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SIMULTANEOUS RP-HPLC METHOD DEVELOPMENT AND IT'S VALIDATION FOR ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN THEIR COMBINED DOSAGE FORM

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ABSTRACT

A new simple and precise simultaneous RP-HPLC method was developed and validated for the identification and analysis of fixed dose combination of Sofosbuvir and velpatasvir in their combined tablet dosage form. The excipients in the tablet form showed no intervention with the analyte chromatograms. The retention time of sofosbuvir was 3.049 minutes and velpatasvir was 4.316 minutes respectively. The developed method was validated for linearity, specificity, accuracy, robustness and ruggedness according to 1CH instructions. The limit of detection were 0.475 ppm and 0.65 ppm and the limit of quantification were 1.44 ppm and 1.98 ppm for Sofosbuvir and Velpatasvir respectively.

The drug content assay in the tablet was closer to 100%. All the validated parameters met the acceptance criteria. The developed method can be used in quality control analysis.

Keywords: Sofosbuvir, Velpatasvir, RP-HPLC, Validation, ICH Guidelines.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major health attack and is the most common cause of liver transplantation. HCV[1] has specific Ribonucleic acid (RNA) sequence variance and based on this HCV is classified in to not less than 6 genotypes. Along with the rising new treatments, including interferon – free regimens, still a better treatment is looked out for HCV infection of genotype 2 and 3 and for severe liver disease. Sofosbuvir[2] (SOF), Fig.-1 has a molecular mass of 529 gram per mol, molecular formula of $C_{22}H_{29}FN_3O_9P$ and has IUPAC name, as:

(S)-Isopropyl-2-((S)-(((2R, 3R, 4R, 5R)-5-(2, 4-dioxo-3, 4-dihydropyrimidin-1 (2H)-yl)-4-fluoro-3-hydroxyl-4-methyltetrahydrofuran-2-yl) methoxy)-(phenoxy) phosphorylamino) propanoate.

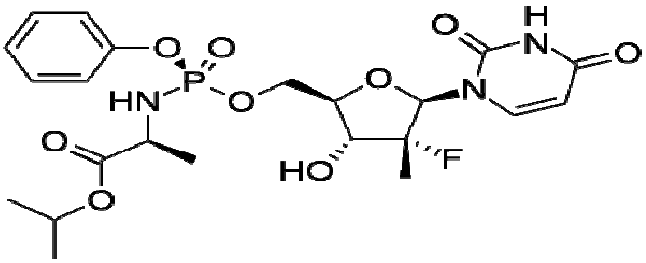

Fig.-1: Sofosbuvir

SOF is a nucleotide precursor drug that is converted into active uridine triphosphate form with in the liver cell. SOF inhibits the activity of HCV nonstructural protein (NS) 5B polymerase nucleotide and thus prevent HCV RNA replication through chain termination.

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Velpatasvir[3] (VEL), Fig.-2, has molecular formula $C_{49}H_{54}N_8O_8$ molecular mass 883.0 gram per mole and has IUPAC name Methyl {(1R)-2-[(2S,4S)-2-(5-{2-[(2S, 5S)-1-{(2S)-2-[(Methoxycarbonyl) amino]-3-methylbutanoyl}-5-methylpyrrolidin-2-yl]-1, 11-dihydro [2] benzopyrano [4¹, 3¹ : 6, 7] naphtho [1, 2-d] imidazole-9-yl}-1H-imidazol-2-yl)-4-(methoxymethyl) pyrrolidin-1-yl]-2-oxo-1–phenyl ethyl} carbamate.

