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Method Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Sofosbuvir and Velpatasvir in Tablet Dosage Form

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Abstract

A simple, accurate and precise stability indicating RP-HPLC method was developed for the simultaneous estimation of the Sofosbuvir and Velpatasvir in tablet dosage form. Chromatogram was run through Discovery C18 (250 x 4.6 mm, 5µm) column. Mobile phase containing buffer 0.1% OPA: acetonitrile taken in the ratio 50:50 v/v was pumped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 240 nm. The method was linear over the concentration range for Sofosbuvir is 100-600µg/ml and for Velpatasvir is 25-150µg/ml. The retention times of Sofosbuvir and Velpatasvir were found to 2.473 min and 3.316 min respectively. %RSD of the Sofosbuvir and Velpatasvir were found to be 0.2 and 0.3 for system precision, 0.4 and 0.5 for repeatability and 0.2 and 0.3 for intermediate precision respectively. %Recovery was obtained as 99.32% and 100.43% for Sofosbuvir and Velpatasvir respectively. LOD and LOQ values obtained from regression equations of Sofosbuvir and Velpatasvir were 0.44, 1.32 and 0.33, 1.01 respectively. Regression equation of Sofosbuvir is y=10179x+3201 and y=16944x+13228 for Velpatasvir respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Sofosbuvir and Velpatasvir in combined tablet dosage form.

Keywords: Sofosbuvir; Velpatasvir; RP-HPLC; Validation

Abbreviations: RP-HPLC: Reverse Phase High Performance Liquid Chromatography; USFDA: US Food and Drug Administration; EMA: European Medicine Agencies; ICH: International Conference on Harmonisation; LOD: Limit of Detection; LOQ: Limit of Quantitation; HCV: Hepatitis C Virus.

Introduction

Sofosbuvir (Figure 1) is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV) [1]. Chemically it is Propan-2-(2S)-2-{[(S)-{[(2R,3R,4R,5R)-5-(2,4-dioxo-

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1,2,3,4-tetrahydropyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy} (phenoxy)phosphoryl]amino}propanoate. Sofosbuvir is nucleotide analog inhibitor, which specifically inhibits HCV NS5B (non-structural protein 5B) RNA-dependent RNA polymerase. As a prodrug nucleotide analog, Sofosbuvir is metabolized into its active form as the antiviral agent 2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate, which acts as a defective substrate for NS5B. NS5B, an RNA-dependent RNA polymerase, is essential for the transcription of Hepatitis C viral RNA and for its high replicative rate and genetic diversity [2].

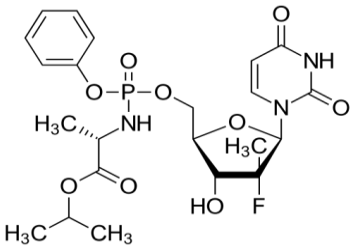

Figure 1: Structure of Sofosbuvir.

Velpatasvir (Figure 2) is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus [3]. Chemically it is (2S)-2-{[hydroxy (methoxy) methylidene] amino}-1-[(2S, 5S)-2-(17-{2-[(2S, 4S)-1-[(2R)-2-{[hydroxy (methoxy) methylidene] amino}-2-phenylacetyl]-4-(methoxy methyl) pyrrolidin-2-yl]-1*H*- imidazol-5-yl}-21-oxa-5, 7-diazapentacyclo [11.8.0.0^ {3, 11}.0^ {4, 8}.0^ {14, 19}] henicosa- 1(13),2,4(8),6,9,11,14(19),15,17-nonaen-6-yl)-5-methylpyrrolidin-1-yl]-3-methylbutan-1-one.

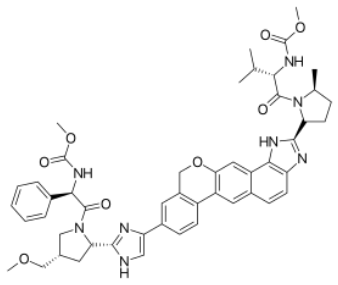

Figure 2: Structure of Velpatasvir.

The mechanism of Velpatasvir is likely similar to other selective NS5A inhibitors which bind domain I of NS5A consisting of amino acids 33-202. NS5A inhibitors compete with RNA for binding at this site. The exact role of NS5A in RNA replication is not yet understood [4]. The fixed-dose combination of Sofosbuvir and Velpatasvir has been approved by the USFDA and EMA for the treatment of patients with chronic hepatitis C virus genotype 1, 2, 3, 4, 5 or 6 infections [5]. Literature survey revealed that few HPLC methods [6-11] were reported for the simultaneous determination of Sofosbuvir and Velpatasvir in pharmaceutical dosage form. The main objective of the present work is to develop an accurate, precise and rapid stability indicating RP-HPLC method for simultaneous estimation of Sofosbuvir and Velpatasvir in combined pharmaceutical dosage form as per ICH guidelines [12,13].

