Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Sofosbuvir, Velpatasvir, and Voxilaprevir in Tablet Formulation

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Abstract

Objective: The present study aimed to develop a stability-indicating reverse-phase high performance-liquid chromatography (RP-HPLC) method for the estimation of Sofosbuvir, Velpatasvir, and Voxilaprevir in tablet dosage form and validated in accordance with ICH guidelines. Methods: The optimized conditions for the developed RP-HPLC method are Agilent C18 (250 mm×4.6mm, 5?) column maintained at 30ºC with a mobile phase consisting of Buffer(0.1

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1 Introduction

epatitis C [1] is a liver infection which is caused by the Hepatitis C virus. The hepatitis C virus is a blood-borne virus and the most common modes of infection are through exposure to small quantities of infected blood. Globally, around 70 million people were suffering from Hepatitis C infection. Antiviral medicines like sofosbuvir, velpatasvir, and voxilaprevir, etc; can cure more than 95% of persons having Hepatitis C infection and reduce the causes of death. As per the literature survey [5][6][7][8][9][10][11], it is learned that very few HPLC methods have been reported for the determination of Sofosbuvir, Velpatavir, and Voxilaprevir individually and in combination by HPLC but there is no method for stability-indicating and simultaneous estimation of all the three drugs.(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxy carbonyl) amino]-2-phenylacetyl}-4-(methoxy methyl) pyrrolidin-2-yl]-1H-imidazol-4-yl}-1,11-dihydro[2] benzo- pyrano[4',3':6,7]naphtho[1,2-d]imidazol-2-yl)-5-methyl- pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl}carbamate.

Therefore, there is a need to develop a rapid and reliable Stability-indicating HPLC method for the simultaneous determination of Sofosbuvir, Velpatasvir, and Voxilaprevir in bulk and pharmaceutical dosage form.

2 II.

3 Materials And Methods

4 a) Reagents and chemicals

Sofosbuvir, Velpatasvir and Voxilaprevir working standards were procured from spectrum pharma research solutions, Hyderabad, as a gift sample. The VOSEVI tablets were supplied by the Medindia Pharma network. All the chemicals used were of AR grade purchased from Merck, Mumbai. All the solvents used were of HPLC grade purchased from Sigma-Aldrich, Mumbai.

5 b) Chromatographic conditions and Instruments

WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector, and an auto sampler integrated with Empower 2 software and Agilent C18 (250mm × 4.6mm, 5µ) column was used for the determination of Sofosbuvir, Velpatasvir, and Voxilaprevir. The optimized conditions included 0.1%

Orthophosphoric acid (OPA) and acetonitrile (55:45%v/v) as mobile phase run on an isocratic mode at flow rate 1.0ml/min. The column was maintained at 300C and detection was done at 220nm. Other equipment used in the method was Ultrasonic bath sonicator (BVK Enterprises) and weighing balance (Denver).

6   c) Preparation of Diluent

A mixture of water and acetonitrile in the ratio of 50:50%v/v was used as diluents.

7   d) Preparation of Mobile phase

A mixture of 0.1% orthophosphoric acid and Acetonitrile in the ratio (55:45%v/v) was used as the mobile phase.

8   e) Preparation of Standard and Sample solutions

20mg, 5mg & 5mg of Sofosbuvir, Velpatasvir and Voxilaprevir working Standards were transferred to 50ml of volumetric flasks separately, 3/4th of diluents (as mentioned) was added to all the three flasks and subjected for sonication for 10 minutes. The final volume was made up with diluents to obtain a final concentration of 400μg/ml of Sofosbuvir, 100μg/ml of Velpatasvir, & 100μg/ml of Voxilaprevir respectively.

