ORIGINAL ARTICLE

New Stability Indicating Ultrafast Liquid Chromatographic Method for the Determination of Sofosbuvir in Tablets

Mukthinuthalapati Mathrusri Annapurna, Gunnam Ravi Teja, Sunkara Mrunal Chaitanya

Department of Pharmaceutical Analysis and Quality Assurance, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam – 530 045, Andhra Pradesh, India

Abstract

Introduction: To develop a new sensitive, precise, simple, and economic stability indicating reverse phase-ultrafast liquid chromatographic (RP-UFLC) method for the estimation of Sofosbuvir (SFB) in bulk and its tablet dosage forms. **Materials and Methods:** Chromatographic separation was achieved through C8 Phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) using acetonitrile: 0.1% Formic acid mixture as the mobile phase. The Shimadzu model CBM-20A/20 Elite high-performance liquid chromatography system was monitored at detection wavelength 259 nm in isocratic mode with flow rate 0.8mL/min and the total run time is 10 min. The method was validated, and forced degradation studies were conducted. **Results and Discussion:** SFB has obeyed Beer-Lambert's law over a concentration range 1–200 µg/mL with correlation coefficient 0.9998. The limit of detection and limit of quantification are found to be 0.2541 µg/ mL and 0.7642 µg/mL, respectively. The percentage relative standard deviation in precision and accuracy studies was found to be <2% and the percentage recovery is 98.11–98.78%. SFB was found to be highly sensitive toward alkaline conditions. **Conclusions:** It is observed that this RP-UFLC method is accurate, precise, sensitive, and reproducible for the estimation of SFB in tablets. The method was validated as per the ICH guidelines and very much specific as the degradants were well separated without interfering the drug peak.

Key words: Sofosbuvir, ultrafast liquid chromatographic, validation

INTRODUCTION

Sofosbuvir (SFB) is a recently approved new drug used for treating hepatitis C virus.[1,2] It is a highly potent NS5B polymerase inhibitor and was proved high efficacy in combination with other drug molecules.[3,4] It is a nucleotide analog and was known as PS-7977 or GS-7977 previously. More attention has been given to this drug due to its high potency, and very high limit to resistance. SFB has been determined in tablet dosage forms[5-11] and human plasma[12,13] using reverse phase-high performance liquid chromatographic (RP-HPLC) methods and spectrophotometric[13] methods. No stability indicating ultrafast liquid chromatographic (UFLC) method has been reported till date for the determination of SFB, and therefore the authors have approached to develop a stability indicating the liquid chromatographic method and validated[14] as per the ICH guidelines. Forced degradation studies[15] were also performed to study the stability of SFB I different environments [Figure 1].

MATERIALS AND METHODS

Chemicals and reagents

SFB was procured from HETERO Labs Ltd., (India). SFB tablets are available with brand names - RESOF (Dr. Reddy's

Address for correspondence:
Mukthinuthalapati Mathrusri Annapurna, Department of Pharmaceutical Analysis and Quality Assurance, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam – 530 045, Andhra Pradesh, India. E-mail: mannapurna.mukthinuthalapati@gitam.edu

Received: 03-03-2018
Revised: 15-03-2018
Accepted: 21-03-2018

Laboratories Ltd., India), HEPCVIR (Cipla Ltd., India) SoviHep (ZYDUS Heptiza, India), MyHep (Mylan, India), etc., with label claim 400 mg. All other chemicals are of AR grade and all solvents are of HPLC grade.

Equipment

The analysis of SFB was performed using Shimadzu Model CBM-20A/20 Elite UFLC system (Shimadzu Co., Kyoto, Japan) equipped with SPD M20A prominence photodiode array detector.

Optimized chromatographic conditions

C_8 column (Phenomenex) (250 mm × 4.6 mm i.d., 5 µm particle size) was employed for the chromatographic study. Chromatography work was performed on isocratic mode using a mixture of 0.1 % formic acid and acetonitrile (40: 60, v/v) as mobile phase with flow rate 0.8 mL/min (ultraviolet [UV] detection at 259 nm). The overall run time was 10 min and the study was observed at ambient temperature (25°C ± 2°C).

Preparation of drug solutions

Stock solution of SFB was prepared by dissolving 25 mg of SFB in a 25 mL volumetric flask with HPLC grade acetonitrile (1000 µg/mL), diluted with mobile phase and filtered through a membrane filter.

