Journal of Medicinal and Chemical Sciences 3 (2020) 329-337

Journal of Medicinal and chemical Sciences

Journal homepage: www.jmchemsci.com

Original Research Article

Development and validation of analytical method for simultaneous estimation of sofosbuvir and velpatasvir by RP-HPLC method in pharmaceutical dosage form

Patel Grishma*, Patel Dhara, Mansuri Reema, Sapra Ritu, Meshram Dhananjay

Assistant Professor, Pioneer Pharmacy Degree College, Nr. Ajwa Crossing, Vadodara, Gujarat, India

ARTICLE INFORMATION

Received: 17 January 2020
Received in revised: 21 April 2020
Accepted: 21 June 2020
Available online: 01 October 2019

DOI: 10.26655/JMCHEMSCI.2020.4.2

KEYWORDS

Sofosbuvir
Velpatasvir
HPLC

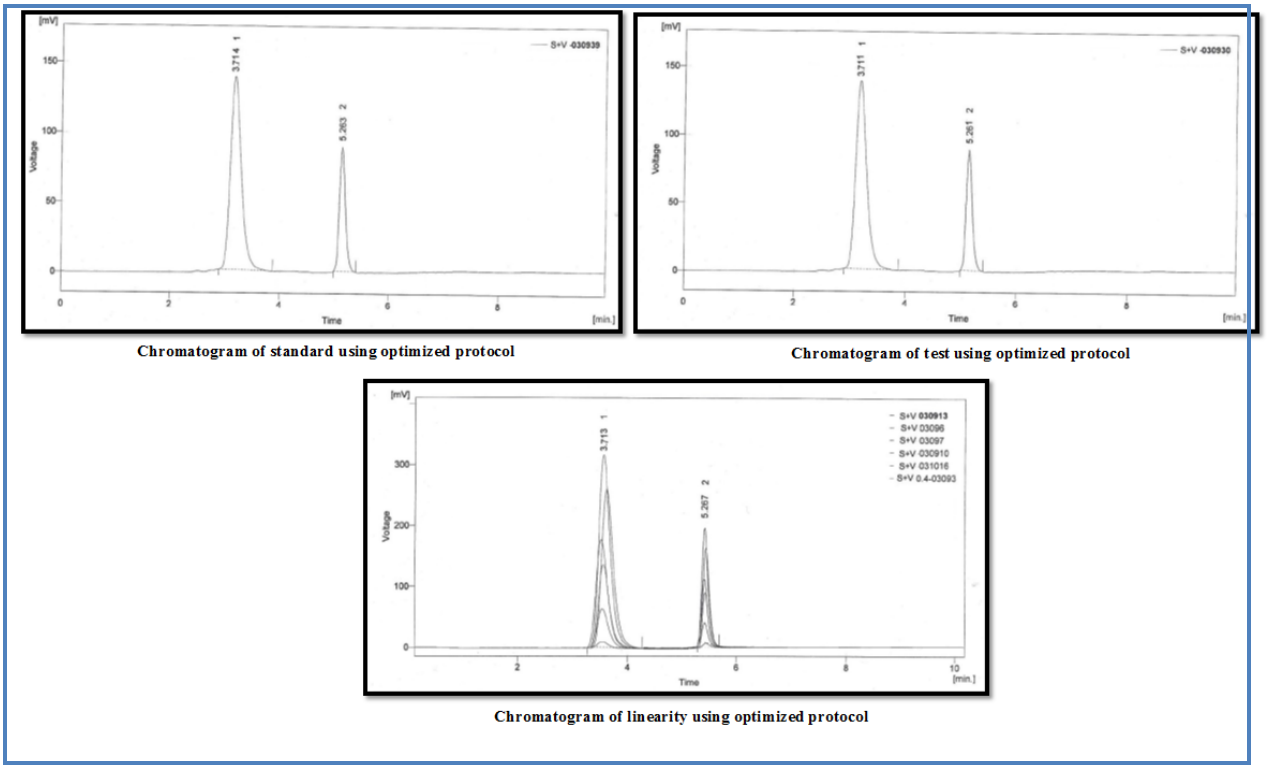
ABSTRACT

In this work, a simple, rapid, accurate, precise, specific, and sensitive RP-HPLC method was developed and validated for the simultaneous estimation of the Sofosbuvir and Velpatasvir in bulk drug and pharmaceutical dosage form. The stationary phase used for the chromatographic separation was Hypersil BDS column C18 column (250 mm× 4.6 mm with the particle size of 5 mm) andthe mobile phase used for the separation was methanol:phosphate buffer (pH3) taken in ratio of 75:25%V/V. The flow rate was 1.0 mL/min at 30 °C. The drugs were detected at the wavelength of 260 nm. The retention time for the Sofosbuvir (SOFO) and Velpatasvir(VELP) were 3.714 and 5.263, respectively. The linearity was performed using the concentration range of 2-12 µg/mL of Sofosbuvir and 0.5-3 µg/mL of Velpatasvir. The correlation coefficient was found to be 0.999 and 0.999, respectively. The % purity of the Sofosbuvir and Velpatasvir was found to be 99.01% and 99.25%, respectively. The proposed method was validated for specificity, linearity, precision, robustness and accuracy were within the range of acceptance limit according to ICH Q2 (B) guidelines and the developed method can be employed for the routine quality control analysis in the bulk and combined pharmaceutical dosage form of Sofosbuvir and Velpatasvir.