From the above stock solution, 1 ml was pipetted out into a 10ml volumetric flask and then the final volume was made with the same diluent. (40μg/ml of Sofosbuvir, 10μg/ml of Velpatasvir and 10μg/ml of Voxilaprevir respectively) 10 Tablets (Vosevi) were weighed accurately and the average weight was calculated. An amount equivalent of 1 tablet was collected into a 50ml volumetric flask; 15ml of diluents was mixed and sonicated for around 30 minutes. It was then subjected to making the volume with diluents.

Filtered the solution and diluted 1ml of the above solution to 10ml with diluents. (40μg/ml of Sofosbuvir & 10μg/ml of Velpatasvir & 10μg/ml of Voxilaprevir).

9   f) Method Validation

The developed method was validated in compliance with International Conference on Harmonization (ICH) guidelines [12,13].

10   g) Specificity

The specificity of the method was determined by comparing the drug solution with the placebo solution and observed for the interference of placebo peak with drug peak.

11   h) Accuracy

The accuracy of the present method was determined by %recovery. The drug solution along with the sample was prepared in three concentration levels 50%, 100%, and 150%. Then the % recovery was calculated.

12   i) Precision

The precision of the method was estimated by injecting the six solutions of the standard into the HPLC system and the % relative standard deviation (%RSD) was calculated.

13   j) Linearity

The linearity of the method was developed by preparing series of dilutions ranging from 12.5μg/ml -75μg/ml for Bictegravir, 50μg/ml-300μg/ml for Emtricitabine and 6.25μg/ml-37.5μg/ml for Tenofovir alafenamide respectively and injecting them into HPLC system.

14   k) Ruggedness

Ruggedness was determined by injecting the six solutions of the standard into HPLC for different days. The % RSD was calculated.

15   l) Robustness

Robustness of the method was determined by varying the optimized analytical conditions such as mobile phase composition by ±5%, flow rate by ±0.1ml/min and column temperature by ±5ºC.

16   m) LOD and LOQ

Calculation of limit of detection as well as Limit of quantification had been done by using standard Equations. LOD= 3.3×?/S, LOQ =10×?/S. Here ? denotes for the standard deviation of intercepts of regression lines, S denotes for slope.

17 n) Solution stability

Solution stability was estimated by analyzing the standard drug solution after storage for 24hrs under laboratory conditions.

18 o) Forced degradation studies

Forced degradation studies [14] were carried out for drug by exposing the drug solution to the various stress conditions such as acidic (2N Hydrochloric acid for 30min at 60ºC), basic (2N Sodium hydroxide for 30min at 60ºC), Oxidation (refluxing the drug solution with 20%H2O2), neutral (refluxing the drug in water for 6h at 60ºC), photolytic (exposing the drug solution to UV light by keeping the solution in UV chamber for 7 days or 200-watt hrs/m2 in photostability chamber), thermal (drug solution was placed in oven at 105ºC for 6hrs) conditions.

19 III.

20 Results

21 DISCUSSION

For the development of a new method for the simultaneous estimation of Sofosbuvir, Velpatasvir, and Voxilaprevir in bulk and pharmaceutical dosage form initially many mobile phases and many columns were tried to elute the drug peak with less tailing factor, more plate count and more resolution.

Waters HPLC Agilent C18 (250mm × 4.6mm, 5µ) column and Buffer: Acetonitrile (55:45%v/v) as mobile phase were selected based on peak parameters. The detection wavelength was found to be 220nm.

Prepared standard solution, sample solution, and blank solution were injected into the HPLC system and system suitability parameters were noted as summarized in Table 1 along with chromatograms as shown in fig. 4, 5, 6 and 7 respectively.

The developed method was found to obey Beer's law in the concentration range of 10-60 µg/ml for Sofosbuvir, 2.5-15 µg/ml for velpatasvir and 2.5-15 µg/ml for Voxilaprevir with a correlation coefficient of 0.999 each respectively. A linear graph was plotted between concentration and peak area as shown in fig. **??**.10 and 11 respectively and results are summarized in Table 1.

