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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN PURE AND PHARMACEUTICAL DOSAGE FORM

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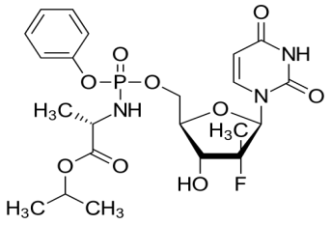
ABSTRACT
A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Sofosbuvir and Velpatasvir, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA pH 4.2 (40:60) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 272 nm. The retention time of the Sofosbuvir and Velpatasvir was 2.781, 4.048 ±0.02min respectively. The method produce linear responses in the concentration range of 7.5-37.5µg/ml of Sofosbuvir and 5-25µg/ml of Velpatasvir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Key word: Sofosbuvir, Velpatasvir, RP-HPLC, validation.

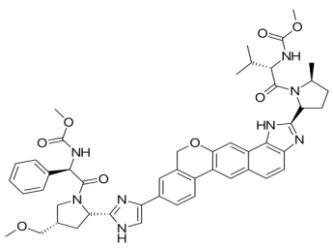
INTRODUCTION

Sofosbuvir is nucleotide analog inhibitor, which specifically inhibits HCV NS5B (non-structural protein 5B) RNA-dependent RNA polymerase. Following intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), sofosbuvir incorporates into HCV RNA by the NS5B polymerase and acts as a chain terminator [Synthesis]. More specifically, Sofosbuvir prevents HCV viral replication by binding to the two Mg2+ ions present in HCV NS5B polymerase's GDD active site motif and preventing further replication of HCV genetic material. Velpatasvir's mechanism of action is likely similar to other selective NS5A inhibitors which bind domain I of NS5A consisting of amino acids 33-202 [1]. NS5A inhibitors compete with RNA for binding at this site. It is also thought that NS5A inhibitors bind the target during its action in replication when the binding site is exposed [2]. Inhibition of NS5A is also known to produce redistribution of the protein to lipid droplets. The exact role of NS5A in RNA replication is not yet understood although it is known to be an important component.

Chemical structure of Sofosbuvir



Chemical structure of Velpatasvir



MATERIALS AND METHODS

Chromatographic conditions

A perominence isocratic HPLC system (waters 2695 HPLC with auto sampler and PDA Detector) column Hypersil C18 (4.6 x 250mm, 5µm). A 10µL Rheodyne injection syringe was used for sample injection. HPLC grade Methanol: TEA pH 4.2 were used for the preparing the mobile phase. A freshly prepared Methanol: TEA pH 4.2 (40:60% v/v) was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1mL/min., column temperature was maintained at room temperature and the detection of the drug was carried out at 272nm.

Preparation of mobile phase:

Accurately measured 650 ml (65%) of Methanol and 350 ml of Phosphate buffer (35%) a were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration

Standard solution preparation:

Accurately weigh and transfer 10 mg of Velpatasvir and 10mg of Sofosbuvir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of Velpatasvir and 0.225ml of Sofosbuvir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Sample solution preparation

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Velpatasvir and Sofosbuvir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.225ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Method validation[3-12]

Linearity

The linearity of the method was demonstrated over the concentration range of 7.5 - 37.5ppm of the Sofosbuvir are get concentration. Aliquots of 7.5,15,22.5,30,37.5µg/ml were prepared from above stock solution, it includes 5-25ppm of Velpatasvir Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Sofosbuvir and Velpatasvir was constructed by plotting peak area versus applied concentration of Sofosbuvir and Velpatasvir A typical chromatogram is shown in Fig 1. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range & it has shown in fig: 2 and 3. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in Table: 1&2 and their calibration parameters were shown in Table: 3&4.

Precision method

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution was made and the response factor of drug peak and% RSD were calculated and present in Table 5&6. The chromatogram was shown in Fig 4. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drug peak and %RSD were calculated shown in Table5&6. From the data obtained, the developed method was found to be precise.

Accuracy

A study of recovery of Sofosbuvir and Velpatasvir from spiked placebo was conducted at three different spike levels i.e.50%, 100% and 150% samples were prepared with Sofosbuvir and Velpatasvir raw material equivalent to about the target initial concentration of Sofosbuvir Velpatasvir Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table 7&8. The mean recoveries of Velpatasvir spiked were found to be in the range of 99.3% - 100.46% and Sofosbuvir from spiked were found to be in the range of 99.3-99.8%.

LOD and LOQ

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The residual standard deviation of the regression lines and slope of the calibration curves were used to calculate the LOD and LOQ (Table no.3&4)

System suitability

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 30µg/ml. The results given in Table9 were within acceptable limits.

