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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR THE ESTIMATION OF SOFOSBUVIR

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Keywords:

Method validation, Sofosbuvir, Stability-indicating, HPTLC, ICH guidelines

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ABSTRACT: A simple stability-indicating high-performance thin-layer chromatographic method which is economic, selective, and precise for analysis of Sofosbuvir (SFB), both as a bulk drug and in formulations, was developed and validated according to ICH guidelines. The method employed HPTLC precoated Merck TLC plates RP-18 F_{254} as the stationary phase while the solvent system prepared by mixing n-Hexane: Ethyl Acetate: Methanol in proportion 5:3:2 v/v. The system was found to give a compact spot for the drug (R_f value of 0.452 ± 0.004). Densitometric analysis of SFB was carried out in the absorbance mode at 261 nm. The linear regression analysis data for the calibration plots showed a good linear relationship, R^2 = 0.9994, with respect to peak area in the concentration range 100-600 ng/band. The LOD and LOQ were 8.009 ng/band and 24.270 ng/band, respectively. SFB was subjected to hydrolysis, oxidation, and thermal degradation, which indicates the drug is susceptible to hydrolysis, oxidation, and heat. The method was validated for precision, recovery, and robustness. Statistical analysis proves that the method is repeatable, selective, and accurate for the estimation of SFB.

INTRODUCTION: Sofosbuvir (SFS) chemically is (S)-isopropyl-2-(S)-(2R, 3R, 4R, 5R)-5-(2, 4 dioxo-3, 4-dihydro pyrimidin-1(2H)-yl)-4-fluoro-3hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-phenoxy) phosphorylamino) propionate. Sofosbuvir is a nucleotide analogue used in combination with other drugs for the treatment of hepatitis virus (HCV) infection [1-2]. Literature review revealed few RP-HPLC [3-7] and UPLC-ESI MS/MS [8-9] methods for estimation of SFB.

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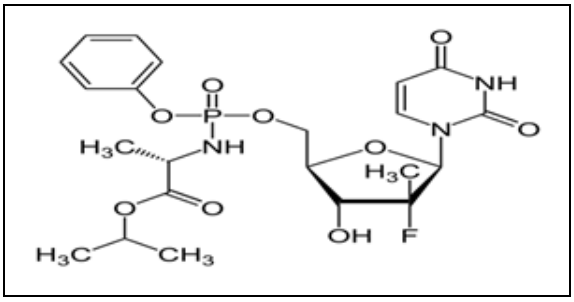

FIG. 1: STRUCTURE OF SOFOSBUVIR

There was no stability-indicating HPTLC method reported. Hence, the purpose of this work was to develop a simple stability-indicating HPTLC method for the determination of SFB in its bulk and pharmaceutical dosage form to provide better scope for further research on the drug. The HPLC method was developed as recommended by ICH guidelines [10-12].

MATERIALS AND METHODS:

Chemical and Reagents: The reagents used in this work were of AR Grade, Methanol, Toluene, N-Hexane, Ethyl Acetate, Hydrochloric Acid, Sodium Hydroxide, Hydrogen Peroxide (30%) purchased from Omkar traders, Mumbai, and double-distilled water from Elga water purification system.

Equipment: The instruments used in the study were Camag HPTLC system comprising of Linomat-5 applicator, Camag TLC Scanner 3, Win CATS software V- 1.4.2, Merck TLC plates RP-18 F_{254} pre-coated plates, Hamilton syringe (100 µl), Shimadzu balance Model AY-120, Hot Air Oven (Kumar Laboratory Oven), Photostability chamber (Make Newtronic, Model IC DAC version 1.2) and calibrated glassware were used for the study.

Selection of Detection Wavelength: From the standard stock solution, further dilutions were done using mobile phase and scanned over the range of 200-400 nm and the spectrum was obtained. It was observed that Sofosbuvir showed considerable absorbance at 261 nm.

Preparation of Standard Stock Solution: Standard stock solution of SFB was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, 1 ml was further diluted to 10 ml with mobile phase to get 100 µg/ml solution of SFB.

