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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN BULK AND TABLET DOSAGE FORMS BY RP-HPLC

M. S. M. Suma*, M. Gowthami, P.V. Madhavi Latha, P. Uma Devi

Department of Pharmacy, Viswanadha Institute of Pharmaceutical Sciences, Affiliated to JNTUK, Vishakhapatnam Andhra Pradesh, INDIA

*Corresponding author E-mail: **sumanava9@gmail.com**

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ABSTRACT

For the simultaneous evaluation of Sofosbuvir and Velpatasvir in tablet dosage form, a simple, precise, detailed technique has been optimized. The mobile phase comprising of methanol and phosphate buffer (60:40 v / v) ratio was injected into a column at a flow rate of 1.0 ml/min. Chromatogram was run through Inertsil-C_{18},BDS column(4.6 x 150 mm, 5µm). Temperature was maintained at 25°C.Optimised wavelength selected at 254nm. Retention time of Sofosbuvir and Velpatasvirwere found to be 3.049 min and 4.317 min. %Recovery was obtained as 99.99% and 99.76% in that order. LOD, LOQ values obtained from regression equations of Sofosbuvir and Velpatasvir were 0.03, 0.09 and 0.15, 0.47 correspondingly. Regression equation of Sofosbuvir is y = 234504x + 9799.3, and y= 31994x + 2049.3 of Velpatasvir. The approach was simple and cost-effective and can be used in industry with the standard consistency check

INTRODUCTION:

Sofosbuvir is a direct acting anti-viral medication used as a part of combination therapy to treat chronic Hepatitis-C, an infectious liver disease caused by infection with Hepatitis-C virus (HCV). Velpatasvir is also direct acting anti-viral medication used as combination therapy to treat chronic hepatitis-C. Sofosbuvir-velpatasvir is a pangenotypic NS5A-NS5B inhibitor single-pill combination regimen that has potent activity against chronic (long-lasting) hepatitis C virus (HCV).[1-2]

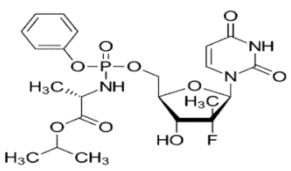
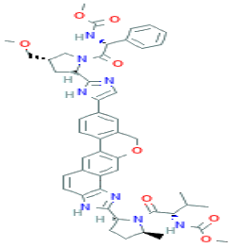


Fig- 1:Structure of Sofosbuvir

Fig-2: Structure of Velpatasvir



MATERIALS AND METHODS

Preparation of phosphate buffer: Phosphate buffer solution of 0.05M was prepared by combining 6.67 gm of potassium di-hydrogen phosphate and 8.55 gm of di potassium hydrogen phosphate in 1 L flask. To this 800ml HPLC grade water was added, sonicated thoroughly, adjusted final volume to 1 L and then filtered through 0.45 microns filter under vacuum filtration.

Adjust the pH to 3.2 with careful addition of Ortho-phosphoric acid.

Preparation of Mobile phase: Accurately measured 600ml of (60%) of HPLC methanol and 400ml ofphosphatebuffer (40%) were mixed and degassed in a digital ultra sonicator for25 minutes and then filtered through 0.45 microns filter under vacuum filtration.[3]

Stock Solution:

Sofosbuvir stock solution: 100 mg of Sofosbuvir API standards were accurately weighed and is transferred into a neat and dry volumetric flask of 100ml. About 80ml of diluent was added and allow for sonicate to remove the complete air bubbles formed in it, which is again make up to mark with same diluent to get 1000 µg/mL.

Velpatasvir stock solution: 100 mg of Velpatasvir API standards were accurately weighed and istransferred into a neat and dry volumetric flask of 100ml. About 80ml of diluentwas added and allow for sonicate to remove the complete air bubbles formed init, which is again make up to mark with same diluent to get 1000 µg/mL.

