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Analytical method development and validation of a RP-HPLC method for determination of Sofosbuvirin Pharmaceutical dosage form

Nagaraju Pappula*, L. Naga Padmini, M. Jahnavi, N. Navya, P. Vasanth, S. Ravi chandra

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur-522002, A.P., India.

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ABSTRACT: Background: Sofosbuvir is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). **Aim:** The main aim of present research work is to develop and validate a new, simple, precise and accurate RP-HPLC method for the determination of sofosbuvirin tablet dosage form. **Method:** The separation was carried out using Discovery C_{18} (250 × 4.6 mm, 5 µm particle size) column, with a mobile phase consisting of OPA (0.1%) and acetonitrilein the ratio of 50: 50 v/v. The flow rate was set at 1 ml/min and detection was monitored at 260 nm. **Results:** The retention time of sofosbuvir is 2.473 min. The linearity coefficient of sofosbuvir was found to be 0.999 and percentage recoveries for sofosbuvir is 99.32. The linearity was found in the concentration range of 100 to 600 µg/ml for sofosbuvir. **Conclusion:** The liquid chromatography method was extensively validated for linearity, accuracy, precision and robustness. All these analytical validation parameters were observed and the % RSD was determined which indicates the usefulness of method for determination of sofosbuvirin tablet formulation.

Corresponding author*
Dr. Nagaraju Pappula
Hindu College of Pharmacy,
Amaravathi Road, Guntur - 522002,
A.P., India.
Tel: +91-9985304304
Mail ID: pappulanagaraju@gmail.com

Keywords: Sofosbuvir, Validation, RP-HPLC, Acetonitrile, Tetrahydrofuran.

INTRODUCTIONS:

Sofosbuvir is chemically known as Propan-2-(2S)-2-{[(S)-{[(2R,3R,4R,5R)-5-(2,4-dioxo-1,2,3,4-tetra hydro pyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl oxolan-2-yl]methoxy} (phenoxy) phosphoryl] amino}propanoate and its empirical formula is $C_{22}H_{29}FN_3O_9P$ with a molecular weight of 529.45 [1-3]. Sofosbuvir is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C. Treatment options for chronic Hepatitis C have advanced significantly since 2011, with the development of direct acting

antivirals (DAAs) such as sofosbuvir. The chemical structure was shown in Figure-1. Literature review revealed that very few methods was reported for determining of sofosbuvir in bulk and pharmaceutical dosage form by spectrophotometric methods [4] and chromatography [5-15]. Hence in the present work an attempt was made to develop simple, precise and accurate analytical method for estimation of sofosbuvir in pharmaceutical dosage form.

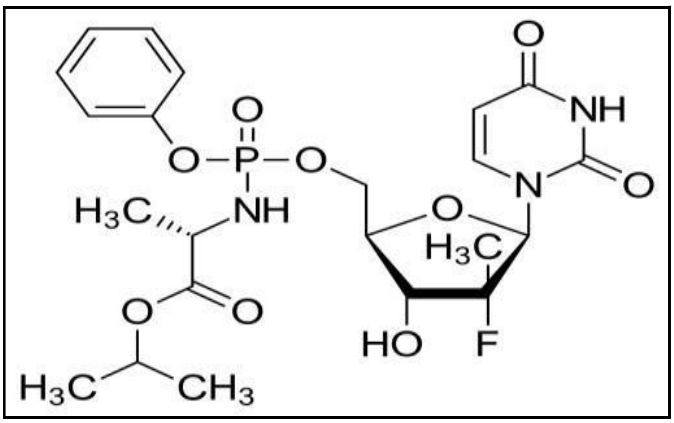

Fig 1. Chemical structure of sofosbuvir.

MATERIALS AND METHOD:

Triple distilled water of HPLC grade, Methanol, acetonitrile and o-phospohricaicd of HPLC grade which were purchased from E. Merck, Mumbai, India. Reference standard sample of sofosbuvir is procured from NATCO Pharma Ltd, Hyderabad, India. Commercial Sofosbuvir tablets (Eparel tablets) were purchased from local market and used in the analysis.

Isocratic RP-HPLC-Shimadzu LC 20 AD (prominence) and the Column specifications is discovery C_{18} column (2), 250 × 4.6 mm, 5µ particle size, Injector-Rheodyne, UV-Visible Spectrophotometer- Perkin Elmer (λ 25).

The reference sample of sofosbuvir was obtained from M/s. Sun Pharma Ltd, Ahmadabad, India. The branded formulation (Tablets) (Resof tablets containing Sofosbuvir) manufactured by M/s. Dr. Reddy's Pharma Ltd, Hyderabad, was procured from the local market. HPLC grade methanol, acetonitrile and analytical grade orthophosphoric acid were obtained from M/s. Rankem Chemicals Ltd, Mumbai, India. Milli-Q water dispensed through a 0.22 µ filter of the Milli-Q water purification system (Millipore, Merck KGaA, Darmstadt, Germany) was used throughout the study.

