WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 6, 1500-1519. Research Article ISSN 2277– 7105

ANALYTICAL METHOD DEVELOPMENT & VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF VELPATASVIR AND SOFOSBUVIR IN BULK AND IT'S DOSAGE FORM BY RP-HPLC

Dr. P. V. Murali Krishna[1]*, G. Gurunath[2], Kalyani Peluri[3] and D. Amulya[4]

[1]*MNR College of Pharmacy, Sangareddy, Hyderabad, Telangana. 502285, India.

[2]Srinivasa College of Pharmacy, Proddatur, A.P.

[3]Swamy Vivekananda College of Pharmacy, Virgonagar, Mandur, Bangalore.

[4]Nirmala College of Pharmacy, Kadapa, AP.

Article Received on
24 March 2020,

Revised on 14 April 2020,
Accepted on 04 May 2020,

DOI: 10.20959/wjpr20206-17533

***Corresponding Author**
Dr. P. V. Murali Krishna
MNR College of Pharmacy,
Sangareddy, Hyderabad,
Telangana. 502285, India.

ABSTRACT

A simple reverse phase liquid Chromatographic method has been developed and subsequently validated for simultaneous determination of Sofosbuvir and Velpatasvir in combination. The separation was carried out using a mobile phase of phosphate buffer (0.05M) pH 7: MeOH (30:70%v/v) (pH was adjusted with orthophosphoric acid) and using methanol as diluent. The column used was Xterra C18 (150 mm x 4.6 mm i.d., 5µm) with flow rate of 1 ml/min using UV detection at 270 nm. The retention times of Sofosbuvir and Velpatasvir were found to be 2.399 and 3.907min respectively. Results of analysis were validated statistically and by recovery studies. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Sofosbuvir and Velpatasvir bulk drug and in its pharmaceutical dosage form.

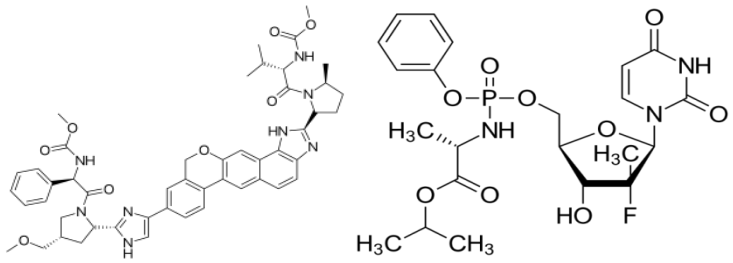
KEYWORDS: Sofosbuvir and Velpatasvir.

INTRODUCTION

Hepatitis C virus was found to be a commonly attacking disease to human beings and was increased day by day. The literature reveals that 72% of the patients were suffered from chronic HCV. In early stage 75% to 85% of the liver is persisted with the virus. These defects have been treated by use of an oral form of these combinational drugs respectively. Velpatasvir (Fig.-1a) is an antiviral drug in the treatment of chronic hepatitis C virus. It is

chemically {(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(Méthoxycarbonyl) amino]-2-phénylacétyl}-4- (méthoxyméthyl)-2- pyrrolidinyl]- 1H-imidazol-4-yl} -1,11- dihydro isochroméno [4',3':6,7] naphto [1,2-d]imidazol-2-yl)-5 -méthyl-1- pyrrolidinyl]-3- méthyl-1-oxo-2-butanyl} carbamate de méthyle having molecular weight 883 and molecular formula is $C_{49}H_{54}N_8O_8$ and Practically insoluble above pH 5, slightly soluble at pH 2 and soluble at pH 1.2 and is slightly soluble in water. Sofosbuvir (Fig.-1b) is an antiviral drug in the treatment of chronic hepatitis C virus. It is chemically propan-2-yl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro -3-hydroxy -4-methyloxolan -2-yl] methoxy-phenoxy phosphoryl] amino] propanoate having molecular weight 529.45. The HPLC technique was used for the development and validation of combinational drugs were reported

As per ICH guidelines the method was developed and validated.



EXPERIMENTAL

List of Equipments

S.No.	Instrument	Model No.	Software	Manufacturer's name
1	HPLC Alliance PDA Detector	Waters 2695 Waters 996	Empower	Waters
2	UV double beam Spectrophotometer	UV 3000	UV Win 5	Lab India
3	Digital weighing Balance	BSA224SCW	-	Satorius
4	pH meter	AD102U	-	Lab India
5	Ultra Sonicator	SE60US	-	-
6	Suction pump	VE115N	-	-

List of Chemicals

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Potassium dihydrogen orthophosphate	Merck	A.R
5	Velpatasvir and Sofosbuvir	-	-

METHOD PROCEDURE

Method development for simultaneous estimation of Velpatasvir and Sofosbuvir in Pharmaceutical dosage forms includes the following steps:

1. Detection wavelength (λ_{max})
2. Column
3. Selection of mobile phase
5. Preparations and procedures

Detection wavelength

10 mg of Velpatasvir and Sofosbuvir 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 for Velpatasvir and Sofosbuvir. The isobestic point was taken as detection wavelength.

