IJBPAS, May, 2024, 13(5): 2388-2395

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ESTIMATION OF SOFOSBUVIR BY UV SPECTROSCOPIC METHOD

SANTHOSH I[1*], PAVAN M[2], SAI LATHA G[2], NIKHILA R[2], ROOPA V[2], UMA CHAITHRA M[2], SURESH CV[3] AND RAO KNV[4]

Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Charlapally, Nalgonda, Telangana-508001

***Corresponding Author: Mr. Santhosh Illendula: E Mail: santoshillendula@gmail.com**

Received 20th May 2023; Revised 25th July 2023; Accepted 30th Aug. 2023; Available online 1st May 2024

https://doi.org/10.31032/IJBPAS/2024/13.5.8033

ABSTRACT

A new simple, accurate, rapid, precise and reproducible spectrophotometric method for the quantitative estimation Sofosbuvir in bulk form. The developed visible spectrophotometric method for the quantitative estimation of Sofosbuvir is based on measurement of absorption at maximum wavelength 260 nm using with acetonitrile**:** methanol (30**:**70) as a solvent. The stock solution of Sofosbuvir was prepared, and subsequent suitable dilution was prepared in diluent to obtained standard curve. The standard solution of Sofosbuvir shows absorption maxima at 260 nm. The drug obeyed beer lambert's law in the concentration range of 5 - 25 µg/ml with regression 0.9991 at 260 nm. The overall % recovery was found to be 99.63% which reflects that the method was free from the interference of the impurities and other excipients used in the bulk form. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for inter-day and intra-day precision was found to be 0.5863 and 0.8896, respectively which is<2% hence proved that method is precise. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of Sofosbuvir in bulk form.

Keywords: Sofosbuvir, UV Visible Spectrophotometry, Method development, Validation, ICH guidelines, Acetonitrile, methanol, Accuracy, Precision

INTRODUCTION [1, 2, 3]

Sofosbuvir Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate

Sofosbuvir inhibits the hepatitis C NS5B protein. Sofosbuvir appears to have a high barrier to the development of resistance. Sofosbuvir is a prodrug of the Protide type, whereby the active phosphorylated nucleotide is granted cell permeability and oral bioavailability. It is metabolized to the active antiviral agent GS-461203 (2'-deoxy-2'-α-fluoro-β-*C*-methyluridine-5'-triphosphate). GS-461203 serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis.

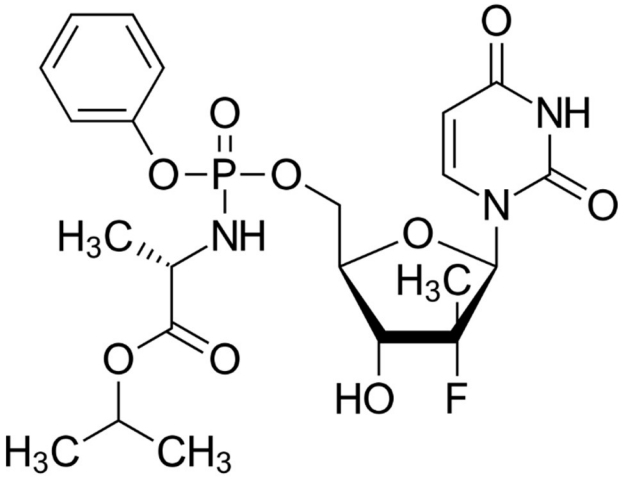

Figure 1: Structure of Sofosbuvir

Although sofosbuvir has a 3' hydroxyl group to act as a nucleophile for an incoming NTP, a similar nucleotide analogue, 2'-deoxy-2'-α-fluoro-β-C-methylcytidine, is proposed to act as a chain terminator because the 2' methyl group of the nucleotide analogue causes a steric clash with an incoming NTP. Sofosbuvir would act in a similar way.

MATERIALS AND METHODS [4, 5]

Chemicals and Reagents: Acetonitrile and Methanol.

Instruments: SHIMADZU UV-1601 UV–Vis spectrophotometer, Electronic Balance **(CITIZEN BALANCE BL-220H),** Ultra Sonicator **(ANALYTICAL),** and P^H Analyzer **(INFRA DIGI IR 501),** Distillation unit **(BOROSIL),** Vaccum filtration unit **(BOROSIL).**

Reagents and Solutions

Diluent preparation: In a 100ml volumetric flask take 30:70 Acetonitrile and Methanol.

Preparation of Standard Solutions

Accurately weighed 100mg of Sofosbuvir was weighed accurately and transferred into 100ml volumetric flask. About 10 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100µg/ml of Sofosbuvir Working standard solution of Sofosbuvir containing 10µg/ml for method. Finally add those above solutions and prepare the final solution is about 10µg/ml.

Preparation of Sample Solutions.

Take 20 Tablets average weight and crush in a mortar by using pestle and weight powder 100 mg equivalent weight of Sofosbuvir sample into a 100ml clean dry volumetric flask, dissolve and make up to volume with diluent. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with diluent.

Determination of wavelength of maximum absorbance for Sofosbuvir

The absorbance of the final solution scanned in the UV spectrum in the range of 200 to 400nm against solvent mixture as blank.