Method validation

The method was validated by evaluating linearity, recovery, precision, accuracy, system suitability, solution stability, limit of detection (LOD), limit of quantification (LOQ), and robustness as per the ICH guidelines for the determination of SFB.

Linearity, precision, accuracy, and robustness studies

Different diluted solutions (1–200 µg/mL) were prepared from the stock, and 20 µL of each solution was injected into the UFLC system, and the peak area of the chromatogram was noted. A graph was plotted using concentration on the X-axis and the mean peak area on the Y-axis. Intraday and interday precisions were studied using three different concentrations of SFB on the same day and on 3 consecutive days, respectively. The accuracy of the method was proved by the standard addition method, and the recovery values were determined. The robustness of an analytical procedure indicates its ability to remain unaffected by small and deliberate changes in method parameters and provides an assurance of its reliability for routine analysis**.** The proposed method was checked for the robustness by slightly changing the optimized conditions such as flow rate (±0.1 mL), mobile phase composition (±2%), pH (±0.2 units), and detection wavelength (254 nm and 264 nm).

Assay of commercial formulations

A total of 20 tablets of available marketed formulations of three brands were procured from the pharmacy store and powdered. The powder equivalent to 25 mg SFB was extracted using the mobile phase, and the solution was sonicated for half an hour and filtered through 0.45 mm membrane filter. 20 µL of solution from each brand was injected into the UFLC system, and the peak areas were noted from the respective chromatograms.

Stability studies

Forced degradation studies were performed to determine the ability of the drug to withstand its properties in the applied stress conditions. SFB was exposed to different stress conditions such as acidic hydrolysis, basic hydrolysis, oxidation, photolytic, and thermal treatment. Acidic degradation was performed by treating the drug solution with 0.1N HCl for 30 min at 60°C in a thermostat and later the solution was cooled, neutralized using sodium hydroxide solution and the solution was made up to volume to the required concentration with the mobile phase. Similarly, the alkaline degradation was performed by treating the drug solution at room temperature with 0.1 N NaOH just for 2 min, neutralized with hydrochloric acid and diluted with mobile phase. Oxidative degradation was performed by treating the drug solution with 30% v/v H_2O_2 at 60° in the thermostat for 1 h. Thermal degradation was performed by heating the drug at 60°C for 1 h. Photodegradation studies were performed by exposing the drug in the solid state to UV light in a photostability chamber for 48 h and then the drug solution was prepared according to the requirement. All the solutions were filtered through Whatman membrane filter No. 45, and 20 µL of each solution was injected into the UFLC system, and the peak area was noted from the corresponding chromatogram.

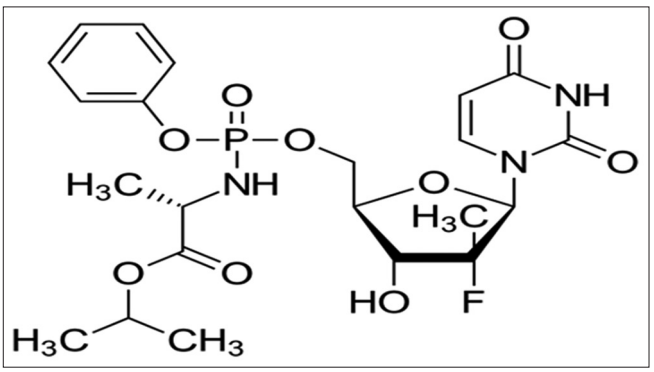

Figure 1: Chemical structure of Sofosbuvir

RESULTS AND DISCUSSION

A simple stability indicating RP-UFLC method has been developed for the determination of SFB in active pharmaceutical ingredient and its tablet dosage forms using C8 Phenomenex column and a mixture of acetonitrile and formic acid as mobile phase. The highlights of the present proposed method were compared with the previously published methods in Table 1.

Method development and optimization

UFLC system was initially optimized using C18 sun fire column with 0.1% formic acid and acetonitrile (A: B: 40: 60, v/v) as mobile phase (flow rate 0.7 mL/min). A low concentration of SFB was injected into the system, and a quite broad chromatogram was eluted with very low theoretical plates (i.e., <2000). Trials were made with different mobile compositions, columns, and flow rates, and the observations were shown in Table 2 and Figure 2. A C8 Phenomenex column (250 mm × 4.6 mm i.d. 5 µm particle size) with mobile phase composition 0.1% formic acid:acetonitrile (40:60, v/v) was found to be more appropriate to satisfy the system suitability parameters and the method was optimized [Table 3] where a sharp drug peak was eluted at 4.393 ± 0.02 min [Figure 3].

