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Research

Quantitative Estimation Of Sofosbuvir And Velpatasvir In Tablet Dosage Forms By Rp-Hplc Method

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Abstract

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Sofosbuvir and Velpatasvir, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA pH 4.2 (40:60) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 272 nm. The retention time of the Sofosbuvir and Velpatasvir was 2.781, 4.048 ±0.02min respectively. The method produce linear responses in the concentration range of 7.5-37.5µg/ml of Sofosbuvir and 5-25µg/ml of Velpatasvir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Sofosbuvir, Velpatasvir, RP-HPLC, validation.

INTRODUCTION

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good sensitivity. High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of

components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

Types of Chromatogphy

Normal-phase chromatography

Mechanism: Retention by interaction with the polar surface of the stationary phase with polar parts of the sample molecules.
Stationary phase: SiO2, Al2O3, -NH2, -CN, -Diol, -NO2, etc.
Mobile phase: Heptane, hexane, cyclohexane, CHCl3, CH2Cl2, dioxane, methanol, etc.
Application: Separation of non-ionic, non-polar to medium polar substances. Disadvantage: Lack of reproducibility of retention times as water or protic organic solvents change the hydration state of the silica or alumina chromatographic media.

Reversed-phase chromatography

Mechanism: Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.
Stationary phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, (CH2)n-CN, (CH2)n-diol, etc.
Mobile phase: Methanol, acetonitrile, water, buffer (sometimes with additives of THF or
Dioxane), etc.
Application: Separation of non-ionic and ion forming non-polar to medium polar substances (carboxylic acids, hydrocarbons). If ion forming substances (as carboxylic acids) are to be separated, a pH control by buffers is necessary.

Reversed-phase ion-pair chromatography

Mechanism: Ionic sample molecules are ionically bound to an ion-pair reagent. The ion- pair reagent contains an unpolar part suitable for interaction with the unpolar hydrocarbon chain of the stationary phase.
Stationary phase: Reversed phase materials (RP-18, RP-8, CN), etc.
Mobile phase: Methanol, acetonitrile, buffer with added ion-pair reagent in the concentration range of 0.001 to 0.01 M, etc.
Application: Ionic substances often show very poor retention in reversed phase chromatography. To overcome this difficulty an ion-pair reagent is added to the eluent.

Ion-exchange chromatography

Mechanism: Retention of reversible ionic bonds on charged groups of the stationary phase
Stationary phase:

	Strong	Weak
Cation exchanger	SO_3^-	COO^-
Anion exchanger	NR_3^+	NHR_2^+

Mobile phase: Aqueous buffer systems.
Application: Separation of substances which can form ions such as inorganic ions, organic acids, organic bases, proteins, nucleic acids.

Advantages of HPLC

1) It provides specific, sensitive and precise method for analysis of the different complicated sample.
2) There is ease of sample preparation and sample introduction.
3) There is speed of analysis.
4) The analysis by HPLC is specific, accurate and precise.
5) It offers advantage over gas chromatography in analysis of many polar, ionic substances, high molecular weight substances, metabolic products and thermolabile as well as nonvolatile substances.

Applications of HPLC

a) Natural Products: HPLC is an ideal method for the estimation of various components in plant extracts which resemble in structure and thus demand a specific and very sensitive method e.g., analysis of digitalis, cinchona, liquorice, and ergot extracts.
b) Stability studies: HPLC is now used for ascertaining the stability of various pharmaceuticals. With HPLC the analysis of the various degradation products can be done and thus stability indicating HPLC systems have been developed.

c) Bioassays and its complementation: Complex molecules as antibiotics and peptide hormones are mainly analysed by bioassay which suffer from high cost, necessity replicates, poor precision and length of time required. Also bioassay gives an overall estimate of potency and gives no guidance about the composition. Thus HPLC can be used to complement bioassays and give an activity profile. It has been used for analysis of chloramphenicol, penicillins, clotrimoxazole, sulfas and peptides hormones.
d) HPLC has also been used in the cosmetic industry for quality control of various cosmetics.

MATERIALS AND METHODS

Velpatasvir & Sofosbuvir Procured from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck.

HPLC METHOD DEVELOPMENT
TRAILS
Preparation of standard solution

Accurately weigh and transfer 10 mg of Velpatasvir and Sofosbuvir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.15ml of Velpatasvir and 0.225ml of Sofosbuvir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer in proportion 40:60 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Symmetry C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	40ºC
Column	:	Symmetry C18 (4.6×150mm, 5µ)
pH	:	4.2
Mobile phase	:	Methanol: TEA buffer pH 4.2 (40:60v/v)
Flow rate	:	1ml/min
Wavelength	:	272nm
Injection volume	:	10 µl
Run time	:	6 min

VALIDATION
PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Triethylamine (TEA) buffer (pH-4.2): Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.5. Fliter and sonicate the solution by vaccum filtration and ultrasonication.