Selection of Mobile Phase and Chromatographic Conditions: Chromatographic separation studies were carried out on the standard working solution of SFB (200ng/band). Initially, trials were carried out using various solvents in various proportions on normal TLC plates, to obtain the desired system suitability parameters. After few trials, n-Hexane: Ethyl Acetate: Methanol (5:3:2 v/v), was chosen as the mobile phase, which gave good resolution and acceptable peak parameters. Other chromatographic conditions like chamber saturation time, run length, sample application volume, sample application positions, the distance between tracks, detection wavelength were optimized to gave reproducible R_f values and symmetrical peak shape for the drug peak.

Preparation of Mobile Phase: Mobile phase was prepared by mixing n-Hexane: Ethyl Acetate: Methanol in proportion 5:3:2 v/v. It was then sonicated on an ultrasonic water bath for 15 min.

Preparation of Sample Solution of Tablets (Assay): Twenty tablets [Myhep 400 mg tablets, Mylan, Each film-coated tablet contains 400 mg of sofosbuvir] were weighed and powdered. Tablet powder equivalent to 10 mg of SFB was weighed and transferred to 10 ml volumetric flask and was diluted with methanol. It was sonicated for 15 min and filtered so as to get a solution having a concentration 1000 µg/ml. 1 ml of this solution was further diluted with mobile phase to get the final concentration of 100 µg/ml SFB.

2 µl of this solution was applied on the plate and analyzed. Six determinations were carried out from homogenous sample to determine % assay.

Stress Degradation Studies of Bulk Drug: Stress degradation studies were carried under condition of acid, base, neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared (Blank and of SFB reference standard). The blank was subjected to stress in the same manner as the drug solution. Dry heat and photolytic degradation were carried out in a solid state.

Alkaline Hydrolysis: One ml working standard solution of SFB (1000 µg/ml) was mixed with 1 ml of 1 N methanolic NaOH. The solution was kept for 24 h in a dark place. The resulting solution was neutralised and diluted with methanol to 10 ml. 4 µl volume of this solution was applied on the TLC plate (400 ng/band). The chromatogram of SFB after alkaline hydrolysis shows 59.79% recovery, R_f 0.45 & R_f of degradant 0.17 & 0.26.

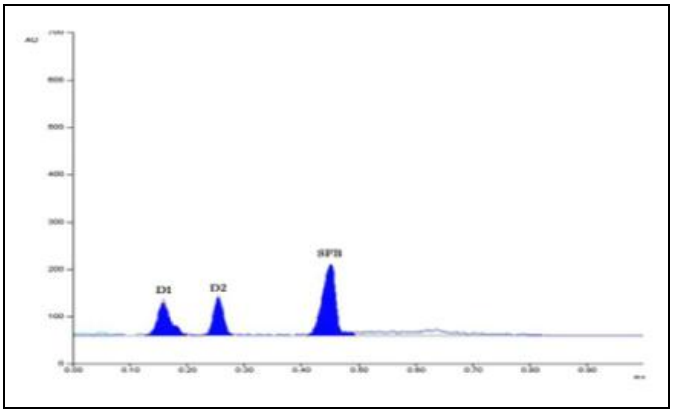

FIG. 2: DENSITOGRAM OF SFB (400 ng/band) AFTER ALKALINE HYDROLYSIS

Acidic Hydrolysis: One ml working standard solution of SFB (1000 µg/ml) was mixed with 1 ml of 1 N methanolic HCl. The solution was kept for 24 h in a dark place. The resulting solution was neutralised and diluted with methanol to 10 ml. 4 µl volume of this solution was applied on the TLC plate (400 ng/band).The chromatogram of SFB after acid degradation shows 91.30 % recovery, R_f 0.45 & R_f of degradant 0.26.

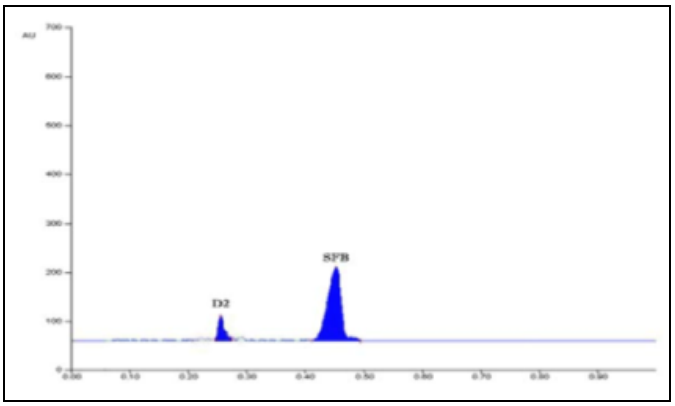

FIG. 3: DENSITOGRAM OF SFB (400 ng/band) AFTER ACID DEGRADATION

Neutral Hydrolysis: One ml working standard solution of SFB (1000 µg/ml) was mixed with 1 ml of distilled water. The solution was kept for 24 h in dark place. The resulting solution was diluted with methanol to 10 ml. 4 µl volume of this solution was applied on the TLC plate (400 ng/band).