Table 1: Linearity results for Sofosbuvir

Conc. (µg / ml)	7.5	15	22.5	30	37.5
Avg. area	88464	166364	237423	319213	401317
Correlation			0.999		

Table 2: Linearity results for Velpatasvir

Conc. (µg / ml)	5	10	15	20	25
Avg. area	80032	162782	241426	326009	417393
Correlation			0.999		

Table 3: Characteristic parameters of Sofosbuvir for the proposed RP-HPLC method

Parameters	**RP-HPLC**
Calibration range (µg/ml)	7.5- 37.5 of Sofosbuvir
Detection wavelength	272nm
Mobile phase (Methanol: TEA pH 4.2)	40:60v/v
Retention time	2.781
Regression equation(Y*)	Y=16592x - 2788
Slope (b)	10552
Intercept (a)	4280
Correlation coefficient (r^2)	0.999
Intraday precision (%RSD*)	0.9
Interday precision (% RSD*)	0.5
Limit of detection (mcg/ ml)	1.1µg/ml
Limit of quantitaion(mcg/ml)	3.5µg/ml

Table 4: Characteristic parameters of Velpatasvir for the proposed RP-HPLC method

Parameters	**RP-HPLC**
Calibration range (mcg/ml)	5-25 of Velpatasvir
Detection wavelength	272nm
Mobile phase (Methanol: TEA pH 4.2)	40:60v/v
Retention time	4.048
Regression equation(Y*)	y = 16592x - 2788.
Slope (m)	16592
Intercept (c)	2788
Correlation coefficient (r^2)	0.999
Intraday precision (%RSD*)	0.5
Interday precision (% RSD*)	0.3
Limit of detection (mcg/ ml)	0.8µg/ml
Limit of quantitaion(mcg/ml)	2.4µg/ml

Table 5: Precision results for Sofosbuvir

Sl no	**Intraday precision (area)**	**Interday precision (area)**
1	2715421	2781856
2	2778540	2761510
3	2754247	2748811
4	2780545	2790831
5	2777021	2785112
6	2780254	2781932
Mean	2764338	2775009
Std Dev	25974	16222.05
% RSD	0.9	0.5

Table 6: Precision results for Velpatasvir

Sl no	**Intraday precision (area)**	**Interday precision (area)**
1	2506927	2536301
2	2504522	2541972
3	2541270	2521259
4	2507885	2537081
5	2504587	2549869
6	2504780	2536301
Mean	2511662	2537131
Std Dev	14572.01	9370.087
% RSD	0.5	0.3

Table 7: Accuracy results for Sofosbuvir

Sample No	**Spike level**	**Amount (ppm) found**	**Amount (ppm) added**	**% Recovery**	**Mean % Recovery**
	50%	11.23	11.25	99. 8	
	50%	11.24	11.25	99.9	
1	50%	11.25	11.25	101	**100.23%**
	100%	22.1	22.5	98.2	
2	100%	22.4	22.5	98.7	**98.80%**

	100%	22.2	22.5	99.5	
	150%	33.73	33.75	99.9	
	150%	33.74	33.75	100.5	
3	150%	33.72	33.75	101	**100.46%**

Table 8: Accuracy results for Velpatasvir

Sample No	Spike level	Amount (ppm) found	Amount (ppm) added	% Recovery	Mean % Recovery
	50%	7.5	7.5	100	
	50%	7.4	7.5	99.8	
1	50%	7.2	7.5	99.6	**99.80%**
	100%	14.8	15	98.6	
	100%	14.9	15	99.8	
2	100%	14.8	15	99.6	**99.30%**
	150%	22.46	22.5	99.8	
	150%	22.45	22.5	98.8	
3	150%	22.43	22.5	99.6	**99.40%**

Table 9: system suitability studies of Sofosbuvir and Velpatasvir by RP-HPLC method

Property	Sofosbuvir Values	Velpatasvir Values	Required limits
Retention time (R_t)	2.781	4.048	RSD ≤ 1%
Theoretical plates (N)	6314	5521	N > 2000
Tailing factor	1.2	1.3	T≤ 2

