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

RP-HPLC Metthod Development and Vaidation for Velpatasvir and Voxilaprevir by Simulatneous Determination in Bulk and Their Pharamceutical Dosage Forms

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A B S T R A C T

Chromatography is the most powerful and versatile technique available to the modern analyst. In a single step process it can separate a mixture into its individual components and simultaneously provide a quantitative estimate of each constituent. Samples may be gaseous, liquid or solid in nature and can range in complexity from a simple blend of two enantiomers to a multi component mixture containing widely differing chemical species. The word chromatography means "color writing" which is a way that a chemist can test liquid mixtures. While studying the coloring materials in plant life, a Russian botanist, M.S. Tswett invented chromatography in 1902.

Keywords: Thermosil C18 column, Velpatasvir and Voxilaprevir, RP-HPLC

A R T I C L E I N F O

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1. Introduction

Chromatography is the most powerful and versatile technique available to the modern analyst. In a single step process it can separate a mixture into its individual components and simultaneously provide a quantitative estimate of each constituent. Samples may be gaseous, liquid or solid in nature and can range in complexity from a simple blend of two enantiomers to a multi component mixture containing widely differing chemical species.

The word chromatography means "color writing" which is a way that a chemist can test liquid mixtures. While studying the coloring materials in plant life, a Russian botanist, M.S. Tswett invented chromatography in 1902.

2. Materials and Methods

Ortho phosphoric acid, KH_2PO_4, Acetonitrile, Methanol, Water, K_2HPO_4

Selection of wavelength:

10 mg of Velpatasvir and Voxilaprevir was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength was 252nm. Velpatasvir and Voxilaprevir. The isobestic point was taken as detection wavelength. The overlay spectrums are shown in Fig.

Optimized Chromatographic Conditions

Trial -(optimized method):

Chromatographic conditions

Column : Thermosil C18 (4.0×125 mm) 5.0µm
Mobile phase ratio: Methanol: Sodium acetate buffer (70: 30 % v/v)
Detection wavelength : 252 nm
Flow rate : 1ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 8min
Retention time : 2.566 & 3.417 mins

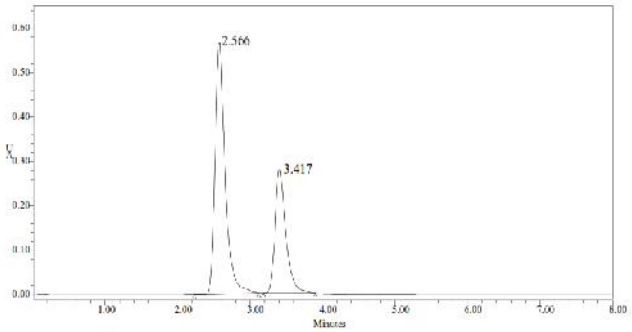

Figure 1: Chromatogram showing trial-5 injection

Observation

The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity

1 mg of Velpatasvir and 10 mg of Voxilaprevir working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 5µg/ml-25µg/ml and 50µg/ml-250µg/ml of Velpatasvir and Voxilaprevir respectively

Accuracy

1mg of Velpatasvir and 10mg of Voxilaprevir working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent

Precision

Repeatability

1mg of Velpatasvir and 10 mg of Voxilaprevir working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Intermediate Precision/ Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Limit of quantification

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Robustness

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

- The flow rate was varied at 0.4ml/min to 0.6 ml/min. Standard solution 15ppm of Velpatasvir and 150 ppm of Voxilaprevir was prepared and analyzed using the varied flow rates along with method flow rate.
- The organic composition in the mobile phase was varied from 65% to75 %standard solution 15 µg/ml of Velpatasvir and 150 µg/ml of Voxilaprevir were prepared and analyzed using the varied mobile phase composition along with the actual mobile phase composition in the method.