Optimization of selection of Solvent

It is well known that the solvents do exerts a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Methanol, Ethanol, Acetonitrile, Isopropyl alcohol, Water, Acetone, etc. First optimize the different solvents. From that solvents Acetonitrile and methanol combination satisfied the all the optimized conditions.

5.4. Wavelength Selection

The standard solutions are preparing by transferring the standard drug in a selected solvent or mixture of solvent and finally diluting with the same solvent or diluent. That prepared solution is scanned in the visible wavelength range of 200-400nm.This has been performed to know the maxima of Sofosbuvir. While scanning the Sofosbuvir solution we observed the maxima at 260 nm. The visible spectrum has been recorded on (SHIMADZU UV-1601) make UV – Vis spectrophotometer model UV-1601.The scanned visible spectrum is attached in the following page. The λ_{max} of the Sofosbuvir was found to be 260 nm in diluents as solvent system.

METHOD VALIDATION [6, 7, 8]

1. Accuracy:

Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Sofosbuvir were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in **Table 1**.

2. Precision:

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Sofosbuvir (API) the percent relative standard deviations were calculated for Sofosbuvir is presented in the **Table 2**.

Intermediate Precision:

Intra-assay & inter-assay:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Sofosbuvir revealed that the proposed method is precise. The results were shown in **Table 3**.

3. Linearity & Range:

The calibration curve showed good linearity in the range of 5-25µg/ml, for Sofosbuvir (API) with correlation coefficient (r^2) of 0.9999 **(Figure 2)**. A typical calibration curve has the regression equation of y = 0.0787x + 0.0107 for Sofosbuvir.

Standard solutions of Sofosbuvir in the concentration range of 5 µg/ml to 25 µg/ml were obtained by transferring (5,10,15,20 and 25 ml) of Sofosbuvir stock solution (100ppm) to the series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 260 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 5 to 25 µg/ml at 260 nm for Sofosbuvir. The results were shown in **Table 4**.

4. Method Robustness:

Robustness of the method was determined by carrying out the analysis under different Wavelength i.e., at 258 nm, and 262 nm. The respective absorbances of 10µg/ml were noted SD < 2%) the developed UV-Spectroscopic method for the analysis of Sofosbuvir (API). The results were shown in **Table 5**.

5. LOD & LOQ:

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.438566 & 1.315698 µg/ml respectively.

RESULTS AND DISCUSSION

The standard solutions of Sofosbuvir with Acetonitrile (10µg/ml) methanol (10µg/ml) subjected to a scan individually at the series of

wavelengths of 200 nm to 400 nm. Absorption maximum of Sofosbuvir was found to be at 260 nm. Therefore, 260 nm was selected as λ_{max} of Sofosbuvir for the present study. The calibration curve of Sofosbuvir was found to be linear in the range of 5-25 µg/ml at 260 nm. Therefore, it was clear that Sofosbuvir can be determined without interference of any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 80%, 100% and 120% of Sofosbuvir standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness etc.

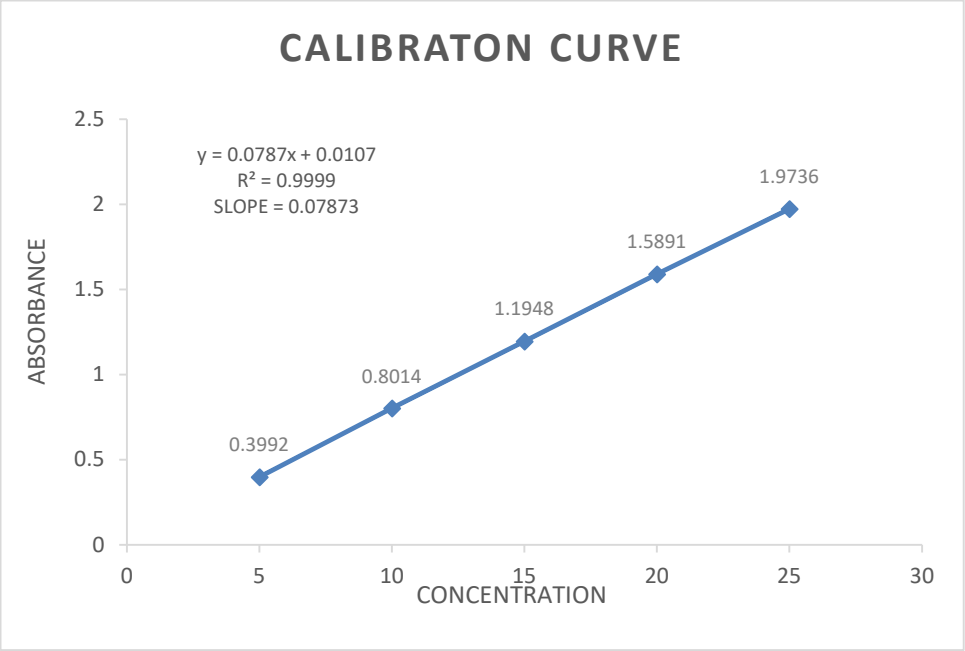

Figure 2: Calibration curve of Sofosbuvir (API).

