



DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UV SPECTROSCOPIC METHOD FOR ESTIMATION OF SOFOSBUVIR

Santosh V. Gandhi^{1*} and Pooja R. Alli¹

AISSMS College of Pharmacy (Affiliated to Savitribai Phule Pune University), Kennedy Road, Near R. T. O., Pune 411001, Maharashtra, India.

*Corresponding Author Email: santoshvgandhi@rediffmail.com

ABSTRACT

A stability-indicating UV Spectrophotometric method has been developed for analysis of the drug in the presence of the degradation products and is validated as per ICH Q2 R1 guidelines. Sofosbuvir in water shows maximum absorbance at 260.5 nm. The data of linear regression analysis indicated a good linear relationship over the range of 10-100 µg/ml concentrations with a correlation coefficient (R^2) of 0.9984. The LOD and LOQ were found to be 0.269 µg/ml and 0.814 µg/ml respectively. A recovery of Sofosbuvir in tablet formulation was observed in the range of 99.524-101.208 %. Percentage assay of Sofosbuvir tablets was found to be in the range of 99.812-101.740 %. Sofosbuvir was subjected to different stress testing conditions. Degradation of Sofosbuvir was mainly found in alkaline condition. The developed method was found to be simple, accurate and precise for analysis of Sofosbuvir and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

KEY WORDS

Method development, Sofosbuvir, Stability indicating method, Ultraviolet spectroscopy, Validation.

INTRODUCTION:

Sofosbuvir is a direct acting pyrimidine nucleotide analog representing the first NS5B HCV polymerase inhibitor. The drug is approved by the US FDA and the European Medicines Agency and has become commercially available for the treatment of Hepatitis C in the US in late 2013 and in several European countries in early 2014. Sofosbuvir is used in the treatment of chronic HCV genotype 1, 2, 3, or 4 infections in adults, including those with hepato cellular carcinoma awaiting liver transplantation and those with HIV co-infection. Limited data is available for treatment of chronic HCV infection caused by genotype 5 or 6. Chemically it is (S)-Isopropyl 2-(((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetra hydrofuran-2-yl) methoxy) -(phenoxy) phosphoryl amino) propanoate.^[1] (www.drugbank.ca/drugs/DB08934).

Literature survey reveals that several analytical methods have been reported for the estimation of Sofosbuvir in pharmaceutical dosage form including spectroscopic methods^[2-4] and high performance liquid chromatography (HPLC) in single as well as in combination with other drugs,^[5-7] high performance thin layer chromatography,^[8] ultra-high performance liquid chromatography^[9-10] and in biological fluids by RP-HPLC.^[11] Although few reports are available on stability indicating HPLC methods in single as well as in combination with other drugs,^[12-16] stability indicating HPTLC methods^[17] but no method is available on stability indicating UV spectroscopy hence we have tried to develop stability indicating UV spectroscopic method for estimation of Sofosbuvir in bulk and pharmaceutical dosage form. The present work describes a simple stability indicating UV spectroscopic method for the determination of Sofosbuvir in bulk and pharmaceutical dosage form (MyHepTM-400mg) according to the

International conference on harmonization (ICH) guidelines.

MATERIALS AND METHODS:

Reagents and chemicals:

The formulation MyHep™ tablets labeled to contain Sofosbuvir 400 mg was procured from local market. AR grade water was collected at college using ELGA water purification system. Hydrochloric acid (HCl), hydrogen peroxide (H₂O₂), and sodium hydroxide (NaOH); all AR grade were purchased from LobaChemie Pvt. Ltd., Mumbai.

Preparation of standard stock solution:

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 10 ml of water to get

concentration of 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with water to get concentration of solution 100 µg/ml. Further 2 ml of this solution was diluted to 10 ml with water to get concentration of solution 20 µg/ml.

Selection of detection wavelength:

From the standard stock solution (1000 µg/ml) further dilutions were made using water and scanned over the range of 200-400 nm and the spectra were obtained. It was observed that the drug showed linear, stable and considerable absorbance at 260.5 nm. Representative UV spectrum of Sofosbuvir is shown in Fig. 1.

