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Development and validation of RP HPLC method for the estimation of Sofosbuvir and related impurity in bulk and pharmaceutical dosage form

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Abstract

Background: The present work is aimed at development and validation of RP HPLC method which is simple, specific, precise, and accurate for estimation of Sofosbuvir and its process-related impurity in bulk and pharmaceutical dosage forms. Extensive literature survey revealed no method for estimation of the above said. The chromatographic separation was achieved on Agilent Eclipse XDB-C18, 4.6 × 250 mm, 5 µm with mobile phase composed of 0.1% trifluoroacetic acid in 1000 ml of water:acetonitrile (50:50) using an isocratic mode of elution. Detection was made using UV detector at 260.0 nm and LC solution software for analysis of data. The developed method was validated according to ICH guidelines.

Results: The linearity of calibration curve for Sofosbuvir in concentration range of 160-480 µg/ml was good. The curve was linear for its process related impurity (Phosphoryl) in concentration range of 10-30 µg/ml. There exists a good correlation between peak area and analyte concentration. Retention time for Sofosbuvir was found to be 3.674 min and its impurity was 5.704 min. Relative standard deviation values for Sofosbuvir is 1.741 and its process related impurity is 0.043. LOD for Sofosbuvir and its impurity was found to be 0.01% (0.04 µg) and 0.03% (0.12 µg) respectively. LOQ for Sofosbuvir and its impurity was found to be 0.50% (0.125 µg) and 1.50% (0.375 µg) respectively.

Conclusion: All the results reveal that the proposed method was found to be highly sensitive, simple, precise, accurate, robust, and fast. Large number of samples can be analyzed in shorter time due to shorter retention times, so it can be successfully applied for routine analysis of Sofosbuvir and related phosphoryl impurity in bulk and pharmaceutical dosage forms.

Keywords: Sofosbuvir, Phosphoryl impurity, Method validation, RP HPLC, Sovaldi

Background

Many drugs are available as the marketed formulations for the treatment of different diseases. So, there is need for control of concentrations of these entities in dosage forms and also in body fluids. Quality assurance and quality control of these marketed formulations are essential for ensuring safety in population. During the course of assay and development of drugs in formulations, there may be interferences caused by a number of sources such as degradation products of the drugs when they are stored for a long time, the presence of other drugs in combination products and the various additives incorporated in formulations have to be kept in view.

HPLC is the most widely used analytical technique. The method is non-destructive and may be applied to thermally labile compounds (unlike GC), so it was most widely used for the analysis of most of the chemicals.

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Due to availability of wide range of detectors, it is also said to be sensitive technique.

Sofosbuvir, sold under the brand name Sovaldi® (en.wikipedia.org/wiki/sofosbuvir/7/01/2021), is used in the treatment of hepatitis C (HCV). It has been recommended in combination with other antiviral drugs like ribavarin, simeprevir, ledipasvir [1, 2]. It is a nucleotide analog inhibitor and acts by blocking hepatitis C NS5B polymerase. It acts by interfering with GDD active site of HCV viral polymerase. Chemically, it is Isopropyl ((((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuranyl)methoxy)(phenoxy)phosphoryl)-L-alaninate. Molecular formula is $C_{22}H_{29}FN_3O_9P$ and molecular weight 529.5 g/mol (Fig. 1) (https://www.acessdata.fda.gov/drugsatfdadocs/label/2015/).

It is a prodrug of 2′deoxy methyluridine monophosphate that is phosphorylated intracellularly to active triposphate [3].

Very few methods were available for determination of sofosbuvir in tablets [4, 5], analysis of combination dosage forms like sofosbuvir, ledipasvir [6], and sofosbuvir, velpatasir in tablet dosage forms [7, 8]. But no method was available in literature for estimation of sofosbuvir and its related impurity in bulk and pharmaceutical dosage forms. So the present work was aimed at development and validation of RP HPLC method for the estimation of sofosbuvir and its related impurity in bulk and pharmaceutical dosage forms.

Methods

Sofosbuvir and its impurity were obtained as a gift sample from Mylan Labs, Hyderabad. Sovaldi tablets were purchased from local pharmacy, Hyderabad. Trifluro acetic acid, acetonitrile (HPLC grade) were procured from Sigma Aldrich, Hyderabad. Water (HPLC grade) was obtained from Merck.