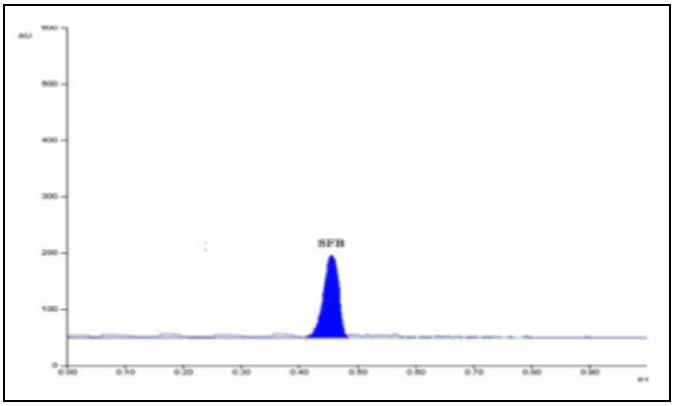

FIG. 4: DENSITOGRAM OF SFB (400 ng/band) AFTER NEUTRAL HYDROLYSIS

Oxidation: One ml working standard solution of SFB (1000 µg/ml) was mixed with 1 ml of 30% H_2O_2 solution. The solution was kept for 24 h in a dark place. The resulting solution was diluted with methanol to 10 ml. 4 µl volume of this solution was applied on the TLC plate (400 ng/band). The chromatogram of SFB after oxidation shows 94.81 % recovery, R_f 0.45 & R_f of degradant 0.71.

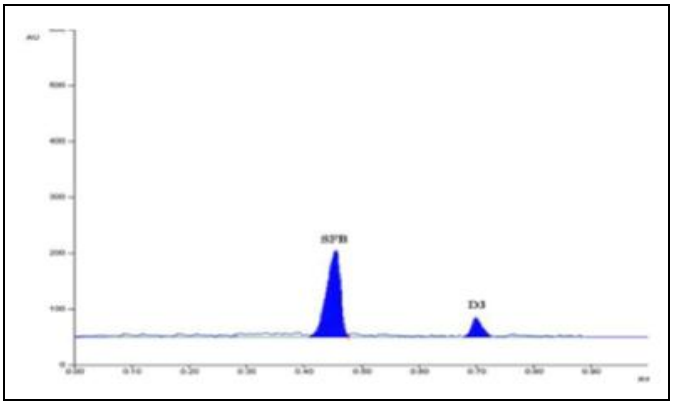

FIG. 5: DENSITOGRAM OF BLANK H_2O_2 AND SFB (400 ng/band) AFTER OXIDATION

Degradation under Dry Heat: Dry heat studies were performed by keeping drug sample in oven (80 ºC) for a period of 24 h. Sample was withdrawn after 24 h and processed as per standard solution preparation procedure mentioned under 1.5 to get 100 µg/ml final concentration. 4 µl volume of this solution was applied on the TLC plate. (400 ng/band). The chromatogram of SFB after exposing to dry heat shows 100.63% recovery, R_f 0.45.

