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UPLC Method Development and Validation for Sofosbuvir, Velpatasvir, and Voxilaprevir Determination

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ABSTRACT

It has been stated that an ideal analytical technique is highly desirable for formulating pharmaceutical prescription. This work describes and validates the new UPLC method for the identification of Sofosbuvir, Velpatasvir, and Voxilaprevir in drug products. Details of developed method was checked for precision, accuracy, linearity, sensitivity and robustness and found it abide ICH guidelines for validation. The chromatographic separation was done on a C18 column and a gradient mobile phase of acetonitrile and buffer at certain conditions. All the analytes were estimated at 260 nm. The selected method displayed a perfect linear response within high concentration levels of the drug more than 0.999 with the merits values for all three drugs. Validation results provided further support to the suitability of the described method in routine quality control analysis where this approach would stabilize pharmaceutical quality assurance in terms of rapidity and cost-effectiveness.

Keywords: *Sofosbuvir, Velpatasvir, Voxilaprevir, UPLC, Method Validation, Pharmaceutical Analysis, ICH Guidelines.*

1. INTRODUCTION

Sofosbuvir, Velpatasvir, and Voxilaprevir are antiviral agents which are administered together with other pharmaceuticals in the regulation of chronic hepatitis C virus (HCV) illness. These agents, with related but distinct functional profiles, act through different manners to different phases of the viral life cycle, thus being a highly synergistic therapeutic modality for all HCV genotypes. It is now widely accepted that these drugs are gaining clinical significance and therefore require proper analytical techniques to determine the quality of these drugs in formulations. UPLC is now widely used as it offers a better resolution, higher speed, and sensitivity over other similar technique – HPLC. The present work proposes to establish and to standardise an UPLC method for the determination of Sofosbuvir, Velpatasvir and Voxilaprevir levels in pharmaceutical preparations. During the validation, regular elements in compliance with ICH Q2(R1) include linearity, linearity range, accuracy, precision, LOD and LOQ. In this way, this paper contributes to the general goal of preserving pharmaceutical quality and adherence by providing a valid and effective technique.

2. MATERIALS AND METHODS:

Instrumentation

The author used Waters UPLC instrument, equipped with a CHS C18 100 x 3 mm, 1.7µm reverse phase column, quaternary pumps and a Tunable UV detector for simultaneous estimation of sofosbuvir, velpatasvir and voxilaprevir. For chromatographic evaluation and data acquisition empower 2 software was employed. Ultrasonic bath sonicator (Labman), Weighing balance (Saritorius), pH meter (Mestar), and vaccum pump (Crompton) was used for present study.

Chemicals

The reference standards of sofosbuvir, velpatasvir and voxilaprevir were provided by Spectrum Pharma Research Solutions, Hyderabad. The chemicals HPLC grade acetonitrile, HPLC grade methanol, HPLC grade water were procured from Rankem, Mumbai, India. potassium dihydrogen phosphate AR grade was purchased from Molychem. Orthophosphoric acid AR grade, hydrochloric acid,sodium hydroxide, hydrogen peroxide were purchased from S.D.Fine chemicals, Hyderabad.

Preparation of pH 4.0 (0.01 N KH_2PO_4) Buffer: 1.36 gm of potassium di-hydrogen ortho phosphate was accurately weighed and transferred to 1000mL volumetric flask, 900mL of milli-Q water was added. The solution was sonicated and the volume was adjusted up to 1000 mL and pH was adjusted to 4.0 with dil. orthophosphoric acid solution. The solution was filtered through 0.22 µ filter.

Preparation of mobile phase:

The pH 4.0 buffer and acetonitrile were mixed in the ratio of 60:40 v/v and sonicated to degas.

Preparation of diluent:

Water and acetonitrile 50:50 % v/v were used as diluent.

Preparation of standard stock solution:

40 mg of sofosbuvir, 10 mg of velpatasvir and 10 mg of voxilaprevir were weighed and transferred to 10 mL volumetric flask and 5mL of diluent was added. The mixture was sonicated for 10 min and made up to the volume with diluent to obtain standard stock solution of sofosbuvir (4000µg/mL), velpatasvir (1000µg/mL) and voxilaprevir (1000µg/mL).

Preparation of working standard solution:

1 mL of standard stock solution was transferred to 10 mL volumetric flask and 5 mL of diluent was added, sonicated and made up to 10 mL with diluent to obtain working standard solution (400 µg/mL of sofosbuvir, 100 µg/mL of velpatavir and 100 µg/mL of voxilaprevir).

Optimization of the chromatogarphic conditions and method development

To develop the method a systematic study was taken up by choosing different columns and mobile phase ratios. A non-polar C_{18} column was chosen as the stationary phase for this study. Different trials were performed by using mixture of frequently used solvents such as water, methanol and acetonitrile, with and without buffer on different columns. The method details and the respective chromatograms of different trails are given in **table 1.2** and **fig 1.4** respectively. After using different combinations of solvents, 0.01 N phosphate buffer (pH 4) and acetonitrile in 60:40 v/v ratio under isocratic conditions at a flow rate of 0.3 mL/min was selected as mobile phase because better separation in terms of good resolution, better peak shape, good efficiency and less tailing was observed. Optimized chromatographic conditions were listed in the **table 1.2**. **Fig 1.5** represents a chromatogram of sofosbuvir, velpatasvir and voxilaprevir.

Table 1.2: Trials for Optimization of chromatographic conditions

Trails	Column and Mobile Phase used	Reasons for rejection
Trail 1	STD HSS C18 (100x2.1mm, 1.8µ) 50:50 Methanol:Water	Sofosbuvir was eluted but Velpatasvir,Voxilaprevir not eluted.
Trail 2	STD HSS C18 (100x2.1mm, 1.8µ) 50:50 Methanol:0.1% OPA	Sofosbuvir was eluted but Velpatasvir,Voxilaprevir not eluted &Sofosbuvir USP platecount was less.
Trail 3	STD X bridge C18 (100x2.1mm, 1.8µ) 50:50 Acetonitrile:0.1% OPA	sofosbuvir,Velpatasvir,Voxilaprevir peaks were eluted,but peak shape was not good,
Trail 4	STD CHS C18 (100x3mm, 1.7µm) 50:50 Acetonitrile: 0.1% OPA	sofosbuvir,Velpatasvir,Voxilaprevir peaks were eluted, but peak shape was not good,& USP plate count is less
Final Trial	STD CHS C18 (100x3mm, 1.7µm) 40:60 Acetonitrile:KH2PO4	peaks having good resolution, tailing Factor, theoretical plate count and resolution

Table 1.3: Optimized chromatographic conditions

S.No	Parameter	Value
1	Column	CHS C18 100 x 3 mm, 1.7 µm.
2	Mobile phase	pH 4 buffer and acetonitrile in the ratio of 60:40 v/v
3	Flow rate	0.3mL/min
4	Run time	3.0 min
5	Column temperature	30^0 C
6	Volume of injection	0.5 µL
7	Detection wave length	260 nm

2.1 Method Validation

The method was validated in compliance with ICH guidelines for its system suitability, linearity, accuracy, precision, robustness, limit of detection and limit of quantification by adopting the following procedures.

System Suitability

To evaluate system suitability parameters (tailing factor, theoretical plates, resolution and % RSD), six injections of the working standard solution were injected as per optimized chromatographic method. Results of system suitability parameters were given in **table 1.4**.

Specificity

Specificity is the extent to which the procedure applies to analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in formulation did not interfere with the drug peaks and thus the method is specific. The UPLC chromatograms recorded for the blank, placebo and the standard shows almost no interfering peaks within retention time ranges. Thus, the proposed UPLC method is selective. Chromatograms were represented as **Fig 1.6-1.8**.

Linearity

Aliquots of standard stock solution were transferred to 10 ml volumetric flask to prepare six different concentrations (n=3) ranging from 25-150% of working standard concentration (100-600 µg/ml of sofosbuvir, 25-150 µg/ml of velpatasvir and 25-150 µg/ml of voxilaprevir). The resulting solutions were analysed. A calibration curve was plotted from the measured peak area (Y-axis) corresponding to the concentration of solution (X-axis). From the graph, slope, y-intercept and R^2 values were determined. The linearity results were given in **Table 1.5-1.6. Fig 1.9-1.11** represents calibration curves of sofosbuvir, velpatasvir and voxilaprevir. From the results it was found that R^2 value was greater than 0.999 for all the three drugs, which indicates that a good linear relation exists between concentration of the drug and peak area.