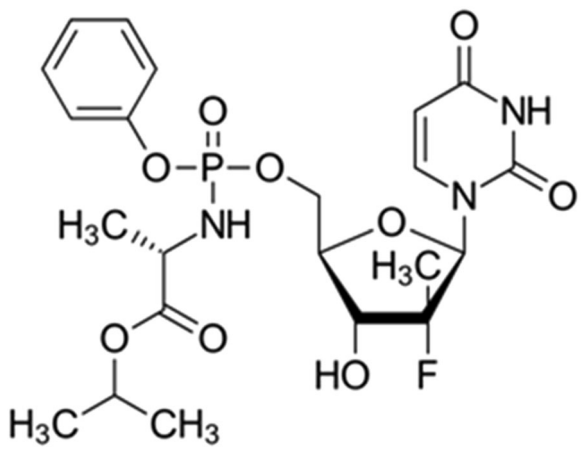

Fig. 1 Chemical structure of sofosbuvir. Molecular formula: $C_{22}H_{29}FN_3O_9P$. Molecular weight: 529.5 g/mol. IUPAC name: Isopropyl ((((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate

Chromatographic equipment and conditions

The development and validation was performed using liquid chromatographic system which is equipped with UV detector and LC solution software. The chromatographic column used for separation was Agilent Eclipse XDB-C18, 4.6 × 250 mm, 5 μm. The mobile phase used for the separation of both API and impurity was 0.1% trifluoroacetic acid in 1000 ml of water: Acetonitrile taken in the ratio of 50:50. Ambient temperature was maintained. Detection was made at a wavelength of 260.0 nm. Validation study was carried out using same optimized condition with suitable preparation of standard and sample solutions.

Preparation of standard solution

Standard solutions of drug and impurity were prepared by dissolving 400 mg of Sofosbuvir and 25 mg of phosphoryl impurity in 100 ml of diluent (water:acetonitrile 50:50).5 ml of the above solution was taken in 50 ml volumetric flask and diluted with diluent up to the mark.

Preparation of test solution

650 mg of Sovaldi formulation was taken in 100 ml volumetric flask, dissolved and diluted to 100 ml with diluent. 5 ml of above solution was taken in 50 ml volumetric flask and diluted with diluent up to the mark.

Validation

Validation was carried out by studying the parameters like specificity, system suitability, accuracy, linearity, precision, limit of detection, limit of quantitation, and robustness as per ICH guidelines Q2 (R1) [9, 10].

Linearity and range

Linearity was checked for standard solutions of drug and also impurity at concentrations of 40%, 60%, 80%, 100%, and 120%. Aliquot solutions of sofosbuvir and phosphoryl impurity were prepared in the range of 160-480 μg/ml and 10-30 μg/ml respectively. The chromatographic system was set to equilibrate and samples of study were injected, keeping the injection volume constant, i.e., 20 μl.

System suitability

System suitability studies form an integral part of method development and ensures adequate performance of chromatographic system. The standard solutions of sofosbuvir (0.4 mg/ml) and phosphoryl impurity (0.025 mg/ml) of about 20 μl were injected under optimized

Table 1 Optimized chromatographic conditions

Parameters	Results
Elution	Isocratic
Mobile phase	0.1 % trifluroacetic acid in 1000 ml water:acetonitrile (50:50)
Column	Agilent Eclipse XDB C18, 4.6 × 250 mm, 5μ
Flow rate	1.0 ml/min
Detection	260.0 nm
Injection volume	20 μl
Temperature	Ambient
Retention time	3.674 min for sofosbuvir and 5.704 min for phosphoryl impurity
Run time	25 min

chromatographic conditions to evaluate the suitability of the system.

Accuracy

Accuracy is defined as the closeness of obtained value to true value. Accuracy studies were done by standard addition method. Accuracy is expressed as % recovery of the standard spiked to previously analyzed test sample of tablet. It was measured in drug products by spiking known amounts (80%, 100%, and 120%) of the analyte into the analyzed tablet powder and each concentration was injected into the column for three times and percent recovered was calculated.