Preparation of orthophosporic acid solution (0.1 %):

About 1 ml of orthophosphoric acid (OPA) was transferred into a 1000 ml flask and 400 ml of Milli-Q water was added and mixed well. Then volume was made up to 1000 ml, sonicated for 5 min, cooled to room temperature and then filtered through a 0.45 µ membrane filter.

Preparation of the mobile phase:

A 50:50 v/v mixture of the above OPA solution and acetonitrile was prepared and used as the mobile phase in the study.

The diluents:

A 50:50 v/v mixture of water and acetonitrile was prepared and used as the diluent in the preparation of drug dilutions.

Preparation of standard solution of sofosbuvir:

About 200 mg of sofosbuvir was accurately weighed and transferred into a 50 ml clean dry volumetric flask containing 30 ml of the diluent. The solution was sonicated for 10 min and then volume was made up to the mark with a further quantity of the diluent to get a concentration of 4000 µg/ml of sofosbuvir (Stock solution). A mixed working standard solution was prepared by further diluting the above stock solution to obtain a concentration of 400 µg/ml of sofosbuvir.

Preparation of the tablet solution:

Twenty tablets of the commercial sample of 'RESOF' were weighed and finely powdered. An accurately weighed portion of powdered sample equivalent to the weight of one tablet (400 mg of sofosbuvir) was transferred into a 50 ml volumetric flask containing 30 ml of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and the volume made up with a further quantity of the diluent. Then, this mixture was filtered through a 0.45 µ membrane filter. Further, 1 ml of the above filtrate was pipetted into a 10 ml volumetric flask and the volume was made up with the diluent.

Calibration curve:

Different volumes of stock solutions were accurately transferred to a 10 ml volumetric flask to 100 to 600 µg/ml concentration range for sofosbuvir. Six replicate solutions in the above range were prepared for each concentration. The calibration curve was constructed by plotting the analyte peak area against concentration.

RESULTS AND DISCUSSION:

Method optimization:

The suitable parameters were chosen after several trails with buffers of different pH values and various

compositions of acetonitrile. However the final concentration was adjusted to achieve good resolution. The trails revealed that with the decrease in acetonitrile concentration, the peak obtained was broad and showed severe tailing. The peak obtained with a composition of acetonitrile and o-phosphoric acid (0.1 %) 50:50 v/v was proved to be most suitable of all the combinations since the peaks obtained were better defined and resolved and free from tailing. To determine the effect of flow rate, the method was performed at different flow rates 0.7, 0.9, 1.1, 1.2 and 1.3 ml/min. The optimum flow rate 1 ml/min was chosen finally. The retention time obtained for sofosbuvir is at 2.473 min and the chromatogram was shown in Fig 2 and 3. Validation was carried out and validation summary was tabulated in Table 1.

Table 1. Validation summary.

Validation parameters	Results
Linearity range, µg/ml	100-600
R_t (min)	2.473
Run time, µg/ml	6

Accuracy:

To previously analyzed sample of sofosbuvir known amounts of standard sofosbuvir corresponding to 50, 100 and 150 % of target concentration were added. The accuracy was expressed as the percentage of the analyte recovered by the proposed method. The percent mean recovery for sofosbuvir was 99.32 and it was within acceptable limit of 98 to 102. The % RSD for sofosbuvir was 0.34 which is within the limit of ≤ 2. Hence, the proposed method was accurate and the results are summarized in Table 2 and Fig 4 to 6.

Table 2. Results of recovery experiments of sofosbuvir.

Preanalysed amount (µg)	Spiked amount (µg)	% Recovered
200	100	99.13
200	100	99.58
200	100	99.10
200	200	99.04
200	200	99.02
200	200	99.10
200	300	99.77
200	300	99.25
200	300	99.91
	MEAN	99.32
	SD	0.304
	%RSD	0.34

Linearity and calibration:

A calibration curve was determined by plotting the peak areas obtained against concentrations. There exists a linear relationship showing concentrations range for sofosbuvir 100 to 600 µg/ml. From the data obtained, correlation coefficient for the sofosbuvir was found to be 0.9987. Linear regression data for calibration curves was shown in the Table 3. The resulting linearity plot was shown in the Fig 7 to 13.

Table 3. Linear regression data for calibration curve.

Drug	Sofosbuvir
Concentration range (µg/mL)	100-600
Slope (m)	10172
Intercept (c)	5976.5
Correlation coefficient (R^2)	0.9987

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The repeatability and intermediate precision were determined by analysing the sample of sofosbuvir. The repeatability and intermediate precision data were assessed by the using the working standard solution of sofosbuvir is summarized in Table 4 and 5 respectively.

Table 4. Result of repeatability of sofosbuvir.

