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Method development and validation of a new RP-UFLC method for the simultaneous estimation of Sofosbuvir and Velpatasvir in tablet dosage form using an internal standard

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Sofosbuvir, Velpatasvir, RP-UFLC, validation, ICH guidelines.

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ABSTRACT

Sofosbuvir is an anti-viral drug used for the treatment of chronic hepatitis C virus infection and Velpatasvir is also an anti-viral drug used for the inhibition of hepatitis C virus and both the drugs acts directly. The combination of Velpatasvir and Sofosbuvir works by stopping the hepatitis C genotype 1, 2, 3, 4, 5, or 6 infection. A new RP-UFLC method has been proposed for the simultaneous estimation of Sofosbuvir and Velpatasvir in tablet dosage forms in presence of an internal standard. Shimadzu Model CBM-20A/20 Alite UFLC system with PDA detector and Zorbox Eclipse Plus C18 column was used for the chromatographic study. Mobile phase mixture consisting of 0.1 % Tri fluoro acetic acid: Acetonitrile (45: 55, v/v) with a flow rate 0.8 ml/min was chosen for the simultaneous determination of Sofosbuvir and Velpatasvir and the method was validated as per ICH guidelines.

INTRODUCTION

Velpatasvir[1] (VL) (Figure 1A) is methyl {(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxycarbonyl)amino]-2-phenylacetyl}-4-(methoxymethyl)-2-pyrrolidinyl]-1H-imidazol-4-yl}-1,11- [4',3':6,7] naphtha [1,2-d] imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate. Sofosbuvir[2] (SF) (Figure 1B) is isopropyl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetra hydro furan-2-yl] methoxy-phenoxy-phosphoryl] amino] propanoate. The combination of Velpatasvir and Sofosbuvir is used for the treatment of chronic hepatitis C.

Literature survey reveals that the combination of Velpatasvir and Sofosbuvir were studied by using RP-HPLC[3-10] and RP-UPLC[11] and in the present study a new RP-UFLC method has been developed for the simultaneous determination of Velpatasvir and Sofosbuvir in tablet dosage forms and the method was validated as per ICH guidelines.

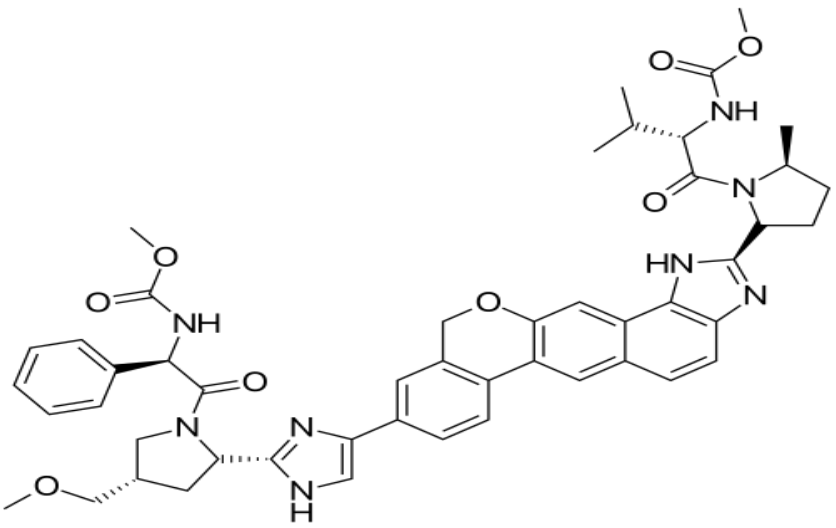


Figure 1A: Chemical structure Velpatasvir (VL)

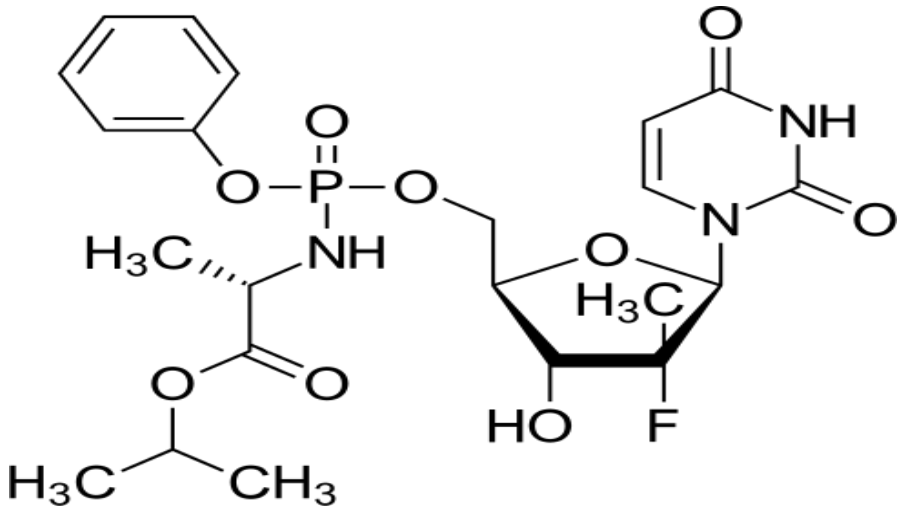


Figure 1B: Chemical structure of Sofosbuvir (SF)

