Research Article

# Development and Validation of a Rapid RP- HPLC Method for the Determination of Sofosbuvir in Bulk and in Pharmaceutical Dosage Form

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**ABSTRACT**

A simple, sensitive, precise, quick, and reproducible reversed-phase high performance liquid chromatographic method has been developed and validated for the determination of Sofosbuvir in pharmaceutical dosage form. Agilent High Pressure Liquid Chromatograph 1260 series with GI311C Quat. pump, Eclipse XDB-$C_{18}$ Colum (5 µm particle size x 4.6 × 250 mm) (made in USA) and diode array detector G1315D was utilized in the study. The mobile phase consisting of methanol and acetonitrile in the proportion of 30: 70 v/v was used for the study. A flow rate of 1 mL/ min with an injection volume of 20 µL was selected for this study. The separation was acquired at a temperature of 30 $^0$C and eluents were observed using by photo diode array detector set at 261 nm. The retention time of Sofosbuvir is found to be 2.440 minutes and the calibration curve was linear in the concentration range of 10 - 30 µg/mL with a correlation coefficient of 0.9998 and also good liner relationship between peak area and concentration in calibration curve. The limit of detection and the limit of quantification were found to be 0.9708 µg/mL and 2.9420 µg/mL respectively. Recovery of Sofosbuvir in pharmaceutical formulation was observed in the range of 99.3 - 99.9 %. Percentage assay of Sofosbuvir was found to be 99.9 ± 0.13 %. Analytical validation parameters such as selectivity, specificity, linearity, precision and accuracy were studied and percentage relative standard deviation (% RSD) value for all key parameters was less than 2 %. Hence the proposed RP-HPLC method was successfully feasible for the determination of Sofosbuvir in bulk and pharmaceutical tablet dosage form.

**Keywords:** RP - HPLC, Sofosbuvir, Validation, ICH guidelines.

## INTRODUCTION

The IUPAC name of Sofosbuvir (SOF) is Propan-2-yl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2, 4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl] methoxy-phenoxy phosphoryl] amino] propanoate. SOF has been get through novel recently approved medication for the treatment of patients with chronic hepatitis C. Hepatitis C virus affects about two to three percent of the global population[1-2]. The drug mostly works as an extremely powerful inhibitor of the NS5B polymerase for Hepatitis C virus (HCV). A thorough quantitative analysis of SOF drug revealed that very few analytical methods were accessible to explain the quantification of SOF by UV- Spectrophotometric method[3], RP-HPLC in human plasma[4], HPLC[5], UPLC-ESI-MS/MS[6], UPLC-MS/MS[7], SPE-LC[8], LC/MS[9] methods have been reported for the estimation of SOF in biological fluids and tablet dosage forms. In recent times Gradient HPLC is extensively employed quantification of drugs. The reported HPLC method for SOF took longer chromatographic run time, lower sensitivity and also not better peak symmetry. Keeping in view the above literature survey the author inclined with an aim to develop a novel Gradent HPLC method to determine simple, precise, accurate, specific, reliable, and economical as well as time saving. The present work describes development of a validated HPLC method for the determination of SOF in tablet dosage form and fig 1a shows the chemical structure of SOF.

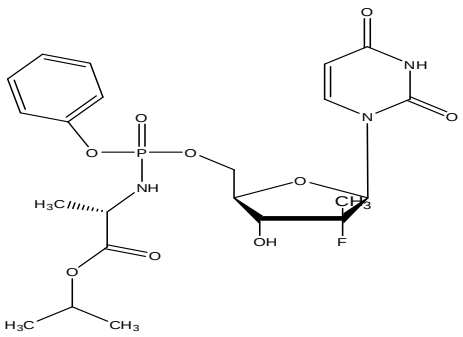

**Figure 1a:** Chemical structure of Sofosbuvir

## MATERIALS AND METHODS

### Materials and reagents

An analytically reference standard SOF was kindly gifted by Hetero Labs Limited, Hyderabad, India. All the chemicals were analytical grade. HPLC grade acetonitrile was obtained from Merck pharmaceuticals private Ltd., Mumbai, India. Methanol and water utilized were of HPLC grade and purchased from Merck specialties private Ltd., Mumbai, India. Commercial tablets of SOF formulation were procured from local pharmacy. Sofovir 400 mg containing with labeled amount of 400 mg per tablet is manufactured by Gilead Pvt Ltd.