Precision

Precision is the agreement between replicate measurements of the same sample. It is expressed as relative standard deviation of replicate measurements. A total of 0.4 mg/ml of sofosbuvir and 0.025 mg/ml of phosphoryl impurity standard solutions were injected six times and the responses were recorded. Sample solution was also injected six times to record the response. The chromatograms were recorded. The peak area and retention time of both solutions under study was determined and relative standard deviation was calculated by the formula % RSD = (S.D/Mean) × 100%.

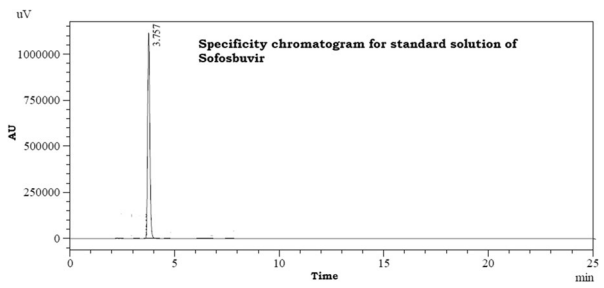

Fig. 2 Chromatogram for standard solution of sofosbuvir

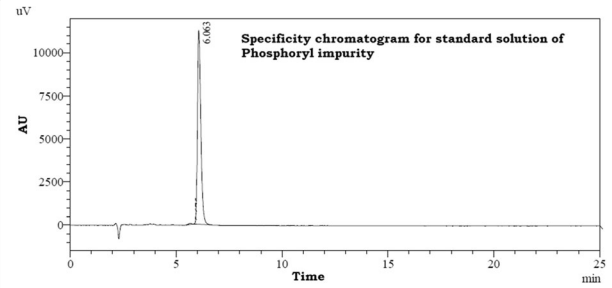

Fig. 3 Chromatogram for standard solution of phosphoryl impurity

Limit of detection and limit of quantitation

Limit of detection (LOD) is the lowest concentration of analyte which can be detected in a sample under the optimized experimental conditions. The limit of quantification (LOQ) was identified as the lowest concentration of the standard curve that could be quantified with acceptable accuracy, precision, and variability. LOD and LOQ were determined based on the signal to noise ratio as per ICH guide lines. Serial dilutions of the standard solutions of drug and impurity were prepared in the dilution levels of 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02%, 0.01%, 0.005% and injected into the chromatographic system. Responses were recorded and LOD and LOQ were determined.

Robustness

Robustness was studied to check whether the drug solution was subjected to small, deliberate changes like flow rate, wavelength, and change in mobile phase ratio. Robustness studies were performed by altering column and variation of flow.

Different column

A total of 0.4 mg/ml of sofosbuvir and 0.025 mg/ml of phosphoryl impurity standard solutions were injected in different column 3 times and responses were recorded. Sovaldi® sample was also injected 3 times and response was recorded.

Variation of flow

A total of 0.4 mg/ml of sofosbuvir and 0.025 mg/ml of phosphoryl impurity were injected 3 times by making changes in the flow rate, and responses were recorded

Table 2 System suitability parameters

Parameters	Sofosbuvir	Phosphoryl impurity
Retention time	3.674 min	5.704 min
Theoretical plates	6144.731	9453.104
Tailing factor	1.366	1.269
Resolution	0.000	9.617
Peak area	5971771	33349

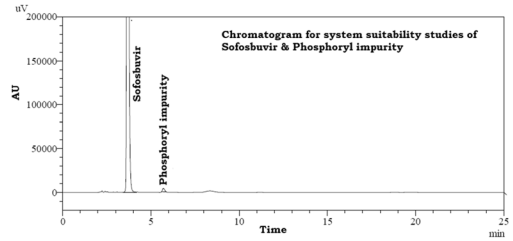

Fig. 4 Chromatogram for system suitability

and same procedure was carried with Sovaldi® sample also.

Results

Method development and optimization

The optimized parameters were listed in Table 1. Chromatogram for standard solutions of sofosbuvir and phosphoryl impurity was presented in Figs. 2 and 3 respectively.

System suitability studies

System suitability parameters were shown in Table 2. Chromatogram for system suitability studies is presented in Fig. 4.

