INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.8 | Issue 3 | Jul - Sep - 2019
Journal Home page: www.ijpar.com

Research article *Open Access*

Method development and validation of combination of sofosbuvir and velpatasvir by RP-HPLC method

Shirisha Bhavani*[1], [2]A. Srinivasa Rao, [1]A. Kavitha, [1]B.Haritha, [1]Burchu Mary Sunitha, [1]Kandi Kanti Manisha, [1]N. Swetha

[1]Department of Pharmaceutical Analysis, Bhaskar Pharmacy College, Yenkapally, Moinabad (M), Ranga Reddy (Dt), Hyderabad – 500 075, Telangana, INDIA
[2]Department of Pharmacy Practice, Bhaskar Pharmacy College, Yenkapally, Moinabad(M), Ranga Reddy (Dt), Hyderabad – 500 075, Telangana, INDIA
Corresponding Author: Shirisha Bhavani

ABSTRACT

Objective
The objective of the present research work was to develop a innovative, simple, and economic method for estimation of Sofosbuvir and Velpatasvir in bulk and dosage form by RP-HPLC.

Methods
The chromatographic conditions were performed on Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. as stationary phase and mobile phase was prepared with a mixture of Potassium Dihydrogen Phosphate buffer (adjusted with 1% Orthophosphoric acid, pH- 3.5) (0.05M) : Acetonitrile with (70:30 v/v), flow 1.0 ml/min, with Injection Volume 10µl, at detection wavelength 257 nm and run time at 10.0 mins

Results
The analytical method is valid for estimation of Sofosbuvir and Velpatasvir over a range of 6 µg/ml – 14 µg/ml and 12 µg/ml – 28 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range.

Conclusion
A specific, sensitive, economic method estimation of Sofosbuvir and Velpatasvir has been developed based on ICH Guidelines with bulk and dosage forms.

Keywords: Sofosbuvir and Velpatasvir, HPLC, Method Development, ICH, Validation, Accuracy, Precision.

INTRODUCTION

Sofosbuvir, sold under the brand name Sovaldi among others, is a medication used for the treatment of hepatitis C. It is only recommended with some combination of ribavirin, peginterferon-alfa, simeprevir, ledipasvir, daclatasvir, or velpatasvir. Cure rates are 30 to 97% depending on the type of hepatitis C virus involved. Safety during pregnancy is unclear; some of the medications used in combination may result in harm to the baby. It is taken by mouth. Common side effects include feeling tired, headache, nausea, and trouble sleeping. Side effects are generally more common in interferon-containing regimens. Sofosbuvir may reactivate hepatitis B in those who have been previously infected in combination with ledipasvir, daclatasvir or simeprevir it is not recommended with amiodarone due to the risk of an abnormally slow heartbeat. Sofosbuvir is in the nucleotide analog family of medication and works by blocking the hepatitis C NS5B protein. Sofosbuvir inhibits the hepatitis C NS5B protein. Sofosbuvir appears to have a high barrier to the development of resistance. Sofosbuvir is a prodrug. It is metabolized to the active antiviral agent GS-461203 (2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate). GS-461203 serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis.

The IUPAC Name of Sofobuvir is propan-2-yl (2S)-2-{[(S)-{[(2R, 3R, 4R, 5R)-5-(2, 4-dioxo-1, 2, 3, 4-tetra hydro Cpyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl] methoxy} (phenoxy) phosphoryl] amino} propanoate.

Velpatasvir is an NS5A inhibitor (by Gilead) which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.[7] Velpatasvir is both an inhibitor and a substrate of the transporter proteins P-glycoprotein (Pgp), ABCG2, OATP1B1 and OATP1B3.[8-10] It is partly degraded by the liver enzymes CYP2B6, CYP2C8 and CYP3A4. Substances that are transported or inactivated by these proteins, or interfere with them, can interact with velpatasvir. [11] In studies, this has been found for the HIV combination efavirenz / emtricitabine / tenofovir, which reduces the area under the curve (AUC) of velpatasvir by about 50%, and the CYP3A4 and Pgp inducer rifampicin, which reduces its AUC by about 80%, rendering it likely ineffective.[12-14] Digoxin is eliminated by Pgp; its AUC is increased by about 30% in combination with velpatasvir and sofosbuvir (although it is not clear which of the two is responsible for this effect). Substances that reduce gastric acid, such as antacids, H2 blockers, and proton pump inhibitors, reduce velpatasvir AUC by 20–40%.[15-17]

