Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 2 Issue 10 October 2018

Research Article

A New Validated Stability Indicating RP-HPLC Method for the Assay of Sofosbuvir in Tablets

Mukthinuthalapati Mathrusri Annapurna*, Pudi Rajesh and Gunnam Ravi Teja

Department of Pharmaceutical Analysis and Quality Assurance, Gitam Institute of Pharmacy, Gitam University Visakhapatnam, Andhra Pradesh, India

***Corresponding Author:** Mukthinuthalapati Mathrusri Annapurna, Department of Pharmaceutical Analysis and Quality Assurance, Gitam Institute of Pharmacy, Gitam University Visakhapatnam, Andhra Pradesh, India.

Received: September 17, 2018; **Published:** September 25, 2018

Abstract

Aim: A new sensitive stability indicating RP-HPLC method was developed for the quantification of Sofosbuvir in tablets.

Materials and Methods: Agilent eclipse XDB C18 (3.5 µm, 6.4 x 150 mm) column (150 mm × 6.4 mm; 3.5 µm) was used with mobile phase 0.1% TEA: Acetonitrile (pH 3.2 adjusted with 10% OPA)/45:55 for the present study on Shimadzu Model CBM-20A/20 Alite HPLC system (Detection wavelength 259 nm) with flow rate 0.6 mL/min. Forced degradation studies were performed and method was validated.

Methodology: A prospective observational study was carried out at Departments of in and out patients at Gandhi hospital and BBR hospital, for a period of 6 months (July 2017 to December 2017). All the patients who are diagnosed with DM complications were included in this study. Patients between ages of 18 - 90 yrs were considered. Patients with comorbidities of DM were excluded in the study.

Results and Discussion: Beer-Lambert's law was obeyed 0.1 - 200 µg/mL and LOD and LOQ were 0.0239 µg/mL and 0.0739 µg/mL respectively. The method was validated as per ICH guidelines.

Conclusion: The stability indicating liquid chromatographic method developed for the determination of Sofosbuvir is specific and can be excellently applied for the determination of Sofosbuvir in tablets.

Keywords: RP-HPLC; Sofosbuvir; Forced Degradation Studies; Validation

Introduction

Sofosbuvir (Figure 1) (SBV) is used for the treatment of hepatitis C virus and it is a highly potent NS5B polymerase inhibitor [1-4]. Sofosbuvir was estimated by liquid chromatographic methods in tablets and biological fluids [5-14]. A new sensitive and selective stability indicating RP-HPLC method was developed by the authors and the method was validated [15]. Stress degradation studies [16] were executed to evaluate the stability of Sofosbuvir

Materials and Methods

HETERO Labs Ltd. (India) supplied gift samples of Sofosbuvir. SoviHep (ZYDUS Heptiza, India), RESOF (Dr. Reddy's Laboratories Ltd., India), MyHep (Mylan, India), HEPCVIR (Cipla Ltd., India) etc. are the brand names available for Sofosbuvir tablets (Label claim

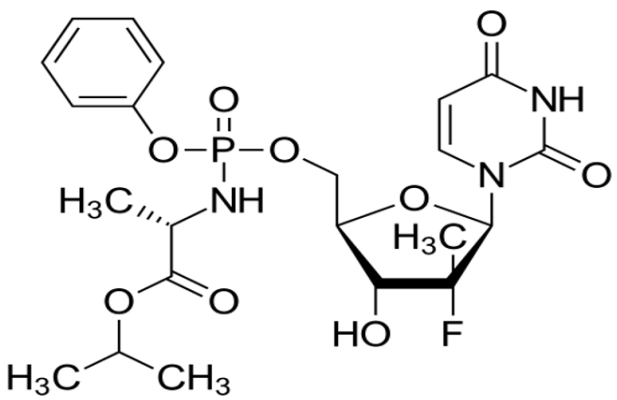

Figure 1: Structure of Sofosbuvir.