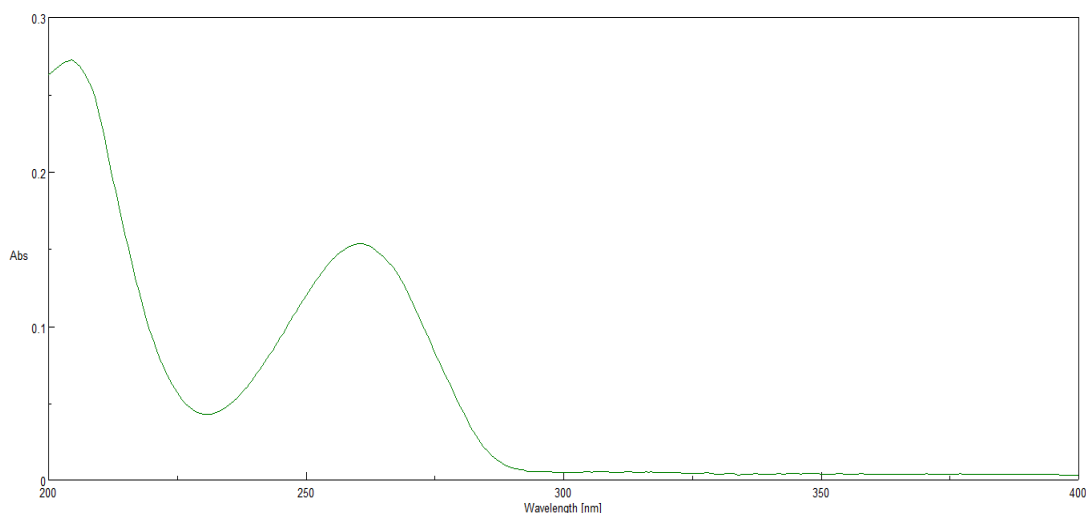


Fig. 1: The UV spectrum of Sofosbuvir (10 µg/ml).

Table 1: Intra-day variation studies data for Sofosbuvir.

Replicates	Conc. (µg/ml)		
	20	30	40
1	0.300	0.454	0.600
2	0.301	0.451	0.600
3	0.302	0.457	0.610
Mean	0.301	0.454	0.603
SD	0.001	0.003	0.006
%RSD	0.419	0.692	0.935

Preparation of sample solution:

20 tablets each containing 400 mg of Sofosbuvir (MyHep™- 400 mg) was weighed and powdered. A quantity of powder equivalent to 10 mg of Sofosbuvir was transferred to a 10 ml volumetric flask containing 5 ml of water. The mixture was ultra-sonicated for 10 min and the resulting sample stock solution was filtered with Whatman filter paper- 41 and the volume was made up

with the water to get concentration of 1000 µg/ml. From this solution 1 ml was diluted to 10 ml with water to get concentration of solution 100 µg/ml. Further dilution in water was done to get concentration 20 µg/ml.

STRESS DEGRADATION STUDIES OF BULK DRUG: ^[18]

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like acidic, alkaline, neutral hydrolysis, oxidation, dry heat and photolytic degradation. Dry heat and photolytic degradation were carried out in the solid state. All studies were carried out at concentration level of 100 µg/ml.

Alkaline hydrolysis:

To 1 ml stock solution of Sofosbuvir (1000 µg/ml), 1 ml of 0.1 N NaOH was added. The above solution was kept for 26 hours at room temperature. After exposure the volume was made up to 10 ml with water to get the concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under alkaline hydrolysis, percent recovery obtained for Sofosbuvir was 70.17%. The representative UV spectrum is shown in Fig. 2.

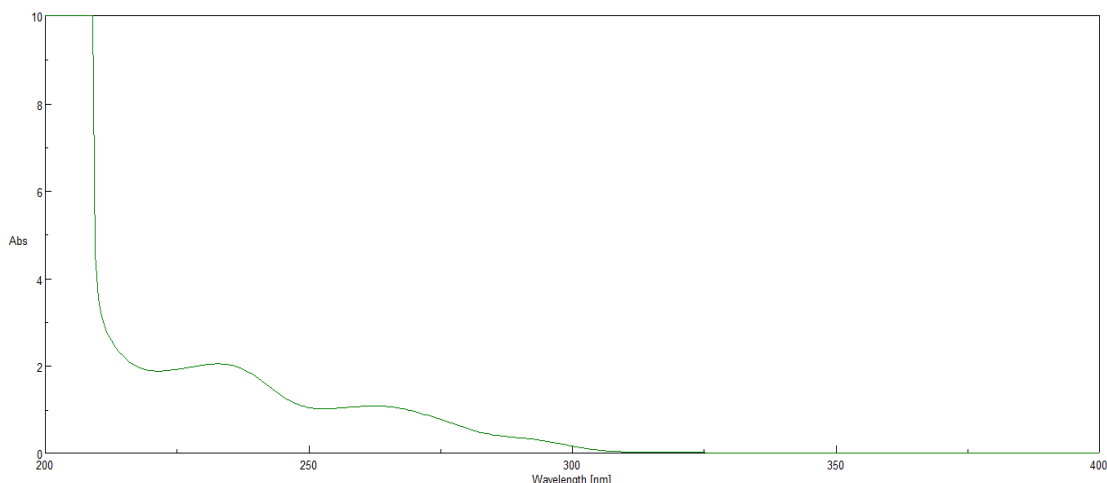


Fig. 2: UV spectrum of Sofosbuvir after alkaline degradation.