The IUPAC Name of Velpatasvir is (2S)-2-{[hydroxy(methoxy)methylidene]amino}-1-[(2S,5S)-2-(17-{2-[(2S,4S)-1-[(2R)-2-{[hydroxy(methoxy)methylidene]amino}-2-phenylacetyl]-4-(methoxymethyl)pyrrolidin-2-yl]-1H-imidazol-5-yl}-21-oxa-5,7-diazapentacyclo [11.8.0. 0 {3,11}.0^{4,8}.0^{14,19}]henicosa-1 (13), 2, 4(8Z), 6, 9, 11, 14(19), 15,17-nonaen-6-yl)-5-methylpyrrolidin-1-yl]-3-methylbutan-1-one.[18]

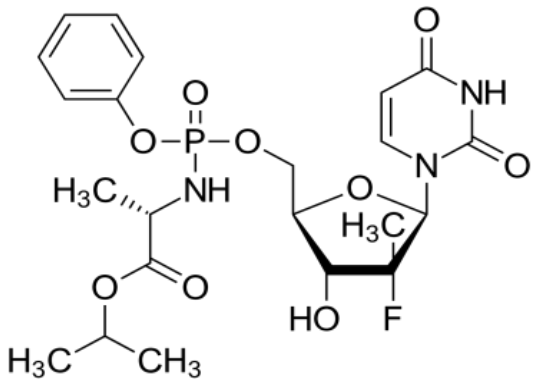


Fig-1: Structure of Sofosbuvir

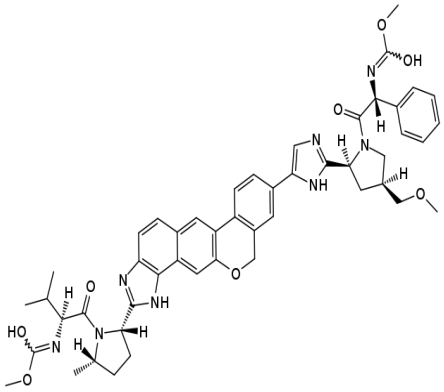


Fig-2: Structure of Velpatasvir

A survey of literature reveals that good analytical methods are not available for Sofosbuvir and Velpatasvir.[19] The present research manuscript describes innovative, simple, economical, accurate, specific, robust, rugged and rapid RP-HPLC method developed in selected solvent system (Mobile Phase) and validated in accordance with International Conference on Harmonization (ICH) Guidelines Q2 (R1), for the estimation of Sofosbuvir and Velpatasvir in bulk drug and in its dosage forms.[20]

EXPERIMENTAL

Materials and Methods

Pharmaceutical grade working standard Sofosbuvir and Velpatasvir were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

Instrumentation

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (T-60 LABINDIA), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

Sample & Standard Preparation for the Analysis

25 mg of Sofosbuvir standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

25 mg of Velpatasvir standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Sofosbuvir and Velpatasvir solution we observed the maxima at 262 nm and 246 nm. The isobestic point for the drugs was found at 257nm.