Linearity

Results are shown in Tables 3 and 4, and the linearity curves are shown in Figs. 5 and 6.

Accuracy

The recovery data for accuracy studies was shown in Tables 5 and 6. The accuracy chromatograms for the respective concentrations were shown in Figs. 7, 8, and 9.

Precision

Results were reported in Tables 7 and 8 and chromatograms were presented in Figs. 10 and 11.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ for sofosbuvir were found to be 0.04 μg and 0.12 μg. LOD and LOQ for phosphoryl impurity were found to be 0.125 μg and 0.375 μg respectively

Table 3 Linearity studies for sofosbuvir

Concentration (μg/ml)	Peak area
160	2579526
240	3700838
320	4859111
400	5959587
480	7216122

Table 4 Linearity studies for phosphoryl impurity

Concentration (μg/ml)	Peak area
10	16048
15	21361
20	28332
25	33741
30	40214

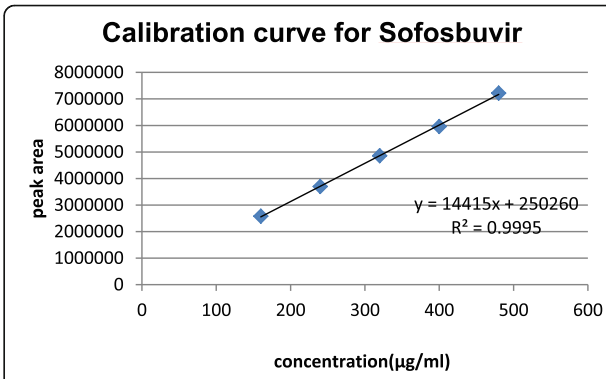

Fig. 5 Calibration curve for sofosbuvir

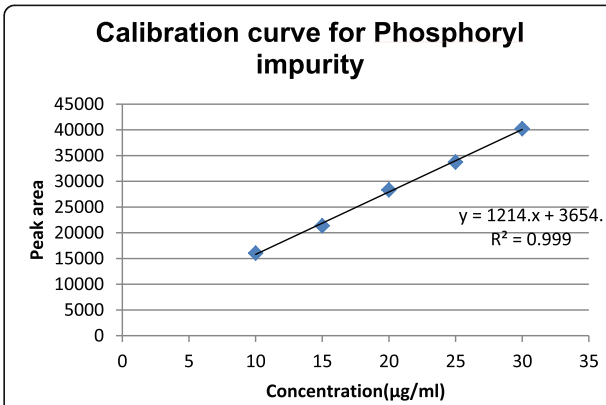

Fig. 6 Calibration curve for phosphoryl impurity

Table 5 Accuracy studies for standard and test solutions of sofosbuvir

S. no	Recovery at 80%		Recovery at 100%		Recovery at 120%	
	Standard	Test	Standard	Test	Standard	Test
1	4902628	5333906	6018345	6479189	7261645	7388812
2	4893568	5333248	6092520	6398900	7342315	7394716
3	4894102	5318437	6014999	6397570	7264303	7394915
Mean	4896766	5328530	6041954.7	6425220	7289421	7392814
SD	5084	8747	43822.81	46743.54	45826.82	3467.55
% RSD	0.104	0.164	0.725	0.728	0.629	0.047
Recovery	102.60% w/v		101.9% w/v		101.90% w/v	

Robustness

Results were reported in Tables 9, 10, 11, and 12.

Chromatograms for robustness studies for standard and test samples of sofosbuvir and impurity were shown in Figs. 12, 13, and 14.

Discussion

Various solvent system combinations for the determination of sofosbuvir and its related impurity (phosphoryl impurity) in bulk and pharmaceutical dosage forms were studied and finally a mixture of 0.1% trifluro acetic acid in 1000 ml of water and acetonitrile (50:50) was selected as mobile phase as it gave better resolution. The effect of flow rate was studied in the range of 0.9 to 1.2 ml/min and 1.0 ml/min was preferred to be effective because the analyte peak obtained was well defined and free from tailing. The retention time (RT) was found to be 3.674 min for sofosbuvir and 5.704 min for phosphoryl impurity.