System suitability

1mg of Velpatasvir and 10 mg of Voxilaprevir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of Velpatasvir and Voxilaprevir from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

3. Results and Discussions

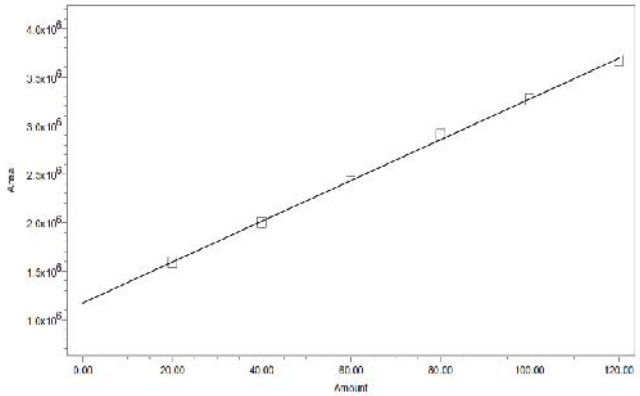

Figure 2: Showing calibration graph for Velpatasvir

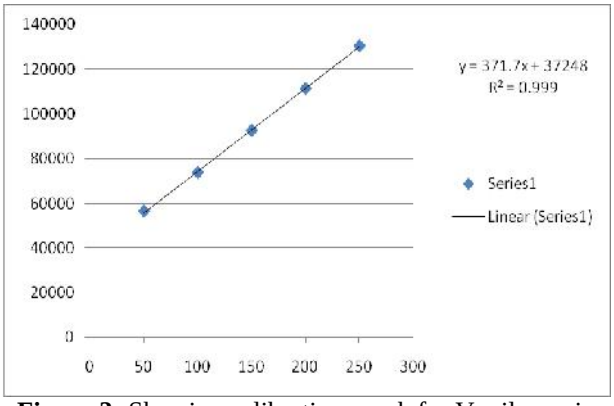

Figure 3: Showing calibration graph for Voxilaprevir

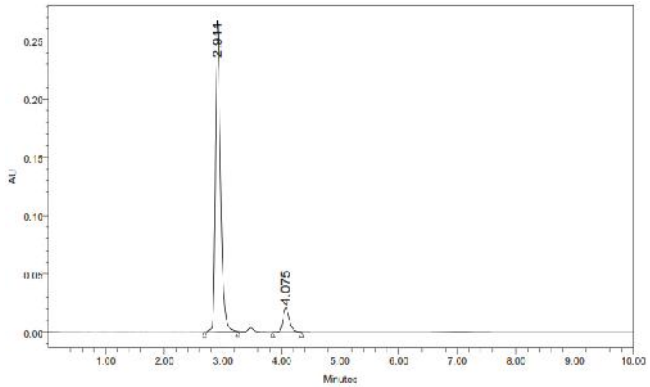

Figure 4: Chromatogram showing less flow rate 0.8ml/min

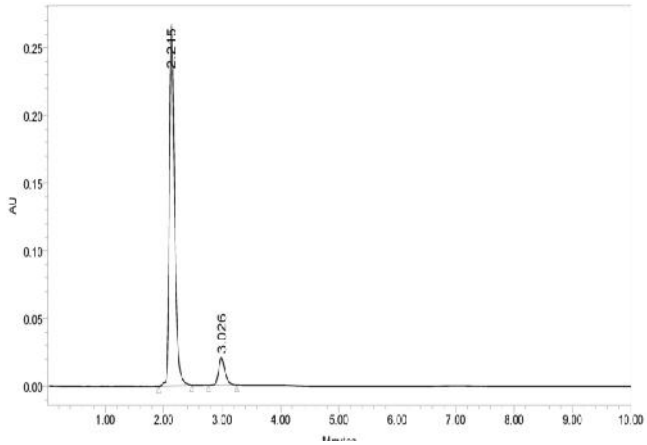

Figure 5: Chromatogram showing less flow rate 1.2 ml/min

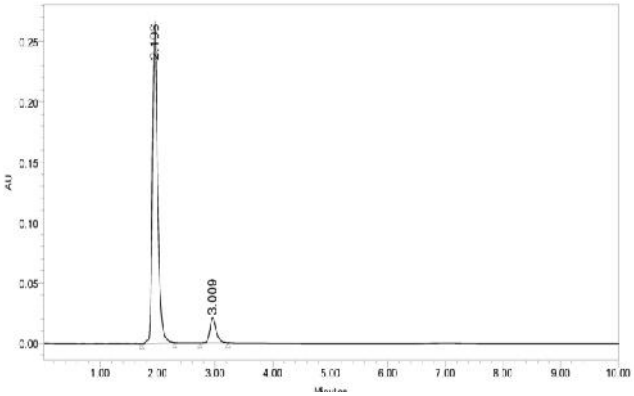

Fig.No.6 Chromatogram showing more organic phase ratio

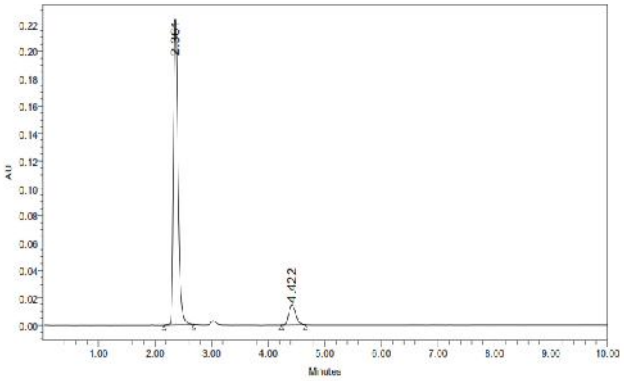

Fig.No.7 Chromatogram showing less organic phase ratio

Table 1: Linearity Results for Voxilaprevir

S.No	Linearity Level	Concentration	Area
1	I	50ppm	56472
2	II	100 ppm	73841
3	III	150ppm	92655
4	IV	200ppm	111541
5	V	250ppm	130567
Correlation Coefficient			0.999

Table 2: Linearity Results for Velpatasvir

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	471543
2	II	10 ppm	656277

3	III	15 ppm	794999
4	IV	20 ppm	946124
5	V	25 ppm	1002139
Correlation Coefficient			0.999

Table 3: Details of Accuracy 50 %

Injection	Peak Name	RT	Area	Height
1	Velpatasvir	2.553	124366	57028
2	Velpatasvir	2.554	124683	48569
3	Velpatasvir	2.564	128376	24157
4	Voxilaprevir	3.397	132846	
5	Voxilaprevir	3.413	136172	24856
6	Voxilaprevir	3.519	134556	86251
Mean			372548.2	
Std.Dev			259486.6	
% RSD			0.515	

Table 4: Details of Accuracy 100 %

Injection	Peak Name	RT	Area	Height