Materials and Methods

Materials

Sofosbuvir and Velpatasvir pure drugs (API) are procured from Spectrum Pharma Research Solutions, Hyderabad, Combination of Sofosbuvir and Velpatasvir tablets were procured from local market. Distilled water, Acetonitrile, Methanol, Potassium dihydrogen ortho phosphate, Ortho phosphoric acid is purchased from Rankem Chemicals Ltd., Mumbai, India.

Instrumentation

The analysis of drugs was carried out on a Waters HPLC 2695 system on a Discovery C18 (250 x 4.6 mm, 5µm). The instrument is equipped with a 2695 pump with inbuilt degasser, 2998 photodiode array detector and a Rheodyne injector with 20µL sample loop. A 20µL Hamilton syringe was used for injecting the samples. Data was analyzed by using Waters Empower 2 software. A double-beam Shimadzu UV-Visible 2450 spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using ultrasonic baths sonicate. A Denver balance was used for weighing the materials.

Mobile phase: A mobile phase consisting of mixture of 0.1% OPA and acetonitrile in the ratio of 50:50, v/v was prepared.

Diluent: Based up on the solubility of the drugs, diluent was selected, acetonitrile and water taken in the ratio of 50:50, v/v.

Preparation of standard stock solutions: Accurately weighed 200 mg of Sofosbuvir, 50 mg of Velpatasvir and transferred to 50 ml volumetric flasks and 3/4th of diluents was added to these flask and sonicate for 10 minutes. Flask were made up with diluents and labeled as standard stock solution (4000 µg/ml of Sofosbuvir and 1000 µg/ml of Velpatasvir).

Preparation of standard working solution (100% solution): 1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluents (400 µg/ml of Sofosbuvir and 100 µg/ml of Velpatasvir).

Preparation of sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50 ml of diluent was added and sonicate for 25 min, further the volume was made up with diluent and filtered by HPLC filters (4000µg/ml of Sofosbuvir and 1000µg/ml of Velpatasvir).

Preparation of sample working solutions (100% solution): 1 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent (400µg/ml of Sofosbuvir and 100µg/ml of Velpatasvir).

Method development and validation

Determination of λ_{max} and optimized wavelength: Overlay spectra of Sofosbuvir and Velpatasvir gave the 240 nm as the optimized wavelength for these two drugs.

Method development: Various trails were performed by using different mobile phases and based on peak parameters the chromatographic conditions were optimized. The optimize chromatogram of Sofosbuvir and Velpatasvir was shown in Figure 3 and results are furnished in Table 1.

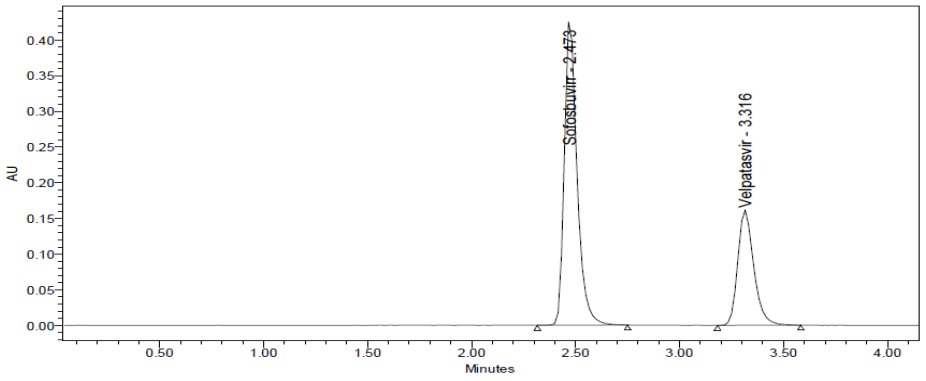

Figure 3: Optimized Chromatogram of Sofosbuvir and Velpatasvir.

Mobile phase	OPA (0.1%): Acetonitrile 50:50, (v/v)
Flow rate	1 ml/min
Column	Discovery C18 (250 x 4.6 mm, 5µm)
Detector wave length	240 nm
Column temperature	30°C
Injection volume	10µl
Run time	8 min
Diluent	Water:acetonitrile in the ratio 50:50(v/v)
Retention time	Sofosbuvir: 2.473 min Velpatasvir: 3.316 min

Table 1: Optimized chromatographic conditions.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Sofosbuvir (400µg/ml) and Velpatasvir (100µg/ml) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The results are shown in Table 2.