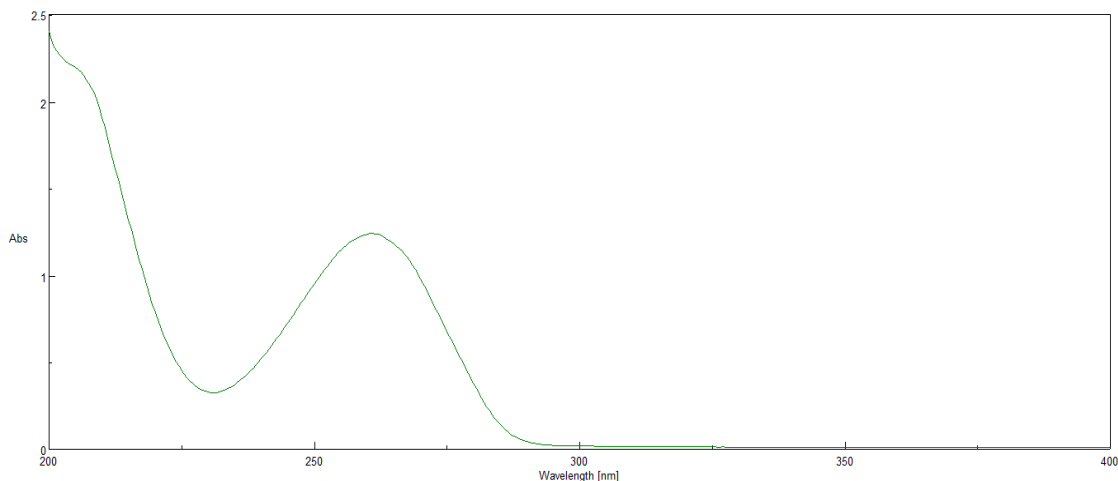


Fig. 3: UV spectrum of Sofosbuvir after acidic degradation.

Acid hydrolysis:

To 1 ml stock solution of Sofosbuvir (1000 µg/ml), 1 ml of 0.1 N HCl was added. The above solution was kept for 26 hours at room temperature. After exposure the volume was made up to 10 ml with water to get the

concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under acid hydrolysis, percent recovery obtained for Sofosbuvir was 81.13%. The representative UV spectrum is shown in Fig. 3.

Table 2: Inter-day variation studies data for Sofosbuvir.

Replicates	Conc. ($\mu\text{g/ml}$)		
	20	30	40
1	0.300	0.455	0.603
2	0.300	0.456	0.600
3	0.303	0.458	0.608
Mean	0.301	0.456	0.603
SD	0.002	0.001	0.004
%RSD	0.617	0.249	0.644

Table 3: Assay of marketed formulation.

Sr. No.	Sofosbuvir		
	Absorbance	Amount Recovered ($\mu\text{g/ml}$)	% Recovery
1	0.303	20.267	101.334
2	0.301	20.161	100.807
3	0.304	20.348	101.740
4	0.301	20.145	100.727
5	0.301	20.163	100.816
6	0.298	19.962	99.812
Mean	0.301	20.175	100.873
SD	0.002	0.130	0.651
%RSD	0.665	0.645	0.645

Table 4: Accuracy of Sofosbuvir.

Level	Conc. of Sample solution ($\mu\text{g/ml}$)	Conc. of Standard solution spiked ($\mu\text{g/ml}$)	Absorbance	Amount recovered ($\mu\text{g/ml}$)	% recovery
50%	20	10	0.451	29.857	100.212
			0.457	30.242	
			0.454	30.092	
			0.606	39.923	
100%	20	20	0.610	40.198	100.352
			0.611	40.302	
			0.758	49.844	
			0.770	50.604	
150%	20	30	0.765	50.249	100.465

Neutral hydrolysis:

To 1 ml stock solution of Sofosbuvir (1000 $\mu\text{g/ml}$), 1 ml of water was added. The above solution was kept for 72 hours at room temperature. After exposure the volume was made up to 10 ml with ELGA water to get the concentration of solution 100 $\mu\text{g/ml}$. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under neutral hydrolysis, percent recovery obtained for Sofosbuvir was 76.97%. The representative UV spectrum is shown in Fig. 4.

Degradation under oxidative condition:

To 1 ml stock solution of Sofosbuvir (1000 $\mu\text{g/ml}$), 1 ml of 30% H_2O_2 was added. The above solution was kept for 26 hours at room temperature. After exposure the volume was made up to 10 ml with water to get the concentration of solution 100 $\mu\text{g/ml}$. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under oxidative degradation, percent recovery obtained for Sofosbuvir was 82.79%. The representative UV spectrum is shown in Fig. 5.

