Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i4.5457

# DEVELOPMENT AND VALIDATION STUDY OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF SOFOSBUVIR

**Zahid Sarfraz[1*], Muhammad Imran[2], Shoomaila Latif[3]**

[1*,2]School of Chemistry, University of the Punjab, Lahore, Pakistan
[3]Department of Chemistry, University of Lahore, Lahore, Pakistan

***Corresponding Author:** Zahid Sarfraz
*School of Chemistry, University of the Punjab, Lahore, Pakistan

## ABSTRACT
A precise, accurate, time saving and cost effective reverse phase high performance liquid chromatographic method was developed for the quantitative determination of sofosbuvir in bulk and in pharmaceutical tablet dosage form. This method was validated with respect to the specificity, accuracy, precision, limits of detection, linearity etc. The Separation was carried on ZORBAX Eclipse Plus C18 column (Length: 150 mm, diameter: 4.6 mm, and particle size of 5 micron) by using 0.05 M phosphoric acid and acetonitrile (66:34% v/v) as mobile phase @ flow rate of 2 mL/min. Total run time of the analysis was kept 6 min and the detection was carried out at the wavelength of 265 nm by using photodiode array detector. Symmetrical peak for Sofosbuvir was obtained with tailing factor of 1.40 at retention time 2.74min. This method gave linear response in the concentration range of 0.003125 mg/mL to 1 mg/mL and value of correlation coefficient ($R^2$) is 0.9999. Precision of method was calculated in terms of repeatability and intermediate precision with relative standard deviation < 2. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.002754 mg/mL and 0.009181 mg/mL respectively. The accuracy of method was calculated in term recovery percentage by spiking 50%, 100% and 150% amount of sofosbuvir in test concentration and result were observed accurate. A verification study for the determination of sofosbuvir in pharmaceutical tablet dosage form was also performed and results were observed very precise and accurate. Hence, the developed method can be safely used for the quantitative determination of sofosbuvir.

**Key words:** RP-HPLC, Sofosbuvir, Method development, validation

## INTRODUCTION
Hepatitis-C is one of the chronic diseases and Hepatitis-C virus is anhepatotropic RNA virus which can causes progressive damage to liver. [2]170 million peoples over the world are chronically infected and about 350000 people die annually due to the Hepatitis-C virus[3-5].
Sofosbuvir (propan-2-yl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy-phenoxyphosphoryl]amino]propanoate) is an antiviral drug used for the treatment of Hepatitis-C viral infections. It is available in the market in both as single and in combination with other antiviral drugs. Sofosbuvir is administrated into the body orally and it is mostly taken with combination of other antiviral drugs like ledipasvir, ribavin, etc.

Sofosbuvir is an off-white to white crystalline solid which is slightly soluble in water and highly soluble in organic solvent like, methanol, acetonitrile etc. and work as nucleotide polymerase inhibitor [6-9]

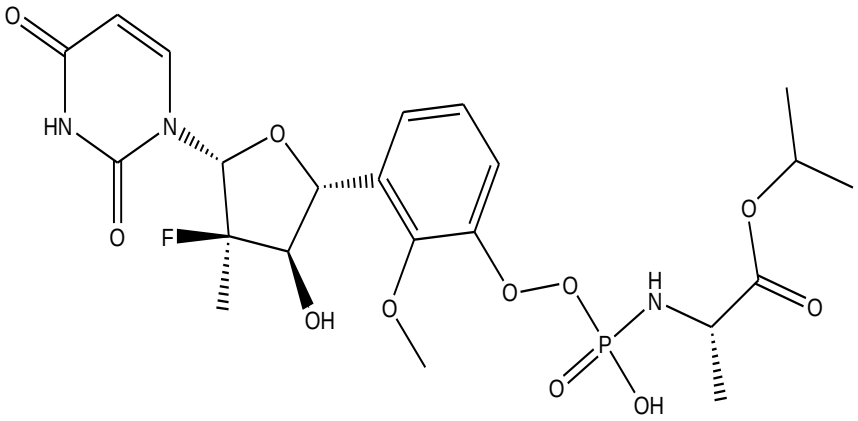



**Fig. 1**: Structure of Sofosbuvir

Analytical method can be a spectral, chromatographic or electrochemical. Analytical method development is the process of selecting an accurate assay procedure to determine the composition of a formulation. Validation study of analytical procedure is the process of proving that the analytical method developed is acceptable for the use in laboratory for the analysis or measure the concentration of subsequent samples [10]