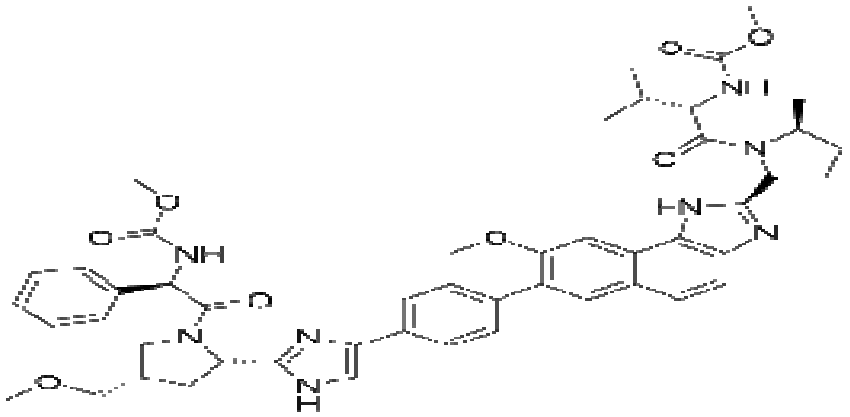


Fig.-2: Velpatasvir

VEL works by preventing the HCV non – structural protein 5A (NS5A) from playing its role both in viral replication and arrangement of HCV virions. SOF and VEL together show significant antiviral properties due to added antiviral interaction of both the drugs and also lack of cross resistance between them.
SOF/VEL is a quick release fixed dose combination tablet containing 400mg SOF and 100mg VEL effective in the treatment of HCV infection in adults.
Previous works involving analytical method development (MD) and its validation using RP-HPLC related to SOF included SOF determination in pure form[4], Estimation and validation of SOF in bulk and tablet form[5], simultaneous determination of SOF along with Ledipasvir in tablet form and also its application to in vitro Dissolution studies[6], simultaneous study of SOF along with simeprevir[7] ultraviolet visible spectroscope method for estimation of Daclatasvir and SOF[8,] Determination of SOF from Human plasma[9,] NO RP-HPLC work is published related to VEL.
The present study concentrates on simultaneous RP-HPLC MD and its validation in a novel fixed dose combination tablet of SOF and VEL following the ICH[10] directions.

EXPERIMENTAL

Chemicals

The Pure active pharmaceutical ingredient (AP1) sample of SOF and VEL were given on request from Jigs chemical. Ahmedabad Sofosvel, a combination tablet of SOF 400 milligram (mg) and VEL 100mg was purchased from the distributor.
Methanol (Met), dipotassium hydrogen phosphate (DPHP), Potassium dihydrogen phosphate (PDHP), Orthophosphoric acid (OPA) all of HPLC grade were purchased from Merck limited.
HPLC grade water was used to prepare the buffer. MP was used as the diluent. All the prepared solutions were microfiltered and agitated in an ultrasonic probe before use in HPLC instrument.

Preparation of Standard Solutions

SOF Stock solution (SS)

100mg of SOF pure AP1 sample was dissolved and diluted with the M.P in a 100ml volumetric flask up to the mark to get a solution of 1000 ppm.

VEL SS

100 mg of VEL pure API sample dissolved and diluted with the M.P in a 100ml volumetric flask up to the mark to get a solution of 1000 ppm.

Working standard (WS) Solution
WS solution of SOF and VEL was prepared by adding 4ml of SOF SS and 1ml of VEL SS together into a single 100ml volumetric flask and diluted up to the mark to get a concentration of 40 ppm SOF and 10 ppm VEL.

Test Sample (TS) Solution
Ten Sofosvel tablets having a dosage of SOF 400mg and VEL 100mg were weighed and average weight of single tablet noted. These tablets were powdered and weight of powder equal to one tablet weight was introduced into a single 100ml flask, dissolved and diluted up to the mark with MP.
The above solution was further diluted in the right proportion to get a concentration of 40 ppm SOF and 10 ppm VEL test sample solution.

Linearity range solutions
Linearity series solutions ranging from 20 ppm to 80 ppm with respect to both the drugs were prepared in combination with SOF 2 microgram per milliliter (µg/ml) to 8 µg/ml and VEL (0.5 µg/ml to 2µg/ml) respectively.

Preparation of Phosphate Buffer
6.67 grams of PDHP and 8.55 grams of DPHP were dissolved and diluted with HPLC grade water in a 1000ml beaker. The pH of the solution was adjusted to 3.2 with OPA.

M.P Preparation
It consisted of degassed Methanol and phosphate buffer in 60: 40 V/V ratio.

