RESEARCH ARTICLE

Development of Reverse Phase HPLC Method and Validation: Sofosbuvir and Velpatasvir Quantification in Bulk and Tablets

S Bhargavi*, B Sailaja

Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India.

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ABSTRACT

A new reverse-phase high-performance liquid chromatography (RP-HPLC) method for measuring the amount of sofosbuvir and velpatasvir in active pharmaceutical ingredients (API) and tablet forms has been developed and the method was also validated. Discovery column C18 150 mm x 4.6 mm, pore size of 5 µm was used for the analysis of drugs. Elution of drugs was achieved with 0.1% OPA Buffer: Acetonitrile mixed in the proportion of 40:60 v/v was used as mobile phase, maintaining 1.0 mL/min as flow rate, column temperature maintained at 30°C and uv detector wavelength was fixed at 272 nm. Sofosbuvir and velpatasvir were eluted at retention time (RT) of 2.183 and 3.038 minutes, respectively. Linearity parameter was performed in the levels from 25 to 150% and the square of correlation coefficient (r^2) was found to be 0.9992. The %recovery obtained for sofosbuvir was 99.6 and for velpatasvir was 99.4. In precision %RSD for sofosbuvir was found to be 0.4 and for velpatasvir was 0.9. Validation was performed for the developed method and all of the validated parameter results obtained was within the ICH Q2 guidelines acceptance range. So, the developed RP-HPLC method can be used in pharmaceutical industry for quantification of sofosbuvir as well as velpatasvir at once in API and tablet dosage form.

Keywords: Sofosbuvir, Acetonitrile, OPA buffer, Velpatasvir.

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INTRODUCTION

The antiviral medication sofosbuvir (Figure 1) has received FDA approval for the treatment and management of chronic hepatitis C viral infection (HCV) in adults as well as in children of age older than three years. Sofosbuvir is an oral nucleotide prodrug with HCV inhibitory action. The drug is an NS5B polymerase enzyme inhibitor of HCV. After oral administration, the drug is metabolized and processed into 2'-deoxy-2'-alpha-fluoro-beta-C-methyluridine-5'-monophosphate. The active triphosphate nucleotide from this molecule is then produced, inhibiting the NS5B polymerase and preventing the spread of viruses. IUPAC name of sofosbuvir is isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl- tetrahydrofuran-2-yl] methoxy-phenoxy-phosphoryl] amino] propanoate. Molecular formula of the drug is $C_{22}H_{29}FN_3O_9P$ and the molecular weight is 529.4 g/mol.[1]

All six primary genotypes of hepatitis C infection are treated with sofosbuvir in combination with the NS5A inhibitor velpatasvir (Figure 2). Velpatasvir is also used for the inhibition of the hepatitis C virus. After being consumed orally and being absorbed inside the cells, the drug seems to form bond with the domain I part of the NS5A protein, a hydrophilic phosphoprotein that binds zinc and is rich in proline, is vital for the replication of RNA of HCV. This stops the generation of viral HCV RNA, disassembles the viral RNA replication complex, and stops viral replication by inhibiting the action of the NS5A protein. IUPAC name of velpatasvir is methyl {(2S) - 1 -[(2S, 5S) – 2 - (9 - { 2 -[(2S,4S) – 1 - {(2R) - 2 -[(methoxycarbonyl) amino] -2 - phenylacetyl} - 4-(methoxymethyl) -2 -pyrrolidinyl] -1 H - imidazol-4-yl} -1, 11 – dihydroisochromeno [4', 3': 6,7] naphtha [1,2-d] imidazol-2-yl) - 5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate with drug molecular formula of $C_{49}H_{54}N_8O_8$ and with drug molecular weight of 883.02 g/mol.[2]

According to a literature survey, few analytical methods including HPLC have been reported for the determination of Sofosbuvir in single,[3] Sofosbuvir in combination with other drugs[4-7] and Sofosbuvir in combination with Velpatasvir[8-12] and other drugs. The primary purpose of current work is to simplify, develop an accurate, precise and fast RP-HPLC technique for the quantification of Sofosbuvir with Velpatasvir in API and tablets.