MATERIALS AND METHODS

Chemicals and reagents

API samples of Sofosbuvir and Velpatasvir were obtained from NATCO Pharma as gift samples. The combination of Sofosbuvir and Velpatasvir is available as tablets with different brand names Sovihep (Zydus Heptiza), MyHep All (Mylan), Velsof (HETERO), Velpanat (NATCO Pharma) etc with label claim: Sofosbuvir (400 mg) and Velpatasvir (100 mg). HPLC grade Acetonitrile, Tri fluoro acetic acid were purchased from Qualigens (India) and of AR grade.

Shimadzu Model CBM-20A/20 Alite UFLC system with PDA detector and Zorbox C_{18} column was employed for the entire chromatographic study. Isocratic elution was performed using 0.1 % Acetic acid and Acetonitrile as mobile phase (UV detection at 259 nm) and the overall run time was 10 min.

40 mg Sofosbuvir and 10 mg Velpatasvir were accurately weighed and transferred in to a 10 ml volumetric flask and dissolved in Acetonitrile to prepare the stock solution and dilutions were made with the mobile phase as per the requirement and filtered through 0.45 µ membrane filter prior to injection. 10 mg Agomelatine, an internal standard (IS) was weighed and transferred in to a separate 10 ml volumetric flask and dissolved in Acetonitrile.

Method validation[12]

Linearity, precision, accuracy and robustness

A series of solutions consisting of Sofosbuvir (4-400 µg/ml) and Velpatasvir (1-100 µg/ml) along with 10 µg/ml of internal standard were prepared from the stock solutions and 20 µl of each of these solutions were injected (n=3) in to the UFLC system and the peak areas were noted from the respective chromatograms obtained. Calibration curves were drawn by taking the concentration of Velpatasvir and Sofosbuvir solutions on the X-axis and the corresponding peak area ratio values (SF/AG and VL/AG) on the Y-axis respectively.

The intra-day precision of the assay method was evaluated by carrying out 9 independent assays for Velpatasvir and Sofosbuvir at three concentration levels (20, 40 and 80 µg/ml) and (5, 10 and 20 µg/ml) respectively (n=3) in presence of the internal standard and the % RSD was calculated from their peak area ratio. The inter-day precision study was also performed in presence of the internal standard on three different days i.e. day 1, day 2 and day 3 at three different concentration levels and the % RSD was calculated. The accuracy of the assay method was evaluated using standard addition method followed by recovery studies (50, 100 and 150%) in presence of the internal standard and that of the robustness study of the method was studied by introducing very small changes in the optimized liquid chromatographic conditions which include detection wavelength, mobile phase composition and flow rate. A 20 µg/ml and 5 µg/ml of SB and VP was used for the Robustness study.

Assay of tablet formulations

Two different brands of tablets of the combination of Sofosbuvir and Velpatasvir of were procured from the local medical store, weighed, crushed in to fine powder and powder equivalent to 40 mg of Sofosbuvir and 10 mg of Velpatasvir was accurately weighed and transferred carefully into a 10 ml volumetric flask and made up to volume with the mobile phase. The contents were sonicated for 30 min and then filtered. The filtrate was diluted as per the requirement with mobile phase and internal standard was added and 20 µl of these solutions were injected into the UFLC system after filtering through 0.45 µ membrane filter and the peak area ratio was calculated from the respective chromatograms obtained.

RESULTS AND DISCUSSION

A new RP-UFLC method has been developed for the simultaneous determination of Velpatasvir and Sofosbuvir in presence of an internal standard in tablet formulations. Some of the important parameters of the previous proposed methods were compared with the present proposed method and discussed in Table 1.