Working Standard Solution: From the stock solution 4ml of Sofosbuvir stock solution and 1ml of Velpatasvir stock solution was pipetted out and transferred in to 100mlvolumetric flask which is again diluted with diluent up to the mark to get 40µg/ml Sofosbuvir and 10µg/ml Velpatasvir.[4]

RESULTS AND DISCUSSION:

Method validation: Specificity, linearity, range, Accuracy, precision, Repeatability, Intermediate precision, limit of detection, limit of Quantification, Robustness.

Method development: Method development was performed by changing various chromatographic conditions like mobile phase ratios, buffers, and flow rates.

SPECIFICITY: The system suitability for specificity was carried out to determine whether there is an interference of any impurities in retention time of analytical peak. The specificity study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak.[5]

LINEARITY: The regular stock solution consists of six linear concentrations (20µg/ml-70µg/ml Sofosbuvir and 5µg/ml-15µg/ml-Velpatasvir). Theywere tabulated in table num-1 and 2.

ACCURACY: Samples are prepared by sample stock solvent at three separate levels: 50%, 100%, and 150 percent each with three preparations. They were tabulated in table num-3 and 4.

PRECISION: The precision of the analytical method was studied by injecting six replicates of standard and sample concentration on the same day and another day. The concentration of Sofusbuvir and Velpatasvirwere injected atintermediate precision and repeatability. The %RSD was calculated and results were reported in table no. 5, 6, 7 and 8.[6]

LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentrations of the drug. The LOD was defined as the concentration which yields a signal-to-noise ratio 3:1 while the LOQ was calculated to be the lowest concentration that could be measured with signal-to-noise ratio 10:1. The LOD & LOQ were calculated by measuring the standard deviation of the response and slope. The result of LOD &LOQ was tabulated in table no.9 .[7]

ROBUSTNESS: There is no established improvement in the process such as the flow rate, the mobile step ratio, and the temperature, but the findings are within the range as per the ICH Guidelines. Robustness condition like flow minus (0.8ml/min), flow plus (1.2ml/min), temperature ambient was maintained and samples were injected in duplicate manner.

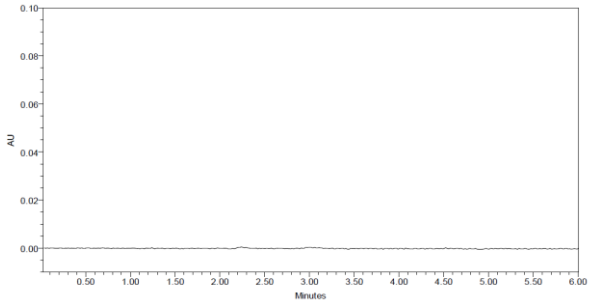


Fig-3:Chromatogramof blank

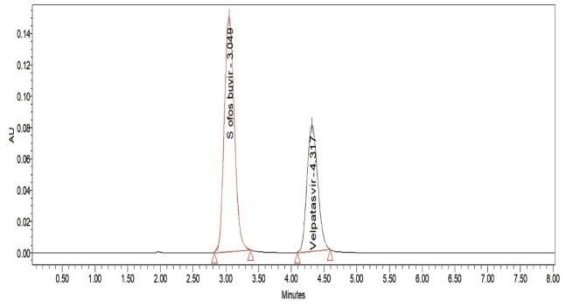


Fig-4: Optimized condition Chromatogram

Table -1:Linearity table for Sofosbuvir

Linearity Level	Concentration (µg/ml)	Peak Area
Level 0	0	0
Level 1	20	4719376
Level 2	30	7079064
Level 3	40	9438751
Level 4	50	11698439
Level 5	60	13841127
Level 6	70	16607815