Preparation of mobile phase: Accurately measured 650 ml (65%) of Methanol and 350 ml of Phosphate buffer (35%) a were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Mobile phase	:	Methanol: TEA pH 4.2 (40:60)
Column	:	Symmetry C18 (4.6×150mm, 5.0 µm)
Flow rate	:	1 ml/min
Wavelength	:	272 nm
Column temp	:	40ºC
Injection Volume	:	10 µl

Run time : 6 minutes

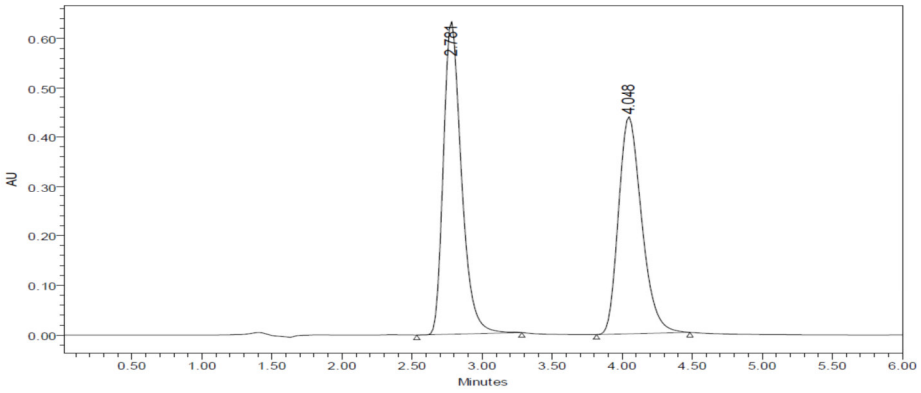

Fig 1: Optimized Chromatogram

Table 1: peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Sofosbuvir	2.781	2774027	299752		1.2	6314
2	Velpatasvir	4.048	2533532	210321	4.6	1.3	5521

From the above chromatogram it was observed that the Sofosbuvir and Velpatasvir peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

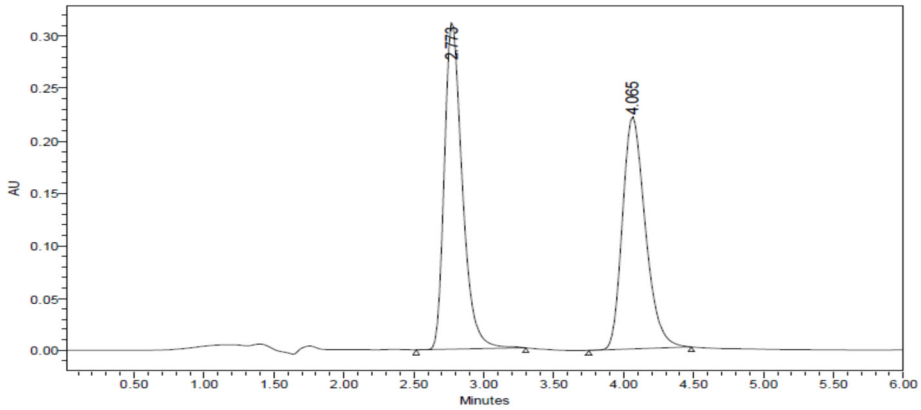

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Sofosbuvir	2.773	2770123	282157		1.6	5011
2	Velpatasvir	4.065	2522041	251068	5.3	1.5	5947

Assay (Standard)

Table 3: Peak results for assay standard of Sofosbuvir

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count
1	Sofosbuvir	2.782	2762937	357421	1.3	6344.7
2	Sofosbuvir	2.766	2774613	388745	1.3	6344.2

3	Sofosbuvir	2.767	2762937	399854	1.3	6300.1
4	Sofosbuvir	2.795	2774613	386542	1.3	6344.7
5	Sofosbuvir	2.768	2776429	364121	1.3	6344.2
Mean			2770306			
Std. Dev.			6767.495			
% RSD			0.2			

