RESEARCH PAPER

UPLC Method Development and Validation of Sofosbuvir in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

The present study aimed to develop and validate a simple, rapid, and robust Ultra Performance Liquid Chromatography (UPLC) method for the quantitative estimation of Sofosbuvir in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using an XBridge Shield RP18 column (3 mm × 50 mm, 2.5 µm) with a mobile phase consisting of ammonium acetate buffer and methanol in the ratio of 55:45 (v/v) at a flow rate of 0.7 mL/min. Detection was carried out at 260 nm, which corresponds to the maximum absorbance (λmax) of Sofosbuvir. The retention time of Sofosbuvir was found to be approximately 2.3 minutes, indicating a rapid analysis. The developed method was validated in accordance with ICH guidelines for parameters such as specificity, linearity, accuracy, precision, robustness, and system suitability. The method showed excellent linearity over the concentration range of 62.5–187.5 µg/mL with a correlation coefficient (R^2) of 0.999. Accuracy studies demonstrated percentage recovery within 98–102%, confirming the reliability of the method. Precision results indicated %RSD values less than 2%, reflecting good repeatability and intermediate precision. The method was found to be robust against small deliberate variations in chromatographic conditions, and solution stability studies confirmed that the analyte remained stable for 24 hours. The proposed UPLC method is simple, accurate, precise, and cost-effective, with reduced solvent consumption and shorter run time. Therefore, it can be successfully applied for routine quality control analysis of Sofosbuvir in bulk and pharmaceutical formulations.

Keywords: Sofosbuvir, UPLC, method development, validation, pharmaceutical analysis, ICH guidelines.

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INTRODUCTION

Sofosbuvir is a potent, orally administered nucleotide analog antiviral drug widely used in the treatment of chronic hepatitis C virus (HCV) infection. It is a phosphoramidate prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine triphosphate analog, which inhibits the NS5B RNA-dependent RNA polymerase enzyme essential for viral replication [1,2]. Due to its high efficacy, favorable safety profile, and pangenotypic activity, sofosbuvir has become a cornerstone in modern antiviral therapy, particularly in combination regimens

for HCV management [3]. Chemically, sofosbuvir is characterized by its complex nucleoside analog structure that enhances its stability and bioavailability. After administration, it is rapidly absorbed and converted into its active metabolite GS-461203, which competes with natural nucleotides and causes premature termination of viral RNA synthesis [2,4]. This targeted mechanism reduces viral load effectively and contributes to high sustained virological response (SVR) rates in patients [3].

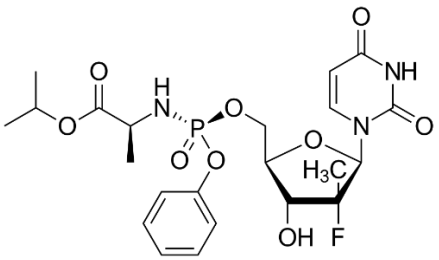

Figure 1: Structure of Sofosbuvir

The increasing clinical use of sofosbuvir has necessitated the development of reliable, sensitive, and rapid analytical methods for its quantification in bulk and pharmaceutical dosage forms. Various analytical techniques such as UV spectrophotometry, HPLC, LC-MS/MS, and UPLC have been reported for the estimation of sofosbuvir alone or in combination with other antiviral agents [5–7]. Among these, Ultra Performance Liquid Chromatography (UPLC) offers significant advantages over conventional HPLC, including higher resolution, faster analysis, improved sensitivity, and reduced solvent consumption, making it highly suitable for routine quality control analysis [8].

Method validation plays a crucial role in ensuring the reliability and reproducibility of analytical results. According to ICH guidelines, parameters such as specificity, linearity, accuracy, precision, robustness, and system suitability must be evaluated to confirm the suitability of the developed method [9]. Several studies have reported validated RP-HPLC and UPLC methods for antiviral drugs, including remdesivir and acalabrutinib, demonstrating the applicability of chromatographic techniques in pharmaceutical analysis [6,7,10]. In the present study, an attempt has been made to develop and validate a simple, rapid, precise, and robust UPLC method for the estimation of sofosbuvir in bulk drug and pharmaceutical dosage forms.

