DOI: 10.55522/jmpas.V11I4.4051 ISSN NO. 2320–7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Review article

Analytical method development and validation of related substances by rphplc of sofosbuvir and velpatasvir tablets

Jay Bhatt*, Dr. Ujashkumar A. Shah, Dr. Hirakkumar V. Joshi

Department of Quality Assurance and Pharmaceutical chemistry, Sakalchand Patel University, Visnagarn, Gujarat, India.

ABSTRACT

The developed method was a simple, accurate, precise, specific and robust method for the validation of Sofosbuvir and Velpatasvir Tablets by reverse phase high pressure liquid chromatography. For Sofosbuvir and Velpatasvir Tablets Chromatography was performed on Agilent 1200 series, UV and PDA Detector, Waters X-bridge C18 (150 mm x 4.6 mm, 3.5 µm) by using Mobile Phase A contains Buffer solution (0.6% Trifluroacetic acid in water adjusted pH to 2.2±0.05) : Acetonitrile (95:5)%v/v and Mobile Phase B contains mixture of purified water, methanol and acetonitrile in the ratio of (20: 30: 50) % v/v/v. at a flow rate of 1.0 mL/min and at 263 nm for Sofosbuvir and 320 nm for Velpatasvir wavelength. The retention times of About 48.0 minutes for Sofosbuvir and About 78.8 minutes for Velpatasvir respectively. Methyl Uridine and Impurity at RRT 0.39 found linear over the range of LOQ - 150 % of target concentration. Method also found precise by spiking impurities at specification level. Accuracy was demonstrate at LOQ - 150 % level by preparing sample in triplicate for each level and found accurate. Hence, the method could be successfully used for the analysis Impurities in Sofosbuvir and Velpatasvir Tablets Tablet.

Keywords: Sofosbuvir, Velpatasvir, HPLC, UV and PDA Detector, Dual Wavelength, Related Substances.

Received - 18-07-2022, Accepted- 23-08-2022

Correspondence: Jay Bhatt ✉ jay.bhatt259.jb@gmail.com, **Orcid Id**: https://orcid.org/0000-0001-6082-4114

Department of Quality Assurance and Pharmaceutical chemistry, Sakalchand Patel University, Visnagar, India.

INTRODUCTION

Antiviral drugs are a class of medication used specifically for treating viral infections rather than bacterial ones. Most antiviral are used for specific viral infections, while a broad-spectrum antiviral is effective against a wide range of viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development [1].

Chemical Name of Sofosbuvir is propan-2-yl (2S)-2-{[(S)-{[(2R,3R,4R,5R)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2yl]methoxy}(phenoxy) phosphoryl] amino}propanoate with nucleotide polymerase inhibitors category. Sofosbuvir has chemical formula $C_{22}H_{29}FN_3O_9P$. Molecular formula is 529.498 g/mol. Sofosbuvir 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). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients.

Velpatasvir acts as a defective substrate for NS5A (Non-structural Protein 5A), a non-enzymatic viral protein that plays a key role in Hepatitis C Virus replication, assembly, and modulation of host immune responses [4].

Chemical Name of Velpatasvir is Methyl {(2S)-1-[(2S,5S)-2-(9-{2[(2S,4S)-1-{(2R)-2-[(methoxycarbonyl)amino]-2-phenylacetyl}-4-(methoxymethyl)-2-pyrrolidinyl]-1H-imidazol-4-yl}1,11-dihydroisochromeno[4',3':6,7]naphtho[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl}carbamate with NS5A inhibitors category. Velpatasvir has chemical formula C49H54N8O8.Molecular formula is 883.019 g/mol. Velpatasvir is a Direct-Acting Antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). HCV is a single stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients [2-3].

MATERIALS AND METHODS

Materials

Sofosbuvir (400 mg) and Velpatasvir (100 mg) Tablets received as gift sample.

Instrumentation

The LC system consisted of an Agilent 1260, DAD,

Shimadzu prominence-I LC-2030C, VWD with 20 µL sample loop. The output signals were monitored and integrated using Chromeleon software.

Table: 1 Following reagents / chemicals are used in the validation study.

Reagents and Chemicals	Make	Analytical grade	% Purity
Sodium hydroxide pellets	ThermoFisher	ExcelaR	98.39
Trifluoroacetic acid	Spectrochem	AR	99.8
Acetonitrile	ThermoFisher	Gradient	99.99
Methanol	Rankem	Gradient	99.94
Tween 80	Merck	Synthesis	NA

Methods

Chromatographic conditions

The elution was Gradient and the mobile phase A contains Buffer solution (0.6% Trifluroacetic acid in water adjusted pH to 2.2±0.05): Acetonitrile (95:5) %v/v and Mobile Phase B contains mixture of purified water, methanol and acetonitrile in the ratio of (20: 30: 50) % v/v/v. The mobile phase was filtered through a 0.45 µm Nylon membrane filter prior to use. Bridge C18, (150 mm x 4.6 mm), 3.5 µm was used for determination. The flow rate was 1.0mL/min and the column was operated at 35°C temperature and Vial thermostat temperature at 5°C. The volume of sample injected was 10 µL. prior to injection of the solutions, column was equilibrated for at least 60min with mobile phase flowing through the system with initial gradient flow. The UV detector was set at wavelength of 263 nm for Sofosbuvir and 320 nm for Velpatasvir [4].

Table: 2 Gradient followed as below.

Time (Minutes)	0	5	15	23	45	60	85	95	110	125
% Mobile phase A	100	100	70	60	60	50	50	25	15	100
% Mobile phase B	0	0	30	40	40	50	50	25	85	0

Diluent

Prepare a mixture water, methanol and Tween 80 in the ratio of (49: 49: 2) % v/v/v

Standard Preparation

Sofosbuvir standard stock solution: Transfer an accurately weighed quantity of about 25 mg of Sofosbuvir standard into a 25 mL volumetric flask, add about 15 mL of diluent and sonicate to dissolve. Make volume up to mark with the diluent and mix.

Velpatasvir standard stock solution: Transfer an accurately weighed quantity of about 25.5 mg of Velpatasvir standard into a 50 mL volumetric flask, add about 5 mL of acetonitrile and sonicate for 5 minutes, add about 30 mL of diluent and sonicate to dissolve. Make volume up to mark with the diluent and mix. Dilute 5.0 mL of this solution to 50.0 mL with diluent and mix.

Standard preparation

(Concentration 20 µg/mL of Sofosbuvir and 5 µg/mL of Velpatasvir): Dilute 2.0 mL of Sofosbuvir standard stock solution and 10.0 mL of Velpatasvir standard stock solution to 100.0 mL with diluent and mix.

Sample preparation

(Concentration 2000 µg/mL of Sofosbuvir and 500 µg/mL of Velpatasvir): Weigh accurately 20 tablets and calculate the average weight. Crush the tablets in to fine powder. Transfer an accurately weighed quantity of the tablet powder equivalent to about 100 mg of Sofosbuvir and 25 mg of Velpatasvir in to a 50 mL volumetric flask. Add 5 mL of acetonitrile and sonicate for 5 minutes, then add about 30 mL of diluent and sonicate with shaking at every 10 minutes for time period of 30 second for 30 minutes with fully twisted arm left and right. Cool and make volume up to mark with diluent and mix. Centrifuge the solution at 5000 RPM for 10 minutes. Filter the solution through 0.45 µm Millipore PVDF filter. Collect the filtrate by discarding first 5 mL of the filtrate [5-6].

Method Validation

The developed method was validated as per ICH guidelines. Parameter Named System suitability, System Precision, Filter Compatibility and Saturation, LOD-LOQ, Method Precision, Stability of analytical solutions, Linearity, Accuracy and Robustness performed as per ICH Guideline Q2 (R1) and Q3B (R2) BY Covering all the concentration.

System suitability and System Precision

System suitability and System Precision are basic parameter of validation as they involves the suitability of the method as well as system on which method is going to be analyze. Several parameter includes %RSD, Theoretical plates, Tailing Factor, S/N ratio, capacity factor etc. are monitored based on the method requirements.

LOD-LOQ

Limit of detection consist of lowest amount of analyte in the sample which can be detected but not necessary to quantify it. Limit of Quantification consist of amount of analyte in the sample which can be detected and necessary to quantify it. It is used particularly for the determination of impurities and/or degradation products.

It is carried out by 3 methods.

- Based on Visual Evaluation
- Based on Signal-to-Noise
- Based on the Standard Deviation of the Blank

Precision

Precision also termed as the repeatability in which same operating conditions applies to multiple sample (i.e 6) over a short interval of time.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

It is also called as trueness.

It was carried out on minimum three different level in triplicate.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

It is most important parameter of the validation as it carried out identification and Purity test of the impurities as well as analyte.