Column

Column is selected based on solubility, polarity and chemical differences among Analytes [Column: xterra C18 (4.6 x 150mm, 5µm, Make: Waters)]

Selection of mobile phase

Phosphate buffer (0.05M) pH 7: MEOH (30:70%v/v) has been selected as mobile phase. Buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved.

Preparations and procedures

Preparation of Phosphate buffer: (PH: 7)

Weighed 0.50 gm of KH_2PO_4 and 0.301 gm of potassium dihydrogen phosphate was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 7 with ortho phosphoric acid.

Preparation of mobile phase

A mixture of pH 7 Phosphate buffer 300 mL (30%), 700 mL of MeOH (70%) are taken and degassed in ultrasonic water bath for 5 min. Then this solution is filtered through

0.45 µ filter under vacuum filtration.

Diluent Preparation

Mobile phase is used as Diluent.

Preparation of the Velpatasvir standard solution

10mg of Velpatasvir standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent.

Preparation of the Sofosbuvir standard preparation

10mg of Sofosbuvir working standard was accurately weighed and transferred into a 10mL clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent.

Preparation of Sample Solution: (Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Sofosbuvir (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluent.

Procedure

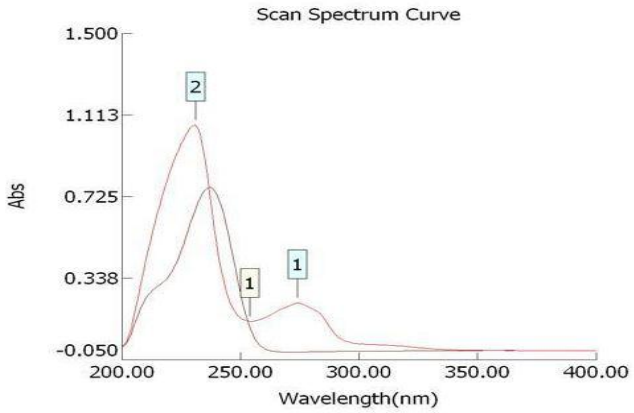
20µL of the standard, sample are injected into the chromatographic system and the areas for Velpatasvir and Sofosbuvir peaks are measured and the %Assay are calculated by using the formulae.

RESULTS AND DISCUSSION

WAVELENGTH DETECTION (OR) SELECTION OF WAVELENGTH

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was

scanned in U.V range from 200-400nm. The overlay spectrum of Velpatasvir and Sofosbuvir was obtained and the isosbestic point of Velpatasvir and Sofosbuvir showed absorbance's maxima at 260 nm.



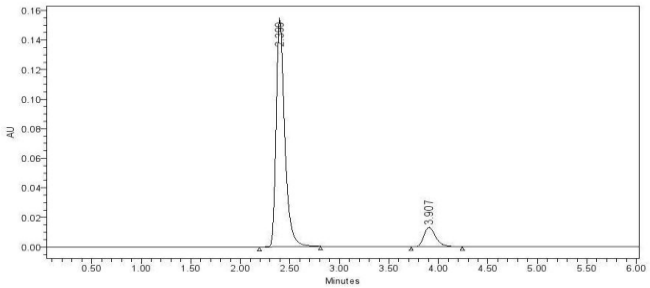
Overlay spectrum of Velpatasvir and Sofosbuvir

System suitability

5 mg of Velpatasvir and 500 mg of Sofosbuvir working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask and add about 20ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further 10 ml of Velpatasvir and Sofosbuvir was pipetted out from the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

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.



Chromatogram of system suitability

Details of System suitability

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Sofosbuvir	2.399	946124	155429	5105	1.3	8.1
2	Velpatasvir	3.907	111541	13239	3788	1.4	

Discussion

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.

Accuracy

Preparation of standard solution (Velpatasvir and Sofosbuvir)

Accurately weighed 10 mg of Velpatasvir and 10mg of Sofosbuvir working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks.

About 7mL and 70ml of Diluents are added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 3ml and 0.3ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted upto the mark with diluents.