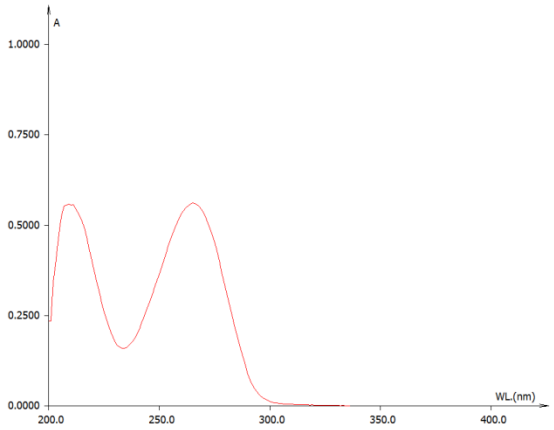


Fig – 3. UV Spectrum for Sofosbuvir

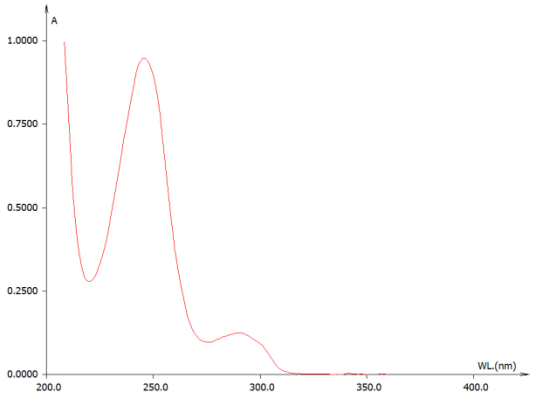


Fig-4. UV Spectrum for Velpatasvir

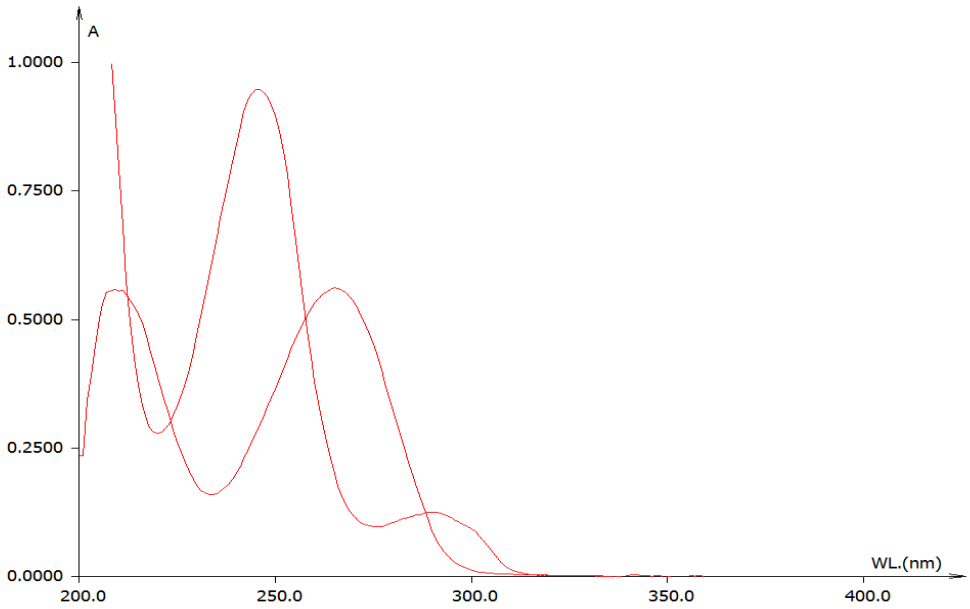


***Fig -5.* Isobestic Point for Sofosbuvir and Velpatasvir**

Method Development

Preparation of Mobile Phase

The mobile phase was prepared with the combination of Potassium Dihydrogen Phosphate buffer (adjusted with 1% Orthophosphoric acid, pH- 3.5) (0.05M) : Acetonitrile at the volume of 1000ml. 700ml of Potassium Dihydrogen Phosphate buffer and 300ml of Acetonitrile were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimized Chromatographic Conditions

Mobile phase	**Potassium Dihydrogen Phosphate buffer (adjusted with 1% Orthophosphoric acid, pH- 3.5) (0.05M) : Acetonitrile = 70:30**
Column	Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	257 nm
Flow rate	1.0 ml/ min.
Run time	10 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic

