International Journal of Medicine and Pharmaceutical Research

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R E S E A R C H A R T I C L E

Method Development and Validation for Simultaneous Estimation of Velpatasvir and Sofosbuvir by Using RP-HPLC in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

A New method was established for simultaneous estimation of Velpatasvir and Sofosbuvir by RP-HPLC method. Chromatographic separations were carried using YMC (4.6 x 150 mm, 5µm) column with a mobile phase composition of Phosphate buffer pH 3 and Acetonitrile (50:50) have been delivered at a flow rate of 1ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 with dual λ absorbance detector 2487 at wavelength 255 nm. The retention time for Velpatasvir and Sofosbuvir were 3.472 and 5.505 minute respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 50-250 µg/ml for Velpatasvir and 200-1000 µg/ml for Sofosbuvir respectively. For accuracy the total recovery was found to be 100.08% and 100.02% for Velpatasvir and Sofosbuvir. The LOD and LOQ for Velpatasvir was found to be 3.00 and 9.98 and LOD and LOQ for Sofosbuvir was found to be 2.98 and 10.00. The force degradation studies were performed and the results are within the limits. The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Velpatasvir and Sofosbuvir in pharmaceutical dosage form.

Keywords: Velpatasvir, Sofosbuvir, RP-HPLC, Simultaneous estimation.

A R T I C L E I N F O

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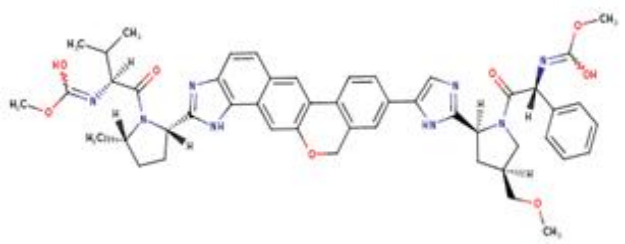
PAPER-QRCODE

A R T I C L E H I S T O R Y: Received 19 Nov 2017, **Accepted** 20 December 2017, **Available Online**18 January2018

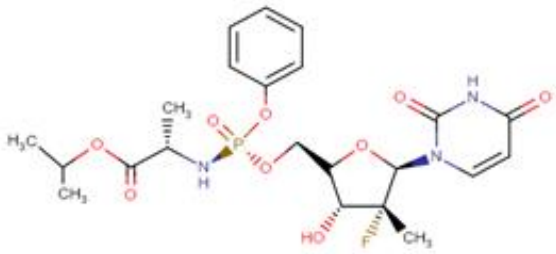
Citation: Durbhakula Pradhathri, *et al*, Method Development and Validation for Simultaneous Estimation of Velpatasvir and Sofosbuvir by Using RP-HPLC in Bulk and Pharmaceutical Dosage Form. *J. Pharm, Biomed. A. Lett., 2018, 6(1): 16-22.*

C O N T E N T S

1. Introduction



Velpatasvir



Sofosbuvir

Analytical methods

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made[6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

2. Materials and Methods

Apparatus

The instrument used for the study was WATERS, software: Empower, 2695 separation module, UV detector.

Mobile phase:

Accurately measured 500 ml (50%) of above buffer and 500 ml of Acetonitrile HPLC (50%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration [9].

Preparation of 0.025M Potassium di hydrogen ortho phosphate buffer:

3.4g of KH_2PO_4 is taken in 1000 ml of HPLC water pH was adjusted with OPA up to 3.0.final solution was filtered through 0.45 µm Membrane filter and sonicate it for 10 mins [10].

Diluent Preparation:

The Mobile phase was used as the diluent.

Optimization Chromatographic trials for Simultaneous Estimation of Velpatasvir and sofosbuvir by RP- HPLC.

Optimization chromatographic conditions

Instrument used : Waters HPLC with auto sampler and UV detector.