Sl. No.	Sofosbuvir		
	Area	USP Plate Count	USP Tailing
1	4136808	6815	1.36
2	4166672	6787	1.42
3	4166672	6683	1.41
4	4146929	6755	1.39
5	4136794	7359	1.34
6	4171736	6837	1.43
Mean	4154269		
SD	15980.6		
% RSD	0.4		

Table 5. Intermediate precision of sofosbuvir.

Sl. No.	Average area (n=6)	USP Plate Count	USP Tailing
Day 1	4221603	6784	1.25
Day 2	4221301	6696	1.36
Overall average	4221452	--	--
SD	10005.1	--	--
% RSD	0.2	--	--

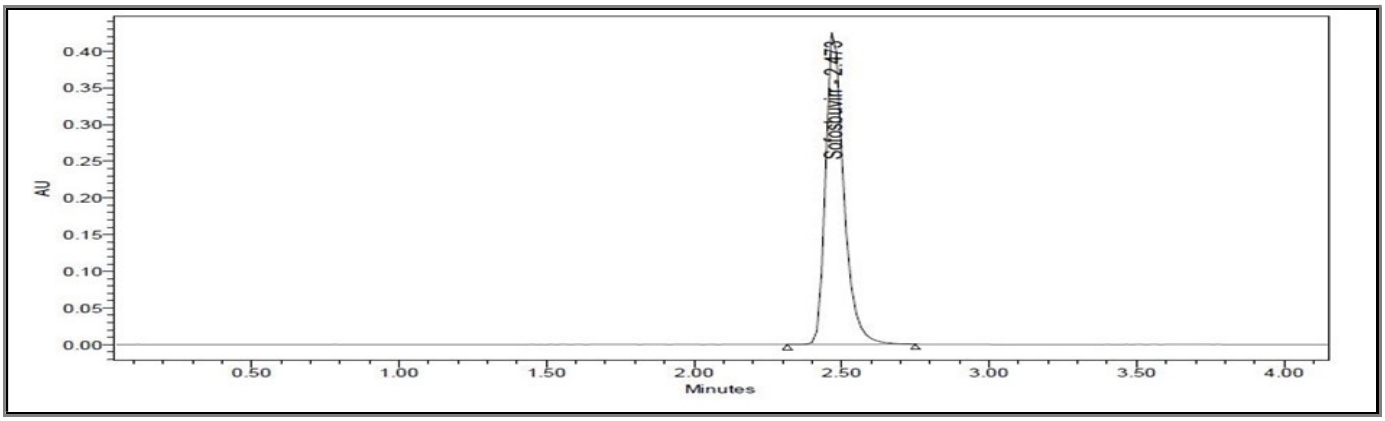

Fig 2. Chromatogram of standard sofosbuvir solution.

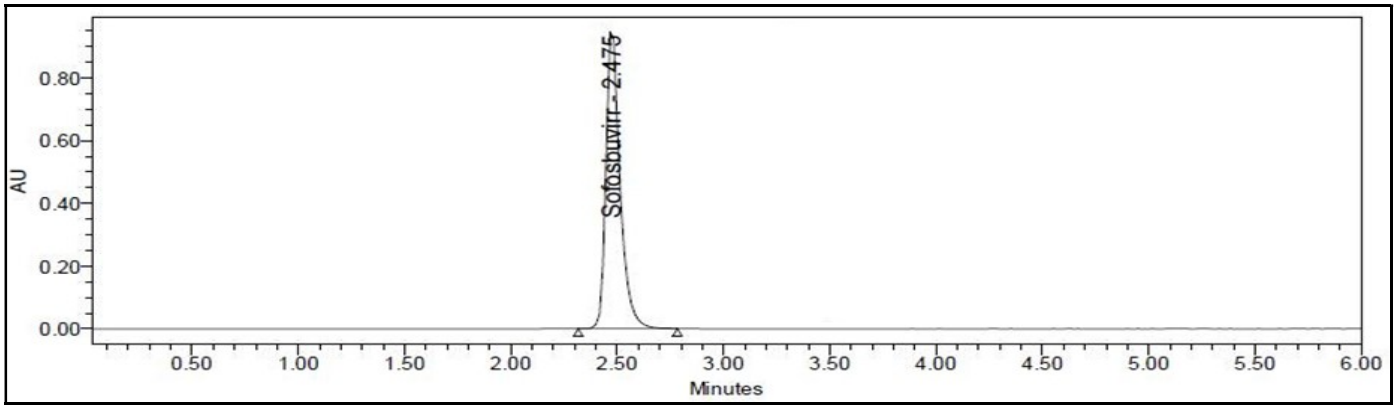

Fig 3. Chromatogram of sample sofosbuvir solution.

