WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 8, Issue 12, 41-51. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR CONCURRENT ASSESSMENT BY RP-HPLC OF SOFOSBUVIR AND VELPATASVIR IN PHARMACEUTICAL DOSAGE FORM

Atif Ali[1]*, Sayed Muzahir Hussain[3], Awais Shabir[1], Muhammad Zubair Khan[2], Tanseer Abbas[4], Muhammad Jamil[5] and Mugheer Pervaiz[1]

[1]Biochemistry Department, Hazara University, Mansehra, Pakistan.

[2]Chemistry Department, Hazara University, Mansehra, Pakistan.

[3]Department of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan.

[4]Faculty of Pharmacy, Gomal University, DI Khan, Pakistan.

[5]Chemistry Department, Peshawar University, Peshawar, Pakistan.

Article Received on
28 August 2019,
Revised on 18 Sept. 2019,
Accepted on 08 Oct. 2019
DOI: 10.20959/wjpr201912-16017

***Corresponding Author**
Atif Ali
Biochemistry Department,
Hazara University,
Mansehra, Pakistan.

ABSTRACT

Purpose: The major purpose of this research is the development of a novel, simple, precise, fast and cheap RP HPLC method for quantifying Velpatasvir and Sofosbuvir in tablet formulation. **Methods:** The separation of these molecules was performed on C18 column (250 x 4.6 mm, 5 µm) of PHENOMENEX. The above process was executed with the help of a mobile phase that contain mixture of phosphate buffer of 0.02 M (pH 5.0) and acetonitrile in a ratio of 450:550 by inducing a 20 µl sample. The wavelength was selected to be 280 nm with 01 ml / min flow rate. **Results:** Velpatasvir and Sofosbuvir retention time values were 8.8 and 3.3 min, and the linearity range was 96-144 µg / ml for Sofosbuvir and 24-36 µg / ml for Velpatasvir respectively. Correlation coefficient of Velpatasvir is 0.9996 and 0.9998 was of Sofosbuvir. **Conclusion:** The method validates specificity, accuracy, precision, linearity, robustness, LOD and LOQ.

KEY WORDS: Velpatasvir, Sofosbuvir, RP-HPLC.

INTRODUCTION

Hepatitis C virus (HCV) brings about contamination of the liver leading to Hepatitis C. It is known to be a Blood borne infection. This Virus is one of the major all-inclusive reasons for death and dismalness[1] and late gauges demonstrated an expansion in its prevalence throughout the most recent decade to 2.8%, relating to more than 185 million contaminations overall.

Past worldwide weight of illness gauges distributed by the World Health Organization (WHO) incorporate just weight from intense HCV disease. The real weight from HCV disease originates from sequelae from unending contamination.[2]

The predominance of unending HCV disease is 39% among individuals who have infused medicates in the previous a year, speaking to an expected 6.1 million individuals with incessant HCV contamination (8% of worldwide diseases).[3] An expected 35% of worldwide HCV diseases are represented by the genotypes 2 and 3 of HCV, influencing approximately 58 million people.[4]

Chronic Hepatitis C is genuine sickness, which may lead to extended haul medical issues, and may even be fatal.

HCV is a wrapped, positive-sense RNA infection of the family Flaviviridae. Normally happening variations of HCV are characterized into six noteworthy genotypes. The utmost common HCV in the United States is the one with genotype-1 that influences 72% of all chronic HCV patients.

As of late, the WHO has distributed rules focusing to enhancements in the counteractive action merely for the continual control and treatment of hepatitis.[5]

Transmission of infected blood and other body liquids are major reason of HBV transmission from person to person. Transcendent methods of conduction fluctuate according to pervasiveness. In zones with immense endemicity of Hepatitis, HBV is basically conveyed steeply from tainted moms to neonates nearby birth. Even the conduction of disease through immediate contact between kids is additionally announced in zones of inordinate and transitional commonness.[6] Conversely, contamination with HBV in low-endemicity territories is primarily obtained amid pre-adulthood and early maturity and is firmly identified with high-chance practices, for example, unprotected sex and infusion tranquilize use.[7]

For a long time, the main treatment alternative for such sort of patients was liver transplantation. In this era, clinical preliminaries of recently affirmed constant acting antiviral operators have demonstrated that it is conceivable to cure HCV contamination securely and adequately in individuals with decompensated cirrhosis.[8]

American Infectious Diseases Society (IDSA) and American Association for the Study of Liver Diseases (AASLD) prescribed Sofosbuvir as the first line treatment together with Velpetasvir to heal all six defective genotypes of hepatitis C in 2016.