Linearity

The linearity of an analytical method is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

This was carried out with minimum 5 concentration and measured by co-relation coefficient, slope and Y-intercept of the particular analyte and impurities.

Robustness

The robustness of the test method carried out by changing into small amount of deliberate variations to identify its reliability in the normal conditions.

- variations of pH in a mobile phase
- variations in mobile phase composition
- different columns (different lots and/or suppliers)
- variations in temperature
- Variations in flow rate.

RESULTS AND DISCUSSION

A reverse-phase column method was developed for the simultaneous estimation of impurities in Sofosbuvir and Velpatasvir dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, by changing in pH of Buffer, by changing in the flow, by changing in column temperature and auto sampler temperature. Different ratios of mobile phase were experimented to optimize the mobile phase. Finally, Buffer solution (0.6% Trifluroacetic acid in water adjusted pH to 2.2±0.05): Acetonitrile (95:5) %v/v and Mobile Phase B contains mixture of purified water, methanol and acetonitrile in the ratio of (20: 30: 50) % v/v/v. Selected as the mobile phase which showed good separation of impurities from each other as well as from analyte. The wavelength of detection selected was 263 nm for for Sofosbuvir and 320 nm for Velpatasvir due to different maxima of both the analyte. for better quantification of impurities dual wavelength was selected. Column temperature is selected 35°C and Vial thermostat temperature is 25°C. By our proposed method the retention time of Sofosbuvir and Velpatasvir were about 46 min. and 76 min. respectively [7-8].

Method Validation

System suitability System Precision

System suitability and precision were demonstrated by injecting sensitivity solution and six replicate injections of standard solution prepared as per the test method and chromatographed into HPLC system. The peak area of Sofosbuvir and Velpatasvir peak for replicate injections of standard solutions were recorded. The signal to noise ratio of Sofosbuvir and Velpatasvir peak were evaluated from sensitivity solution. The tailing factor and theoretical plates for the Sofosbuvir and Velpatasvir peak were evaluated from standard solution. The precision was evaluated by computing the relative standard deviation for the Sofosbuvir and Velpatasvir peak area in these replicate injections.

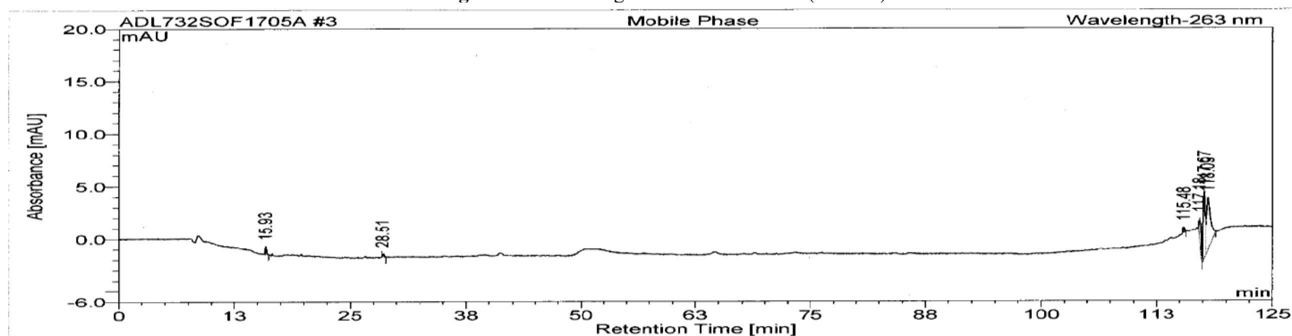
Table: 3: The observations are tabulated below

Injection No.	Peak area	
	Sofosbuvir	Velpatasvir
1	198.610	132.623
2	201.282	134.627
3	201.978	134.002
4	204.402	133.843
5	202.158	133.690
6	201.986	135.697
Average	**201.736**	**134.080**
% RSD	**0.9**	**0.8**
Theoretical plates	**45389**	**251066**
Tailing factor	**1.0**	**1.1**

The signal to noise ratio of Sofosbuvir peak: 14.0

The signal to noise ratio of Velpatasvir peak: 17.3

Figure 1: Chromatogram of Mobile Phase (260 nm)



Acceptance criteria

The signal to noise ratio of Sofosbuvir and Velpatasvir peak should not be less than 10. The theoretical plates for Sofosbuvir peak and Velpatasvir peak should not be less than 5000.

The tailing factor for Sofosbuvir peak and Velpatasvir peak should not be more than 2.0.

The relative standard deviation for six replicate standard injections should not be more than5.0 % for Sofosbuvir peak at 263 nm and Velpatasvir peak at 320 nm

Conclusion

The result obtained meets the system precision and system suitability requirement, which indicates that the system is suitable and precise for analysis.

Figure 2: Chromatogram of Mobile Phase (320 nm)

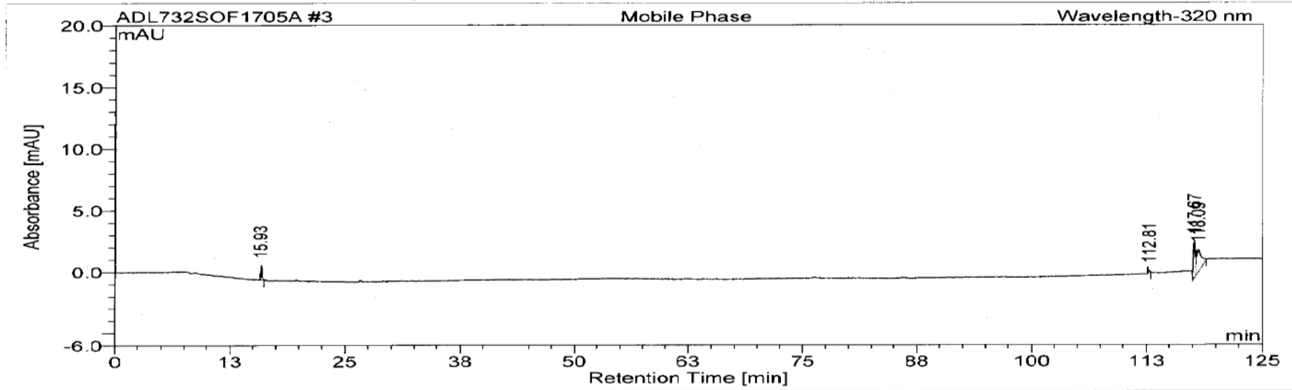


Figure 3: Chromatogram of Diluent (260 nm)

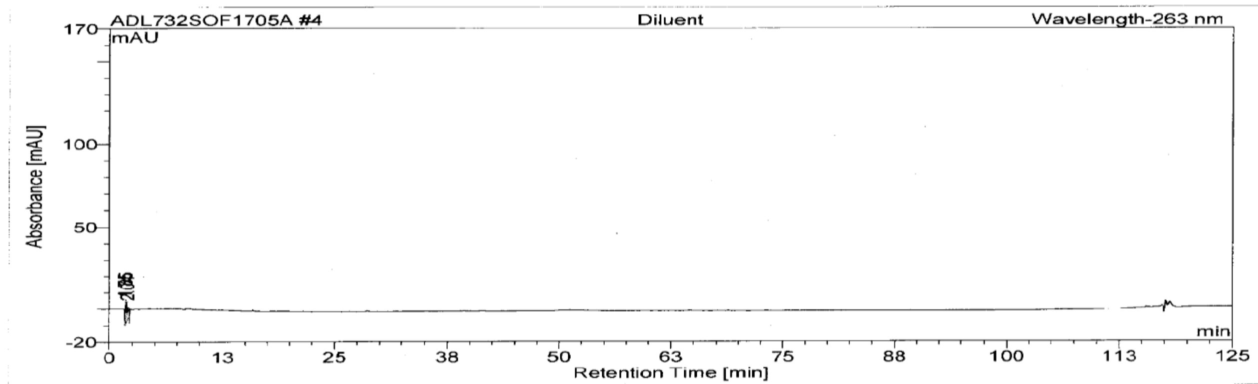


Figure 4: Chromatogram of Diluent (320 nm)