Preparation of Sample solutions

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

Accurately 5mg of Velpatasvir and 5mg of Sofosbuvir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Velpatasvir and Sofosbuvir stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

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

Accurately 10mg of Velpatasvir and 10mg of Sofosbuvir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Velpatasvir and Sofosbuvir stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

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

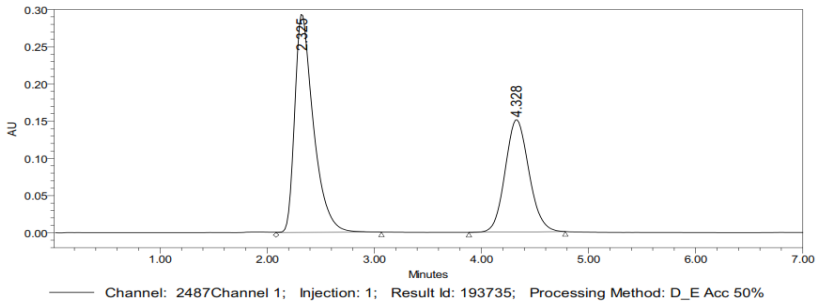
Accurately 15mg of Velpatasvir and 15mg of Sofosbuvir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Velpatasvir and Sofosbuvir stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

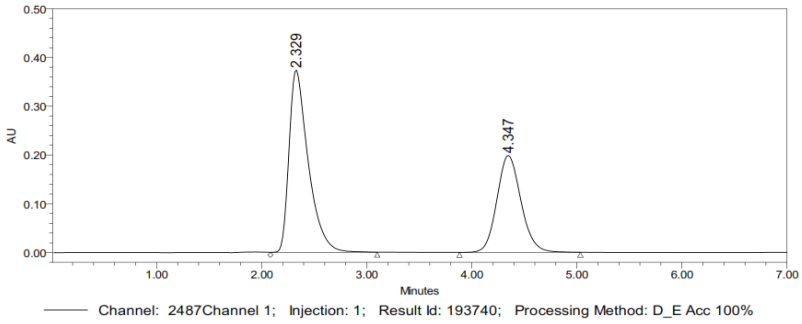
Procedure

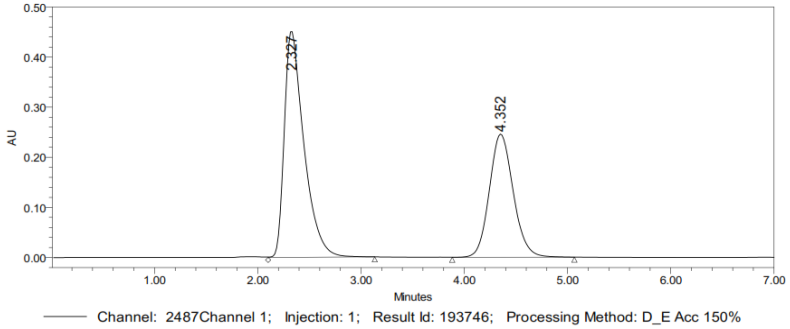
The standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions were injected. The Amount found and Amount added for Velpatasvir & Sofosbuvir and the individual recovery and mean recovery values were calculated.

Acceptance criteria

 Correlation coefficient should be not less than 0.999.







Accuracy results of Velpatasvir

%Concentration (at specification level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	353867	5	5.0	101.3%	100.0%
100%	4735088	10	9.94	99.4%	
150%	5911798	15	14.8	99.2%	

Table 15: Accuracy results of Sofosbuvir.

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovey	Mean Recovery
50%	2332744	5	5.10	101.8%	**100.5%**
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

Discussion: The accuracy study was performed for % recovery of Sofosbuvir and Velpatasvir. The % recovery was found to be 100.5% and 100.0% respectively (NLT 98% and NMT 102%).

Precision

A) Repeatability

Preparation of standard stock solution

Accurately 10 mg of Velpatasvir and 10mg of Sofosbuvir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further it was pipette (3ml and 0.3ml) into a 10ml volumetric flask and diluted up to the mark with diluents.

Procedure

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

Acceptance criteria

 The % RSD for the area of five standard injections results should not be more than 2.

Repeatability results of Sofosbuvir

	Name	RT	Area
1	Sofosbuvir	2.321	2235319
2	Sofosbuvir	2.317	2240678
3	Sofosbuvir	2.323	2249490
4	Sofosbuvir	2.32	2245822
5	Sofosbuvir	2.324	2251694
Mean			2244601
Std. Dev			6656.8
%RSD			0.30

Table 10: Repeatability results of Velpatasvir.

	Name	RT	Area
1	Velpatasvir	4.304	1501417
2	Velpatasvir	4.300	1486940
3	Velpatasvir	4.308	1490656
4	Velpatasvir	4.310	1487329
5	Velpatasvir	4.314	1490384
Mean			1491345
Std. Dev			5881.4
%RSD			0.39

Discussion: The Method precision study was performed for the %RSD of Velpatasvir and Sofosbuvir was found to be 0.30 and 0.39 (NMT 2).