Drug Profile

Sofosbuvir

Sofosbuvir with the name of IUPAC (S)- Isopropyl 2-((S)- (((2R,3R,4R,5R)- 5-(2,4-dioxo3,4-dihydropyrimidin-1(2H)- yl)- 4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2yl) methoxy)- (phenoxy)phosphorylamino) propionate, with molecular formula C22H29FN3O9P and atomic weight 529.453 g/mol. Sofosbuvir is an immediate acting antiviral drug casted off as a foremost ration in the blend treatment of incessant Hepatitis C- an ailment caused by the severe contamination of liver by the Hepatitis C Virus (HCV).

The development of sofosbuvir, has significantly enhanced therapy replacements for ongoing hepatitis C since 2011. As a simple prodrug nucleotide, Sofosbuvir is used as a 2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate antiviral operator in its vibrant assembly, which acts that function as a defective substratum for NS5B (non-basic protein 5B). NS5B, and RNA-subordinate polymerase RNA, is rudimentary for hepatitis C viral RNA elucidation and for its high replicative and hereditary assorted variety.[9] To cure Hepatitis C infection, Sofosbuvir and other direct acting antiviral drugs in a combination, act as extremely strong substitutes. They depict an immense interference in the improvement of resistance.

Velpatasvir: Velpatasvir has the IUPAC name methyl {(2S)- 1-[(2S,5S)- 2-(9-{2-[(2S,4S)-1-{(2R)- 2-[(methoxycarbonyl)amino]-2-phenylacetyl}-4-(methoxymethyl)pyrrolidin-2-yl]-1H-imidazol-4-yl}-1,11-dihydro[2]benzopyrano[4',3':6,7]naphtho[1,2-d]imidazol-2-yl)- 5-methylpyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl}carbamate. Its molecular formula is C49H54N8O8 and molecular weight is 883.019 g/mol. It is also a direct-acting antiviral (DAA) drug that used as a component of a blend treatment to cure constant hepatitis C. It acts as an inadequate substrate for NS5A (Non-Structural Protein 5A), a non-enzymatic viral protein have important role in the duplication, collection and tweaking of invulnerable

reactions of the host.[10] Alongside the advancement of Direct Acting Antivirals (DAAs, for example, velpatasvir, the treatment varieties for constant Hepatitis C have advanced profoundly since 2011. Significantly, velpatasvir has a substantially increased obstacle to opposition to the original NS5A inhibitors, making it a very strong and reliable treatment option for incessant hepatitis C (Myers RP *et al*., 2015).

Economically accessible item is Epclusa in which both drugs are present. Epclusa was first endorsed in June of 2016 and is the leading product available in combine formulation, suggested for therapeutic management for all Hepatitis C genotypes with or without cirrhosis. Epclusa is furthermore as of now the most influential HCV antiviral prescription available with a sustained virologic reaction (SVR). American and Canadian guidelines recommend Epclusa for all HCV genotypes as a first-line treatment.[11]