Journal of Medicinal and Chemical Sciences: http://www.jmchemsci.com/

Corresponding author, email: grishmapatel80@yahoo.com (P. Grishma).
Tel.: + 8469551282

Graphical Abstract



Introduction

Sofosbuvir (SOFO) is chemically known as Isopropyl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2, 4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl] methoxy-phenoxyphosphoryl] amino] propionate (Figure 1) [1-3]. Velpatasvir (VELP) is chemically known as 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-dihydroisochromeno [4', 3':6, 7] naphtho [1, 2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3- methyl-1-oxo-2 butanyl} carbamate (Figure 2) [4]. Sofosbuvir and Velpatasvir are used in combination in treatment of hepatitis C. Sofosbuvir and Velpatasvir acting as a NS5B and NS5A inhibitor respectively [5-8]. The deep literature survey revealed that, various Spectrophotometric and chromat graphic methods are available for the estimation of SOFO [9-13] and VELP alone and in combination with other drugs like daclatasvir [14] and ledipasvir [15-18]. There is no official method available in pharmacopieas for estimating of both the drugs simultaneously and it was also found that there is no reported chromatographic methods available for simultaneous estimation of the SOFO and VELP in combined dosage form. Therefore, simple, rapid and reliable method for simultaneous estimation of these drugs in combined dosage form seemed to be necessary. The purpose of this study was to determine and validate both the drugs concurrently by simple, accurate, rapid and precise chromatographic method for routine analysis [19].

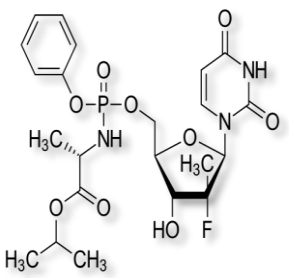


Figure 1. Structure of Sofosbuvir

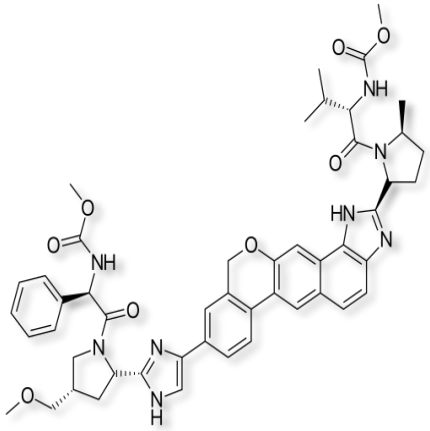


Figure 2. Structure of Velpatasvir

Methods

The chromatographic technique performed on a UV detector SPD-20A and LC solutions software, reversed phase Hypersil BDS column C18 column (250 mm × 4.6 mm with the particle size of 5 mm) as stationary phase, shimadzu ATX 224 digital balance, vacuummicrofiltration unit with 0.45 µm membrane filter was used in the study.