The method was found to be accurate as the % recovery was 98.50%-101.50% for all the three drugs and was within the limits. The %RSD was found to be less than 1 for all the three drugs indicates that the method was precise. The method was found to be specific, as there is no interference of retention time of placebo peak with that of drug peak. The placebo chromatogram was shown as fig. **??**.

Forced degradation studies results indicate that the drug was found to be stable in various stress conditions as net degradation was found to be within the limits. The chromatograms were shown in fig. 12-17 and results were summarized in Table 2.
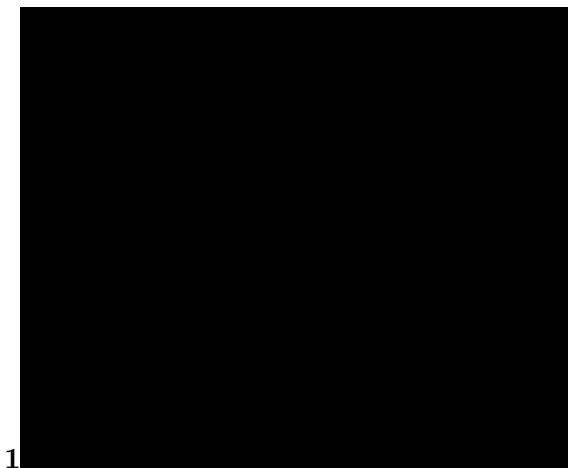
V.