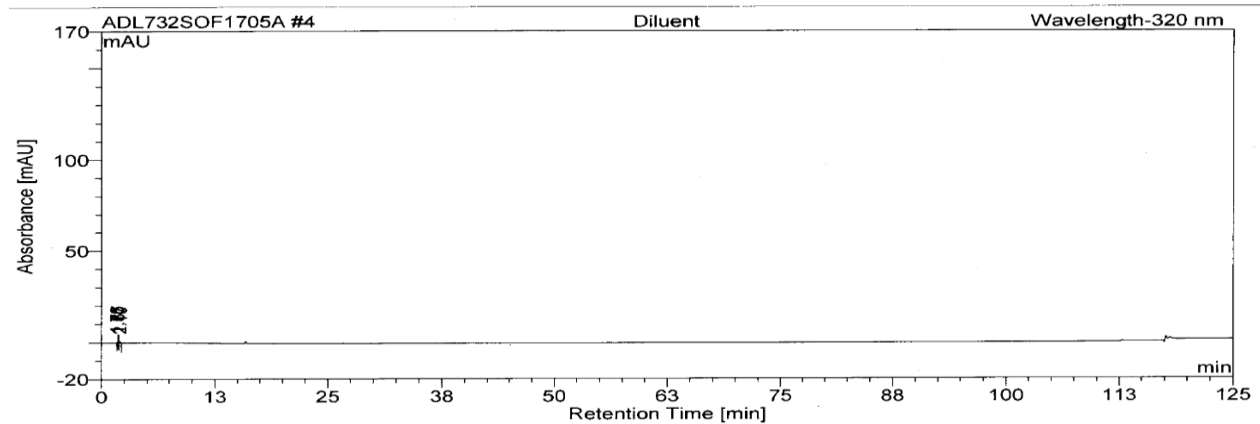
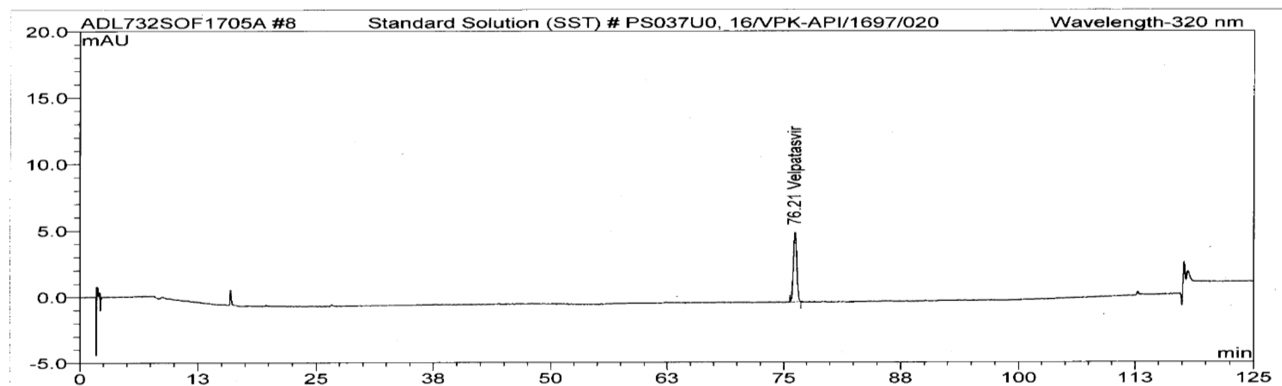
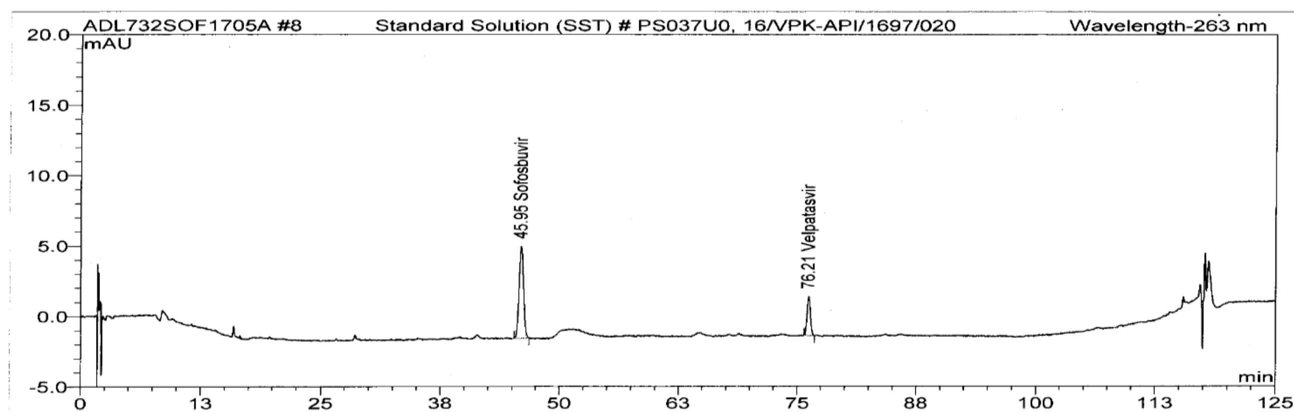


Figure 5; Chromatogram of Standard (320 nm)



DOI: 10.55522/jmpas.V11I4.4051 ISSN NO. 2320–7418

Figure 6: Chromatogram of Standard (260 nm)



Filter Compatibility and Saturation

Filter Compatibility and saturation study were carried out using Millipore PVDF 0.45 µm filter by altering the filtration volume. The filter compatibility and saturation study were performed by preparing sample solution as per the test method, in which known impurities (i.e., Methyl Uridine and Impurity at RRT 0.39) were spiked at 0.5 % level.

Table 2: sample solution as per the test method

Volume of sample Discarded (mL)	% Known impurities (Sofosbuvir)			% Maximum individual unknown impurity		% Total impurities
	Methyl Uridine	Impurity at RRT 0.39	RP Isomer	Bis Impurity		
Unfiltered	0.51	0.53	0.05	BQL	BQL	1.1
3	0.51	0.54	0.05	BQL	BQL	1.1
5	0.51	0.53	0.05	BQL	BQL	1.1
7	0.51	0.53	0.05	BQL	BQL	1.1

Volume of sample Discarded (mL)	Difference from initial results (Sofosbuvir)					
	Known impurities				Maximum individual unknown impurity	Total impurities
	Methyl Uridine	Impurity at RRT 0.39	RP isomer	Bis Impurity		
3	0.00	0.01	0.00	NA	NA	0.0
5	0.00	0.00	0.00	NA	NA	0.0
7	0.00	0.00	0.00	NA	NA	0.0

Volume of sample Discarded (mL)	% Known impurity	% Maximum individual unknown impurity	% Total impurities
	S-Isomer		
Unfiltered	0.06	0.12	0.54
3	0.06	0.12	0.55
5	0.07	0.12	0.46
7	0.06	0.12	0.44

Volume of sample Discarded (mL)	Difference from initial results (Velpatasvir)		
	Known impurity	Maximum individual unknown impurity	Total impurities
	S-Isomer		
3	0.00	0.00	0.01
5	0.01	0.00	0.08
7	0.00	0.00	0.10

Volume of sample Discarded (mL)	% Total impurities (Sum of Sofosbuvir total impurities + Sum of Velpatasvir total impurities)	Difference from initial results
Unfiltered	1.6	NA
3	1.7	0.1
5	1.6	0.0
7	1.5	0.1

Acceptance criteria

The difference between the % calculated results of unfiltered and filtered samples should not differ by more than ± 0.05 for each individual impurity and ±0.1 for total impurities.

Conclusion

From the established data the Millipore PVDF 0.45 µm filter proved compatible for all discarded volume for related substance method, hence Millipore PVDF 0.45 µm can be used for the analysis. Based on established data it is recommended that first 5 mL of filtrate will be discarded for the analysis.

LOD-LOQ

Limit of detection and quantitation for known impurities (i.e., Methyl Uridine and Impurity at RRT 0.39) and unknown impurities (in terms of Sofosbuvir and Velpatasvir) were established based on residual standard deviation and slope of the linearity data. From the linearity data the limit of detection and quantitation were calculated using the following formula.

$$\text{Limit of detection} = \frac{3.3\,\sigma}{S} \qquad \text{Limit of Quantitation} = \frac{10\,\sigma}{S}$$

Where,

σ -- Residual standard deviation of regression line

S -- Slope of regression line

Table 3: LOD-LOQ Peak area

Injection No.	Peak area (LOD)			
	Methyl Uridine	Impurity at RRT 0.39	Sofosbuvir	Velpatasvir
1	7.129	3.567	3.177	2.760
2	7.666	3.492	3.562	2.882
Average	**7.398**	**3.530**	**3.370**	**2.821**

Injection No.	Peak area (LOQ)			
	Methyl Uridine	Impurity at RRT 0.39	Sofosbuvir	Velpatasvir
1	21.181	10.074	11.663	7.434
2	20.290	9.993	11.102	7.557
3	20.992	10.019	12.153	7.915
4	21.995	10.467	11.244	7.655
5	22.262	10.034	13.710	7.967
6	21.679	10.026	11.571	7.434
Average	**21.400**	**10.102**	**11.907**	**7.660**
% RSD	**3.4**	**1.8**	**8.0**	**3.0**
S/N Ratio	**57.0**	**68.2**	**11.3**	**13.2**

Acceptance criteria

The detector response should be positive for LOD solution and the RSD at LOQ level for impurities should not be more than 10.0 %.