400 mg). All solvents are HPLC grade only. Shimadzu Model CBM-20A/20 Alite system (Shimadzu Co., Kyoto, Japan) was employed with PDA detector.

Stock solution preparation and assay of sofosbuvir

25 mg of Sofosbuvir was dissolved in HPLC grade acetonitrile (1000 µg/mL) and diluted with mobile phase. Three different brands of Sofosbuvir were bought and 20 tablets from each brand were extracted with mobile phase as per the general procedure and diluted as per the necessity to conduct assay.

Optimized chromatographic conditions

The present study was performed on Agilent eclipse XDB C18 (3.5 µm, 6.4 x 150 mm) column (150 mm × 6.4 mm; 3.5 µm) using 0.1% TEA: Acetonitrile (pH 3.2 adjusted with 10% OPA)/45:55 as mobile phase (flow rate 0.6 mL/min) (UV detection at 259 nm).

Method validation

The method was validated - linearity, system suitability, limit of detection (LOD), Limit of quantification (LOQ), precision, accuracy and robustness (ICH guidelines).

Linearity, precision, accuracy and robustness studies

0.1 to 200 µg/mL Sofosbuvir solutions were prepared and 20 µL was injected from each concentration (n = 3) to the system and mean peak area was noted from the corresponding chromatogram. Calibration graph was drawn to evaluate the linearity and simultaneously Intraday and inter-day precision were also studied along with accuracy. Robustness of the study was evaluated by introducing minor changes in the chromatographic parameters.

Forced degradation studies

Forced degradation studies were performed with the applied stress conditions. Sofosbuvir was exposed to different stress conditions such as acidic hydrolysis, basic hydrolysis, oxidation, photolysis and thermal treatment. Sofosbuvir exposed to acidic (0.1N HCl/60°C/30 min) and alkaline 0.1N NaOH/25°C/10 mins treatment and neutralized after diluted. Oxidative degradation was performed by treating with 30% w/v H_2O_2 at 70° for 1 hour in the thermostat. Thermal degradation was performed by heating the drug solution at 60°C for 1 hour. Photolysis was performed by exposing the drug in solid state to UV light in a photo stability chamber for 7 days.

Results and Discussion

A new sensitive stability indicating liquid chromatographic method was developed for the assay of Sofosbuvir in its tablet dosage forms. The present method was compared with the previously published methods of the literature in table 1.

Mobile phase/ Flow rate/ Detection (% v/v)/ (mL/min)/ (nm)	Column	Linearity (µg/mL)	Comments	Ref
Methanol: Water (60:40) 1/ 235	Agilent C18	320 - 480	Very low linearity range	5
Methanol: 0.1% TFA (60:40) / 1/ 260	Phenomenex prodigy DS-3V	100 - 600	pH maintenance is required	6
O-phosphoric acid: Acetonitrile (55:45) / 1/ 260	Kromasil C18	100 - 600	pH maintenance is required	7
O-phosphoric acid: Acetonitrile (pH 2.0); (68:32)/ 1/ 228	Kromasil	0.05 - 2.0	Human plasma	8
Methanol (100) /1/ 265	Hypersil C18	20 - 100	Complete organic phase	9
Methanol: Acetonitrile (30:70) / 1/ 261	Eclipse XDB-C18	10 - 13	Very low linearity range	10
Acetonitrile: Phosphate buffer (pH 2.5); (55:45) / 1/ 260	Eclipse XDB-C18	140 - 420	pH maintenance is required	11
0.1 % Formic acid: Acetonitrile (40:60)/ 0.8/ 259	C8 Phenomenex	1 - 200	Stability indicating UFLC High linearity range	12
Acetonitrile: Water (pH adjusted to 2.4) (80:20)/ 0.7/ 260	C18 (PRIMESIL)	20 - 100	pH maintenance is required	13
Methanol: Acetonitrile (90:10) / 1/ 260	Hypersil™ ODS C18	5 - 40	Complete organic phase	14
0.1% TEA: Acetonitrile (pH 3.2 adjusted with 10% OPA) / 45:55	Agilent eclipse XDB C18	1.1 - 200	Wide linearity range Stability indicating (PDA)	Present method

Table 1: Comparison of present study with the published methods.

