An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

A NEW UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR USING BULK AND PHARMACEUTICAL DOSAGE FORMS

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ARTICLE INFO

Key Words

UPLC, Sofosbuvir, Velpatasvir

ABSTRACT

A simple and selective UPLC method is described for the determination of Velpatasvir and Sofosbuvir in tablet dosage forms. Chromatographic separation was achieved on a Methanol: Acetonitrile: Water using mobile phase consisting of a mixture of 50 volumes of Methanol, 30 volumes of acetonitrile and 20 volumes of Water with Chromatographic peak was detection at 266 nm. Linearity was observed in the range 50-175 µg/ml for Velpatasvir (r^2 =0.998) and 50-175 µg /ml for Sofosbuvir (r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Sofosbuvir and Velpatasvir was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future

INTRODUCTION

Sofosbuvir is nucleotide analog inhibitor, which specifically inhibits HCV NS5B polymerase by binding to the two Mg2+ ions present in HCV NS5B polymerase's GDD active site motif. Its molecular formula and molecular weight is $C_{22}H_{29}FN_3O_9P$ and 529.45g/mol respectively.

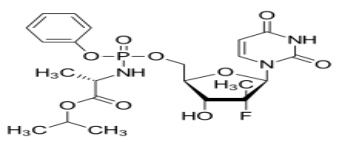

Figure: 2.2 Molecular structure of Sofosbuvir

Velpatasvir is an inhibitor of the Hepatitis C Virus (HCV) NS5A protein required for RNA replication and assembly of HCV virions. Its molecular formula and molecular weight is $C_{49}H_{54}F_2N_8O_6$ and 888.999 respectively.

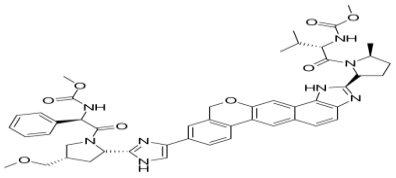

Figure: 2.1 Molecular structure of Velpatasvir

MATERIALS AND METHODS:

Sofosbuvir and Velpatasvir sample were obtained from Chandra labs, Hyderabad, India and local pharmacy. All reagents used were HPLC/AR grade, nylon membrane filters of 0.45µ pore size were used to filter the mobile phase and its components.

INSTRUMENTATION:

Analysis was carried out in waters acquity with binary UPLC pump equipped with PDA detector. Separation has been carried out using Agilent 1290 Infinity C18 (50x2.1mm ID), 1.8 µ column.

METHOD DEVELOPMENT:

Various analytical development trials has been performed by using different chemicals and reagents, organic solvents at different pH ranges and strengths in different proportions of buffer and Organic solvents to separate the three peaks with acceptable resolution and with good peak shape. Various stationary phases of multiple makes were used to check the chromatography with acceptable peak shape, tailing factor and plate count for reproducibility at room temperature (20-25^{0}C). Based on the observations and conclusions obtained from the number of chromatographic trials performed on UPLC, a particular set of chromatographic conditions were optimized to be suitable for estimation of the Sofosbuvir and Velpatasvir in the tablets. The optimized chromatographic conditions which are found to be suitable for the estimation of the Sofosbuvir and Velpatasvir are given below Table no.1.

Preparation of Diluent: Prepared dilution of water and Acetonitrile in the ration 30:20 (v/v). Mixed well.

Preparation of Mobile Phase: Prepared dilution of Methanol and Acetonitrile in the ratio of 50:30 (v/v). Mixed well. Sonicated for 10mins.

Preparation of Sofosbuvir standard stock solution: 10 mg of Sofosbuvir was weighed in 100ml volumetric flask and dissolved in water and then dilute up to mark with water and prepare 30 µg/ml of solution by diluting 3ml to 10ml with water.

Preparation of Velpatasvir standard stock solution: 10 mg of Velpatasvir was weighed and transferred in to 100ml volumetric flask and dissolved in water and then make up to the mark with water and prepare 40 µg/ml of solution by diluting 4ml to 10ml with water.

Preparation of Standard solution: Weigh accurately 10mg of Velpatasvir and Sofosbuvir in 100ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of Velpatasvir and Sofosbuvir is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

METHOD VALIDATION:

System suitability: It is assessed by injecting the six replicate into a system. Results are given in Table no.1 & 2.

Calibration curve: A linear relationship was evaluated across the range of the analytical procedure and demonstrated directly on the drug substance. The test results were evaluated by calculation of regression line by the method of least square. The respective component concentrations were given below.

Sofosbuvir – 50, 75, 100, 150, 175 and Velpatasvir – 50, 75, 100, 150,175

All the above prepared solutions of respective individual component were

analyzed to calculate the correlation coefficient of the individual components.

Accuracy: Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated.

Sample stock preparation: 10 tablets **(EPCLUSA -** 400 mg sofosbuvir and 100 mg velpatasvir) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Sofosbuvir and Velpatasvir were prepared by dissolving weight equivalent to 10 mg of Sofosbuvir and Velpatasvir and dissolved in 10ml mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase.