Table-1: Instrumentation

HPLC Instrument	Waters 2695 Alliance HPLC system
Detector	996 Photodiode Array detector
Data acquisition	Empower software
Electronic balance	Sartorius
Ultrasonic probe	Fast clean

Simultaneous RP HPLC MD
MP was eluted isocratically at ambient temperature on a C-18 column maintaining 1ml / min flow rate for 20µl sample volume and total runtime of 8 minutes.
The data analysis report showed Retention time (RT) of 3.048 min and 4.316 min for SOF and VEL in the API standard and in the TS the RT for SOF and VEL were 3.049 min and 4.316 min respectively.

Table-2: Optimized Chromatographic Conditions

Column	Inertsil C-18 (250 × 4.6mm, 5 micron)
Mobile Phase (MP)	Methanol : Buffer (60 : 40) V/V volume ratio
Flow rate (FR)	1 milliliter per minute (ml / min)
Run time	8 min
Column temperatures (temp)	Ambient
Sample volume	20 microliters (µl)
Maximum absorbance wavelength	254 nanometers (nm)
Micro Filter pore size	0.45 micrometers (µm)
Buffer pH	3.2

Method Validation
The developed method was validated as per the ICH instructions for system suitability (ST), specificity, linearity, Accuracy (Recovery), precision, Ruggedness, Robustness, Limit of detection (LOD) and limit of Quantification (LOQ).

RESULTS AND DISCUSSION

S.T (SOF)

WS solution of SOF and VEL were injected five times into the HPLC system and the ST parameters were evaluated from the standard chromatograms. The percent relative standard deviation (% RSD) for RT and PA were calculated. All the ST validation data for SOF (Table-3) and VEL (Table-4) met the criteria.

Table-3: S.T (SOF)

Injection	RT (min)	PA	Theoretical plate count	Tailing factor
1	3.048	9438247	11023.845712	1.14721
2	3.049	9436021	11010.547812	1.13384
3	3.047	9431581	11036.874214	1.18742
4	3.048	9432036	11027.254178	1.16547
5	3.047	9433819	11084.658952	1.17485
Mean	3.0478	9434755	11036.825171	1.1852313
SD	0.000837	3358.178	-	-
% RSD	0.027451	0.270438		

Specificity

The Blank chromatogram (Fig.-3) depicted no peak at the RT of the analytes. The chromatogram of the AP1 (Fig.-4) and TS (Fig.-5) depicted almost identical RT for SOF and VEL. The excipients present in the tablet dosage form showed no interfering peaks. The developed simultaneous RP – HPLC method was specific.

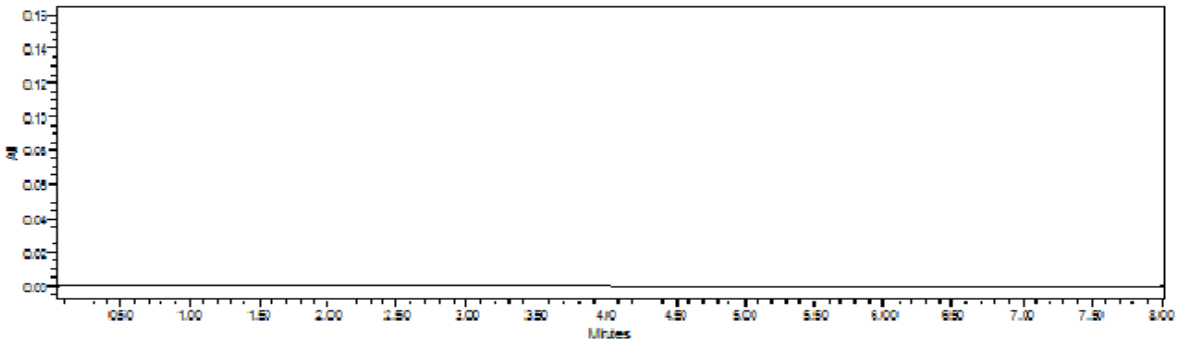


Fig.-3: Blank Chromatogram

Table-4: S.T (VEL)

