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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN TABLET DOSAGE FORM

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Keywords:

Sofosbuvir, Ultra Performance Liquid Chromatography (UPLC), Velpatasvir, Validation, Simultaneous estimation

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ABSTRACT: The objective of the method was to develop a simple, rapid, sensitive, precise, accurate and validated Ultra Performance Liquid Chromatographic (UPLC) method for the simultaneous estimation of Sofosbuvir and Velpatasvir in tablet dosage form. Chromatographic separation was achieved on an acquity UPLC HSS C18, 2.1 × 100 mm, 1.8µ column with a mobile phase composed of orthophosphoric acid buffer and acetonitrile in the ratio of 45:55 at a flow rate of 0.2 ml/min and 1 µl injection volume. The effluents were detected at a wavelength of 250 nm using TUV detector. The retention times of Sofosbuvir and Velpatasvir were found to be 1.425 and 1.767 min respectively. The method was validated with respect to specificity, accuracy, linearity, precision, robustness. The correlation coefficient for Sofosbuvir and Velpatasvir were found to be 0.9992 and 0.9995 respectively. Recovery of Sofosbuvir and Velpatasvir in formulation was found to be 99.48% and 99.6% respectively. Due to simplicity, high precision and rapidness the method can be successfully applied for simultaneous estimation of Sofosbuvir and Velpatasvir in combined dosage form.

INTRODUCTION: Sofosbuvir (SBR), chemically Isopropyl (2*S*) -2 -[[[(2*R*, 3*R*, 4*R*, 5*R*)-5-(2, 4-dioxopyrimidin -1 -yl) -4-fluoro -3-hydroxy -4-methyl- tetrahydrofuran -2 -yl] methoxy-phenoxy phosphoryl]amino] propanoate is a nucleotide prodrug that potently inhibits genotype 1 to 6 hepatitis c virus (HCV) RNA replicons *in-vitro* and has demonstrated high sustained virologic response (SVR) rates [1, 2, 3]. The structure of Sofosbuvir was shown in **Fig. 1**.

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Velpatasvir is a novel HCV non-structural protein 5A (NS5A) inhibitor that is being developed in combination with Sofosbuvir and other direct acting antivirals for the treatment of HCV infection. The chemical name of Velpatasvir is Methyl {(1*R*)-2-[(2*S*,4*S*) -2-(5 -{2-[(2*S*, 5*S*) -1-{(2*S*)-2-[(methoxy carbonyl) amino] -3 -methylbutanoyl} -5-methyl pyrrolidin-2-yl] -1, 11 -dihydro [2] benzopyrano [4',3':6,7] naphtha [1,2-*d*] imidazol-9-yl} -1*H*-imidazol-2-yl)-4(methoxymethyl) pyrrolidin-1-yl]-2-oxo-1-phenylethyl}carbamate[4]. The structure of Velpatasvir was shown in **Fig. 2**. Due to the additive antiviral interaction and lack of cross-resistance observed *in-vitro* between Sofosbuvir and Velpatasvir, the administration of these two drugs as a film-coated tablet is expected to provide significant antiviral activity and a favourable resistance profile.

The literature survey revealed that there are few stability indicating RP-HPLC [5, 6, 7, 8, 9, 10] and UV [11] spectrophotometric methods are available for the simultaneous estimation of Sofosbuvir and Velpatasvir in tablet dosage form. Few analytical methods for Sofosbuvir and other HCV drugs in biological fluids by UPLC-MS/MS have been reported [12, 13, 14, 15, 16]. However, a stability indicating UPLC method was not available. Hence, present work focused on the development and validation of a simple, rapid, robust and economical stability indicating UPLC [17, 18] method. To the best of our knowledge the anticipated method is the first UPLC method to allow simultaneous estimation of Sofosbuvir and Velpatasvir in tablet dosage form.