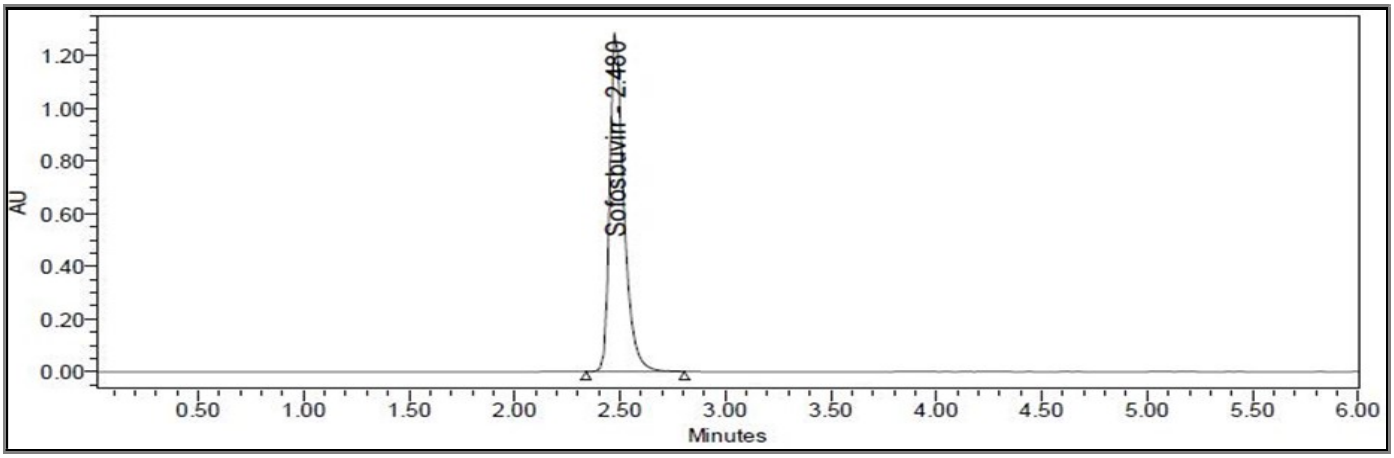

Fig 4. Accuracy chromatogram (50 % level) of sofosbuvir.

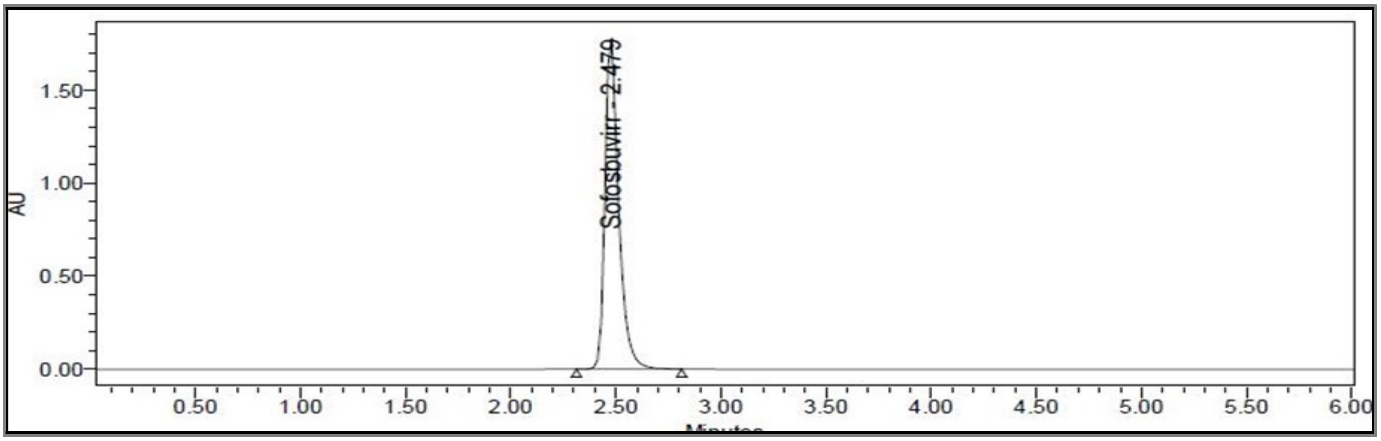

Fig 5. Accuracy chromatogram (100 % level) of sofosbuvir.

Repeatability:

Six replicate injections of sofosbuvir were analyzed on the same day for assessing repeatability. The % RSD for sofosbuvir was found to be 0.4. This value was found to be within acceptable limit of ≤2 and hence, the method is reproducible. The corresponding results are shown in the Table 4.