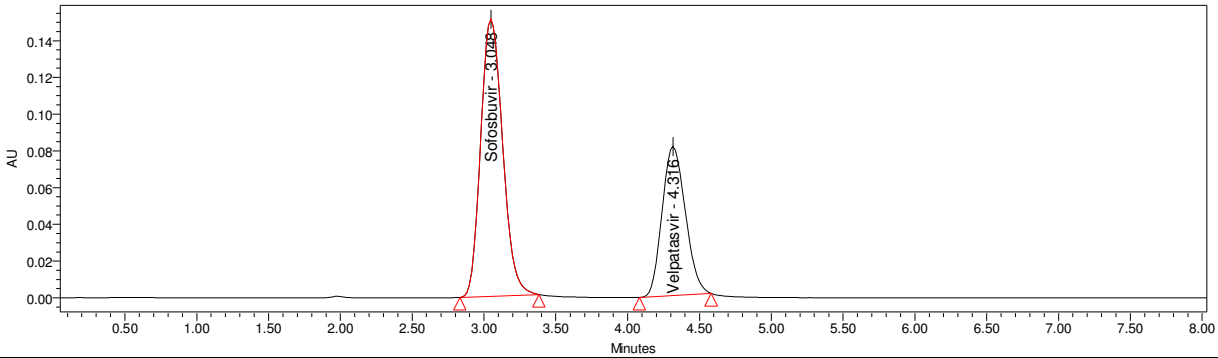
Injection	RT (min)	PA	Theoretical plate count	Tailing factor
1	4.316	323209	8325.874512	1.284572
2	4.316	323181	8384.547862	1.254872
3	4.312	323028	8314.875424	1.278451
4	4.317	323915	8372.784518	1.287451
5	4.313	324059	8392.084512	1.298745
SD	0.002168	1588.8	-	-
% RSD	0.50244	0.289823		

Linearity

Linearity was studied in the range of 20ppm to 80 ppm with respect to both SOF and VEL (Table-5). For each linearity level solution 3 chromatograms were recorded. A good linear relationship was observed between the average PA and the solution concentration in ppm within the range (2µg/ml to 8µg/ml) for SOF (Fig.-6) and (0.5 µg/ml to 2µg/ml) for VEL (Fig.-7). Strong linear relationship was proved by high value of correlation coefficient (r) which was 0.999 for SOF and VEL.

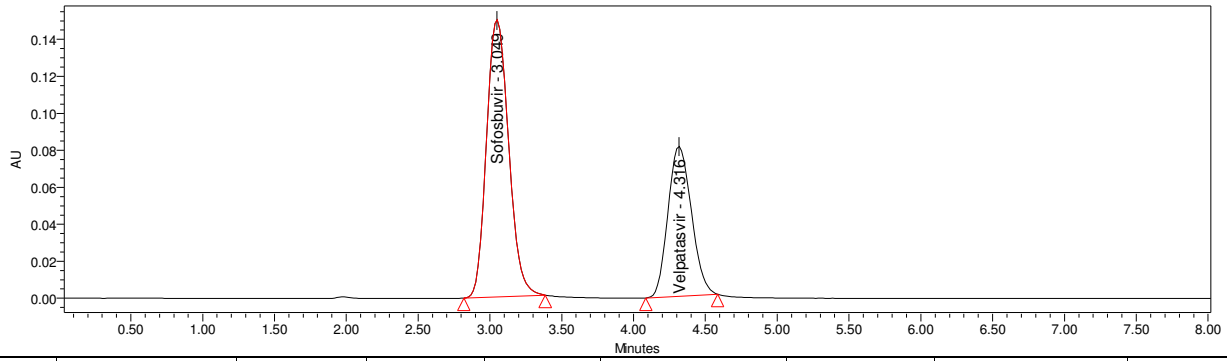
Table-5: Linearity

Concentration (ppm)	Average PA (SOF)	Average PA (VEL)
0	0	0
20	4719376	161774
30	7079064	242661
40	9438751	323547
50	11798439	404434
60	14158127	485321
70	16517815	566208
80	18477503	637095
Slope	23306	8016
Y – intercept	77193	1929
	0.999	0.999



	Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	Asymmetry Factor	USP Plate Count
1	Sofosbuvir	3.048	9438247	63.71	150828		1.14721	1.087451	11023.845712
2	Velpatasvir	4.316	323209	36.29	81160	4.342819	1.1284572	1.087541	8325.874512

Fig.-4: Standard Chromatogram of SOF/VEL



	Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	Asymmetry Factor	USP Plate Count
1	Sofosbuvir	3.049	9436021	63.71	149923		1.13384	1.089624	11010.547812
2	Velpatasvir	4.316	323181	36.29	80992	4.297313	1.254872	1.087632	8384.547862

Fig.-5: TS chromatogram SOF / VEL

Accuracy

Drug assay was conducted thrice as per test method for each spike level solution which consisted of SOF and VEL concentration equal to 50%, 100% and 150% of the label amount. The average % recovery of

SOF and VEL were calculated from each chromatogram. The % recovery from all the three level solutions for SOF and VEL were closer to 100 and met the acceptance limits (Table-6, Fig.-8, and Fig.-9).