Author for Correspondence: bhrgv.sakala@gmail.com

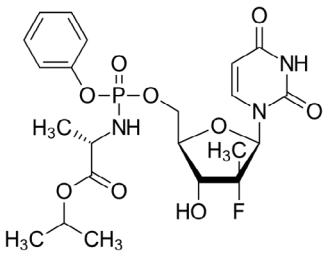

Figure 1: Sofosbuvir

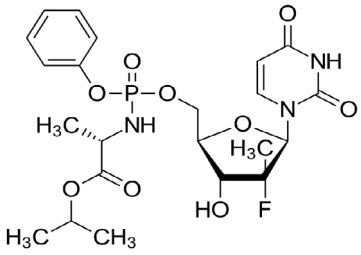

Figure 2: Velpatasvir

MATERIALS AND METHODS

Reagents and Chemicals

Sofosbuvir and velpatasvir drug samples were gifted from Spectrum Pharma Pvt. Ltd. Marketed tablet formulation named EPCLUSA manufactured by Gilead Sciences, Inc was used for analysis. Solvents like HPLC grade water, HPLC grade Acetonitrile and, chemicals like orthophosphoric acid were purchased from Merck Specialties Private Limited.

Equipment Used

Waters e2695 system, equipped with a pump having quaternary ports for solvent supply, an automated sample injector, a column thermostat, PDA detector 2996 module and the software is Empower 2 was used to carry out the study.

Chromatographic Conditions

For analytical separation, Discovery C18 column of dimensions length 150 mm x 4.6 mm, pore size of 5 µm was used. The mobile phase used is 0.1% ortho-phosphoric acid buffer solution and acetonitrile mixed in 40:60% v/v ratio. The flow rate throughout the analysis maintained was 1-mL/min. The temperature of the sample compartment was set to ambient and of the column compartment was set to 30°C. Injection volume was 10 µL and the Samples were detected at 272 nm.

0.1% OPA buffer solution preparation

Pipetted 1-mL of ortho phosphoric acid and transferred to a volumetric flask of 1000 mL capacity containing 500 mL of water, mixed and diluted up to the final mark with water.

Mobile phase preparation

Buffer: Acetonitrile in a proportion of 40:60 v/v was mixed and sonicated for 10 minutes.

Diluent preparation

Water and acetonitrile mixed in a proportion of 50:50 v/v and sonicated for 10 minutes.

Standard solution

Weighed 40 mg of Sofosbuvir as well as 10 mg of velpatasvir standards and transferred into the 10 mL volumetric flask. Diluent was added to 3/4th volume of the volumetric flask, mixed and degassed for 5 minutes and diluent was added upto the mark. The solution and 1-mL of the above-prepared standard was pipetted into the 10 mL volumetric flask and diluent diluted was added upto the mark and mixed. (400 PPM of Sofosbuvir and 100 PPM of Velpatasvir).

Sample solution preparation

Ten tablets that were bought from the market were weighed on the balance and the tablet average weight was calculated. With a mortar and pestle, tablets were crushed until fine powder was formed. A quantity of tablet powder equivalent to 400 mg and 100 mg of sofosbuvir and velpatasvir respectively was weighed on the balance and transferred into a 100 mL volumetric flask. To the volumetric flask, diluent was added upto 3/4th volume and then sonicated for about 25 minutes in a sonicator. After sonication, diluent was added upto final volume. Mixed the solution and filtered using PVDF filters with 0.45 µm pore size. One mL of the above-prepared sample solution was pipetted and transferred into a 10 mL volumetric flask and the diluent was added up to final mark and mixed.

Method development and optimization

For elution and resolution of the drugs with good peak shape having less peak tailing and for high response in short run time the chromatographic variables that need to be optimized are mobile phase, flow rate, column and temperature. At various flow rates and different mobile phases, development was tried using Hypersil BDS C18 (250 mm x 4.6 mm, 5 µm), Inertsil BDS C18 (150 mm x 4.6 mm, 5 µm), and Discovery C18 (150 x 4.6 mm, 5 µm) columns. HPLC grade Acetonitrile and 0.1% OPA as mobile phase in the proportion of 40:60 v/v maintaining flow rate of 1.0 mL/min and run time of 6 minutes on Discovery C18 150 mm x 4.6 mm, 5 µm column supported inefficient separation of sofosbuvir and velpatasvir. The wavelength 272 nm was fixed by using overlay spectra of sofosbuvir and velpatasvir. The optimized chromatogram is shown in Figure 3.

Method Validation[13]

System suitability

The suitability of HPLC system was determined by injecting blank one time and standard six times. %RSD for peak area of sofosbuvir and velpatasvir from six injections of standard preparation is determined. Other parameters like plate count, tailing, and %RSD have been evaluated to determine system suitability.

Specificity

Specificity is determined by injecting a blank solution in a single injection. The study is carried out to monitor the blank interference at the peaks of sofosbuvir and velpatasvir.