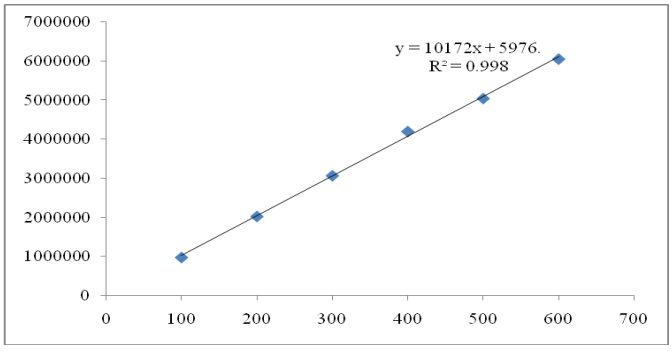

Fig 7. Linearity plot of sofosbuvir.

Intermediate Precision:

Six replicate injections of the same dilution were analysed on two different days for verifying the variation in the precision. The % RSD of the results for sofosbuvir was found to be 0.2, which are within acceptable limit of ≤2. Hence, the method is reproducible on different days. This indicates that the method is precise. The results are shown in the Table 5.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in the values of method parameters and provides an indication of its reliability during normal usage. The parameters included slight variation in flow rate of the mobile phase, composition of the mobile phase and column temperature.

The robustness study was performed by slight modifications in flow rate of the mobile phase, composition of the mobile phase and column temperature. The sample of sofosbuvir containing 100 µg/ml of sofosbuvir concentration is analysed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrates that the developed method is robust in nature. The results of robustness as shown in Table 6.

Stability of formulation solution:

The sample solutions were analysed at 0 h (comparison sample) and after 24 h (stability sample) by keeping at ambient room temperature. Stability was determined by determining % RSD for the formulation sample solutions. The sample solution injected after 24 h by keeping at room temperature (30°C) did not show any appreciable change. The deviation in the assay was not more than 2 and the results are shown in Table 7.

Recovery studies:

Determination of accuracy by direct comparison to reference standard is a preferred technique. Recovery studies were performed by spiking the blank matrix of the sample at different levels of the known level in the sample. Average recovery of the analyte was found to be in the range of 99.8 to 100.1 % at different levels of spiking. The developed RP-HPLC method utilizes acetonitrile and o-phosphoric acid (0.1%) in the ratio of (50:50 v/v) as a mobile phase and discovery C_{18} column as a stationary phase. The method precision and system precision were performed and found to be within the limits. The recovery study reveals the accuracy and precision of the method employed for the present studies and the results are shown in Table 8 and Fig 14.

Table 7. Stability data of sofosbuvir.

Drug	% Assay at 0 h*	% Assay at 24 h*	Deviation
Sof.	99.56	99.01	0.55

***n=6 for each parameter. Sof – Sofosbuvir.**

Table 8. Results of assay and recovery studies.

Sample	Amount claim	Amount Found	% Recovery (n=6)
1	400	398.4	99.6±0.16

Values are expressed as mg/tablet.

CONCLUSION:

It is clear from the present study that the prescribed method of analysis is simple, accurate, specific and precise in operation and can be employed for routine batch analysis of sofosbuvirin tablets.

ACKNOWLEDGEMENT:

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Table 6. Robustness study for sofosbuvir.

Condition	Mean area	% assay	% difference
Optimized	4213158	99.65	-----
Flow rate at 0.9, 1.1 ml/min	4210341, 4213265	99.04, 99.79	0.61, 0.14
Mobile phase: Buffer-acetonitrile - 55:45, 65:35	4214210, 4214231	100.11, 100.15	0.46, 0.50
Column Temperature: at 25, 35°C	4210335, 4212844	99.03, 99.59	0.62, 0.06

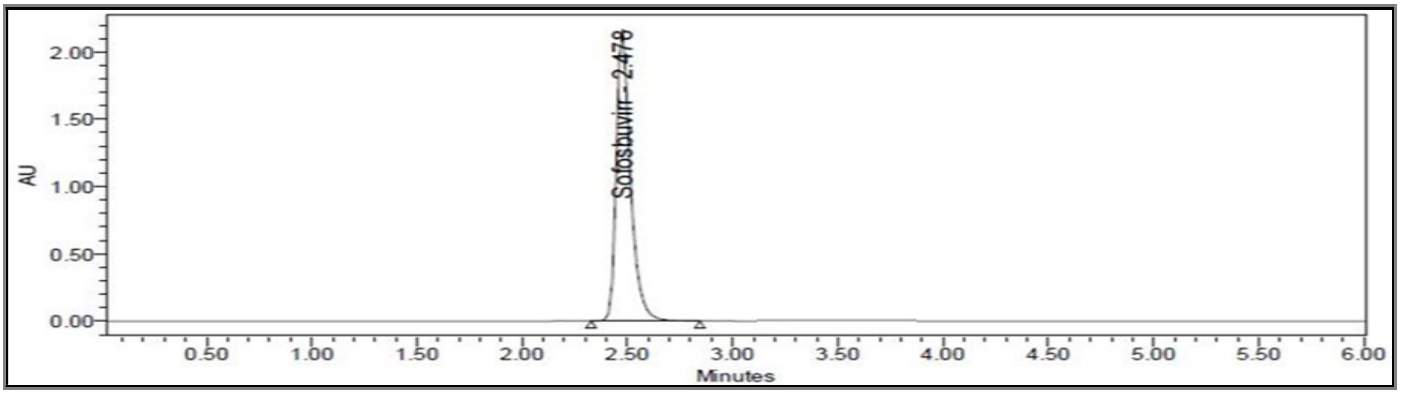

Fig 6. Accuracy chromatogram (150 % level) of sofosbuvir.

