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HPLC METHOD FOR ESTIMATION OF DRUG RELEASE OF SOFOSBUVIR IN PHARMACEUTICAL FORMULATION

Kaushlendra Singh [*1, 2], Sachin Bhatt [1] and Ramesh Prasad [1, 2]

Department of Chemistry [1], Sarvepalli Radhakrishnan University, Bhopal - 462026, Madhya Pradesh, India.
Macleods Pharmaceuticals Limited [2], Mumbai - 400093, Maharashtra, India.

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Correspondence to Author:
Kaushlendra Singh

Team Leader,
Macleods Pharmaceuticals,
Hiral Green Building, Flat no. - 120,
E-wing, Mira Road (East) - 401107,
Maharashtra, India.

E-mail: kaushlendra0707@gmail.com

ABSTRACT: Pharmaceutical industry and quality control laboratories needs of robust methods for analysis of drugs used in the treatment of life-threatening diseases such as hepatitis C. Using HPLC technique, a rapid, selective, precise and accurate method was developed and validated for the estimation of % drug release of Sofosbuvir in a pharmaceutical formulation. Stability indicating HPLC method was developed using Zorbax eclipse plus C18 (100 × 4.6 mm), 3.5 µ as analytical column and a combination of ammonium acetate buffer pH 5.3 and methanol in the ratio (45: 55) was used as mobile phase in isocratic mode. UV detection was carried out at 260 nm, column temperature was maintained at 25 °C, and the flow rate was 1.5 ml/min. The method was validated as per internationally accepted ICH guideline and found to be specific for blank and placebo solution, precise, robust, accurate and linear in range 9.2 to 69.0 µg/ml of Sofosbuvir. This method can be used for routine analysis of pharmaceutical formulation in any quality control laboratory leading to delivery of good quality healthcare solution.

INTRODUCTION: Hepatitis C disease is a disease of the liver which is caused by the hepatitis C virus. Sofosbuvir is helpful in the treatment of chronic hepatitis C (CHC) infection. Sofosbuvir (SOF) is a hepatitis C virus (HCV) nucleotide analog NS5B polymerase inhibitor indicated for the treatment of genotype 1, 2, 3 or 4 chronic hepatitis C virus (HCV) infection in adults. Sofosbuvir is absorbed with a peak plasma concentration observed at, 0.52 h post-dose. Following absorption, Sofosbuvir is metabolized in hepatocytes, where it is converted to the active nucleoside triphosphate form [1].

Very few research papers are available for estimation of the content of Sofosbuvir in a pharmaceutical formulation. A reverse phase HPLC method is reported for the estimation of the content of Sofosbuvir in tablets [2, 3], RP-HPLC method is available for simultaneous estimation of Sofosbuvir and Ledipasvir in tablet dosage form [4], RP-HPLC method is available for simultaneous estimation of Sofosbuvir and Velpatasvir in tablet dosage form [5, 6]. But no HPLC method is available for determination of *in-vitro* drug release of Sofosbuvir in a pharmaceutical formulation.

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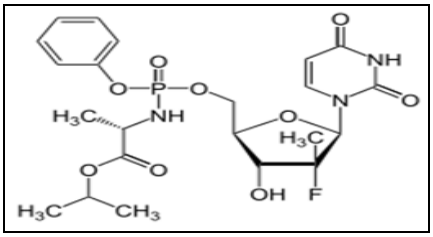


FIG. 1: CHEMICAL STRUCTURE OF SOFOSBUVIR

In this research work, HPLC is used for estimation of drug release of Sofosbuvir (S)-Isopropyl 2-((S)-(((2R, 3R, 4R, 5R)-5 -(2, 4-dioxo-3, 4-dihydro pyrimidine-1 (2H) -yl)- 4- fluoro- 3- hydroxy-4-methyl tetrahydrofuran-2-yl) methoxy)-(phenoxy) phospho- rylamino) propanoate **Fig. 1**.