Table 1: Literature survey

Mobile phase	Method / Column	Linearity (µg/mL)	Ref
0.1% ortho-phosphoric acid: Acetonitrile (45:55)	RP-HPLC Kromasil C18	100-600 (SB) 25-150 (VP)	3
Acetonitrile: Water (70:30)	RP-HPLC Hypersil C18	20-100 (SB) 10-50 (VP)	4
Methanol: Acetronitrile: Ammonium acetate (40:40:20)	RP-HPLC Agela C18	10-60 (SB) 1-6 (VP)	5
0.1% *O*-phosphoric acid: Acetonitrile (55:45)	RP-HPLC Discovery C18	25-150 (SB) 12.5-75 (VP)	6
0.1% ortho phosphoric acid: Acetonitrile (60:40)	RP-HPLC Discovery C18	100.34-1003.39 (SB) 25.29-252.93 (VP)	7
0.5 mM disodium hydrogen phosphate buffer (pH adjusted to 6.5 with ortho phosphoric acid and Methanol (78:22)	RP-HPLC Waters C18	125-375 (SB) 32.5-97.5 (VP)	8
0.1% Trifluoro acetic acid: Methanol (42:58)	RP-HPLC	80-240 (SB)	9

	X Terra C18	20-60 (VP)	
Acetonitrile: 0.025M KH_2PO_4 (pH adjusted to 3.0 with OPA) (50:50)	RP-HPLC YMC	200-1000 (SB) 50-250 (VP)	10
Ortho phosphoric acid buffer: Acetonitrile (45:55)	RP-UPLC Acquity UPLC HSS C18	20-600 (SB) 0-150 (VP)	11
0.1 % Tri fluoro acetic acid: Acetonitrile (45: 55)	RP-UFLC Zorbox Eclipse Plus C18	4-400 (SB) 1-50 (VP)	Present method

Method validation

Linearity, precision, accuracy and robustness

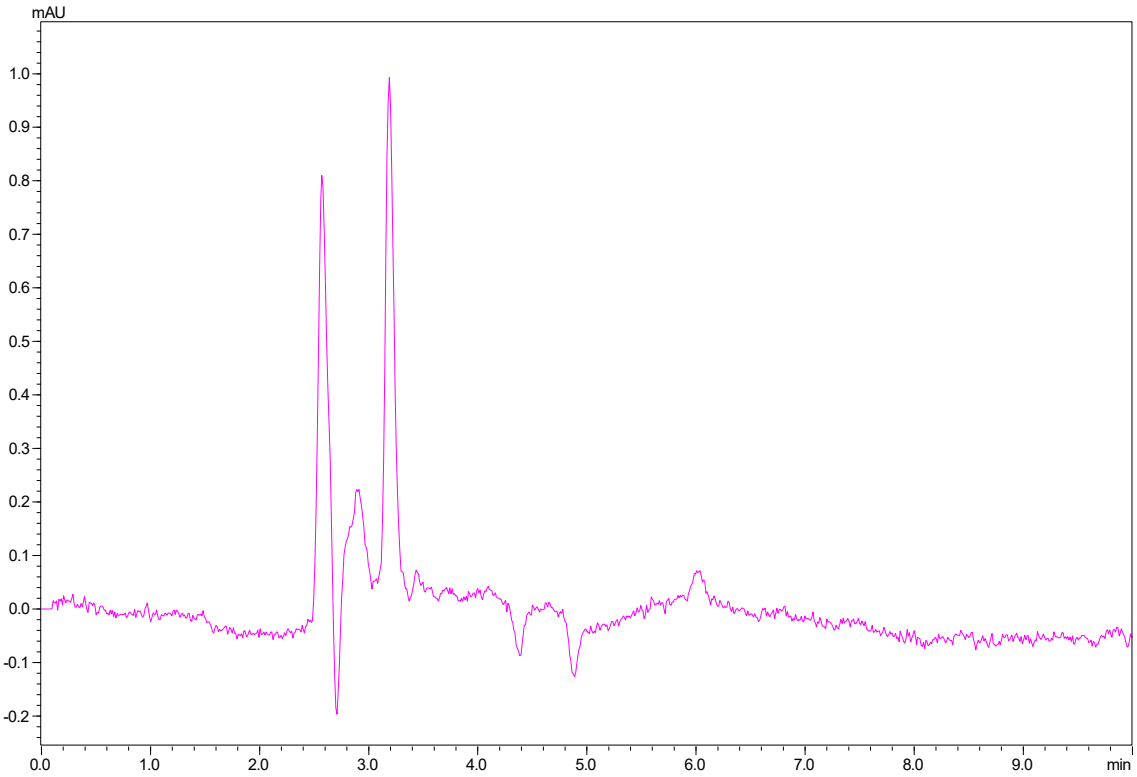
Shimadzu Model CBM-20A/20 Alite UFLC system with PDA detector and Zorbox Eclipse Plus C18 were used for the present chromatographic study. Mobile phase mixture consisting of 0.1 % Tri fluoro acetic acid: Acetonitrile (45: 55, v/v) with a flow rate 0.8 ml/min was chosen for the simultaneous determination of Sofosbuvir and Velpatasvir (Detection wavelength 259 nm) in presence of an internal standard, Agomelatine.