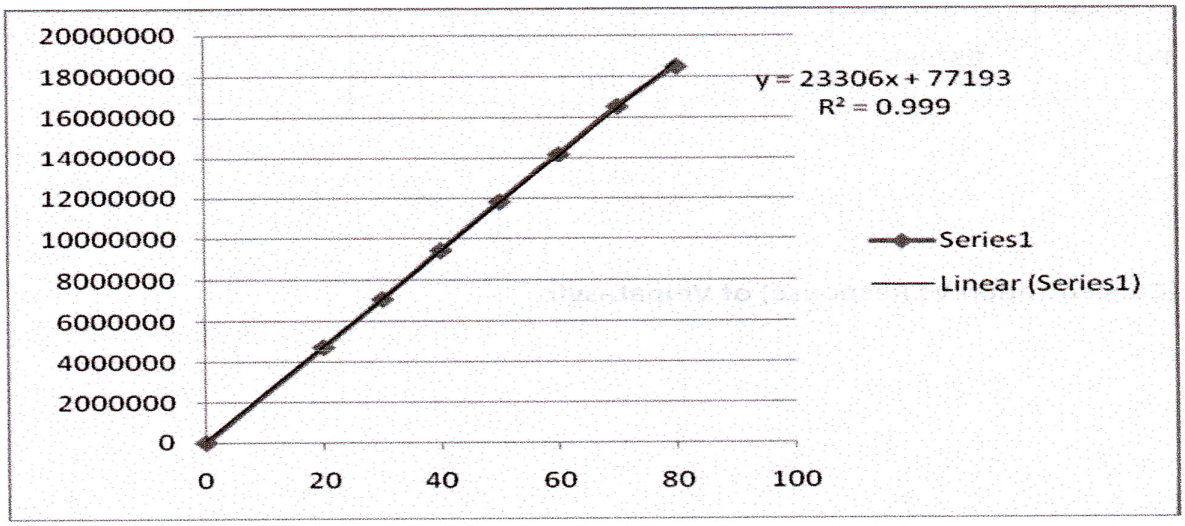


Fig.-6: Linearity Graph (SOF)

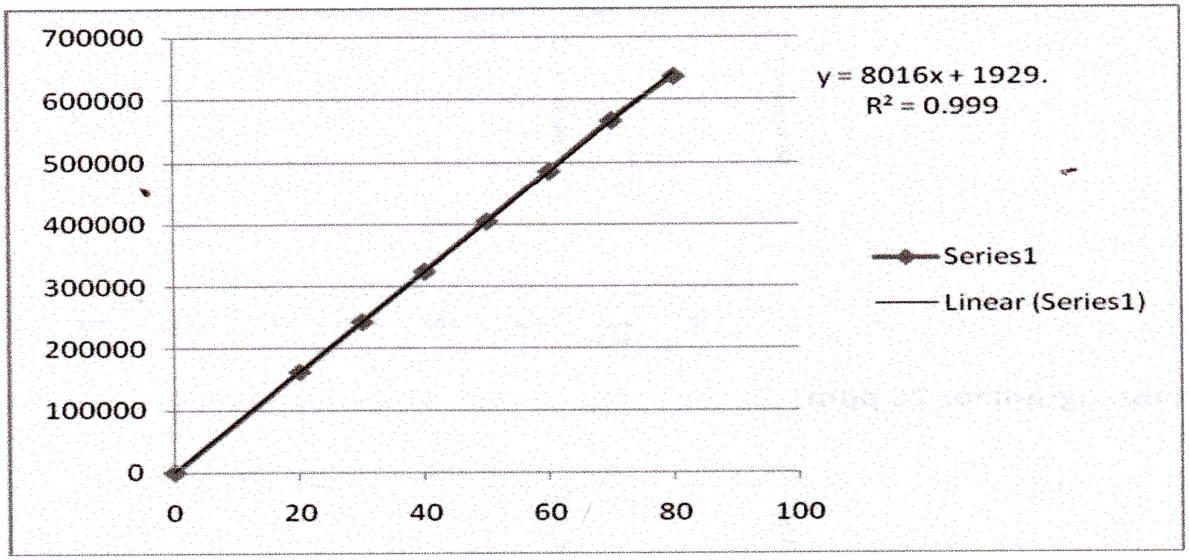


Fig.-7: Linearity Graph (VEL)