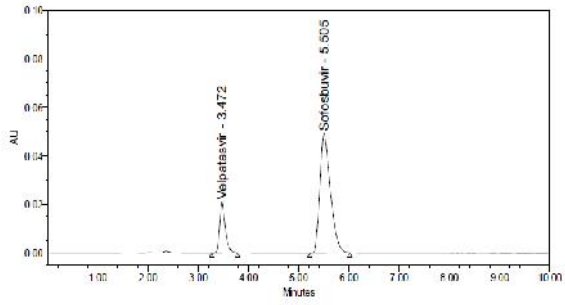
Temperature : Ambient (25° C)

Mode of separation : Isocratic mode

Column : YMC column (4.6*150mm, 5µ)

Buffer : weigh 3.4g of potassium dihydrogen ortho phosphate in 1000 ml of HPLC water Ph was adjusted with OPA up to 3.0.

pH	:	3.0
Mobile phase	:	50% buffer 50% Acetonitrile
Flow rate	:	1 ml per min
Wavelength	:	255 nm
Injection volume	:	20 µl
Run time	:	10 min.



Optimization Chromatogram

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Method Validation Parameters

Linearity

Preparation of stock solution:

Accurately weigh and transfer 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I:

0.5 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – II :

1 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluents [11].

Preparation of Level – III :

1.5 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – IV :

2 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent

Preparation of Level – V :

2.5 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Accuracy

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution[12]**:**

Accurately weigh and transfer 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 50 mg of Velpatasvir and 200 mg of Sofosbuvir working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents [13].

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 150 mg of Velpatasvir and 600 mg of Sofosbuvir working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Velpatasvir & Sofosbuvir and calculate the individual recovery and mean recovery values [14].

PRECISION

Preparation of stock solution:

Accurately weigh and transfer 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory[15].

Preparation of stock solution:

Accurately weigh and transfer 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits[16].

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.
A. The flow rate was varied at 0.9 ml/min to 1.1ml/min. Standard solution 150 ppm of Velpatasvir & 600 ppm of Sofosbuvir was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

B. The Organic composition in the Mobile phase was varied from ±10%.

Standard solution 150 ppm of Velpatasvir & 600 ppm of Sofosbuvir was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%. Organic composition in the mobile phase affected the method significantly. Hence it

Indicates that the method is robust even by change in the Mobile phase ±10

Limit of Detection: (for Velpatasvir)

Preparation of 150µg/ml solution:

Accurately weigh and transfer 100 mg of Velpatasvir working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it c ompletely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents [17].

Preparation of 0.81 µg/ml solution:

Further pipette 0.054ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of Detection: (for Sofosbuvir)

Preparation of 600 µg/ml solution:

Accurately weigh and transfer 400 mg of Sofosbuvir working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents[18].

Preparation of 1.38 µg/ml solution:

Further pipette 0.023 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Limit of Quantification: (for Velpatasvir)

Preparation of 150 µg/ml solution:

Accurately weigh and transfer 100 mg of Velpatasvir working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 2.70 µg/ml solution:

Further pipette 0.18 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of Quantification: (for Sofosbuvir)

Preparation of 600 µg/ml solution: Accurately weigh and transfer 400 mg of Sofosbuvir working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 4.62 µg/ml solution:

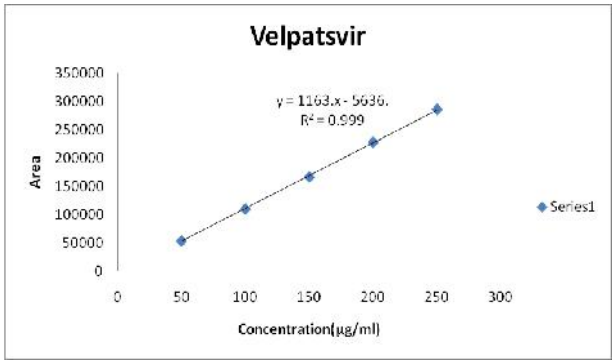
Further pipette 0.077 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