Method validation

The proposed method was validated by linearity, precision, accuracy, and robustness as per the ICH guidelines. The calibration curve was drawn by taking a concentration of SFB on X-axis and the corresponding mean peak area values on the Y-axis. SFB obeys Beer-Lamberts law over the concentration range 1–200 µg/mL [Table 4] with linear regression equation

Table 1: Highlights of present study over the previously published methods

Mobile phase/flow rate/detection (%v/v)/(mL/min)/(nm)	Column	Linearity (µg/mL)	Comments	Ref
Methanol: Acetonitrile (30:70)/1/261	Eclipse XDB-C18	10–13	Very low linearity range; Very costly	5
O-phosphoric acid: acetonitrile (55:45)/1/260	Kromasil C18	100–600	pH maintenance is required	6
Methanol (100)/1/265	Hypersil C18	20–100	Complete organic phase; Very costly	7
Acetonitrile: phosphate buffer (pH 2.5); (55:45)/1/260	Eclipse XDB-C18	140–420	pH maintenance is required	8
Methanol: water (60:40) 1/235	Agilent C18	320–480	Very low linearity range	9
O-phosphoric acid: acetonitrile (pH 2.0); (68:32)/1/228	Kromasil	0.05–2.0	Human plasma; Very low linearity range	10
Methanol: 0.1% TFA (60:40)/1/260	Phenomenex prodigy ODS-3V	100–600	pH maintenance is required	11
Acetonitrile: water (pH adjusted to 2.4) (80:20)/0.7/260	C18 (PRIMESIL)	20–100	pH maintenance is required	12
Methanol: acetonitrile (90:10)/1/260	Hypersil™ ODS C18	5–40	Very low linearity range; Very costly	13
0.1% Formic acid acetonitrile (40:60)/0.8/259	C8 Phenomenex	1–200	Stability indicating UFLC; High linearity range; no pH maintenance; Economical.	Present method

Table 2: Observations made in trials during optimization

Trails	Column	Mobile phase (A: B)(v/v)	Flow rate (mL/min)	Rt (min)	Comments	Figure
1	C18 sun fire	40:60	0.7	2.794	Theoretical plates<2000, broad peak tailing factor>1.5	2a
2	C18 sun fire	40:60	0.8	3.986	Theoretical plates<2000, fronting,	2b
3	C18 sun fire	55:45	0.8	3.167	Theoretical plates<2000, peak tailing	2c
4	C8 Phenomenex	55:45	0.8	2.634	Broad peak, tailing factor>1.5	2d
5	C8 Phenomenex	45:55	0.8	4.848	Rt is more	2e
6	C8 Phenomenex	40:60	0.8	4.393	Method optimized	2f

Table 3: Optimized conditions for determination of SFB

Parameter	Optimized chromatographic conditions
Mobile phase	0.1% Formic acid and acetonitrile (40: 60 v/v)
Stationary phase	C_8 (Phenomenex) column (250 mm × 4.6 mm i.d., 5 µm particle size)
Flow rate	0.8 mL/min
Detection range	259
Column temperature	(25°±2°C)
Injection volume	20 µL
Detector	SPD M20A prominence photodiode array detector
Elution	Isocratic mode
Total runtime	10 min
Retention time	4.848 ± 0.02 min

SFB: Sofosbuvir

Table 4: Linearity of SFB

Concentration (µg/mL)	*Mean peak area	% RSD
1	26431	0.36
5	129435	0.41
10	264870	0.23
20	539740	0.87
40	1099436	0.81
100	2748701	0.37
120	3296431	1.01
150	4223896	0.93
200	5637372	0.89

*Mean of three replicates. RSD: Relative standard deviation, SFB: Sofosbuvir

Table 5: Intraday precision study of SFB

Concentration (µg/mL)	*Mean peak area	Statistical analysis *Mean±SD (% RSD)
20	539740	538838.33±1832.23 (0.34)
20	536730	
20	540045	
50	1349350	1347904.33±3327.98 (0.25)
50	1350265	
50	1344098	
100	2728701	2731743.33±2703.42 (0.10)
100	2733870	
100	2732659	

*Mean of three replicates. RSD: Relative standard deviation, SFB: Sofosbuvir

y = 28131 x – 22947 correlation coefficient 0.999 [Figure 4]. The LOD and LOQ are found to be 0.2541 µg/mL and 0.7642 µg/mL, respectively.