Precision

Precision is the degree of repeatability of an analytical method under normal operating conditions. Intraday precision (Repeatability) and Inter-day precision (Intermediate precision) were measured by preparing and injecting six replicas of working standard solution on same day and on different day respectively under same operating conditions. % RSD of six injections was calculated. **Table 2.7** represents the results of intraday precision and **Table 2.8** represents inter-day precision

results.

Accuracy

The accuracy of the method was determined by calculating the % recovery of the drug that was spiked. It was carried at three different concentration levels (50%,100% and 150%) and three injections from each concentration level was analyzed. The mean % recovery for each level was calculated. The results were given in **table 1.8-1.10.**

LOD and LOQ

Limit of Detection (LOD) is defined as lowest amount of analyte which can be detected but not necessarily quantified. Limit of Quantification (LOQ) is defined as lowest amount of analyte which can be quantified with suitable accuracy and precision. LOD and LOQ values were represented in **table 1.11.**

Robustness

A study was performed to test the robustness of an analytical method by varying small deliberate changes in method parameters such as mobile phase composition, flow rate and column temperature. The organic content in mobile phase was changed from ±10% of actual value. Similarly flow rate was varied from ±10% of actual value. Temperature was varied from ±5^{0}C of actual value % RSD of replicate injections were measured and it should be not more than 2.0%. **Table 1.12-1.14** represents the results of robustness.

2.2 Estimation of The Drug From Dosage Forms

The author applied the developed method to determine the quantity of drugs sofosbuvir, velpatasvir and voxilaprevir in pharmaceutical formulation (VOSEVI).

Preparation of sample solution and recovery study

Accurately five tablets were weighed and crushed into powder form. Weight equivalent to one tablet was taken and transferred to a 100 ml volumetric flask, 75 ml of diluents were added. The mixture was sonicated for 25 min and final volume was adjusted. The resulting solution was filtered through 0.22 µ filter. 1ml of this solution was diluted up to 10 ml with diluent to obtain sample solution. The results of recovery study were presented in **table 1.15-1.17.**

2.3 Forced degradation studies

Preparation of standard stock solution:

40 mg of sofosbuvir, 10 mg of velpatasvir and 10 mg of voxilaprevir were weighed and transferred to 10 mL volumetric flask added 5mL of diluent and sonicated for 10 min and made up to the volume with diluent to obtain standard stock solution of sofosbuvir (4000µg/mL), velpatasvir (1000µg/mL) and voxilaprevir (1000µg/mL).

Acid degradation:

1mL of above standard stock solution was transferred into 10mL volumetric flask, added 1mL of 2 N HCl and treated at 60°C for 30 min, allow to attain the room temperature and neutralized with 1N NaOH. Make up the volume with diluent to obtain a solution of 400 µg/mL of sofosbuvir, 100 µg/mL of velpatasvir and 100µg/mL of voxilaprevir. Injected 0.5µL of solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation:

1mL of above standard stock solution was transferred into 10mL volumetric flask, added 1mL of 2 N NaOH and treated at 60°C for 30 min, allow to attain the room temperature and neutralized with 1N HCl. Make up the volume with diluent to obtain a solution of 400 µg/mL of sofosbuvir, 100 µg/mL of velpatasvir and 100µg/mL of voxilaprevir. Injected 0.5µL of solutions were injected into the UPLC system and the chromatograms (**Fig 1.17**) were recorded to assess the stability of sample.

Oxidative degradation:

1mL of above standard stock solution was transferred into 10mL volumetric flask, added 1mL of 20% hydrogen peroxide, the solution was kept at room temperature for 30 min and make up the volume with diluent to obtain a solution of 400 µg/mL of sofosbuvir, 100 µg/mL of velpatasvir and 100µg/mL of voxilaprevir. Injected 0.5µL of solutions were injected into the UPLC system and the chromatograms (**Fig 1.18**) were recorded to assess the stability of sample.

Dry Heat Degradation Studies

1mL of above standard stock solution was transferred into 10mL volumetric flask and placed in oven at 105^{0}C for 6 h, allow to attain room temperature and make up the volume with diluent to obtain a solution of 400 µg/mL of sofosbuvir, 100 µg/mL of velpatasvir and 100µg/mL of voxilaprevir. Injected 0.5µL of solutions were injected into the UPLC system and the chromatograms (**Fig 1.19**) were recorded to assess the stability of sample.

Photo Stability Studies

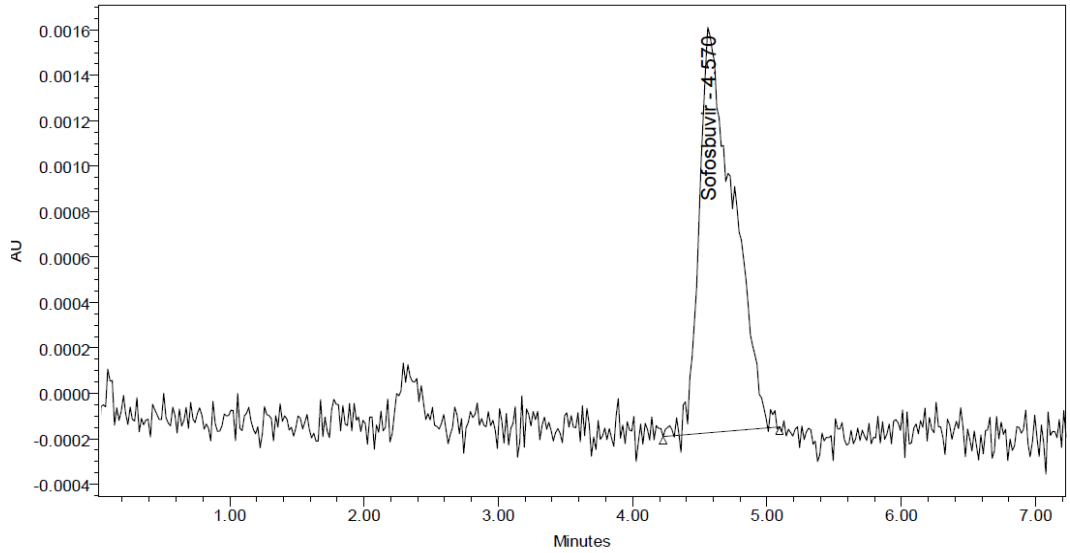
1mL of above standard stock solution was transferred into 10mL volumetric flask and kept in UV chamber and exposed to UV light for 7 days, and make up the volume with diluent to obtain a solution of 400 µg/mL of sofosbuvir, 100 µg/mL of velpatasvir and 100µg/mL of voxilaprevir. Injected 0.5µL of solutions were injected into the UPLC system and the chromatograms (**Fig 1.20**) were recorded to assess the stability of sample.

Neutral Degradation Studies

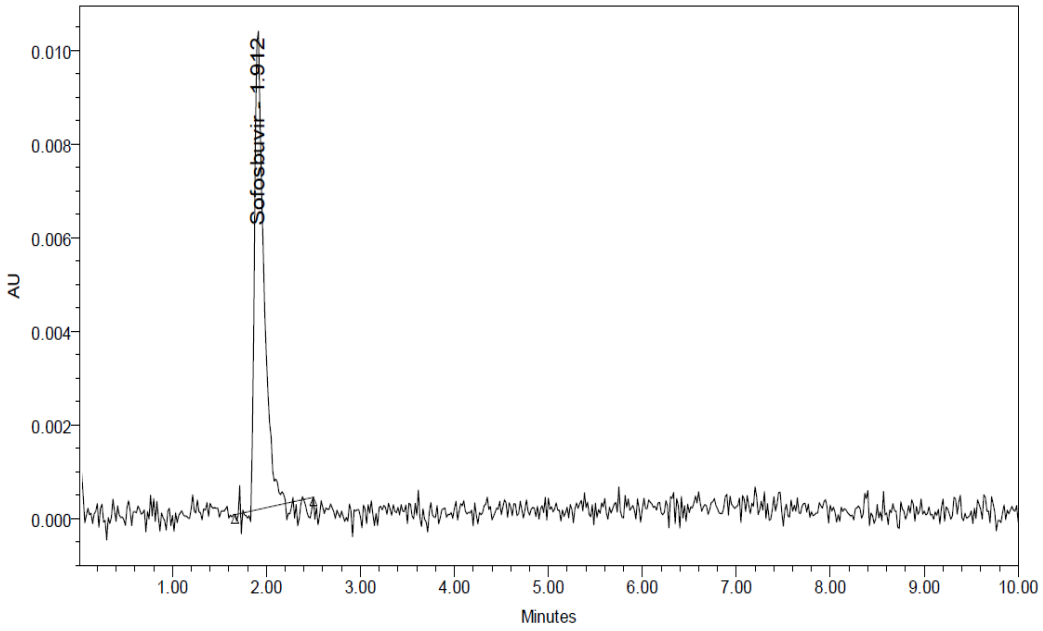
1mL of above standard stock solution was transferred into 10mL volumetric flask, added 1mL of water, the solution was heated at 60^0C for 30 min and make up the volume with diluent to obtain a solution of 400 µg/mL of sofosbuvir, 100 µg/mL of velpatasvir and 100µg/mL of voxilaprevir. Injected 0.5µL of solutions were injected into the UPLC system and the. chromatograms (**Fig 1.21**) were recorded to assess the stability of sample

When the drugs were subjected to different stress conditions, there were found to degrade significantly in acid and base stress conditions. When treated with acid, degradation of about 6.98 % for sofosbuvir, 6.34 % for velpatasvir and 5.47 % for voxilaprevir was observed. The % of degradation was found to be less than 7 % when stressed under various conditions.