22 CONCLUSION

A specific, precise, stability-indicating method was developed for the determination of Sofosbuvir, Velpatasvir, and Voxilaprevir in pure and tablet dosage form using RP-HPLC. The method was validated by using various validation parameters and the method was found to be linear, precise, accurate, specific and robust. From the degradation, studies conducted it is concluded that Sofosbuvir, Velpatasvir, and Voxilaprevir were stable at high concentrations of Acid, Base, Peroxide, Thermal, UV and Water stress study conditions. The run time was 5min which enables rapid quantitation of many samples in routine and quality control analysis of tablet formulations.
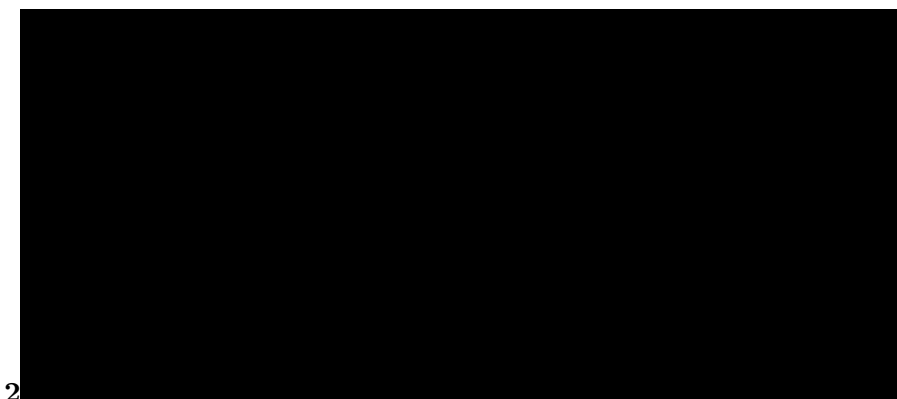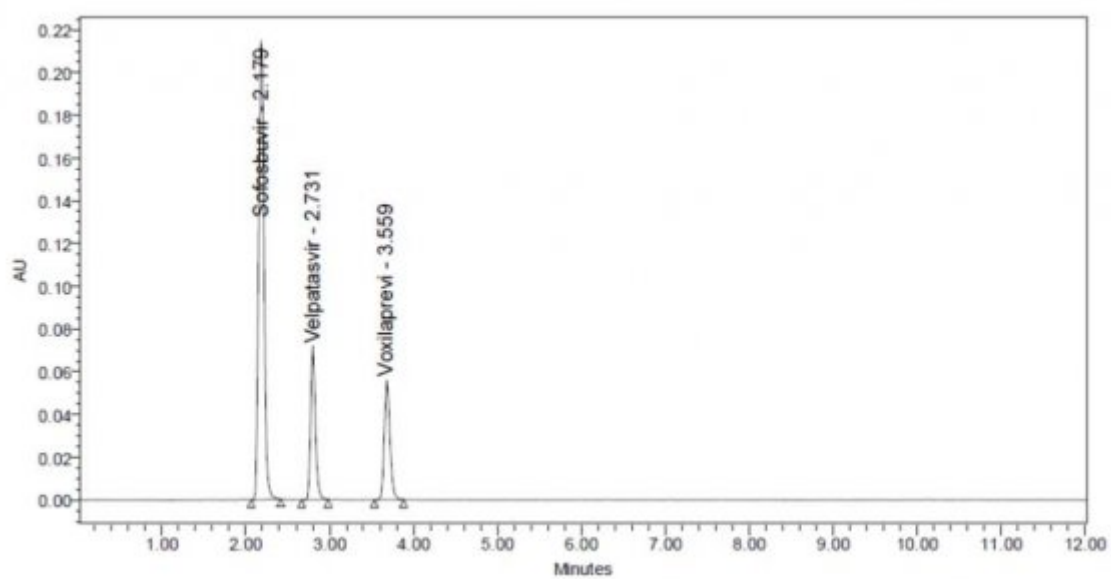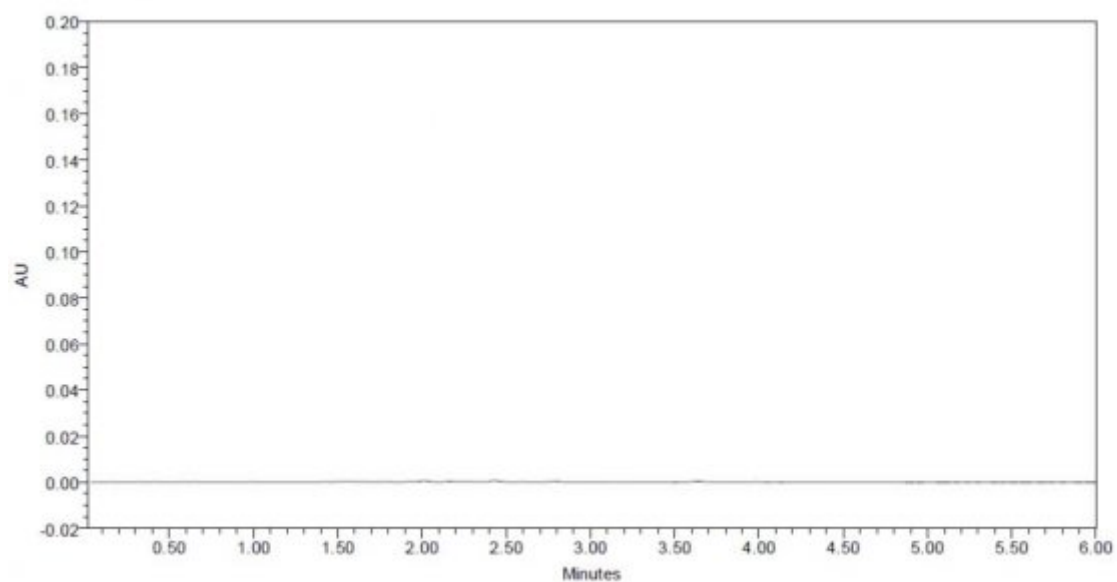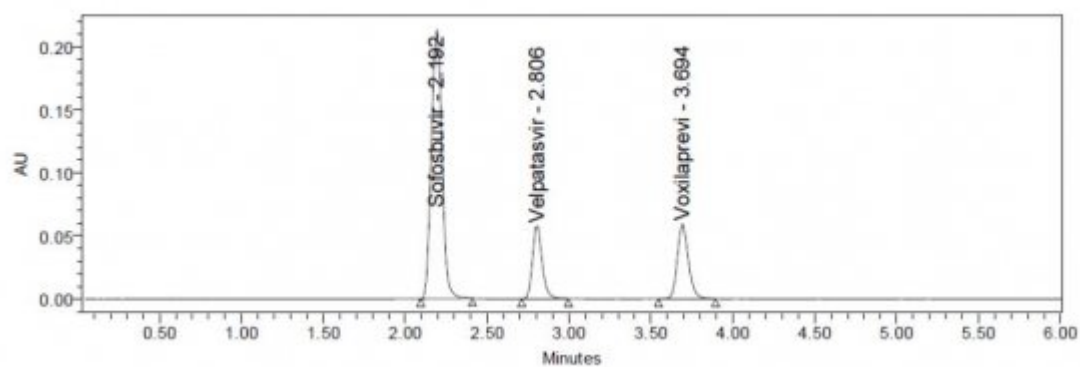
Figure 1:

