Validated UV and HPLC method development for the estimation of Sofosbuvirin marketed formulation

Vandana Sinha, Priyanka Chaturvedi✉, Manish Sharma, Anurag Singh, Surendra Jain

Sagar Institute of Research and Technology, Ayodhya Bypass,
Bhopal, Madhya Pradesh - 462041,
India

✉**Corresponding author:**
E-mail address: chaturvedi2506@gmail.com

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ABSTRACT

In the present research work, a successful attempt was made for Validated UV and HPLC method which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling. The objective of the research work of estimation of the drug in marketed formulation. Proposed UV method was found to be linear in the range of 5-25 µg/ml Sofosbuvir with the correlation coefficient near to one (0.998). HPLC method was found to be linear in the range of 1-5 µg/ml Sofosbuvir with the correlation coefficient near to one (0.999) respectively. The isocratic mobile phase consisted of methanol: Acetonitrile in the ratio of 30:70 v/v at a flow rate of 1.0 ml min-1. A thermo C-18 column (4.6 x 250mm, 5µ particle size) was used as the stationary phase, 260.0 nm was selected as the detection wavelength for UV-vis. detector. The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and % RSD less than 2), Precise and can be successfully employed in the routine analysis of those drugs in bulk drug also as in tablet dosage.

Keywords: SOFB, UN, HPLC, SD, RSD

1. INTRODUCTION

The developments of analytical methods for the determination of medicine in bulk, in dosage forms or in body fluids have received a substantial attention in recent years due to their importance in quality control, bioavailability and pharmacokinetic study etc [1]. The notion of drug treatment, previously "right drug for the right person" is now changing from "right action for the right person" to "right time for the right person". It is necessary to find the concept of each drug either in bulk or single or combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage for treatment [2]. The scope of developing and validating and analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise [3]. The main objective for that is to improve the condition and Parameters, Which should be followed in the development and validation [4]. The development of analytical method for the determination of medicine in bulk, in dosage forms or in body fluids have received attention in recent years due to their importance in quality control, bioavailability and pharmacokinetic study [5-7].

1. The line up and objective of the present work is to develop new simple, sensitive and Validated U.V. and RP-HPLC method for estimation of Sofosbuvir [8] in marketed formulation [2, 9].
2. Validation of developed Analytical method according to ICH guideline [10, 11].

2. MATERIALS AND METHODS

2.1. Material

The gift sample of Sofosbuvir (Sovaldi) was received from Aurobindo Pharma Limited, and chemical reagent Methanol, Water, Acetonitrile HPLC grade was received from Merck specialties pvt, Ltd., Mumbai.

2.2. Preparation

2.2.1. Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from1-5 µg/ml for SOFB was prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 260 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived [9].

2.2.1.1. Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 10 mg of each drug in 8ml distilled water in 10ml volumetric The flask was sonic at end for about 10 min to solubilize the drug and the volume was made up to the mark 10ml with distilled water to get a concentration of 1000 µg/ml (Stock-A) for both.

2.2.1.2. Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of SOFB transferred into 25 ml volumetric flask separately and diluted up to 25 ml with distilled water that gave concentration of 100 3 Preparation of Working Standard Solution.

2.2.1.3. Preparation of Working Standard Solution

10.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with distilled water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for SOFB. [12].

2.2.1.4. Selection of wavelength for linearity

Solutions of 10µg/ml of was prepared separately. The solution was scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of SOFB was observed at 260.0 nm. SOFB showed linearity in the concentration range of 5-25µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration [13].

2.3. Validation of developed Method

2.3.1. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 1 to 5 µg/ ml for SOFB) and areas for each concentration were recorded three times and mean area was calculated. The regression equation and correlation coefficient of

curve are given and the standard calibration curve of the drug is shown in figure 6.10. The response ratio (response factor) was found by dividing the AUC with respective concentration [5].

2.3.2. Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

2.3.3. Accuracy

Replicating studies were performed to calculate the precision of developed method to preanalysed test solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery.