**Instrumentation**

The high pressure liquid chromatographic system used was an Agilent High Pressure Liquid Chromatograph 1260 series with GI311C Quat. pump, Eclipse XDB-$C_{18}$ column (5 µm particle size x 4.6 × 250 mm) (made in USA) and diode array detector G1315D was used for data acquisition. Digital pH meter (Systronics model - 802), An electronic balance (Shimadzu TX223L), a sonicator (spectral lab, model UCB 40) and UV- Visible Spectrophotometer (Systronics model-2203) were used in this study.

**Chromatographic conditions**

Chromatographic separation was achieved on Eclipse XDB-$C_{18}$ column (250 mm x4.6 mm, 5 µm particle size) utilizing mobile phase composition of methanol and acetonitrile in the ratio of 30: 70 v/v. Mobile phase was filtered through 0.22 mm filter paper and degassed. Flow rate was set at 1 mL/ min at ambient temperature, injection volume of 20 µL and using diode array detector to monitor the effluents at wavelength of 261 nm. (fig. 1b).

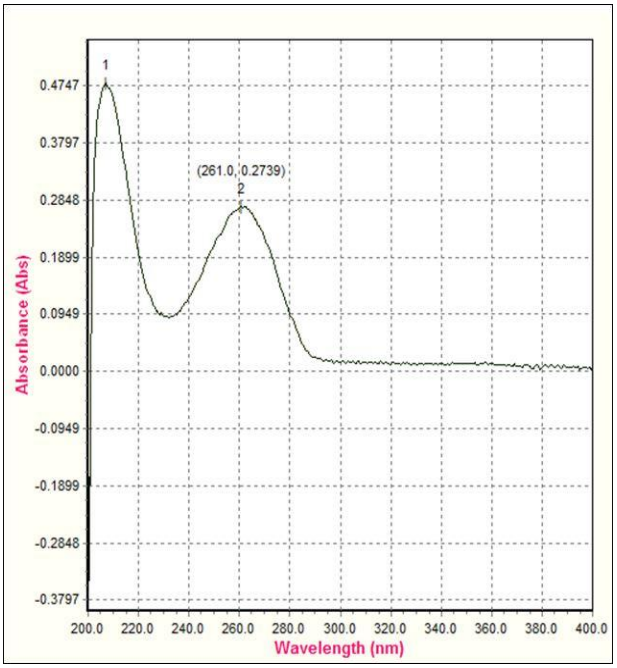


**Figure 1b:** UV spectrum of SOF (maximum lambda max at 261 nm)

**Preparation of mobile phase**

Mobile phase was prepared by mixing methanol and acetonitrile (HPLC grade) in the ratio of 30:70 v/v and sonicated for 15 minutes and filtered through 4.5 mm millipore filter paper and degassed before use.

**Preparation of standard stock and working standard of drug solution**

1000 µg/ml standard SOF solution was prepared by dissolving 10 mg of drug in to 10 mL of mobile phase and sonicated for 5 minutes then filter with vacuum filtration kit through 0.45 µ millipore filter paper and required concentrations solutions containing 10, 15, 20, 25 and 30 µg/mL of SOF were prepared eventually.

**Preparation of sample solution for tablets assay**

Twenty tablets of Sofovir were correctly weighed, crushed and finely powdered. A portion of the powder equivalent to the weight of 10 mg was accurately weighed and shifted into 100 ml volumetric flask and 20 mL of mobile phase was added to flask and sonicated for 20 minutes to complete dissolution of drug. It was filtered through whatman filter paper no.42 to remove insoluble materials. The volume of filtrate was diluted to 100 ml with mobile phase (100 µg/mL). The above prepared solution was further diluted to get required concentrations then analyzed following the proposed procedures. The content of the tablet was calculated from plotted calibration graph or using regression equation.

**Analytical method validation**

The proposed RP-HPLC method of analysis was validated in pursuance of ICH Q2 (R1) guide lines[10-11] for the parameters like system suitability, specificity, linearity, precision, accuracy, and robustness, limit of detection (LOD) and limit of quantitation (LOQ).

**System suitability**

The chromatographic systems utilized for analysis must pass system suitability limits before sample analysis can begin. Set up the chromatographic system allow the HPLC system to stabilize for forty minutes. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters like, tailing factor (NMT 1.5), theoretical plate count (NLT 3000) and retention time. The % RSD for peak area of six replicate injections of SOF standard NMT 2.0. The parameters such as tailing factor, % RSD and theoretical plates were studied and found satisfactory.