Method Precision: The Precision of the method was determined by sample preparation. Calculated % of assay using formula.

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation and AT: Peak area due to assay preparation

WS: Standard Weight of Sofosbuvir/ Velpatasvir in mg

WT: Weight of sample in assay preparation and DT: Dilution of assay preparation

DS: Dilution of standard preparation and P: Purity of Sofosbuvir/Velpatasvir

AW: Average weight of tablets in mg

LC: Labelle claim of Sofosbuvir/ Velpatasvir

RESULTS AND DISCUSSIONS:

Method Optimization: The developed method was optimized after many trials. The optimized method developed on C18 (50mm x 2.1mm ID), 1.8µm as stationary phase. Using Methanol and Acetonitrile in the ratio 50:30% v/v as mobile phase. The column was maintained at room temperature (25˚C). Mobile phase pumped with a flow rate of 0.5ml/min and injection volume is 10µl.

System suitability: All system suitability parameters were passed which include the theoretical plates, tailing factor, resolution for Sofosbuvir and Velpatasvir respectively TableNo.2.

Linearity: The best fit line was obtained with regression coefficient between the peak area vs concentration. Results are given below Table No.03 and Fig No.1a and 1b.

Specificity: It was evaluated by injecting blank and placebo along with drug product, no interference was found at the components respective retention timings. Chromatogram depicted below Fig No.2a and 2b.

Accuracy: Prepared accuracy at 3 levels in triplicate at 50%, 100% and 150% with matrix and achieved satisfactory results and at each level of recovery was calculated. Results are given below table No.4a and 4b.

Method Precision: The Precision of the method was determined by injecting Sofosbuvir and Velpatasvir with sample solution 6 times respectively. Method precision was expressed in terms of % RSD. Results are given in table no.05.

Table 1: Chromatographic condition

Mobile phase	Methanol: Acetonitrile: Water
Ratio	50: 30: 20
Column	Waters AcquityC18 (50mm x2.1 mm ID) 1.8µm
Flow rate	0.5ml/min
Column temperature	Room temperature(20-25ºC)
Sample temperature	Room temperature(20-25ºC)
Wavelength	266 nm
Injection volume	10 µl
Run time	5min

Table 2: System suitability

S.NO	Name of the component	Retention time	Area	Theoretical plates	Theoretical factor	Resolution
1	Sofosbuvir	1.960	33575783	8624	1.19	-
2	Velpatasvir	3.780	54112192	7895	1.28	11.8

Table 3: Linearity Parameters

S. No	Parameter	Velpatasvir	Sofosbuvir
1	**Correlation coefficient**	**0.998**	**0.999**
2	Slope	29.69	4.214
3	Intercept	6646	566.0