MATERIALS AND METHODS:

Reagents and Materials: All reagents used for method development purpose were of Analytical Reagent Grade. Acetonitrile (Merck) and methanol (Merck), orthophosphoric acid (Rankem), ammonium acetate (Merck) sodium hydroxide (Rankem) and potassium dihydrogen orthophosphate (Merck) were used for solution and mobile phase preparations. Milli-Q water was used for all solution preparations. Working standards of Sofosbuvir were obtained from Macleods Pharmaceuticals Limited, Mumbai, India.

Determination of Wavelength Maxima: Diluted solutions of Sofosbuvir were prepared, and the absorption spectrum was obtained in the UV range using UV-Vis Spectrophotometer. λmax of Sofosbuvir was determined from the respective absorption spectrum of the drugs. 260 nm was found to be suitable based on UV spectrum **Fig. 2**.

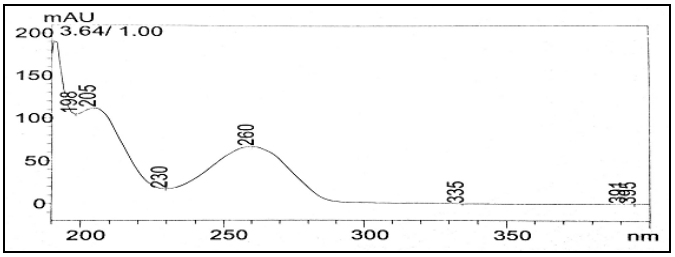


FIG. 2: UV ABSORPTION SPECTRA OF SOFOSBUVIR

Chromatographic System: Shimadzu HPLC with UV-Visible detector and quaternary gradient pump was used for this study. Zorbax eclipse plus C18 (100 × 4.6 mm), 3.5 µ HPLC column was used for chromatographic separation. Ammonium acetate buffer with pH 5.3 and methanol was used as mobile phase in an isocratic mode in the ratio (45:55) respectively. The flow rate was 1.5 ml/min, and detection was carried out at 260 nm. HPLC was equipped with Lab solutions software for data collection.

Solution Preparation:

Standard Preparation:

Sofosbuvir Standard Solution: About 46 mg of Sofosbuvir was accurately weighed, dissolved in the diluent and made up to volume with dissolution medium in a 50 ml volumetric flask. 5 ml of Sofosbuvir standard solution was further diluted to 100 ml with dissolution medium and mixed.

Sample Preparation: In dissolution tester, 900 ml of dissolution medium was poured in each dissolution vessel. To obtain 37 °C ± 0.5 °C sufficient time was allowed for the dissolution medium. USP type II (paddle) was used as a stirring element and speed adjusted to 75 rpm. At the end of specified time, 10 ml aliquot was withdrawn using a sampling cannula and filtered immediately through Whatman GF/C filter paper, discarding first 5 ml of the filtrate. Further diluted 5 ml to 50 ml with dissolution media, mixed.

Dissolution Parameter Selection: Based on USFDA recommendation for Sofosbuvir tablets, dissolution parameters should be set as 900 ml of phosphate buffer pH 6.8 with paddle apparatus rotating at 75 rpm. Same was selected for the present research work.

Mobile Phase Optimisation: Mobile phase was optimized to achieve shorter run time and longer HPLC column life. Ammonium acetate buffer at pH 5.3 was evaluated and found to be suitable.

HPLC Column Optimisation: Zorbax eclipse plus C18 (100 mm × 4.6 mm), 3.5 µ was the column of choice. 100 mm column was chosen to achieve a shorter run time with sharper peak shapes.

Selection of HPLC Pump Mode: Sofosbuvir retain more with a low solvent mobile phase. Hence a mixture of ammonium acetate pH 5.3 and methanol in the ratio (45:55) respectively with a flow rate of 1.5 ml/min was chosen to elute Sofosbuvir within 5 min run time.

Diluent Optimisation: Due to higher solubility and longer stability of Sofosbuvir, water, and acetonitrile in the ratio of 60:40 was chosen as diluent.