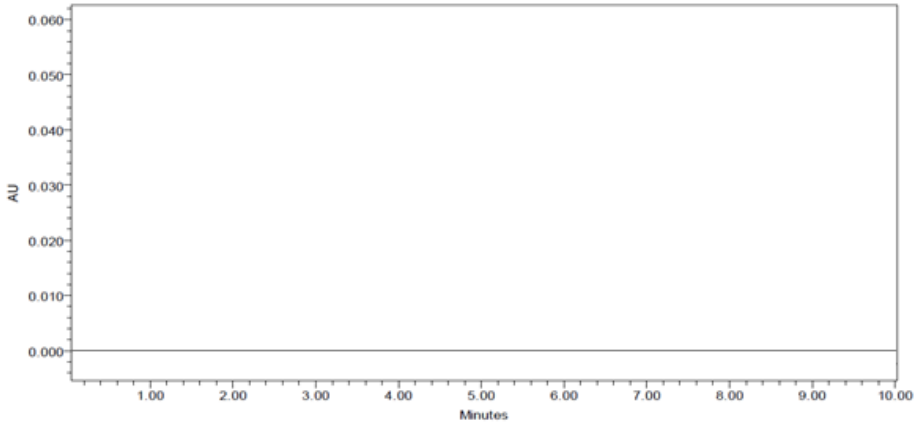
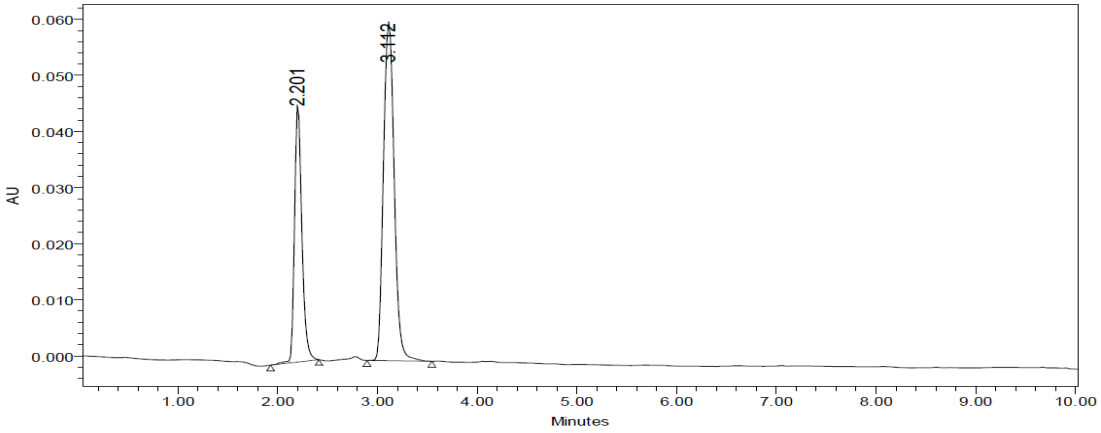


Fig-6: Chromatogram for Blank Preparation



Fig–7: Chromatogram of Sofosbuvir and Velpatasvir in Optimized Condition

METHOD VALIDATION

Linearity & Range

Calibration standards at five levels were prepared by appropriately mixed and further diluted standard stock solutions in the concentration ranges from 6-14µg/ml and 12-28µg/ml for Sofosbuvir and Velpatasvir. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs. Chromatograms of each solution were recorded.