Aim

The aim of the present research work was to develop an innovative analytical method for the estimation of Sofosbuvir in capsule dosage form by Ultra high performance liquid chromatography (UPLC). The developed method will be validated as per ICH Guidelines Validation parameter for the developed method as per ICH Guidelines

MATERIALS AND METHODS

Table 1: List of the chemicals and reagents

Materials	Make
Sofosbuvir capsule	NATCO Pharma Ltd.
Mfg. Date: Feb 2025 Exp. Date: Jan 2027	
Working standard for Sofosbuvir	Synpharma research lab, Hyderabad.
Ammonium acetate	Sd fine- Chem ltd, Mumbai, India
Methanol (UPLC grade water)	Loba Chem, Mumbai, India

Table 2: Equipments

Equipments Used	Make
UV-Spectrophotometer	Shimadzhu
UPLC	Thermo Fisher Scientific
Ultrasonicater	Labindia
Analytical balance	Sartorius
PH meter	Labindia

Method development

Selection of Detection Wavelength

20 mg of Sofosbuvir was accurately weighed and dissolved in 10 mL of DMSO, and the volume was made up with the mobile phase to obtain a suitable concentration. The resulting solution was scanned in the UV region between 200–400 nm using a UV–Visible spectrophotometer. The obtained spectrum was analyzed to determine the wavelength of maximum absorbance (λmax). Sofosbuvir exhibited a strong and distinct absorbance peak at 260 nm in the UV region. Therefore, 260 nm was selected as the detection wavelength for further chromatographic analysis, as it provides optimum sensitivity and accurate quantification of the drug.

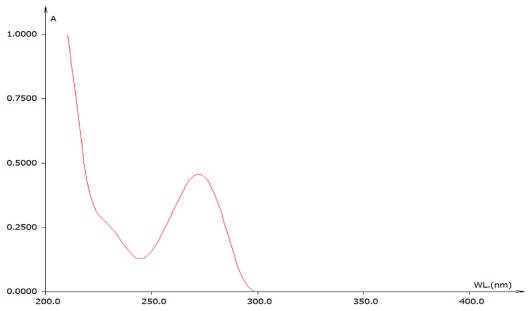

Figure 2: UV spectrum of Sofosbuvir

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Table 3: Selection of Mobile Phase and Column

Trail No	Column	Mobile phase composition	λMax (nm)	Flow rate (ml/min)	Observation
1	Luna 3UC18100A (50mm x 3mm, 3µm)	Ammonium acetate buffer : Methanol (55:45v/v)	260	0.3	Poor peak shape
2	Thermo Scientific (50 mm x4.6 mm, 5 µm)	Ammonium acetate buffer : Methanol (55:45v/v)	260	0.5	Fronting is observed
3	Xterra MS C-18 (50mm x 4.6mm, 3.5µm)	Ammonium acetate buffer : Methanol (55:45v/v)	260	0.7	Less theoretical plate count
4	ACQUITY CSH C-18 (50mm x 2.1mm, 1.7µm)	Ammonium acetate buffer : Methanol (55:45v/v)	260	0.7	The peak was eluted at 3.683 min an attempt was made reduce the retention time
5	XBridge Shield RP18 (3mmX 50mmX2.5µm)	Ammonium acetate buffer : Methanol (55:45v/v)	260	0.7	good separation peaks symmetry are found to be satisfactory

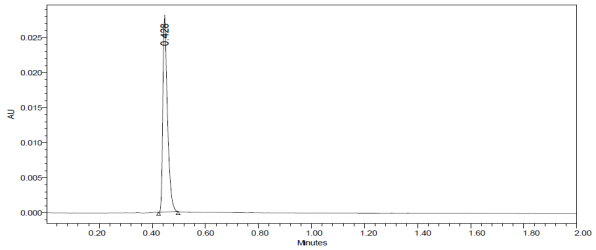

Figure 3: Chromatogram of optimized method

Name	RT	Area
Sofosbuvir	2.391	6023.448

Method validation

Preparation of solution

Preparation of Buffer Solution

0.5mM Ammonium acetate buffer was prepared by dissolving 38.5 g of Ammonium acetate in 1000 mL distilled water. The solution was filtered through 0.45 µ nylon filter.