The signal to noise ratio at LOQ concentration should not be less than 10.

Table 4: noise ratio at LOQ concentration

Name of Impurity	LOD		LOQ	
	µg/mL	%	µg/mL	%
Methyl Uridine	0.3365	0.02	1.0095	0.05
Impurity at RRT 0.39	0.3353	0.02	1.0058	0.05
Sofosbuvir (For unknown impurity)	0.3363	0.02	1.0090	0.05
Velpatasvir (For unknown impurity)	0.0829	0.02	0.2448	0.05

Conclusion

From the above-established data, it can be concluded that the test method is capable of detecting and quantifying the impurities, if present in the sample, to the extent that mentioned in table

Method Precision

Method precision was demonstrated by preparing sample as such as per the test method and six samples, in which the known impurities (i.e., Methyl Uridine and Impurity at RRT 0.39) were spiked at 0.5 % level, representing a single batch. The impurities were quantified for each of these samples. The precision of the method was evaluated by computing the percentage relative standard deviation for each of known impurities, unknown impurities and total impurities.

Table 5: Total impurities

Sample Name	% Known impurities (Sofosbuvir)						% Maximum individual unknown impurity	% Total impurities
	Methyl Uridine	Impurity at RRT 0.39	RP Isomer	Bis Impurity	Methyl ester impurity	Sofosbuvir N-Hydroxy impurity		
Sample as such	BDL	BQL	BQL	BQL	BDL	BDL	BQL	NA

Sample Name	% Known impurity	% Maximum individual unknown impurity	% Total impurities
	S-Isomer (Velpatasvir)		

Sample as such	0.06	0.12	0.53

Sample Set.	% Known impurities						% Maximum individual unknown impurity	% Total impurities
	Methyl Uridine	Impurity RRT 0.39	RP Isomer	Bis Impurity	Methyl ester impurity	Sofosbuvir N-Hydroxy		
	0.51	0.53	0.05	BQL	BDL	BDL	BQL	1.1
	0.51	0.54	0.05	BQL	BDL	BDL	BQL	1.1
	0.51	0.54	0.05	BQL	BDL	BDL	BQL	1.1
	0.51	0.53	0.06	BQL	BDL	BDL	BQL	1.1
	0.51	0.53	0.05	BQL	BDL	BDL	BQL	1.1
	0.51	0.54	0.05	BQL	BDL	BDL	BQL	1.1
Average	**0.51**	**0.54**	**0.05**	**NA**	**NA**	**NA**	**NA**	**1.1**
% RSD	**0.0**	**1.0**	**8.2**	**NA**	**NA**	**NA**	**NA**	**0.0**

Sample Set	% Known impurity	% Maximum individual unknown impurity	% Total impurities
	S-Isomer		
	0.07	0.12	0.56
	0.07	0.12	0.58
	0.06	0.12	0.55
	0.06	0.11	0.50
	0.06	0.11	0.52
	0.07	0.12	0.57
Average	**0.07**	**0.12**	**0.55**
% RSD	**7.8**	**4.3**	**5.6**

Sample Set	% Total impurities (Sum of Sofosbuvir total impurities + Sum of Velpatasvir total impurities)
	1.7
	1.7
	1.7
	1.6
	1.6
	1.7
Average	**1.7**
% RSD	**3.0**

Table 6: Impurity level

Impurity levels	% RSD
0.05 % to 0.10 %	Not more than 25.0 %
0.11 % to 0.50 %	Not more than 15.0 %
0.51 % to 1.0 %	Not more than 10.0 %
More than 1.0 %	Not more than 5.0 %

Linearity

The linearity of detector response of known impurities (i.e., Methyl Uridine and Impurity at RRT 0.39) were demonstrated by preparing solutions of Methyl Uridine, Impurity at RRT 0.39 over the range of LOQ to 150 % of shelf life specification limit. These solutions were injected into the HPLC system and response of the same was recorded. A plot of concentration vs. impurity peak area was done.

Acceptance criteria

The correlation coefficient should be not less than 0.980.

The study proves that the response for known impurities (i.e., Methyl Uridine and Impurity at RRT 0.39) are linear over the range of LOQ to 150 % of shelf life specification limit.

Table 7: concentration vs. impurity peak area

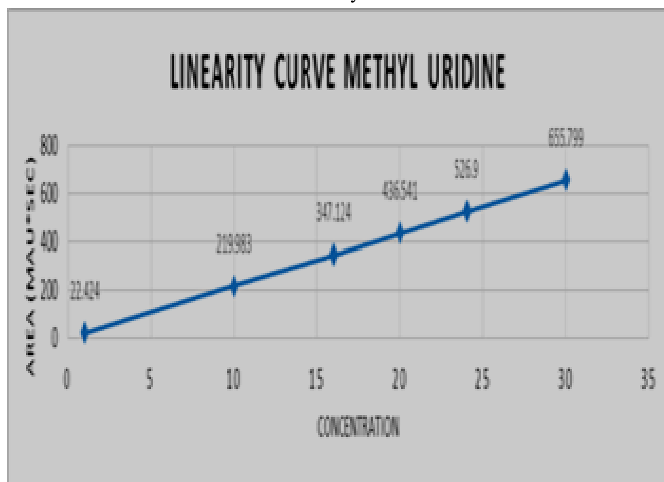
Linearity Level	Concentration of Methyl Uridine (µg/mL)	Peak area	RRF
LOQ	1.0007	22.424	NA
50 %	10.0075	219.983	0.48
80 %	16.0120	347.124	0.49
100 %	20.0150	436.541	0.48
120 %	24.0180	526.900	0.48
150 %	30.0225	655.799	0.48
Average			**0.48**

Conclusion

Table 8: Concentration of Impurity at RRT 0.39 (µg/mL)

Linearity Level		Peak area	RRF
LOQ	1.0000	9.929	NA
50 %	9.9998	100.450	1.05
80 %	15.9997	159.634	1.06
100 %	19.9997	200.901	1.05
120 %	23.9996	241.252	1.05
150 %	29.9995	300.701	1.05
Average			**1.05**

Figure 7: Linearity Curve Methyl Uridine

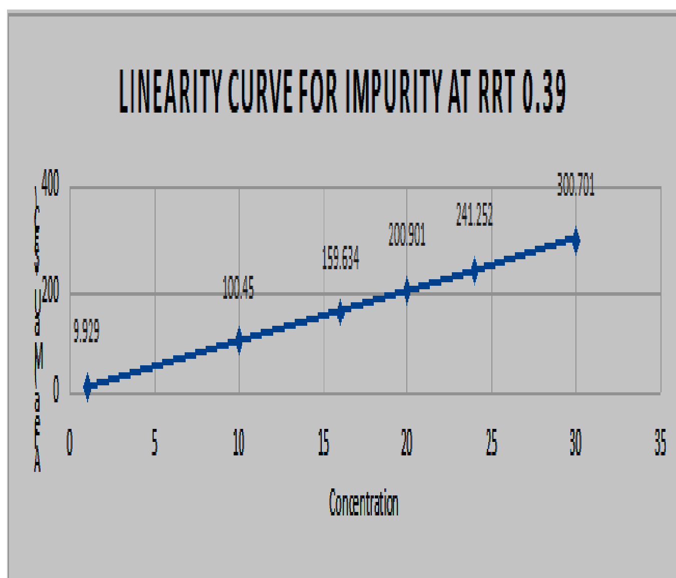


The plot was found linear with a correlation co-efficient of 0.99997.

Table 9: 50% Recovery for Impurity

50% Level Recovery for Impurity AT RRT 0.39			
Sample No.	**Amount Spiked (mg)**	**Amount Recovered (mg)**	**% Recovery**
1	0.2009	0.2014	101.0
2		0.2026	100.6
3		0.1982	99.2
Average			**99.9**

Figure 8: Linearity Curve for Impurity at RRT 0.39



The plot was found linear with a correlation co-efficient of 0.99999.