Materials

Pharmaceutically pure sample of Sofosbuvir and Velpatasvir was obtained as a gift sample from Zydus health care centre, Ahmadabad and hetero drugs pvt, Ltd. Hyderabad, India.High performance liquid chromatography–grade methanol was from the Samir tech chem. Pvt. Ltd (AR grade).

Chromatographic conditions

The drugs separation was achieved by the Hypersil BDS column C18 column (250 mm × 4.6 mm with the particle size of 5 mm), added by mobile phase mixture of methanol:phosphate buffer (pH 3) (75:25, %v/v). The flow rate was 1.0 mL/min and UV detection is at 260 nm, injection volume is 20 µL and at 30 °C.

Preparation of mobile phase

Buffer preparation

3.4 g of potassium dihydrogen ortho phosphate and 2 mL of trethylamine in 800 mL of water adjust the pH to 3 with orthophosphoric acid and add sufficient waterto produce 1000 mL with distilled water. Mobile phase was prepared by mixing 75 mL of methanol with 25 mL of 25mM phosphate buffer having pH 3 mixed well and sonicated. Thenthe mobile phase was filtered with 0.45 µm membrane filter.

Preparation of standard solution

Preparation of stock solution

40 mg of SOFO and 10 mg of VELP were weighed and transferred to 100 mL volumetric flask. This stock solution was prepared in methanol sonicated for 15 min,The volume was adjusted up to the mark with same solvent. Then, the solution was filtered through thewhattman filter paper No.41. This stock solution contained 400 µg/mL and 100 µg/mL of SOFO and VELP, respectively.

Calibration standards

From the primary stock solution 0.05 mL, 0.1 mL, 0.15 mL, 0.2 mL, 0.25 mL, and 0.3 mL taken in 10 mL volumetric flaskand diluted up to mark to give concentration of 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, 10 µg/mL, and 12 µg/mL of SOFO and 0.5 µg/mL, 1 µg/mL, 1.5 µg/mL, 2 µg/mL, 2.5 µg/mL, and 3 µg/mL of VELP.

Sample solution

Twenty tablets (each containing 400 mg SOFO and 100 mg VELP) were accurately weighed and finely powdered. A quantity of powder equivalent to 40 mg SOFO and 10 mg VELP was weighed and transferred to 100 mL volumetric flask. This stock solution was prepared in methanol sonicated for 15 minthen the volume was adjusted up to the mark with same solvent. Later on, the solution was filtered using the whattman filter paper No.41. This stock solution contained 400 µg/mLand 100 µg/mL. 0.1 mL solution was pipette out in 10 mL volumetric flask, this solution contained 4 µg/mL SOFO and 1 µg/mL VELP.

Method validation

System suitability

The values for evaluatingthe system suitability of the chromatographic procedure were relative standard deviation (RSD) <2%, tailing factor<1.5 and theoretical plates >1500. The retention time, resolution, theoretical plates, and tailing factor were evaluated for the system.

Linearity and range

Fresh aliquots were prepared from the stock solution of SOFO (100 µg/mL) the range of 2-12 µg/mL and VELP (100 µg/ml) the range of 0.5-3 µg/mL and they were transferred in to 10 mL volumetric flask and diluted up to 10 mL using the methanol as a solution. The Peak area of the solution was then measured at 260 nm. The calibration curve was constructed by plotting peak area vs. concentration and the decay coefficient equation was calculated.

Precision

The precision was checked by preparing sixreplicates of Sofosbuvir (4 µg/mL) and Velpatasvir (1 µg/mL) and peak area was measured for SOFO and VELP, respectively, without altering the parameters of the proposed method.

