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Simultaneous determination and validation of third generation antiviral drugs by RP-HPLC method

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ABSTRACT

A simple reverse phase high pressure liquid chromatography (RP-HPLC) method has been developed and validated for simultaneous determination of Sofosbuvir, Velpatasvir and Voxilaprevir in tablet dosage forms. The drugs were separated on Discovery C18 (150 x 4.6 mm, 5µ) column using 0.1% othophosphoric acid and Acetonitrile ratio (60:40%v/v) as the mobile phase at a buffer having pH 2.2. The mobile phase is pump into the column at flow rate of 1ml/min and column oven temperature is maintained at 30°C. The drugs were detected at a wavelength 220nm. The retention time for Sofosbuvir, Velpatasvir and Voxilaprevir were found to be 2.120min, 3.164 min and 3.800min. %RSD. The percentage recovery drugs were found to be in range of 99.08%, 98.97% and 99.29% respectively. The Limit of detection, Limit of quantification were 0.21ppm, 0.62ppm, 0.03ppm, 0.09ppm and 0.27ppm, 0.81ppm respectively. Regression equation of concentration over there peak area were found to be Sofosbuvir was y =28579.x + 23225, Velpatasvire was y = 38719x +11703and of Voxilaprevir was y = 38712.x + 74459, Y is the peak area and X is the concentration of drug. The method is useful in the simultaneous determination of third generation antiviral drugs by RP-HPLC.

Keywords: RP-HPLC, Sofosbuvir, Velpatasvir and Voxilaprevir

INTRODUCTION

Hepatitis C is a disease caused by a virus that infects the liver. The virus, called the Hepatitis C virus or HCV for short, is just one of the Hepatitis viruses .The virus can cause both acute and chronic hepatitis, ranging in severity from a mild illness lasting a few week to a serious, lifelong illness. The literature reveals that 72% of the patients were suffered from chronic HCV. These defects have been treated by use of an oral form of these combination drugs respectively.

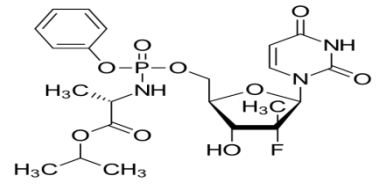
Sofosbuvir (Fig.-1a) is an antiviral drug in the treatment of chronic hepatitis C virus. It is chemically isopropyl (2s)-2[[[(2R,3R,4R,5R)-5-(2,4-dioxop[yrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl- tetra hydro furan-2-yl] methoxy-

phenoxy-phosphoryl] amino] propanoate. Mainly Sofosbuvir is activated in the liver to the triphosphate by hydrolysis of the carboxylate ester. Molecular formula $C_{22}H_{29}FN_3O_9P$ and Weight was 529.458.

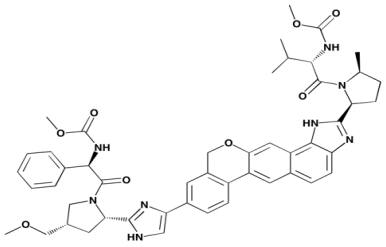
Velpatasvir (Fig.-1b) is an NS5A inhibitor which acts on hepatitis C virus. Velpatasvir is chemically Methyl{(2S)-1-[(2S, 5S) – 2 - (9 - { 2 -[(2S ,4S) – 1 - {(2R) - 2 -[(methoxycarbonyl) amino] -2 - phenylacetyl} - 4- (methoxymethyl) - 2 - pyrrolidinyl] -1 H - imidazol-4-yl} -1, 11 – dihydroisochromeno [4', 3': 6,7] naphtha [1,2-d] imidazol-2-yl) - 5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate used as an anti-cholinergic and anti-spasmodic. Molecular formula $C_{49}H_{54}N_8O_8$ and weight is 883.019.

Voxilaprevir (Fig.-1c) it is also a protease inhibitor and acts as a transporter of polypeptide. Voxilaprevir is chemically (1R,18R,20R,24S,27S,28S)-N-[(1R,2R)-2-(Difluoromethyl)-1-{[(1-methylcyclopropyl) sulfonyl] carbamoyl} cyclopropyl]-28-ethyl-13,13-difluoro-7-methoxy-24-(2-methyl-2-propanyl)-22, 25-dioxo-2,21-dioxa-4,11,23,26-tetra aza penta cyclo nonacosa-3(12),4,6,8,10-pentaene-27-carboxamide.Molecular formula $C_{40}H_{52}F_4N_6O_9S$ and weight is 868.94 .