3. RESULTS:



Trial 1



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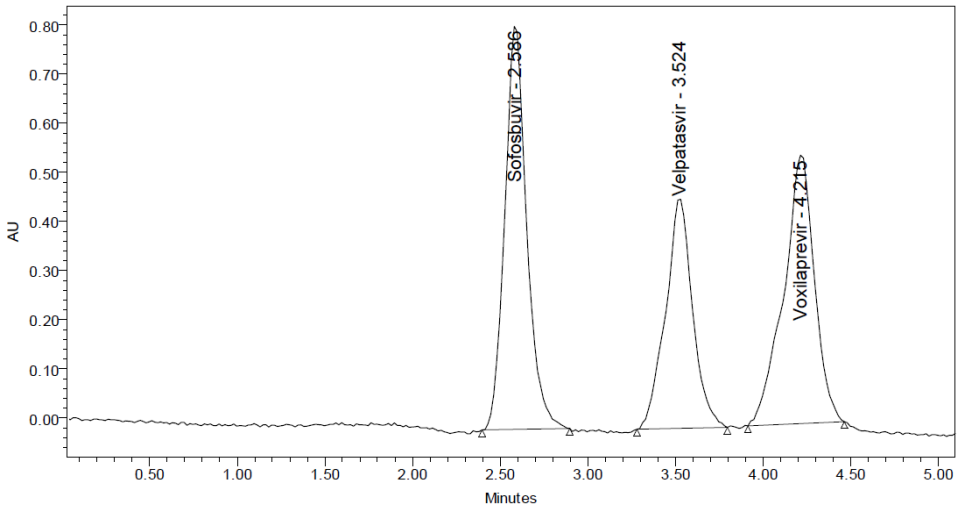

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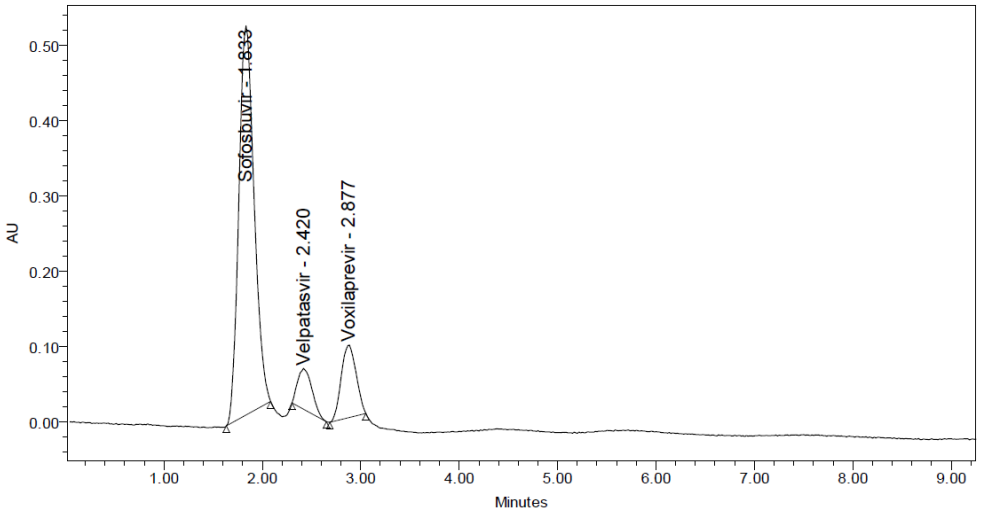
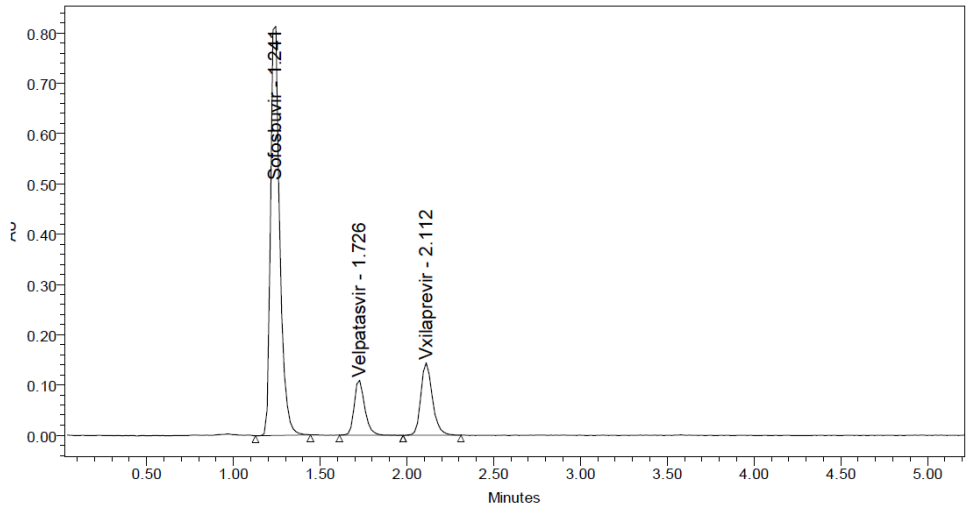


Trial 4



Trail 5 (Optimized chromatogram)

Fig 2.4 Trial Chromatograms for optimization of analytical method

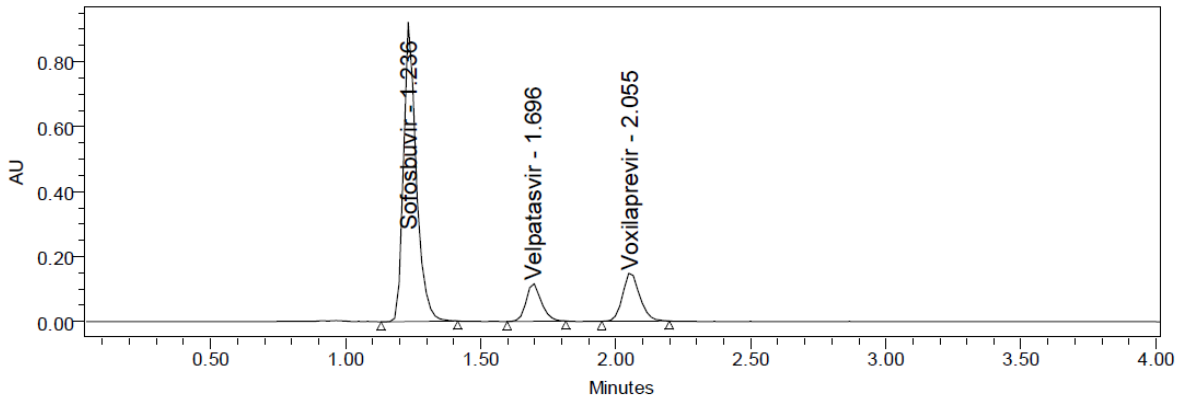


Fig 1.5: A representative chromatogram of working standard solution

Table1.4: System suitability results of sofosbuvir, velpatasvir and voxilaprevir

Parameters	sofosbuvir	velpatasvir	voxilaprevir
Retention time	1.236	1.696	2.055
Peak Area	3150070	458443	615411
Resolution	--	4.6	3.2
Theoretical plates (n)	3009	3887	4979
Tailing factor (T)	1.25	1.21	1.15
% RSD (n=6)	0.8	0.9	0.3