Method optimization

Initially C18 Sunfire column and C8 Phenomenex column were tried but Sofosbuvir couldn't elute properly and the results were not within the acceptable criteria and therefore Agilent eclipse XDB C18 column was introduced where the drug was eluted at about 3.1 min with the given mobile phase mixture (0.1% TEA: Acetonitrile (pH 3.2 adjusted with 10% OPA) / 45:55). The optimized conditions were shown in table 2 and Figure 2 shows the resulted chromatogram of Sofosbuvir.

Method validation

The proposed method was validated and in this regard the calibration curve shows that Sofosbuvir follows linearity 0.1 - 200 µg/mL (Table 3) with linear regression equation y = 35847x - 24354

Parameters	Values/Conditions
Mobile Phase/ Ratio	0.1% TEA: Acetonitrile (pH 3.2-adjusted with 10% OPA)/45:55
Column	Agilent eclipse XDB-C18 (3.5 µm - 6.4 x 150 mm)
Retention Time	3.168 minutes
Tailing Factor	1.373
Theoretical Plates	5894.345
HPLC System	Shimadzu Model CBM-20A/20 Alite UFLC system with SPD M20A prominence (PDA)
Column Temperature	(25° ± 2°C)
Total Run Time	10 min

Table 2: Optimized chromatographic conditions.