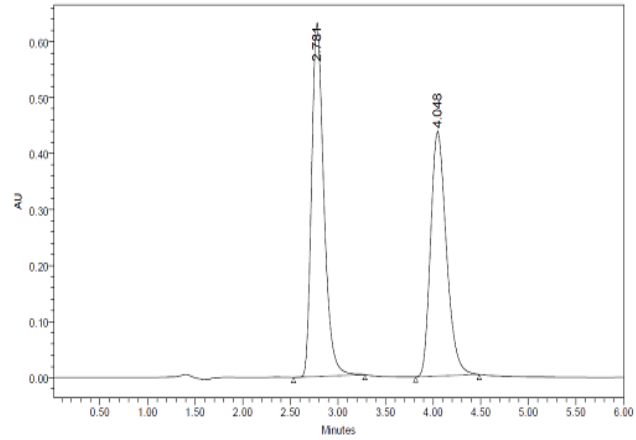


Fig: 1: Chromatogram of Sofosbuvir and Velpatasvir at 272nm

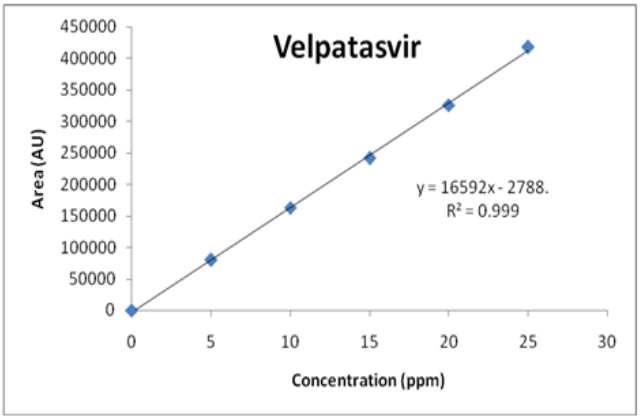


Fig.3: Calibration curve of Velpatasvir

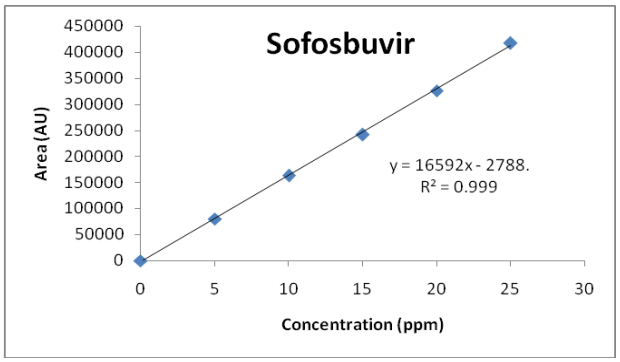


Fig.2:. Calibration curve of Sofosbuvir

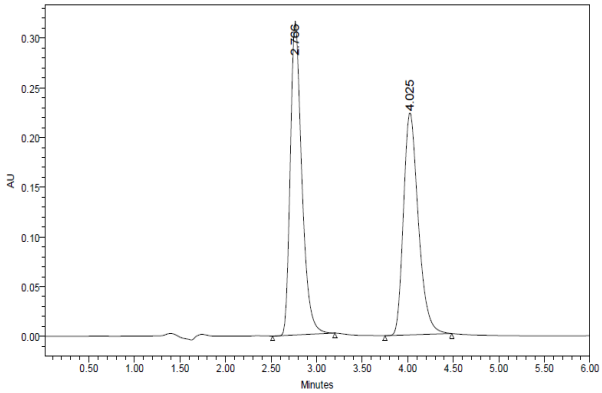


Fig.4: Chromatogram of precision

RESULTS AND DISCUSSION

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Sofosbuvir and Velpatasvir in bulk dug and pharmaceutical dosage form by using the most commonly employed Symmetry C-18 column with PDA-detection.

The run time was set at 6 min and the retention time for Sofosbuvir and Velpatasvir was 2.781, 4.048 respectively. Each sample was injected 5 times and the retention times were same. When the concentrations of Sofosbuvir and Velpatasvir and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r^2=0.999) was observed between the concentration of Sofosbuvir and Velpatasvir and the respective peak areas in the range 7.5-37.5µg /ml of Sofosbuvir 5-25µg/ml of Velpatasvir. The regression equation was used to estimate the amount of Sofosbuvir and Velpatasvir, either in tablet formulations or in validation study (precision and accuracy). For the proposed RP-HPLC method, characteristic parameters were shown in Table: 2.

To analyse tablet formulations, RP-HPLC method has been developed. Sofosbuvir and Velpatasvir tablets were analyzed as per the procedure described above. The low % RSD values (≤ 2) indicated that the method was precise and accurate. The mean recoveries found in the range of 99.3% – 100.46%. No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Sofosbuvir and Velpatasvir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Sofosbuvir and Velpatasvir was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA pH 4.2 (40:60) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Sofosbuvir and Velpatasvir in bulk drug and in Pharmaceutical dosage forms.

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