Intraday and interday precisions were studied using three different concentrations of SFB on the same day and on 3 consecutive days, respectively. The percentage relative standard deviation (RSD) was found to be 0.10–0.34 and 0.46–1.05, respectively (<2.0%) demonstrating that the method is precise [Tables 5 and 6]. The accuracy of the method was proved by the standard addition method, and the recovery values were determined. The percentage recovery of SFB and its results of the method are reported in Table 7. The percentage RSD was found to be 0.21–0.60 (<2.0%) with a recovery of 98.11–98.78%. The percentage RSD was found to be 0.46–0.89 (<2.0%) in robustness study. The system suitability and solution stability were evaluated, and the percentage RSD was <2%.

Assay of commercial formulations

SFB has shown 99.64–99.78 [Table 8] recovery in the marketed formulations and the chromatogram obtained in one of the marketed formulations was shown in Figure 3.

Stress degradation studies

SFB was exposed to various stress conditions such as thermal, acidic, oxidative, photo, and alkaline hydrolysis. In acidic hydrolysis, SFB was eluted at 4.398 min along with degradation products at 3.136 and 5.693 min. About 11.65% of degradation was observed, and it may be due to the amino moiety attached to the phenoxy phosphoryl part of the SFB drug molecule. While performing alkaline hydrolysis 1 ml of o.1 N sodium hydroxide was used initially, and the drug peak was totally vanished. Therefore, the study was modified by decreasing the alkaline concentration as well as the time of treatment, i.e., the drug solution was treated with alkali for a time of 2 min and immediately neutralized and injected where the drug was eluted at 4.397min and degradant peaks at 3.569, 5.076, 7.488, and 8.129 min with 27.03% degradation.

The instantaneous degradation of SFB may be definitely due to the propionic acid moiety present in it.

Due to oxidation different degradation peaks were observed at 3.281, 3.654, 3.778, 7.223, and 8.264 min along with the drug peak at 4.396 min (drug degradation 32.85%). The peak observed at 3.281 is only due to the oxidative agent, i.e., hydrogen peroxide. In the given peak, the drug degradation was around this might be due to the hydroxyl present in the drug molecule. In photolytic and thermal conditions, the drug peak eluted at 4.398 min and no degradants were reported. It is confirmed that the drug is highly sensitive toward alkaline conditions and more sensitive toward oxidation. In all the degradation studies, it was found that the drug peak was well separated among the degradants indicating that the method is selective and specific. The system suitability parameters were