S. No.	Parameters	Sofosbuvir	Velpatasvir
1	Linearity (µg/ml)	100-600	25-150
2	Correlation coefficient	0.999	0.999
3	Retention time (min.)	2.473	3.316
4	Resolution	-	6.1
5	Tailing factor	1.37	1.34
6	Theoretical plates (N)	6915	8432
7	LOD (µg/ml)	0.44	0.33
8	LOQ (µg/ml)	1.32	1.01

Table 2: System suitability parameters for Sofosbuvir and Velpatasvir.

Specificity: Specificity is the parameter used to check the interference in the optimized method.

Linearity: Accurately weighed 200 mg of Sofosbuvir, 50 mg of Velpatasvir and transferred to 10 ml volumetric flask. Add 3/4th of diluent to these flasks and sonicate for

10 minutes. Flasks were made up with diluent and labeled as standard stock solution.

a. 25% Standard solution: 0.25 ml each from two standard stock solutions was pipetted out and made up to 10 ml (100 µg/ml of Sofosbuvir and 25 µg/ml of Velpatasvir).
b. 50% Standard solution: 0.5 ml each from two standard stock solutions was pipetted out and made up to 10 ml (200 µg/ml of Sofosbuvir and 50 µg/ml of Velpatasvir).
c. 75% Standard solution: 0.75 ml each from two standard stock solutions was pipetted out and made up to 10 ml (300 µg/ml of Sofosbuvir and 75 µg/ml of Velpatasvir).
d. 100% Standard solution: 1.0 ml each from two standard stock solutions was pipetted out and made up to 10 ml (400 µg/ml of Sofosbuvir and 100 µg/ml of Velpatasvir).
e. 125% Standard solution: 1.25 ml each from two standard stock solutions was pipetted out and made up to 10 ml (500 µg/ml of Sofosbuvir and 125 µg/ml of Velpatasvir).
f. 150% Standard solution: 1.5 ml each from two standard stock solutions was pipetted out and made up to 10 ml (600 µg/ml of Sofosbuvir and 150 µg/ml of Velpatasvir).

The results were furnished in Table 3 and calibration curves were shown in Figure 4 & Figure 5.

Sofosbuvir		Velpatasvir	
Conc (µg/ml)	**Peak area**	**Conc (µg/ml)**	**Peak area**
0	0	0	0
100	978961	25	439545
200	2029601	50	873317
300	3072642	75	1278714
400	4205215	100	1728204
500	5050574	125	2120573
600	6060361	150	2548041
Slope	10179	Slope	16944
Intercept	3201	Intercept	13228
R^2	0.999	R^2	0.999

Table 3: Linearity results for Sofosbuvir and Velpatasvir.

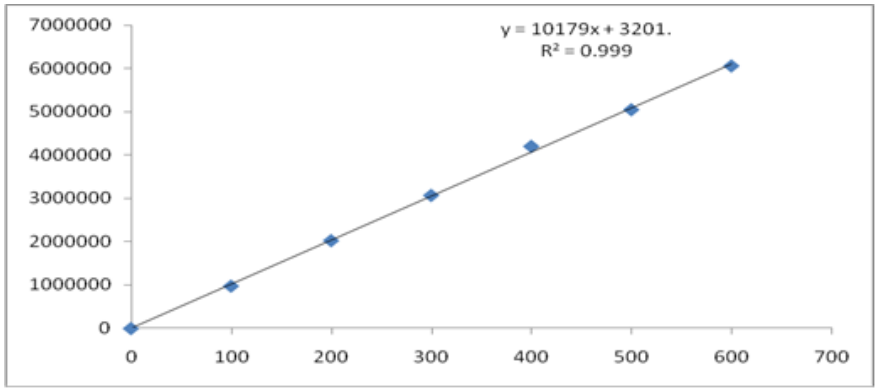

Figure 4: Calibration curve of Sofosbuvir.