Precision

Six samples were prepared using sample preparation technique and 10 µL of each sample preparation was injected under

same experimental conditions into HPLC and the %assay was calculated from the areas obtained. The method precision is assessed by the mean assay and the %RSD for six assay results.

Linearity

Six linearity solutions, varying in concentration from 25 to 150% of the standard were prepared and injected in triplicate. 10 µL of each sample preparation was injected with same chromatographic conditions. Retention time and peak area of sofosbuvir and velpatasvir were determined. Calibration curves were constructed with a concentration (PPM) on the X-axis and the corresponding area on the Y-axis for sofosbuvir and velpatasvir separately. From the calibration curves, the square of the correlation coefficient was determined.

Accuracy

Accuracy describes the percentage recovery of the analyte. It was determined using the conventional addition approach. The standard added to a sample solution was used to conduct recovery studies at 50% level, 100% level and 150% level in triplicate. For each level, three sample preparations were prepared and analyzed. Retention time and peak area of sofosbuvir and velpatasvir were determined. %drug found was calculated from the areas obtained. %recovery is calculated by dividing the %drug found by the %drug added. The average %recovery is also determined.

LoD and LoQ

From the calibration curves, slopes and y-intercepts were calculated. Standard deviation value for y-intercepts and average of slopes from three calibration curves were calculated. From the obtained values LoD as well as LoQ were calculated.

Robustness

Standards were analyzed with changed method-specified conditions and system suitability parameters like %RSD of peak areas, plate count, retention time and tailing. Flow rate variation (± 0.1 mL/min), mobile phase variation, the temperature variation (± 2˚C) were determined in robustness.

RESULTS AND DISCUSSIONS

Method Development Trails

Initially, method was tried with BDS C8 150 X 4.6, 5 µ columns using 0.1% perchloric acid and acetonitrile in the ratio of 55:45v/v. But peak shape was not good and plate count was also found to be less than 2000 (Figure 3).

The next trial was performed using BDS C8 150 X 4.6 mm, 5 µ column using 0.1% orthophosphoric acid buffer and acetonitrile mixed in the proportional ratio of 55:45 v/v. But peak shape was not good and the plate count was also found to be less than 2000 (Figure 4).

So changed the trail with column to Discovery C18 150 x 4.6 mm, 5 µ using 60:40 v/v of 0.1% orthophosphoric acid and ACN (Figure 5). Broad Peak shape was observed but plate count, tailing and resolution was found to be satisfactory.

In the next trail changed the mobile phase composition to 0.1% orthophosphoric acid and HPLC grade ACN in the ratio of 40:60% v/v and found satisfactory (Figure 6).

Because retention times and run times were reduced, the method developed was simple and economical. The use of HPLC-grade acetonitrile and OPA as the mobile phase reduced the cost of the method while also eliminating the need for buffers and increasing the life of the column.

Method Validation Results

System suitability

%RSD for six standard injections peak area for sofosbuvir was found to be 0.5 and for velpatasvir was found to be 0.5. The plate count for Sofosbuvir was 3438 and for velpatasvir was 4426, tailing for sofosbuvir was found to be 1.23 and for velpatasvir was 1.34 respectively. The resolution between the two peaks was 5.1 (Table 1).

Specificity

At the sofosbuvir and velpatasvir RT, there is no interference from the blank. Since there was no blank interference with the main peak's elution, the HPLC method was specific (Figure 7).

Precision

Sofosbuvir and velpatasvir were reported to have %Assay ranging from 99 to 102% and the %RSD for sofosbuvir is 0.4

