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Research Article

New RP-HPLC Method for Simultaneous Estimation of Sofosbuvir and Velpatasvir in Combined Pharmaceutical Dosage Forms

K. Revathi, G. Sowndarya*, V. Swathi

Department of Pharmaceutical Analysis, Sri Sivani College of pharmacy, Srikakulam, Andhra Pradesh – 532410, India.

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ABSTRACT

For simultaneous estimation of SOF & VEL in bulk and pharmaceutical dosage form, a quick, rapid, reliable, precise and sensitive RP-HPLC method has been developed and validated. On Discovery C18 (4.6 x250 mm, 5 µm), the chromatographic separation was achieved using a mobile phase buffer (60 %; 0.01 N KH_2PO_4: 40 % acetonitrile, 1.0 ml/min fluid at a room temperature, detection at 260 nm). SOF & VEL had retention periods of 2.373 and 2.967 min. Linearity for both of drugs was conducted at a concentration range of 100-600 µg / mL, 25-150 ppm, and the correlation range was 0.999 and 0.999 respectively in SOF & VEL. SOF & VEL have respectively been shown to be pure at a rate of 99.30 and 99.83 %. The proposed method is validated for the specificities, lines, range, precision and robustness of SOF & VEL in bulk and the combined pharmaceutical dosage form according to the guidelines of ICH Q2 (B) and can be employed for the routine quality test.

1. Introduction

Sofosbuvir (SOF) is a drug for hepatitis C therapy[1,2]. Only a mixture of ribavirin, peginterferon alpha, simeprevir, ledipasvir or daclatasvir is recommended. Depending on the type of hepatitis C virus involved, cure rates are from 30 to 97 percent. Pregnancy safety is unclear; although some combinations of medications may damage the infant. Take it by mouth. IUPAC SOF is called propanoate, molecular formulation C22H29FN3O9P, molecular weight, 529.4 g / mol. IUPAC name is Isopropyyl[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidine-1-yl)-4-fluoro-3-hydroxide-4-methyltetrahydrofurane-2-yl] amino]. The analysis of the literature shows that HPLC and UV spectrophotometry have been validated[2].

Velpatasvir (VEL) is an inhibitor of NS5A used with sofosbuvir for the treatment of infection of hepatitis C of all six major genotypes[3,4]. Chemically speaking, VEL is a non-cholinergic and anti- spasmodic[5,9] carbamate of Methyl} ~(2S), 1-{ [(2S, 5S)-2 -[(2S, 3S), 1-{ (2S, 5S, 2) – {(2S)- {(1,2S)-{ 2}-{ (12,2S)-{ 1]-{ (2S), 3 {(1,2S)-2-pyrrolidinyle}-2, 11 – dihydroisochromen [51, 7] naphtha [1,2-d] imidazol-2-yl]-5-methyl-1-oxo-2. Molecular formula $C_{49}H_{54}N_8O_8$, Molecular weight 883.02 g/mol. The main objective of the present work is for the simultaneous estimating of SOF r and VEL in pharmaceutical tableting form[10-15] to develop a simple, fast, precise and sensitive inverted HPLC methodology. And the method was validated as per ICH Q2 (B) guidelines in terms of specificity, Robustness, Accuracy, Linearity, Limit of detection (LOD), and Limit of Quantification (LOQ).

2. Materials & Methods

2.1. Chemicals:

Sofosbuvir and Velpatasvir pure drugs (API), Combination Sofosbuvir and Velpatasvir tablets (epclusa), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rakem and analytical grade.

2.2. HPLC Instrumentation and Chromatographic Condition:

The column used for the study was Discovery C_{18} (4.6 x250 mm, 5 µm) for analytical separation, fitted with quaternary pumps, a Photo Diode Array detector and an Auto Sample integrated with Empower 2 Software. The mobile process is 0.01 N KH_2PO_4: 60:40 percent v/v

* *Corresponding author*. Tel.: +919703623165.
E-mail address: chinnisoundy@gmail.com

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acetonitrile. The flow has been changed to 1 ml/min. The tool was kept at room temperature. The amount of injection was 10 µL. The isosbestic point in the **Fig.1** was achieved with a UV detection of 260 nm. Both tops are well resolution, tail factor, counting and resolution of theoretical plates. SOF and VEL were respectively high at 2.373 and 2.967 min. The counting plate and tail factor were very effective, thus optimising and validating this method.

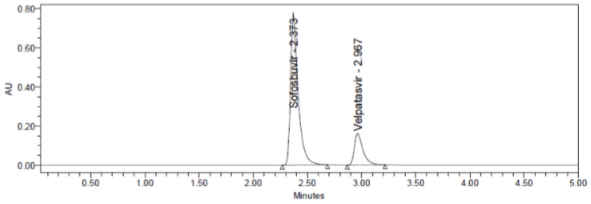

Fig.1: Optimization method of SOF and VEL

2.3. Method Validation

To obtain the present analytical method a new, responsive, convenient method has been developed to simultaneously estimate SOF and VEL from a bulk dose type. For parameter, including precision, device suitability, specificity, linearity, accuracy, detection and quantification limits, robustness and robustness according to ICH-1996 and USP-30 guidelines, the experimental method has been validated.