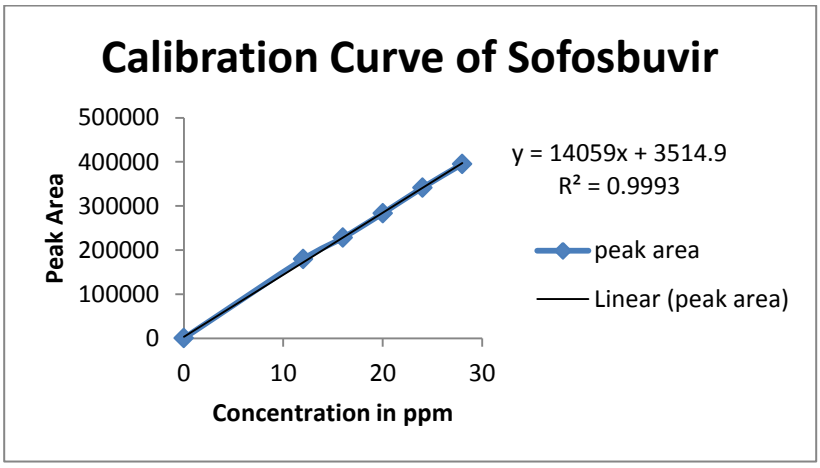


Fig-8: Standard curve for Sofosbuvir

Table-2: Linearity Readings for Sofosbuvir

CONC. (µg/ml)	AUC (n=6)
0	0
6	119571
8	167873
10	211264
12	255428
14	299987

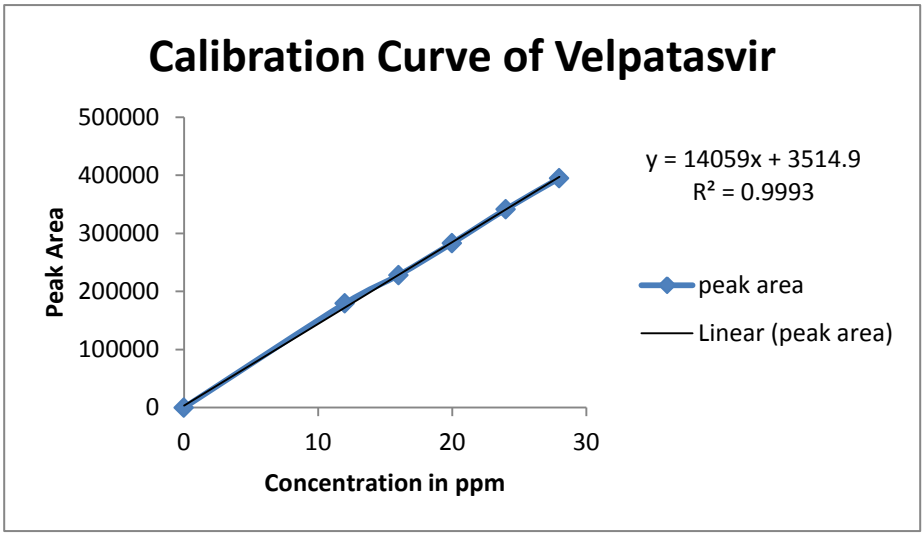


Fig-9: Standard curve for Velpatasvir

Table-3: Linearity Readings for Velpatasvir

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	179371
16	227893
20	283264
24	341428
28	394987

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Sofosbuvir and Velpatasvir were taken and added to the pre-analyzed formulation of concentration 10µg/ml and 20µg/ml. From that percentage recovery values were calculated. The results were shown in table-4 and 5.

Table-4: Accuracy Readings of Sofosbuvir

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S1 : 80 %	8	7.997368	115949	99.9671	Mean= 100.7003%
S2 : 80 %	8	8.106622	117485	101.3328	S.D. = 0.6884036

S3 : 80 %	8	8.064087	116887	100.8011	% R.S.D.= 0.683616%
S4 : 100 %	10	9.904901	142767	99.04901	Mean= 100.36157%
S5 : 100 %	10	10.02966	144521	100.2966	S.D. = 1.346221
S6 : 100 %	10	10.17391	146549	101.7391	R.S.D.= 1.3413706%
S7 : 120 %	12	12.01807	172476	100.1506	Mean= 100.183756%
S8 : 120 %	12	11.88079	170546	99.00657	S.D. = 1.19411
S9 : 120 %	12	12.16729	174574	101.3941	% R.S.D. = 1.19191%

Table-5: Accuracy Readings of Velpatasvir

Sample ID	Concentration (µg/ml)		Peak Area	%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered			
S1 : 80 %	16	16.08685	229679	100.5428	Mean= 100.54488%
S2 : 80 %	16	15.93079	227485	99.56745	S.D. = 0.97847%
S3 : 80 %	16	16.2439	231887	101.5244	R.S.D.= 0.9731%
S4 : 100 %	20	20.07632	285767	100.3816	Mean= 99.97095%
S5 : 100 %	20	19.98769	284521	99.93847	S.D. = 0.395406
S6 : 100 %	20	19.91856	283549	99.59279	% R.S.D.= 0.39552%
S7 : 120 %	24	23.75432	337476	98.97634	Mean= 100.27718%
S8 : 120 %	24	24.11494	342546	100.4789	S.D. = 1.21262
S9 : 120 %	24	24.33032	345574	101.3763	% R.S.D. = 1.20927%

PRECISION

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Sofosbuvir and Velpatasvir (API). The percent relative standard deviation was calculated for Sofosbuvir and Velpatasvir are presented in the Table-6.