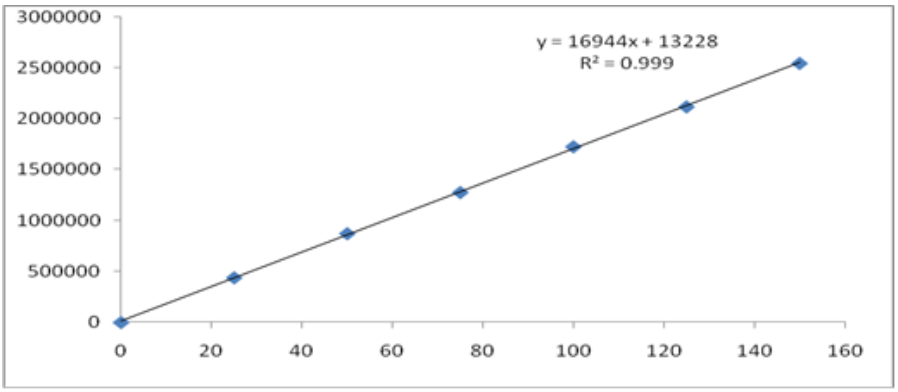

Figure 5: Calibration curve of Velpatasvir.

Precision: Precision of method was studied by performing system precision, repeatability and intermediate precision by injecting the 6 replicates of standard solution. The results are shown in Table 4, Table 5 and Table 6. Calculate the %RSD and it should not be more than 2.0.

S. No.	Area of Sofosbuvir	Area of Velpatasvir
1.	4216525	1718783
2.	4206222	1719066
3.	4198374	1727920
4.	4206891	1719017
5.	4209707	1720259
6.	4215261	1710021
Mean	4208830	1719178
SD	6651.8	5685.7
%RSD	0.2	0.3

Table 4: System precision results of Sofosbuvir and Velpatasvir.

S. No.	Area of Sofosbuvir	Area of Velpatasvir
1.	4136808	1676927
2.	4166672	1674769
3.	4166672	1659724
4.	4146929	1665289
5.	4136794	1681692
6.	4171736	1669206
Mean	4154269	1671268
SD	15980.6	8077.2
%RSD	0.4	0.5

Table 5: Repeatability results of Sofosbuvir and Velpatasvir.

S. No.	Area of Sofosbuvir	Area of Velpatasvir
1.	4225458	1724714
2.	4221386	1721664
3.	4238541	1729585
4.	4213158	1722424
5.	4210536	1736772
6.	4219334	1724328
Mean	4221402	1726581
SD	10005.1	5707.9
%RSD	0.2	0.3

Table 6: Intermediate precision results of Sofosbuvir and Velpatasvir.

Accuracy: The accuracy of the method was established by calculating percentage recovery of Sofosbuvir and Velpatasvir by the method of addition. Known amount of Sofosbuvir and Velpatasvir at 50%, 100%, and 150% was added to a pre quantified sample solution. The recovery studies (Table 7 & Table 8) were carried out in the tablet in triplicate each in the presence of placebo. The % Recovery for each level should be between 98 to 102%.

% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery
50%	200	198.264	99.13	99.32%
	200	199.1661	99.58	
	200	198.2094	99.10	
100%	400	396.1633	99.04	
	400	396.0603	99.02	
	400	396.4142	99.10	
150%	600	598.6467	99.77	
	600	595.4805	99.25	
	600	599.4535	99.91	

Table 7: Accuracy results of Sofosbuvir.

% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery
50%	50	49.532	99.06	100.43%
	50	49.748	99.50	
	50	50.181	100.36	
100%	100	100.572	100.57	
	100	100.525	100.53	
	100	100.155	100.16	
150%	150	151.872	101.25	
	150	150.643	100.43	
	150	150.339	100.23	

Table 8: Accuracy results of Velpatasvir.

a. Preparation of 50% Spiked Solution: 0.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out and made up to the mark with diluent.
b. Preparation of 100% Spiked Solution: 1.0 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out and made up to the mark with diluent.
c. Preparation of 150% Spiked Solution: 1.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

LOD sample preparation: 0.25 ml each from two standard stock solutions was pipetted out and transferred to two separate 10 ml volumetric flasks and made up with diluent. From the above solutions 0.1 ml each of Sofosbuvir and Velpatasvir solutions were transferred to 10 ml volumetric flasks and made up with the same diluent.

LOQ sample preparation: 0.25 ml each from two standard stock solutions was pipetted out and transferred to two separate 10 ml volumetric flasks and made up with diluent. From the above solutions 0.3 ml each of Sofosbuvir and Velpatasvir solutions were transferred to 10 ml volumetric flasks and made up with the same diluent.

Robustness: Small deliberate changes in method like flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH guidelines. Robustness conditions like flow minus (0.9 ml/min), flow plus (1.1 ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner.