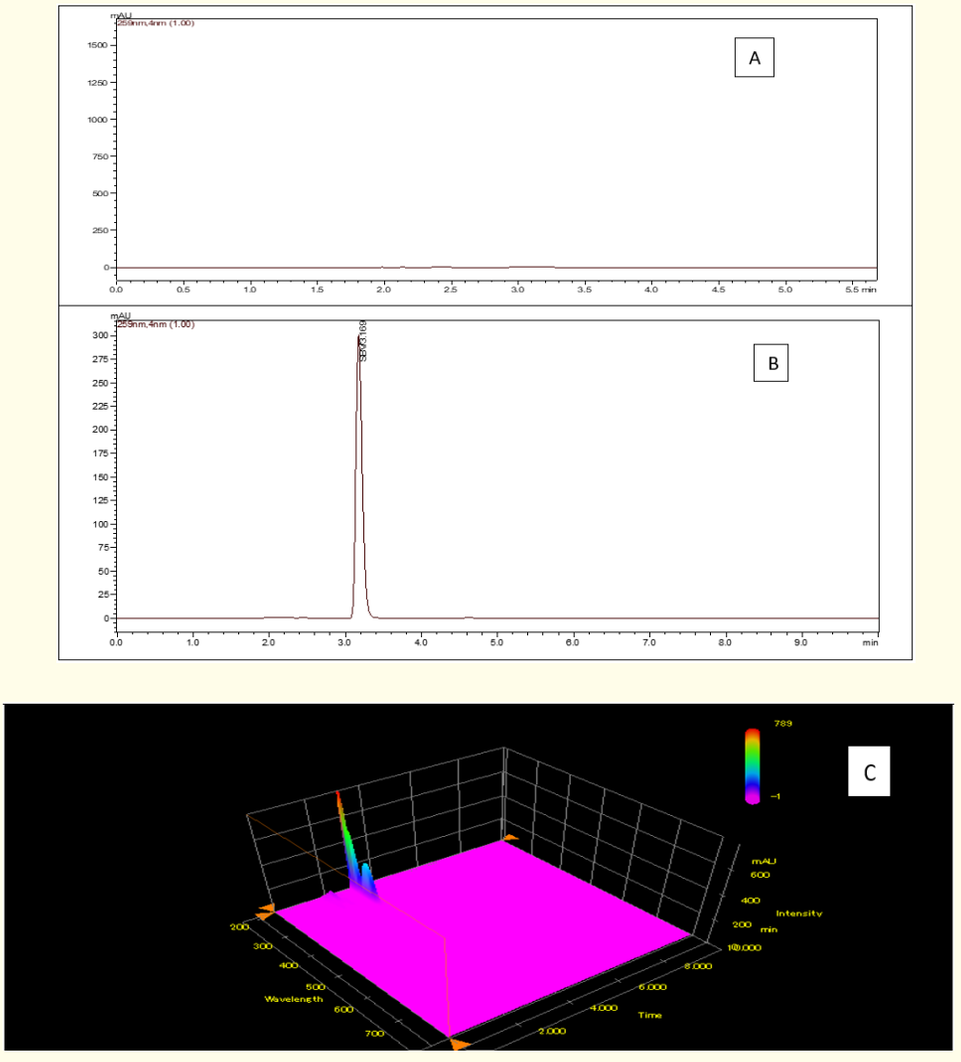

Figure 2: Chromatograms of A) Blank B) Sofosbuvir standard (50 µg/mL) C) 3D chromatogram of Sofosbuvir standard.

(correlation coefficient 0.9995 (Figure 3). The LOD and LOQ were 0.0239 µg/mL and 0.0739 µg/mL respectively. The % RSD in intra-day and inter-day precision was found to be 0.0919 - 0.2454 and 0.21 - 0.42 respectively (< 2.0 %) proving that the method is precise (Table 4 and Table 5) where as in accuracy it was 0.42 - 0.57 with recovery 99.13 - 99.83%. The percentage RSD was 0.46-0.89 (< 2.0 %) in robustness study.

Conc. (µg/ml)	*Mean peak area	Retention Time (min)
0.1	4139	3.159
1	38164	3.168
10	377473	3.168
20	731320	3.172
50	1731593	3.150
100	3577658	3.163
150	5366487	3.166
200	7055316	3.171

Table 3: Linearity of Sofosbuvir.

*Mean of three replicates

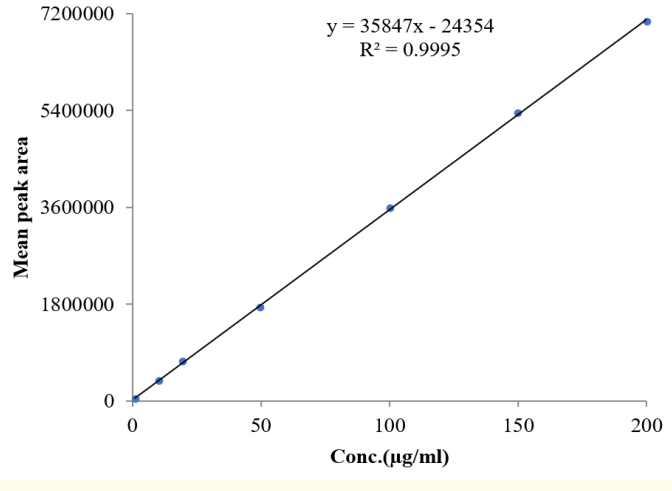

Figure 3: Calibration curve of Sofosbuvir.

Assay of Sofosbuvir tablets

Three marketed brands were analyzed by the proposed stability indicating RP-HPLC method. The assay of Sofosbuvir was found to be 99.31-99.73 (Table 8) in the three available brands.

Conc. (µg/ml)	*Mean peak area	Statistical Analysis	
		Mean peak area ± SD	% RSD
10	367473	366943 ± 689.109	0.1878
10	366164		
10	367192		
20	736620	736089.333 ± 676.181	0.0919
20	735328		
20	736320		
50	1729593	1728214 ± 4240.16	0.2454
50	1723456		
50	1731593		

Table 4: Intraday precision.