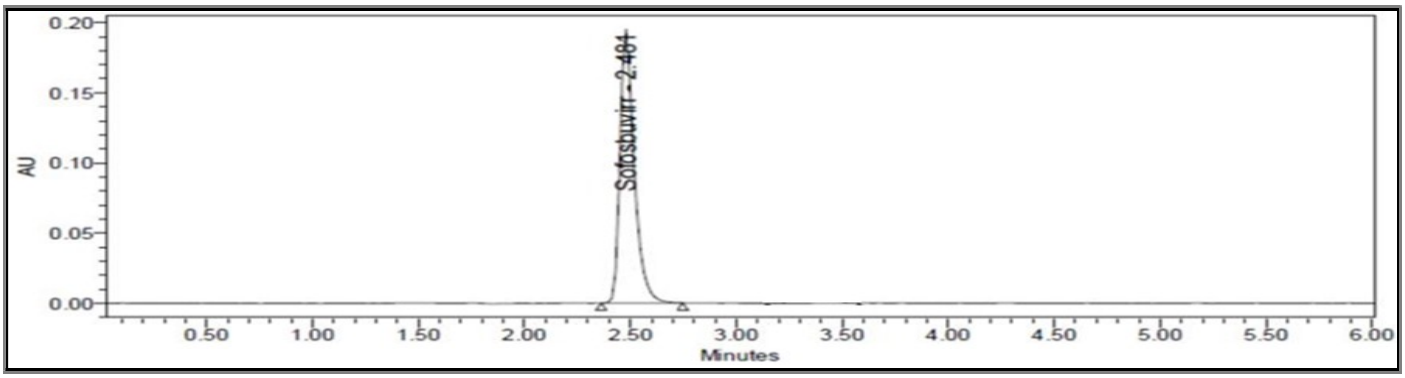

Fig 8. Linearity chromatogram (25 % level) of sofosbuvir.

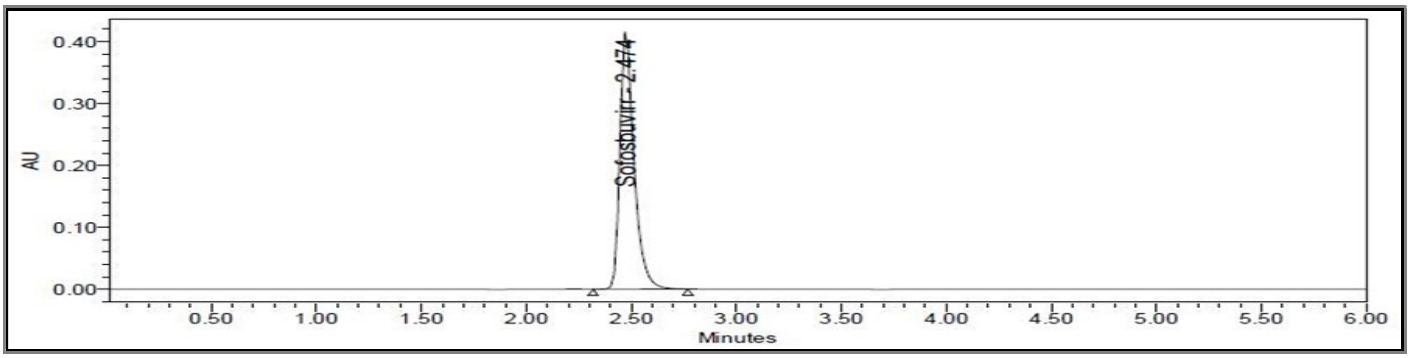

Fig 9. Linearity chromatogram (50 % level) of sofosbuvir.