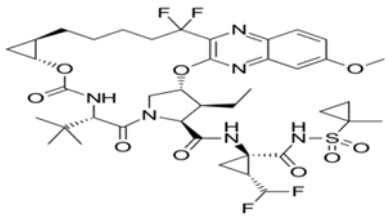
Literature review revealed few RP-HPLC methods for simultaneous determination of Sofobuvir , Velpatasvir and Voxilaprevir in tablet dosage form .The purpose of this work was to develop a simple, rapid, precise and sensitive reverse phase HPLC method developed and validated for the simultaneous determination of Sofobuvir , Velpatasvir and Voxilaprevir in pharmaceutical tablet dosage form. And the method was validated as per ICH guidelines in terms of specificity, Robustness, Accuracy, Linearity, Limit of detection (LOD), and Limit of Quantification (LOQ).



Sofosbuvir



Velpatasvir



Voxilaprevir

Fig.1 The Chemical Structur of Sofosbuvir, Velpatasvir and Voxilaprevir

MATERIAL AND METHODS

Instrumentation and chromatographic conditions

The present assay was carried out on a Waters HPLC system [Model: 2695] equipped with Photo Diode array detector, auto sampler integrated with empower software and a column inertsil (150 x 4.6 mm, 5µ) column using 0.1% othophosphoric acid and Acetonitrile ratio (60:40%v/v) as the mobile phase at a buffer having pH 2.2 The mobile phase is pump into the column at flow rate of 1ml/min and column oven temperature is maintained at 30°C. The drugs were detected at a wavelength 220nm.The other instrument used were PH meter, Ultrasonicator.

Chemicals and Reagents

Sofosbuvir, Velpatasvir and Voxilaprevir standard drug were supplied as gift samples by Spectrum labs,Hyderabad. The chemicals used for development of the method were of AR grade and purchased from Ranchem. The solvents used were of HPLC grade and purchased from Ranchem.

Preparation of 0.1% OPA Buffer Solution

1ml of Ortho phosphoric acid was pipette out and dissolved in a 900ml of Milli-Q water taken in a 1000ml Volumetric flask and final volume was made up to the mark with Milli-Q water.

Preparation of standard Solutions

Sofosbuvir Stock Solution

40mg of Sofosbuvir pure API sample was taken in 10ml volumetric flask and dissolved in ¾ ml Methanol, with the help of sonication for about 10min.The the volume was made up to the mark using methanol to get the solution of 4000ppm .

Velpatasvir Stock Solution

10mg of Velpatasvir pure APL sample was taken in a 10ml volumetric flask and dissolved in ¾ ml Methanol, with the help of sonication for about 10min. The volume was made up to the mark using methanol to get the solution of 1000ppm.

Voxilaprevir Stock Solution

10mg of Voxilaprevir pure API sample was taken in a 10ml volumetric flask and dissolved in ¾ ml Methanol, with the help of sonication for about 10min. The volume was made up to the mark using methanol to get the solution of 1000ppm.

Working Standard Solution

Working solution of Sofosbuvir, Velpatasvir and Voxilaprevir was prepared by adding 1ml of Sofosbuvir stock solution, 1ml Velpatasvir stock solution and ml of Voxilaprevir stock solution added into a single 100ml volumetric flask and diluted up to the mark to get a concentration of 400ppm Sofosbuvir, 100ppm Velpatasvir and 100ppm Voxilaprevir.

Preparation Sample Solution

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 10 mL volumetric flask, 25mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered.

RESULTS AND DISCUSSION

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of Sofosbuvir, Velpatasvir and Voxilaprevir. In order to get the optimized RP-HPLC method, various mobile phases and columns were used. From several trials final method is optimized with the following conditions.

Method Development

The mobile phase consists of 0.1% orthophosphoric acid buffer and acetonitrile in ratio 60:40 and column was used Octadecyl column C18 (150mm×4.6mm, 5µm particle size) as the mobile phase at a buffer having pH 2.2. The flow rate was adjusted to 1ml/min. The instrument was operated at an 30°C temperature. The UV detection was achieved at 220nm.