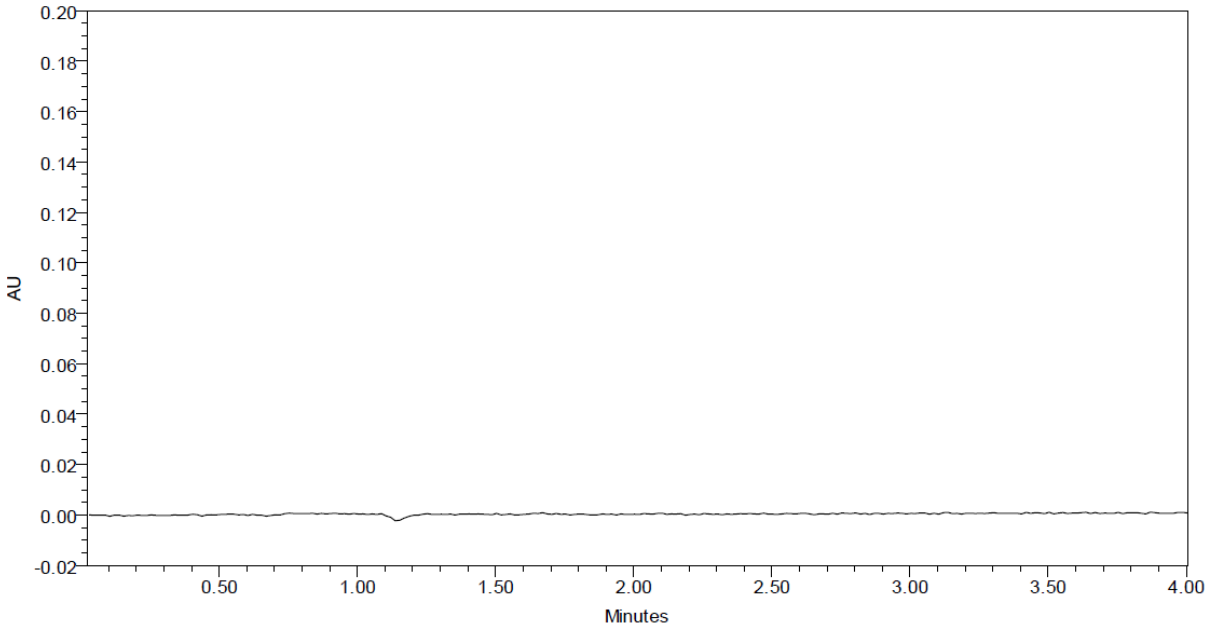


Fig 1.6 : Chromatogram of Blank solution

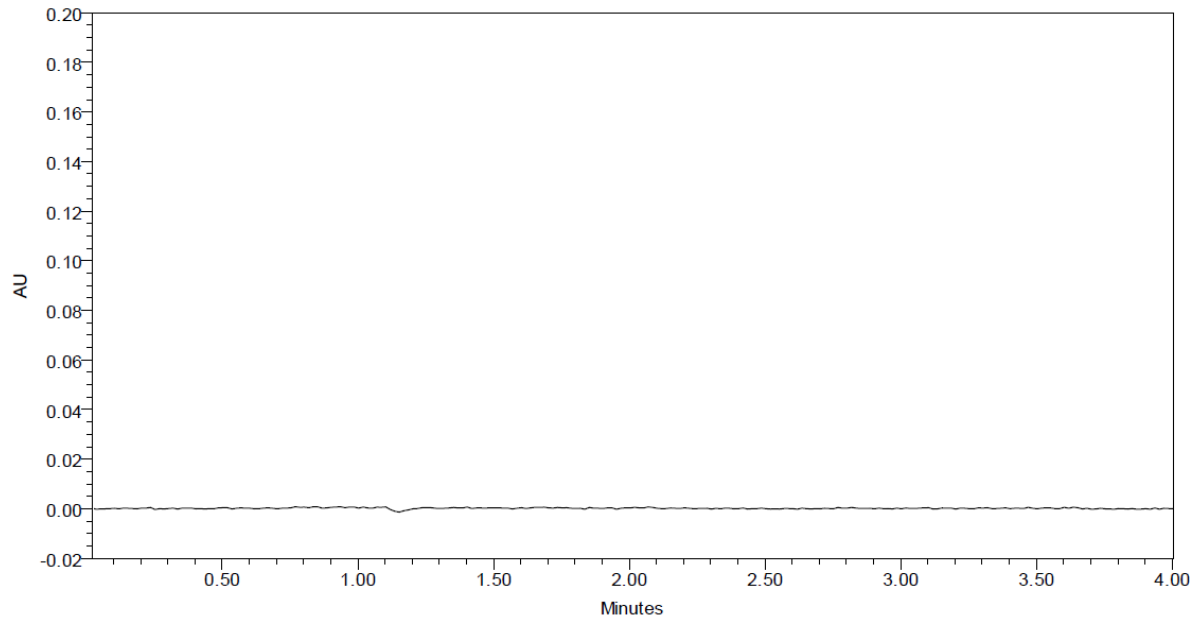


Fig1.7 : Chromatogram of placebo solution

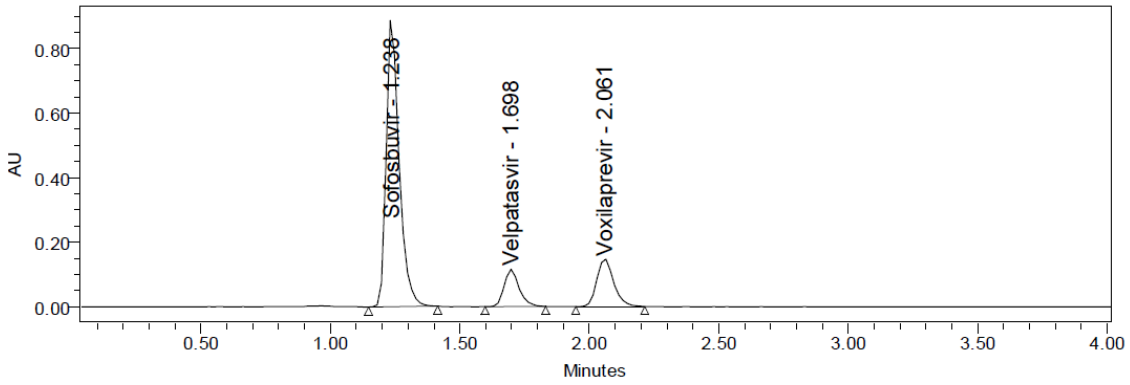


Fig 1.8 : Chromatogram of working standard solution

Table 1.5 Linearity data of sofosbuvir,velpatasvir and voxilaprevir

sofosbuvir.			**velpatasvir**			**voxilaprevir**		
Conc (µg/mL)	**Mean area***	**%RSD**	**Conc (µg/mL)**	**Mean area***	**%RSD**	**Conc (µg/mL)**	**Mean area***	**%RSD**
100	765133	0.37	25	124780	0.55	25	152671	0.52
200	1581340	0.41	50	232147	0.34	50	306663	0.58
300	2284745	0.06	75	346272	0.11	75	453323	0.35
400	3128314	0.81	100	457494	0.35	100	617570	0.59
500	3877596	0.27	125	588672	0.25	125	755692	0.71
600	4594711	0.06	150	705094	0.45	150	914507	0.51