*Mean of three replicates

Conc. (µg/mL)	*Mean peak area			*Mean ± SD (% RSD)
	Day 1	Day 2	Day 3	
50	1709230	1734998	1727633	1723953.67 ± 8447.37 (0.49)
100	3583997	3546398	3500430	3543608.33 ± 23742.17 (0.67)
150	5232357	5278469	5216997	5242607.67 ± 28310.08 (0.54)

Table 5: Inter day precision.

*Mean of three replicates

Stress degradation studies

During oxidation, photolysis and thermal degradation studies Sofosbuvir has shown very slight degradation i.e. < 2% specifying that the drug is highly stable towards those stress conditions. During oxidation two degradant products were observed at 2.110 and 2.958 minutes. During acidic hydrolysis Sofosbuvir has shown degradants at 1.888, 2.154 and 3.708 minutes and about 37.943% drug was decomposed, and it may be due to the basic amino moiety present in the drug chemical structure while in alkaline hydrolysis more than 90% the drug was disappeared within no time at room temperature itself representing that the drug is highly sensitive and the degradants were appeared at 2.055 and 3.705 min. The method is selective and specific and none of the degradants interfere with the drug peak. The system suitability parameters were well in the acceptance criteria (Table 9). The resulting chromatograms and the 3D diagrams obtained during the studies were shown in figure 4 and figure 5.

Conc. (µg/ml)			*Mean peak area	Statistical analysis	Drug found (µg/ml)	% Recovery
Formulation	Pure Drug	Total		*Mean ± SD (% RSD)		
20	16	36	1326474	1320218 ± 7526.87 (0.57)	36.14	100.41
20	16	36	1311865			
20	16	36	1322315			
20	20	40	1453467	1445965.3 ± 6843.93 (0.47)	39.65	99.14
20	20	40	1444367			
20	20	40	1440062			
20	24	44	1601246	1598663.3 ± 6872.16 (0.42)	43.91	99.81
20	24	44	1603870			
20	24	44	1590874			

Table 6: Accuracy.

*Mean of three replicates

Parameter	Condition	*Mean peak area	Statistical analysis	
			*Mean peak area ± SD	% RSD
Flow rate (± 0.1 ml/min)	0.5	1748621	1748611 ± 17063.50	0.97
	0.6	1731543		
	0.7	1765670		
Detection wavelength (± 2 nm)	257	1754973	1757035 ± 21549.59	1.22
	259	1736590		
	261	1779541		
Mobile phase composition (± 2% v/v) (0.1% Formic acid: Acetonitrile)	43:57	1788043	1763733 ± 25186	1.42
	45:55	1737754		
	47:53	1765402		

Table 7: Robustness.

Formulation	Label claim (mg)	*Amount found (mg)	Recovery (%)
Brand I	400	397.24	99.31
Brand II	400	398.32	99.58
Brand III	400	398.92	99.73

Table 8: Assay of Sofosbuvir tablets.

*Mean of three replicates

Stress condition	Retention Time (R_t)	% Recovery	% Drug degradation	Theoretical plates	Tailing factor
Sofosbuvir standard	3.167	100	------	5916.659	1.375
Acidic hydrolysis 0.1N HCl/60°C/30min	3.167 1.888 2.154 3.708	62.05	37.943	5471.474	1.386
Alkaline hydrolysis 0.1N NaOH/25°C/10 mins	3.168 2.055 3.705	7.39	92.66	5274.450	1.368
Oxidation 30% H_2O_2/70°C/1hr	3.177 2.110 2.958	96.36	3.63	5744.756	1.329
Photolysis/7days	3.157	98.345	1.655	5758.363	1.373
Thermal degradation/60°C/1hr	3.169	98.97	0.69	5916.659	1.375

Table 9: Stress degradation results of Sofosbuvir.