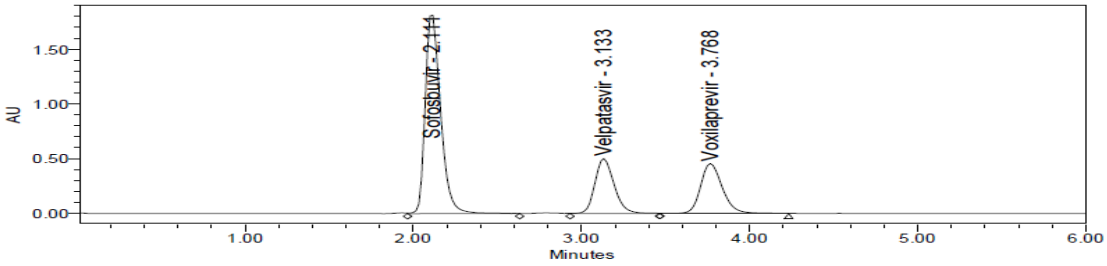


Fig.2 Standard Chromatogram of Sofosbuvir, Velpatasvir And Voxilaprevir

Method Validation

Specificity

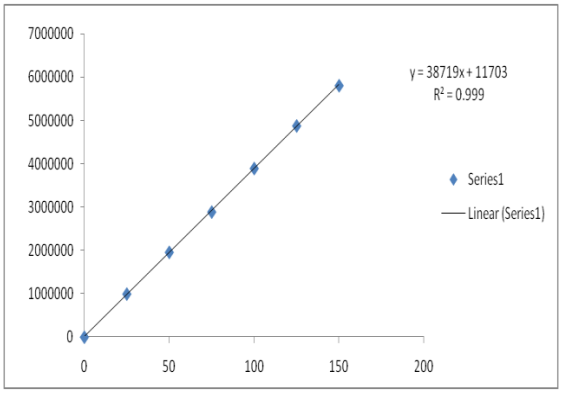
The specificity was carried out to determine whether checking of the interference in the optimized method and not found interfering peaks in blank and placebo at retention times of these drugs in this method.

Linearity

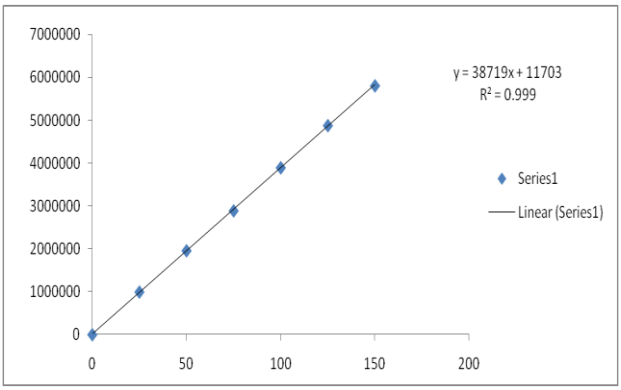
The linearity was determined as linearity regression of the claimed analyte concentration of the range 100 to 600ppm Sofosbuvir and 25ppm to 150ppm for Velpatasvir and Voxilaprevir respectively. The coefficient was found to be 0.999, 0.999 and 0.999 for Sofosbuvir, Velpatasvir and Voxilaprevir respectively. Hence the results were obtained within the limit. A typical HPLC chromatogram of the standard mixture is shown in Fig.3.

Table-1: Linearity result for Sofosbuvir, Velpatasvir and Voxilaprevir

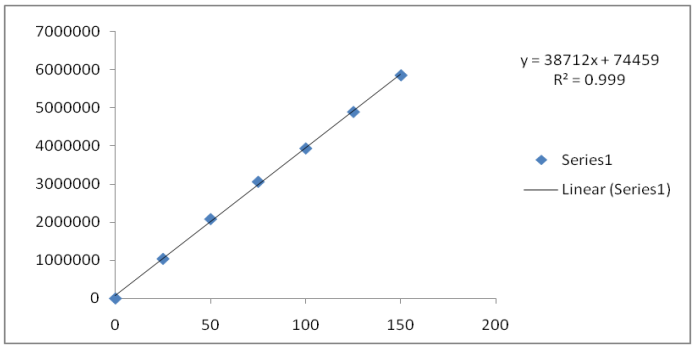
S.NO	Sofosbuvir		Velpatasvir		Voxilaprevir	
	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
1	100	3123690	25	994937	25	1038319
2	200	6055377	50	1957144	50	2080285
3	300	8979425	75	2887701	75	3057414
4	400	11763472	100	3892277	100	3931486
5	500	14599764	125	4874253	125	4888835
6	600	17120594	150	5803255	150	5848760