Preparation of Mobile Phase

Prepare a mixture of buffer and methanol in the ratio of 55:45 Filter through 0.45µ Membrane filter and degas

Preparation of standard solution:

Standard stock preparation

Stock was prepared by 25 mg of Sofosbuvir transferred in 20 ml volumetric flask add 10ml of DMSO and makeup with mobile phase (1250 µg/mL).

Standard preparation

Pipette out 2 ml of standard stock preparation into 20ml volumetric flask and makeup with mobile phase (125µg/mL).

Preparation of sample solutions

20 capsules were accurately weighed and average weight was found. Weigh accurately about 146.22 mg (25 mg of Sofosbuvir) transferred into 20 mL volumetric flask. About 10 mL of DMSO was added and sonicate in an ultrasonic bath for 15 min and then volume make up with mobile phase. Then pippete out 2ml of above solution and volume makeup with mobile phase. The solution filtered through 0.45µm nylon syringe filter. The amount of Sofosbuvir present in capsule were calculated by using the following formula: Area of Sample X Weight of STD X Potency of STD X Dilution Factor.

Validation of developed method

The developed method was validated according to ICH guidelines. The method was validated in terms of specificity, system suitability, linearity, precision, accuracy and robustness.

System suitability

Suitability-001

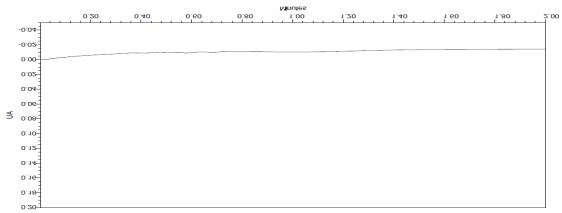

Figure 4: Chromatogram of system suitability-001

Injection ID	Sofosbuvir Theoretical plates	Rt	Area
1	7971.38	2.391	6094.831
2	7964.02	2.391	6130.308
3	7973.15	2.391	6047.247
4	7960.40	2.392	6144.261
5	7968.71	2.392	6161.495

Average 2.391 6115.628
SD 0.0005 45.4081
%RSD 0.0229 0.7425
Report: The % RSD for the area of five injections results should not be more than 2%

Specificity

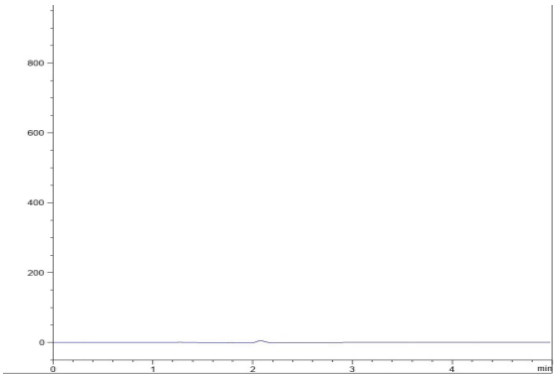

Figure 5: Specificity Chromatogram of Blank

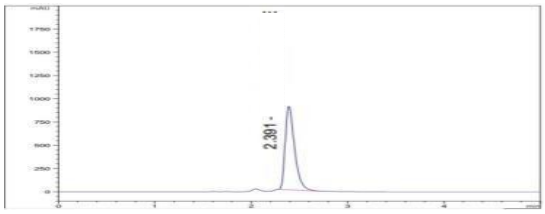

Figure 6: Specificity Chromatogram of Sofosbuvir

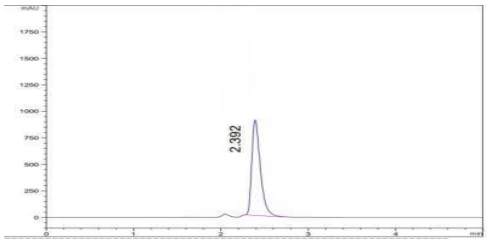

Figure 7: Specificity Chromatogram of Sofosbuvir

Accuracy

Table No 5: Results of Accuracy-Sofosbuvir (50%)