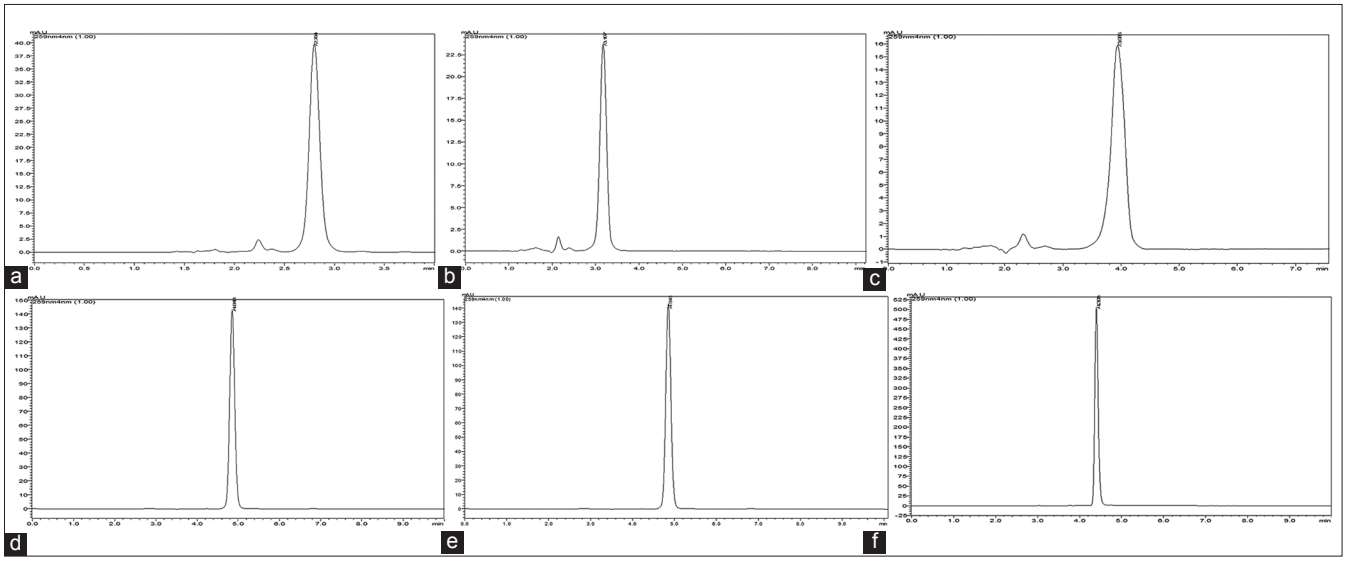

Figure 2: (a-f) Chromatograms observed during method optimization of Sofosbuvir (trial runs)

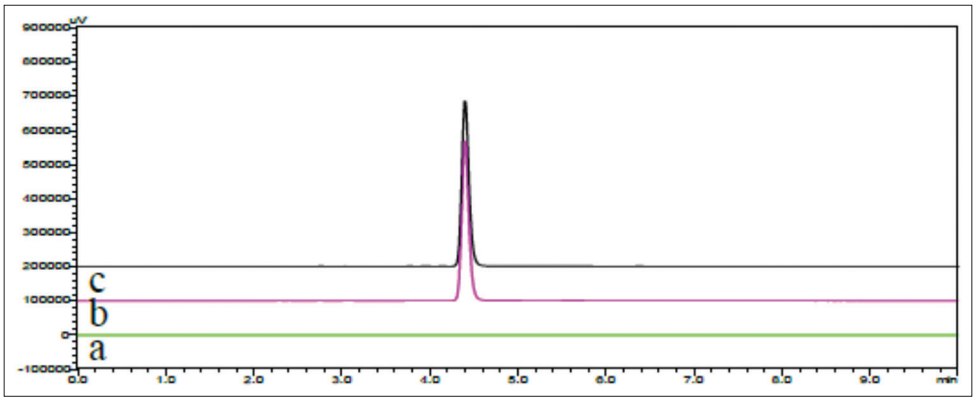

Figure 3: Typical chromatograms (a) Blank (b) Sofosbuvir (SFB) standard (100 µg/mL) (c) SFB tablets (100 µg/mL)

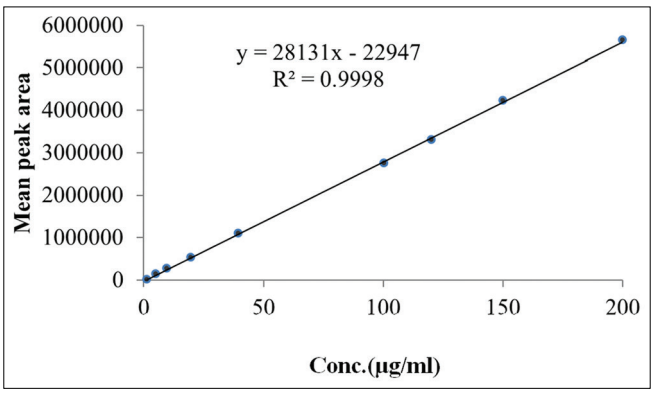

Figure 4: Calibration of Sofosbuvir

Table 6: Interday precision study of SFB

Concentration (µg/mL)	*Mean peak area			*Mean±SD(% RSD)
	Day 1	Day 2	Day 3	
20	539740	549276	539145	542720.33±5685.16 (1.05)
50	1350265	1349314	1339114	1346231.00±6181.82 (0.46)
100	2733870	2699245	2689105	2707406.67±23472.02 (0.87)

*Mean of three replicates. RSD: Relative standard deviation, SFB: Sofosbuvir

Table 7: Accuracy study of SFB

Concentration (µg/mL)			*Mean±SD (%RSD)	% recovery
Formulation	Pure drug	Total		
20	18	38	37.539±0.2029 (0.54)	98.40
20	18	38		98.56
20	18	38		99.39
20	20	40	39.435±0.2379 (0.60)	98.07
20	20	40		98.45
20	20	40		99.23
20	22	42	41.206±0.0872 (0.21)	98.22
20	22	42		97.87
20	22	42		98.23