1	Velpatasvir	2.563	12486	78248
2	Velpatasvir	2.561	12364	78542
3	Velpatasvir	2.559	12458	72345
4	Voxilaprevir	3.431	13744	32815
5	Voxilaprevir	3.467	13784	32894
6	Voxilaprevir	3.431	13887	32577
Mean			254827.4	
Std.Dev			3542781.2	
% RSD			0.522	

Table 5: Details of Accuracy 150 %

Injection	Peak Name	RT	Area	Height
1	Velpatasvir	2.574	126647	75842
2	Velpatasvir	2.573	125742	74224
3	Velpatasvir	2.644	128522	72148
4	Voxilaprevir	3.436	126411	73421
5	Voxilaprevir	3.439	127541	72648
6	Voxilaprevir	3.537	128461	78412
Mean			475826.4	
Std.Dev			2758462.2	
% RSD			0.543	

Table 6: Showing accuracy results for Velpatasvir

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	2630409	5	4.96	99.91%	99.56%
100%	5277055	10	9.98	99.18%	
150%	7514836	15	15.02	99.60%	

Table 7: Showing accuracy results for Voxilaprevir

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1366666	0.5	0.99	99.53%	99.47%
100%	2777487	1.0	1.05	99.38%	
150%	4151234	1.5	1.495	99.52%	

Table 8: Showing %RSD results for Velpatasvir

	Peak Name	Rt	Area	Height
1	velpatasvir	2.755	5223559	541538.3
2	velpatasvir	2.687	5208511	485548.5
3	velpatasvir	2.632	5323569	574440.4
4	velpatasvir	2.612	5259147	557413.5
5	velpatasvir	2.616	5273463	565020.1
6.	velpatasvir	2.613	5276431	587260.1
mean			5257650	
Std.dev			45206.4	
%RSD			0.86	

Table 9: showing% RSD results for Voxilaprevir

	Peak Name	Rt	Area	Height
1	Voxilaprevir	3.616	2742453	238643.4
2	Voxilaprevir	3.634	2762750	271543.5
3	Voxilaprevir	3.460	2797670	281711.6
4	Voxilaprevir	3.446	2793578	274499.8
5	Voxilaprevir	3.437	2778483	276713.0
6	Voxilaprevir	3.438	2778485	246712.0
mean			27854628	
Std.dev			758206.4	
%RSD			0.82	