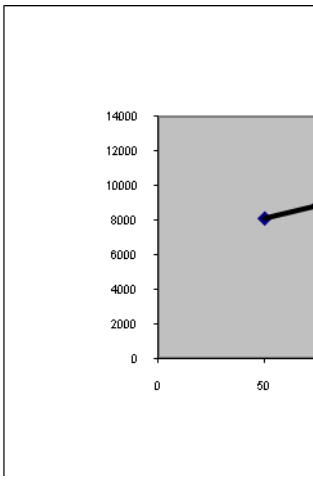

Fig.1 (a): Linearity graph of VELPATASVIR

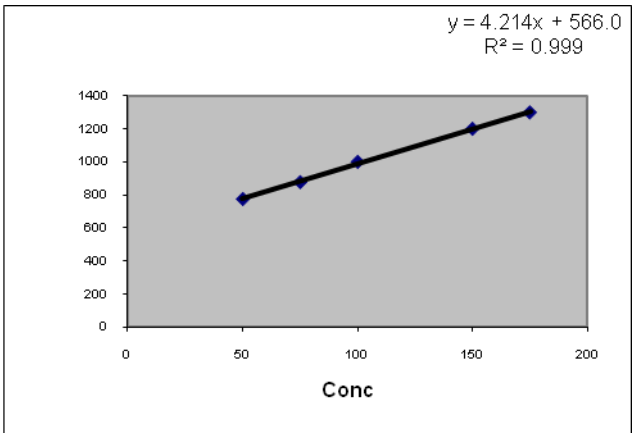

Fig. 1(b): Linearity graph of SOFOSBUVI

Specificity

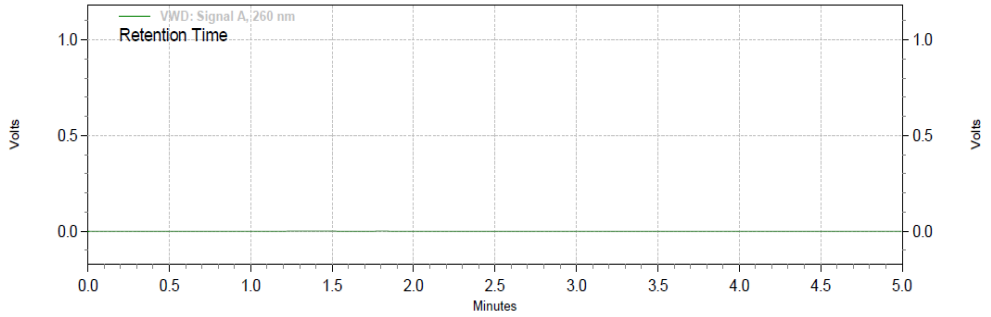

Fig 2 (a): Chromatogram of Sofosbuvir and Velpatasvir Blank

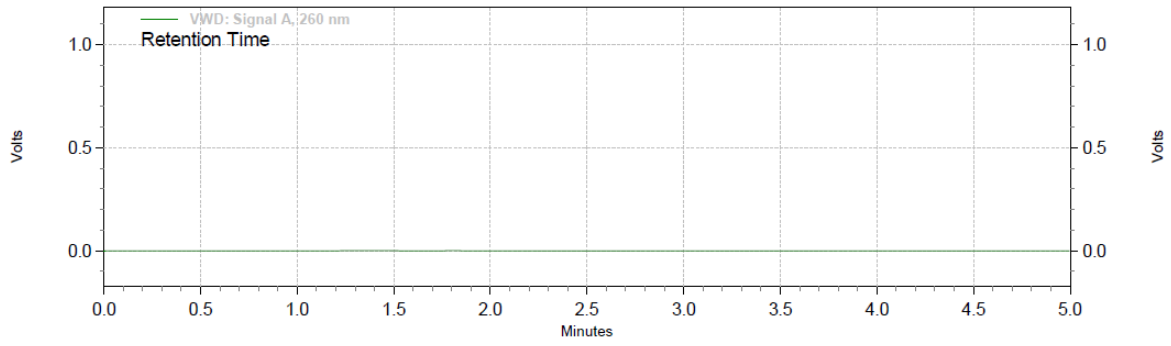

Fig 2(b): Chromatogram of Placebo

Table 4 (a): Recovery results for Velpatasvir

%Recovery	Amount present (µg/ml)	Percent Recovery *	% Mean Recovery
50%	5	99.80	100.17%
100%	10	99.32	
150%	15	100.63	

Table 4 (b): Recovery results for Sofosbuvir

%Recovery	Amount present (µg/ml)	Percent Recovery *	% Mean Recovery
50%	10	99.29	100.61
100%	20	100.04	
150%	30	99.80	

Table 5: Precision of Sofosbuvir and Velpatasvir

Injection	SOFOSBUVIR		VELPATASVIR	
	Area	**%Assay**	**Area**	**%Assay**
1	33942217	99.3	54335283	99.5
2	33512713	98.0	53884296	98.7
3	33507949	98.0	54091715	99.1
4	33519794	98.0	54660522	100.1
5	33564969	98.2	54144218	99.2
6	33367398	98.6	54219865	99.3
Average	-	98.2	-	99.3

SD	-	0.6	-	0.5
%RSD	-	0.6	-	0.5

Table 6: Robustness

Table 6: Robustness of Velpatasvir and Sofosbuvir

Chromatographic changes		**Theoretical Plates**		**Tailing factor**		**Resolution**
		SOFOSBUVIR	VELPATASVIR	SOFOSBUVIR	VELPATASVIR	Between SOFOSBUVIR and VELPATASVIR
Flow rate (mL/min)	0.4	4236	7185	1.18	1.48	11.9
	0.6	3579	6558	1.35	1.16	11.2
Temperature(°C)	25	3864	6541	1.39	1.18	11.4
	35	3939	6882	1.35	1.18	11.6

Robustness: Measure of the method capability to remain unaffected by small variations of such as flow rate, mobile phase, temperature. Results are given below Table no.06.

CONCLUSION:

A new precise, accurate, rapid method has been developed for the simultaneous estimation of Sofosbuvir and Velpatasvir in pharmaceutical dosage form by RP-HPLC. The optimum wavelength for the determination of Velpatasvir and Sofosbuvir was selected at 266 nm on the basis of isobestic point. Various trials were performed with different mobile phases in different ratios, but Phosphate Buffer pH 3.5: Acetonitrile (50:30) %v/v) were selected as good peak symmetry and resolution between the peaks was observed. The different analytical performance parameters such as linearity, precision, accuracy, and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 50-175 µg/ml For Velpatasvir and 50-175 µg/ml for Sofosbuvir. From linearity the correlation coefficient R^2 value was found to be 0.998 for Velpatasvir and 0.999 for Sofosbuvir. The proposed HPLC method was also validated for system suitability, system precision and method precision. The %RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be less than 2000, which indicates efficient performance of the column. The percentage recovery for Sofosbuvir was

found to be 100.17% and for Velpatasvir was found to be 100.61% respectively shows that the proposed method is highly accurate. Tailing factors are within limits and small variation in flow rate and wavelength.

Hence the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of Sofosbuvir and Velpatasvir in Educational institutions and Quality control laboratories.

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