Accuracy

Table 10: LOQ Level Recovery for Methyl Uridine

LOQ Level Recovery for Methyl Uridine			
Sample No.	**Amount Spiked (mg)**	**Amount Recovered (mg)**	**% Recovery**
	0.0201	0.0199	108.5
		0.0212	112.4
		0.0218	111.4
Average			110.8

Table 11: 100% Recovery for Methyl Uridine

100% Level Recovery for Methyl Uridine			
Sample No.	**Amount Spiked (mg)**	**Amount Recovered (mg)**	**% Recovery**
1	0.4018	0.3975	98.6
2		0.3970	98.5
3		0.4010	99.5
Average			**98.9**

Table 12: 150% Recovery for Methyl Uridine

150% Level Recovery for Methyl Uridine			
Sample No.	**Amount Spiked (mg)**	**Amount Recovered (mg)**	**% Recovery**
1	0.6026	0.5996	99.2
2		0.5986	99.0
3		0.6018	99.6
Average			**99.3**

Table 13: 50% Level Recovery for Impurity

LOQ Level Recovery for Impurity AT RRT 0.39			
Sample No.	**Amount Spiked (mg)**	**Amount Recovered (mg)**	**% Recovery**
1	0.0201	0.0218	99.0
2		0.0226	105.5
3		0.0224	108.5
Average			**104.3**

Table 14: 100% Level Recovery for Impurity AT RRT 0.39

100% Level Recovery for Impurity AT RRT 0.39			
Sample No.	**Amount Spiked (mg)**	**Amount Recovered (mg)**	**% Recovery**
	0.4030	0.3965	98.6
		0.3965	98.5
		0.4002	99.5
Average			**99.0**

Table 15: 150% Level Recovery for Impurity AT RRT

150% Level Recovery for Impurity AT RRT 0.39			
Sample No.	**Amount Spiked (mg)**	**Amount Recovered (mg)**	**% Recovery**
1	0.6045	0.6012	99.8
2		0.5967	99.0
3		0.6017	99.9
Average			**99.6**

Acceptance Criteria

Table 16: Impurity levels Recovery

Impurity levels	% Recovery
Up to 0.10 %	Between 50.0% to 150.0%
0.11 % to 0.50 %	Between 70.0% to 130.0%
0.51 % to 1.0 %	Between 80.0% to 120.0%
More than 1.0 %	Between 90.0% to 110.0%

Conclusion

As the recovery results obtained for known impurities (i.e., Methyl Uridine and Impurity at RRT 0.39) were within the acceptable limits of recovery, the study proves that the method is accurate for quantification of these impurities in the range of LOQ to 150 % of shelf-life specification level.

Robustness

Conditions

Column Temperature was changed by ± 5°C (i.e., 30°C and 40°C).

Organic phase ratio of mobile phase was changed by ± 2 % absolute. [i.e., Buffer solution: Acetonitrile (98.5:1.5) % v/v for mobile phase A for – 1 % and Purified water: Methanol: Acetonitrile (21:29.5:49.5) % v/v/v for mobile phase B for – 1 % and Buffer solution: Acetonitrile (96.5:3.5) % v/v for mobile phase A for + 1 % and Purified water: Methanol: Acetonitrile (19:30.5:50.5) % v/v/v for mobile phase B for + 1 %].

Flow rate was changed by ± 10 % (i.e., 0.9 mL/min and 1.1 mL/min).

Mobile phase A buffer pH was changed by ± 0.1 units (i.e., pH 2.1 and pH 2.3).

Table 17: Organic phase ratio of mobile phase Conditions (For Sofosbuvir)

Conditions (For Sofosbuvir)	% RSD	Tailing factor	Theoretical plates	Retention Time
Normal	1.0	1.1	48307	50.25
Column temperature was changed by – 5°C (i.e., 30°C)	0.7	1.1	55323	53.09
Column temperature was changed by + 5°C (i.e., 40°C)	0.7	1.1	41853	45.95
Flow rate was changed by – 10 % (i.e., 0.9 mL/ min)	0.4	1.0	56672	52.69
Flow rate was changed by +10 % (i.e., 1.1 mL/ min)	0.7	1.1	45693	45.63
Mobile phase A buffer pH was changed by – 0.1 units (i.e., pH 2.1)	1.0	1.0	44527	48.46
Mobile phase A buffer pH was changed by + 0.1 units (i.e., pH 2.3)	0.3	1.1	47920	49.66
Organic phase ratio of the mobile phase was changed by – 2 %	0. 2	1.1	52267	50.60
Organic phase ratio of the mobile phase was changed by + 2 %	0.5	1.1	54296	40.18

Assessment of robustness study

Since the system suitability requirement i.e., the theoretical plates for Sofosbuvir peak and Velpatasvir peak should not be less than 5000, the tailing factor for Sofosbuvir peak and Velpatasvir peak should not be more than 2.0 and the relative standard deviation for six replicate standard injections should not be more than 5.0 % for Sofosbuvir peak at 263 nm and Velpatasvir peak at 320 nm is met for all the above mentioned changed conditions, it proves that the method is robust.

Table 18: Organic phase ratio of mobile phase Conditions (For Velpatasvir)

Conditions (For Velpatasvir)	% RSD	Tailing factor	Theoretical plates	Retention Time
Normal	0.4	1.1	282749	79.27
Column temperature was changed by – 5°C (i.e., 30°C)	0.8	1.1	281541	80.43
Column temperature was changed by + 5°C (i.e., 40°C)	0.8	1.1	220174	76.60
Flow rate was changed by –10 % (i.e., 0.9 mL/ min)	0.4	1.1	299631	80.47
Flow rate was changed by +10 % (i.e., 1.1 mL/ min)	0.8	1.1	206903	76.04
Mobile phase A buffer pH was changed by – 0.1 units (i.e., pH 2.1)	0.7	1.1	230688	77.57
Mobile phase A buffer pH was changed by + 0.1 units (i.e., pH 2.3)	0.5	1.1	300458	81.10
Organic phase ratio of the mobile phase was changed by – 2 %	0.5	1.1	332393	81.44
Organic phase ratio of the mobile phase was changed by + 2 %	0.5	1.1	123633	69.41

Specificity

Interference from placebo with analyte and known impuritieswere studied by preparing the following specificity samples.

1. By preparing placebo in triplicate equivalent to the sample weight.
2. By preparing individual known impurities solution.
3. By preparing individual excipients solution.
4. By spiking placebo with known impurities.
5. By spiking test solution with known impurities.
6. By preparing API (Sofosbuvir and Velpatasvir) solution.
7. By preparing test solution as per test method.

The impurities peak were well separated. There were no interference from placebo with analyte and known impurities.

The peak purity of individual known impurities solution were found as follows

Table 19: Name of impurity (Peak purity)

Name of impurity	Peak purity
Methyl Uridine	1000
Impurity at RRT 0.39	1000
Methyl ester impurity	999
Ethyl ester impurity	998
RP Isomer of Sofosbuvir	990
Sofosbuvir N-Hydroxy impurity	993
Alpha Isomer	993

DOI: 10.55522/jmpas.V11I4.4051 ISSN NO. 2320–7418

Pentafluoro Phenol	1000
Bis impurity	1000
Phosphoramidate compound	998
Velpatasvir S-Isomer	999
Velpatasvir D. Boc compound (N-1)	999

Acceptance criteria

There should no interference from placebo with analyte and known impurities, the peak purity of known impurities and analyte in sample solution should not less than 990.

Figure 9: Chromatogram of Placebo (263 nm)

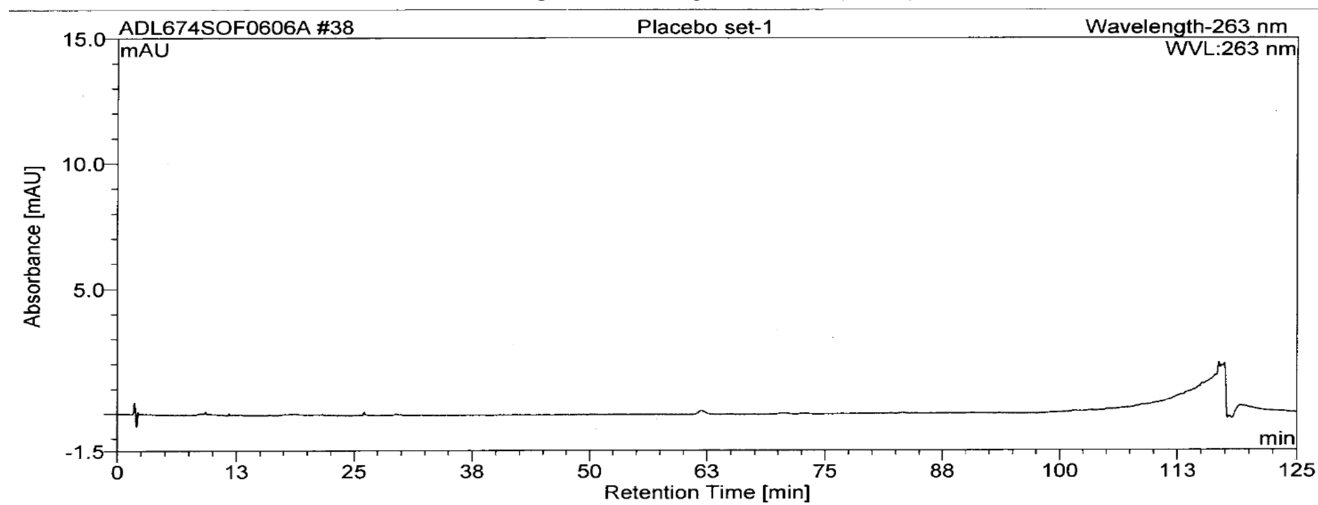


Figure 10: Chromatogram of Methyl Uridine (263 nm)