Table 10: Showing results for intermediate precision of Velpatasvir

	Peak name	Rt	Area	Height
1	Velpatasvir	2.756	5698542	539568.1
2	Velpatasvir	2.688	5682534	536985.4
3	Velpatasvir	2.633	5695846	539584.1
4	Velpatasvir	2.613	5689452	534569.8
5	Velpatasvir	2.617	5636591	534985.5
6	Velpatasvir	3.439	5634595	535875.6
mean			5600593	
Std.dev			203577.3	
%RSD			0.44	

Table 11: Showing results for Intermediate precision of Voxilaprevir

	Peak name	Rt	Area	Height
1	Voxilaprevir	3.617	2624315	231325.6
2	Voxilaprevir	3.635	2623598	231315.4
3	Voxilaprevir	3.461	2623541	231250.1
4	Voxilaprevir	3.447	2624987	231342.6
5	Voxilaprevir	3.438	2635698	231765.2
6	Voxilaprevir	3.439	2635699	264818.2
mean			2626428	
Std.dev			5215.58	
%RSD			0.19	

Table 12: Results for system suitability of Velpatasvir

Injection	**RT(min)**	**Peak area**	**TP**	**TF**
1	2.526	124652	1554.31	1.28
2	2.767	127376	1634.55	1.31
3	2.764	122803	1623.37	1.31
4	2.808	125382	1622.73	1.23
5	2.789	122153	1460.39	1.32
6	2.799	122155	1634.88	1.27

Mean		123634	-	-
SD		631.0	-	-
%RSD		0.6	-	-

Table 13: Results for system suitability of Voxilaprevir

Injection	RT(min)	Peak area	TP	TF
1	3.901	434308	4315.31	1.17
2	3.904	436736	4232.73	1.17
3	3.905	436821	4372.54	1.17
4	3.907	435350	4354.17	1.17
5	3.908	425462	4322.22	1.17
6	3.909	435465	4328.19	1.18
Mean		44531.3	-	-
SD		1257.3	-	-
%RSD		0.3	-	-

Table 14: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Velpatasvir	373625.50	581075863	3.17
Voxilaprevir	5772.40	476579210	0.0172

Table 15: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Velpatasvir	372727.80	574265980	5.80
Voxilaprevir	5761.30	478828490	0.212

4. Conclusion

A new method was established for simultaneous estimation of Velpatasvir and Voxilaprevir by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Velpatasvir and Voxilaprevir by using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Velpatasvir and Voxilaprevir was found to be 101.27% and 99.24% respectively. The system suitability parameters for Velpatasvir and Voxilaprevir such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Velpatasvir and Voxilaprevir was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.47%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.80, and LOQ value was 0.0172 and 0.212 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Velpatasvir and Voxilaprevir in API and Pharmaceutical dosage form.

5. References

[1] J. Sandya Rani A New RP-HPLC Method Development and Validation for Simultaneous Estimation of Sofosbuvir and Velpatasvir in Pharmaceutical Dosage Form International Journal of Engineering Technology Science and Research IJETSR, 2017, 4(11), 145-152.

[2] Sarath Nalla. A Stability Indicating RP-HPLC Method For Simultaneous Estimation Of Velpatasvir And Sofosbuvir In Combined Tablet Dosage Forms Journal of Pharmacy and Pharmaceutical Sciences. 2017, 6(9), 1596-1611.

[3] Hyock Joo Kwon Method Development And Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Sofosbuvir and Velpatasvir in bulk and its Pharmaceutical Formulations. Eurasian Journal of Analytical Chemistry 2016, 11(4), 197-210

[4] Anusha Tiyyagura et al, Method Development and Validation for The Simultaneous Estimation of Velpatasvir and Sofosbuvir in Pharmaceutical Dosage Form by RP-HPLC, *IJPCBS, 2012, 3(1), 44-54.*

[5] V. Rama Koteswara Rao, Nanda Kishore Agarwal, K.Haritha Pavami, B. Prem Kumar, R. Mallikarjuna, Analytical method development and validation for the simultaneous estimation of Levamisole and Mebendazole in bulk & tablet formulation by RP-HPLC method, *Indian Journal*

of Research In Pharmacy And Biotechnology. 2013, 1(5): 665-669.

[6] Ganta Srinivas. Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Determination of Dutasteride and Tamsulosin in Bulk As Well As In Pharmaceutical Dosage Form by using PDA Detector, Asian J Pharm Clin Res, 2014, 7(2): 105-113.