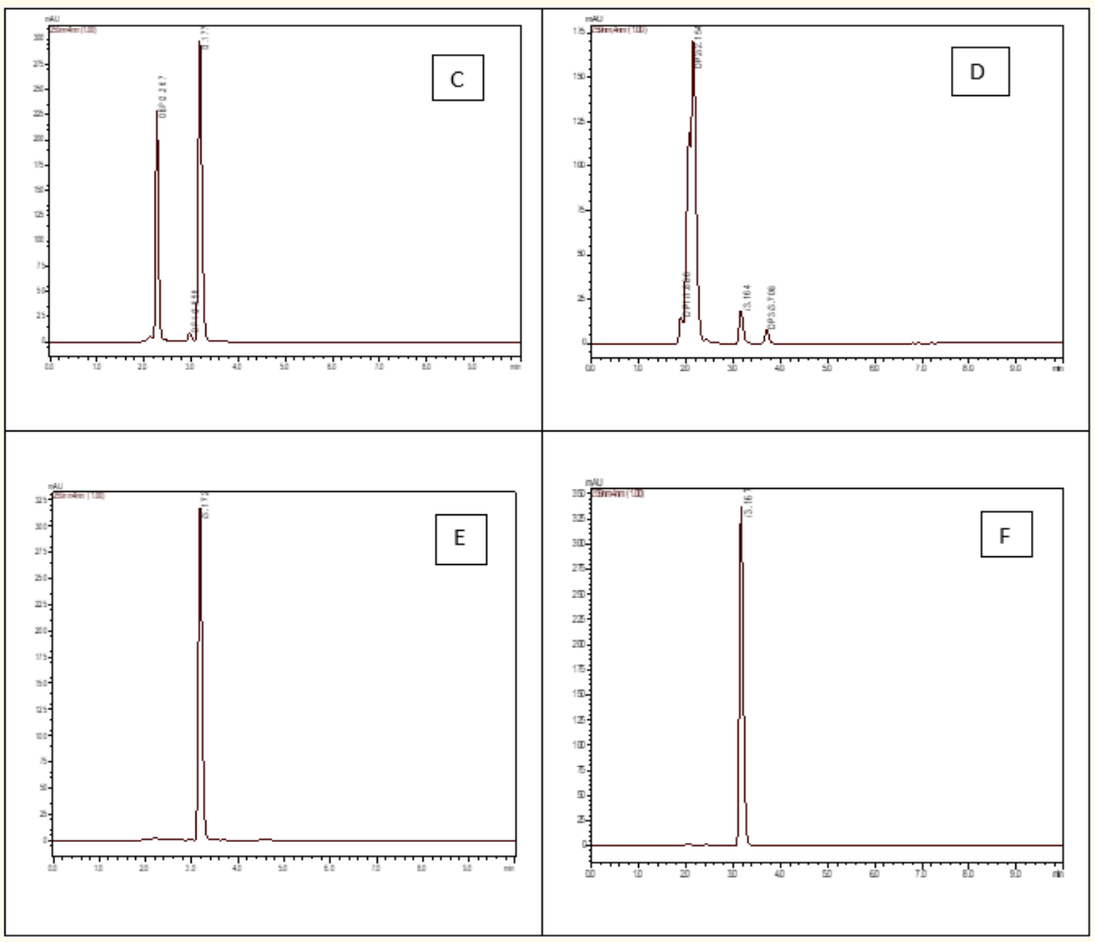

Figure 3: Typical chromatograms of Sofosbuvir during forced degradation studies.