B) INTERMEDIATE PRECISSION (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.

Ruggedness results of Sofosbuvir

	Name	RT	Area	Height (µV)
1	Sofosbuvir	2.328	2194758	189693
2	Sofosbuvir	2.326	2195700	190025
3	Sofosbuvir	2.327	2196191	189862
4	Sofosbuvir	2.326	2195326	190700
5	Sofosbuvir	2.331	2200951	189426
Mean			2196585	
Std. Dev			2496.0	
%RSD			0.11	

Table 12: Ruggedness results of Velpatasvir.

	Name	RT	Area	Height (µV)
1	Velpatasvir	4.335	1456296	95623
2	Velpatasvir	4.336	1457422	95150
3	Velpatasvir	4.334	1456513	95165
4	Velpatasvir	4.337	1454579	95298
5	Velpatasvir	4.340	1451483	95251
Mean			1455259	
Std.Dev			2347.6	
%RSD			0.16	

Discussion: The intermediate precision was performed for %RSD of Velpatasvir and Sofosbuvir was found to be 0.11 and 0.16 respectively (NMT 2).

Linearity

Preparation of stock solution

Accurately 10 tablets were weighed & crushed in mortar and pestle and weight equivalent to 10 mg of Velpatasvir and Sofosbuvir (marketed formulation) sample were transferred into a 10mL clean dry volumetric flask and about 7mL of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (20ppm of Velpatasvir & 10 pm of Sofosbuvir)

1ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – II (40ppm of Velpatasvir & 20 ppm of Sofosbuvir)

2ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – III (60ppm of Velpatasvir & 30 ppm of Sofosbuvir)

3ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – IV (80ppm of Velpatasvir & 40 ppm of Sofosbuvir)

4ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – V (100ppm of Velpatasvir & 50 ppm of Sofosbuvir)

5ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluent.

Procedure

Each level was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated.

Acceptance criteria

 Correlation coefficient should be not less than 0.999.

Linearity results of Sofosbuvir

Sno:	Sample Name	Name	RT	Area	Height
1	Linearity 1	Sofosbuvir	2.309	1810101	145957
2	Linearity 2	Sofosbuvir	2.322	2044287	176935
3	Linearity 3	Sofosbuvir	2.324	2367133	206622
4	Linearity 4	Sofosbuvir	2.336	2602279	228576
5	Linearity 5	Sofosbuvir	2.345	2869778	259346

Table 14: Linearity results of Velpatasvir.

Sno:	Sample Name	Name	RT	Area	Height
1	Linearity 1	Velpatasvir	4.307	1164173	75128
2	Linearity 2	Velpatasvir	4.317	1342535	87703
3	Linearity 3	Velpatasvir	4.323	1555931	101999
4	Linearity 4	Velpatasvir	4.340	1777973	117084
5	Linearity 5	Velpatasvir	4.340	1942319	129409

Discussion

Correlation coefficient should be not less than 0.999.

Plotting of calibration graphs

The resultant areas of linearity peaks are plotted against Concentration.

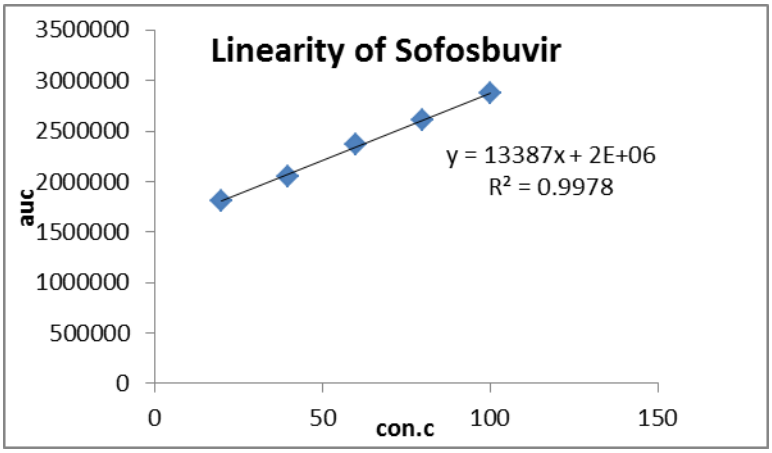


Fig. 7.2.6.5: Calibration curve of Sofosbuvir.