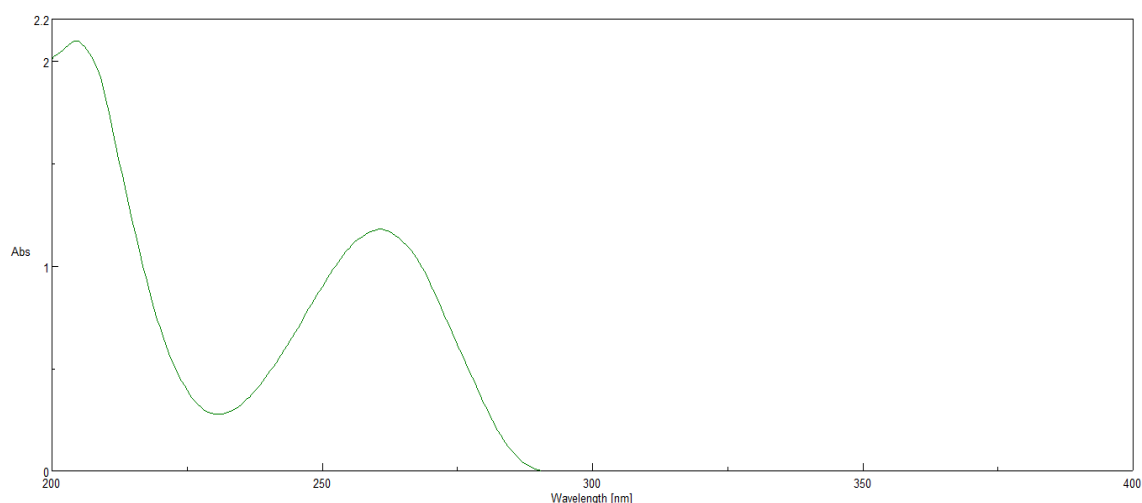


Fig. 4: UV spectrum of Sofosbuvir after neutral degradation.

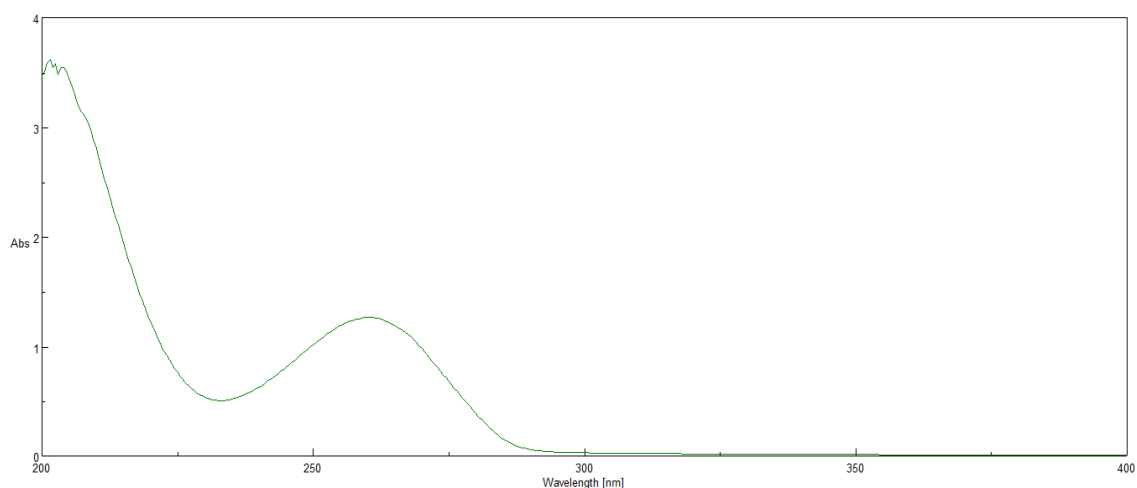


Fig. 5: UV spectrum of Sofosbuvir after oxidative degradation.

Table 5: Robustness study.

% RSD Found For Robustness Study (Absorbance)		
DETECTION WAVELENGTH (± 1 nm)		
259.5	260.5	261.5
1.394	0.501	1.363

Degradation under dry heat:

Dry heat studies were performed by keeping drug sample in oven (80°C) for a period of 72 hours. A sample was withdrawn and transferred to 10 ml volumetric flask then dissolved in water to get concentration of solution 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with

water to get concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under dry heat degradation condition, percent recovery obtained for Sofosbuvir was 88.65%. The representative UV spectrum is shown in Fig. 6.