Accuracy

The accuracy studies were carried out by spiking of standard at three different concentrations i.e. 50%, 100%, 150%. The recovery studies were carried out by adding known amount of standard solution of three different levels.

Limit of detection and limit of quantification

The LOD and LOQ were separately determined based on the standard deviation of the Y-intercept and the slop of the calibration curve.

LOD and LOQ were calculated by application of following formula:

$LOD = 3.3\ \sigma/S$

$LOQ = 10\ \sigma/S$

where, σ=standard deviation of response

S=slope of calibration curve

Robustness

Robustness was performed by deliberate changes in method parameters such as flow rate, detection wavelength on assay of analyte of interest. Here, the detection varied ±2 nm and flow rate varied ±1.0 mL.

Result and Discussion

System suitability

System suitability was checked by repeated preparations for 4 µg/mL of SOFO and 1 µg/mL of VELP. The typical values for evaluating the system suitability of a chromatographic procedure are RSD <2%, tailing factor<1.5 and theoretical plates>1500. The retention time, peak area, theoretical plates, and tailing factor were assessed for the system suitability data of Sofosbuvir and Velpatasvir, the data are presented in Table 1 and Figure 3 and 4.

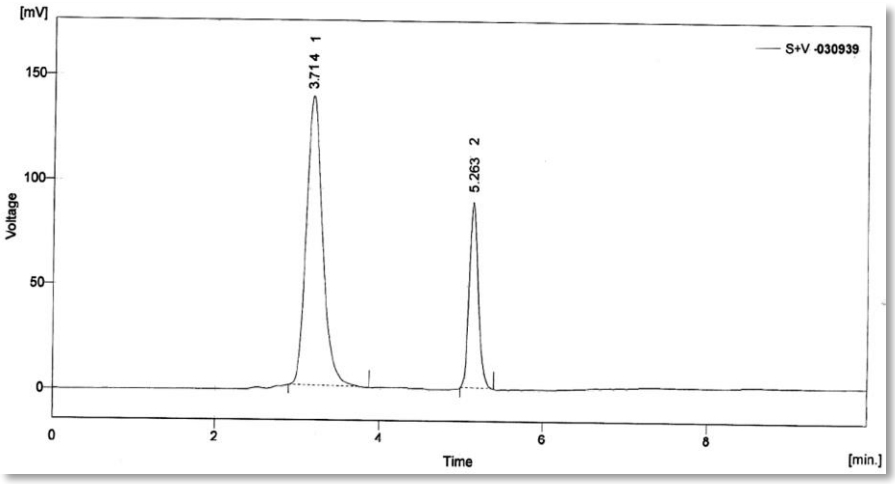


Figure 3. Chromatogram of standard using optimized protocol

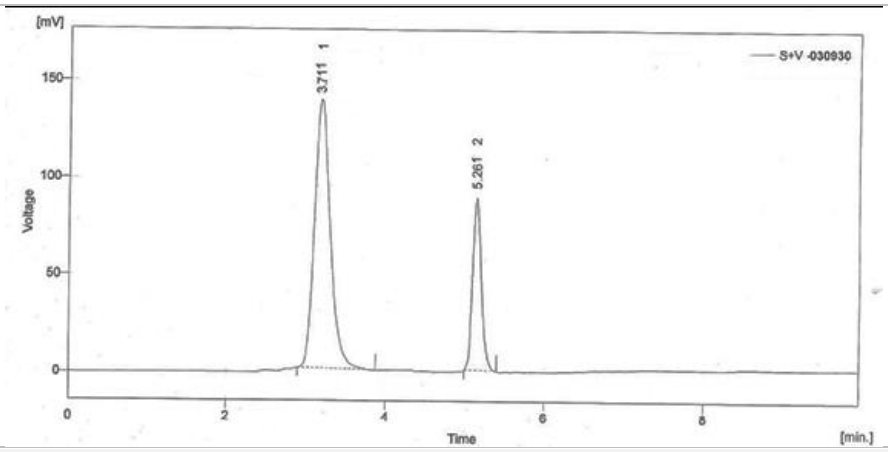


Figure 4. Chromatogram of test using optimized protocol

Table 1. System suitability parameters for Sofosbuvir and Velpatasvir

Parameters	Observed results±SD (n=6)		%RSD		Acceptance criteria
	SOFO	VELP	SOFO	VELP	
Retention time (Rt)	3.714±0.004	5.23±0.020	0.10	0.39	%RSD<2
Peak area	1889.25±7.62	639.77±1.48018	0.40	0.23	%RSD<2
Theoretical plates (N)	7328.66±10.26	4851.33±13.05	0.14	0.26	>2000
Tailing factor(N)	1.498±0.025	1.48±0.024	1.68	1.62	T≤2
Resolution(Rs)	9.9±0.15		0.29		>2

Linearity and Range

Linearity was studied by analyzing six standard solution covering the range of 2-12 µg/mL for SOFO and 0.5-3 µg/mL for VELP. From the primary stock solution 0.05 mL, 0.1 mL, 0.15 mL, 0.2 mL, 0.25 mL and 0.3 mL taken in 10 mL volumetric flask and diluted up to mark to give concentration of 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, 10 µg/mL and 12 µg/mL of SOFO and 0.5 µg/mL, 1 µg/mL, 1.5 µg/mL, 2 µg/mL, 2.5 µg/mL and 3 µg/mL of VELP. A calibration curve with concentration versus peak areas was plotted by injecting the above prepared concentration. Correlation coefficient values for Sofosbuvir and Velpatasvir are 0.999 and 0.999 for respectively. The linearity data for Sofosbuvir and Velpatasvir are illustrated in Figure 6 and 7.