Many methods are already reported for the analysis of Sofosbuvir, which include both chromatographic methods and spectrophotometric method [11-18]. However, we report here the development and validation study of RP-HPLC method for quantitative analysis of Sofosbuvir. The main purpose of this work is to obtain cost effective and time saving method for the quantitative analysis of sofosbuvir in active pharmaceutical ingredient and pharmaceutical tablet dosage forms

## EXPERIMENTAL WORK

### Chemicals:

All chemicals used in the analysis are of high quality. Sofosbuvir standard was obtained from the Genix pharma (drug's manufacturer in Pakistan) with claimed potency of 99.1% and used without further purification. Samples of sofosbuvir were obtained from market with brand name of Cure-C, Sofos, Sofohil and Sofiget manufactured by Global pharma pvt. Limited, Genix pharma, Hilton pharma pvt. Limited, and Gets pharma pvt. Limited respectively. Acetonitrile and methanol used were of HPLC grade. Orth-phosphoric acid used in preparation of mobile phase was obtained from sigma Aldrich**.**

### Instruments and Equipments:

Two HPLC system shimadzu separation module SPD-M20A coupled with PDA detector SPD-M20A and alliance separation module waters 2695e coupled with PDA detector were used. For weighing purpose shimadzu analytical weighing balance was used and all apparatus used in the experiment work was made up of Pyrex and dried before use.

### Method Development

**Chromatographic Conditions**: Separation was carried out on Agilent ZORBAX Eclipse Plus C18 column (length:150, dia: 4.6mm and particle size: 5µm) and Waters spherisob C18 column (length:150, dia: 4.6mm and particle size: 5µm) by using mobile phase 0.0 5M orthophosphoric acid and HPLC grade acetonitrile at the ratio of 66:34%. The flow rate was kept 2 mL/min. and detection was carried out at the wave length of 265 nm by using photo diode array detector. The injection volume of sample was 20 uL and total run time of the analysis was kept 6 min.

**Preparation of Mobile phase & diluent: A.** 0.05M phosphoric acid (Prepared by adding 3.37 mL of orthophosphoric acid in 1000 mL of solution and filtered by using Filter paper/ Micropore membrane disc having diameter: 47 mm, and Pore size 0.2 µm)
**B**: Acetonitrile

**Diluent**: Diluent for sample and standard preparation was made by mixing mobile phase A and mobile phase B at the ratio of 66:34%.

**Preparation of standard stock solution**: 1 mg/mL stock solution of sofosbuvir was prepared taking 100 mg of sofosbuvir standard (GENixQC-F-046) obtained from Genix pharma Pvt. Limited. The amount of sofosbuvir was weighed by using Shimadzu analytical weighing balance and added into 100 mL of volumetric flask. About 40 mL of diluent was added into the flask, sonicated for 10 minutes and made volume upto the mark.

**Preparation of 0.1 mg/mL Standard:** 0.1 mg/mL standard solution was prepared from 1 mg/mL stock solution by taking 2.5 mL of standard stock solution and made volume upto 25mL.

**Validation of Method:**
**Linearity:** Linearity of the method was calculated by preparing three replicates of each concentration in the range of 0.003125 to 1 mg/mL i.e.1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.1 mg/mL, 0.05 mg/mL, 0.025 mg/mL, 0.0125 mg/mL, 0.003125 mg/mL. All concentration solution were prepared from above prepared standard stock solution through serial dilutions

**Precision:** Precision of method was calculated in term of repeatability and intermediate precision by analyzing the six replicates of 0.1mg/mL standard solution on same day, different days and different equipment as well.

**Detection limit and Quantification Limit:** Detection limit and quantification limits of method were calculated from linearity curve by using the relation;
$LOD = 3\ \sigma/S$
$LOQ = 10\ \sigma/S$
Where,
$\sigma$ = standard deviation of the response
S= Slope of the calibration curve

**Robustness**: Robustness of the method was verified by changing the composition of mobile phase, the flow rate of mobile phase and temperature of the column.