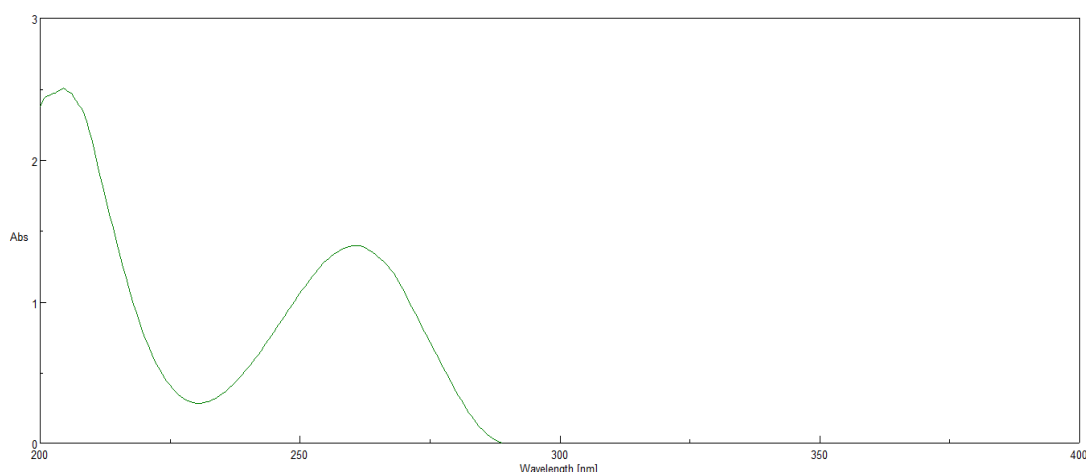


Fig. 6: UV spectrum of Sofosbuvir after dry heat degradation.

Table 6: Summary of Validation Parameters.

Sr. No.	Validation parameters	Sofosbuvir
1.	Detection wavelength (nm)	260.5
	Linearity equation	$y = 0.0154x - 0.0092$
2.	R^2	$R^2 = 0.9984$
	Range	10-100 $\mu\text{g/ml}$
	Precision (%RSD)	
3.	Intra-day	0.669
	Inter-day	0.492
4.	Assay	$100.873\% \pm 0.645$
5.	Accuracy	Mean $\pm$ %RSD
	50	$100.212\% \pm 0.645$
	100	$100.352\% \pm 0.487$
	150	$100.465\% \pm 0.757$
6.	Limit of detection	0.269 $\mu\text{g/ml}$
7.	Limit of quantitation	0.814 $\mu\text{g/ml}$
8.	Robustness	Robust

Photo-degradation studies:

1. UV illumination for solid drug sample:

The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200-watt hr/m^2 . After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with water to obtain concentration of solution 1000 $\mu\text{g/ml}$. From this

solution 1 ml was taken in 10 ml volumetric flask and volume was made up with water to get concentration of solution 100 $\mu\text{g/ml}$. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under photo degradation study by UV light, percent recovery obtained for Sofosbuvir drug solid sample was 82.46%. The representative UV spectrum is shown in Fig. 7.

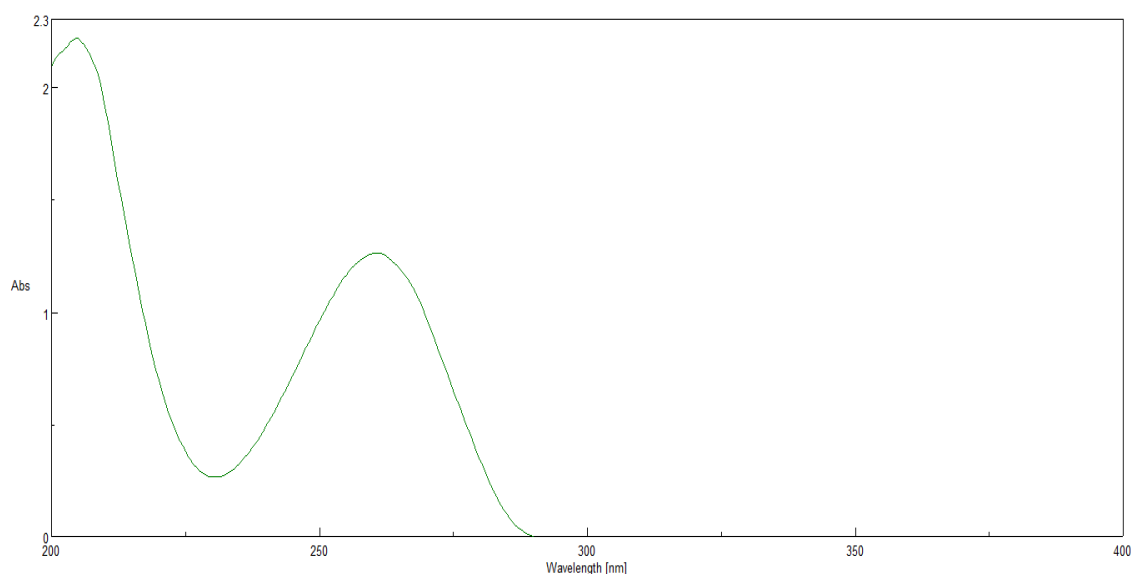


Fig. 7: UV spectrum of Sofosbuvir after photolytic UV degradation of solid drug sample.

2. UV illumination for drug solution:

From stock solution (1000 $\mu\text{g/ml}$) of Sofosbuvir 1 ml was taken in 10 ml volumetric flask and volume was made up with water to obtain concentration of solution 100 $\mu\text{g/ml}$. And expose this drug solution to UV light providing illumination of NLT 200-watt hr/m^2 . After

exposure 2 ml of resulting solution was taken in cuvette and absorbance was recorded. Under photo degradation study by UV light, percent recovery obtained for Sofosbuvir drug solution was 76.06%. The representative UV spectrum is shown in Fig. 8.