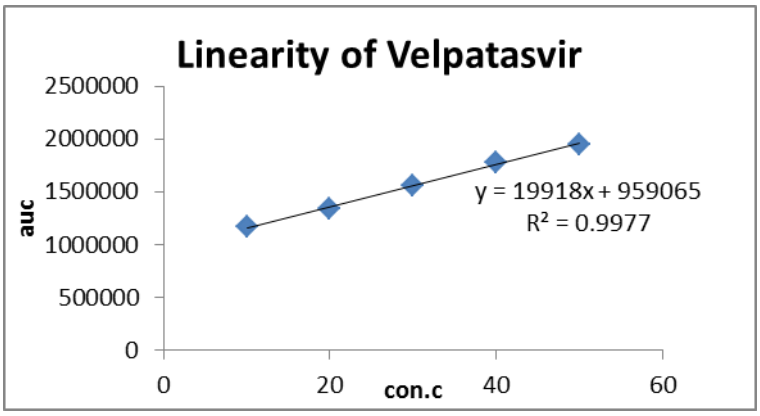


Fig. 7.2.6.6: Calibration curve of Velpatasvir.

Discussion: The linearity study was performed for concentration range of 20µg-100µg/ml and 10µg - 50µg/ml of Sofosbuvir and Velpatasvir and the correlation coefficient was found to be 0.999 and 0.999. (NLT 0.999).

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 1 - 5 µg and 100 - 500 µg of Sofosbuvir and Velpatasvir respectively.

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.

Formula

$$LOD = 3.3 \text{ X } \frac{\sigma}{S}$$

Where

σ - Standard deviation (SD) S – Slope

Sofosbuvir

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank: 41µV Signal

Obtained from LOD solution: 125 µV

S/N = 125/41= 3.04

Acceptance Criteria

S/N Ratio value shall be 3 for LOD solution.

Velpatasvir

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank: 41 µV Signal Obtained from LOD solution: 121 µV

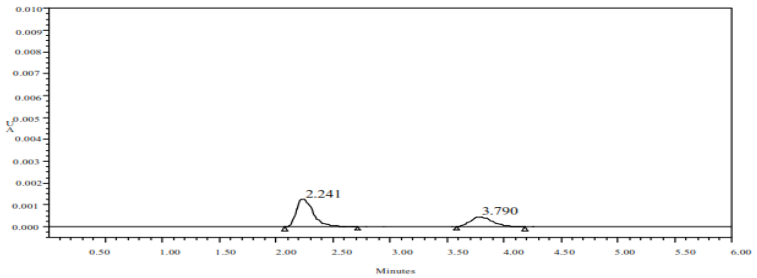
S/N = 121/41 = 2.95

Acceptance Criteria

S/N Ratio value shall be 3 for LOD solution.

The LOD was performed for Velpatasvir and Sofosbuvir was found to be 2.95and 3.04 respectively.

LOD



Chromatogram of LOD

Discussion

The LOD was performed for Velpatasvir and Sofosbuvir was found to be 2.95 and 3.04 respectively.

LOQ

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.

Formula

LOQ = 10 σ /Slope

Where

σ - Standard deviation

S – Slope

Sofosbuvir

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 41 µV Signal Obtained from LOQ solution : 412µV

S/N = 412/41 = 10.0

Acceptance Criteria

S/N Ratio value shall be 10 for LOQ solution.

Velpatasvir

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 41 µV Signal Obtained from LOQ solution : 405µV

S/N = 405/41 = 9.87

Acceptance criteria

S/N Ratio value shall be 10 for LOQ solution.

The LOQ was performed for Velpatasvir and Sofosbuvir was found to be 9.87 and 10 respectively**.**

LOQ

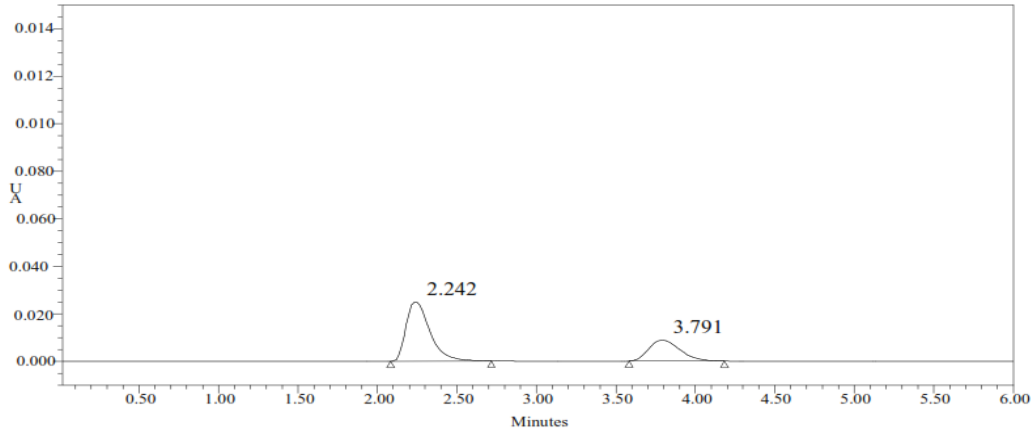


Fig. 7.2.9: Chromatogram of LOQ.