**Linearity**

Under proposed experimental conditions, the relationship between the area and concentration of SOF was studied. Linearity was checked by preparing standard solutions at 5 different concentration levels of SOF. Standard solutions (10, 15, 20, 25, 30 µg/mL) of SOF were injected into the HPLC system to get the chromatograms. The average peak area and retention time were recorded.

**Specificity**

The intention of this study is to determine whether the effect of excipients and other additives that are usually present in the pharmaceutical formulations of SOF are interfering with the peaks of the analytes or not in optimum chromatographic conditions. The blank solution was prepared by mixing of the excipients in the mobile phase without the drug.

**Table 1:** Optimum chromatographic conditions and system suitability parameters

| Parameter | Chromatographic conditions |
|---|---|
| **Instrument** | Agilent High Pressure Liquid Chromatograph 1260 series with GI311C Quat. Pump. |
| **Column** | Eclipse XDB model $C_{18}$ column (4.6 mm i.d.X250 mm, 5µm particle size). |
| **Detector** | Diode array detector G1315D |
| **Diluents** | Methanol: Acetonitrile (30 : 70 v/v) |
| **Mobile phase** | Methanol: Acetonitrile (30 : 70 v/v) |
| **Flow rate** | 1 mL/min. |
| **Detection wave length** | UV at 261 nm. |
| **Run time** | 10 minutes |
| **Temperature** | Ambient temperature (25 °C) |
| **Volume of injection loop** | 20 µL |
| **Retention time ($t_R$)*** | 2.440 minutes |
| **Theoretical plates [th.pl]*** | 11903 |
| **Tailing factor (asymmetry)*** | 1.150. |

* = number of determinations (n=6).