S.No	Sample ID	Conc. Spiked (µg/ml)	Peak Area	Retention Time	Calculated conc'n (µg)	% Recovery
1	C 1	62.5	3028.192	2.391	62.41	99.85
2	C 2	62.5	3050.525	2.391	62.25	99.65
3	C 3	62.5	3078.419	2.392	61.32	98.11
Mean			**3052.379**	**2.391**	**61.99**	**99.20**
Std dev			**25.16476**	**0.0005**	**0.588586**	**0.95212**
%RSD			**0.8244**	**0.0241**	**0.949434**	**0.959767**

Table No 6: Results of Accuracy-Sofosbuvir (100%)

S.No	Sample ID	Conc. Spiked (µg/ml)	Peak Area	Retention Time	Calculated conc'n (µg/ml)	% Recovery
1	C 1	125	6206.661	2.391	124.52	99.65
2	C 2	125	6185.477	2.392	124.23	99.23
3	C 3	125	6213.884	2.391	124.63	99.54
MEAN			**6202.007**	**2.391**	**124.46**	**99.47**
STD DEV			**14.7642**	**0.0005**	**0.2066**	**0.21**
% RSD			**0.2381**	**0.0241**	**0.1660**	**0.22**

Table No 7: Results of Accuracy-Sofosbuvir (150%)

S.No	Sample Id	Conc. Spiked (µg/ml)	Peak Area	Retention Time	Calculated conc'n (µg/ml)	% Recovery
1	C 1	187.5	9358.491	2.391	186.46	99.44
2	C 2	187.5	9364.791	2.392	186.91	99.68
3	C 3	187.5	9419.931	2.392	187.18	99.23
Std Dev			**33.8008**	**0.0005**	**0.363731**	**0.225167**
% RSD			**0.3603**	**0.0241**	**0.194665**	**0.226412**

Precision

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analytical method is usually expressed as the standard deviation and relative standard deviation.

Table 8: Precision results of Sofosbuvir

S.No	Peak Retention Time	Peak Area
1	2.391	6169.336
2	2.391	6076.421
3	2.391	6112.390
4	2.392	6183.911
5	2.392	6210.565
6	2.391	6137.234
MEAN	**2.391**	**6148.31**
STD DEV	**0.0005**	**49.3278**
%RSD	**0.02**	**0.802299**

Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method precision as performed on different day by using different make column and different analyst.

Table 9: Results of Analyst Variation for Sofosbuvir

S.No	Peak Retention Time	Peak Area
Injection 1	2.391	6016.362
Injection 2	2.391	6022.849
Injection 3	2.392	6039.673
Injection 4	2.392	6068.921
Injection 5	2.391	6034.285
Mean	**2.3914**	**6036.418**
Std Dev	**0.0005**	**20.3589**

Linearity and range

Preparation of Standard Solution

The linearity of the method was performed by preparing the concentration range of 62.5-187.5µg/mL for Sofosbuvir, from standard stock solution. Calibration curves were constructed by plotting concentration versus area of Sofosbuvir.

Table 10: Linearity results of Sofosbuvir

Concentration (µg/ml)	Peak Retention Time	Peak Area
62.5	2.391	3030.221
93.75	2.392	4455.754
125	2.391	6127.145
156.25	2.391	7703.001
187.5	2.392	9415.958

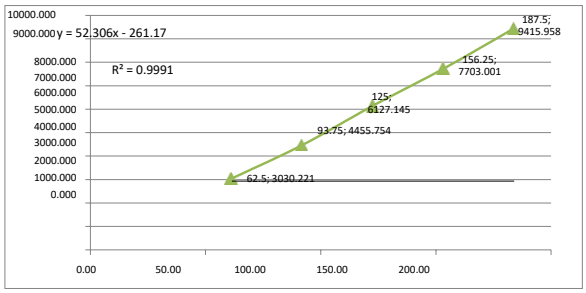

Figure 8: Linearity graph of Sofosbuvir

Linearity result for sofosbuvir

Result: The developed method for Sofosbuvir was found to linear between the range.