Table-6: Repeatability Readings of Sofosbuvir and Velpatasvir

HPLC Injection Replicates	AUC for Sofosbuvir	AUC for Velpatasvir
Replicate – 1	113568	241022
Replicate – 2	113241	240137
Replicate – 3	115408	242911
Replicate – 4	117412	245245
Replicate – 5	112541	241941
Replicate – 6	112546	240444
Average	**114119.3333**	**241356.6667**
Standard Deviation	**1925.83838**	**1416.95812**
% RSD	**1.68756**	**0.58708**

Intermediate precision

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Sofosbuvir and Velpatasvir revealed that the proposed method is precise.

Table-7: Results of Intra-Assay & Inter-Assay for Sofosbuvir

Conc. Of Sofosbuvir (API) (µg/ml)	Observed Conc. Of Sofosbuvir (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
8	8.12	0.45	8.34	0.34
10	10.13	0.27	10.76	0.15
12	12.36	0.53	12.43	0.23

Table-8: Results of Intra-Assay & Inter-Assay for Velpatasvir

Conc. Of Velpatasvir (API) (µg/ml)	Observed Conc. of Velpatasvir (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
16	16.16	0.46	16.24	0.28
20	20.12	0.25	20.43	0.35
24	24.16	0.51	24.87	0.27

Method Robustness

Influence of little changes in optimized chromatographic conditions like changes in flow rate (± 0.1ml/min), mobile phase ratio (±2%), Wavelength of detection (±2nm) and organic phase (±5%) studied to measure the robustness of the method are also in favour of (Table-9 & 10, % RSD < 2%) the developed RP-HPLC method for the analysis of Sofosbuvir and Velpatasvir (API).

Table-9: Results of Method Robustness Test for Sofosbuvir

Change in parameter	% RSD
Flow (0.8 ml/min)	0.29
Flow (1.2 ml/min)	0.35
More Organic	0.88
Less Organic	0.81
Wavelength of Detection (264 nm)	0.89
Wavelength of detection (260 nm)	0.72

Table-10: Results of Method Robustness Test for Velpatasvir

Change in parameter	% RSD
Flow (0.8 ml/min)	0.23
Flow (1.2 ml/min)	0.29
More Organic	0.42
Less Organic	0.31
Wavelength of Detection (258 nm)	1.65
Wavelength of detection (254 nm)	0.69

LOD & LOQ

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The LOD was found to be 0.07 µg/ml and 0.05 µg/ml for Sofosbuvir and Velpatasvir respectively. The LOQ was found to be 0.21 µg/ml and 0.15 µg/ml for Sofosbuvir and Velpatasvir respectively.

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test

parameters were established. The data are shown in Table-11.

Table-11: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs> 2	3.65
2	Asymmetry	T ≤ 2	Sofosbuvir = 0.35 Velpatasvir = 0.23
3	Theoretical plate	N > 2000	Sofosbuvir = 3771 Velpatasvir = 2437

Estimation of Sofosbuvir and Velpatasvir in Tablet Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Finally the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicated to degas the mobile phase (Solvent system). From this above stock solution (1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system (Mobile phase). The prepared solutions were injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection (Blank Solution) of the standard solution also injected into the HPLC system and the chromatograms and peak areas were recorded and calculated.

Table-12: Assay of SOFOSBUVIR and VELPATASVIR Tablets

Brand name of Tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
MyHep All (Mylan Pharmaceutical Company)	400/100	399.13 (±0.07) / 99.28(±0.05)	99.7825 (±0.85) / 99.28 (±0.63)

RESULTS

The optimized chromatographic conditions were Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. as stationary phase and mobile phase was prepared with a mixture of Potassium Dihydrogen Phosphate buffer (adjusted with 1% Orthophosphoric acid, pH- 3.5) (0.05M) : Acetonitrile = (70:30), flow 1.0 ml/min, with Injection Volume 10µl, at detection wavelength 257 nm and run time at 10.0 min. In these chromatographic conditions the peak was pure, sharp, symmetric and found a greater number of theoretical plates.