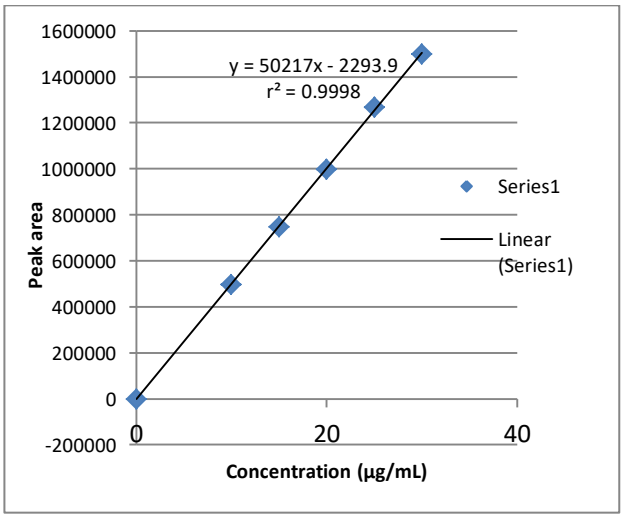


**Figure 2:** Calibration curve of Sofosbuvir

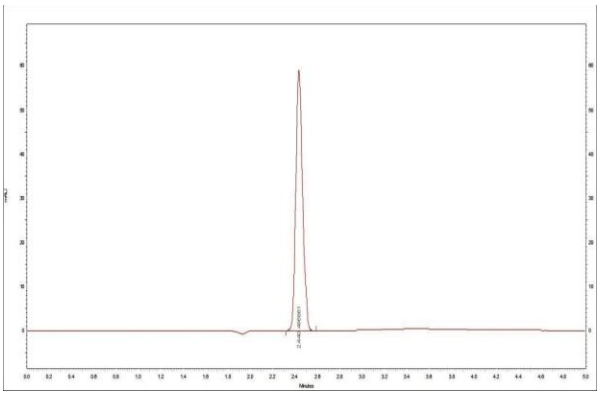

**Figure 3a:** Standard chromatogram of Sofosbuvir (10 µg/mL)

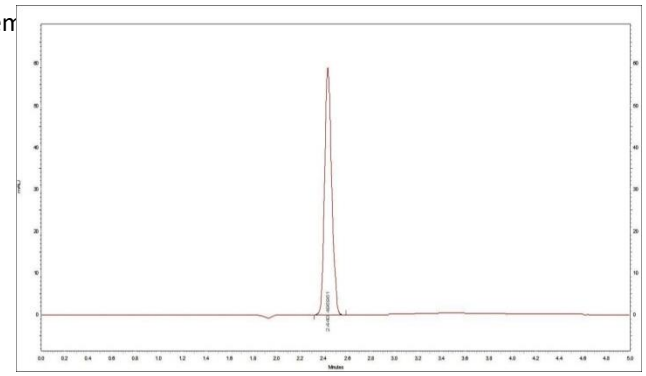

**Figure 3b:** Standard chromatogram of Sofosbuvir (15 µg/mL)

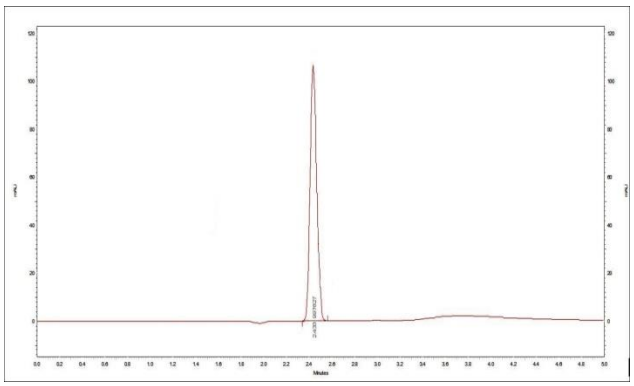

**Figure 3c:** Standard chromatogram of Sofosbuvir (20 µg/mL)

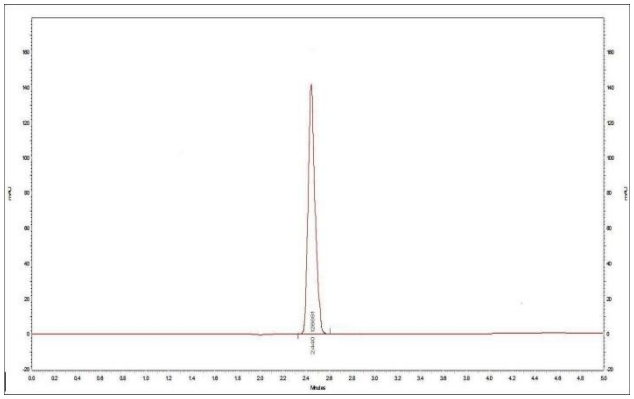

**Figure 3d:** Standard chromatogram of Sofosbuvir (25 µg/mL)

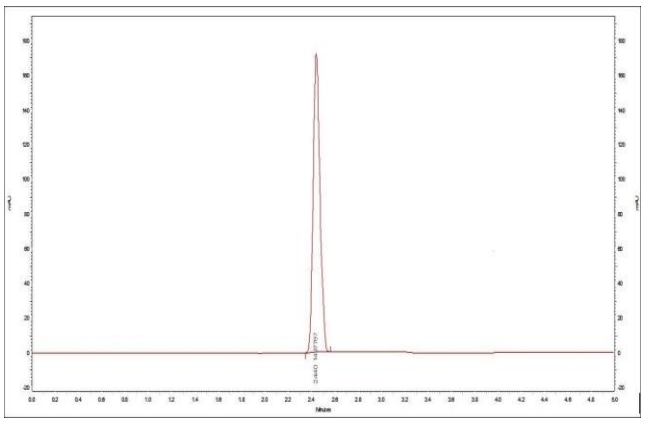

**Figure 3e:** Standard chromatogram of Sofosbuvir (30 µg/mL)

**Precision**

To check the reproducibility of the method. Precision was determined by intra-day and inter-day study. The repeatability (intra assay precision) was studied by repeating the assay 3 times in the same day. Inter-day precision (Intermediate precision) was studied by repeating the assay 3 times on 3 different days (consecutive days) triplicate on each day and % RSD (% relative standard deviation) was calculated.

**Accuracy/Recovery**

The accuracy of the method was found out by standard addition method. A known amount of standard drug was added at 50 %, 100 % and 150 % level. The concentrations were re-analyzed with the above described procedure. The percent recovery of the triplicate solutions was determined and average of the percent recovery was calculated.

**Robustness**

Robustness of the method is its ability to remain unaffected by small changes in variety of parameters such as the slight variation in acetonitrile percentage composition of the mobile phase, flow rate, detection wavelength.