System suitability

The system suitability method acceptance criteria set in each validation run were tailing factor ≤ 2.0 and theoretical plates > 2000. In all cases, the relative standard deviation for analyte peak area < 2.0% as per ICH guidelines [9]. All the parameters like retention time (RT), number of theoretical plates (N), tailing factor (T), and resolution were within the acceptable limits, so the optimized method is suitable for analysis of both compounds.

Linearity

A calibration curve was obtained by plotting a graph between peak area and concentration. Excellent correlation was obtained between peak area and concentration with R^2 = 0.999 for sofosbuvir and 0.999 for phosphoryl impurity as per the limit R^2 > 0.999 [11].

Accuracy

Percent recovery was found to be 102.6%, 101.9%, and 101.90% for drug and 107.80%, 118.9%, and 104.60% for related substance at 80%, 100%, and 120% respectively. All experimental results are in the acceptable criteria, i.e., 97-102% for drug and 80-120% for related substance [11, 12] and the method was found to be accurate.

Precision

The % RSD values for the peaks were found within the limits, RSD ≤ 2 [11] as shown in results, so the method was found to be precise.

Table 6 Accuracy studies for standard and test solutions of phosphoryl impurity

S. no	Recovery at 80%		Recovery at 100%		Recovery at 120%	
	Standard	Test	Standard	Test	Standard	Test
1	27190	28254	39498	45640	47723	52126
2	26587	28277	38997	45902	45723	51823
3	26247	28920	39364	45414	46869	51861
Mean	26675	28484	39286	45652	46772	51937
SD	478	378	259.37	199.41	1003.55	165.06
% RSD	1.790	1.327	0.660	0.437	2.146	0.318
Recovery	107.80% w/v		118.9% w/v		104.60% w/v	

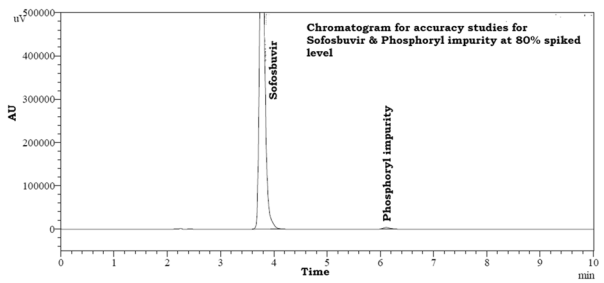

Fig. 7 Chromatograms for accuracy studies at 80% spiked level

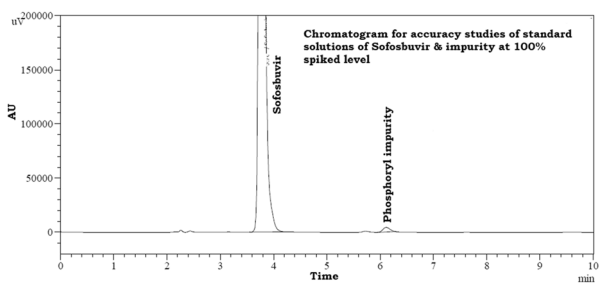

Fig. 8 Chromatograms for accuracy studies at 100% spiked level

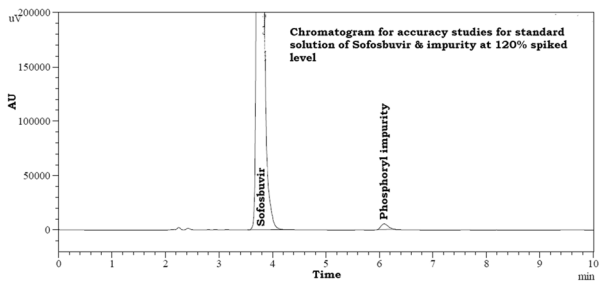

Fig. 9 Chromatograms for accuracy studies at 120% spiked level

Table 7 Precision studies for standard and test solutions of sofosbuvir

S. no.	Retention time (min)		Peak area	
	Standard	Test	Standard	Test
1	3.756	3.757	6012506	5971170
2	3.761	3.755	5972456	5972988
3	3.763	3.755	5972404	5969403
4	3.832	3.755	6231754	5967112
5	3.756	3.757	5967803	5973215
6	3.755	3.755	5967236	5973758
Mean	3.771	3.756	6020693	5971274
% RSD	0.807	0.026	1.741	0.043
SD	0.030	0.001	104812	2597