**is average of three determinations*

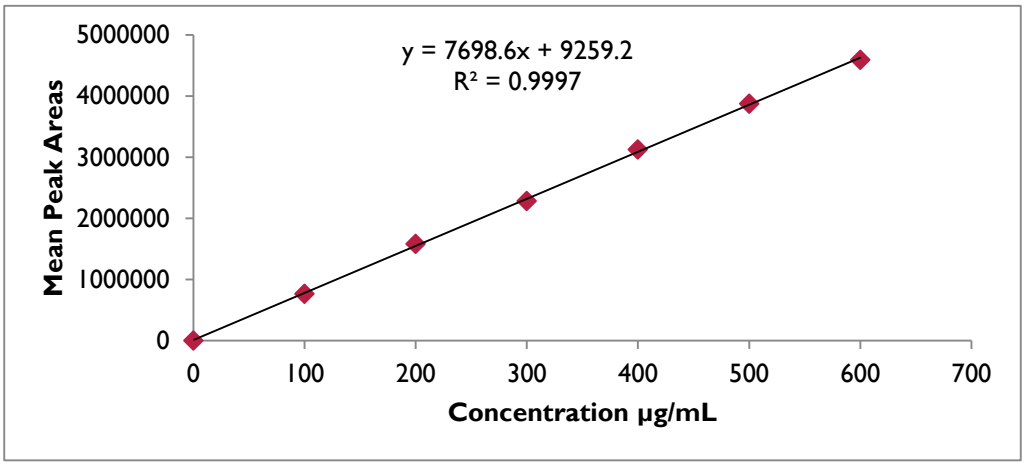


Fig 1.9: Calibration curve of sofosbuvir

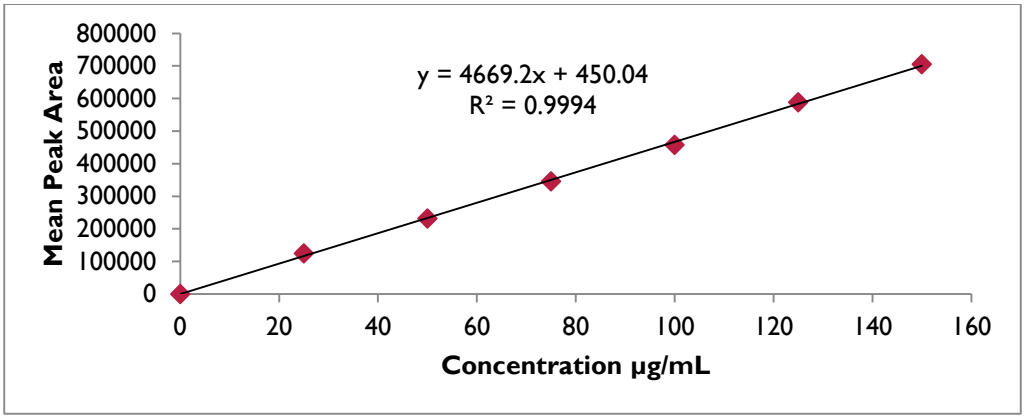


Fig 1.10: Calibration curve of velpatasvir

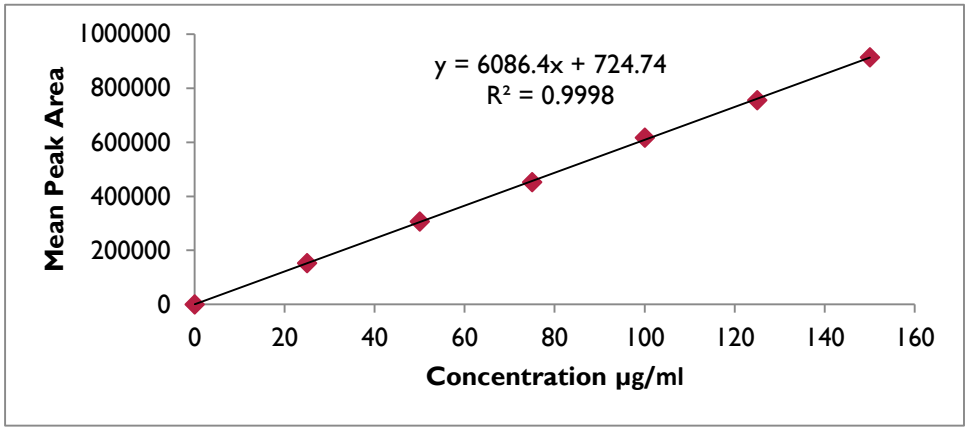


Fig 2.11: Calibration curve of voxilaprevir

Table 1.6 Regression characteristics of the linearity plot of sofosbuvir, velpatasvir and voxilaprevir

Parameters	**Sofosbuvir**	**Velpatasvir**	**Voxilaprevir**
Linearity Range (µg/mL)	100-600µg/ml	25-150 µg/ml	25-150 µg/ml
Regression coefficient R^2	0.999	0.999	0.999
Slope(m)	7698	4669	6086.4
Intercept(c)	9259.2	450.04	724.74
Regression equation (Y=mx+c)	y =7698x + 9259.2	y = 4669.2x +450.04	y = 6086.4x + 724.74

Table 1.8: Accuracy data of sofosbuvir.

% Level	**Amount Spiked (µg/mL)**	**Peak Area**	**Amount recovered (µg/mL)**	**% Recovery**	**Mean % Recovery**

50%	200	4604515	196.942	98.47	99.10
	200	4613963	198.169	99.08	
	200	4623906	199.461	99.73	
100%	400	6153175	398.118	99.53	99.12
	400	6152590	398.042	99.51	
	400	6115966	393.285	98.32	
150%	600	7691877	598.002	99.67	99.10
	600	7625812	589.420	98.24	
	600	7679590	596.406	99.40	

Table 1.11 Limit of Detection and Limit of Quantification

Parameters	**sofosbuvir**	**velpatasvir**	**voxilaprevir**
LOD	1.02	0.23	0.24
LOQ	3.10	0.68	0.72

Table 1.12: Robustness data of sofosbuvir.

Variations	Chromatographic Parameters				
	RT	Peak Area	Tailing factor	Theoretical Plates	Resolution
36% of ACN in the mobile phase	1.246	3204186	1.25	2747	-
44% of ACN in the mobile phase	1.233	3248926	1.26	2969	-
Flow rate at 0.27 mL/min	1.240	3264870	1.29	2986	-
Flow rate at 0.33 mL/min	1.197	3126604	1.23	2854	-
Temperature at 25^0C	1.235	3293110	1.25	2943	-
Temperature at 35^0C	1.245	3406564	1.35	2727	-

Table 1.15 Assay data of sofosbuvir

S.no	**Area of standard solution**	**Area of formulation**	**% Assay**
1	3137149	3133880	99.60
2	3156000	3127588	98.80
3	3101698	3135849	100.80

4	3157898	3127969	98.76
5	3164945	3131331	98.64
6	3169808	3150516	99.09
Avg	3150070	3134522	99.28
SD	25243.9	8475.7	0.819
% RSD	0.8	0.3	0.8

Table 1.18: Forced degradation studies data of sofosbuvir.

Degradation type	**Stress condition**	**sofosbuvir**		
		Standard Area	**Sample Area**	**% Assay**
Acidic	2 N HCl refluxed at 60^0C for 30 min	3147916	2937106	93.02
Basic	2 N NaOH refluxed at 60^0C for 30 min	3147916	2995607	94.88
Peroxide	20 % H_2O_2 bench top 30 min	3147916	3016070	95.52
Thermal	Heated at 105^0C for 6 h	3147916	3061946	96.98
UV	UV chamber at 200 Watts h/m^2 for 7 days	3147916	3098422	98.13
Neutral	Water refluxed at 60^0C for 30 min	3147916	3121205	98.85

S.NO	Peak Areas		
	sofosbuvir	velpatasvir	voxilaprevir
	Intra day	Intra day	Intra day
1	3137149	454455	615941
2	3156000	456120	615496
3	3101698	463143	618626
4	3157898	454647	612261
5	3164945	462945	615487
6	3169808	459350	614657
Mean	3150070	458443	615411
SD	25243.9	3972.2	2055.3
% RSD	0.8	0.9	0.3

Table 2.7: Intraday precision results

S.NO	**Peak Areas**					
	sofosbuvir		**velpatasvir**		**voxilaprevir**	
	Day1	**Day2**	**Day1**	**Day2**	**Day1**	**Day2**
1	3137149	3154572	454455	445229	615941	597872
2	3156000	3124700	456120	447042	615496	598377
3	3101698	3134726	463143	446991	618626	601544
4	3157898	3138672	454647	442352	612261	587886

5	3164945	3112147	462945	448590	615487	593342
6	3169808	3117002	459350	451955	614657	605164
Mean	3150070	3130303	458443	447027	615411	597364
SD	25243.9	15598.3	3972.2	3219.3	2055.3	6092.7
% RSD	0.8	0.5	0.9	0.7	0.3	1.0

Table 2.5: Inter-day precision results

Table 2.9 Accuracy table of velpatasvir

% Level	Amount Spiked (µg/mL)	Peak Area	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	701886	50.2326	100.47	100.08
	50	701242	50.0947	100.19	
	50	699824	49.791	99.58	
100%	100	929118	98.901	98.90	99.53
	100	936521	100.486	100.49	
	100	930512	99.199	99.20	
150%	150	1155704	147.431	98.29	98.97
	150	1160741	148.51	99.01	
	150	1165119	149.447	99.63	

Table 2.10 Accuracy table of voxilaprevir

% Level	Amount Spiked (µg/mL)	Peak Area	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	912963	49.891	100.02	99.87
	50	915929	50.378	99.19	
	50	910772	49.531	99.51	
100%	100	1213175	99.219	99.22	99.45
	100	1209590	98.631	98.63	
	100	1220966	100.50	100.50	
150%	150	1521877	149.943	99.96	99.84
	150	1520812	149.768	99.85	
	150	1519590	149.567	99.71	