Table-6: Accuracy

Spike level concentration		SOF			VEL		
	Injections	Amount added (ppm)	Amount Recovered (ppm)	% Recovery	Amount added (ppm)	Amount Recovered (PPM)	% Recovery
50%	1	20	20.01	100.05	20	20.45	102.25
	2	20	19.91	99.5	20	19.84	99.2
	3	20	20.08	100.4	20	20.07	100.35
100%	1	40	40.03	100.07	40	39.45	98.62
	2	40	39.98	99.95	40	40.07	100.17
	3	40	39.91	99.77	40	39.93	99.82
150%	1	60	60.02	100.03	60	60.02	100.03

	2	60	60.07	100.11	60	59.98	99.96
	3	60	60.04	100.06	60	60.07	100.11
			Mean	99.993			100.05
			%RSD	0.68			0.327

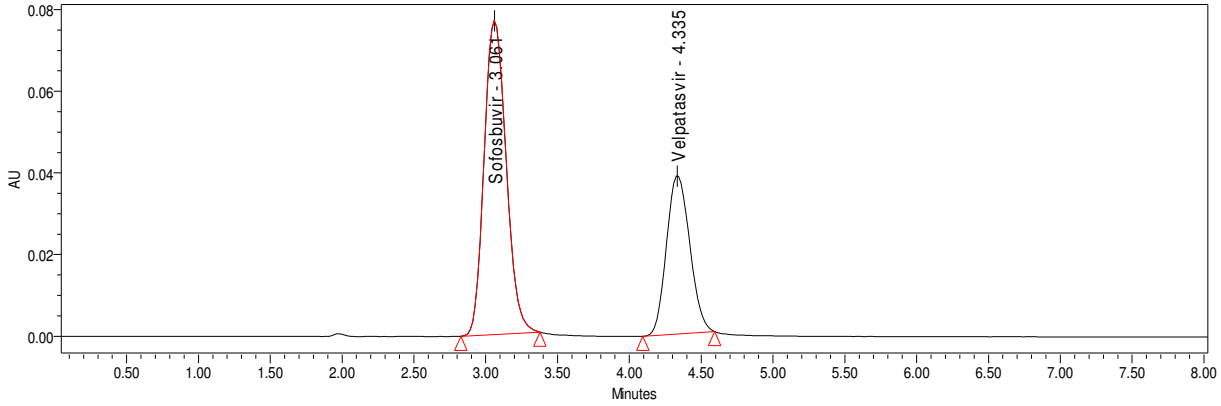


Fig.-8: Accuracy (50%) Chromatogram

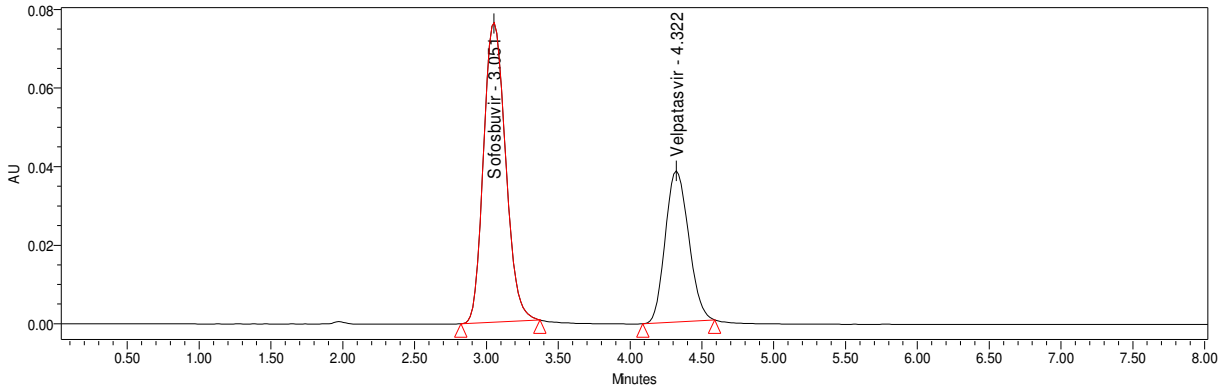


Fig.-9: Accuracy (150%) chromatogram

Precision

System Precision

System precision was conducted to make sure that the HPLC system was perfect. WS Solution at 40 ppm with respect to both SOF and VEL together in five replicates were injected and the chromatograms were analyzed. The % RSD for PA and % Assay for SOF and VEL were with in the acceptance limit of not more than 2 (Table-7).

Table-7: System Precision

Trial	SOF		VEL	
	PA	% Assay	PA	% Assay
1	9437784	99.74	323112	99.98
2	9437412	99.14	323452	99.30
3	9430257	99.62	323742	99.60
4	9438431	99.72	323047	99.84
5	9438754	99.42	323087	99.72
Mean	94367079	99.13	3231472	99.71
SD	10475.12	0.24746	7452.4712	0.425
% RSD	0.7842	0.31713	0.752411	0.240

Method Precision

TS solution at 40 ppm with respect to both SOF and VEL was injected in six replicates and chromatograms studied.

The %RSD for PA for SOF and VEL were less than 2. The individual assay of SOF and VEL were within the limits of not less than 98% and not more than 102%. (Table-8)

Table-8: Method Precision

Trial	SOF		VEL	
	PA	% Assay	PA	% Assay
1	9432571	99.25	323584	99.54
2	9438475	99.12	323054	99.72
3	9434752	98.12	323847	99.31
4	9430487	99.52	323751	99.84
5	9436547	98.84	323814	99.42
6	9437841	99.54	323745	99.32