Structure

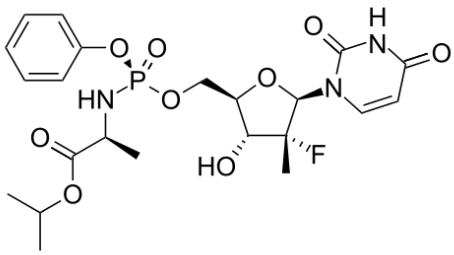

Figure. Sofosbuvir

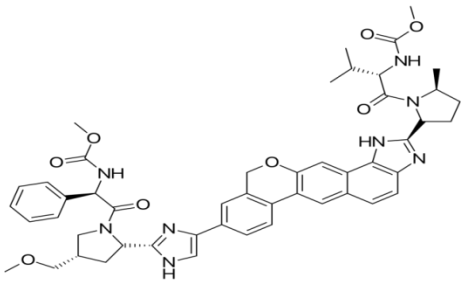

Figure. Velpatasvir

MATERIALS AND METHODS

Chemicals and Reagent: The commercially accessible Epclusa formula containing Velpatasvir 100mg and Sofosbuvir 400 mg bought from the US market. Global Pharmaceuticals provides the reference work standards for both drugs. E.Merck Ltd, Pakistan acquired potassium dihydrogen phosphate, phosphoric acid and acetonitrile (HPLC grade).

Instrument used: A Hitachi, Model (5110-5410), Adwa pH meter, Model AD 1020.

Solutions preparation

Diluent: Acetonitrile and distilled water were used as a diluent in the ratio of 80:20.

Preparation of Stock Solution

Solution A (Sofosbuvir): In a 50 ml volumetric flask, the Sofosbuvir weighted working standard was correctly acclimatized, equivalent to 60 mg Sofosbuvir, supplemented with diluent.

The solution has a sofosbuvir concentration of 1200 µg / ml.

Solution B (Velpatasvir): The Velpatasvir weighted working standard was accurately acclimated in a 50 ml volumetric flask, equivalent to 15 mg Velpatasvir, supplemented with diluent.

The solution has a Velpatasvir concentration of 300 µg / ml.

Standard Prepration: Transferred 5ml in 50ml volumetric flask from solution A and solution B and volume was made up with diluent. Final solution having a concentration of sofosbuvir 120 µg/ml and velpatasvir 30 µg/ml.

Sample Preparation: After weighing, 20 tablets of Epclusa was pulverized into a fine powder, and a precisely weighted the fine powder equivalent to 60 mg of Sofosbuvir and 15 mg of Velpatasvir was added to a volumetric flask of 50 ml, 30 ml of a diluent was added. After 15 min of stirring of this solution, volume was made up by diluent.

5 ml from the above solution was transferred to a 50 ml volumetric flask, diluent was used for volume makeup. This solution contains120 µg/ml Sofosbuvir and 30 µg/ml Velpatasvir.

Mobile phase: Mobile phase was prepared by mixing Acetonitrile and Phosphate buffer having pH 5.0(0.02M Potassium Phosphate) in the ratio of 55:45. Mobile phase was filtered by using 0.4 µm membrane filter paper and sonicate for 5 minute.

Chromatographic condition: Separation and quantification of the two drugs was achieved by using a C18 column of PHENOMENEX (250 x 4.6 mm x 5 µm). A 20 µl solution sample prepared in a diluent (water-acetonitrile mixture 20:80) was injected. Flow rate was set to be 1 ml / min and absorbance was measured at 280 nm. Column temperature has been adjusted to 30 ° C.

RESULTS AND DISCUSSIONS

Method Development: Drug analysis is the main role of drug production and development. There are no analytical procedures for new drugs in the Pharmacopoeia, so a simple, precise, and specific linear method of analysis needs to be developed.

The choice of mobile phase was based on the separation of two drugs with ideal resolution and spikes of Sofosbuvir and Velpatasvir, which were achieved on a PHENOMENEX C18-HPLC column.

Method validation: The advanced technique like system suitability, specificity, accuracy linearity, robustness, LOD and LOQ has been certified according to ICH and USP rules.

System Suitability Test: According to the USP guideline, suitability tests were performed prior to running samples for verification. The RSD of six sample of both drugs was 0.21% and 0.49%, indicating that the HPLC system has good precision. Table 1 shows the results obtained by suitability.

Table 1(a): System Suitability of Sofosbuvir

%RSD (<2.0)	Tailing Factor	Theoretical Plates
0.216	1.07	89800

Table 1(b): System Suitability of Velpatasvir.