Sofosbuvir and Velpatasvir have shown linearity over the concentration range 4-400 µg/ml and 1-100 µg/ml with linear regression equations, $y = 1243x + 0.4016$ ($R^2 = 0.9996$) and $y = 1659x + 0.0415$ ($R^2 = 0.9999$) respectively (Table 2). The chromatograms of mobile phase (Blank) (Figure 2A), Velpatasvir (Figure 2B), Sofosbuvir (Figure 2C) were shown in Figure 2. The chromatogram of Sofosbuvir and Velpatasvir together was shown in Figure 2D. The calibration curves drawn by plotting the concentration of the drug on the X-axis and the corresponding peak area ratio values on the Y-axis were shown in Figure 3. The LOD and LOQ were found to be 1.2685 and 3.8451 for Sofosbuvir and 0.3202 µg/ml and 0.9724 µg/ml for Velpatasvir respectively.

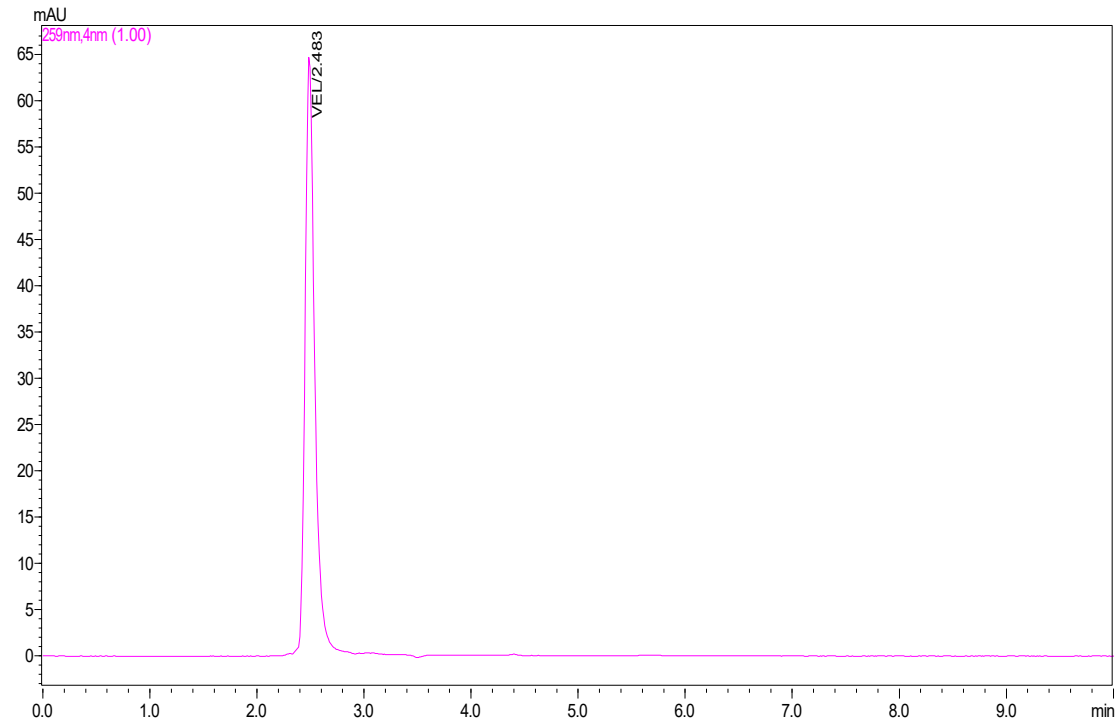
The % RSD was found to be 0.2443-0.8628 µg/ml for Sofosbuvir and 0.1221-0.7478 µg/ml for Velpatasvir which is less than 2.0 indicating that the method is precise (Table 3). The % RSD in accuracy study was found to be 0.821-0.942 (% Recovery 99.87-99.91) for Sofosbuvir and 0.547-0.712 (% Recovery 99.88-99.93) for Velpatasvir which is less than 2.0 indicating that the method is accurate (Table 4) and the % RSD in robustness study was found to be 0.57-1.06

for Sofosbuvir and 0.69-0.92 µg/ml for Velpatasvir which is less than 2.0 indicating that the method is precise (Table 5).