Figure 2: Figure 1 :

Figure 3: Figure 2 :
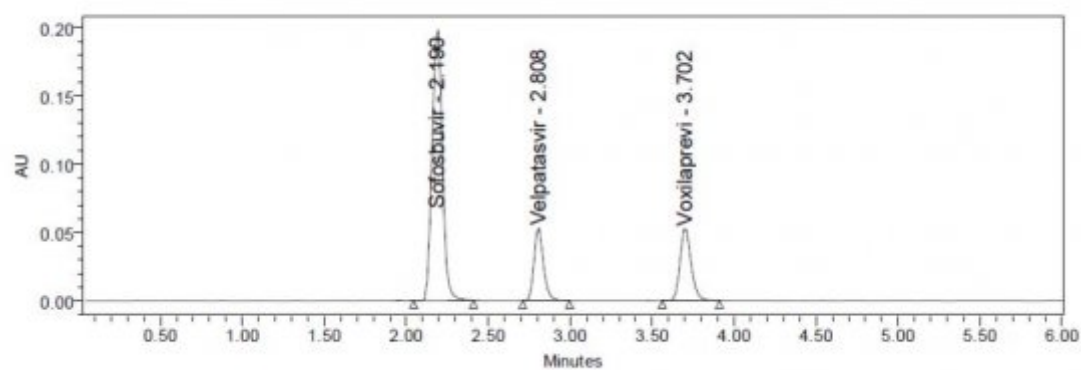
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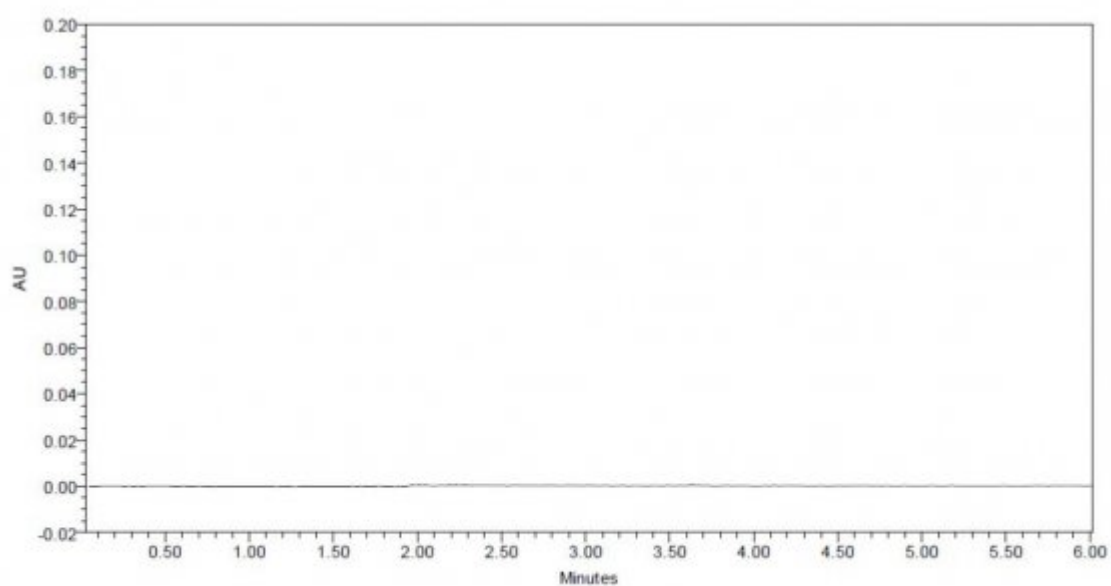Figure 4: Figure 3 :