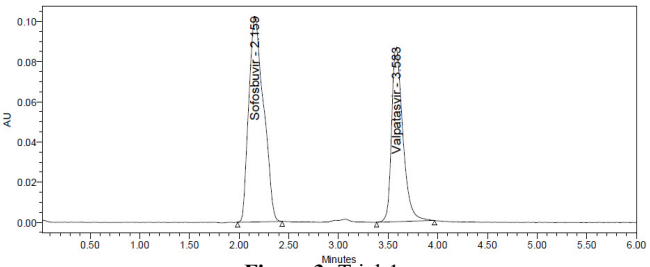

Figure 3: Trial-1

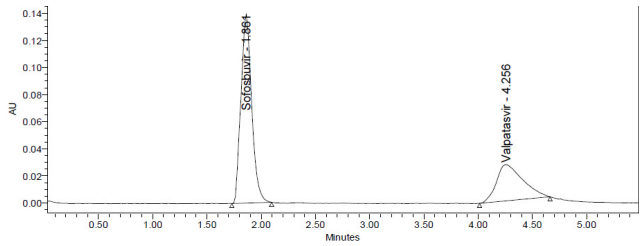

Figure 4: Trail-2

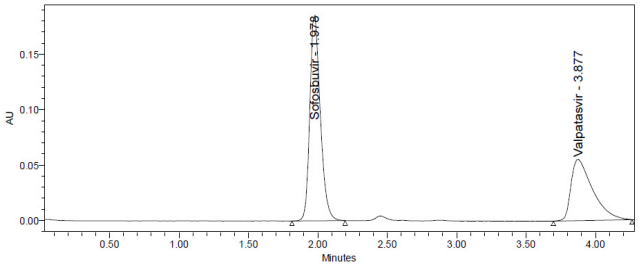

Figure 5: Trail-3

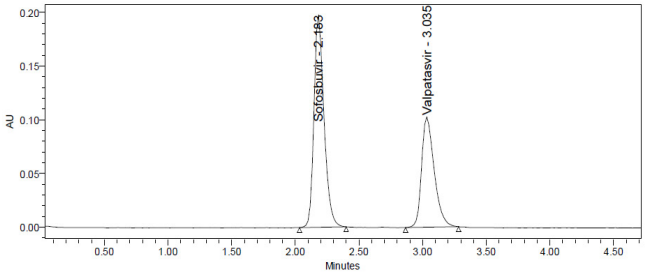

Figure 6: HPLC chromatogram under optimized conditions

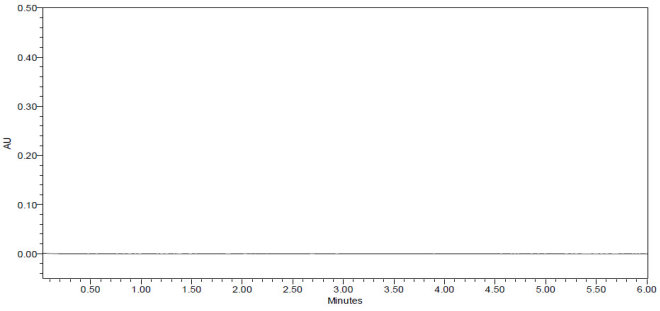

Figure 7: Chromatogram of blank

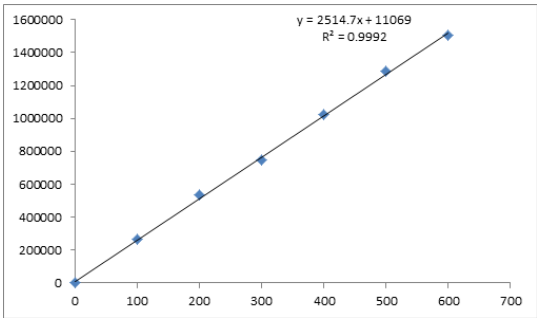

Figure 8: Calibration curve of sofosbuvir

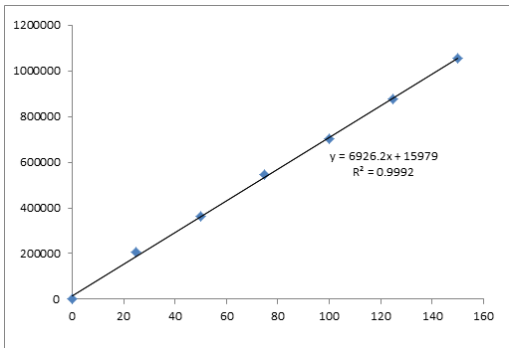

Figure 9: Calibration curve of velpatasvir

Table 1: System suitability data

Parameter	*Sofosbuvir*	*Velpatasvir*
%RSD	0.5	0.5
Plate count	3438	4426
Tailing factor	1.23	1.34
Resolution	5.1	NA

Table 2: Precision data

S. No	*% Assay of Sofosbuvir*	*% Assay of Velpatasvir*
1	99.0	99.8
2	99.1	99.4
3	100.1	101.4
4	99.8	99.0
5	99.4	99.3
6	99.1	99.4
Mean	99.4	99.7
SD	0.4424	0.8538
%RSD	0.4	0.9

Table 3: Linearity data

Percentage	*Sofosbuvir*		*Velpatasvir*	
	Concentration (PPM)	*Peak area*	*Concentration (PPM)*	*Peak area*
25	100	265965	25	204626
50	200	532110	50	363975
75	300	747318	75	544496
100	400	1024271	100	703271
125	500	1285008	125	876882
150	600	1503604	150	1054834
Regression equation	y = 2514.7x + 11069		y = 6926.2x + 15979	
r2	0.9992		0.9992	