Table 8 Precision studies for standard and test solutions of phosphoryl impurity

S. no	Retention time (min)		Peak area	
	Standard	Test	Standard	Test
1	6.099	6.092	32597	32112
2	6.103	6.090	32581	32219
3	6.104	6.091	32591	32238
4	6.181	6.089	32310	31488
5	6.092	6.091	31998	31327
6	6.091	6.088	32286	31941
Mean	6.112	6.090	32447	31888
% RSD	0.562	0.022	0.980	1.223
SD	0.034	0.001	318	390

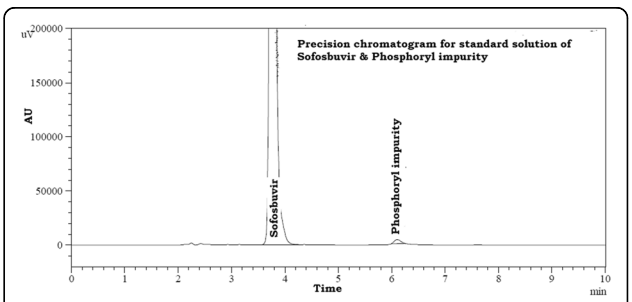

Fig. 10 Precision chromatogram for standard solution of sofosbuvir and phosphoryl impurity

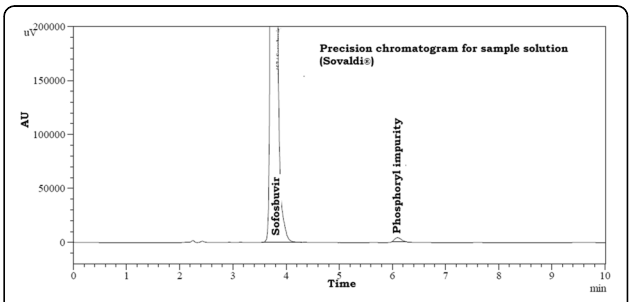

Fig. 11 Chromatogram for precision studies for sample solution (Sovaldi®)

Table 9 Robustness studies (variation of flow) for standard and test solutions of sofosbuvir

	Peak area (AU)		Retention time (min)	
	Standard	**Test**	**Standard**	**Test**
Increased flow rate (1.1 ml/min)				
1	5508834	5678165	3.346	3.340
2	5517126	5693588	3.344	3.338
3	5517268	5671572	3.342	3.339
Mean	**5514409**	**5681108**	**3.344**	**3.339**
Std. deviation	4829	11299	0.002	0.001
% RSD	0.088	0.199	0.060	0.022
% Assay	99.90%			
Decreased flow rate (0.9 ml/min)				
1	6800653	6652974	4.076	4.081
2	6809922	6648743	4.078	4084
3	6802508	6658755	4.081	4.085
Mean	**6804361**	**6653490**	**4.078**	**4.083**
Std. deviation	4904	5025.96	0.002	0.002
% RSD	0.072	0.076	0.058	0.052
% Assay	99.91%			

Table 10 Robustness studies (different column) for standard and test solutions of sofosbuvir

Injection no.	Peak area (AU)		Retention time (min)	
	Standard	**Test**	**Standard**	**Test**
1	5992287	6004074	3.766	3.770
2	6001913	5996533	3.766	3.769
3	5998929	6007984	3.768	3.768
Mean	**5997710**	**6002864**	**3.767**	**3.769**
Std. dev	4927.48	5820.66	0.001	0.001
% RSD	0.082	0.097	0.028	0.027
% Assay	99.60			

Table 11 Robustness studies (variation of flow) for standard and test solutions of phosphoryl impurity