%RSD (<2.0)	Tailing Factor	Theoretical Plates
0.49	1.30	90150

Specificity: It is the capacity to determine the interference between analyte and other compound. Figure 1 shows no interference between analyte and other compound like mobile phase and placebo, indicating the specificity of analytical technique.

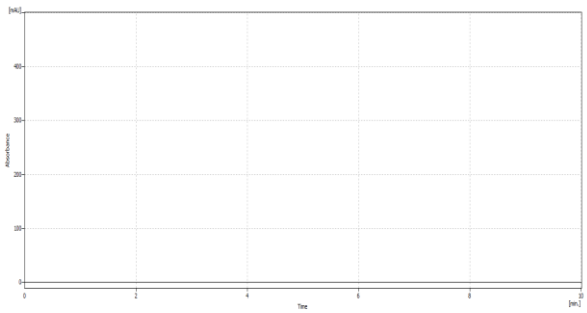

Fig 1(a): Chromatogram of Placebo

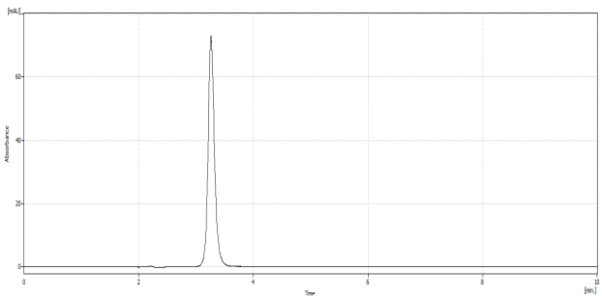

Fig 1(b): Chromatogram of Sofosbuvir

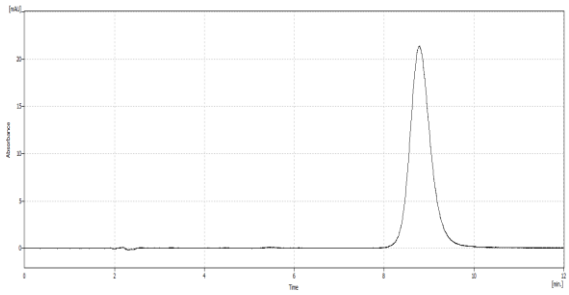

Fig 1 (c): Chromatogram of Velpatasvir

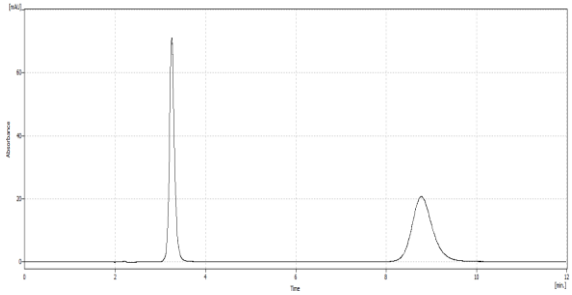

Fig 1(d): Chromatogram of both drugs

Linearity: Linearity is the relation between absorbance and concentration of drugs. That was shown by plotting the X-axis and Y-axis plots at concentrations from 96 ug / ml to 144 ug / ml Sofosbuvir and 24 ug / ml to 36 ug / ml Velpatasvir. The LOD and LOQ of Sofosbuvir are 2.633ug / ml and 7.978ug / ml, respectively, and Velpatasvir is 1.116ug / ml and 3.382ug / ml respectively. Results are shown in Figure 2(a) and 2(b).