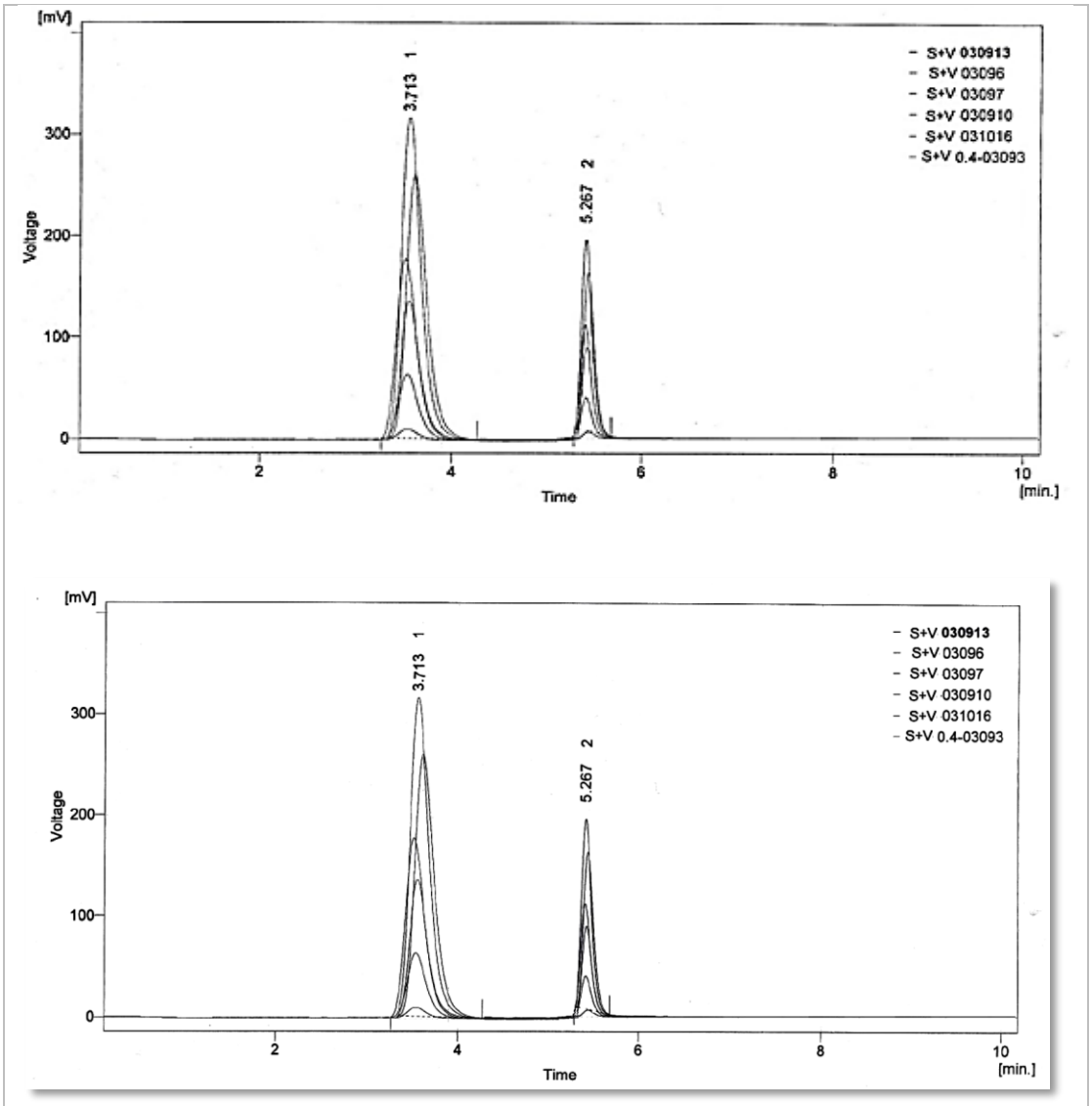


Figure 5. Chromatogram of linearity using optimized protocol

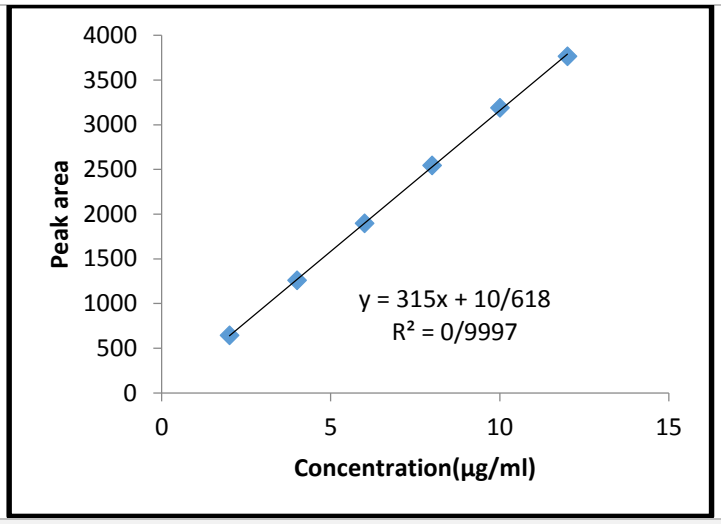