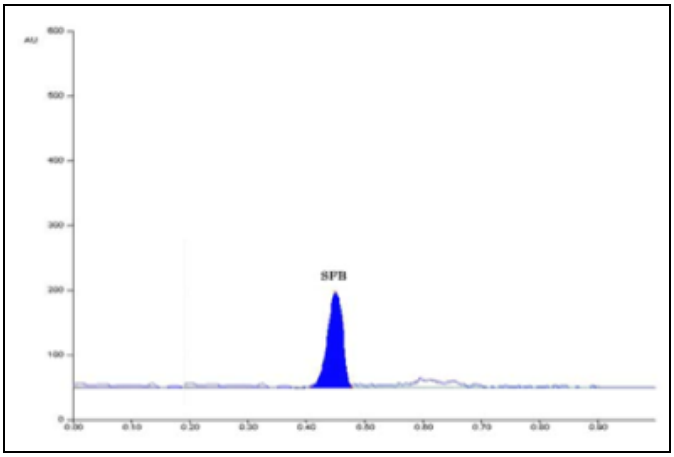

FIG. 6: DENSITOGRAM OF SFB (400 ng/band) AFTER EXPOSING TO DRY HEAT

Photo-Degradation Studies: Photolytic studies were also carried out by exposure of the drug to UV light up to 200 watt-hours/square meter and subsequently to cool fluorescent light to achieve an illumination 1.2 million Lux. Hr.

The sample was withdrawn after exposure and processed as per standard solution preparation procedure mentioned under 1.5 to get 100 µg/ml final concentration. 4 µl volume of this solution was applied on the TLC plate (400 ng/band). The chromatogram of SFB after photodegradation shows 99.81% recovery, R_f 0.45.

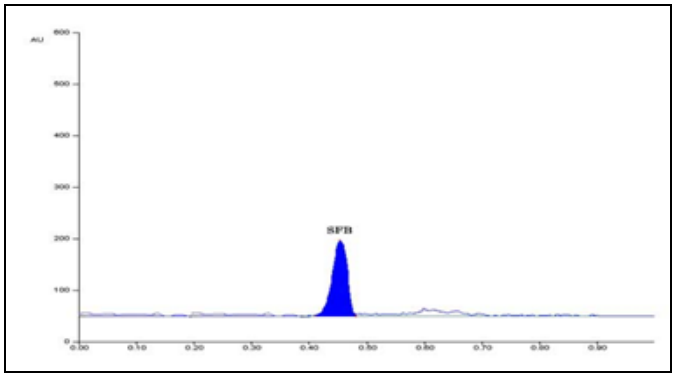

FIG. 7: DENSITOGRAM OF SFB (400 ng/band) AFTER PHOTO DEGRADATION

RESULTS AND DISCUSSION:

Validation of Analytical Methods:

Linearity and Range: From the standard stock solution (1000 µg/ml) of SFB, further dilutions were made with methanol to get a solution having a concentration of 100 µg/ml. Different volumes were applied on TLC plate to obtain a linear range. Six replicates per concentration were applied. The linearity (relationship between peak area and concentration) was determined over the concentration range 100-600 ng/band.

TABLE 1: LINEARITY STUDY OF SFB

Replicates	Concentrations of SFB					
	100 ng/band	200 ng/band	300 ng/band	400 ng/band	500 ng/band	600 ng/band
	Peak Area					
1	933.1	1844.2	2733.1	3686.8	4625.6	5678.9
2	952.1	1806.9	2730.4	3653.2	4537.4	5581.2
3	922.7	1836.2	2744.3	3666.3	4555.6	5638.5
4	944.2	1825.3	2738.2	3672.3	4566.3	5531.5
5	934.5	1833.5	2736.3	3670.2	4570.5	5655.7
Mean	937.32	1829.22	2736.46	3669.76	4571.08	5617.16
Std. Dev.	11.24	14.19	5.30	12.07	33.05	59.97
%RSD	1.20	0.78	0.19	0.33	0.72	1.07

Precision: The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the intraday studies, 3 replicates of 3 different concentrations (200, 400, 600 ng/band) of SFB were analyzed in a day, and percentage RSD was calculated. For the inter-day variation studies, 3 replicates of different concentrations were analyzed on 3 consecutive days, and %RSD was calculated.

TABLE 2: PRECISION STUDY OF SFB

Intraday Precision			Interday Precision		
Conc. (µg/ml)	% Recovery	S.D.	Conc. (µg/ml)	% Recovery	S.D.
200	101.36	1.56	200	101.33	0.62
400	99.91	0.73	400	99.16	0.46
600	100.47	1.20	600	100.23	0.64

Accuracy: To check the accuracy of the method, recovery studies were carried out by adding a standard drug to the sample at three different levels 50, 100, and 150%. The basic concentration of the sample chosen was 10 µg/ml of SFB from tablet solution. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of SFB were calculated by using the linearity equation of SFB.