Discussion

The LOQ was performed for Velpatasvir and Sofosbuvir was found to be 9.87 and 10 respectively.

Robustness

Flow Rate

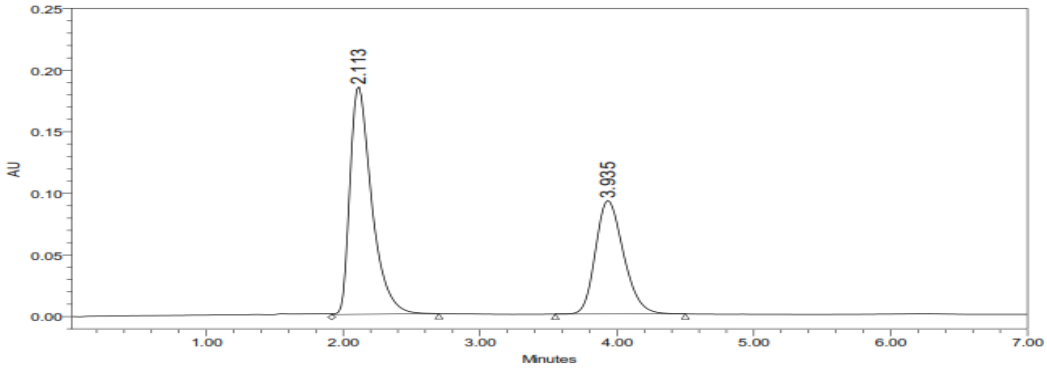
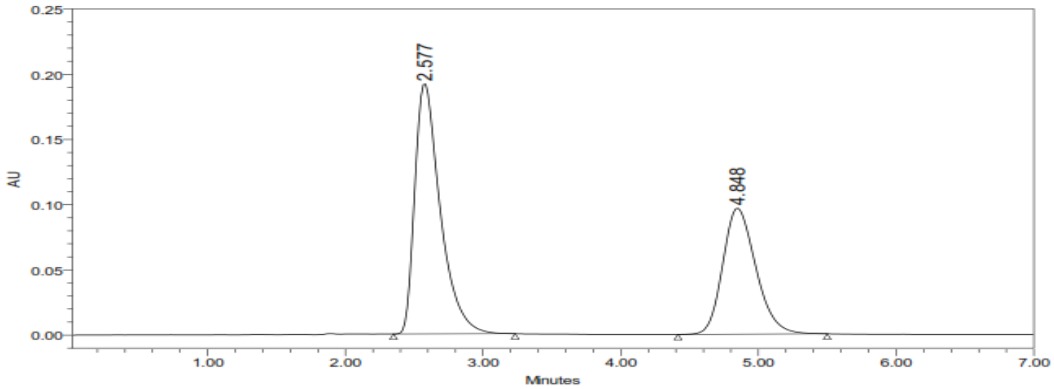


Fig 7.2.10: Chromatogram for Robustness more flow.



Chromatogram for Robustness less flow

System suitability results For Sofosbuvir (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	1748.5	1.22
2	1.0	1548.2	1.2
3	1.2	1948.0	1.2

Table 17: System suitability results for Velpatasvir (Flow rate).

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	883.3	1.56
2	1.0	1234.0	1.1
3	1.2	969.2	1.6

Mobile Phase

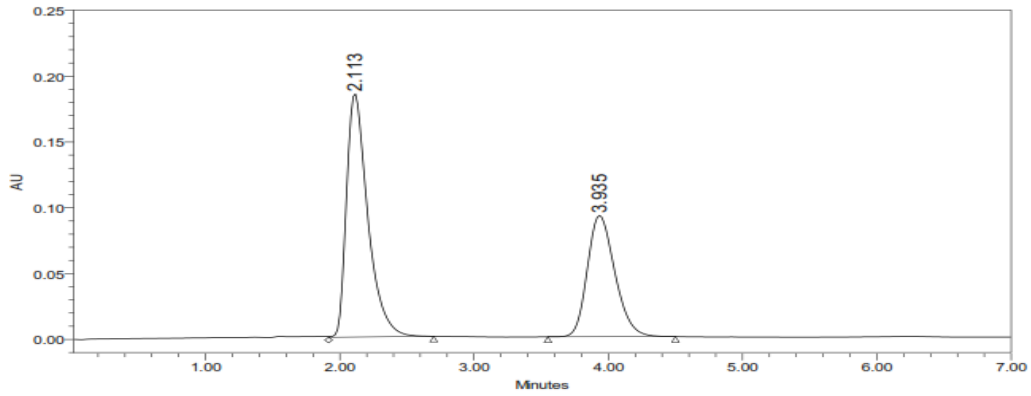


Fig: 7.2.1.2: Chromatogram for Robustness more organic.