**Accuracy:** Accuracy of the method was calculated in term of recovery percentage by spiking 50 %, 100% and 150 % of analyte in target concentration. 0.1mg/mL target conc. was prepared from standard stock solution and for spiking of 50%, 100% and 150% a 1.25 mg, 2.5 mg and 3.75 mg of analyte was added in 25 mL of target concentration respectively.

**Verification of method:**
Developed method was verified for the identification and quantification of sofosbuvir in pharmaceutical dosage forms. For this purpose, four different brands of sofosbuvir were obtained from the local market. For the preparation of homogeneous sample solution, four tablets containing sofosbuvir 400 mg each were weighed. The total weight of four tablets were 4110.9 mg, 4096.8 mg, 4071.4 mg, and 4072.3 mg for cure-C, Sofos, Sofohil and Sofiget respectively. The weighed amount of tablets is grinded by using pestle and mortar to bring it in powder form. The 0.1 mg/mL sample solution of sofosbuvir were prepared by taking equivalent amount 64.3 mg, 64.0 mg 63.6 mg, 63.6 mg of grinded powder. Grinded powder was weighed by using analytical weighing

balance and added into 25 mL of volumetric flask, dissolved in about 15 mL of diluent and made volume upto the mark after sonication for 10 minutes. Before injecting the sample in the column, the sample was filtered through syringe nylon filter.

## RESULTS AND DISCUSSION

### Method Development

The aim of the present study was to develop a precise, accurate, and cost effective method for the analysis of sofosbuvir in bulk and pharmaceuticals tablet dosage form. In order to achieve the best results, different trials by varying mobile phase and flow rate were proceeded. The best results having symmetrical peak, low retention time and minimum tailing factor were achieved by using 0.5 M $H_3PO_4$ and acetonitrile in the ratio of 64:36 % v/v as a mobile phase at the flow rate of 2mL/min.

A very symmetrical chromatographic peak was obtained for the sofosbuvir at the retention time of 2.748 min with tailing factor of 1.40.

**Table 1:** System suitability parameters

| **Conc.** | **Replicates** | **Peak Area** | Mean Peak Area | 1179038.833 |
|---|---|---|---|---|
| 0.1 mg/mL | Replicate-1 | 1183387 | | |
| | Replicate-2 | 1178064 | SD | 3503.192454 |
| | Replicate-3 | 1174892 | %RSD | 0.297122737 |
| | Replicate-4 | 1176189 | Tailing Factor | 1.40 |
| | Replicate-5 | 1178657 | RT | 2.74 |
| | Replicate-6 | 1183044 | Theoretical Plate count | 5310 |

### Method validation:

To ensure the validity of result, validation of method is as important as the development of the method, so the developed method is validated according to pharmacopeia and international conference of harmonization (ICH) guidelines. Validation study of method is performed for the parameters linearity, precision, limit of detection, limit of quantification, robustness and accuracy and results were observed as below.

### Linearity

Linearity of the analytical procedure is the ability of procedure to obtain the test results (within given range) which are directly proportional to the concentration of the analyte in the sample. Linearity of the method was calculated by preparing samples solutions of sofosbuvir in concentration range of 0.003125 to 1 mg/mL i.e.1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.1 mg/mL, 0.05 mg/mL, 0.025 mg/mL, 0.0125 mg/mL, and 0.003125 mg/mL. Three replicates of each prepared concentration were analyzed and results obtained from analysis are tabulated in Table 4.3.

**Table 4.2:** Linearity Calculation

| **Conc. of STD** | **Peak Area** | | | |
|---|---|---|---|---|
| | **Replica 1** | **Replica 2** | **Replica 3** | **Average** |
| 0.003125 mg/mL | 19793 | 18114 | 19805 | 19237.33 |
| 0.0125 mg/mL | 71549 | 72132 | 71657 | 71779.33 |
| 0.025mg/mL | 320475 | 319432 | 320098 | 320001.66 |
| 0.05 mg/mL | 607915 | 612029 | 611089 | 610344.33 |
| 0.1 mg/mL | 1183387 | 1178064 | 1174892 | 1178781 |
| 0.25 mg/mL | 2618741 | 2623712 | 2595188 | 2612547 |
| 0.5 mg/mL | 5653273 | 5661477 | 5565417 | 5626722.33 |
| 1 mg/mL | 10693701 | 10736271 | 10586957 | 10672309.7 |
| Correlation Coefficient ($r^2$) | | 0.9999 | | |
| Intercept | | 21060.5514 | | |
| Slope | | 5033938 | | |
| Standard Error of Y-intercept | | 4621.845698 | | |

The calibration curve was drawn between the concentration of sample and the mean peak area of all prepared concentrations. The calibration curve was observed linear with correlation coefficient of 0.999, which is in acceptable range according to international standards.

