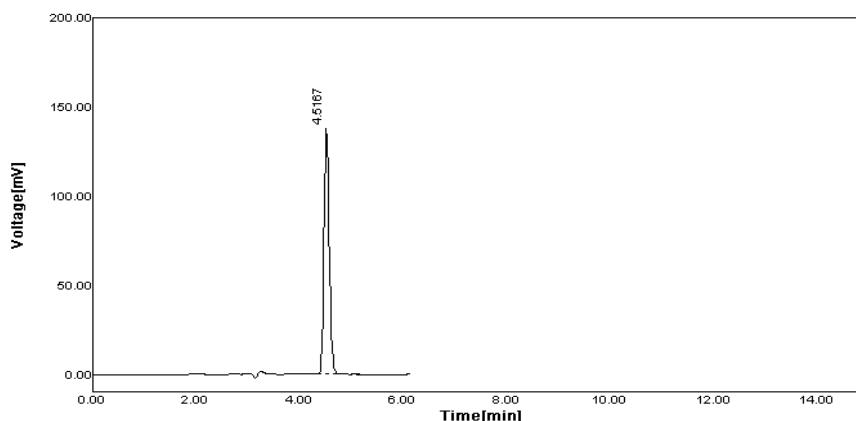
**Fig No: 17. Chromatogram of Robustness (Mobile Phase Composition-79:21)**



**Fig No: 18. Chromatogram of Robustness (Mobile Phase Composition-81:19)**



**Fig No: 19. Chromatogram of Robustness (Wavelength 259)**



**Fig No: 20. Chromatogram of Robustness (Wavelength 261)**

### 6) LOQ AND LOD

Limit of detection (LOD) is the minimum quantity of analyte in sample that can be detected.

LOD is calculated from the formula =  $3.3 \sigma/S$

$\sigma$  = Standard deviation of the response, S = slope of the calibration curve, Sofosbuvir = 0.53

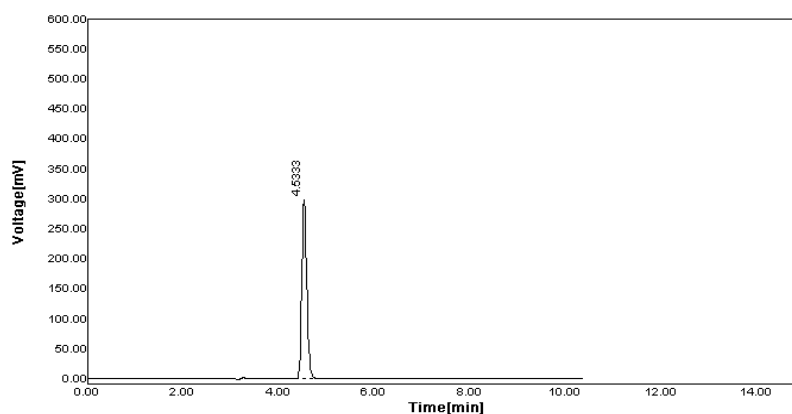
Limit of Quantitation (LOQ) It is the lowest amount of analyte in a sample which can be quantitatively determine with suitable precision and accuracy.

LOQ is calculated from the formula =  $10 \sigma/S$

$\sigma$  = Standard deviation of the response, S = slope of the calibration curve, Sofosbuvir = 1.62

### **TABLET ASSAY**

The standard (2 replicates) and sample (2 replicates) solutions were injected into the chromatographic system having the optimized method conditions. The samples were injected into the column; chromatograms recorded are shown in Fig. no. 21. The results of assay were shown in the Table 10.



**Fig No: 21. Chromatogram of Assay of sofosbuvir**

**Table No: 10. Analysis of marketed formulation**

Sr No.	Concentration µg/ml	Amt. found	% Label claim
1	60	59.49	99.15
2	60	60.01	100.02
	Mean	24.85	99.58
	SD	0.37	0.2
	%RSD	1.48	0.2

## CONCLUSION

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of Sofosbuvir from pure and its tablet 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, precision, Repeatability, robustness, LOD and LOQ, 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 and tablet dosage form and also can be used for dissolution or similar studies.

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