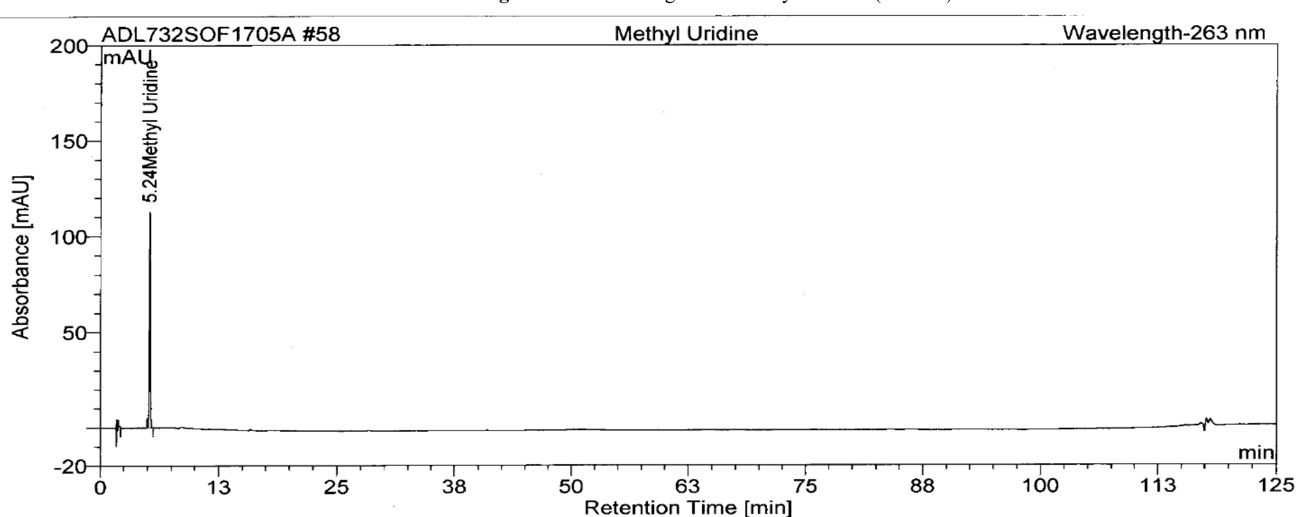
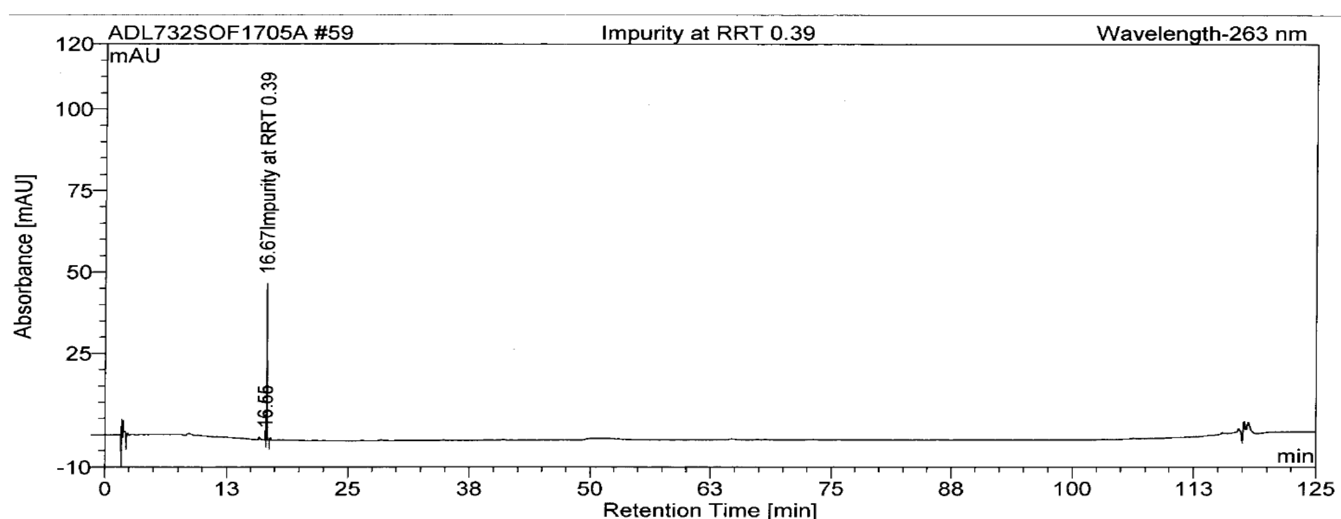


Figure 11: Chromatogram of Impurity at RRT0.99 (263 nm)



DOI: 10.55522/jmpas.V11I4.4051 ISSN NO. 2320–7418

Figure 12: Chromatogram of Methyl Ester (263 nm)

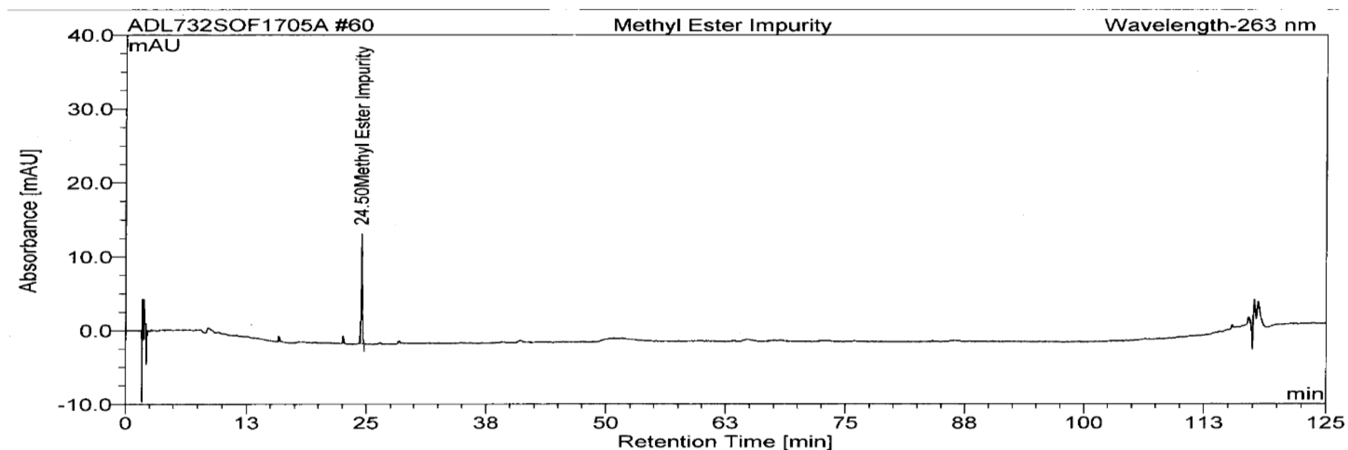


Figure 13: Chromatogram of Ethyl Ester Imp. (263 nm)

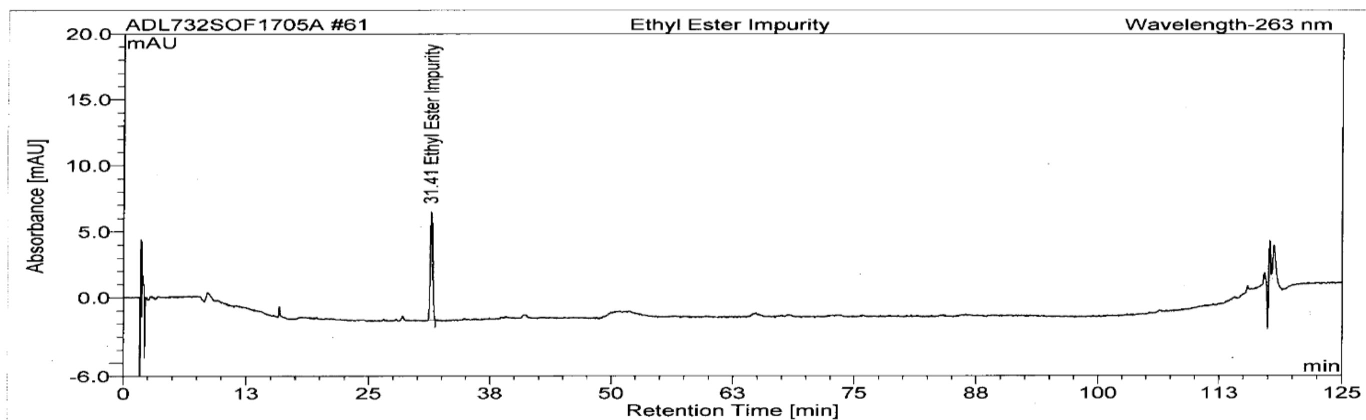


Figure 14: Chromatogram of RP Isomer of Sofosbuvir (263 nm)