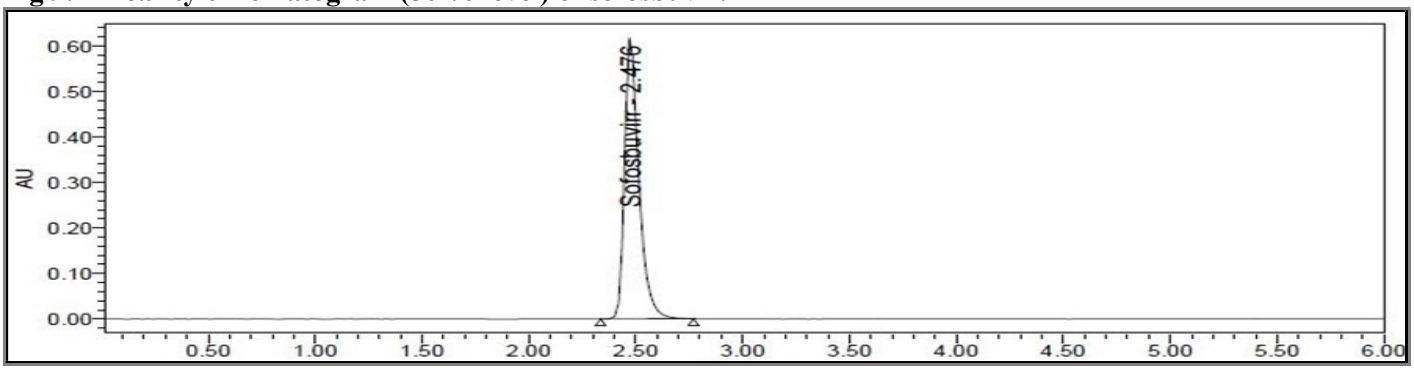

Fig 10. Linearity chromatogram (75 % level) of sofosbuvir.

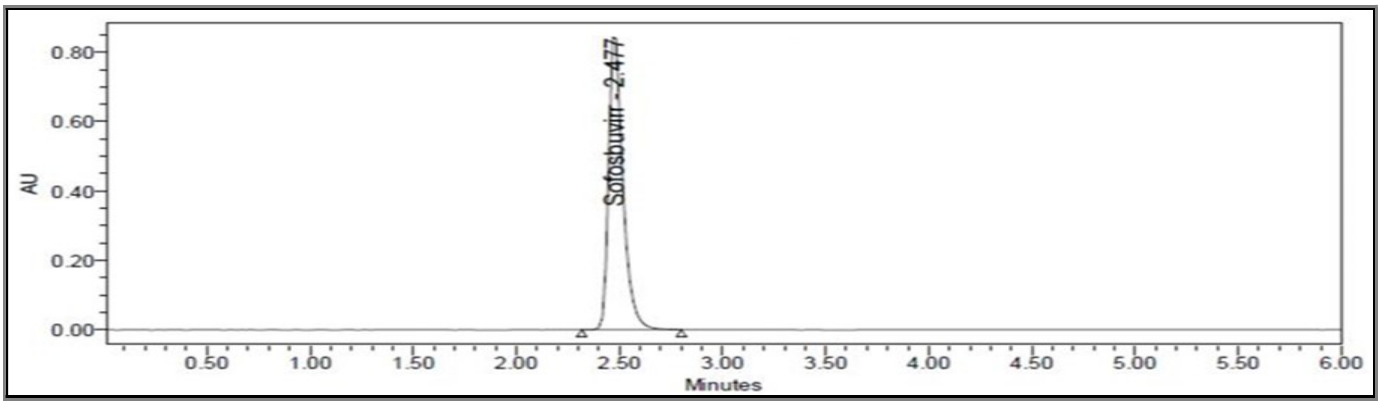

Fig 11. Linearity chromatogram (100 % level) of sofosbuvir.

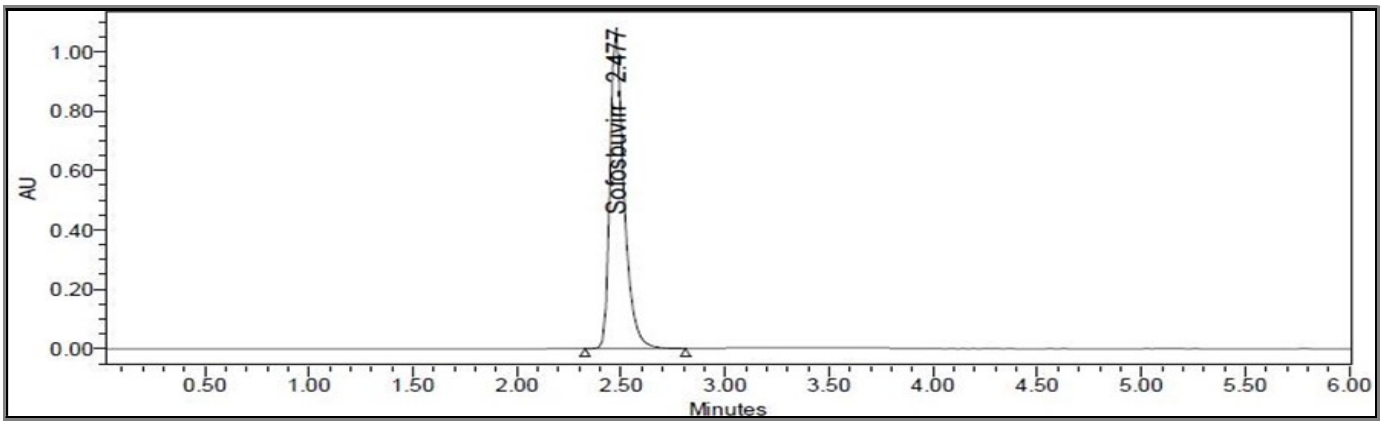

Fig 12. Linearity chromatogram (125 % level) of sofosbuvir.