Robustness

Table 11: Robustness results for Sofosbuvir

Drug name	Parameter	Chromatographic condition
Sofosbuvir	Flow rate Change	
	0.6ml/min	2.485 / 6156.326
	0.7ml/min	2.391 / 6085.354
	0.8ml/min	2.289 / 6056.239
	Wavelength change ±2%	
	229 nm	2.392 / 6181.546
	231 nm	2.391 / 6074.523
	233 nm	2.391 / 6065.236

Solution stability

Table 12: Solution stability for Standard

Time in hours	% Assay	% Difference
Initial	99.88	-
24 Hours	99.63	0.25

Table 13: Solution stability for Sample

Time in hours	% Assay	% Difference
Initial	99.32	-
24 Hours	98.99	0.33

Assay of proposed method

Preparation of sample:

20 capsules were accurately weighed and average weight was found. Weigh accurately about 146.22 mg (25 mg of Sofosbuvir) transferred into 20 mL volumetric flask. About 10 mL of DMSO was added and sonicated in an ultrasonic bath for 15 min and then volume make up with mobile phase. Then pippete out 2ml of above solution and volume makeup with mobile phase. The solution filtered through 0.45µm nylon syringe filter.

Table 14: Assay results

Label claim	Average weight	Area	Percentage assay
25mg	146.22 mg	6051.247	99.86

Test result is showing that the test method is precise. The percentage assay of Sofosbuvir is found to be 99.86 %. Results are within the limits.

RESULTS AND DISCUSSION

The primary objective of this study was to develop a simple, rapid, and reliable UPLC method for the quantitative estimation of sofosbuvir in bulk and pharmaceutical dosage forms. Various chromatographic conditions were optimized to achieve better resolution, peak symmetry, and reduced retention time. During method development, different columns and mobile phase compositions were evaluated. The optimized chromatographic condition consisted of ammonium acetate buffer and methanol in the ratio of 55:45 (v/v), which provided a sharp and symmetric peak with satisfactory resolution. Detection was carried out at 231 nm, corresponding to the maximum absorbance (λmax) of sofosbuvir, ensuring high sensitivity of the method. The retention time of sofosbuvir was found to be approximately 2.391 minutes, indicating a rapid analytical run suitable for high-throughput analysis.

System suitability parameters such as theoretical plates, retention time, and peak area were found to be within acceptable limits, confirming the efficiency of the chromatographic system. The %RSD values for peak area were less than 2%, demonstrating good repeatability and system precision.

The developed method exhibited excellent linearity over the concentration range of 62.5–187.5 µg/mL, with a correlation coefficient (R^2) of 0.999, indicating a strong linear relationship between concentration and peak area. This confirms that the method follows Beer-Lambert's law within the specified range. Accuracy studies were performed at three concentration levels (50%, 100%, and 150%), and the percentage recovery values ranged between 98% and 102%, which are within the acceptable limits as per ICH guidelines. This demonstrates that the method is accurate and free from interference by excipients present in the formulation. Precision of the method was evaluated in terms of repeatability and intermediate precision. The %RSD

values obtained were less than 2%, indicating that the method is highly precise. Ruggedness studies carried out by different analysts and on different days also showed consistent results, confirming the reliability of the method.

Robustness studies were conducted by making deliberate variations in chromatographic conditions such as flow rate and wavelength. No significant changes were observed in retention time, peak area, or system suitability parameters, indicating that the method is robust and unaffected by small variations in experimental conditions. Solution stability studies demonstrated that both standard and sample solutions remained stable for up to 24 hours, with % difference well within acceptable limits. This ensures the practicality of the method during routine laboratory analysis. The assay results showed that the percentage purity of sofosbuvir in the pharmaceutical dosage form was within the range of 90–110%, confirming the suitability of the method for quantitative analysis.

The overall performance of the developed UPLC method is comparable to previously reported analytical methods for antiviral drugs, including remdesivir and other nucleoside analogs [6,7]. The advantages of the present method include shorter run time, reduced solvent consumption, and improved sensitivity, making it cost-effective and environmentally friendly. Similar findings have been reported in earlier studies where UPLC methods demonstrated superior efficiency over conventional HPLC techniques [8,10]. Thus, the developed method is accurate, precise, linear, robust, and suitable for routine quality control analysis of sofosbuvir in bulk and pharmaceutical dosage forms.

CONCLUSION

Overall, the method proves to be reliable, reproducible, and efficient for the estimation of Sofosbuvir in bulk and pharmaceutical dosage forms. Its compliance with ICH validation guidelines ensures its applicability in regulatory and industrial settings.

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