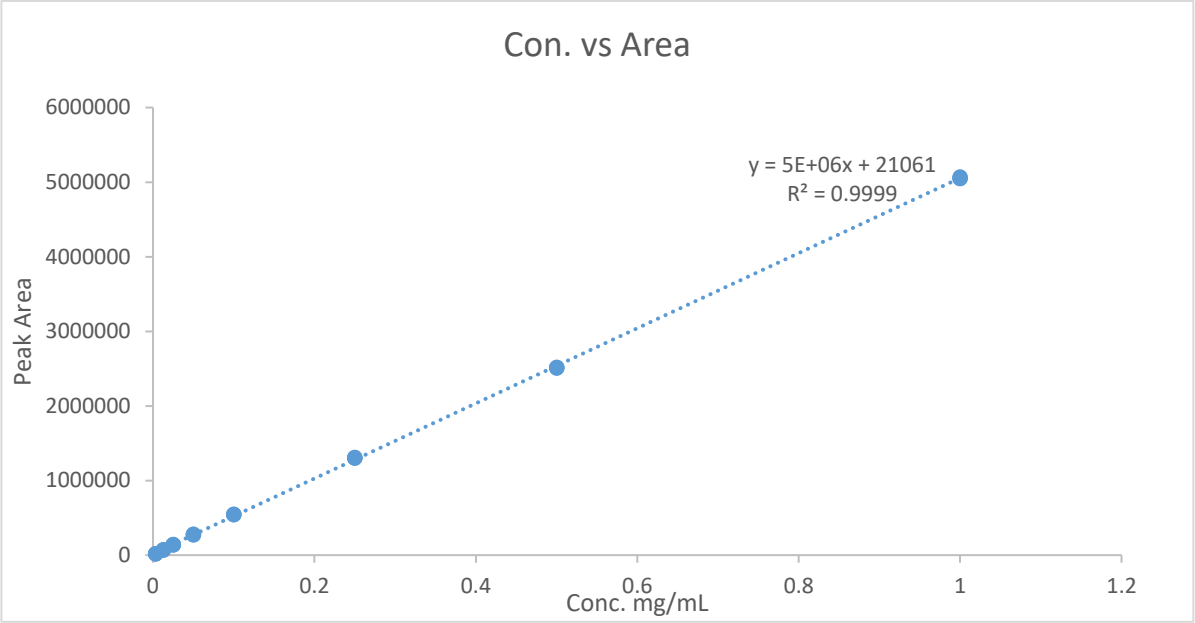



**Fig. 2:** Linearity curve for Sofosbuvir

**Limit of Detection & Limit of Quantification**

The detection limit (LOD) of the analytical procedure is the lowest amount of the analyte which may be detected but not necessary to quantitated by using that procedure and it was calculated from regression data.

LOD = 3 σ/S = 0.002754 mg/mL

Quantification limit of the analytical procedure is the lowest amount of the analyte in the sample which can be quantitatively determined with suitable accuracy and precision and it was also calculated from regression data.

LOQ = 10 σ/S = 0.009181 mg/mL

**Precision**

Precision of method was calculated in term of repeatability and intermediate precision as required by ICH guidelines for method validation by analyzing the sample on different days and different equipment's. The values for both repeatability and intermediate precision were in expectable range according to USP pharmacopeia.