Figure 6. Calibration curve of SOFO (2-12 µg/mL)

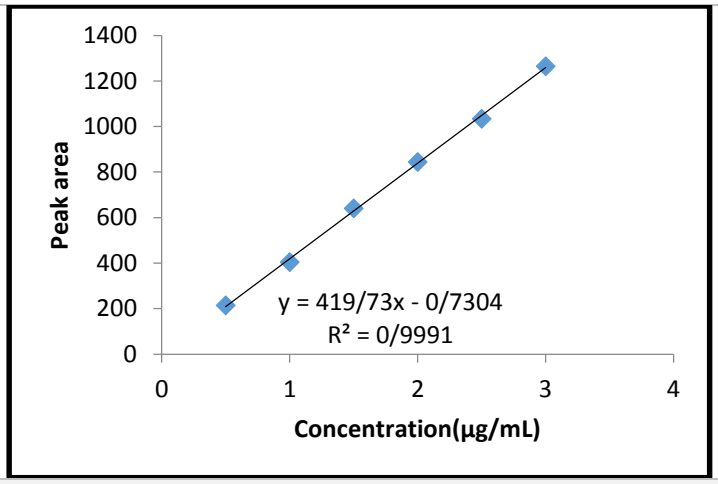


Figure7. Calibration curve of VELP (0.5-3 µg/mL)