**Table 2:** Robustness results of Sofosbuvir

| S. No | Parameter | Optimized | Used | Retention time ($t_R$), min | Plate count$^{\$}$ | Peak asymmetry$^{\#}$ | Remark |
|---|---|---|---|---|---|---|---|
| 1. | Flow rate (±0.2 mL/min) | 1.0 mL/min | 0.8 mL/min | 2.430 | 11,639 | 1.290 | *Robust |
| | | | 1.0 mL/min | 2.440 | 11,915 | 1.25 | *Robust |
| | | | 1.2 mL/min | 2.450 | 11,660 | 1.210 | *Robust |
| 2. | Detection wavelength (±5 nm) | 261 nm | 266 nm | 2.440 | 11,915 | 1.25 | Robust |
| | | | 261 nm | 2.440 | 11,915 | 1.25 | Robust |
| | | | 256 nm | 2.440 | 11,915 | 1.25 | Robust |
| 3. | Mobile phase composition (Methanol: Acetonitrile) | 30:70 v/v | 35:65 v/v | 2.447 | 11,649 | 1.203 | *Robust |
| | | | 30:70 v/v | 2.440 | 11,915 | 1.25 | *Robust |
| | | | 45:55 v/v | 2.440 | 11,640 | 1.203 | *Robust |

Acceptance criteria (Limits): $^{\#}$Peak Asymmetry < 1.5, $^{\$}$ Plate count > 3000, * Significant change in Retention time.

**Ruggedness**

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective peak areas were noted.

**LOD and LOQ**

The purpose of this study is to evaluate the sensitivity of the proposed method. Limit of Detection (DL) is the lowest concentration in a sample that can be detected, but not necessarily quantified under exact value. The limit of quantitation (QL) is the lowest concentration of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated based on using following formula.

DL= 3.3(SD)/S and QL= 10 (SD)/S, where SD = Standard deviation response.

S= Slope of the calibration curve.

**Analysis of marketed formulation**

Using the developed RP-HPLC chromatographic method, assay of SOF in tablet was carried out as mentioned in the experimental section. Six replicate determinations were made. Satisfactory results were obtained and were good agreement with the label claim.

**Table 3:** Summary of Validation parameters

| Validation Parameters | Results |
|---|---|
| **Linearity range (µg/mL)** | 10-30 |
| **Linear regression equation** | Y = 50217x-2293.9 |
| **Correlation coefficient ($r^2$)** | 0.9998 |
| **Specificity** | Specific |
| **Repeatability (% RSD, n=6)** | 0.011 |
| **Intraday precision (% RSD, n=3)** | 0.021 |
| **Inter day precision ( % RSD, n=3)** | 0.025 |
| **% Recovery ±SD (accuracy, n=6)** | 99.3-99.9 % |
| **Limit of Detection (µg/mL)** | 0.9708 |
| **Limit of Quantitation (µg/mL)** | 2.9420 |
| **Robustness** | Robust |

**Table 4:** Assay results of Sofosbuvir formulation

| Brand name | Final concentration | Concentration* Found ±SD | Assay ± % RSD |
|---|---|---|---|
| **Sofovir 400 mg tablet (Gilead Laboraties Pvt Ltd India).** | 20 µg/mL | 19.98 µg/mL ±0.14 | 99.9 ± 0.13 |
| **Sofosbuvir bulk drug** | 20 µg/mL | 19.89 µg/mL ±0.17 | 99.45 ± 0.16 |