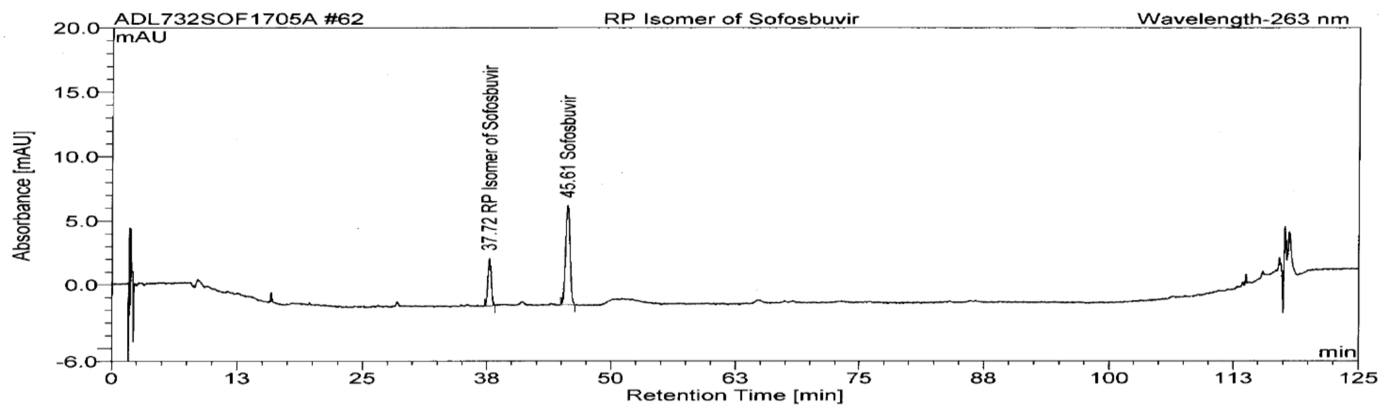


Figure 15: Chromatogram of Sofosbuvir N-Hydroxy Imp. (263 nm)

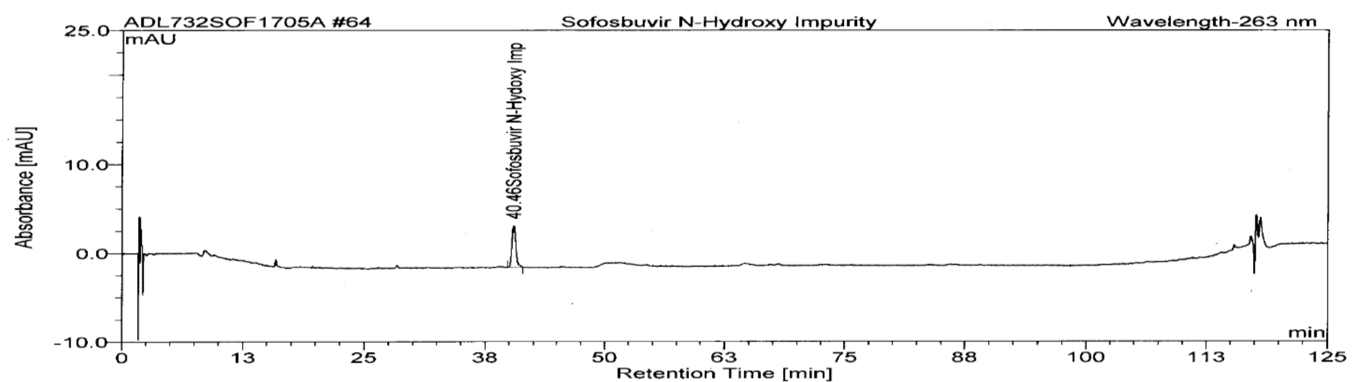


Figure 16: Chromatogram of Alpha Isomer of Sofosbuvir (263 nm)

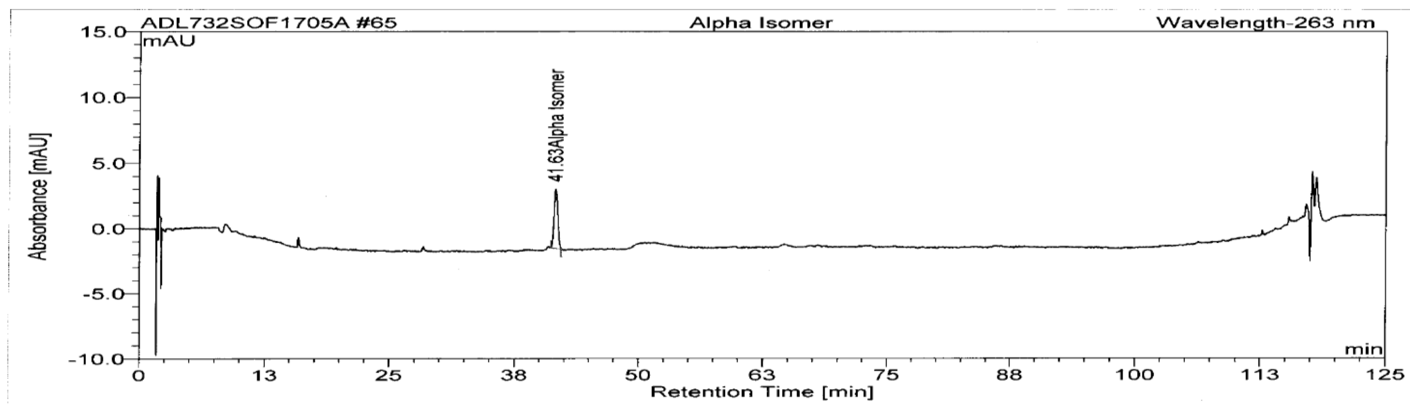


Figure 17: Chromatogram of Pentafluoro Phenol (263 nm)

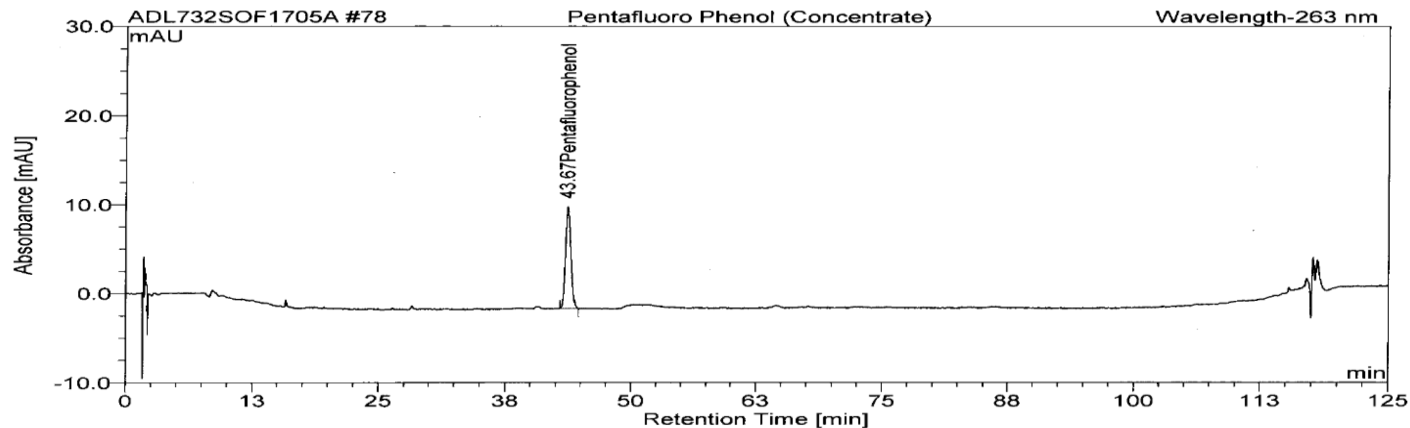


Figure 18: Chromatogram of Velpatasvir S-Isomer (320 nm)