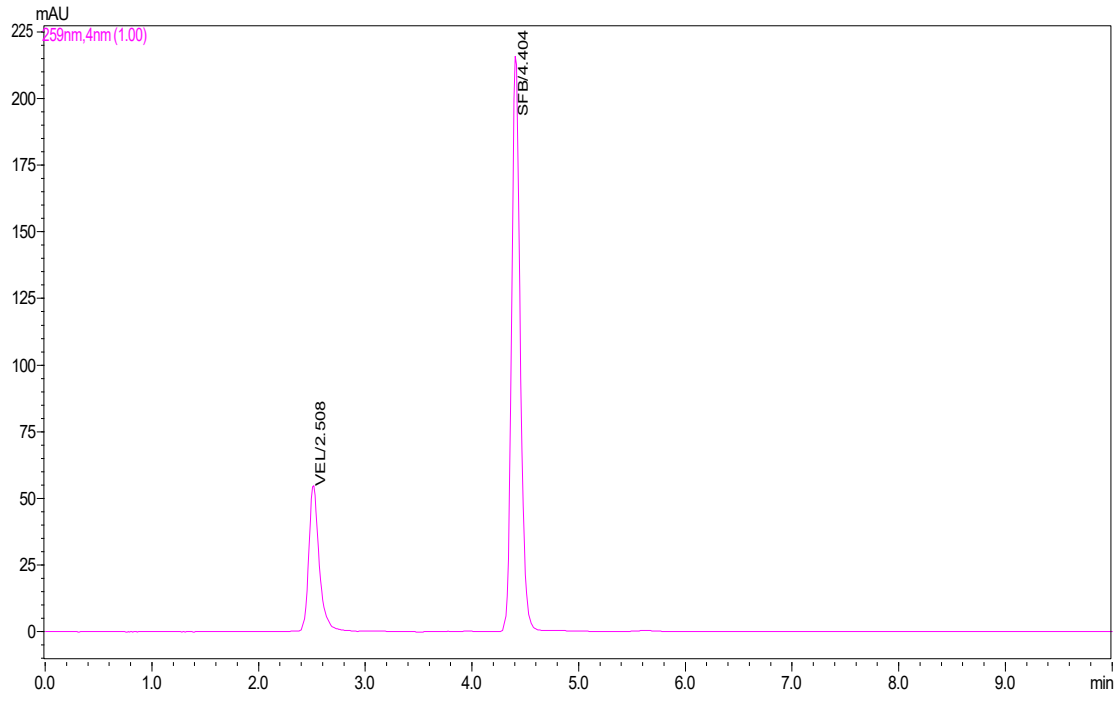
µg/ml for Sofosbuvir and 0.74-1.01 µg/ml for Velpatasvir which is less than 2.0 indicating that the method is robust (Table 5).