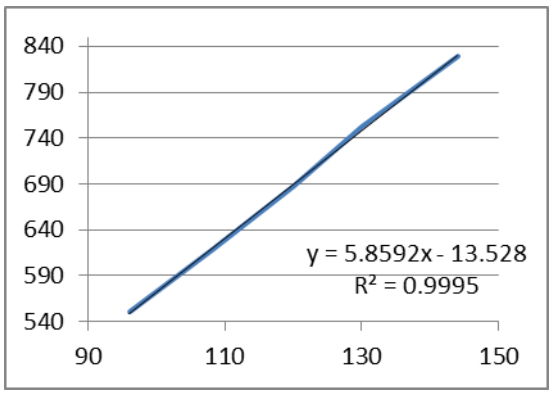

Fig 2 (a): Sofosbuvir Linearity plot

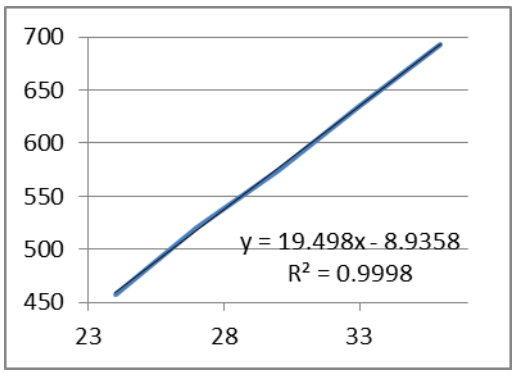

Fig 2 (b): Velpatasvir Linearity plot

Recovery: After spiking 80%, 100% and 120% sample concentration of Velpatasvir and Sofosbuvir in Placebo, Recovery was achieved by injecting replicate samples.

Table 4 shows spiked and retrieved values.

Table 4: Recovery of Sofosbuvir and Velpatasvir.

Recovery Level	Drugs	Amount Spiked (mg/ml)	Amount Recovered (mg/ml)	Recovery (%age)	Standard Deviation	RSD (%)
80%	Sofosbuvir	320 320 320	318.44 316.24 318.88	99.32	1.41	0.44
	Velpatasvir	80.0 80.0 80.0	79.92 81.48 81.48	101.20	0.90	1.11
	Sofosbuvir	400	399.84	99.86	2.24	0.56

100%		400 400	397.04 401.48			
	Velpatasvir	100.0 100.0 100.0	101.79 101.53 101.99	101.77	0.23	0.22
120%	Sofosbuvir	480 480 480	477.07 478.08 475.52	99.35	1.29	0.27
	Velpatasvir	120.0 120.0 120.0	122.06 122.11 121.57	101.59	0.29	0.24
			Sofosbuvir	Velpatasvir		
Overall Mean			99.51%	101.52%		
Overall Standard Deviation			1.64	0.47		
Overall % RSD			0.41	0.52		

Precision: Precision of analytical method shows the degree of scattering between samples. Proposed precision by evaluating the six sample replicates. Assay of each duplicate and calculation of the percentage RSD of the sample. The results obtained are in table 5.

Table 5: Precision results of Sofosbuvir and Velpatasvir.

Drugs	Peak Areas of Replicate	Average Peak Areas of each replicate	Assay %	Average	Standard Deviation	%RSD
Sofosbuvir	581.021	578.09	99.86	100.18	0.45	0.4517
	575.168					
	575.005	581.79	100.50			
	588.589					
	577.448	581.45	100.44			
	585.454					
Velpatasvir	699.771	692.28	100.16	100.66	0.43	0.4320
	684.804					
	687.800	697.19	100.87			
	706.594					
	696.708	697.72	100.95			
	698.739					

Robustness: It is the little variation in different perimeter of analysis, to check sample stability. Variations such as flow rate and column temperature do not affect the performance of the method. Table 6 shows the results obtained.

Table 6 (a): Robustness result of Velpatasvir.

Level		Peak Areas of Replicate		Theoretical plates	Tailing factor	Resolution	Standard Deviation	%RSD
100% conc.	Change in Flow rate	0.8ml/min	835.480	44536	1.303	11.909	14.49	1.71
			855.975	44293	1.326	11.883		
		1.0ml/min	724.043	37564	1.343	11.057	10.83	1.51
			708.723	37317	1.315	11.029		
		1.2ml/min	582.716	32929	1.327	10.384	3.09	0.53
			578.340	32929	1.320	10.384		
	Change in column Temp.	25°C	708.725	33430	1.347	11.031	1.18	0.17
			710.417	33388	1.341	11.018		
		30 °C	713.926	37578	1.346	8.644	5.73	0.81
			705.824	37578	1.351	8.783		
		35 °C	738.395	37607	1.329	11.067	14.00	1.92
			716.485	37564	1.340	11.057		

Table 6 (b): Robustness result of Sofosbuvir.