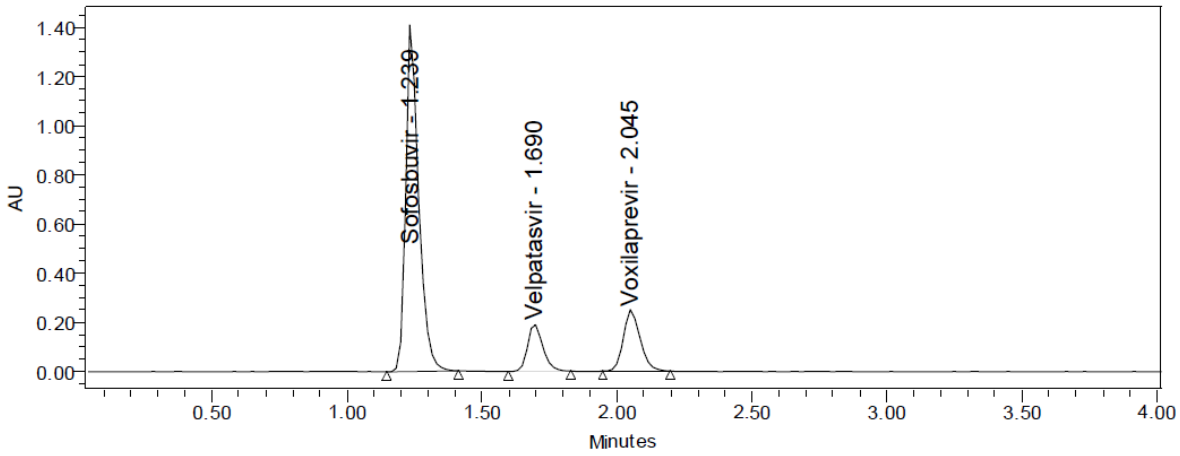


Fig 1.12: A representative chromatogram of sofosbuvir,velpatasvir and voxilaprevir at Accuracy 50%

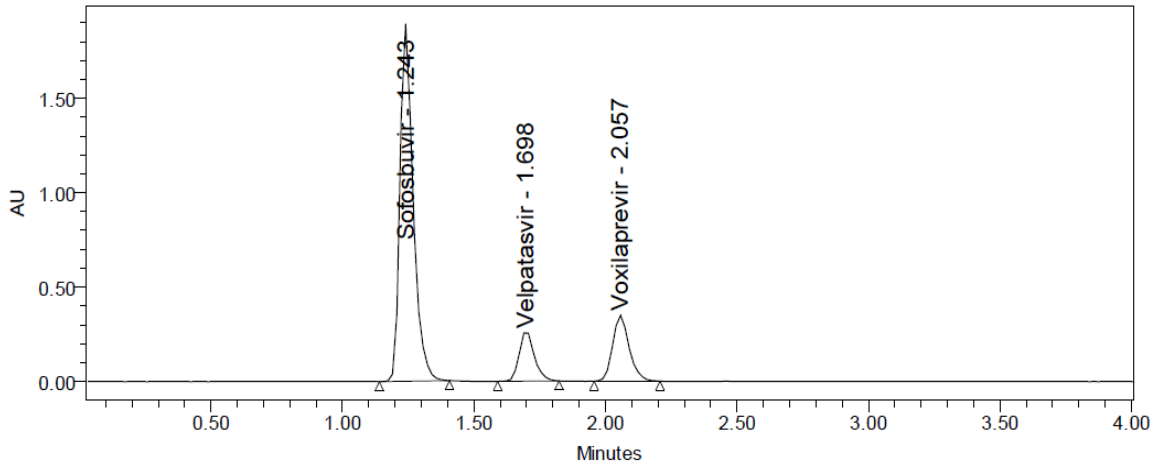


Fig 1.13: A representative chromatogram of sofosbuvir,velpatasvir and voxilaprevir at Accuracy 100%

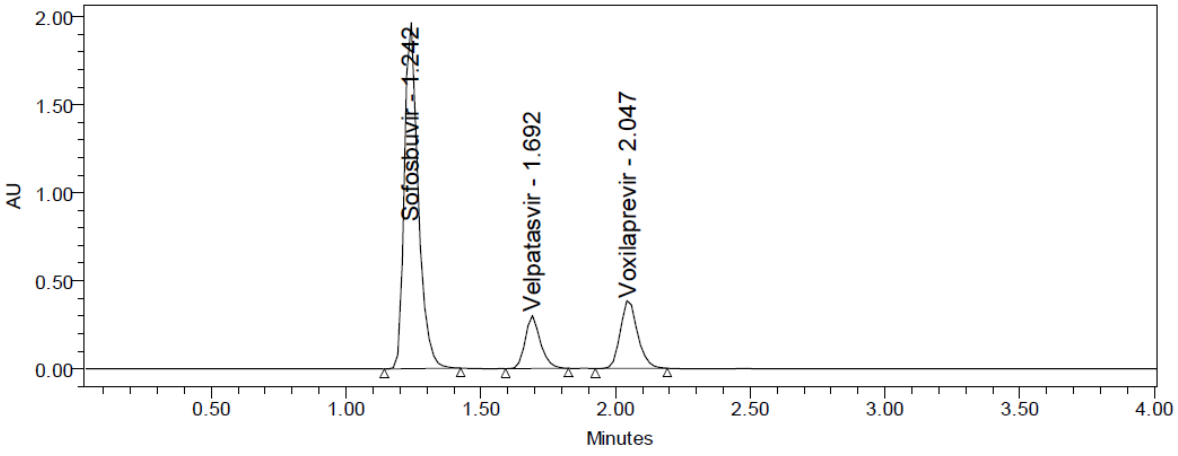


Fig 1.14: A representative chromatogram of sofosbuvir,velpatasvir and voxilaprevir at Accuracy 150%

Table 2.13: Robustness data of velpatasvir

Variations	Chromatographic Parameters

	RT	Peak Area	Tailing	Theoretical Plates	Resolution
36% of ACN in the mobile phase	1.773	492669	1.18	4228	5
44% of ACN in the mobile phase	1.619	497902	1.20	4260	4
Flow rate at 0.27 mL/min	1.693	496026	1.14	4095	4.5
Flow rate at 0.33 mL/min	1.628	484258	1.16	3975	4.5
Temperature at 25^0C	1.649	510359	1.16	4279	4.3
Temperature at 35^0C	1.734	511753	1.21	3932	4.7

Table 2.14: Robustness data of voxilaprevir

Variations	Chromatographic Parameters				
	RT	Peak Area	Tailing	Theoretical Plates	Resolution
36% of ACN in the mobile phase	2.176	705423	1.17	5326	3.4
44% of ACN in the mobile phase	1.935	719216	1.18	5002	3
Flow rate at 0.27 mL/min	2.047	713291	1.15	5226	3.1
Flow rate at 0.33 mL/min	1.966	695508	1.15	5067	3.1
Temperature at 25^0C	1.989	730327	1.15	4851	3.2
Temperature at 35^0C	2.122	736015	1.16	5030	3.4

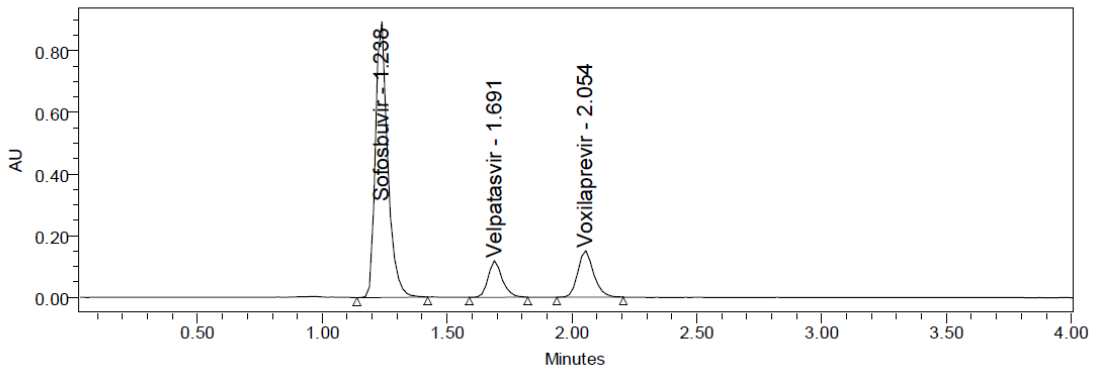


Fig 1.15: Chromatogram of marketed formulation

Table 2.16 Assay data of velpatasvir

S.no	Area of standard solution	Area of formulation	% Assay
1	454455	458678	100.73
2	456120	452362	98.43
3	463143	454739	97.99
4	454647	457349	100.39
5	462945	456487	98.41
6	459350	457270	99.35
Avg	458443	456148	99.22
SD	3972.2	2259	1.14
% RSD	0.9	0.5	1.1