Degradation studies

Acid degradation studies: To 1 ml of stock solutions of Sofosbuvir and Velpatasvir, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 400 µg/ml & 100 µg/ml solution and 10 µl solutions into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies: To 1 ml of stock solutions of Sofosbuvir and Velpatasvir, 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60^0C. The resultant solution was diluted to obtain 400µg/ml & 100 µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies: To 1 ml of stock solutions of Sofosbuvir and Velpatasvir, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For HPLC study, there resultant solution was diluted to obtain 400 µg/ml & 100 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry heat degradation studies: The standard drug solutions were placed in oven at 105°C for 1hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 400µg/ml & 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo stability studies: The photochemical stability of the drug was studied by exposing the standard drug solutions to UV Light by keeping the beaker in UV Chamber for 1 day or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 400µg/ml & 100µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hr at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 400µg/ml & 100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. The results of degradation studies were computed in Table 9 & Table 10.

S. No.	Degradation condition	% Drug degraded	Purity angle	Purity threshold
1	Acid	4.66	1.339	1.460
2	Alkali	4.04	1.493	1.548
3	Oxidation	3.77	1.493	1.548
4	Thermal	2.86	1.439	1.460
5	UV	2.83	1.146	1.810
6	Neutral	2.83	1.646	1.812

Table 9: Degradation data of Sofosbuvir.

S. No.	Degradation condition	%Drug degraded	Purity angle	Purity threshold
1	Acid	4.92	0.120	0.329
2	Alkali	4.24	0.121	0.321
3	Oxidation	3.18	0.121	0.321
4	Thermal	2.97	0.120	0.329
5	UV	2.68	0.122	0.317
6	Neutral	0.94	0.122	0.317

Table 10: Degradation data of Velpatasvir.

Results and Discussion

In the present work, a simple, accurate and precise stability indicating HPLC method has been optimized, developed and validated for the simultaneous estimation of Sofosbuvir and Velpatasvir in pharmaceutical formulations with UV detector by using Discovery C18 (250 x 4.6mm I.D., 5µm particle size) in isocratic mode with mobile phase composition of 0.1% OPA and acetonitrile in the ratio of 50:50, v/v. The use of OPA buffer and acetonitrile in the ratio of 50:50, v/v resulted in peak with good shape and resolution. The flow rate was 1.0 mL/min and the drug component was measured with UV detector at 240 nm. Specificity the chromatograms of Sofosbuvir and Velpatasvir should not find any interfering peaks in blank, placebo, standard and sample at retention times of these drugs in this method. So this method was said to be specific.

The method was linear in the range of 100-600 µg/ml for Sofosbuvir with correlation coefficient of 0.999 and 25-100 µg/ml for Velpatasvir with correlation coefficient of

0.999. The %RSD for system precision of Sofosbuvir and Velpatasvir were found to be 0.2 and 0.3, %RSD for repeatability of Sofosbuvir and Velpatasvir were found to be 0.4 and 0.5 and %RSD for intermediate precision of Sofosbuvir and Velpatasvir were found to be 0.2 and 0.3 respectively, which indicate the method is precise. The % recoveries of Sofosbuvir were found in the range of 99.02-99.91% and the % mean recovery was found to be 99.32% and Velpatasvir were found in the range of 99.06-101.25% and the % mean recovery was found to be 100.43% respectively, which indicate the method is accurate. The retention times of Sofosbuvir and Velpatasvir were found to 2.473 min and 3.316 min respectively, cuts down on overall time of sample analysis and the method was more cost effective as it utilizes very less quantity of mobile phase. The number of theoretical plates was 6915 and tailing factor was 1.37 for Sofosbuvir and number of theoretical plates was 8432 and tailing factor was 1.34 for Velpatasvir respectively, which indicates efficient performance of the column.

Selectivity of the method was demonstrated by the absence of any interfering peaks at the retention times of the two drugs. The limit of detection and limit of quantification for Sofosbuvir were found to be 0.44µg/ml and 1.32µg/ml and for Velpatasvir were found to be 0.33µg/ml and 1.01µg/ml respectively, which indicate the sensitivity of the method. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. Validated method was applied for the determination of Sofosbuvir and Velpatasvir in commercial formulations. The mean % assay was found to be 99.60% for Sofosbuvir and 99.47% for Velpatasvir respectively.

HPLC studies of Sofosbuvir and Velpatasvir under different stress conditions indicated the degradation behavior. The results revealed that both the drugs are stable in described conditions.

Conclusion

The present method was proposed for the simultaneous estimation of the Sofosbuvir and Velpatasvir by using RP-HPLC in tablet dosage form is found to be simple, accurate, rapid and precise. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be applied in regular quality control tests in industries.

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