Table 1: Results of accuracy

Level of Recovery	Sample Conc. (µg/ml)	Recorded conc. (µg/ml)	Absorbance	% Recovery	Mean % Recovery
80%	8	7.900	0.632	98.75	99.93
80%	8	8.110	0.649	101.37	
80%	8	7.975	0.638	99.68	
100%	10	9.893	0.7915	98.93	99.30
100%	10	9.877	0.7902	98.77	
100%	10	10.02	0.8019	100.2	
120%	12	11.862	0.949	98.85	99.71
120%	12	12.062	0.965	100.51	
120%	12	11.975	0.958	99.79	

Acceptance criteria: correlation coefficient should not be less than 0.990

Table 2: Results of Repeatability

Sr.No.	Conc. (µg/ml)	Wavelength (nm)	Absorbance
1	10	260	0.7981
2	10	260	0.8021
3	10	260	0.7898
4	10	260	0.7976
5	10	260	0.7936
6	10	260	0.7908
Mean □ S.D.			0.7953
Standard Deviation			0.0047
% RSD			0.5971

Table-3: Results of intra-Day & inter-Day

Conc. taken (µg/mL)	Observed Conc. Of Sofosbuvir (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Absorbance	Statistical Analysis	Con. found (µg/mL)	Statistical Analysis
10	0.7911	Mean = 0.7943 SD = 0.00706611 %RSD = 0.8896	0.8041	Mean = 0.7998 SD = 0.004689 %RSD =0.5863
10	0.8024		0.8005	
10	0.7894		0.7948	

Table 4: Results of Linearity

Concentration(µg/ml)	Absorbance(n=6)
5	0.3992
10	0.8014
15	1.1948
20	1.5891
25	1.8936

Acceptance criteria: correlation coefficient should not be less than 0.990

Table-5: Result of Method Robustness Test

Wavelength (-2) (258nm)

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.7886	Mean = 0.7882 SD = 0.005308 % RSD = 0.6734
10	0.7813	
10	0.7949	

Wavelength (+2) (262nm)

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.7914	Mean = 0.7876 SD = 0.005308 % RSD = 0.6739
10	0.7893	
10	0.7821	

CONCLUSION

From the experimental studies it can be concluded that first UV-Spectroscopic method is developed for Sofosbuvir in marketed pharmaceutical dosage form. The developed method for the drug (Sofosbuvir) was found to be accurate and precise.

The great features of spectrophotometric methods are their simplicity, economical and rapidity. In this method Acetonitrile and methanol is used as diluent. The results of method validation showing that the developed analytical procedure is suitable for its intended purpose and meets the Guidelines given by the ICH.

The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Sofosbuvir in different formulations.

ACKNOWLEDGEMENT

The authors are grateful to the management of Nalanda College of Pharmacy, Nalgonda for providing the facilities to carry out the present research work

REFERENCES

[1] Swathi P *et al*. RP HPLC method development and validation for estimation of sofosbuvir in pure and tablet dosage forms. Asian J Pharm Tech 2017 sep 27; 7(3):153–156. DOI:10.5958/2231-5713.2017.00025.3

[2] Rajeswar Dutt, Thandu Venkateshwarlu, Santhosh Illendula. RP-HPLC method for the simultaneous estimation of Sofosubvir and velpatasvir in combined dosage form. Indo American Journal of Pharmaceutical Sciences (IAJPS) 2019 Volume 06, Issue 7, 13890-13902.

[3] Santhosh Illendula, Naveen Kumar Singhal; A Review: Novel analytical method development & validation for the determination of selected anti cancer & antiviral drugs, World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS) 2022 jul 1; 11(07) 2022, 533-566. DOI:10.20959/wjpps20227-22475.

[4] Santhosh Illendula *et al*; Method development and validation of Axitinib in bulk and pharmaceutical dosage form by UV spectroscopic method, Indo American Journal of Pharmaceutical Sciences (IAJPS) 06(03) 2019, 6221-6227.

[5] Santhosh Illendula, Thota Sumanjali, D. Sandhya, Method Development and Validation of Afatinib in Bulk and Pharmaceutical Dosage Form by UV-Spectroscopic method. Indo American Journal of Pharmaceutical Sciences (IAJPS) 2018 Volume 05, Issue 3, 1569-1575.

[6] Ganji s *et al*. Development and validation of RP HPLC method for the estimation of Sofosbuvir and related impurity in bulk

and pharmaceutical dosage form. *Futur J Pharm Sci* **7**, 154 (2021).

[7] Sandhya Rani J; A new RP HPLC method development and validation for simultaneous estimation of sofosbuvir and velpatasir in pharmaceutical dosage form. Int J Eng Technol Sci & Res2017 4(11):145–15.

[8] Hanuman T, Sivakkumar T, Sridhar S. New Analytical Method Development and Validation for Simultaneous Estimation of Sofosbuvir and Velpatasvir in Bulk and Pharmaceutical Dosage Form by RP-HPLC Method. JDDT. 15Sep.2020 [cited 13May2023];10(5):143-8.