System Suitability

In order to determine such system suitability and efficacy, the testing parameters have been repeatedly tested, in the case of SOF , VEL , and 100 ppm, by injecting freshly prepared standard storage products at the concentration level of 400 or 100ppm.

Specificity

The specificity was conducted to decide whether any impurities interfere in the analytical peak retention duration. By detecting an interference with potential impurities and excipients, the specificities of the system were determined.

Linearity

By evaluating a variety of different concentrations for each of the two analytes, the linearity of the proposed HPLC approach was tested and found that the peak ranges were proportional to the analyses. A 1000 ppm stock solution was developed with diluent from two analytes. Various standard work solutions for SOF and VEL were developed and injected into HPLC in the range 100 to 600 ppm and 25 to 150 ppm, respectively. The selected medicines were seen to have a defined linearity. A replicate analysis (n=3) of all concentration levels provided the calibration plot (peak area versus concentration) and the linear relationship was tested using a smaller square procedure.

Accuracy

The precision analysis for SOF and VEL was conducted in terms of recovery by 50, 100 and 150 %. Triplicate and percent retrievals of SOF and VEL were measured on the HPLC method for regular and sample solutions. In the estimation of percent recovery, the region of each degree has been used.

Precision

The method 's accuracy was calculated by means of the actual measurement of six 400ppm and 100ppm replicates of Sofosbuvir, Velpatasvir. In the peak areas in a sequence of medication solutions, in three days the specificity of the test was also calculated in terms of intra- and inter-day variance. Intra- and inter-day variations were determined from relative standard deviation (RSD) in the drug solution's peak area.

Limit of Detection and Quantification

The SOF and VEL detection values were respectively 0.29 ppm and 1.15 ppm. SOF and VEL were 0.88 ppm and 3.47 ppm, respective quantification values reduced.

Robustness

The robustness of the system was tested by testing the effect of minor deliberate adjustments in procedural variables such as flow rate (±5%) and wave length shift (±5 nm). For fluctuations of 0.8 ml / min to 1.2 ml / min the robustness has been performed and the procedure is robust only in less flow condition and with mobile phase changes even ±5 percent.

3. Results and Discussion

The current study mentioned is the development and validation of SOF and VEL simultaneous estimates of a new RP-HPLC process. Various mobile phases and columns have been used to achieve the optimized RP-HPLC process. A variety of experiments are used to refine the end system for the conditions developed: The mobile step consists of the Kromacil C18 column (250 to 4.6 mm, 5 µm particle sizes) of orthophosphoric and acetonitrile buffers and columns. The flow rate has been increased to 1 ml/min. The tool was kept at room temperature. At 260 nm, the UV was detected. The amount of injection was 10µL.

3.1. Method Validation

Since the other compounds are not interfering in the analytical peak retention time. This approach was then claimed to be a particular one. The machine suitability parameters, such as theoretical plates (N), tailoring factor (T), were measured and found to be no more than 2000 and no longer than 2. Table 1 displays the results.

Table 1: System suitability of Sofosbuvir and Velpatasvir method

Parameter	Sofosbuvir	Velpatasvir
Retention time (Rt)	2.373 min	2.967 min
Theoretical plates (N)	5887.66	7131.167
Tailing factor (T)	1.58	1.52

The linearity of the claimed analyte concentration of 100-600 ppm was calculated as linearity regression, with Sofosbuvir and Velpatasvir from 25 ppm up to 150 ppm, respectively. For Sofosbuvir and Velpatasvir respectively, the correlation coefficient was 0.999 and 0.999. Therefore, the results within the limit were obtained. The linearity data is shown in **Table 2** and shown in **Figure 2** & **3** graphically.

Standard methods of addition dictated the precision of the method produced. This approach adds to the previously analysed sample solution the known quantities of SOF and VEL, and then compares experimental and true values. The nominal analytical concentration was calculated in three ranges corresponding to 50, 100 and 150 %. The percentage of SOF and VEL recovery was 99.56 %, and the results were tabled in **Table 3** & **4**. The results were shown.