Table 4: Recovery studies

Spiked Level (%)	*Sofosbuvir*		*Velpatasvir*	
	%Recovery	*Mean*	*%Recovery*	*Mean*
50	99.9		98.1	
50	101.0	100.4	99.6	98.8
50	100.4		98.6	
100	98.5		99.3	
100	99.5	99.0	98.6	98.9
100	98.8		98.8	
150	100.5		100.9	
150	99.6	99.5	100.8	100.4
150	98.3		99.5	
Average	NA	99.6	NA	99.4

and for velpatasvir is 0.9. The %RSD was determined to be less than 2%, signifying the method was precise (Table 2).

Linearity

The square of correlation coefficients for sofosbuvir was 0.9992 and for velpatasvir was 0.9992, respectively, showing that the HPLC method is linear in the level from 25 to 150%. (Table 3, and Figures 8 and 9)

Accuracy

Individual %recovery and average were found in between 98 to 101% (Table 4).

Table 5: Robustness data

Parameter	*Sofosbuvir*				*Velpatasvir*				*Resolution*
	%RSD	*Rt*	*Plate count*	*Tailing*	*%RSD*	*Rt*	*Plate count*	*Tailing*	
Flow (1.1 mL/min)	0.7	2.071	3560	1.29	0.9	2.927	4477	1.38	5.3
Flow (0.9 mL/min)	0.5	2.307	3465	1.15	1.2	3.235	4917	1.29	4.2
M.P (35:65 v/v)	0.5	2.192	3558	1.26	1.2	3.099	4517	1.35	5.4
M.P (45:55 v/v)	0.4	2.174	3681	1.25	0.9	3.037	4725	1.33	5.2
Temp (28˚C)	0.7	2.178	3583	1.25	0.9	3.050	4604	1.35	5.3
Temp (32˚C)	0.4	2.181	3525	1.24	0.9	3.075	4711	1.35	5.3

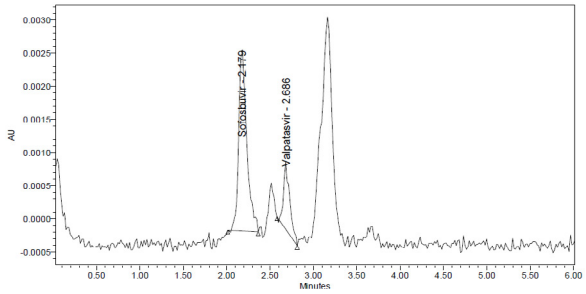

Figure 10: LoD Chromatogram

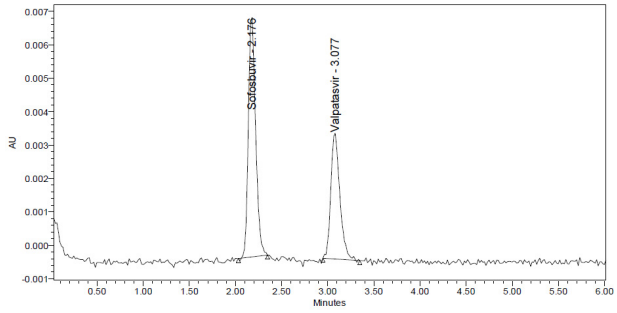

Figure 11: LoQ Chromatogram

Table 6: LoQ and LoD data

Parameter	*Sofosbuvir*	*Velpatasvir*
LoD	0.91	2.75
LoQ	0.34	1.03

Robustness

%RSD for six injections of the standard solutions for sofosbuvir and velpatasvir was found to be less than 2%. Plate count was found to be more than 2000 and USP tailing was found to be less than 2.0 for both drugs, respectively. The resolution between the two peaks was found to be more than 2.0 for the varied chromatographic conditions, reflecting that the method is robust (Table 5).

Limit of detection and limit of quantitation

Sofosbuvir limit of detection (LoD) is 0.91 µg/mL and velpatasvir LoD is 2.75 µg/mL (Figure 10). Sofosbuvir limit of quantitation (LoQ) is 0.34 µg/mL and velpatasvir LoQ is 1.03 µg/mL (Figure 11, and Table 6).

The proposed method for determining sofosbuvir, velpatasvir in API and tablets was found to be simple, precise, accurate, and fast. All the validated parameters were within limits as defined by ICH Q2 criteria. The method is simple to employ in pharmaceutical industry in quality control analysis since interference from excipients prevalent in pharmaceutical preparations is not seen.

ACKNOWLEDGEMENT

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