	Peak area		Retention time (min)	
	Standard	**Test**	**Standard**	**Test**
Increased flow rate (1.1 ml/min)				
1	31293	32276	5.249	5.209
2	31460	33894	5.234	5.198
3	31629	33877	5.221	5.200
Mean	**31461**	**33349**	**5.235**	**5.203**
Std. deviation	168.00	929	0.014	0.006
% RSD	0.534	2.787	0.273	0.111
% Assay	99.65			
Decreased flow rate (0.9 ml/min)				
1	36149	37515	6.313	6.364
2	36950	37386	6.331	6.375
3	37261	37369	6.351	6.384
Mean	**36787**	**37423**	**6.332**	**6.374**
Std. deviation	573.71	79.84	0.019	0.010
% RSD	1.560	0.213	0.303	0.157
% Assay	99.69			

Table 12 Robustness studies (different column) for standard and test solutions of phosphoryl impurity

Injection no.	Peak area (AU)		Retention time (min)	
	Standard	**Test**	**Standard**	**Test**
1	33533	35324	6.095	6.102
2	33964	33955	6.096	6.105
3	34743	33980	6.103	6.106
Mean	**34080**	**34420**	**6.098**	**6.105**
Std. dev	613.28	783.28	0.004	0.002
% RSD	1.800	2.276	0.069	0.032
% Assay	99.77			

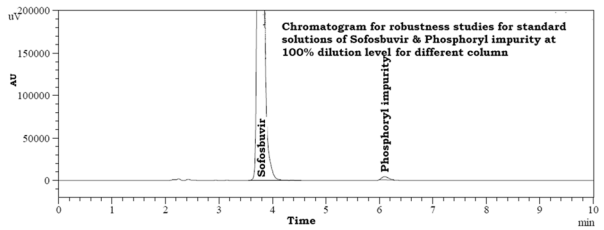

Fig. 12 Chromatogram for robustness studies of standard solution of sofosbuvir and impurity at different column

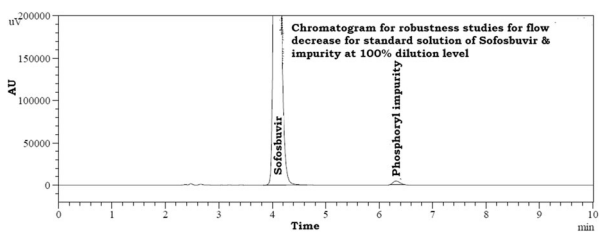

Fig. 13 Chromatogram for robustness studies for standard solution of sofosbuvir and impurity at flow decrease

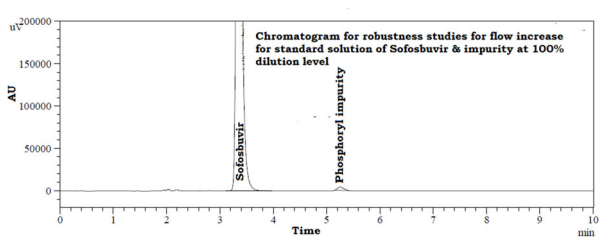

Fig. 14 Chromatogram for robustness studies for standard solution of sofosbuvir and impurity at flow increase

Robustness

No prominent changes were observed by altering column and flow rate. Hence, the method was found to be robust.

Conclusion

The method proposed for the analysis of sofosbuvir and related impurity in bulk and pharmaceutical dosage forms was found to be specific, precise, accurate, fast, and economical. The developed method was validated in terms of accuracy, linearity, robustness, and precision in accordance with ICH guidelines. Short retention time enabled analysis of sofosbuvir and phosphoryl impurity with minimal amount of mobile phase. The method was found to be precise and accurate. Due to low detection and quantitation limits, the method was said to be sensitive. Robustness data indicate that the method is unaltered due to small changes in chromatographic conditions. This method can be applied successfully for the determination of sofosbuvir and its related phosphoryl impurity in bulk and pharmaceutical dosage forms.

Abbreviations

API: Active pharmaceutical ingredient; LOD: Limit of detection; LOQ: Limit of quantitation; RSD: Relative standard deviation

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Authors' contributions

DS designed the plan of work; SG and ARN jointly contributed for doing all the research work and analyzed the data; GS drafted the manuscript, made critical revision, and approved final version. The authors read and approved the final manuscript. All authors are willing to get their work published in your esteemed journal.

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Availability of data and materials

All data and materials are available on request.

Declarations

Ethics approval and consent to participation

Not applicable.

Consent of publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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