Table 4: Peak results for assay standard of Velpatasvir

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Velpatasvir	4.049	2540214	236741	4.6	1.3	5937.7
2	Velpatasvir	4.025	2541284	226745	4.7	1.3	5008.8
3	Velpatasvir	4.029	2534375	210326	4.6	1.3	5937.7
4	Velpatasvir	4.067	2526189	226741	4.7	1.3	5008.8
5	Velpatasvir	4.030	2546248	231494	4.7	1.3	5990.7
Mean			2537662				
Std. Dev.			7677.647				
% RSD			0.3				

%RSD of five different sample solutions should not more than 2. The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample)

Table 5: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	Sofosbuvir	2.764	2732203	294531		1.3	6314	1
2	Velpatasvir	4.012	2507543	216321	4.6	1.3	5954	1
3	Sofosbuvir	2.767	2751843	286473		1.3	6369	2
4	Velpatasvir	4.016		216354	4.6	1.3	5944	2
5	Sofosbuvir	2.764	2744776	312684		1.3	6329	3
6	Velpatasvir	4.013	2515628	206571	4.6	1.3	5990	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Sofosbuvir in pharmaceutical dosage form was found to be 99.2%.

LINEARITY
SOFOSBUVIR

Concentration µg/ml	Average Peak Area
7.5	88464
15	166364
22.5	237423
30	319213
37.5	401317

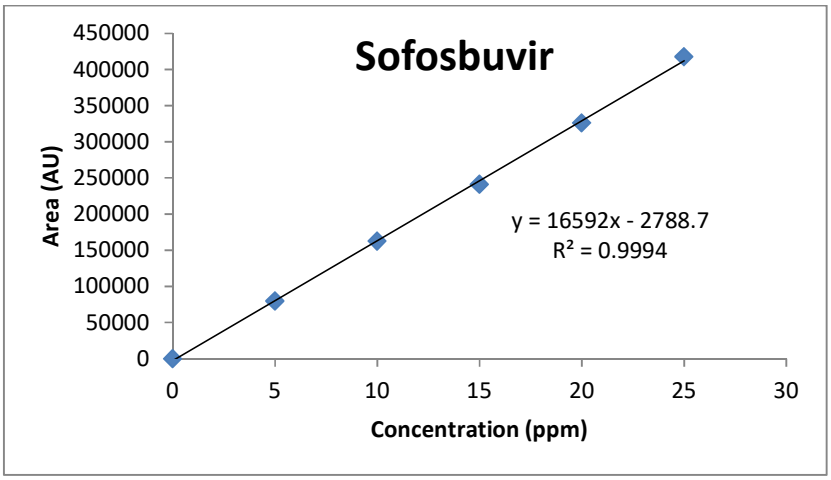

Fig 3: Calibration graph for Sofosbuvir

VELPATASVIR

Concentration µg/ml	Average Peak Area
5	80032
10	162782
15	241426
20	326009
25	417393

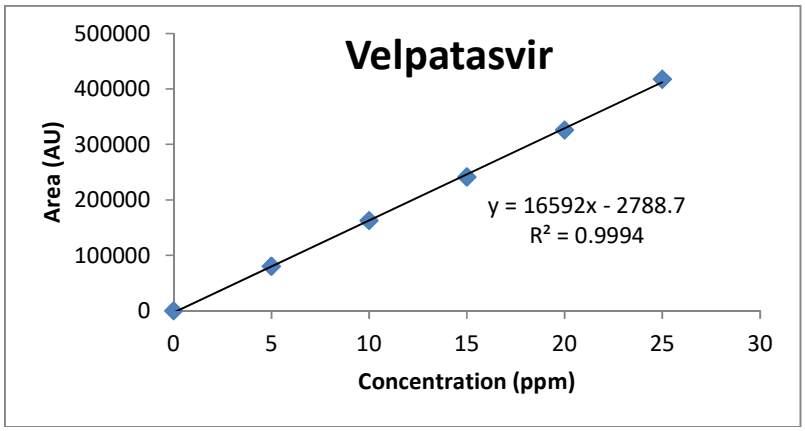

Fig 4: calibration graph for Velpatasvir

REPEATABILITY

Table 6: Results of repeatability for Sofosbuvir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sofosbuvir	2.766	2766870	294578	6684	1.3
2	Sofosbuvir	2.774	2771971	286541	6347	1.3
3	Sofosbuvir	2.770	2771958	302657	6674	1.3
4	Sofosbuvir	2.772	2780299	293412	6451	1.3
5	Sofosbuvir	2.771	2789695	283154	6678	1.3
Mean			2776159			
Std. Dev			8969.896			
% RSD			0.3			