**Table 4.5:** Calculation of precision

| **Concentration** | **Replicates** | **Equipment 1** | | **Equipment 2** |
|---|---|---|---|---|
| | | **Intra Day** | **Inter day** | |
| | | **Peak Area** | | |
| 0.1mg/mL | Replicates 1 | 1183387 | 1157843 | 549295 |
| | Replicates 2 | 1178064 | 1139050 | 545368 |
| | Replicates 3 | 1174892 | 1160988 | 550128 |
| | Replicates 4 | 1176189 | 1152978 | 549295 |
| | Replicates 5 | 1178657 | 1138855 | 545368 |
| | Replicates 6 | 1183044 | 1144011 | 550128 |
| Mean | | 1179038.833 | 1148954.167 | 548263.6667 |
| SD | | 3503.192454 | 9638.841412 | 2273.699423 |
| %RSD | | 0.297122737 | 0.838923056 | 0.414709119 |

## Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage

| Parameter | | RT | Tailing factor | Remarks |
|---|---|---|---|---|
| Change in Mobile Phase Composition | ACN : 0.05 M $H_3PO_4$ 36:64 % | 2.73 | 1.399 | Robust |
| | ACN : 0.05 M $H_3PO_4$ 34:66% | 2.75 | 1.408 | |
| | ACN :0.05 M $H_3PO_4$ 32:68% | 2.76 | 1.414 | |
| Change in Flow Rate | 1.9 mL/min. | 2.77 | 1.399 | Robust |
| | 2.0 mL/min | 2.75 | 1.408 | |
| | 2.1 mL/min | 2.73 | 1.410 | |
| Change in Temperature | 28 ºC | 2.75 | 1.381 | Robust |
| | 25 ºC | 2.75 | 1.408 | |
| | 22 ºC | 2.75 | 1.418 | |

## Accuracy

Accuracy of an analytical procedure is the closeness of observations with the conventional true value or an accepted reference value. The accuracy of analytical procedure was calculated in term of recovery percentage by adding known amount of analyte in the target concentration. According to ICH guidelines of the analytical procedure, accuracy should be determined by using at least nine measurements over minimum three concentrations which cover specified range. i.e. three replicates of three concentration [19]. So for determination of accuracy, three different concentrations were prepared by adding 50%, 100% and 150% to the target concentration of the analyte. Nine measurements were taken from three replicates of each prepared concentrations.

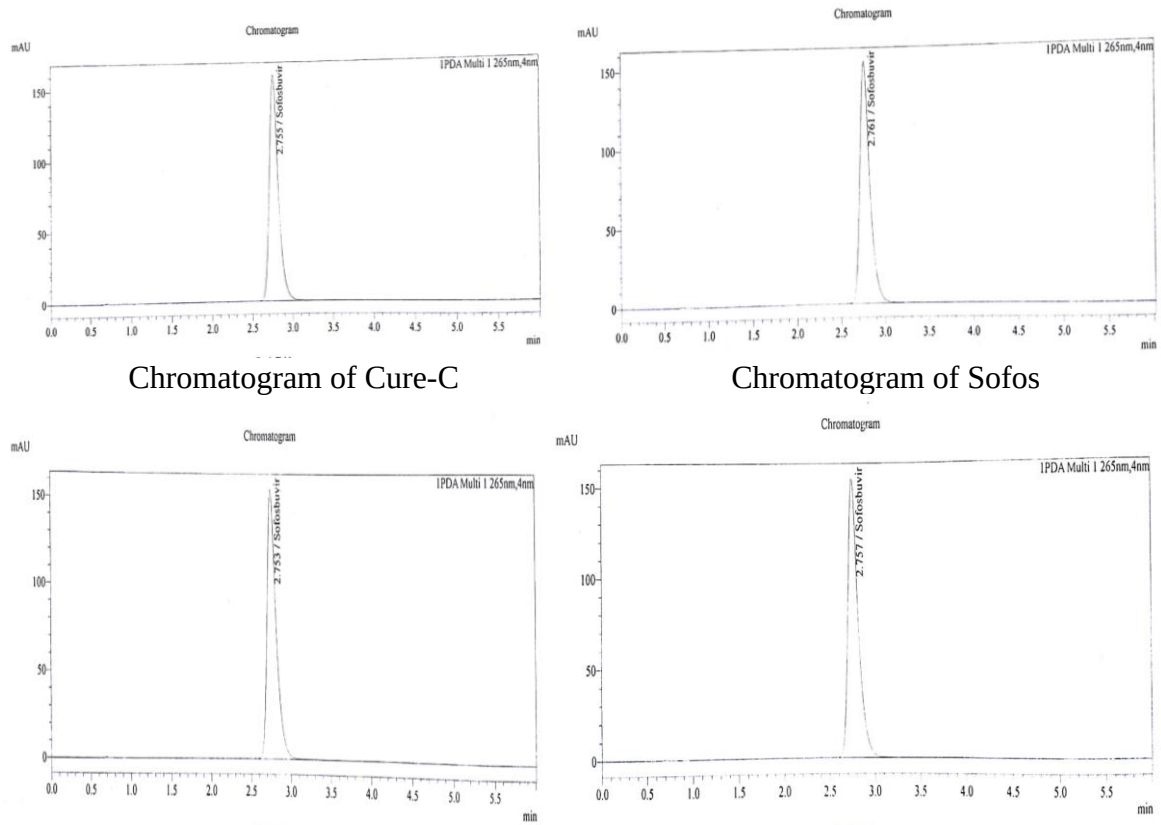
**Table 4.6:** Calculation of percentage recovery

