CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.3270795

Available online at: http://www.iajps.com ***Research Article***

RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN COMBINED DOSAGE FORM

Dr. Rajeswar dutt[*1], Thandu venkateshwarlu[1], Santhosh illendula[1], V.Shirisha[1]

[1]Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Cherlapally(v), Nalgonda(Dt), Telangana (St), India, 508001.

Article Received: May 2019 **Accepted:** June 2019 **Published:** July 2019

Abstract:

A simple, accurate, precise method was developed for the simultaneous estimation of the Sofosbuvir and Velpatasvir in tablet dosage form. Chromatogram was run through Denali 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.01NKH_2PO_4 (4.8 pH) : Acetonitrile taken in the ratio 65:35 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.01N KH_2PO_4 (4.8PH) buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 220.0 nm. Retention time of Sofosbuvir and Velpatasvir were found to 2.30 min and 3.187 min. %RSD of the Sofosbuvir and Velpatasvir were found to be 0.4 and 0.6 respectively. %Recovery was obtained as 99.85% and 99.76% for Sofosbuvir and Velpatasvir respectively. LOD, LOQ values obtained from regression equations of Sofosbuvir and Velpatasvir were 0.33, 0.99 and 0.43, 1.29 respectively. Regression equation of Sofosbuvir is y = 4401x+18039, y = 3121x+2113 of Velpatasvir. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in industries.

Key words : *Sofosbuvir, Velpatasvir, RP-HPLC.*

Corresponding author:

Dr. Rajeswar dutt,

Department of Pharmaceutical Analysis, Nalanda College of Pharmacy. Cherlapally (v), Nalgonda(Dt), Telangana (St), India, 508001.

pharmadutt@gmail.com

QR code

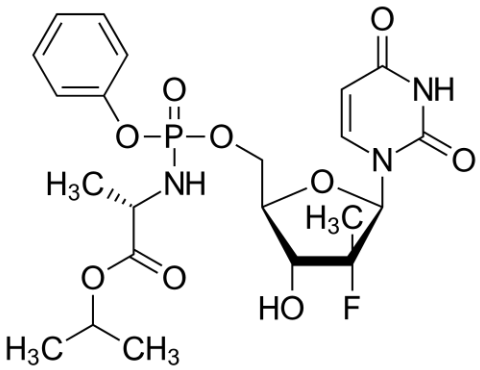
Please cite this article in press Rajeswar dutt et al., ***RP-HPLC Method for the Simultaneous Estimation of Sofosbuvir and Velpatasvir in Combined Dosage Form.,*** *Indo Am. J. P. Sci, 2019; 06(07).*

INTRODUCTION:

Sofosbuvir

Sofosbuvir may be a direct acting antiviral medication used as a part of combination medical aid to treat chronic viral hepatitis, associate degree infectious disease caused by infection with viral hepatitis Virus (HCV).

Structure:



CAS number: 1190307-88-0
Weight: Average: 529.458
Chemical Formula: $C_{22}H_{29}FN_3O_9P$

IUPAC Name:

propan-2-yl (2S)-2-{[(S)-{[(2R,3R,4R,5R)-5- (2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy} (phenoxy)phosphoryl]amino}propanoate.

Mechanism of action:

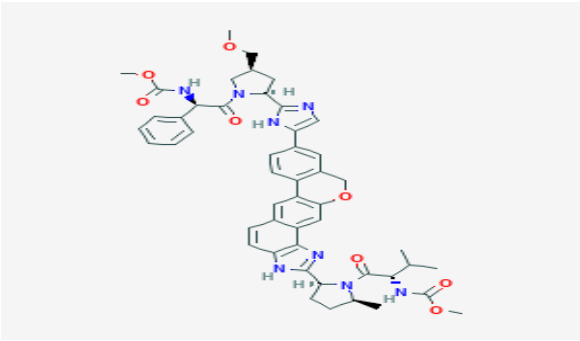
Sofosbuvir is nucleotide analog inhibitor, which specifically inhibits HCV NS5B (non-structural protein 5B) RNA dependent RNA polymerase. Following intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), sofosbuvir incorporates into HCV RNA by the NS5B polymerase and acts as a chain terminator. More specifically, Sofosbuvir prevents HCV viral replication by binding to the two Mg2+ ions present in HCV NS5B polymerase's GDD active site motif and preventing further replication of HCV genetic material

Physical State: Solid
Storage: Store at -20° C
Melting Point: 100-106 °C

Velpatasvir:

Velpatasvir is a Direct-Acting Antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV).

Structure:



CAS number: 1377049-84-7
Weight: Average: 883.019
Chemical Formula: $C_{49}H_{54}N_8O_8$

IUPAC Name:
(2S)-2-{[hydroxy(methoxy)methylidene]amino}-1-[(2S,5S)-2-(17-{2-[(2S,4S)-1-[(2R)-2-{[hydroxy(methoxy)methylidene]amino}-2-phenylacetyl]-4-(methoxymethyl)pyrrolidin-2-yl]-1H-imidazol-5-yl}-21-oxa-5,7-diazapentacyclo[11.8.0.0^{3,11}.0^{4,8}.0^{14,19}]henicosa-1(13),2,4(8),6,9,11,14(19),15,17-nonaen-6-yl)-5-methylpyrrolidin-1-yl]-3-methylbutan-1-one

Purity: 99% min.
Appearance: white Powder.