The results obtained in method validation were

Linearity & Range: The calibration curve showed good linearity in the range of 6-14 µg/ml and 12-28 µg/ml, for Sofosbuvir and Velpatasvir (API) with correlation coefficient (r2) of 0.999 and 0.999. A typical calibration curve has the regression equation of y = 14059x + 3514 and y = 14059x + 3514 for Sofosbuvir and Velpatasvir.

Accuracy

The mean recoveries were found to be 100.7003, 100.36157, 100.183756% for Sofosbuvir and 100.54488, 99.97095, 100.27718% Velpatasvir. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Repeatability

The repeatability study which was conducted on the solution having the concentration of about 10µg/ml and 20µg/ml for Sofosbuvir and

Velpatasvir showed %RSD of 1.68756% and 0.58708%. It was concluded that the analytical technique showed good repeatability.

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) are 0.07 µg/ml and 0.05 µg/ml for Sofosbuvir and Velpatasvir. The quantified (LOQ) were found to be 0.21 µg/ml and 0.15 µg/ml respectively.

Assay

The assay in MyHep All Tablet containing Sofosbuvir and Velpatasvir was found to be 99.7825% and 99.28%.

DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Sofosbuvir and Velpatasvir, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.

S.Nageswararao, et al. achieved separation by using Methanol: 0.01M Potassium dihrogen orthophosphate buffer in proportion of 55:45(v/v) with pH adjusted to 7±0.5 by using triethyl amine as mobile phase.[21] Sarath Nalla, et al developed method by using a mobile phase in combination of 60:40 v/v mixture of 0.1% orthophosphoric acid in water and acetonitrile (ACN) but we have used Potassium Dihydrogen Phosphate buffer (adjusted with 1% Orthophosphoric acid, pH- 3.5) (0.05M) : Acetonitrile = (70:30).[22] As per Jaimin P. Patel, et al. used Inertsil ODS C18 column (150mm x 4.6mm, 5µm) with a mobile phase composed of 0.05M Potassium Dihydrogen Phosphate buffer (pH- 3.5, adjusted with 1% Orthophosphoric acid) and Acetonitrile in the ratio of 60:40 % v/v.[23] Uppalapati.Jyothi, et al. used C18 column (XTerra RP18 150*4.6, 5um) by using the mobile phase with 0.1%v/v Trifluoro acetic acid in water: Methanol (42:58) in isocratic mode, maintained at ambient temperature, is used as stationary phase applied for pharmaceutical dosage form.[24]

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Sofosbuvir and Velpatasvir in formulations.

CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Sofosbuvir and Velpatasvir API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Sofosbuvir and Velpatasvir indicated that the developed method is specific for the estimation of Sofosbuvir and Velpatasvir. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

REFERENCE

[1]. "Sofosbuvir". The American Society of Health-System Pharmacists. Archived from the original on 2016.
[2]. Divya Rajagopal for the Economic Times. Sept 12, 2015. Can Indian generic makers find gold with a blockbuster Hepatitis C drug?
[3]. "Sofosbuvir (Sovaldi) - Treatment - Hepatitis C Online". www.hepatitisc.uw.edu. Archived from the original on 23 December 2016. Retrieved 8 January 2017.
[4]. FDA, News Release (June 28, 2016). "FDA approves Epclusa for treatment of chronic Hepatitis C virus infection". Archived from the original on June 3, 2017.
[5]. "Sovaldi 400 mg film coated tablets - Summary of Product Characteristics (SPC) - (eMC)". www.medicines.org.uk. Archived from the original on 31 January 2017. Retrieved 8 January 2017.
[6]. "Sofosbuvir label" (PDF). FDA. August 2015. Archived (PDF) from the original on 2016-09-27.
[7]. "Direct-Acting Antivirals for Hepatitis C: Drug Safety Communication - Risk of Hepatitis B Reactivating". FDA. 4 October 2016. Archived from the original on 6 October 2016. Retrieved 6 October 2016.
[8]. "Sovaldi 400 mg film coated tablets - Summary of Product Characteristics". UK Electronic Medicines Compendium. September 2016. Archived from the original on 10 November 2016. Retrieved 10 November 2016.