Precision

The precision of the method was checked by repeating the preparation (n=6) of 4 µg/mL of SOFO and 1 µg/mL of VELP. The %RSD was found to be <2% demonstrating good repeatability. The values for the Sofosbuvir and Velpatasvir are shown in Table 2.

Table 2. Method precision data for Sofosbuvir and Velpatasvir

Conc.(µg/mL)		Mean of Peak area		± SD (n=6)		% RSD	
SOFO	VELP	SOFO	VELP	SOFO	VELP	SOFO	VELP
4	1	1257.91	404.55				
4	1	1256.83	404.41				
4	1	1258.84	403.67				
4	1	1258.92	403.71				0.13
4	1	1258.61	404.89	0.85	0.55	0.06	
4	1	1257.35	404.91				

LOD and LOQ

LOD and LOQ were found to be 0.021 µg/mL and 0.065 µg/mL for SOFO and VELP and LOQ was found to be 0.013 µg/mL and 0.040 µg/mL for SOFO and VELP (Table 3.).

Accuracy

The accuracy studies were carried out by spiking of standard at three different concentrations i.e. 50%, 100%, 150%. The recovery studies were carried out by adding known amount of standard solution of three different levels. The accuracy data for the Sofosbuvir and Velpatasvir are revealed in Table 4.

Table 3. LOQ and LOQ data for Sofosbuvir and Velapatsvir

Parameters	Sofosbuvir	Velpatasvir
LOD	0.021	0.065
LOQ	0.013	0.040

Table 4. Results of accuracy

Drugs	Level	Amount present(µg/mL)	Amount added (µg/mL)	Total amount of drug (µg/mL)	Amount found (µg/mL)	%Recovery±SD (n=3)	%RSD
	50%		2	6	5.98	99.80 ± 0.13	0.13
SOFO	100%	4	4	8	8.05	100.66±0.05	0.05
	150%		6	10	10.08	100.89±0.09	0.09
	50%		0.5	1.5	1.52	101.82±0.19	0.19
VELP	100%	1	1	2	2.01	100.93±0.30	0.30
	150%		1.5	2.5	2.46	98.74 ± 0.51	0.52

Robustness

Robustness was performed by deliberate changes in method parameters such as flow rate, detection wavelength on assay of analyte of interest. Here, the detection varied ±2 nm and flow rate varied ±1.0 mL. The robustness data for Sofosbuvir and Velpatasvir are shown in Table 5.

Table 5. Results of robustness

Sr.No	Parameters	Variation	Mean Area		Retention time		Tailing factor	
			SOFO	VELP	SOFO	VELP	SOFO	VELP
1.	Flow rate (1±0.2 mL/min)	0.8	1252	402	3.68	5.00	1.52	1.25
		1.0	1305	417	3.72	5.23	1.49	1.34
		1.2	1310	428	3.71	5.23	1.32	1.38
2.	Mobile phase (%v/v)	73:27	1238	384	3.83	5.07	1.40	1.31
		75:25	1282	413	3.75	5.10	1.40	1.64
		77:23	1365	423	3.71	5.18	1.43	1.71
3.	pH	2.8	1164	387	3.59	5.15	1.27	1.41
		3	1235	420	3.60	5.22	1.48	1.40
		3.2	1268	426	3.62	5.24	1.57	1.48

Conclusion

From the experimental result the data for Accuracy was found to be between 98-102%, in the precision % RSD was found to be less then 2, all system suitability parameter was found within the acceptance criteria and Robustness

was performed by deliberate changes in method parameters such as flow rate, mobile phase and pH but result obtained was within the acceptance criteria so it was concluded that, this newly developed method for the Sofosbuvir and Velpatasvir was found to be simple, precise, accurate, robust and high resolution and shorter retention time makes method more acceptable and cost effective. In addition, it can be effectively applied for the routine analysis in research institution, quality control department and approved testing laboratories.