Fig-5: Calibrationcurveof Sofosbuvir

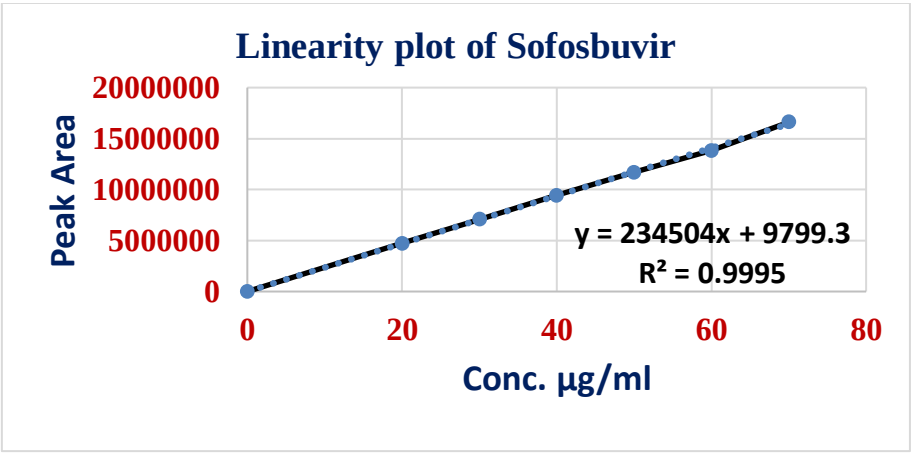


Table -2: Linearity table for Velpatasvir

Linearity Level	Concentration (µg/ml)	Peak Area
Level 0	0	0
Level 1	5	161774
Level 2	7.5	242661
Level 3	10	323547
Level 4	12.5	404434
Level 5	15	485321
Level 6	17.5	556208

Fig-6: Calibration curve of Velpatasvir

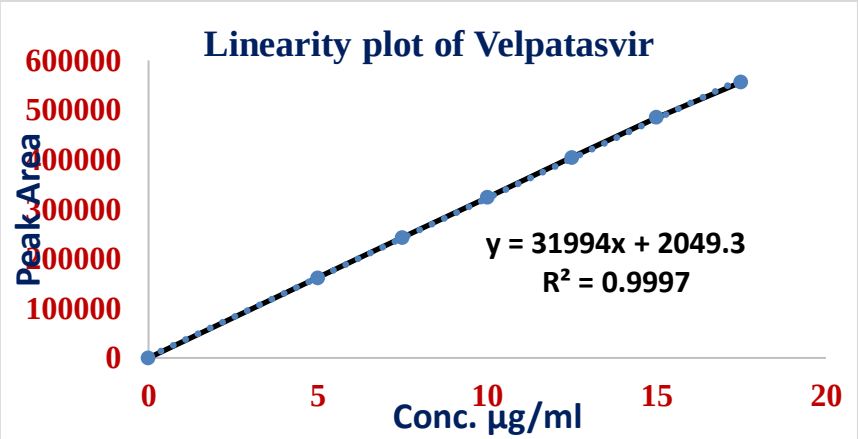


Table- 3: Accuracy table of Sofosbuvir

Concentration of sample taken (µg/ml)	% of spiked level	Amount added(µg)	Amount found(µg)	% Recovery	Statistical Analysis of % Recovery
40µg/ml	50%Injection 1	20	20.01	100.05	MEAN = 99.98 %RSD = 0.45
	50%Injection 2		19.91	99.5	
	50%Injection 3		20.08	100.4	
	100 % Injection 1	40	40.03	100.07	MEAN = 99.93 %RSD = 0.15
	100 %Injection 2		39.98	99.95	
	100%Injection 3		39.91	99.77	
	150% Injection 1	60	60.02	100.03	MEAN = 100.06 %RSD = 0.04
	150%Injection 2		60.07	100.11	
	150%Injection 3		60.04	100.06	

Table:4- Accuracy table of Velpatasvir

Concentration of sample taken(µg/ml)	% of spiked level	Amount added(µg)	Amount found(µg)	% Recovery	Statistical Analysis of % Recovery
10µg/ml	50%Injection 1	5	4.96	99.20	MEAN = 99.66 %RSD = 0.51
	50%Injection 2		4.98	99.60	
	50%Injection 3		5.01	100.20	
	100 %Injection 1	10	9.93	99.30	MEAN = 99.86 %RSD = 0.60
	100 %Injection 2		10.05	100.50	
	100%Injection 3		9.98	99.80	
	150%Injection 1	15	14.96	99.73	MEAN = 99.77 %RSD = 0.34
	150%Injection 2		14.92	99.46	
	150%Injection 3		15.02	100.13	