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Figure 5: Figure 4 :
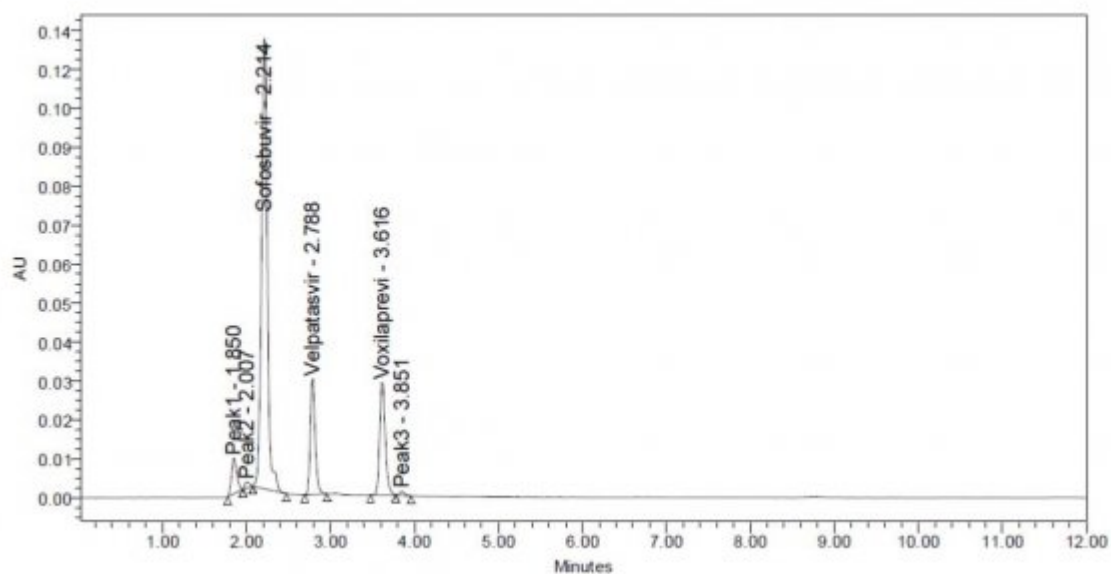
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Figure 6: Figure 5 :Figure 6

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Figure 7: Figure 7 :Figure 8 :Figure 9 :Figure 10 :