2.3.4. Precision

The stock solution was prepared. The precision are established in three differences.

2.3.5. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5 g/ml for SOFB indicates the precision under the same operating condition over short [14].

2.3.6. Intermediate Precision

2.3.6.1. Day to Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in three replicate at five concentrations. Results of day to day intermediate precision for SOFB reported.

2.3.6.2. Robustness

As per ICH standards, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (70:30 % v/v) to (75:25 % v/v) [15].

2.3.6.3. Analysis of both the drug in Tablet Sample

Twenty tablets were precisely weighed and their mean weight was determined. The tablets were grinded to fine powder; an accurately weighed quantity of powder equivalent to 10mg of SOFB was transferred to 10 ml volumetric flask. The solution was sonicated for 25 min and the final volume was made with mobile phase. The emulsion was then filtered through a 0.45µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 10µg/mL SOFB respectively. The amounts of SOFB in tablets formulation were calculated by extrapolating the rate of area from the calibration curve. Analysis procedure was repeated six times with formulation [5, 16].

2.4. Identification and characterization of drugs

2.4.1. Physical characterization of drug

The drug SOFB was physically characterized on the beginning of appearance, color and odor. All these parameter were recorded and compared with the literature.

2.4.2. Melting point determination

The melting point determined used for the strength of mind of melting point of SOFB by the open capillary methods. The melting point of drug was recorded and compared with literature values. The Melting point of SOFB was found 120-125^{0}C [8].

3. RESULT AND DISCUSSION

Method I: Analytical method development and validation using UV [11, 17, 18].

3.1. Linearity

The linearity of analytical method was implemented to check its ability to evoke test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the outcome was recorded. The reports of linearity are reported in table 1.

Table 1. Results of linearity of SOFB

PARAMETER	SOFB
Concentration (µg/ml)	5-25
Correlation Coefficient (r^2)*	0.998
Slope (m)*	0.013
Intercept (c)*	0.006

*value of five replicate

3.2. Accuracy

The validity and reliability of preferred methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 2.

Table 2: results of recovery study

% LEVEL	% MEAN±SD*
	SOFB
80%	99.07±0.697
100%	98.89±0.855
120%	99.13±0.748

***** Value of three replicate and five concentrations.

3.3. Precision

Precision was determined by repeatability and Intermediate precision of antidote. Replicability result indicates the precision under the same operating condition over short interval time. The intermediate clarity study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in table 3.

Table 3: Results of Precision

PARAMETER	% MEAN±SD*
	SOFB
Repeatability	98.960±0.090
Intermediate precision	
Day to day precision	98.602±0.119
Analyst-to-Analyst	98.443±0.105
Reproducibility	98.381± 0.119

***** Value of five replicate and five concentrations

3.4. Assay of tablet formulation

The results of the analysis of tablet formulation were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drug [12, 19].

Table 4: Assay of tablet formulation

Conc. present (µg/ml)	Replicate-1	
	Conc.found (µg/ml)	% Conc. found
SOFB	**SOFB**	**SOFB**
10	9.98	99.80
10	9.95	99.50
10	9.65	96.50

10	9.78	97.80
10	9.69	96.90

Method II Analytical method development and validation for estimation of Sofosbuvir (SOFB) using RP-HPLC.
The RP-HPLC method was developed for estimation of Sofosbuvirin marketed formulation by isocratically using Acetonitrile: Methanol (pH 7 with TEA) in the ratio of 70:30v/v as mobile phase, Prontosil C-18 column 6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 260nm. Then developed method was validated by using various parameters [20, 21]

3.5. System suitability

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result [4]. The result of system suitability parameter is reported in table 5.

Table 5: Results of system suitability parameters

PARAMETERS	**% MEAN±SD***
	SOFB
No. of Theoretical Plates	2539.000±42.010
Tailing Factor	1.167±0.061
Retention time	4.564±0.003

3.6. Linearity

The linearity of logical method was implemented to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of quality solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table 6.