[9]. Gounder, Celine (9 December 2013). "A Better Treatment for Hepatitis C". The New Yorker. Archived from the original on 20 September 2016.
[10]. "WHO Model List of Essential Medicines (19th List)" (PDF). World Health Organization. April 2015. Archived (PDF) from the original on 13 December 2016. Retrieved 8 December 2016.
[11]. "Epclusa (sofosbuvir and velpatasvir) Tablets, for Oral Use. Full Prescribing Information" (PDF). Gilead Sciences, Inc. Foster City, CA 94404. Retrieved 1 August 2016.
[12]. FDA Approves Epclusa, Drugs.com
[13]. Haberfeld, H, ed. (2016). Austria-Codex (in German). Vienna: Österreichischer Apothekerverlag. Epclusa 400 mg/100 mg Filmtabletten.
[14]. Ascher DB, Wielens J, Nero TL, Doughty L, Morton CJ, Parker MW: Potent hepatitis C inhibitors bind directly to NS5A and reduce its affinity for RNA. Sci Rep. 23(4), 2014, 4765. doi: 10.1038/srep04765.
[15]. Targett-Adams P, Graham EJ, Middleton J, Palmer A, Shaw SM, Lavender H, Brain P, Tran TD, Jones LH, Wakenhut F, Stammen B, Pryde D, Pickford C, Westby M: Small molecules targeting hepatitis C virus-encoded NS5A cause subcellular redistribution of their target: insights into compound modes of action. J Virol. 85(13), 2011, 6353-68. doi: 10.1128/JVI.00215-11. Epub 2011.
[16]. 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. 24, 2017, 61(5). pii: e02084-16. doi: 10.1128/AAC.02084-16. Print 2017 May.
[17]. Lawitz E, Freilich B, Link J, German P, Mo H, Han L, Brainard DM, McNally J, Marbury T, Rodriguez-Torres M: A phase 1, randomized, dose-ranging study of GS-5816, a once-daily NS5A inhibitor, in patients with genotype 1-4 hepatitis C virus. J Viral Hepat. 22(12), 2015, 1011-9. doi: 10.1111/jvh.12435. Epub 2015 Jul 16.
[18]. 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. 29(1), 2015, 19-34. Epub 2015 Jan 13.
[19]. Lawitz EJ, Dvory-Sobol H, Doehle BP, Worth AS, McNally J, Brainard DM, Link JO, Miller MD, Mo H: Clinical Resistance to Velpatasvir (GS-5816), a Novel Pan-Genotypic Inhibitor of the Hepatitis C Virus NS5A Protein. Antimicrob Agents Chemother. 60(9), 2016, 5368-78. doi: 10.1128/AAC.00763-16. Print 2016 Sep.
[20]. Hill A, Simmons B, Gotham D, Fortunak J. "Rapid reductions in prices for generic sofosbuvir and daclatasvir to treat hepatitis C". Journal of Virus Eradication. 2 (1), 2016, 28–31.
[21]. S.Nageswararao.*, V.Rajashekar, M.Deepthishalini, New analytical method development and validation for the simultaneous estimation of velpatasvir and sofosbuvir in pharmaceutical dosage forms, International Journal of Pharmacy and Industrial Research, 08(03), 123-128
[22]. Sarath Nalla* and Seshagiri Rao J. V. L. N., A Stability Indicating Rp-Hplc Method For Simultaneous Estimation Of Velpatasvir And Sofosbuvir In Combined Tablet Dosage Forms, World Journal Of Pharmacy And Pharmaceutical Sciences, 6(9), 1596-1611
[23]. Jaimin P. Patel* and Ankit B. Chaudhary, Stability Indicating Rp-Hplc Method Development And Validation For Simultaneous Estimation Of Sofosbuvir And Velpatasvir In Tablet, World Journal Of Pharmacy And Pharmaceutical Sciences, 7(5), 813-828
[24]. Uppalapati.Jyothi*, Dr.Parimi.Umadevi, Analytical Method Development And Validation For The Simultaneous Estimation Of Sofosbuvir And Velpatasvir Drug Product By Rp-Hplc Method, Indo American Journal of Pharmaceutical Research, 7(8), 401-409.