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Figure 8: Figure 11 :Figure 12 :
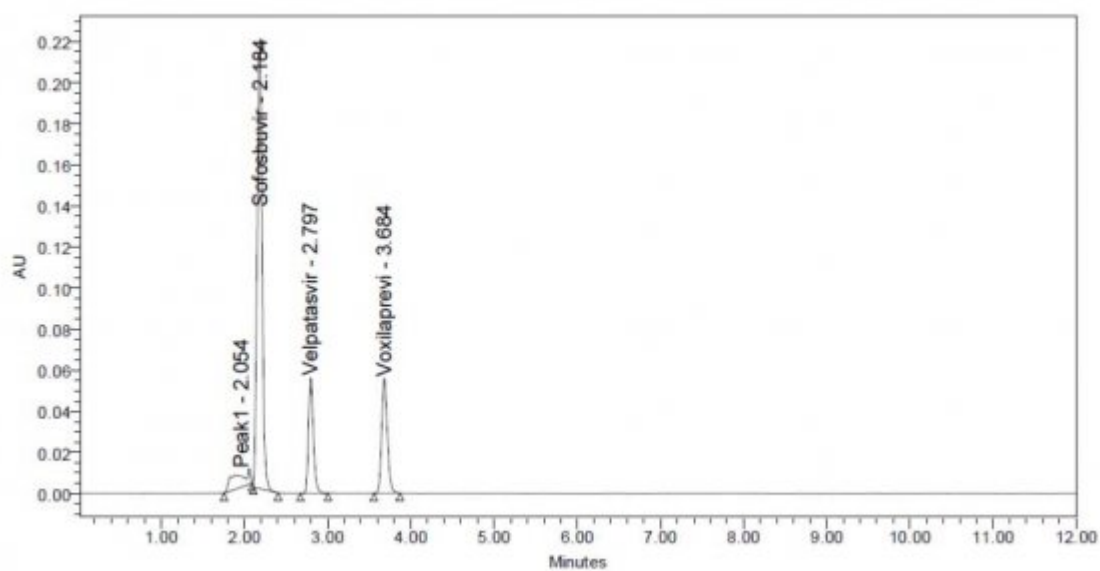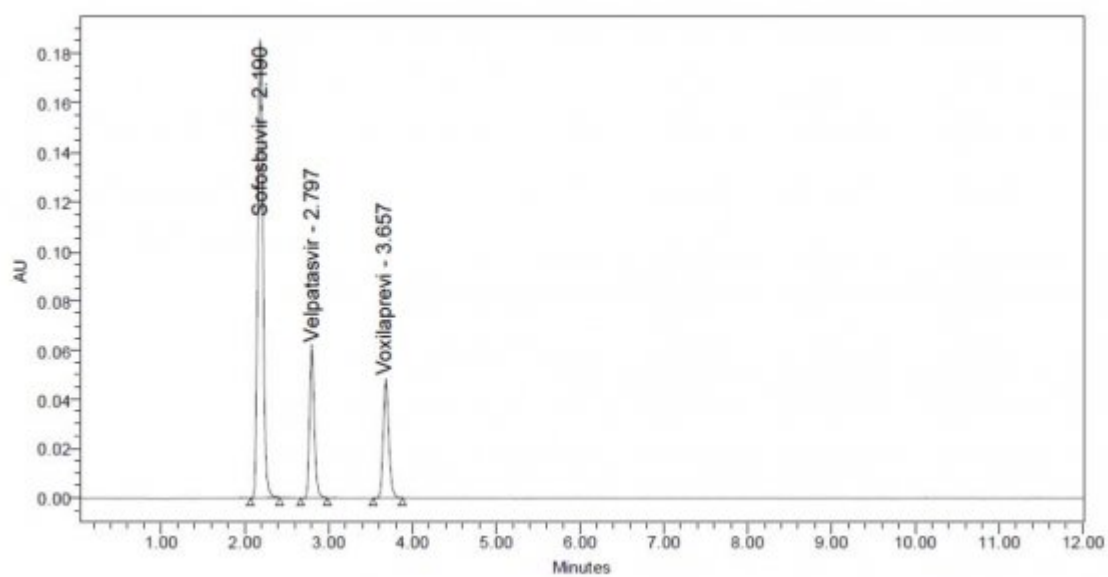
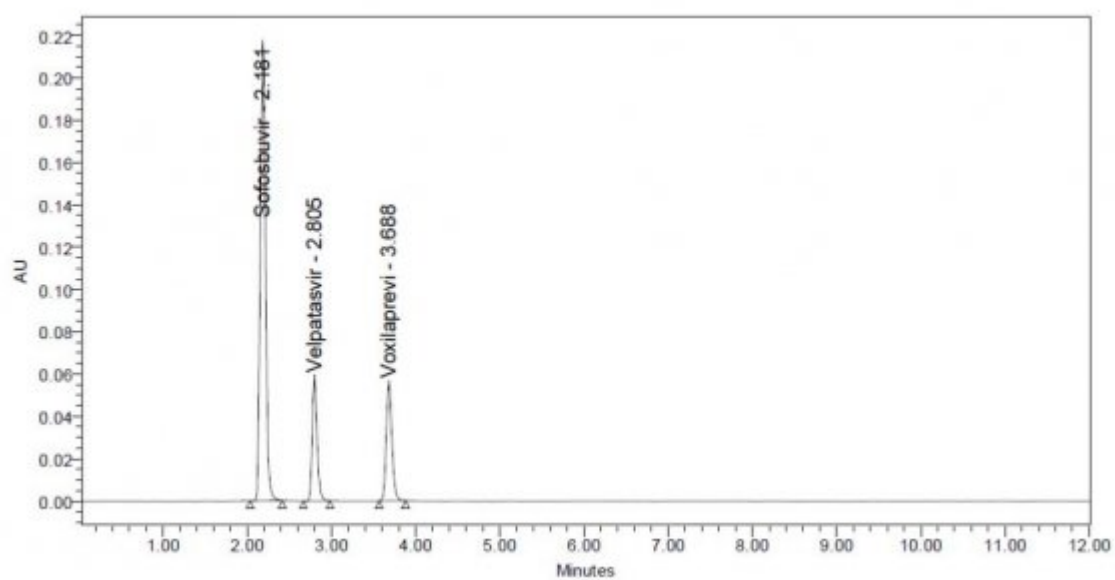Figure 9: Figure 13 :Figure 14 :Figure 15 :