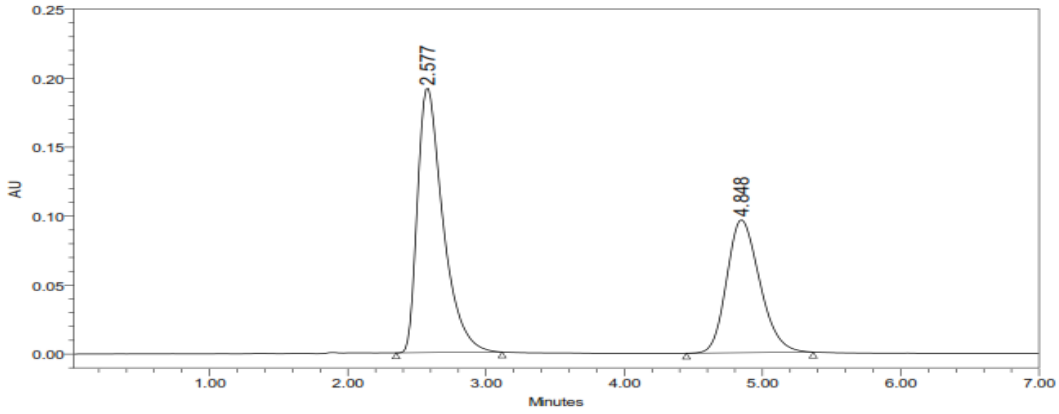


Fig: 7.2.10.3 Chromatogram for Robustness less organic Table: 18.

Table 19: System suitability results for Velpatasvir (Mobile phase).

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	883.3	1.56
2	Actual	1234.0	1.1
3	10% More	969.2	1.6

Discussion: Results for actual Mobile phase composition (55:45Buffer: ACN) have been considered from Accuracy standard.

System suitability results for Sofosbuvir (Mobile phase)

	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10% More	1948.0	1.2

Discussion: Results for actual Mobile phase composition (45:55Buffer: ACN) have been considered from Accuracy standard.

ASSAY

Repeatability results of Sofosbuvir

S. No	Name	Area	% Assay
1	Sofosbuvir	2235319	99.59
2	Sofosbuvir	2240678	99.83
3	Sofosbuvir	2249490	100.22
4	Sofosbuvir	2245822	100.05
5	Sofosbuvir	2251694	100.32
6	Sofosbuvir	2244601	100.00
Average		2244600.67	100.00
STDEV		5953.98	0.27
% RSD		0.27	0.27

Table 10: Repeatability results of Velpatasvir.

S. No	Name	Area	% Assay
1	Velpatasvir	1501417	100.68
2	Velpatasvir	1486940	99.70
3	Velpatasvir	1490656	99.95
4	Velpatasvir	1487329	99.73
5	Velpatasvir	1490384	99.94
6	Velpatasvir	1491345	100.00
Average		1491345.17	100.00
STDEV		5260.72	0.35
% RSD		0.35	0.35

Discussion: The Assay was performed and the %RSD of Velpatasvir and Sofosbuvir was found to be 0.27 and 0.35 (NMT 2).

Assay calculation

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT\ P}{WT} \times \frac{Avg.}{100} \times \frac{Wt.}{Label\ Claim} \times 100$$

For Velpatasvir

$$= \frac{1314011}{1311967} \times \frac{10}{10} \times \frac{3}{10} \times \frac{10}{25} \times \frac{10}{3} \times \frac{99.8}{100} \times \frac{125}{50} \times 100 = 99.95\%$$

For Sofosbuvir

$$= \frac{125260}{124581} \times \frac{10}{100} \times \frac{0.3}{10} \times \frac{10}{25} \times \frac{10}{3} \times \frac{99.7}{100} \times \frac{125}{0.5} \times 100 = 100.24\%$$

Where:

AT = average area counts of sample preparation.

As = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = LABEL CLAIM mg/ml.

OBSERVATION

The system suitability parameters for Velpatasvir and Sofosbuvir such as theoretical plates and tailing factor were found to be 5117.5, 1.3 and 3877.3, 1.4. Resolution was 8.1. The % purity of Velpatasvir and Sofosbuvir in pharmaceutical dosage form was found to be 100.7% and 101.4% respectively.