| Sr. # | Conc. | Peak Area | Mean | Det. Conc. | Spiked amount | S-US | % Rec. |
|---|---|---|---|---|---|---|---|
| 2 | 50 % spiked | 1504960 | 1616002 | 0.140 | 0.05 | 0.0503 | 100.43 |
| | | 1648659 | | 0.153 | | | |
| | | 1694387 | | 0.157 | | | |
| 3 | 100% Spiked | 2169705 | 2135879 | 0.202 | 0.1 | 0.0986 | 98.68 |
| | | 2062332 | | 0.191 | | | |
| | | 2175600 | | 0.202 | | | |
| 4 | 150% Spiked | 2806974 | 2706101.3 | 0.261 | 0.15 | 0.1517 | 101.15 |
| | | 2552168 | | 0.237 | | | |
| | | 2759162 | | 0.257 | | | |

*Det. = Determined *US = Unspiked *S-US = Spiked –Unspiked

## Verification of method

Method developed for the analysis of sofosbuvir in active (API) form provides very precise results. As in pharmaceutical dosage form, other compounds like excepient and preservative etc. may be present which may affect the quality of test results. So the developed method was applied for the quantitative analysis of sofosbuvir in pharmaceutical tablet dosage form to verify the validity of method for the analysis of sofosbuvir in pharmaceutical formulations. For this purpose, four samples of different brands of sofosbuvir Cure-C, Sofos, Sofohil and Sofiget which were easily available were taken from the market and analyzed by using developed method. No interference of excepients or impurities observed. Typical chromatogram obtained for all sample by using developed procedure are shown in Figure 1.




Chromatogram of Cure-C Chromatogram of Sofos

Chromatogram of Sofohil Chromatogram of Sofiget

**Fig. 4.3** Chromatogram of samples

**Table 4.7: Results for Samples**

| Concentration | Replicates | Cure-C | Sofos | Sofohil | Sofiget | Std. Sol |
|---|---|---|---|---|---|---|
| 0.1mg/mL | Replicate-1 | 1183387 | 1183387 | 1183387 | 1183387 | 1230331 |
| | Replicate-2 | 1178064 | 1178064 | 1178064 | 1178064 | 1210048 |
| | Replicate-3 | 1174892 | 1174892 | 1174892 | 1174892 | 1210011 |
| Mean | | 1178781 | 1178781 | 1178781 | 1178781 | 1216797 |
| SD | | 3504.932 | 3504.932 | 3504.932 | 3504.932 | 9570.231 |
| %RSD | | 0.297335 | 0.297335 | 0.297335 | 0.297335 | 0.78651 |

## Conclusion:

A very precise, accurate, time saving and cost effective method is developed for the quantitative analysis of sofosbuvir in bulk and pharmaceutical dosage form. Method is also validated according to ICH guidelines for the parameters linearity, range, precision, limit of detection, limit of quantification, accuracy and robustness. All results were found in specified limits which were described in most of the international guide lines and international pharmacopeias. Developed method is also verified for the quantitative determination of sofosbuvir in pharmaceutical dosage forms by taking different brands of sofosbuvir from the market. Very fine results were obtained without interference of the excepients or other impurities which may present in the sample. So the develop method can be used for the quantitative analysis of the sofosbuvir in bulk and pharmaceutical formulations at both industrial levels as well as in quality control laboratories.