%RSD for sample should be NMT 2. The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 7: Results of method precession for Velpatasvir

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Velpatasvir	4.025	2534539	193240	5761	1.3	4.7
2	Velpatasvir	4.040	2539247	201647	5489	1.3	4.6
3	Velpatasvir	4.032	2544661	193472	5367	1.3	4.6
4	Velpatasvir	4.041	2548839	196475	5845	1.3	4.6
5	Velpatasvir	4.036	2558822	201394	5347	1.3	4.7
Mean			2545222				
Std. Dev			9329.852				
% RSD			0.3				

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Day-1

Table 8: Results of Intermediate precision for Sofosbuvir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sofosbuvir	2.781	2715421	294651	6647	1.3
2	Sofosbuvir	2.780	2778540	284123	6781	1.3
3	Sofosbuvir	2.782	2754247	274561	6984	1.3
4	Sofosbuvir	2.780	2780545	281241	6475	1.3
5	Sofosbuvir	2.782	2777021	286471	6647	1.3
6	Sofosbuvir	2.774	2780254	294512	6489	1.3
Mean			2764338			
Std. Dev			25974			
% RSD			0.9			

%RSD of Six different sample solutions should not more than 2

Table 9: Results of Intermediate precision for Velpatasvir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Velpatasvir	4.048	2506927	211541	5495	1.4	4.6
2	Velpatasvir	4.050	2504522	206141	5694	1.4	4.6
3	Velpatasvir	4.049	2541270	198641	5785	1.4	4.7
4	Velpatasvir	4.050	2507885	206741	5947	1.4	4.6
5	Velpatasvir	4.049	2504587	209487	5742	1.4	4.6
6	Velpatasvir	4.040	2504780	193481	5914	1.4	4.6
Mean			2511662				
Std. Dev			14572.01				
% RSD			0.5				

%RSD of Six different sample solutions should not more than 2. The %RSD obtained is within the limit, hence the method is rugged.

Day 2:

Table 10: Results of Intermediate precision Day 2 for Reserpine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Reserpine	2.078	370979	42978	3083.0	1.9
2	Reserpine	2.082	371041	42568	3583.2	1.8
3	Reserpine	2.080	371386	42211	3533.2	1.8
4	Reserpine	2.089	369246	42277	1537.8	1.6
5	Reserpine	2.083	370840	42065	1489.3	1.6
6	Reserpine	2.089	369246	42277	1537.8	1.6
Mean			370456.3			
Std. Dev			954.6004			
% RSD			0.25			

%RSD of five different sample solutions should not more than 2

Table 11: Results of Intermediate precision for Dihydralazine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dihydralazine	5.077	3841404	246818	5208.0	2.1	10.1
2	Dihydralazine	5.151	3885014	242854	5127.6	2.1	10.0
3	Dihydralazine	5.112	3743003	242955	5269.7	2.2	10.2
4	Dihydralazine	5.133	3743003	242955	5269.7	2.2	10.2
5	Dihydralazine	5.203	3885014	242854	5127.6	2.1	10.0
6	Dihydralazine	5.133	3743003	242955	5269.7	2.2	10.2
Mean			3806740				
Std. Dev			71613.47				
% RSD			1.8				

%RSD of five different sample solutions should not more than 2. The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY

The accuracy results for Sofosbuvir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1382603	11.25	11.23	99. 8	99.3**%**
100%	2777270	22.5	22.1	98.2	
150%	41448756	33.75	33.73	99.9	

The accuracy results for Velpatasvir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1306990	7.5	7.5	100	99.4**%**
100%	2510628	15	14.8	98.6	
150%	3777999	22.5	22.46	99.8	

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 12: Results for Robustness

Sofosbuvir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2774027	2.781	6314	1.2
Less Flow rate of 0.9 mL/min	2884521	3.327	6199	1.4
More Flow rate of 1.1 mL/min	2542012	2.516	6234	1.4
Less organic phase	2888515	3.326	6298	1.4
More organic phase	2541550	2.416	6287	1.2

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Velpatasvir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	2533532	4.048	5521	1.3
Less Flow rate of 0.9mL/min	2750214	5.319	5643	1.6
More Flow rate of 1.1 mL/min	2254107	3.649	5782	1.5
Less organic phase	2754017	5.318	5309	1.4
More organic phase	2215870	3.233	5580	1.51

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Sofosbuvir and Velpatasvir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Sofosbuvir and Velpatasvir was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA pH 4.2 (40:60) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Sofosbuvir and Velpatasvir in bulk drug and in Pharmaceutical dosage forms.

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