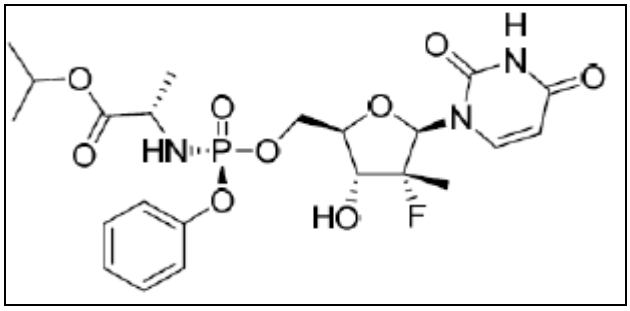

FIG. 1: CHEMICAL STRUCTURE OF SOFOSBUVIR

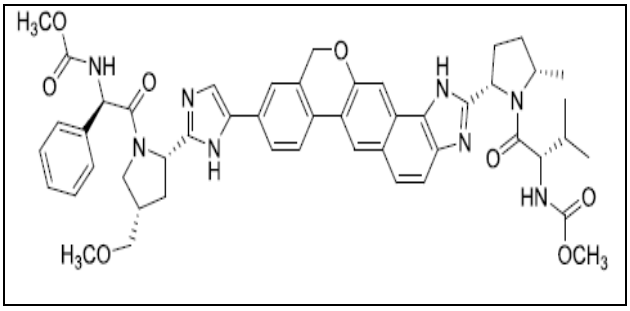

FIG. 2: CHEMICAL STRUCTURE OF VELPATASVIR

MATERIALS AND METHODS

Instrumentation: The separation was carried on Waters Acquity UPLC 2996 with Empower 2 software that consisted of a binary solvent manager equipped with automatic sampler. An acquity UPLC HSS C18, 2.1 ×100 mm, 1.8 µ column was used for separation of active ingredients. Analytes were monitored with TUV detector at a wavelength 250 nm. Ultrasonicator was used to remove dissolved gases and air bubbles in the mobile phase.

Chemicals and Reagents: Sofosbuvir and Velpatasvir standard samples were obtained as gift samples from Spectrum Labs, Hyderabad. HPLC grade water and methanol were purchased from Merck Ltd., Mumbai. Analytical grade acetonitrile and orthophosphoric acid were obtained from Rankem, Avantor Performance Material India Ltd. Marketed formulation of combination was purchased from local market.

Chromatographic Conditions: Separation of analytes was achieved with a mobile phase consisting of orthophosphoric acid buffer and acetonitrile at a ratio of 45:55 delivered at a flow rate of 0.2ml/min through column kept at 25 ºC. The volume of injection was 1 µl and runtime was 3 min. The eluents were detected at a wavelength 250 nm. Chromatograms of standard and optimized method were shown **Fig. 3** and **4**.

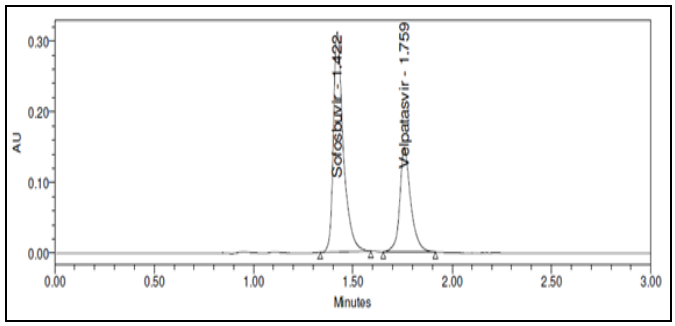

FIG. 3: CHROMATOGRAM OF OPTIMIZED METHOD

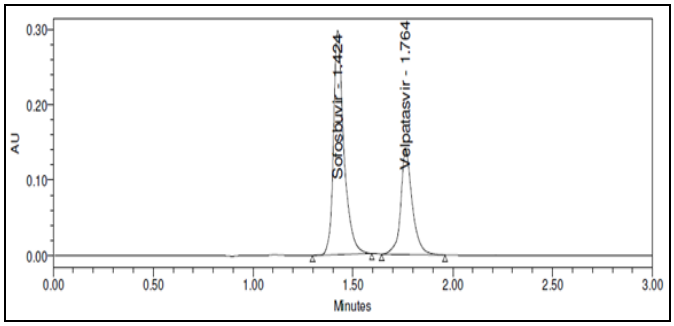

FIG. 4: CHROMATOGRAM OF STANDARD PREPARATION

Preparation of Standard Solution: Accurately weighed and transferred 40 mg of Sofosbuvir and 10 mg of Velpatasvir working standards into a 10 ml clean and dry volumetric flask. 3/4th volume of diluent was and sonicated for 5 min. Final volume was made with diluents. From above stock solution 1 ml from the above two stock solutions was taken into a 10 ml volumetric flask and made up to 10 ml.