*Mean of three replicates. RSD: Relative standard deviation, SFB: Sofosbuvir

Table 8: Assay of available marketed formulations (tablets)

Formulation	Label claim (mg)	*Amount found (mg)	*Recovery (%)
Brand I	400	398.653	99.66
Brand II	400	399.134	99.78
Brand III	400	398.569	99.64

* Mean of three replicates

Table 9: Stress degradation studies of SFB

Stress condition Medium/temperature/ duration	Rt (min)	% recovery	% Drug degradation	Theoretical plates	Tailing factor
Standard drug	4.398	100	-	11179.419	1.298
Acidic hydrolysis 0.1N HCl/60°C/30 min	4.398 3.136 5.693	88.35	11.65	10768.607	1.335
Alkaline hydrolysis 0.1N NaOH/25°C/2 min	4.397 3.569 5.076 7.488 8.129	72.97	27.03	11176.479	1.332
Oxidative 30%H_2O_2/70°C/1h	4.396 3.281 3.654 3.778 7.223 8.264	67.15	32.85	10745.856	1.335
Photolytic/7 days	4.398	99.149	0.851	11109.198	1.331
Thermal/60°C/1 h	4.398	98.97	1.03	11225.475	1.331

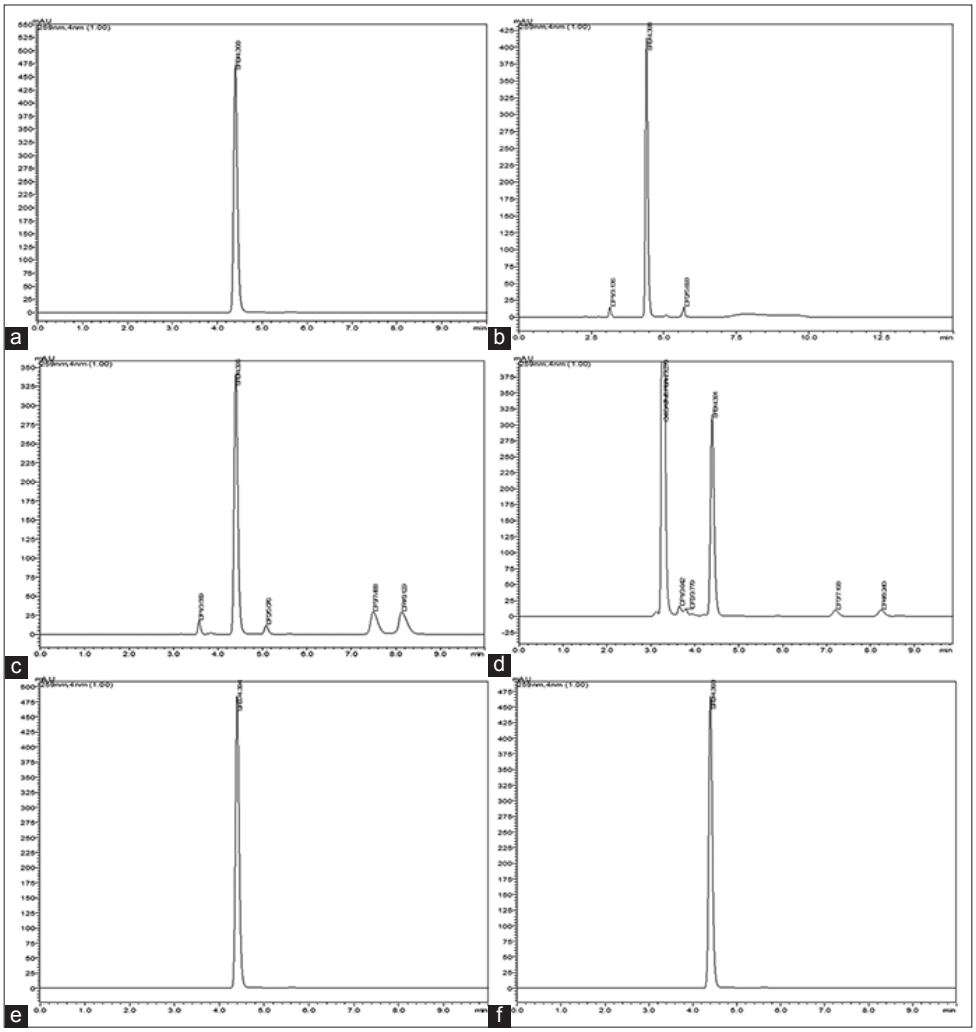

Figure 5: Typical chromatograms of Sofosbuvir (100 µg/mL) (a) standard, (b) acidic hydrolysis, (c) alkaline hydrolysis, (d) oxidation, (e) photolysis, (f) thermal degradation

well in the acceptance criteria [Table 9]. The representative chromatograms obtained during degradation studies were shown in Figure 5.