References

[1] Mohan H. *Text Book of Pathology*; 5th Edn; Jaypee Brothers Medical Publishers Limited, 2008; p 608-625

[2] Walker R., Whittlsea C. *Clinical Pharmacy and Therapeutics*; 4thEdn; Elsevier, 2008; p 215-230

[3] Bennett P.N., Brown M.J. *Clinical Pharmacology*; 10thEdn; Elsevier, 2008, 582-590

[4] Lawitz E., Mangia A., Wyles D., Rodriguez-Torres M., *N. Engl. J. Med.,* 2013, **368**:1878

[5] Jacobson I.M., Gordon S.C., Kowdley K.V., Yoshida E.M. *N. Engl. J. Med.*, 2013, **368**:1867

[6] Zeng Q.L., Zhang J.Y., Zhang Z. *World. J. Gastroenterol.,* 2013, **19**:3199

[7] Herbst D.A., Reddy K.R. *Expert. Opin. Investig. Drugs.,* 2013, **20**:527

[8] Gentile I., Borgia F., Buonomo A.R. *Curr. Med. Chem.,* 2013, **20**:3733

[9] Soriano V., Vispo E., Labarga P. *Expert. Opin. Pharmacothe.*, 2013*r*, **14**:1161

[10] Tripathi K.D. *Essentials of Medical Pharmacology*; 7th Edn; Jaypee Brothers Medical Publication, New Delhi, 2013, p 803-805

[11] Sofosbuvir, Velpatasvir-Generic Drug Details", accessed on 27th July 2019, https://www.drugpatentwatch.com/p/generic-api/SOFOSBUVIR%3B+VELPATASVIR

[12] Bhatia H.K., Singh H. *J. Pharmacol. Pharmacother.,* 2014, **5**:278

[13] Ghayathri V.R., Vinodhini C., Chitra K., *World. J. Pharm. Pharm. Res.,* 2014, **3**:1411

[14] Sofosbuvir", accessed on 23rd July 2019, https://www.drugbank.ca/drugs/DB08934 "Velpatasvir", accesed on 23rd July2019, https://www.drugbank.ca/drugs/DB11613

[15] ICH and ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Q2 (R1): Validation of Analytical Procedures: Text and Methodology. 2005

[16] Lough W.J., Wainer I.W. *High Performance Liquid Chromatography', Fundamental Principle and Practice*; 1st Edn; Blackie Academic and Professional Publication, Glasgow, 1995, p 97-113

[17] Skoog D.A., Hollar F.J., Crounch S.R. *Instrumental analysis*; 3rd Edn; Cengage Learning India Pvt. Ltd, New Delhi, 2009, p 378-392

[18] Lindsay Sandie. HPLC by open learning; 2nd Edn; Chapman and Hall LondonJhoneWiley and Sons, London, 1992, p 149-187

[19] Hamilton R.J., Sewell P.A. *Introduction to HPLC*; 2nd Edn; Chapman and Hall London, 1982, p 189.

How to cite this manuscript: Patel Grishma*, Patel Dhara, Mansuri Reema, Sapra Ritu, Meshram Dhananjay. Development and validation of analytical method for simultaneous estimation of sofosbuvir and velpatasvir by RP-HPLC method in pharmaceutical dosage form. *Journal of Medicinal and Chemical Sciences*, 2020, 3(4), 329-337. DOI: 10.26655/JMCHEMSCI.2020.4.2