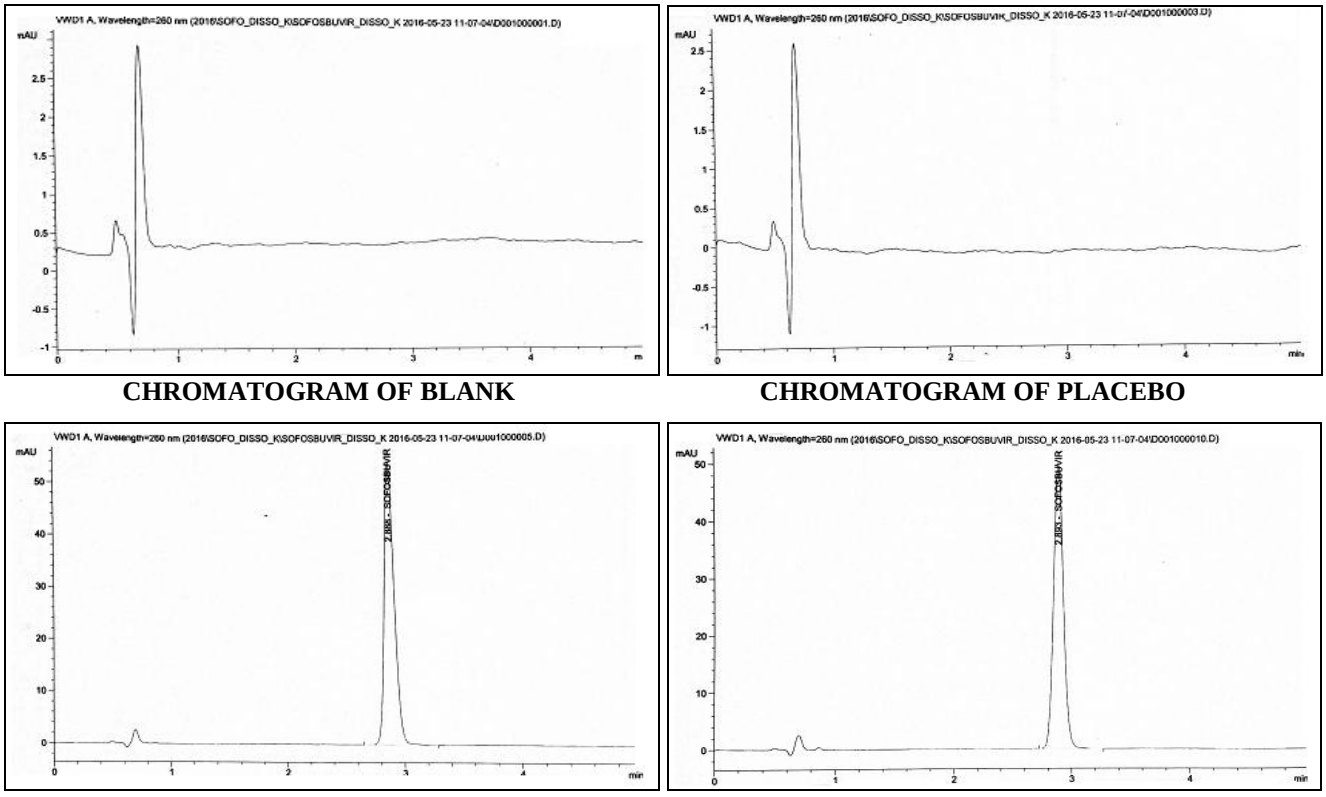
RESULTS AND DISCUSSION:

Method Validation: Upon achieving optimum separation, HPLC method was validated as per ICH guidelines [7] to ensure its suitability for routine use in estimation of % drug release of Sofosbuvir in a pharmaceutical formulation. Validation parameters adopted are presented below:

Specificity: Specificity was carried out by injecting blank solution, placebo solution, Sofosbuvir standard and sample solutions **Fig. 3**. Result tabulated in **Table 1** shows no interference due to blank and placebo at a retention time of Sofosbuvir. Hence, a method is specific.