Table 2: Linearity table for Sofosbuvir and Velpatasvir

Sofosbuvir		Velpatasvir	
Conc. (µg/mL)	**Peak area**	**Conc. (µg/mL)**	**Peak area**
0	0	0	0
100	9096466	25	2220104
200	18744528	50	4378691
300	29537745	75	6396903
400	37738839	100	8848297
500	47542510	125	11028521
600	56458883	150	12932587

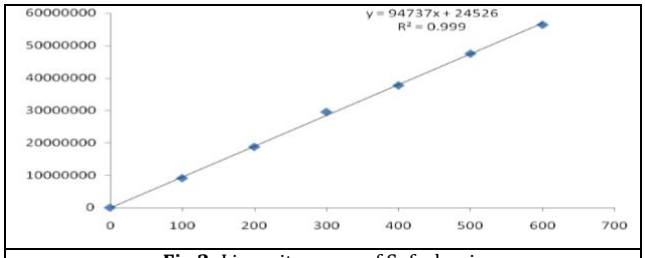

Fig 2: Linearity curve of Sofosbuvir

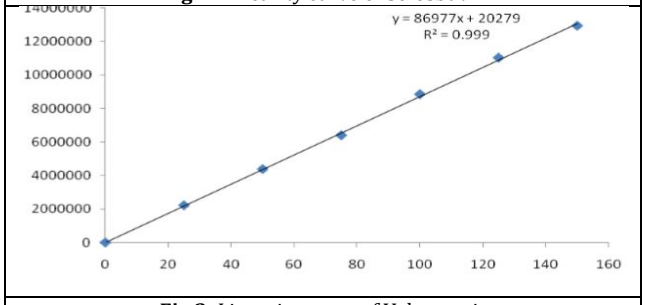

Fig 3: Linearity curve of Velpatasvir

Table 3: Accuracy table of Sofosbuvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
	200	198.6161	99.31	
50	200	199.0136	99.51	
	200	199.7203	99.86	
	400	398.4814	99.62	
100	400	398.0113	99.50	99.56
	400	399.2566	99.81	
	600	599.6482	99.94	
150	600	594.9732	99.16	
	600	595.745	99.29	

Table 4: Accuracy table of Velpatasvir

% Level	Amount Spiked (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean %Recovery
	50	49.48392	98.97	
	50	49.78271	99.57	
50	50	49.38216	98.76	
	100	99.19113	99.19	
100	100	99.30132	99.30	99.43
	100	99.78098	99.78	
	150	149.755	99.84	
150	150	149.2158	99.48	
	150	149.5561	99.70	

The method's precision was measured by assessing the peak areas of six sample solutions replicates. The rate of RSD for process precision for SOF and VEL was 0.4 and 0.4 and results were shown in **Table 5**.

Table 5: System and Intermediate precision table of Sofosbuvir and Velpatasvir

S.No	System (Area)		Intermediate (Area)	
	SOF	**VEL**	**SOF**	**VEL**
1	37197212	8778942	36816329	8575817
2	37024091	8781361	36326955	8548326
3	37115514	8771452	3644266	8565826
4	37224620	8792194	36363062	8549691
5	37240519	8793093	36279865	8540453
6	37071903	8811296	36330180	8569720
Mean	37145643	8788056	36410110	8558306
S.D	88338.5	14044.0	200913	14042.9
% RSD	0.2	0.2	0.6	0.2

The SOF and VEL detection values were respectively 0.29 ppm and 1.15 ppm. SOF and VEL were constrained in quantification by 0.88 ppm and 3.47 ppm, respectively. **Table 6** showed the values.

Table 6: Sensitivity table of Sofosbuvir and Velpatasvir

Molecule	LOD	LOQ
Sofosbuvir	0.29	0.88
Velpatasvir	1.15	3.47

Samples were injected in a duplicate manner and were maintained under the conditions of flow minus (1.1 mm/min), flow plus (1.3 mm/min), phase minus mobile (60B:40A), phase plus mobile (50B:50A), temperature minus (25 °C) and tempping plus (35 °C). The fitness parameters of the device were not very affected and all parameters were passed. It was within the limit for percent RSD. Results were tabulated in **Table 7**.

Table 7: Robustness data for Sofosbuvir and Velpatasvir.

Condition	% RSD of SOF	%RSD of VEL
Flow rate (-) 1.1ml/min	0.1	0.7
Flow rate (+) 1.3ml/min	0.2	0.5
Mobile phase (-) 60B:40A	1.0	0.2
Mobile phase (+) 50B:50A	0.6	1.4
Temperature (-) 25°C	0.1	0.1
Temperature (+) 35°C	0.6	0.3

4. Conclusion

For simultaneous estimation of SOF and VEL in tablet form, a quick, accurate and accurate approach has been established. SOF and VEL were found to be 2.373 and 2.967 minutes in retention time. The RSD was and were found respectively 0.2 and 0.2 % for SOF and VEL. Recovery for SOF and VEL respectively was achieved at 99.56 and 99.48 %. The values of LOD, LOQ from regression equations of 0.29, 1.15 and 0.88, 3.47, both from SOF and VEL. SOF regression equation is y = 94737x + 24526, y = 87090x + 14840 VEL regression equation. Retention times have been decreased and time taken down such that a quick and cost-effective method is developed which can be applied to industry regular quality control tests.

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Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

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