SUMMARY AND CONCLUSION

A new method was developed for simultaneous estimation of Velpatasvir and Sofosbuvir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Velpatasvir and Sofosbuvir by using Xterra C18 5µm (4.6*150mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 7: MeOH

(30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 270nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.

The retention times were found to be 2.399mins and 3.907mins. The % purity of Sofosbuvir and Velpatasvir was found to be 100.7% and 101.4% respectively. The system suitability parameters for Velpatasvir and Sofosbuvir such as theoretical plates and tailing factor were found to be 1.3, 5117.5 and 1.4, 3877.3 the resolution was found to be 8.0.

The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Velpatasvir and Sofosbuvir was found in concentration range of 10µg-50µg and 20µg-100µg and correlation coefficient (r2) was found to be 0.997 and 0.997, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Velpatasvir and Sofosbuvir in API and Pharmaceutical dosage form.

REFERENCES

1. G.R.Chatwal, S.K.Anand, "Text book of Instrumental Methods of Chemical Analysis", Himalaya Publishing House; 5th Edn, 2002; 2.566-2.570.
2. G.W.Ewing, "Text book of Instrumental Methods of Chemical Analysis", Mc Graw-Hill Book Company; 5th Edn; 375-385.
3. B.K. Sharma, "Textbook of Instrumental Methods of Chemical Analysis", GOEL publishing house, Meerut; 23rd Edn; 288-289.
4. G.Vidyasagar, "Textbook of Instrumental Methods of Drug Analysis", Pharmamed Press, 2009; 106-120.
5. H. H Willard, L.L Merritt, J. A Dean, and F. A Settle, "Textbook of Instrumental Methods of Analysis", CBS publishers and distributors, New Delhi; 7th Edn, 1986; 592-596.
6. H.H.Tackett, J.A.Cripe, G.Dyson, Positive displacement reciprocating pump fundamentals-power and direct acting types, Proceedings of the twenty-fourth international pump user's symposium, 2008; 45-58.
7. D.A.Skoog, F.J.Holler, S.R.Crouch, "Textbook of Instrumental Analysis, Brooks/Cole", Cengage Learning India Private Limited, 2007; 900-906.

8. R. E. Schirmer, "Textbook of Modern Methods of Pharmaceuticals", CRC press, 2nd Edn; 242-244.
9. LR.Snyder, JJ Kirkland, LG.Joseph, "Practical HPLC Method Development", Wiley Inter Science, New York; 2nd Edn, 1997; 1-56.
10. Ranjith singh, " J Pharm. Educ". Res., 4; 2013; 26-33.
11. ICH: Q2B, "Analytical Validation"– Methodology, 1996.
12. Raghu Ram Jampala, V. Kiran Kumar, Appala Raju Nemala, Development and Application of Liquid Chromatographic Method for Simultaneous Determination of Elvitegravir, Tenofovir Disoproxil Fumarate, Emtricitabine, and Sofosbuvir in Fixed Dosage Form. "Pharmaceutical Methods", Jan-Jun 2014; 5(1).
13. V.V. Raveendra Babu, Pankaj K. Sharma and I. Singhvi, A New Gradient Liquid Chromatographic Method for Simultaneous Estimation of Tenofovir Disoproxil Fumarate, Cobicistat, Emtricitabine and Velpatasvir in Bulk Drug and Tablet Dosage Form. "Asian J. Chem"., 2014; 26(18): 6233-6237.
14. Urooj Fatima, T. Mamatha and Rajesh Goud Gajula, A novel RP-HPLC method development and validation of Sofosbuvir in bulk drug and tablet dosage form. "Der Pharmacia Sinica", 2014; 5(5): 99-105.
15. Chandni Saha, Md.Nazeeruddin Ahmed, Development And Validation Of A Simple Uv Spectrophotometric Method For The Determination Of Sofosbuvir In Its Bulk Form. "Indo Americal Journal of Pharmaceutical Research", 2014; 4(12): 5792-5796
16. Padigela Swetha, V. V. S. Rajendra Prasad, M. Bhagavan Raju, N. Suresh Kumar, Estimation Of Velpatasvir In Tablet Dosage Forms By Rp-Hplc. "Indo Americal Journal of Pharmaceutical Research", 2013; 3(6): 4697-4705.
17. Swati S.Pardhi, Kiran B.Aher, Girija B.Bhavar, S.R.Chaudhari, Development And Validation Of Uv Spectrophotometric Method For The Estimation Of Velpatasvir In Bulk And Pharmaceutical Formulations. "International Journal of Pharma Sciences and Research", 2012; 6(01).
18. www.wikipedia.com