Preparation of Sample Solution: 20 tablets were weighed, powdered and then accurately weighed the powder equivalent to 40 mg of Sofosbuvir and

10 mg of Velpatasvir into a 100 ml volumetric flask, 50 ml of diluent added and sonicated for 25 min, further the Volume was made up with diluent and filtered through 0.45 µ nylon filter. From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent.

System Suitability Studies: The system suitability test is used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard solutions. The results were shown in **Table 1**.

Method Validation:

TABLE 1: SYSTEM SUITABILITY RESULTS OF SOFOSBUVIR AND VELPATASVIR

S. no.	System Suitability Parameters	Observations		Proposed Acceptance Criteria
		Sofosbuvir	Velpatasvir	
1	% Relative Standard Deviation of six replicate injections of analyte peak in the standard solution	0.9	0.9	Should be not more than 2.0%
2	Tailing factor for analyte peak in standard solution	1.4	1.2	Should be not more than 2.0
3	Plate counts for analyte peak in the standard solution	4267	5254	Should be not less than 2000

Specificity: The specificity is the ability of an analytical method to assess unequivocally the analyte of interest in the presence of components that may be expected to be present, in the sample matrix.

Linearity: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given linearity. The linearity of the chromatographic method was tested by plotting peak area against concentrations (minimum of 5 concentration levels). The concentrations were prepared in a range of 25-600 ppm (25%-150%). Linear regression equation and correlation coefficient (R^2) were employed to statistically evaluate the linearity results. The calibration curves were shown in **Fig. 5** and **6**.

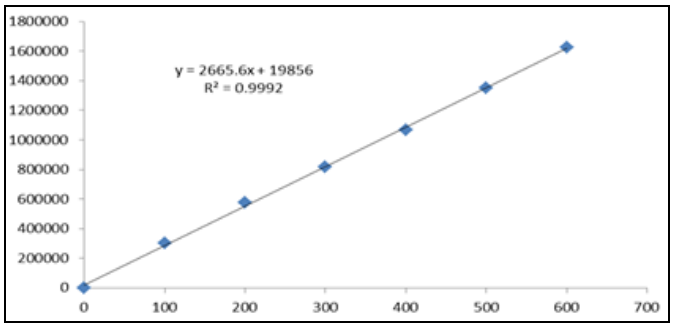

FIG. 5: LINEARITY PLOT OF SOFOSBUVIR

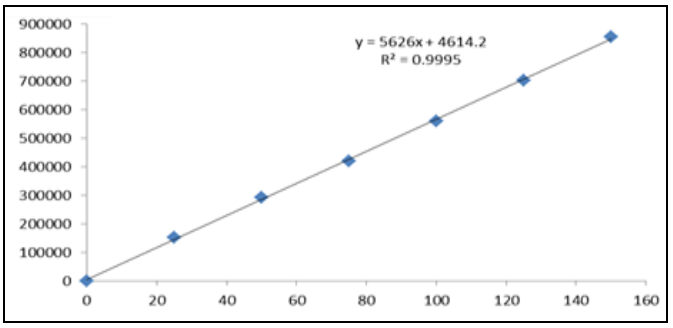

FIG. 6: LINEARITY PLOT OF VELPATASVIR

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was carried by determining the recovery studies at three different concentration levels (50%, 100% and 150%) repeated three times. The percentage recovery and mean were calculated. The results of three concentration levels were shown in **Table 2**.