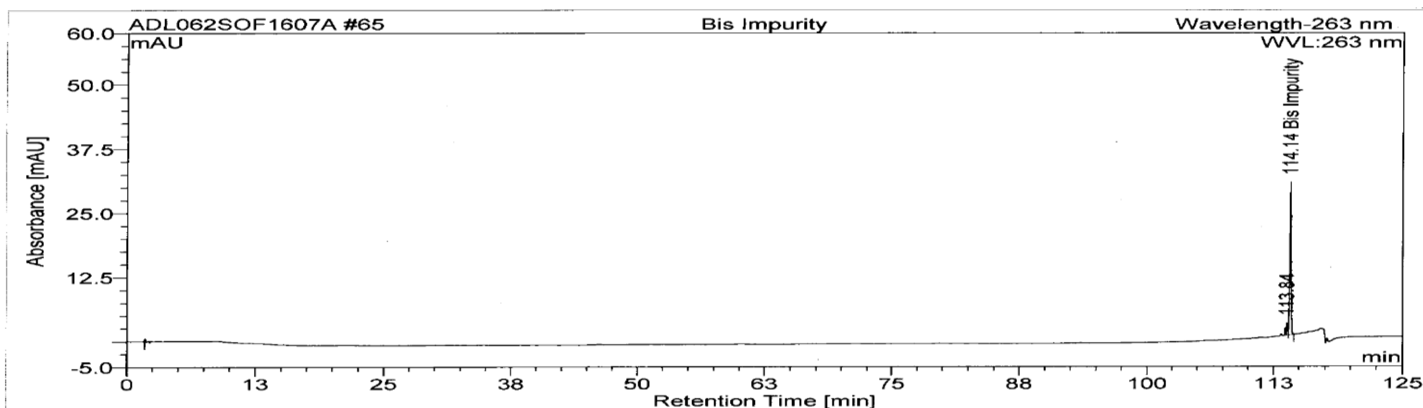


Figure 19: Chromatogram of Test Solution spiked with known impurities (263 nm)

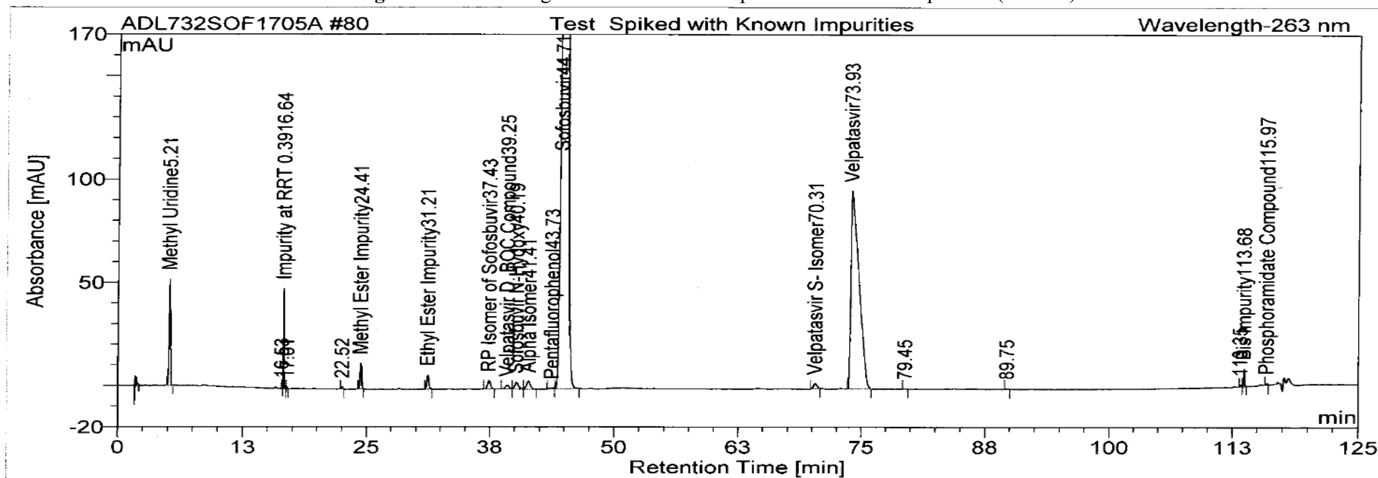
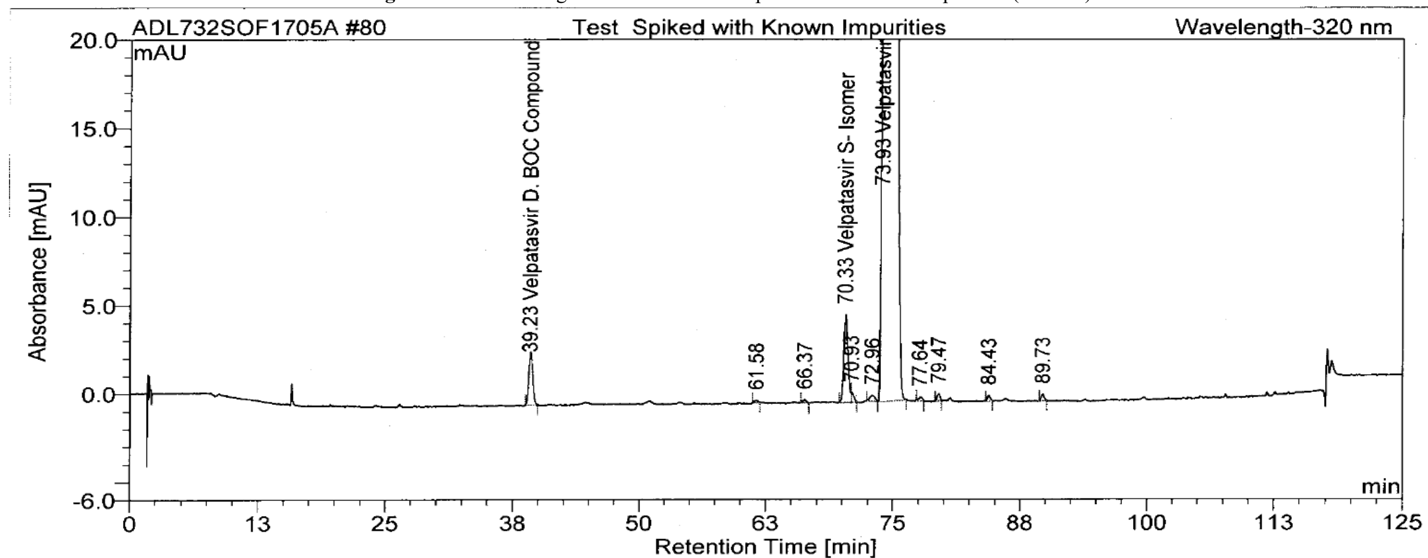


Figure 20: Chromatogram of Test Solution spiked with known impurities (320 nm)



REFERENCES

1. Jay Bhatt, Ujashkumar A Shah, Hirak kumar V. Joshi et al, 2021. Analytical method development and validation of related substances by rphplc of emtricitabine and tenofovir disoproxil fumarate tablets. Jour. of Med. P'ceutical & Allied. Sci. V - 10 - I 5, 1184, P-3656-3665. doi: 10.22270/ jmpas.V10I5.1184
2. Raj kumar B, Subrahmanyam KV et al. ,a new validated rp-hplc method for the simultaneous determination of simeprevir and sofosbuvir in pharmaceutical dosage form.Indo american journal of pharmaceutical research, 2016; 6(2).
3. Mohan Vikas P, Satyanarayana T, et al, development and validation of new rp-hplc method for the determination of sofosbuvir in pure form.2016, 5(5):775-781.
4. Ravikumar Vejendla, C.V.S. Subramanyam, G. Veerabhadram estimation and validation of sofosbuvir in bulk and tablet dosage form by rp-hplc International Journal of Pharmacy Int J Pharm 2016; 6(2):121-127.
5. Kanthale SB, Thonte SS, Mahapatra DK, 2019. Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Ivabradin and Metoprolol in Bulk and Tablet Formulation. J Appl Pharm Sci. 9(4), 137-144.
6. Prakash O, Mahapatra DK, Singh R, et al, 2018. Development of a New Isolation Technique and Validated RP-HPLC method for Quercetin and Kaempferol from Azadirachta indica leaves. Asian J Pharm Anal. 8(3), 164-168.
7. Kanthale SB, Thonte SS, Mahapatra DK, 2019. Development of Validated Stability Indicating RP-HPLC Method for the Estimation of Glecaprevir and Pibrentasvir in Bulk and Pharmaceutical Dosage Form. J Appl Pharm Sci. 9(6), 52-60.
8. Deodhe S, Dhabarde DM, Kamble MA, et al, 2017. Development and validation of a novel stability indicating RPHPLC method for the estimation of entecavir in tablet formulation. Eur J Anal Chem. 12(3), 223-235.

How to cite this article

Jay Bhatt, Dr. Ujashkumar A. Shah, Dr. Hirakkumar V. Joshi, 2022. Analytical method development and validation of related substances by RP-HPLC of sofosbuvir and velpatasvir tablets. Journal of Medical Pharmaceutical and allied Science V 11 - I 4, Pages - 5201 – 5213. Doi: 10.55522/jmpas.V11I4.4051.