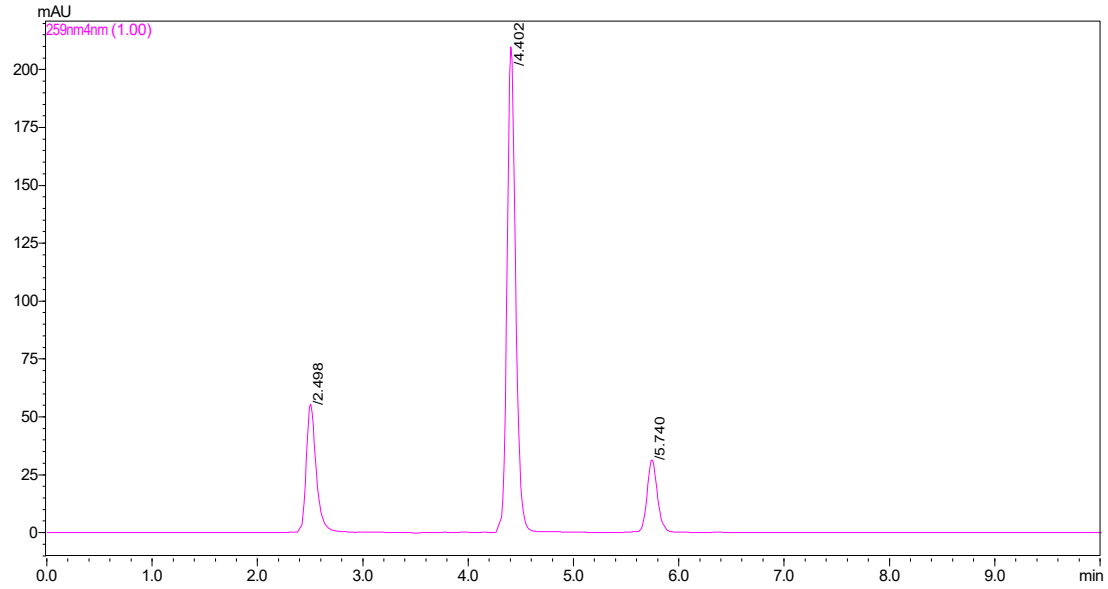
A) Blank



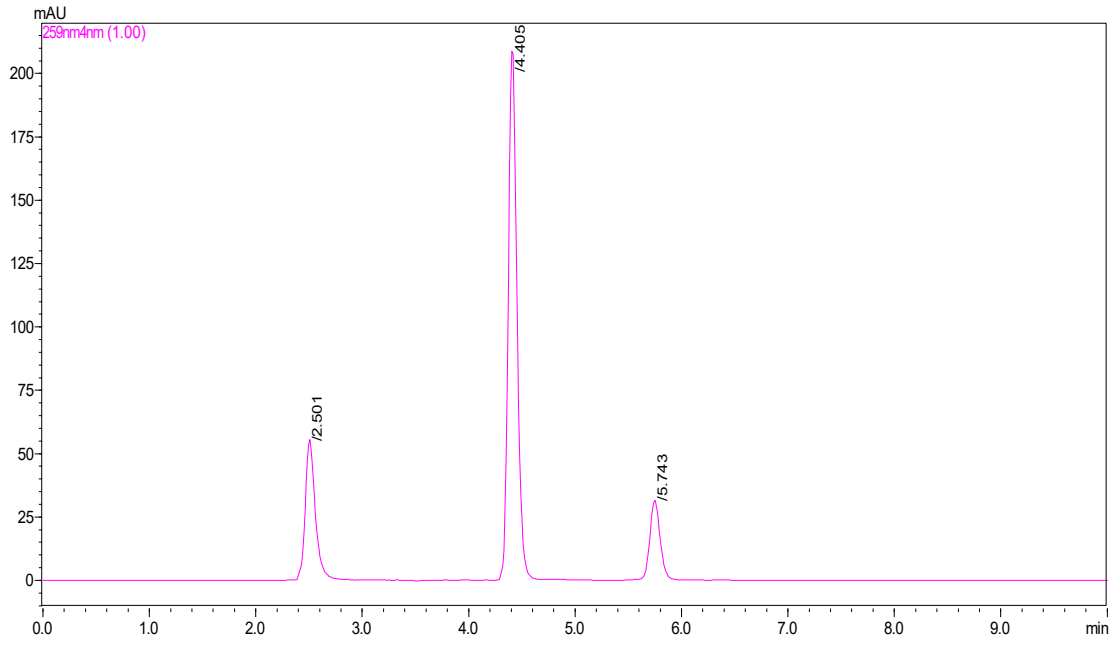
B) Representative chromatogram of Velpatasvir (Rt 2.483 min)



C) Representative chromatogram of Sofosbuvir (Rt 4.404 min) and Velpatasvir (Rt 2.508 min)



D) Representative chromatogram of Sofosbuvir (Rt 4.402 min) and Velpatasvir (Rt 2.498 min) in presence of internal standard (Rt 5.740 min)