A) Sofosbuvir tablets B) Alkaline hydrolysis C) Oxidation D) Acidic hydrolysis E) Thermal degradation F) Photolysis

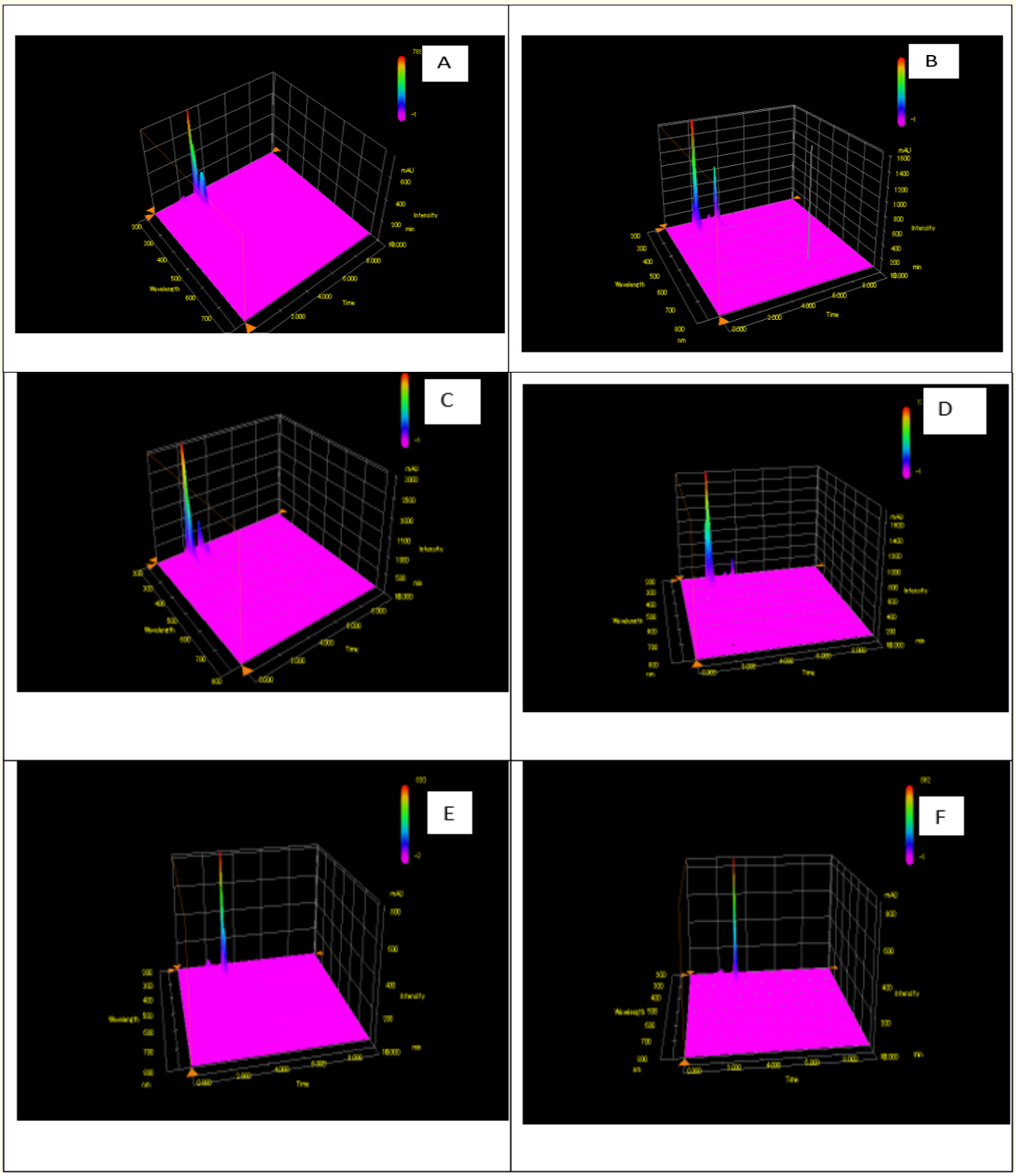

Figure 5: 3D chromatograms of Sofosbuvir during forced degradation studies.