## REFERENCES

1. Mahoney, A. and J. Evans (2008). Comparing drug classification systems. AMIA.Annual Symposium proceedings. AMIA Symposium.
2. Manns, M. P. and M. Cornberg (2013). "Sofosbuvir: the final nail in the coffin for hepatitis C" The Lancet Infectious Diseases **13**(5): 378-379.

3. Organization, W. H. (2014). Hepatitis C. Fact sheet no. 164. Geneva: World Health Organization; 2014
4. Sterling, R. and J. Lim (2012). "Chronic Hepatitis C Virus: Advances in Treatment, Promise for the Future." Gastroenterology **143**(6): 1688
5. Nakano, T., G. M. Lau, G. M. Lau, M. Sugiyama and M. Mizokami (2012). "An updated analysis of hepatitis C virus genotypes and subtypes based on the complete coding region." Liver international **32** (2): 339-345.
6. Evans, W. E. and H. L. McLeod (2003). "Pharmacogenomics—drug disposition, drug targets, and side effects." New England Journal of Medicine **348** (6): 538-549.
7. Herbst Jr, D. A. and K. R. Reddy (2013). "Sofosbuvir, a nucleotide polymerase inhibitor, for the treatment of chronic hepatitis C virus infection." Expert opinion on investigational drugs **22**(4): 527-536.
8. Eltahla, A. A., F. Luciani, P. A. White, A. R. Lloyd and R. A. Bull (2015). "Inhibitors of the hepatitis C virus polymerase; mode of action and resistance." Viruses**7** (10): 5206-5224.
9. Nemade, R., M. Dole and S. Sawant (2017). "development and validation of uv-spectrophotometric method for estimation of sofosbuvir in bulk form by absorbance maxima method." world journal of pharmacy and pharmaceutical sciences Volume 6, Issue 7, 749-757
10. Toomula, N., A. Kumar, D. Kumar and V. Bheemidi (2012). "Biological databases-integration of life science data." J. Comput. Sci. Syst. Biol **4**: 87-92.
11. Patel, S. B., B. G. Chaudhary, M. K. Buch and A. B. Patel (2009). "Stability indicating RP-HPLC method for simultaneous determination of valsartan and amLodipine from their combination drug product." International Journal of ChemTech Research **1**(4): 1257-1267.
12. Vikas, P. M., P. M. Vikas, D. T. Satyanarayana, D. V. Kumar, E. Mounika, M. S. Latha, R. Anusha and Y. Sathish (2016). "Development and validation of new RP-HPLC method for the determination of sofosbuvir in pure form." World Journal of pharmacy and pharmaceutical Sciences **5**(5): 775-781
13. Vejendla, R., C. Subramanyam and G. Veerabhadram (2016). "Estimation and validation of sofosbuvir in bulk and tablet dosage form by RP-HPLC." International Journal of Pharmacy **6**(2): 121-127.
14. Swathi, P., K. R. Dutt, K. Vrao and M. A. Raja (2017). "RP-HPLC Method Development and Validation for Estimation of Sofosbuvir in Pure and Tablet Dosage Form." Innovat International Journal of Medical & Pharmaceutical Sciences **2**(4).
15. Rai, S. P. Y., Y. Prajapati and P. Patni (2017). "development and Validation of RP-HPLC and UV Spectroscopic methods for Simultaneous Estimation of Sofosbuvir and Ledipasvir in their combined tablet dosage forms." An International journal of Pharmaceutical Sciences **8**(2).
16. Nalla, S. and J. Seshagiri Rao (2017). "A Stability indicating RP-HPLC method for simultaneous estimation of Velpatasvir and Sofosbuvir in combined tablet dosage forms." World Journal of Pharmacy and Pharmaceutical Sciences **6**(9): 1596-1611.
17. Mansour, F. R. (2018). "A new innovative spectrophotometric method for the simultaneous determination of sofosbuvir and ledipasvir." Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy **188**: 626-632.
18. Jayavarapu, K. R., G. Isaac, R. S. Dharani, S. Prabha, S. Raziya, T. Satyanarayana and S. Parimalakrishnan (2017). "Accurate, simple and quantitative UV-Spectroscopic method for the estimation of Sofosbuvir in pure and marketed formulations." Indian Journal of Research in Pharmacy and Biotechnology **5**(5): 333-336.
19. Guideline, I. H. T. (2005). "Validation of analytical procedures: text and methodology." Q2 (R1)**1**