A. Linearity Curve For Sofosbuvir



(B)Linearity Curve For Velpatasvir



(C)Linearity Curve of Voxilaprevir
Fig.3: Calibration Curve for Drugs (A, B, C)

Accuracy

Accuracy study was performed for 50%, 100% and 150% for Sofosbuvir, Velpatasvir and Voxilaprevir in term of % recovery. Standard and sample solution were injected into HPLC system and percentage recoveries of Sofosbuvir, Velpatasvir and Voxilaprevir were calculated. The area of each level was for calculation of % recovery. The results are table no.2.

Table -2: Accuracy table of Sofosbuvir, Velpatasvir And Voxilaprevir

Drug Name	% Level	Amount Spiked(ug/ml)	Amount Recovery	% Recovery	Mean % Recovery
Sofosbuvir	50%	50	50.0096	100.02	98.97%
		50	49.6204	99.24	
	100%	50	49.9728	99.95	
		100	98.0464	98.05	
		100	98.1369	98.14	
	150%	100	98.1974	98.20	
		150	148.100	98.73	
		150	149.036	99.36	
		150	148.621	99.08	
Velpatasvir	50%	50	50.0096	100.02	98.97%
		50	49.6204	99.24	
	100%	50	49.9728	99.95	
		100	98.0464	98.05	
		100	98.1369	98.14	

Voxilaprevir	150%	100	98.1974	98.20	99.29%
		150	148.100	98.73	
		150	149.036	99.36	
		150	148.621	99.08	
	50%	50	49.300	98.60	
		50	49.526	99.05	
		50	49.774	99.55	
	100%	100	99.129	99.13	
		100	99.192	99.19	
		100	99.123	99.12	
	150%	150	148.521	99.01	
		150	149.409	99.61	
		150	150.490	100.33	