TABLE 1: RESULTS OF SPECIFICITY

S. no.	Sample details	Retention time (min)
1	Blank	No interference observed
2	Placebo solution	No interference observed
3	Sofosbuvir	2.95



CHROMATOGRAM OF BLANK **CHROMATOGRAM OF PLACEBO**

CHROMATOGRAM OF SOFOSBUVIR STANDARD **CHROMATOGRAM OF SOFOSBUVIR SAMPLE**

FIG. 3: CHROMATOGRAMS OF SPECIFICITY

Solution Stability: Solution stability was calculated and evaluated by storing standard and sample solutions at 25 °C to 24 h. The absolute difference between solutions after storing for 24 h at 25 °C concerning the initial solution was within the acceptance criteria of not more than 2. Results are tabulated in **Table 2**.

TABLE 2: RESULTS OF SOLUTION STABILITY

Time (h)	Sofosbuvir standard		Sofosbuvir Sample	
	Area	Absolute difference	Area	Absolute difference
0	315215	-	272977	-
16	314454	0.2	272540	0.2
24	314277	0.3	271756	0.4

Filter Compatibility: Sample solution was prepared by spiking Sofosbuvir into placebo powder containing equivalent to one dosage unit. At filtration stage, a solution of Sofosbuvir tablets was filtered through Whatman GF/C filter (25 mm). First 5.0 ml of filtrate was discarded. The filtrate was collected for further analyzed. The unfiltered sample solution was centrifuged. The absolute difference between the results obtained for the centrifuged solution and filtered solution of Sofosbuvir tablets is tabulated in **Table 3**. The absolute difference between the results obtained for the centrifuged sample solution and filtered sample solution was within acceptance criteria of NMT 2.

TABLE 3: RESULTS OF FILTER COMPATIBILITY STUDY

Filter type	Sofosbuvir sample	
	Area	Absolute difference
Centrifuge	298302	-
Whatman GF/C filter	294504	1.3

Hence, Whatman GF/C (25 mm) filter is considered suitable for sample filtration.

Filter Saturation: At the filtration stage, three filtrates were obtained using three separate Whatman GF/C filters (25 mm) by discarding 1 ml, 3 ml, and 5 ml respectively. The filtrates were collected for further analysis. The absolute difference between the results obtained for subsequently filtered solutions is tabulated in **Table 4**.

TABLE 4: RESULTS OF FILTER SATURATION STUDY

Volume discarded	Sofosbuvir sample	
	Area	Absolute difference
1 ml	292812	2.2
3 ml	293553	2.0
5 ml	294504	1.3

The absolute difference at 5 ml for filtered solutions was found to be within acceptance criteria of NMT 2. Hence, 5 ml volume was considered as sufficient to saturate the filter.

Accuracy: Accuracy study was performed at 50% of 100% and 120% of the working concentration of active ingredient. Recovery solutions were prepared by spiking Sofosbuvir stock solution prepared to placebo powder in a specified volume of dissolution medium. Results tabulated in **Table 5** show that method is accurate.

TABLE 5: RESULTS OF ACCURACY STUDY

	Sofosbuvir	
Level	Area	% Recovery
50 %	193570	99.2
	193592	99.3
	192973	99.2
100%	314281	99.5
	315074	99.7
	315183	99.8
120%	382451	100.9
	384022	101.3
	382814	101.0
Mean % Recovery		100.0

Linearity: By using standard stock solution a series of diluted solutions were prepared to obtain solutions at 20% to 150% of the working concentration of active ingredient **Fig. 4**. Each solution was injected, and the peak area was recorded. Slope, Y-intercept and Correlation coefficient of the regression line were calculated. Results are tabulated in **Table 6**. The correlation coefficient is well within acceptance criteria of not less than 0.999.