TABLE 3: RECOVERY STUDY OF SFB

Level	Conc. (ng/band)		Area	% Recovery	Mean	% RSD
	Sample	Std				
50%	200	100	2779.7	100.648	99.266	1.330
			2706.3	98.017		
			2737.4	99.132		
100%	200	200	3649.0	98.849	98.590	0.829
			3663.8	99.247		
			3605.3	97.674		
150%	200	300	4611.4	99.771	99.759	0.149
			4617.4	99.900		
			4603.6	99.604		

Limit of Detection (LOD): LOD is calculated from the formula:

$$LOD = 3.3\ \sigma / S = 8.009\ ng/band$$

Where, σ = standard deviation of response for the lowest conc. in the range, S = slope of the calibration curve.

Limit of Quantification (LOQ): The quantitation limit is expressed as:

$$LOD = 10\ \sigma / S = 24.270\ ng/band$$

Where, σ = standard deviation of response for the lowest conc. in the range, S = slope of the calibration curve.

Robustness: The robustness of the method was determined by carrying out the analysis under conditions during which chamber saturation time, time form application to development, and time form development to scanning are altered, and the effect on the area was noted.

TABLE 4: ROBUSTNESS STUDY OF SFB

Drug	% RSD Found For Robustness Study								
	Mobile phase saturation time (± 10 %) min			Time from application to development (min)			Time from development to scanning (min)		
SFB	13.5	15	16.5	10	20	30	30	60	90
	0.808	0.622	0.979	0.870	1.737	0.890	1.355	1.360	1.498

DISCUSSION: Stability indicating HPTLC method for the determination of SFB was developed. Linearity for SFB was found in the range of 100-600 ng/band with a regression coefficient (R^2) of 0.999, it indicates that the proposed method is found to be linear. LOD and LOQ values were 8.009 ng/band and 24.27ng/band, respectively. The RSD values for intraday and interday precision studies were found to be less than 2%. This low value of RSD indicates that the proposed method is precise. Degradation of SFB was found to occur under acidic condition (1N HCl, 24 h), alkaline condition (1N NaOH, 24 h), and oxidative condition (30% H_2O_2, 24 h), SFB was considerably stable in neutral (24 h), dry heat (80 ºC for 24 h) and photostability [UV, 200-watt hrs/square meter Florescence, 1.2 million Lux. hrs].

SUMMARY:

TABLE 5: SUMMARY OF STRESS DEGRADATION STUDY OF SFB RS

S. no.	Stress Conditions	% Recovered for SFB (%)	R_f of SFB	% Degradation	R_f of Degradant
1	Base (1 N NaOH, kept for 2 hr)	59.79	0.45	40.21	0.17, 0.26
2	Acid (1 N HCl, Kept for 24 hr)	91.30	0.45	8.70	0.26
3	Neutral (kept for 24 hr)	98.83	0.46	--	--
4	H_2O_2, 30% (kept for 24 hr)	94.81	0.45	5.19	0.71
5	Dry Heat (80^0C for 24 hr.)	99.96	0.45	--	--
6	Photo stability [UV, 200 watt hrs/square meter Florescence , 1.2 million Lux. Hrs]	99.81	0.45	--	--

TABLE 6: SUMMARY OF VALIDATION STUDY

S. no.	Validation Parameter	Results SFB
1.	Linearity Equation	y = 9.302 x – 28.97
2.	Range	100 – 600 ng/band
3.	Assay (Mean ± % RSD)	100.46 ± 1.04
	Precision	Mean ± % RSD
4.	Intraday precision	100.584 ± 0.724
	Interday precision	100.245 ± 1.084
	Accuracy	Mean ± % RSD
5.	50 %	99.266 ± 1.330
	100 %	98.590 ± 0.829
	150 %	99.759 ± 0.149
6.	LOD	8.009 ng/band
7.	LOQ	24.270 ng/band

CONCLUSION: In the present work, stability-indicating HPTLC methods for the estimation of SFBwere developed and validated as per ICH guidelines. The standard deviation and % RSD (<2 %) is within the limit, indicating a high degree of precision of the methods.

The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Hence, it can be concluded that the developed methods are simple, accurate and precise, reproducible, and economical.

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CONFLICTS OF INTEREST: The authors do not have any conflict of interest.

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