Table-5: Intermediate precision data for Sofosbuvir

Injection	Retention Time	Area of the peak	Height of the Peak	Peak plate count	Peak tailing
1	3.045	9432571	13918	11038.64	1.15
2	3.048	9438475	14842	10986.37	1.14
3	3.049	9434752	14967	11051.68	1.18
4	3.048	9430487	13984	11028.29	1.16
5	3.047	9436547	13967	10967.53	1.16
6	3.042	9437841	14737	11019.75	1.17
Mean		9438845			
SD		147205			
% RSD		0.74			

Table-6: Intermediate precision data for Velpatasvir

Injection	Retention Time	Area of the peak	Height of the Peak	Peak plate count	Peak tailing
1	4.309	323584	7842	8406.17	1.27
2	4.316	323054	8018	8297.38	1.28
3	4.049	323847	8103	8364.28	1.26
4	4.048	323751	7469	8341.19	1.27
5	4.312	323814	7684	8403.27	1.28
6	4.309	323745	7923	8365.28	1.27
Mean		323875			
SD		3240.54			
% RSD		0.54			

Table-7: Repeatability table for Sofosbuvir

Injection	Retention Time	Area of the peak	Height of the Peak	Peak plate count	Peak tailing
1	3.043	9437784	15068	10923.12	1.13
2	3.045	9437412	14942	11064.31	1.16
3	3.045	9430257	14637	10934.16	1.14
4	3.043	9438431	15018	11034.26	1.18
5	3.043	9438754	14936	10981.34	1.17
6	3.045	9436128	15213	11034.18	1.15
Mean		9436461			
SD		3147.71			
% RSD		0.03			

Table-8: Repeatability table for Velpatasvir

Injection	Retention Time	Area of the peak	Height of the Peak	Peak plate count	Peak tailing
1	4.310	323112	7936	8364.28	1.28
2	4.312	323452	8028	8316.29	1.28
3	4.309	323742	7853	8324.31	1.27
4	4.308	323047	8061	8391.25	1.29
5	4.307	323087	7954	8365.27	1.26
6	4.310	323094	7919	8357.21	1.28
Mean		323255			
SD		280.65			
% RSD		0.09			

Table-9: LOD and LOQ data for Sofosbuvir and Velpatasvir

Drug	Average slope	Average intercept	Standard deviation of the intercept	Regression coefficient (R^2)	LOD (µg/ml)	LOQ (µg/ml)
Sofosbuvir	234504	9799.3	2663.18	0.999	0.03	0.09
Velpatasvir	31994	2049.3	1528.04	0.999	0.15	0.47

Table-10: Robustness data for Sofosbuvir

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	9416963	1.16		9436039	1.17		9456257	1.16

Table-11: Robustness data for Velpatasvir

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	321727	1.14		323472	1.12		324794	1.13

System suitability parameters were not much affected and all the parameters were passed %RSD was found to be within the limits and results were tabulated in table no. 10 and 11.[8-9]

CONCLUSION:

A simple, fast, accurate and specific RP-HPLC method has been developed for the simultaneous quantification of Sofosbuvir and Velpatasvir in bulk and tablet dosage form by studying different parameters. The maximum absorbance was found to be at 254nm for Sofosbuvir and 254nm for Velpatasvir. The common wavelength observed was 254nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was INERTSIL – C18, BDS column, 150 x 4.6mm, 5µ and resulted good peak shape. 25°C temperature was found to besuitable for the nature of drug solution. The flow rate was fixed at 1.0ml/minbecause of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 60:40 (Methanol : Phosphate buffer) was fixed due to good symmetrical peaks andgood resolution. So, this mobile phase was used for the proposed study.

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