Table 6: Results of linearity of SOFB

PARAMETER	**SOFB**
Concentration (µg/ml)	1-5
Correlation Coefficient (r^2)*	0.999
Slope (m)*	142.7
Intercept (c)*	3.807

*value of six replicate

3.7. Specificity

Specificity of the method was carried out to assess unambiguously the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

3.8. Accuracy

The rationality and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 7.

Table 7: Results of recovery study

% LEVEL	**% MEAN±SD***
	SOFB
80%	99.35±0.214
100%	97.76±0.917
120%	105.83±11.314

***** Value of three replicate and three concentrations.

3.9. Precision

Precision decided by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under an equivalent operating condition over short interval time. The halfway precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in table 8.

Table 8: Results of precision

PARAMETER	% MEAN±SD*
	SOFB
Repeatability	98.204±0.049
Intermediate precision	
Day to day precision	98.667±0.046
Analyst to analyst	98.811±0.031

***** Value of five replicate and five concentrations

3.10. Robustness

The robustness of developed method was checked by changing within the deliberate variation in solvent. Result of robustness shown in table 9.

Table 9: Results of robustness

PARAMETER	% MEAN±SD*
	SOFB
Robustness	98.078±0.050

***** Value of five replicate and five concentrations

3.11. LOD AND LOQ [3]

Name	LOD (µg/ml)	LOQ (µg/ml)
SOFB	0.25	0.80

ASSAY OF TABLET

The results of the analysis of synthetic mixture were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drugs [2].

Table 10: Analysis of tablet sample

	SOFB*
Label Claim (mg)	**400mg**
% Found (mg)	399.78
% Assay	99.94
% RSD	0.045

*Average of three determination

Identification by IR:

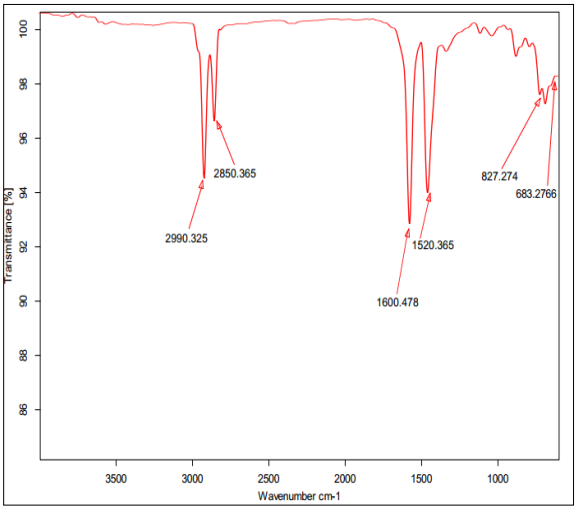

Figure 1: IR spectra of Sample Sofosbuvir

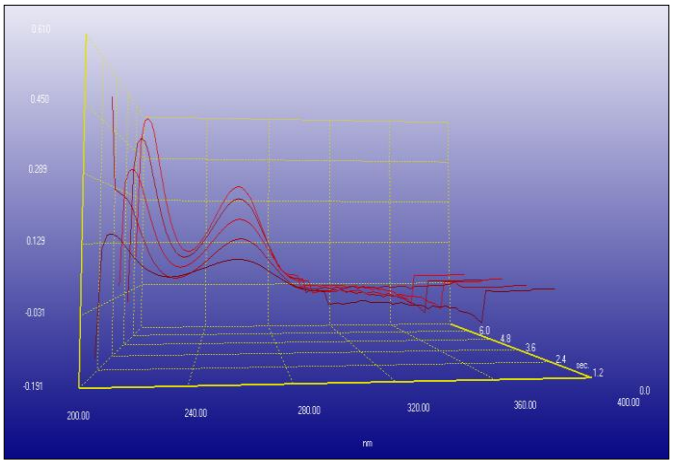

Figure 2: 3D spectra of Calibration curve of Sofosbuvir

Interpretation of Sofosbuvir:

The FTIR spectra of sofosbuvir showed prominent peaks at wave numbers 1520.365 cm-1 (RCH2CH3), 1600.478 cm-1 (RCOO-), 827.274 cm-1 (R2NH), 276 cm-1 for alkyl halides 2990cm-1 (RCH).