CONCLUSIONS

The validated stability indicating method developed for the determination of Sofosbuvir is specific and selective and more economical. Sofosbuvir is known to be more sensitive toward the basic environment. This method can be excellently applied for the determination of Sofosbuvir in tablets.

ACKNOWLEDGMENT

The authors are grateful to M/s GITAM (Deemed to be University) for providing research facilities and to HETERO Labs Ltd. (India) for providing the gift samples of Sofosbuvir

REFERENCES

1. Herbst DA, Reddy KR. Sofosbuvir, a nucleotide polymerase inhibitor, for the treatment of chronic hepatitis C virus infection. Exµpert Opin Investig Drugs 2013;22:527-36.
2. Gentile I, Borgia F, Buonomo AR, Castaldo G, Borgia G. A novel promising therapeutic option against hepatitis C virus: An oral nucleotide NS5B polymerase inhibitor Sofosbuvir. Curr Med Chem 2013;20:3733-42.
3. Soriano V, Vispo E, de Mendoza C, Labarga P, Fernandez-Montero JV, Poveda E, *et al*. Hepatitis C therapy with HCV NS5B polymerase inhibitors. Expert Opin Pharmacother 2013;14:1161-70.
4. Bhatia HK, Singh H, Grewal N, Natt NK. Sofosbuvir: A novel treatment option for chronic hepatitis C infection. J Pharmacol Pharmacother 2014;5:278-84.
5. Ravisankar P, Sivaparvathi Y, Chandana K, Ayesha Ameen SK, Kiranmai D, Sri Ram R, *et al*. Development and validation of a rapid RP-HPLC method for the determination of Sofosbuvir in bulk and in pharmaceutical

dosage form. Int J Pharm Sci Rev Res 2017;44:245-50.
6. Jeyabaskaran M, Rambabu, Rajinikanth. A new RP-HPLC method development and validation of Sofosbuvir in bulk and pharmaceutical dosage forms. J Pharm 2014;1:125-33.
7. Swathi P, Dutt KR, Rao KN, Raja MA. RP-HPLC method development and validation for estimation of Sofosbuvir in pure and tablet dosage form. Int J Med Pharm Sci 2017;2:7-9.
8. Sasidhar B, Sankar GG. High performance liquid chromatographic method for the determination of Sofosbuvir in pharmaceutical dosage forms. J Comp Pharm 2016;3:136-42.
9. Ramesh G, Madhukar A, Kannappan N, Ravinder A, Sravanthi K. Analytical method development and validation of Sofosbuvir tablets by RP-HPLC. J Pharm Res 2016;5:161-3.
10. Madhavi S, Rani AP. Bioanalytical method development and validation for the determination of Sofosbuvir from human plasma. Int J Pharm Pharm Sci 2017;9:35-41.
11. Ravikumar V, Subramanyam CV, Veerabhadram G. Estimation and validation of Sofosbuvir in bulk and tablet dosage form by RP-HPLC. Int J Pharm 2016;6:121-7.
12. Patil JA, Patil ST, Rane BR, Pawar SP, Development and validation of RP-HPLC Method for determination of Sofosbuvir by pure and tablet dosage form. World J Pharm Res 2017;6:891-905.
13. Sherif Abdel-Naby Abdel-Gawad. Simple chromatographic and spectrophotometric determination of Sofosbuvir in pure and tablet forms. Eur J Chem 2016;7:375-9.
14. ICH Harmonized Tripartite Guidelines. Validation of Analytical Procedures: Methodology (Q2B). Geneva: ICH Harmonized Tripartite Guidelines; 1996.
15. International Conference on Harmonization of Technical REQUIREMENT FOR THE Registration of Pharmaceutical for Human use: Validation of Analytical Procedures, Text and Methodology- Q2(R1); 2005.\

Source of Support: Nil. **Conflict of Interest:** None declared.