Level		Peak Areas of Replicate		Theoretical plates	Tailing factor	Resolution	Standard Deviation	%RSD
100% conc.	Change in Flow rate	0.8ml/mint.	697.914	97944	1.361	11.909	9.84	1.40
			711.829	98025	1.361	11.883		
		1.0ml/mint	570.832	91022	1.350	11.057	7.94	1.39
			566.605	91022	1.350	11.029		
		1.2ml/mint.	468.602	81173	1.302	10.384	1.64	0.35
			466.279	81173	1.302	10.384		
	Change in column Temp.	25°C	573.368	88221	1.344	11.031	3.68	0.64
			578.577	88312	1.328	11.018		
		30 °C	577.469	88403	1.350	8.644	3.35	0.58
			572.738	91022	1.350	8.783		
		35 °C	592.741	91022	1.350	11.067	11.55	1.98
			576.404	91022	1.350	11.057		

CONCLUSION

In this study, quantitative method of analysis in tablet dosage forms for Sofosbuvir and Velpatasvir were developed. Resolution between Sofosbuvir and Velpatasvir gives good results. USP and ICH guidelines were used to validate the method based on the above experimental results. The validated method can therefore be used in a tablet dosage form for numerical analysis of Sofosbuvir and Velpatasvir.

Abbreviations

AASLD	American Association for the Study of Liver Diseases
DAA	Direct Acting Antivirals
HBV	Hepatitis B virus

HCV	Hepatitis C virus
IDSA	American Infectious Diseases Society
NS5A	Non-Structural Protein 5A
RNA	Ribo Nucleic Acid
SVR	Sustained Virologic Reaction
WHO	World Health Organization

REFERENCES

1. Cooke GS, Lemoine M, Thursz M, Gore C, Swan T, Kamarulzaman A, DuCros P, Ford N. Viral hepatitis and the Global Burden of Disease: a need to regroup. J Viral Hepat, 2013; 20: 600–601.
2. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol, 2006; 45: 529-538.
3. Degenhardt L, Peacock A, Colledge S, Global prevalence of injecting drug use and sociodemo graphic characteristics and prevalence of HIV, HBV, and HCV in people who inject drugs: a multistage systematic review. Lancet Glob Health, 2017; 5: 1192–207.
4. Gower E, Estes C, Blach S, Razavi- Shearer K, Razavi H, Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol, 2014; 61: S45-S57.
5. Wiersma ST, McMahon B, Pawlotsky JM, Thio CL, Thursz M, Lim SG. Treatment of chronic hepatitis B virus infection in resource-constrained settings: expert panel consensus. Liver Int., 2011; 31: 755–61.
6. Beasley RP, Hwang LY. Postnatal infectivity of hepatitis B surface antigen-carrier mothers. J Infect Dis., 1983; 147: 185–90.
7. Trepo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet, 2014; 384: 2053–63.
8. Forns X, Charlton M, Denning J, Sofosbuvir compassionate use program for patients with severe recurrent hepatitis C after liver transplantation. Hepatology, 2015; 61: 1485-94.
9. Simmonds P: Genetic diversity and evolution of hepatitis C virus-15 years on. J Gen Virol., 2004 Nov; 85: 3173-88.
10. Mogalian E, German P, Kearney BP, Yang CY, Brainard D, Link J, McNally J, Han L, Ling J, Mathias A: Preclinical Pharmacokinetics and First-in-Human Pharmacokinetics, Safety, and Tolerability of Velpatasvir, a Pangenotypic Hepatitis C Virus NS5A Inhibitor, in Healthy Subjects. Antimicrob Agents Chemother, 2017 Apr 24; 61.

11. Myers RP, Shah H, Burak KW, Cooper C, Feld JJ: An update on the management of chronic hepatitis C: 2015 Consensus guidelines from the Canadian Association for the Study of the Liver. Can J Gastroenterol Hepatol, 2015 Jan-Feb; 29(1): 19-34.