Table 2.17 Assay data of voxilaprevir

S.no	Area of standard solution	Area of formulation	% Assay
1	615941	615069	99.76
2	615496	613105	99.51
3	618626	612010	98.83
4	612261	615635	100.45
5	615487	614077	99.67
6	614657	616046	100.13
Avg	615411	614324	99.72
SD	2055.3	1558.2	0.554
% RSD	0.3	0.3	0.6

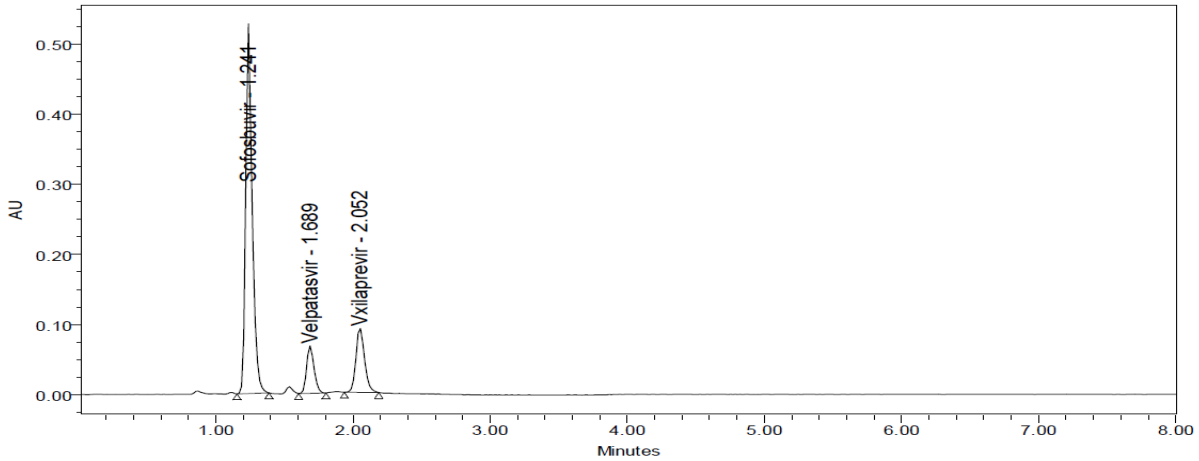


Fig 1.16 : Chromatogram of Acid degradation.

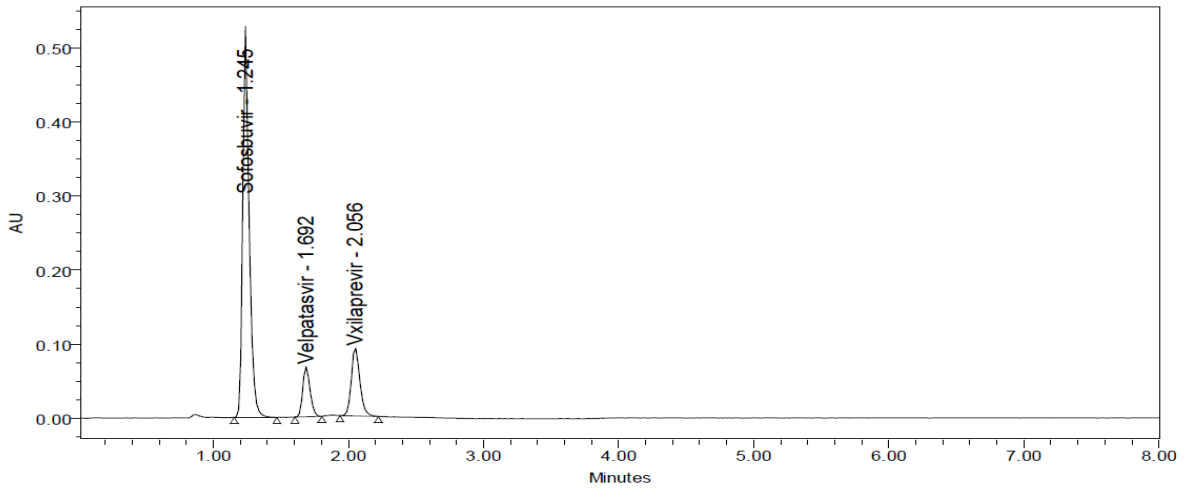


Fig 1.17 : Chromatogram of base degradation.

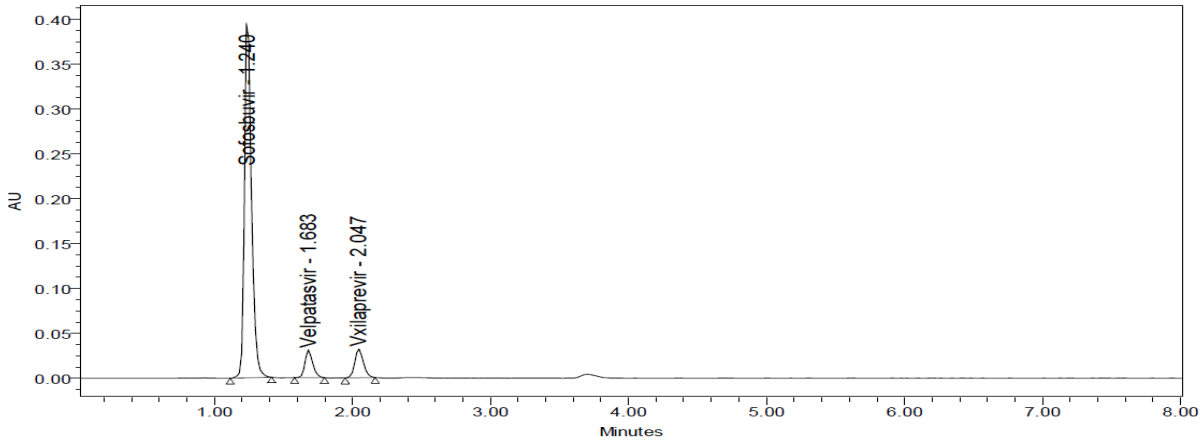


Fig .18 : Chromatogram of oxidative degradation.