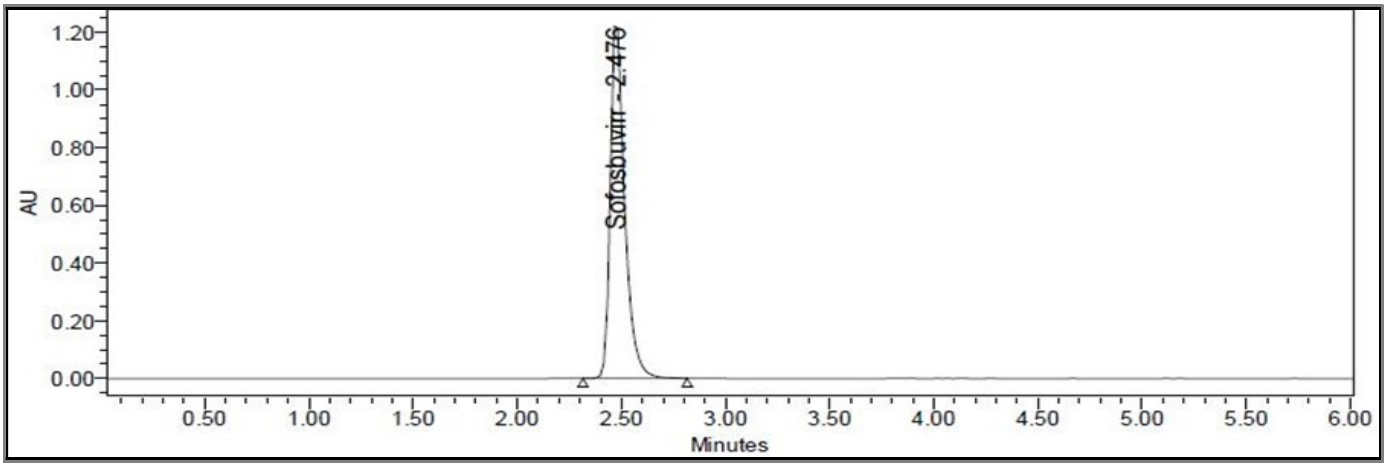

Fig 13. Linearity chromatogram (150 % level) of sofosbuvir.

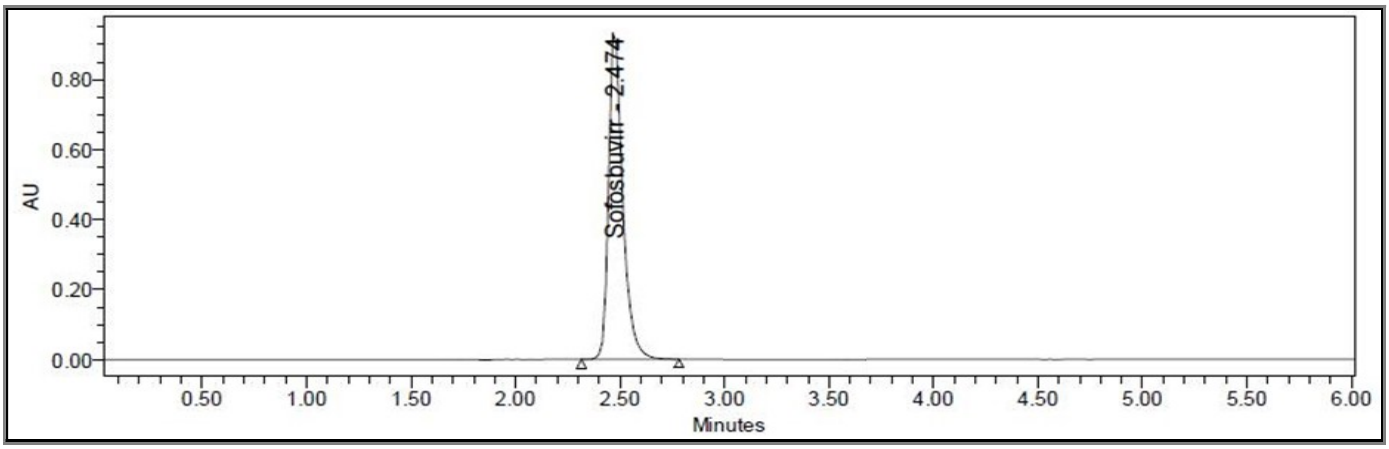

Fig 14. Chromatogram showing separation of sofosbuvir from tablet formulation.

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