Figure 10:

Figure 11:

Figure 12:

8

1

Parameter	Result		
USP Plate count	4088	10175	126558
USP Tailing factor	1.0	1.2	1.2
USP Resolution	4.5	-	7.2
Precision (%RSD)	0.9	0.8	0.4
Accuracy	98.50-101.50	98.50-101.50	98.50-101.50
Specificity	Specific, No interference		
Linearity range (µg/ml)	10-60	2.5-15	2.5-15
Correlation coefficient, r 2	0.999	0.999	0.999
LOD (µg/ml)	0.11	0.10	0.06
LOQ (µg/ml)	0.34	0.29	0.17
Ruggedness (%RSD)			
Day1	0.9	0.8	0.4
Day2	0.9	1.9	0.6
Robustness (%RSD)			
Flow rate -	1.2	1.1	1.0

Figure 13: Table 1 :

2

Stress condition	Sofosbuvir		Velpatasvir		Voxilaprevir	
	%Assay	%D	%Assay	%D	%Assay	%D
Acid	94.23	5.77	94.04	5.96	94.14	5.86
Base	95.35	4.65	95.10	4.9	94.49	5.51
Neutral	99.37	0.63	99.51	0.49	99.67	0.33
Peroxide	95.95	4.05	95.58	4.42	96.61	3.39
Photolytic	97.73	2.27	97.58	2.42	99.08	0.92
Thermal	98.22	1.75	96.43	3.57	98.71	1.29

%D-Percentage Degradation

Figure 14: Table 2 :

3.4	3.7	3.7
2.8	2.7	2.9
2.2	2.4	2.2
3.8	3.4	3.2
2.4	2.7	2.4
1.0	0.6	1.0
0.9	1.0	0.6

Figure 15:

.1 Acknowledgment

.2 Conflict of Interests

The authors claim that they have no conflict of interest. It has not meant to publish elsewhere. Moreover, it has not meant simultaneously presented for publication elsewhere.

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