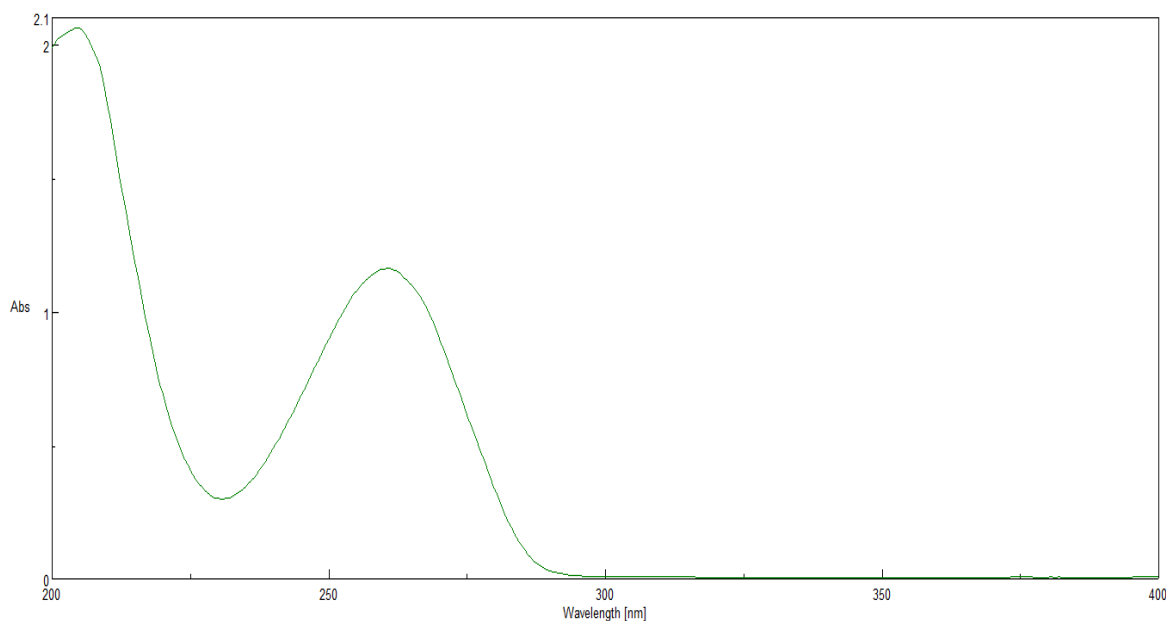


Fig. 8: UV spectrum of Sofosbuvir after photolytic UV degradation of drug solution.

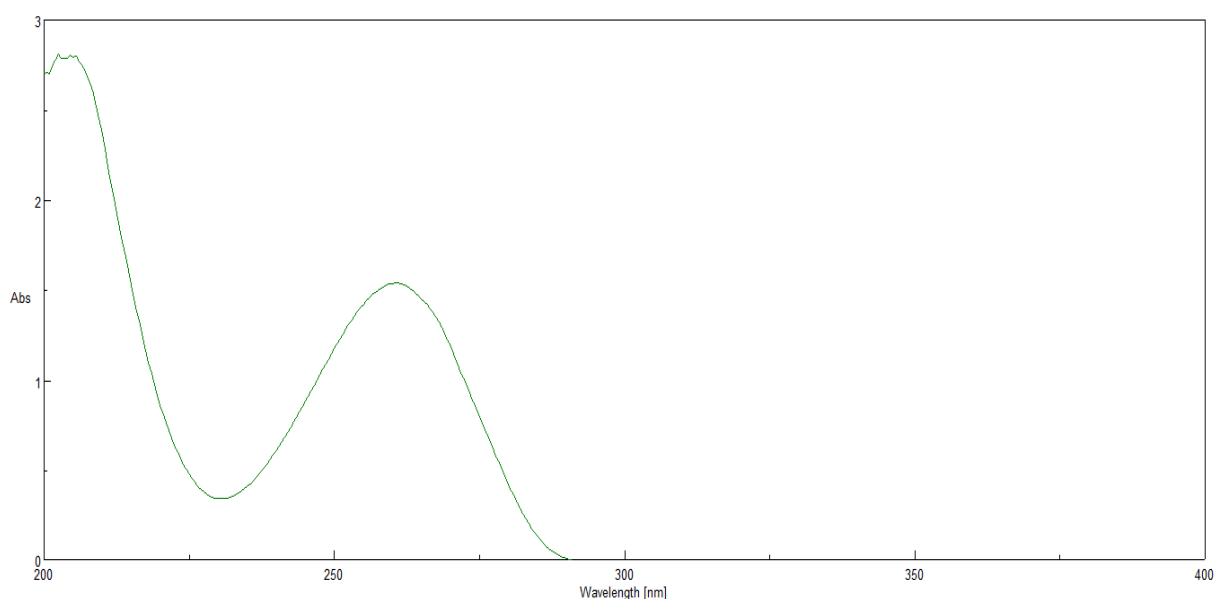


Fig. 9: UV spectrum of Sofosbuvir after photolytic fluorescent light degradation.