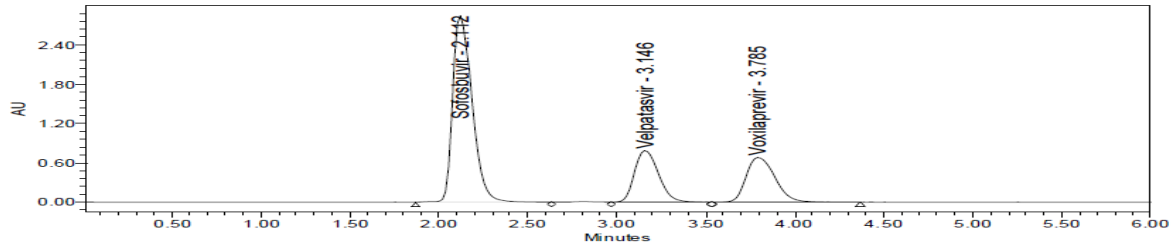


Fig .4 Accuracy 50% chromatogram

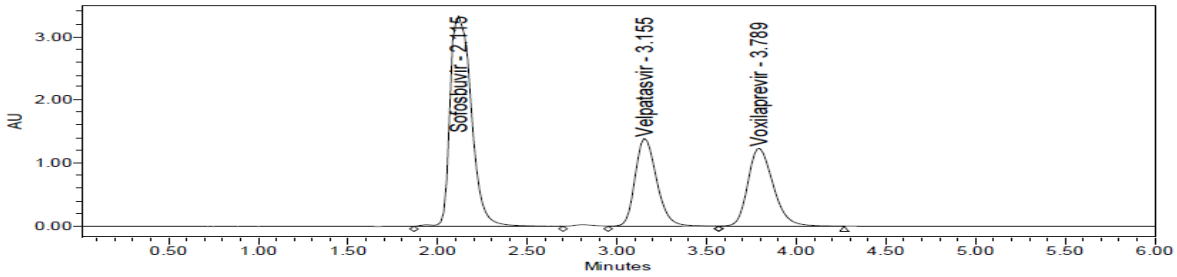


Fig .5 Accuracy 50% chromatogram

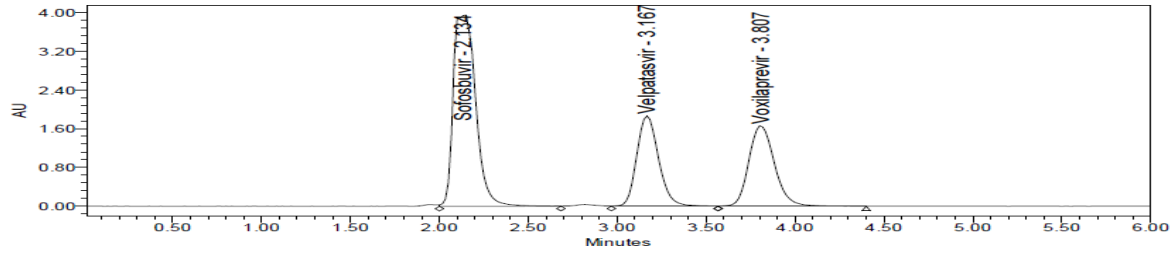


Fig .6 Accuracy 150% chromatogram

The precision of the method was ascertained from determination of peak areas of six replicates of sample solution. The %RSD for method precision was found to be0.7,0.8 and 0.7 for Sofosbuvir, Velpatasvir and Voxilaprevir respectively. The results were tabulated in table no.3.

Intermediate Precision

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table.

Average area, standard deviation and % RSD were calculated for three drugs and obtained as 0.6%, 0.8% and 0.7% respectively for Sofosbuvir, Velpatasvir and Voxilaprevir.

Table .3: System precision table of Sofosbuvir, Velpatasvir and Voxilaprevir.

S. No	Area of Sofosbuvir	Area of Velpatasvir	Area of Voxilaprevir
1.	11424079	3860628	3971632
2.	11599503	3887016	3984936
3.	11643923	3811993	3977776
4.	11652304	3882101	3962332
5.	11569081	3871588	3937278
6.	11625497	3818774	3910770
Mean	11585731	3855350	3957454
S.D	84850.3	32334.2	28211.8
%RSD	0.7	0.8	0.7

Table .4: LOD and LOQ values of SOF, VEL and VOX

Drug name	LOD	LOQ
Sofosbuvir.	0.21 µg/ml	0.62 µg/ml
Velpatasvir	0.03 µg/ml	0.09µg/ml
Voxilaprevir	0.27 µg/ml	0.81 µg/ml

The robustness was carried out with minor but deliberate changes in parameters as presented by in Table no. 5

Table .5: Robustness data for Sofosbuvir, Velpatasvir and Voxilaprevir

Chromatographic Conditions	%RSD Sofosbuvir.	%RSD Velpatasvir	%RSD Voxilaprevir
Flow rate (+) 0.9ml/min	0.4	0.4	0.3
Flow rate (+) 1.1ml/min	1.7	0.9	0.9
Mobile phase (-) 65B:35A	0.5	0.2	0.8
Mobile phase (+) 55B:45A	0.9	0.6	0.2
Temperature (-) 25°C	0.4	0.9	0.3
Temperature (+) 35°C	1.7	0.8	0.9

The system suitability parameters like theoretical plates, tailing factor were calculated and were found to be more than 2000 , tailing factor should be less than 2 and resolution must be more than 2. Ascertained that proposed RP-HPLC method was accurate and precise.

Table .6: System suitability parameters for Sofosbuvir, Velpatasvir, and Voxilaprevir

Parameter	Sofosbuvir	Velpatasvir	Voxilaprevir
Retention Time	2.132	3.170	3.802
Theoretical Plates	2974	3237	3592
Tailing factor	1.32	1.19	1.21

CONCLUSION

Simultaneous determination of Sofosbuvir, Velpatasvir and Voxilaprevir by using RP-HPLC method for tablet dosage form was developed and validated in accordance with the ICH guidelines. All the validation parameters met the acceptance limits. The developed method can be used for regular quality control analysis.

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