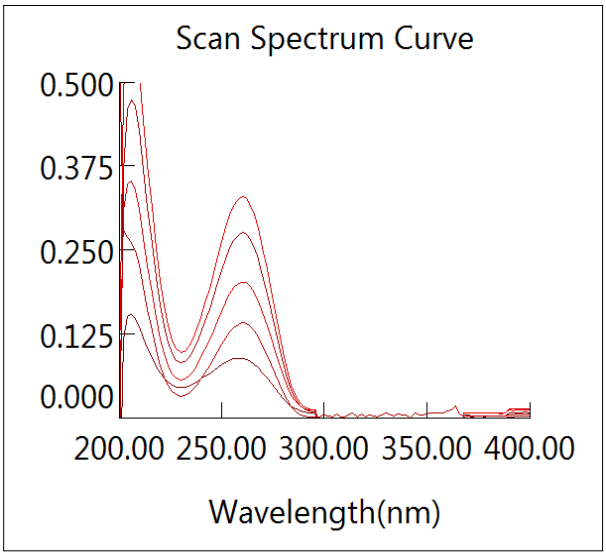

Figure 3: Calibration curve of Sofosbuvir

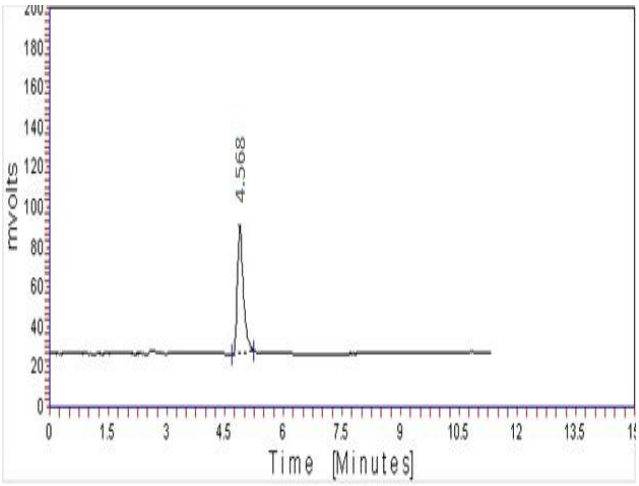

Figure 4: Chromatogram of Sofosbuvir

4. CONCLUSION

In the present research work, a successful attempt was made for Proposed UV method was found to be linear in the range of 5-25 µg/ml Sofosbuvir with the correlation coefficient near to one (0.998). HPLC method was found to be linear in the range of 1-5 µg/ml Sofosbuvir with the correlation coefficient near to one (0.999) respectively. The validation and the reliability of proposed method were assessed by recovery study. The recovery of added standards (80%, 1000%) was ranging near to one for Sofosbuvir respectively.

Liquid chromatographic system from waters comprising of manual injector, Waters 515 binary pump for continuous flow and constant pressure delivery and U.V. detector connected to data ace software controlling the instrumentation as well as processing the data generated were used. The isocratic mobile phase consisted of methanol: Acetonitrile in the ratio of 30:70 v/v at a flow rate of 1.0 ml min-1. A thermo C-18 column (4.6 x 250mm, 5µ particle size) was used as the stationary phase, 260.0 nm was selected as the detection wavelength for UV-vis. detector.

The proposed methods were found to be linear in the range of 5-25µg/ml by U.V and 1-5 µg/ml by HPLC with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing within the deliberate variation in solvent.

The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and % RSD less than 2), Precise and can be effectively employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage.

The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the target of this research work.

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Peer-review

External peer-review was done through double-blind method.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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