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Analytical Method Development and Vaidation for Velpatasvir and Sofosbuvir in combined Dosage Form by RP-HPLC

K.Nithiyananthan[1*], K.V.S.Prasadarao[2]

[1]*Research Scholar, Acharya Nagarjuna University, Guntur, Andhra Pradesh 522510.*
[2]*Principal, Rahul Institute of Pharmaceutical Sciences and Research, Chirala, Andhra Pradesh 523157.*

A B S T R A C T

The estimation of Velpatasvir and Sofosbuvir was done by RP-HPLC. The Phosphate buffer was pH 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C18 column C_{18} (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 225nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Velpatasvir and Sofosbuvir were found to be from 100-500 µg/ml of Velpatasvir and 1-5µg/ml of Sofosbuvir. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Velpatasvir and Sofosbuvir. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements.

Keywords: Velpatasvir and Sofosbuvir, Phosphate buffer, Inertsil C_{18} column etc.

A R T I C L E I N F O

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***Corresponding Author**
K. Nithiyananthan
Research Scholar, Acharya Nagarjuna University, Guntur, Andhra Pradesh 522510.
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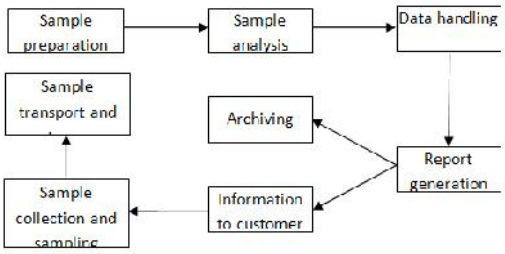
1. Introduction

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Chemical composition is the entire picture (composition) of the material at the chemical

scale and includes geometric features such as molecular morphologies and distributions of species within a sample as well as single dimensional features such as percent composition and species identity.1

- To be effective and efficient, analyzing samples requires expertise in
- The chemistry that can occur in a sample.
- Analysis and sample handling methods for a wide variety of problems (the tools-of-the-trade).
- Accuracy and precision of the method.
- Proper data analysis and record keeping.

The major stages of an analytical process are described as follows:



Steps in analytical cycle

The pharmaceutical analysis comprises the procedures necessary to determine the "identity, strength, quality and purity" of such compounds. It also includes the analysis of raw material and intermediates during manufacturing process of drugs.

Types

Qualitative analysis: Qualitative inorganic analysis seeks to establish the presence of a given element or inorganic compound in a sample. Qualitative organic analysis seeks to establish the presence of a given functional group or organic compound in a sample.

Quantitative analysis: Quantitative analysis seeks to establish the amount of a given element or compound in a sample.

- Methods of detecting analytes
- Physical means
- Mass
- Color
- Refractive index
- Thermal conductivity

With electromagnetic radiation (Spectroscopy):

- Absorption
- Emission
- Scattering

By an electric charge:

- Electrochemistry
- Mass spectrometry

2. Materials and Methods

Instrumentation: HPLC-auto sampler –UV detector, Separation module2695, UV.detector2487, Empower-software version-2, Waters, U.V double beam spectrometer, UV 3000+, U.V win software, Lab India. Digital weighing balance (sensitivity 5mg), pH meter, Sonicator.

Chemicals:

Velpatasvir and Sofosbuvir, Ortho phosphoric acid, Acetonitrile, Methanol, Water, KH_2PO_4, K_2HPO_4

Chromatographic Conditions:

Mobile phase : Phosphate buffer pH 3.0: Methanol (30:70%v/v)
Column : Inertsil C18 5µm (4.6*250mm)
Flow rate : 0.8 ml/min
Wavelength : 260 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume: 10 µl

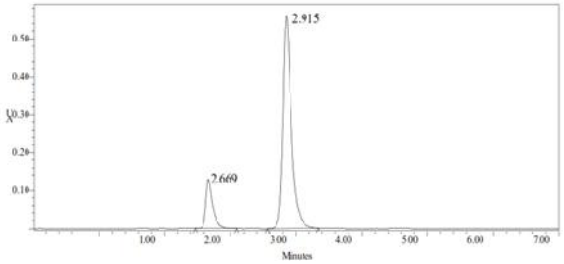


Figure 1: Optimized chromatogram for Velpatasvir and Sofosbuvir

3. Results and Discussion

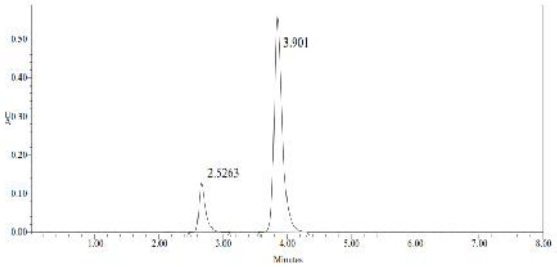


Figure 2: Chromatogram for system suitability

Validation Parameters

Precision: Precision of The Method Was Carried Out For Standard Solutions As Described Under Experimental Work.

Intermediate Precession (Ruggedness):

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Limit of Detection for Velpatasvir and Sofosbuvir:

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Limit of Quantification (LOQ):

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Robustness: The standard and samples of Velpatasvir and Sofosbuvir were injected by changing the conditions of chromatography.

4. Conclusion

The estimation of Velpatasvir and Sofosbuvir was done by RP-HPLC. 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C18 column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 225 nm.

Table 1: System suitability results for Velpatasvir and Sofosbuvir

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Velpatasvir**	2.5	124505	213642		1.2	4673.4
2	**Sofosbuvir**	3.9	1308495	154566	6 0	1.3	6090.3

Table 2: Method precession for Velpatasvir and Sofosbuvir

Injection	Area	
	Velpatasvir	**Sofosbuvir**
Injection-1	1302729	123149
Injection-2	1302947	123766
Injection-3	1303236	124271
Injection-4	1303977	124691
Injection-5	1309759	124956
Average	1304529.8	124162.7
Standard Deviation	2961.1	725.6
%RSD	0.2	0.6

Table 3: Results of Intermediate precision for Velpatasvir

Injection	Area	
	Velpatasvir	**Sofosbuvir**
Injection-1	1300148	122487
Injection-2	1304520	122626
Injection-3	1305937	122632
Injection-4	1306476	122702
Injection-5	130871	122962
Average	1305070.2	122681.8
Standard Deviation	3061.8	174.8
%RSD	0.2	0.1

Table 4: Accuracy (recovery) data for Velpatasvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 5: Accuracy (recovery) data for Sofosbuvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table 6: Area of different concentration of Velpatasvir

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.997

Table 7: Area of different concentration of Sofosbuvir

S.No.	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

Table 8: Analytical performance parameters of Velpatasvir and Sofosbuvir

Parameters	Velpatasvir	Sofosbuvir
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table 9: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Velpatasvir	52	152	2.9
Sofosbuvir	52	156	3

Table 10: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Velpatasvir	52	522	10.03
Sofosbuvir	52	524	10.1

Table 11: Flow Rate (ml/min) data for Velpatasvir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 12: Flow rate (ml/min) data for Sofosbuvir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	7063.3	1.3
2	0.8	6090.3	1.2
3	1.0	6998.0	1.3

Table 13: Variation of Mobile Phase data for Velpatasvir

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 14: Change in Organic Composition in the Mobile Phase for Sofosbuvir

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

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