3. Results and Discussion

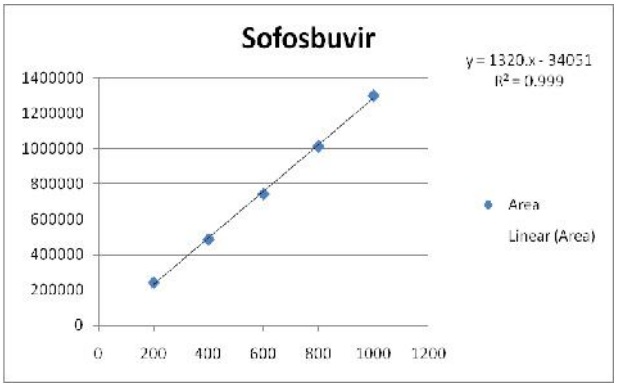
Method Validation Parameters

1. Linearity:

The linearity study was performed for the concentration of 50 ppm to 250ppm for Velpatasvir and 200ppm to 1000ppm for Sofosbuvir and chromatograms are shown below.



Calibration graph of Velpatasvir



Calibration graph of Sofosbuvir

Table 1: Linearity Results for Velpatasvir

S. No	Linearity Level	Concentration	Area
1	I	50	53953
2	II	100	110011
3	III	150	166601
4	IV	200	227887
5	V	250	285840
Correlation Coefficient			0.999

Linearity Results for Sofosbuvir

S. No	Linearity Level	Concentration	Area
1	I	200	243401
2	II	400	488042
3	III	600	744612
4	IV	800	1013904
5	V	1000	1300811
Correlation Coefficient			0.999

Accuracy results for Velpatasvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	80505	50	49.72	99.43	
100%	161649	100	99.82	99.82	
150%	245309	150	151.49	100.99	100.08

Accuracy results for Sofosbuvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	373486	200	199.69	99.84	
100%	746639	400	398.74	99.68	
150%	1129342	600	603.12	100.52	100.02

System Precision

Injection	Area for Velpatasvir	Area for Sofosbuvir
Injection-1	161345	747339
Injection-2	161232	746432
Injection-3	161671	747131
Injection-4	161999	747399
Injection-5	162898	747018
Injection-6	164679	747649
Average	162304	747161.3
Standard Deviation	1308.1	419.3
%RSD	0.8	0.1

Intermediate Precision

Injection	Area for Velpatasvir	Area for Sofosbuvir
Injection-1	162345	744533
Injection-2	162432	747232
Injection-3	162971	744531
Injection-4	162899	744399
Injection-5	162898	744018

Injection-6	162333	744689
Average	162646.3	744900.3
Standard Deviation	305.8	1164.7
%RSD	0.2	0.2

Results for variation in flow for Velpatasvir: System suitability results for Velpatasvir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4353.29	1.30
2	1	4822.40	1.36
3	1.1	4543.29	1.40

System suitability results for Sofosbuvir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3433.94	1.42
2	1	4722.40	1.26
3	1.1	3863.94	1.44

Results for variation in mobile phase composition for Velpatasvir

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4543.29	1.40
2	*Actual	4822.40	1.36
3	10% more	4543.29	1.40

System suitability results for Sofosbuvir

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	3863.94	1.44	9.50
2	*Actual	3115.92	1.12	9.50
3	10% more	3863.94	1.44	9.50

Limit of Detection for Velpatasvir and Sofosbuvir

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Velpatasvir	58	174	3.00
Sofosbuvir	58	173	2.98

Limit of Quantification for Velpatasvir and Sofosbuvir

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Velpatasvir	58	579	9.98
Sofosbuvir	58	580	10.00

4. Conclusion

The estimation of Velpatasvir and Sofosbuvir was done by RP-HPLC. The assay of Velpatasvir and Sofosbuvir was performed with tablets and the % assay was found to be 100.08 and 99.97 which shows that the method is useful for routine analysis. The linearity of Velpatasvir and Sofosbuvir was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.1for Velpatasvir and Sofosbuvir which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.2 and 0.2 for Velpatasvir and Sofosbuvir which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.08% and 100.02% for Velpatasvir and Sofosbuvir. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criterion for LOD and LOQ is 3 and 10.The LOD and LOQ for Velpatasvir was found to be 3.0 and 9.98 and LOD and LOQ for Sofosbuvir was found to be 2.98 and10.00. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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