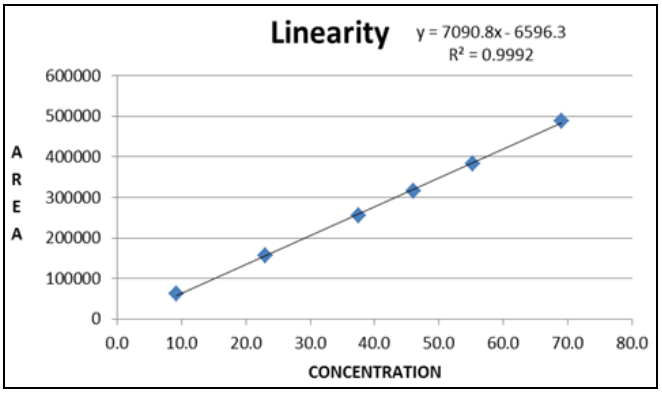


FIG. 4: LINEARITY CHART OF SOFOSBUVIR

TABLE 6: RESULTS OF LINEARITY

% Level	Sofosbuvir	
	Concentration (ppm)	Area
20	9.2	63119
50	23.0	156498
80	37.5	255015
100	46.0	315530
120	55.2	383053
150	69.0	489072
Slope		7090.8
Y-Intercept		6596.3
Correlation coefficient		0.9992

Precision: Precision test was carried out at 100 % level of target concentration. Six sample preparations were prepared and injected. The mean and relative standard deviation of the results was calculated. The results obtained for % release are tabulated in **Table 7**.

TABLE 7: RESULTS OF PRECISION

	Sofosbuvir	
	Area	% Release
Sample-1	297555	99.3
Sample-2	290301	96.7
Sample-3	290210	96.5
Sample-4	278905	93.2
Sample-5	283512	94.4
Sample-6	294110	97.8
Mean		96.3
% RSD		2.3

Intermediate Precision: For intermediate precision of Sofosbuvir dissolution method, the analysis was carried out on a different day, using a different HPLC and different dissolution apparatus. Intermediate precision was calculated and tabulated in **Table 8**. The absolute difference between the mean % release results obtained in precision and intermediate precision was found to be within the acceptance criteria of not more than 5.0. Results are tabulated in **Table 9**.

TABLE 8: RESULTS OF INTERMEDIATE PRECISION

	Sofosbuvir	
	Area	**% Release**
Sample-1	293.316	99.3
Sample-2	296.167	96.7
Sample-3	292.319	96.5
Sample-4	300.315	93.2
Sample-5	286.069	94.4
Sample-6	295.569	97.8
Mean		96.6
% RSD		1.7

TABLE 9: COMPARATIVE RESULTS OF PRECISION AND INTERMEDIATE PRECISION

Content	Mean % release in precision	Mean % release in intermediate precision	Absolute difference
Sofosbuvir	96.3	96.6	0.3

Based on the above results, it was concluded that the method is precise.

Robustness: Robustness of the method was evaluated by making the following alterations in the analytical parameters.

- Changing the flow of mobile phase (1.3 ml/ min, 1.7 ml/min)
- Changing the rpm of the dissolution medium (73 rpm, 77 rpm)

Results for analysis with the above-mentioned alterations are tabulated in **Table 10**. Comparative results tabulated in **Table 11** shows that the method is robust for deliberate changes in methodology.

TABLE 10: RESULTS OF ROBUSTNESS

Unit	% Release of Sofosbuvir				
	Unaltered	**Dissolution 73 rpm**	**Dissolution 77 rpm**	**Flow 1.3 ml/min**	**Flow 1.7 ml/min**
1	99.3	97.5	98.2	96.0	97.0
2	96.7	97.0	96.3	96.0	95.1
3	96.5	94.0	95.8	92.8	94.3
4	93.2	97.0	92.8	95.5	91.8
5	94.4	94.9	93.8	93.4	92.7
6	97.8	95.8	97.4	94.5	96.3
Mean	96.3	96.0	95.7	94.7	94.5
% RSD	2.3	1.4	2.2	1.4	2.1

TABLE 11: COMPARATIVE RESULTS OF ALTERED *vs.* UNALTERED CONDITION

S. no.	Changed parameter	Sofosbuvir	
		Mean % release	**Absolute difference**
1	Unaltered	96.3	-
2	RPM 73	96.0	0.3
3	RPM 77	95.7	0.6
4	Flow 1.3	94.7	1.6
5	Flow 1.7	94.5	1.8

CONCLUSION: HPLC method developed for dissolution of Sofosbuvir tablets has been proved to be simple, accurate, precise and robust to changes in analytical methodology. This method can be used for estimation of % release of Sofosbuvir in the pharmaceutical formulation in quality control laboratory of any pharmaceutical industry.

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