A) Sofosbuvir standard B) Alkaline hydrolysis C) Oxidation D) Acidic hydrolysis E) Thermal degradation F) Photolysis

Conclusion

The stability indicating liquid chromatographic method developed for the determination of Sofosbuvir is specific and selective. Sofosbuvir is highly sensitive towards basic environment and the study is very much interesting where a great challenge for the impurity profiling study as well as the study of metabolites in both *in vivo* and *in vitro* in R and D of pharmaceutical industries. This method can be excellently applied for the determination of Sofosbuvir in tablets.

Acknowledgement

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.

Bibliography

1. Soriano V., *et al*. "Hepatitis C therapy with HCV NS5B polymerase inhibitors". *Expert Opinion on Pharmacotherapy* 14 (2013): 1161-1170.

2. Gentile I., *et al*. "A novel promising therapeutic option against hepatitis C virus: An oral nucleotide NS5B polymerase inhibitor Sofosbuvir". *Current Medicinal Chemistry* 20 (2013): 3733-3742.

3. Harmeet Kaur Bhatia., *et al*. "Sofosbuvir: A novel treatment option for chronic hepatitis C infection". *Journal of Pharmacology and Pharmacotherapeutics* 5.4 (2014): 278-284.

4. Herbst DA and Reddy KR. "Sofosbuvir, a nucleotide polymerase inhibitor, for the treatment of chronic hepatitis C virus infection". *Expert Opinion Investigational Drugs* 22 (2013): 527-536.

5. Ramesh G., *et al*. "Analytical method development and validation of Sofosbuvir tablets by RP-HPLC". *Journal of Pharmacy Research* 5.7 (2016): 161-163.

6. Ravikumar V., *et al*. "Estimation and validation of Sofosbuvir in bulk and tablet dosage form by RP-HPLC". *International Journal of Pharmaceutics* 6.2 (2016): 121-127.

7. Jeyabaskaran M., *et al*. "A new RP-HPLC method development and validation of Sofosbuvir in bulk and pharmaceutical dosage forms". *Journal of Pharma creations* 1.4 (2014): 125-133.

8. Madhavi S and Prameela Rani A. "Bioanalytical method development and validation for the determination of Sofosbuvir from human plasma". *International Journal of Pharmacy and Pharmaceutical Sciences* 9.3 (2017): 35-41.

9. Swathi P., *et al*. "RP-HPLC method development and validation for estimation of Sofosbuvir in pure and tablet dosage form". *Asian Journal of Pharmacy and Technology* 2.4 (2017): 7-9.

10. Ravisankar P., *et al*. "Development and validation of a rapid RP-HPLC method for the determination of Sofosbuvir in bulk and in pharmaceutical dosage form". *International Journal of Pharmaceutical Sciences Review and Research* 44.1 (2017): 245-250.

11. Sasidhar B and Girija Sankar G. "High performance liquid chromatographic method for the determination of Sofosbuvir in pharmaceutical dosage forms". *Journal of Comprehensive Pharmacy* 3.4 (2016): 136-142.

12. Mathrusri Annapurna M., *et al*. "New stability indicating ultrafast liquid chromatographic method for the determination of Sofosbuvir in tablets". *Asian Journal of Pharm*aceutics 12.1 (2018): S151-S158.

13. Jayesh A Patil., *et al*. "Development and validation of RP-HPLC Method for determination of Sofosbuvir by pure and tablet dosage form". *World Journal of Pharmacy Research* 6.5 (2017): 891-905.

14. Sherif Abdel-Naby Abdel-Gawad. "Simple chromatographic and spectrophotometric determination of Sofosbuvir in pure and tablet forms". *European Journal of Chem*istry 7.3 (2016): 375-379.

15. ICH validation of analytical procedures: text and methodology Q2 (R1), International Conference on Harmonization (2005).

16. ICH stability testing of new drug substances and products Q1A (R2), International Conference on Harmonization (2003).

Volume 2 Issue 10 October 2018