3. Fluorescent light:

The photo degradation study of the drug was studied by exposing the drug to fluorescent light providing illumination of NLT 1.2×10^6 Lux hr of fluorescent light. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with water to obtain concentration of solution 1000 $\mu\text{g/ml}$. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with water to get concentration of solution 100 $\mu\text{g/ml}$. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under photo degradation study by fluorescent light, percent recovery obtained for Sofosbuvir was 87.47%. The representative UV spectrum is shown in Fig. 9.

VALIDATION OF ANALYTICAL METHOD: [19]

Linearity:

From the standard stock solution (1000 $\mu\text{g/ml}$) of Sofosbuvir, solution was prepared containing 100 $\mu\text{g/ml}$ of Sofosbuvir in water. This solution was further diluted with water to get range of solution containing different concentrations 10-100 $\mu\text{g/ml}$. Absorbance was taken at λ_{max} 260.5 nm. The linearity (relationship between absorbance and concentration) was determined by analyzing ten solutions over the concentration range of 10-100 $\mu\text{g/ml}$. The equation of calibration curve was found to be $y = 0.0154x - 0.0092$. The absorbance of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 10.

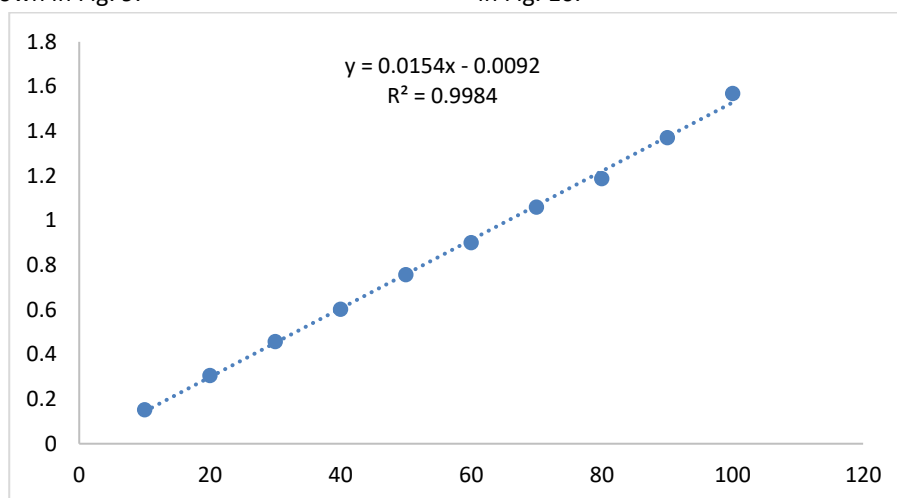


Fig. 10: Linearity curve of Sofosbuvir (10-100 $\mu\text{g/ml}$).

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter-day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intra-day and inter-day variations are shown in Table 1 and Table 2.

Limit of detection (LOD) and limit of quantitation (LOQ):

From the linearity data the LOD and LOQ was calculated, using the formula $LOD = 3.3 \sigma/S$ and $LOQ = 10 \sigma/S$, where σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. The LOD and LOQ was found to be 0.269 $\mu\text{g/ml}$ and 0.814 $\mu\text{g/ml}$, respectively.

Assay:

MyHep™- 400 mg tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Sample solution was taken in cuvette and absorbance was recorded. Basic concentration of sample chosen was 20 $\mu\text{g/ml}$ from tablet solution. Procedure was repeated for six times. Concentration and % recovery was determined from linear equation. The results obtained are shown in Table 3.

Accuracy:

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the MyHep™- 400 mg tablet sample solution, at three different levels around 50, 100 and 150%. Basic concentration of sample solution chosen was 20 $\mu\text{g/ml}$ of Sofosbuvir. % recovery was determined from linearity equation. The results obtained are shown in Table 4.

Robustness:

Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength ($\pm 1 \text{ nm}$) was altered and the effects on the absorbance was noted. The method was found to be robust. The results obtained are shown in Table 5.

RESULTS AND DISCUSSION:

The developed method was found to be simple, sensitive, specific, accurate and repeatable for analysis of Sofosbuvir in bulk and pharmaceutical dosage form without any interference from the excipients. The

results indicated the suitability of the method to study stability of Sofosbuvir under various forced degradation conditions.