Mean	9438845	99.56	323875	99.87
SD	147205	0.54213	3240.5412	0.7845
% RSD	0.7451	0.412	0.54721	0.874654

Intermediate Precision

TS at 40 ppm with respect to both SOF and VEL was taken and six replicate trials were run on two different days. The chromatograms collected for method precision were considered as day one chromatograms. The %RSD and % Assay calculated from the chromatograms on two different days for individual SOF and VEL met the acceptance limits. The method developed was thus precise.

Robustness

Small variation in the experimental conditions (Table-9) showed little or no effect. The %RSD for PA and RT were within the acceptance limits (Table-10).

Table-9: Experimental conditions

Changed Value	I	II	III
M.P FR (ml/min)	0.8	1.0	1.2
Temp (ºC)	20	25	30
Buffer pH	3.1	3.2	3.3

Table-10: Robustness Data

	Value	MP FR			Temp			Buffer pH		
		0.8	1.0	1.2	20	25	30	3.1	3.2	3.3
SOF	Mean PA(n=3)	9416963	9436039	9456257	9376469	9454160	9546386	9341702	947503	9562431
	SD	1525.937	2597.38	4338.7	8493.9	4845.5	28271.8	17083	14889	13982.2
	% RSD	0.0162	0.0275	0.045	0.0905	0.0512	0.2961	0.1828	0.15714	0.1462
VEL	Mean PA(n=3)	321727.7	323472	324794.7	318496	32624.1	336844	314742	327722 .3	332496.3
	SD	161.97	395.186	75.566	562.3	2826.4	2575.6	2486.2	2264.3	2845.466
	% RSD	0.0503	0.12217	0.0232	0.176	0.8663	0.7646	0.7899	0.6973	0.855789

LOD

From the regression line the LOD for SOF and VEL were calculated as 0.475 ppm and 0.65 ppm respectively.

LOQ

From the regression line the LOQ for SOF and VEL were calculated as 1.44 ppm and 1.98 ppm respectively.

CONCLUSION

Simultaneous RP– HPLC method for estimation of SOF and VEL in their combined tablet dosage form was developed and validated in accordance with the ICH instructions. All the validation parameters met the acceptance limits. The developed method can be used for regular quality control analysis.

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