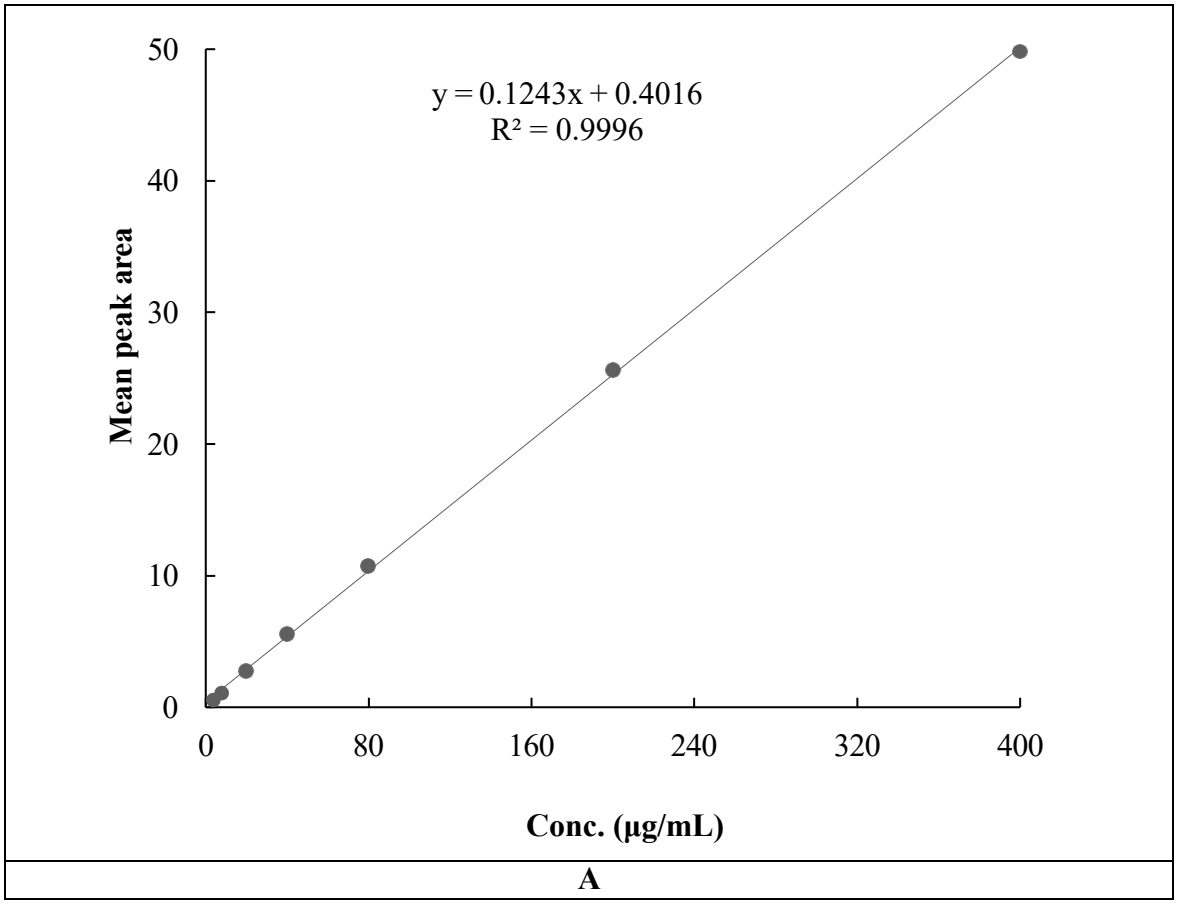
E) Representative chromatogram of Sofosbuvir (Rt 4.405 min) and Velpatasvir (Rt 2.501 min) in presence of internal standard (Rt 5.743 min)

Figure 2: Chromatograms of A) Blank B) Velpatasvir C) Sofosbuvir and Velpatasvir (API) D) Sofosbuvir and Velpatasvir in presence of internal standard D) Sofosbuvir and Velpatasvir in presence of internal standard in tablet formulation

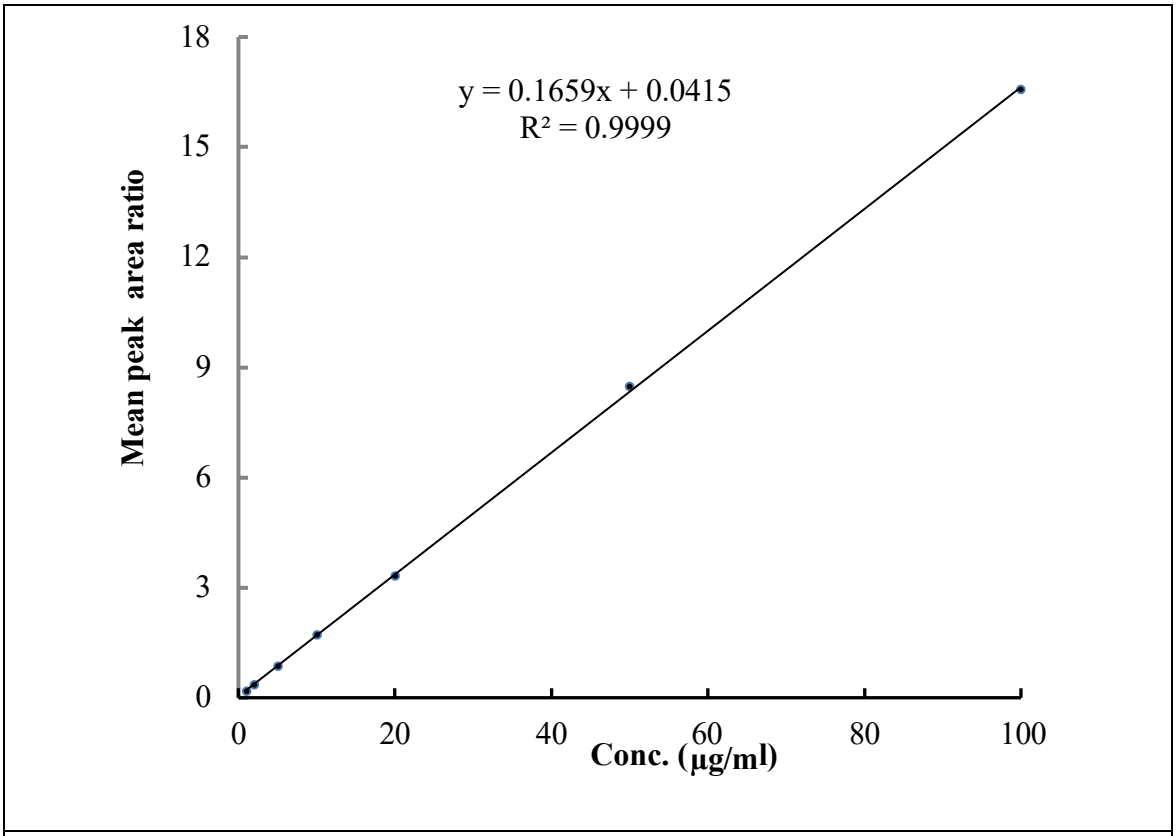
Table 2: Linearity study in presence of internal standard