TABLE 2: ACCURACY RESULTS OF SOFOSBUVIR AND VELPATASVIR

S. no.	Spike level	Amount added (ppm)		Amount recovered (ppm)		% Recovery	
		Sofosbuvir	Velpatasvir	Sofosbuvir	Velpatasvir	Sofosbuvir	Velpatasvir
1	50%	200	50	201.8221	49.66509	100.91	99.33
2	50%	200	50	202.2289	50.25556	101.11	100.51
3	50%	200	50	200.4169	49.58173	100.21	99.16
4	100%	400	100	393.5689	98.67949	98.39	98.68
5	100%	400	100	392.331	100.4797	98.08	100.48
6	100%	400	100	398.3006	100.8867	99.58	100.89
7	150%	600	150	595.0886	147.1157	99.18	98.08
8	150%	600	150	594.4394	149.682	99.07	99.79
9	150%	600	150	592.4702	149.1795	98.75	99.45

Precision: The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogenous sample. The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurement. The precision of the proposed method was determined by intraday and inter day precision. For intra-day precision six replicates of test preparations were injected on the same day into chromatographic system and calculated the percentage assay and percentage RSD. For inter-day precision six replicate test samples of same concentrations were injected on two different days. The percentage assay and percentage RSD were calculated.

Limit of Detection and Limit of Quantification (LOD and LOQ): The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of quantitation is the lowest injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography. The quantitative limit is particularly used for the determination of impurities and degradation products. The results were shown in **Table 3**.

TABLE 3: LOD AND LOQ RESULTS OF SOFOSBUVIR AND VELPATASVIR

Parameters	Sofosbuvir	Velpatasvir
Number of samples	6	6
Correlation range (µg /mL)	100-600 (µg/mL)	25-150 (µg/mL)
Regression coefficient	0.9992	0.9995
Limit of Quantification (µg/mL)	0.29	1.250
Limit of Detection (µg/mL)	0.10	0.412

Robustness: The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The method was evaluated for robustness by changing different parameters like variation in temperature by ± 5% (20 °C and 30 °C) and variation in flow rate ± 0.1% (0.1 ml/min and 0.3 ml/min). The results implied that there was no marked change in system suitability parameters which makes the developed UPLC method a robust method.

Forced Degradation Studies: Forced degradation studies were conducted to know the stability of the method. The degradation studies were carried out by applying various stress conditions for the product like acid stress, base stress, UV stress, humidity stress, thermal stress and oxide stress. Degradation peaks were observed only in acid stress and peroxide stress and all degradation peaks were well resolved from analyte peaks.

TABLE 4: RESULTS OF FORCED DEGRADATION STUDIES

Samples	Sofosbuvir		Velpatasvir	
	% Assay	% Degradation	% Assay	% Degradation
Control sample	99.08	-	100.48	-
Acid stress	95.52	3.56	95.06	5.42
Base stress	96.97	2.1	97.25	3.23
Peroxide stress	98.13	0.95	98.14	2.34
Thermal stress	99.03	0.05	99.34	1.14
UV stress	99.27	-0.19	99.19	1.29
Humidity stress	99.45	-0.37	99.04	1.44

The results of forced degradation studies were shown in **Table 4**.

RESULTS AND DISCUSSION: Results of system suitability parameters shown uniformity and % RSD was 0.9 for both Sofosbuvir and Velpatasvir which implies the system is suitable for the proposed method. The specificity of the method was determined by standard chromatogram and formulation chromatogram. There was no interference of placebo or excipient peaks with standard or analyte peaks. Therefore the developed method was specific. Accuracy of the method was determined by recovery studies.

The mean recoveries of Sofosbuvir and Velpatasvir were found to be 98% to 100% (limit 98%-102%) which indicated a good accuracy for the analysis of two drugs. The linearity of the method was determined by plotting a calibration curve for concentration and area.

The correlation coefficient values for Sofosbuvir and Velpatasvir were 0.9992 and 0.9995 respectively. The precision was determined by carrying Intra-day and inter-day variations in terms of % RSD and the values were within limits (NMT 2%) which revealed that the method was precise. Robustness was determined by making small changes in chromatographic conditions and the results showed the method was robust. Degradation studies were performed under different conditions and there was no marked degradation except in acid stress. The degradation studies implied that there is no interference of degradants with the analytes peak.

CONCLUSION: The developed UPLC analytical method provides an ecofriendly, reliable, reproducible, simple, rapid, sensitive, accurate, precise and specific assay method for the simultaneous estimation of Sofosbuvir and Velpatasvir in pharmaceutical formulations. Degradation studies reveal that the developed method was stability indicating. Hence the proposed method can be conveniently used for the routine analysis of Sofosbuvir and Velpatasvir in pure and pharmaceutical dosage forms.

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CONFLICT OF INTEREST: All authors have none to declare

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