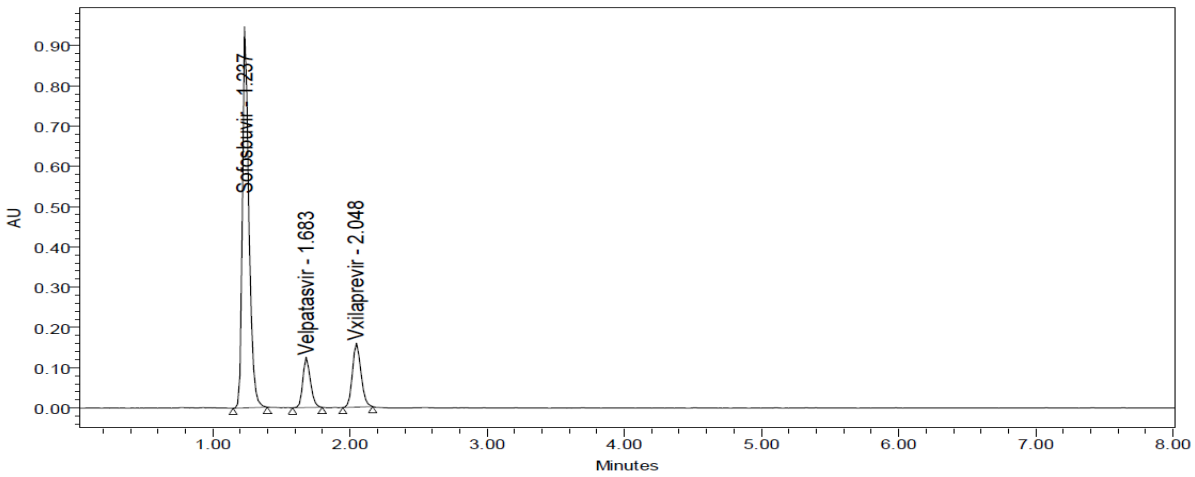


Fig 1.19: Chromatogram of thermal degradation

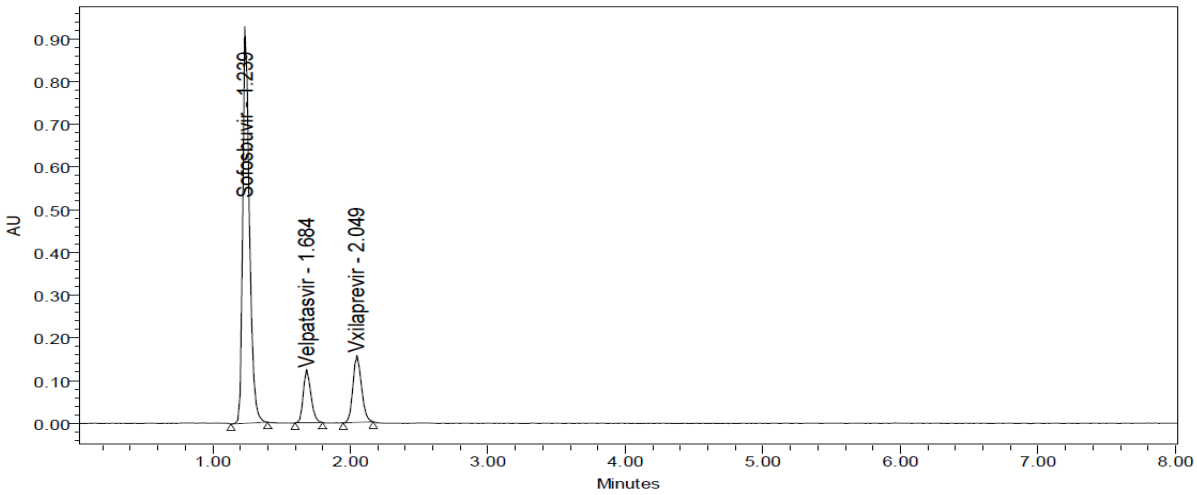


Fig 1.20: Chromatogram of photolytic degradation

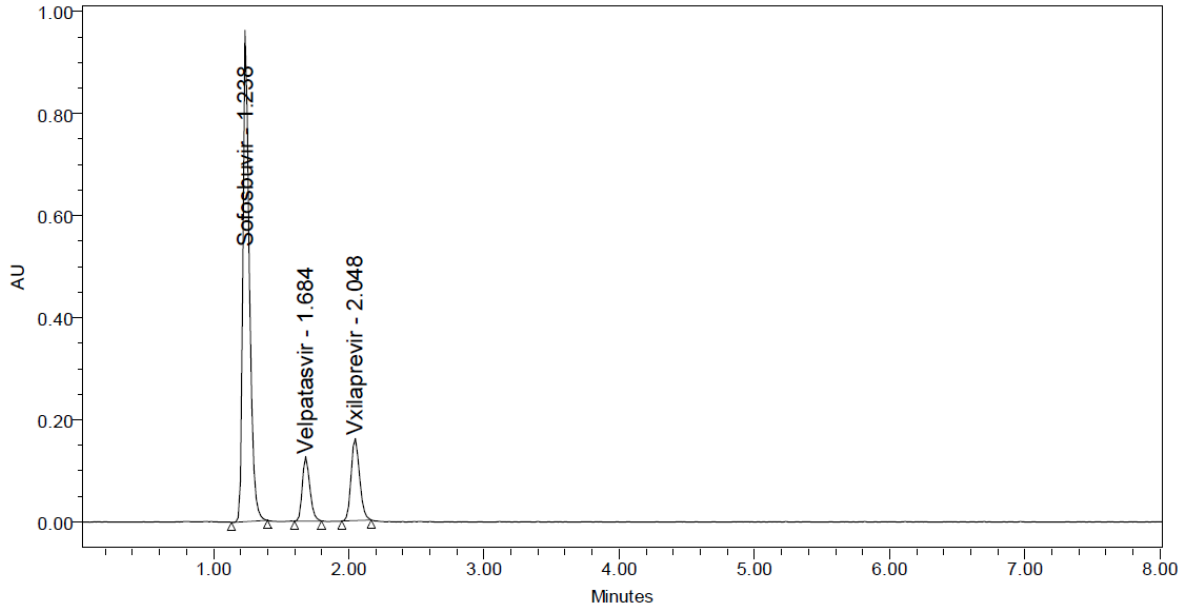


Fig 1.21: Chromatogram of neutral degradation

Table 2.19: Forced degradation studies data of velpatasvir.

Degradation type	Stress condition	velpatasvir		
		Standard Area	Sample Area	% Assay
Acidic	2 N HCl refluxed at 60^0C for 30 min	458443	429805	93.66
Basic	2 N NaOH refluxed at 60^0C for 30 min	458443	431805	94.10
Peroxide	20 % H_2O_2 bench top 30 min	458443	439335	95.74
Thermal	Heated at 105^0C for 6 h	458443	441437	96.19
UV	UV chamber at 200 Watts h/m^2 for 7 days	458443	450473	98.16

Neutral	Water refluxed at 60^0C for 30 min	458443	457051	99.60

Table 2.20: Forced degradation studies data of voxilaprevir.

Degradation type	Stress condition	voxilaprevir		
		Standard Area	Sample Area	% Assay
Acidic	2 N HCl refluxed at 60^0C for 30 min	615411	582310	94.53
Basic	2 N NaOH refluxed at 60^0C for 30 min	615411	586053	95.13
Peroxide	20 % H_2O_2 bench top 30 min	615411	593289	96.31
Thermal	Heated at 105^0C for 6 h	615411	599143	97.26
UV	UV chamber at 200 Watts h/m^2 for 7 days	615411	604531	98.13
Neutral	Water refluxed at 60^0C for 30 min	615411	615464	99.91

4. CONCLUSION

The purpose of this research was to develop a specific, accurate and precise UPLC method for the estimation of sofosbuvir, velpatasvir and voxilaprevir in bulk and in pharmaceutical dosage. The method was developed by studying different parameters and chromatographic separation was optimized using CHS C18 column (100 x 3mm, 1.7µ). An isocratic elution was employed with pH 4.0 phosphate buffer and acetonitrile in the ratio of 60:40 v/v as mobile phase. The flow rate of the mobile phase was 0.3 ml/min and the column temperature were maintained at 30^0C. The detection wavelength was optimized at 260nm. Retention times were found to be 1.236 min, 1.696 min and 2.055 min for sofosbuvir, velpatasvir and voxilaprevir respectively.

The developed method was validated as per ICH guidelines. The system performance was checked by injecting six replicas of working standard solution, same retention time was observed for all the cases. No significant interferences due to diluents, excipients and degradants at the retention time of drugs was observed. Hence the method was said to be specific. The method was found to be precise, the % RSD values for intraday precision and inter-day precision experiments were less than 2.0%. The linear relationship (R^2> 0.999) was observed between peak areas of drugs and the corresponding concentrations over 100-600 µg/ml of sofosbuvir, 25-100 µg/ml of voxilaprevir and velpatasvir. The mean linear regression equation observed was y=7698x+9259.2 for sofosbuvir, y=4669.2x+450.04 for velpatasvir and y=6086.4x+724.74 for voxilaprevir. The LOD values for sofosbuvir, voxilaprevir and velpatasvir was found to be 1.02 µg/ml ,0.23 µg/ml and 0.24 µg/ml respectively. The LOQ values for sofosbuvir, voxilaprevir and velpatasvir was found to be 3.10 µg/ml,0.68 µg/ml and 0.72 µg/ml respectively. The mean % recovery was calculated at three different concentration levels (50%,100% and 150%) to determine accuracy of the method. The overall mean % recovery for sofosbuvir, velpatasvir and voxilaprevir was found to be 99.10%, 99.52% and 99.72% respectively. The method was found to be accurate as % mean recovery was within the specified limits. Robustness was tested by varying minor changes in flow rate, organic content in mobile phase and temperature. The system suitability parameters remain unaltered when flow rate was 0.3±0.03 ml/min, organic content in mobile phase was 40±4% and temperature was 30 ± 5^0C.

The developed UPLC method was specific, precise, accurate, sensitive and robust for the determination of sofosbuvir, velpatasvir and voxilaprevir in bulk and dosage form. The run time was less than 5min.So faster analysis and less amounts of solvents were consumed. The method can be applicable for routine analysis of these drugs in quality control laboratories.

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