Conc.(µg/ml)		Peak area		Retention time		Mean peak area ratio	
VL	SF	VL	SF	VL	SF	VL/AG	SF/AG
1	4	40090	121057	2.52	4.399	0.1855	0.5602
2	8	73982	233327	2.513	4.401	0.3439	1.0847
5	20	185589	601960	2.502	4.395	0.8608	2.7920
10	40	374103	1218158	2.508	4.404	1.7181	5.5945
20	80	714825	2312595	2.507	4.396	3.3233	10.7517
50	200	1829352	5537373	2.513	4.388	8.4850	25.6839
100	400	3579852	10767964	2.508	4.384	16.5662	49.8302

*Mean of three replicates



A



B

Figure 3: Calibration curve of A) Sofosbuvir and B) Velpatasvir

Table 3: Precision study in presence of internal standard

Conc. (µg/ml)		Peak area ratio		*Mean peak area ratio ± SD (% RSD)	
VL	SF	VL/AG	SF/AG	VL/AG	SF/AG
5	20	0.8592	2.7851	0.860267 ± 0.00105 (0.122101)	2.789033 ± 0.006813 (0.244269)
5	20	0.8613	2.7851		
5	20	0.8603	2.7969		
10	40	1.7069	5.6541	1.721767 ± 0.012875 (0.747773)	5.635567± 0.048625 (0.86283)
10	40	1.7292	5.5804		
10	40	1.7292	5.6722		
20	80	3.2967	10.7042	3.2937 ± 0.014731 (0.447245)	10.69767 ± 0.062258 (0.581974)
20	80	3.3067	10.7564		
20	80	3.2777	10.6324		

*Mean of three replicates

Table 4: Accuracy study in presence of internal standard

Conc. (µg/ml)							% Recovery (% RSD)	
Formulation		Pure Drug		Total		IS		
SF	VL	SF	VL	SF	VL	AG	SF	VL
40	10	20	5	60	15	10	99.89 (0.821)	99.92 (0.547)
40	10	20	5	60	15	10		
40	10	20	5	60	15	10		
40	10	40	10	80	20	10	99.91 (0.942)	99.93 (0.712)
40	10	40	10	80	20	10		
40	10	40	10	80	20	10		
40	10	60	15	100	25	10	99.87 (0.883)	99.88 (0.659)
40	10	60	15	100	25	10		
40	10	60	15	100	25	10		

*Mean of three replicates

Table 5: Robustness study in presence of internal standard

Parameters	Conditions	% Recovery (% RSD)	
		VP	SB
Flow rate (± 0.1 ml/min)	0.9	99.83 (0.74)	99.84 (0.86)
	0.8		
	0.7		
Detection wavelength (± 2 nm)	257	99.87 (0.92)	99.92 (0.57)
	259		
	261		
Mobile phase (± 2 v/v) 0.1 % Tri fluoro acetic acid: Acetonitrile	43: 57	99.91 (0.69)	99.85 (1.06)
	45: 55		
	47: 53		

*Mean of three replicates

Assay of tablet formulations

The assay of Sofosbuvir and Velpatasvir tablets was carried out by applying the above proposed method for two different marketed tablet formulations in presence of internal standard and the % Assay was calculated from the linear regression equation. The percentage of purity was found to be 99.42-99.86 and 99.89-99.92 % (Table 6) for Sofosbuvir and Velpatasvir respectively and the corresponding chromatogram of one of the formulations was shown in Figure 2E.

Table 6: Assay of tablet formulations

Formulation	Labeled claim (mg)		Amount found* (mg)		Assay* (%)	
	VP	SB	VP	SB	VP	SB
Brand I	100	400	99.86	399.57	99.86	99.89
Brand II	100	400	99.42	399.69	99.42	99.92

* Mean of three replicates

CONCLUSION

The proposed RP-UFLC method was validated as per ICH guidelines and can be used for the analysis of combined dosage forms of Sofosbuvir and Velpatasvir.

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