CONCLUSION:

A simple, precise, accurate, reproducible and stability indicating UV spectroscopic method without interference from the excipients or from degradation products has been developed and validated for the determination of Sofosbuvir as bulk drug and in tablet dosage form. Drug found to be degraded under alkaline condition evident from change in UV spectrum pattern of drug. The developed method can be used for quantitative analysis of Sofosbuvir in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

ACKNOWLEDGEMENT:

Authors are thankful to the Principal and Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

REFERENCES:

1. Keating G. M., Vaidya A., Sofosbuvir: first global approval. *Drugs*, 74(2): 273-282, (February 2014)
2. Abdel S., Abdel-Gawad N., Simple chromatographic and spectrophotometric determination of sofosbuvir in pure and tablet forms. *European Journal of Chemistry*, 7(3): 375-379, (2016)
3. Baker M. M., El-Kafrawy D. S., Mahrous M. S., Belal T. S., Validated spectrophotometric and chromatographic methods for analysis of the recently approved hepatitis C antiviral combination ledipasvir and sofosbuvir. *Annales Pharmaceutiques Francaises*, 76: 16-31, (2018)
4. Mansuri R., Patel D., Patel K., Meshram D., Development and validation of three novel UV spectrophotometric methods for determination of newly discovered combination for the treatment of hepatitis C and their comparison using ANOVA. *International Journal of Pharmaceutics & Drug Analysis*, 6(3): 391-399, (2018)
5. Swathi P., Dutt K. R., Rao K. N. V., Alagar Raja M., RP-HPLC method development and validation for estimation of sofosbuvir in pure and tablet dosage form. *Asian J. Pharm. Tech.*, 7(3): 153-156, (2017)
6. Nagaraju T., Vardhan S. V. M., Ravi Kumar D., Ramachandran D., A new RP-HPLC method for the simultaneous assay of sofosbuvir and ledipasvir in combined dosage form. *International Journal of ChemTech Research*, 10(7): 761-768, (2017)

7. Uppalapati J., Dr. Parimi U., Analytical method development and validation for the simultaneous estimation of sofosbuvir and velpatasvir drug product by RP-HPLC method. *Indo American Journal of Pharmaceutical Research*, 7(8): 401-409, (2017)
8. Salama F. M., Khalid A. A., Ahmed A. A., Ahmed E., Ebrahim A., Application of TLC densitometric method for simultaneous estimation of the newly co-formulated antiviral agents ledipasvir and sofosbuvir in their tablet dosage form. *Analytical Chemistry Letters*, 7(2): 241-247, (2017)
9. Pottabathini V., Gugulothu V., Kaliyaperumal M., Battu S., Identification, isolation and structure confirmation of forced degradation products of sofosbuvir. *American Journal of Analytical Chemistry*, 7: 797-815, (2016)
10. Shaik J. S., Muniappan M., Manikanta K. A., Muralidaran K., Ramulu Y., Rao S. V., Estimation of sofosbuvir with validated ultra-high-performance liquid chromatographic (UHPLC) method in its bulk and formulations. *Der Pharmacia Sinica*, 8(2): 10-15, (2017)
11. Madhavi S., 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): 35-41, (2017)
12. Nebesen M., Elzanfaly E. S., Stability-indicating method and LC-MS-MS characterization of forced degradation products of sofosbuvir. *Journal of Chromatographic Science*, 54(9): 1631-1640, (2016)
13. RoopaRani S., Dr. Shobha rani S., Stability indicating method development and validation for simultaneous determination of sofosbuvir and simeprevir by RP-HPLC in bulk form. *Journal of PharmaResearch*, 6(2): 70-76, (2017)
14. Bhavsar K. S., Khandhar A., Dr. Patel P. U., Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of sofosbuvir and daclatasvir dihydrochloride in solid dosage form. *World Journal of Pharmacy and Pharmaceutical Sciences*, 7(3): 1246-1271, (2018)
15. Reddy M. M. M., Sankar D. G., Rao J. V. L. N. S., A novel method development and validation of RP-HPLC method and stress degradation study of determination of sofosbuvir and velpatasvir in bulk and combined tablet dosage form. *European Journal of Biomedical and Pharmaceutical sciences*, 5(1): 490-501, (2018)
16. Naazneen S., Sridevi A., Development of assay method and forced degradation study of ledipasvir and sofosbuvir by RP-HPLC in tablet formulation. *Indo American Journal of Pharmaceutical Research*, 7(9): 480-489, (2017)
17. Gadade V. G., Darkunde S. L., Nanda R. K., Bhujbal S. S., Bhole R. P., Stability indicating normal phase HPTLC method for estimation of sofosbuvir in bulk drug and pharmaceutical dosage form. *International Journal of Current Medical and Pharmaceutical Research*, 3(10): 2528-2532, (October 2017)
18. ICH guidelines, for stability testing of new drug substances and products Q1A(R2), 2004.
19. ICH guidelines for validation of analytical procedures: text and methodology Q2(R1) 2005.

Received:04.05.18, Accepted: 07.06.18, Published:01.07.2018

***Corresponding Author:**

Santosh V. Gandhi*

Email: santoshvgandhi@rediffmail.com