**SD:** standard deviation, RSD: Relative standard deviation, *n=6.

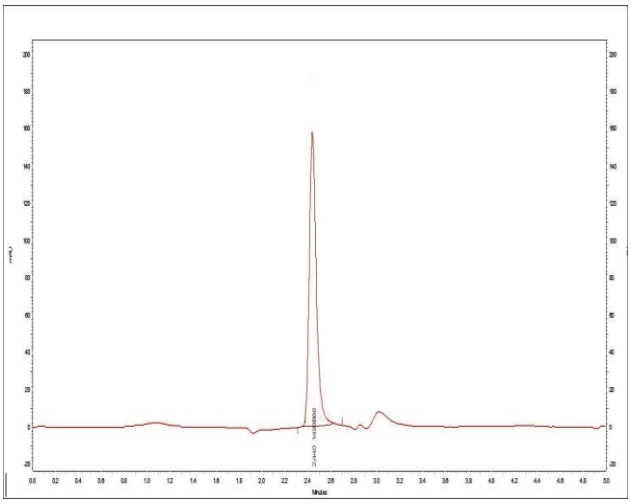

**Figure 4:** Sample chromatogram of Sofosbuvir

## RESULTS AND DISCUSSION

The present study was aimed at developing a precise, sensitive, rapid and accurate reversed phase HPLC method for the analysis of SOF in bulk drug and in pharmaceutical dosage forms. In order to achieve extraordinary retention time and peak asymmetry, various mobile phase containing ACN, Methanol, HPLC grade water in different ratios with different flow rates were tested. Good symmetrical sharp peak, minimum tailing factor with short run time was obtained with mobile phase composing MeOH: ACN in the ratio (30: 70 v/v) at a flow rate of 1mL/minute with maximum lambda max at 261 nm.

All the system suitability parameters were computed at the optimized chromatographic conditions and retention time of 2.440, Plate number of 11,903 and tailing factor of 1.150 were obtained for SOF. The obtained values of the entire system suitability parameters are within the limits of agreeable range which shows that the proposed method is fit for detection of SOF in the tablet form. The optimum chromatographic conditions and system suitability parameters are tabulated in table 1.

The calibration curve was constructed between concentrations versus peak area by the prepared in the concentration range of 10-30 µg/mL of stock solution. The linearity range was found to be 10 - 30 µg/mL. The calibration graph of SOF is presented in fig 2. The regression equation was found to be Y= 50217 x - 2293.9. The correlation coefficient of SOF $r^2$ was noted as 0.9998 which states that the method was good linear to the concentration versus peak area responses. The Results show that a phenomenal relationship between peak area and concentration of drug in calibration curve. The standard chromatograms of SOF are depicted in fig 3a to fig 3e.Regarding specificity, the commonly used tablet excipients did not interfered with this method. It shows that the method is specific. Furthermore the well-shaped peaks also indicate the specificity of the method. It was noted that the percentage RSD values of system precision was 0.01. Precision for intra-day and inter-day precision was 0.021 and 0.025 respectively. Intra-day and inter-day % RSD values (< 2.0) clearly showed that the method was found to be fairly precise and reproducible. Regarding accuracy a known amount of the standard drug was added to the fixed amount of preanalyzed sample solution. % recovery was calculated by comparing the area before and after addition of the std. drug. The standard addition method was performed at 50 %, 100 % and 150 % levels. High value of recoveries (99.3 - 99.9 %) obtained for SOF indicates that the proposed method was found to be accurate. The robustness of the developed method was evaluated by small deliberate changes in method parameters such as flow rate (± 0.2 ml/min), detection wavelength (± 5 nm) and mobile phase composition (± 2%). The % RSD values of robustness which is less than 2 % reveals that the proposed method is robust. The results of robustness study are shown in table 2. Even though the small changes in the conditions did not significantly effect on the peak asymmetry, plate count and retention time of SOF. Regarding ruggedness, the % RSD values of ruggedness for SOF is less than 2 % which indicates that the method is quite rugged. The developed method had high sensitivity with LOD of 0.97987 µg/mL and LOQ of 2.9420 µg/mL. The results of LOD and LOQ supported the sensitivity of the developed method. Table 3 shows the summary of all validation parameters. The developed method was successfully applied for the determination of SOF in bulk drug and tablet dosage form. The assay results show that the amount of drug was in good agreement with the labeled value of the formulation. Table 4 represents the assay of the formulation. The representative sample chromatogram of SOF is shown in Fig 4.

## CONCLUSION

A simple, accurate, specific and precise RP-HPLC method has been developed and validated for estimation of SOF in bulk and tablet dosage form. The successful experimental results evolved in this novel method

assertively concluded that this analytical method was simple, specific, linear, precise, accurate and robust. Statistical results of the analysis obviously showed that the procedure adopted with HPLC developed method possessed best precision, accuracy. The method was completely free from other active ingredients and additives used in the tablet formulation. The final results of the analysis denotes that this new method confirmed to be sensitive, reproducible, short run time, excellent peak symmetry use of lesser sample volumes as well inexpensive. Therefore this newly invented method can be concluded that the proposed method is aexcellent approach for obtaining reliable results and found to be suitable for the routine analysis of SOF in pharmaceutical dosage forms.

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