Mechanism of action: Velpatasvir's inhibits vie with polymer for binding at this website. it's additionally thought that NS5A inhibitors bind the target throughout its action in replication once the binding website is exposed [2]. Inhibition of NS5A is additionally illustrious to provide distribution of the supermolecule to lipide droplets. the precise role of NS5A in polymer replication isn't nevertheless understood though it's illustrious to be a vital element.
Half life: 15 hrs

MATERIALS AND METHODS:

Materials:

Material	Source
Reference sample(API)	Spectrum Pharmalabs, Hyderabad, Telangana
Test sample (**epclusa** Formulation)	Local pharmacy
HPLC grade :Acetonitrile, Methanol and water	Merck chemical division, Mumbai
AR grade: Potassium dihydrogen ortho phosphate, Ortho-phosphoric acid and sodium dihyrogen Ortho phosphate	Rankem, avantor performance material india limited

Instruments:

Instrument	Manufacturing company
Electronics Balance	Denver
Digital pH meter 7007	Digisun Electronics Hyderabad
Ultrasonicator	Labman
HPLC 2695 SYSTEM with PDA detector integrated with Empower 2 Software	WATERS
UV-VIS spectrophotometer integrated with UV win 6 Software	PG Instruments T60
Vacuum pump	Crompton
Hot Air Oven	Servewell Instrument PVT LTD, Bangalore.

METHODS:

Diluent: Mixer of Acetonitrile and water taken in the ratio of 50:50 v/v.

Preparation of Standard stock solutions: Correctly weighed 200 mg of Sofosbuvir, 50 mg of Velpatasvir and poured in to 50ml graduated flasks and 25ml of diluents was added to this flask and sonicated for 10 minutes. Flask were made up with diluent and labeled as Standard stock solution.1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.

Preparation of Sample stock solutions: 5 tablets were weighed and the typical weight of every one tablet was calculated, then the weight equivalent to 1 tablet was poured into a 100ml volumetric flask, add 50ml of diluent and sonicated for 25 min, additional volume was made up with diluent and filtered by HPLC filters .From this 1ml of filtered and poured into 10ml volumetric flask and made up with diluent.

Preparation of buffer:
0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

RESULT AND DISCUSSION:

Optimized wavelength was choose to 220 nm.
Optimized technique:
Mobile phase : 65 % OPA (0.1%): 35% Acetonitrile
Flow rate : 1 m

l/min
Colum n : Denali C18 (150 x 4.6 mm, 5µm)
Detector wave length : 220n m
Column temperature : 30° C
Injection volume : 10µ L
Run time : **7** min
Diluent : Water and Acetonitrile (50:50v/v)
Results : The two peaks have great goals, tailing component, theoretical plate count and resolution.

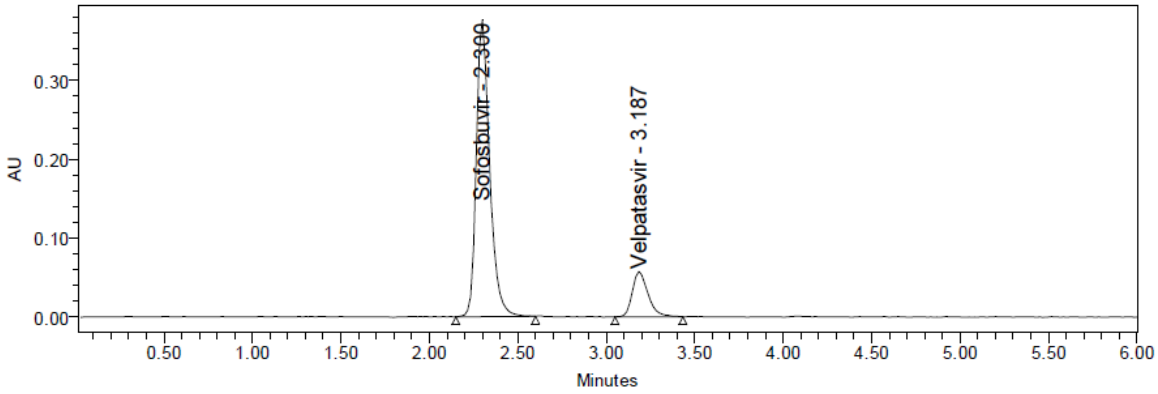


Fig. 1 Optimized Chromatogram

METHOD VALIDATION:

Accuracy: To determine the accuracy of the projected technique, recovery studies were distributed by adding totally different amounts (50%, 100%, and 150%). From that proportion recovery values were calculated.

Table. 1 Accuracy table of Sofosbuvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	200	199.52579	99.76	99.85%
	200	200.00591	100.00	
	200	200.84935	100.42	
100%	400	398.21041	99.55	
	400	396.21881	99.05	
	400	403.44922	100.86	
150%	600	598.59714	99.77	
	600	597.1052	99.52	
	600	598.24358	99.71	

Table.2 Accuracy table of Velpatasvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	49.649	99.30	99.76%
	50	49.722	99.44	
	50	49.748	99.50	
100%	100	99.469	99.47	
	100	99.411	99.41	
	100	99.771	99.77	
150%	150	150.487	100.32	
	150	149.880	99.92	
	150	151.045	100.70	

Discussion: 3 levels of Accuracy tests were set up by standard expansion strategy. Triplicate infusions were given for each dimension of precision and mean % Recovery was 99.85% and 99.76% for Sofosbuvir and Velpatasvir separately.

Precision:

System Precision:

Table.3 System precision table

S. No	Area of Sofosbuvir	Area of Velpatasvir
1.	1864864	328019
2.	1874623	325058
3.	1879810	320949
4.	1864901	322567
5.	1872070	322304
6.	1867676	322821
Mean	1870657	323620
S.D	5951.1	2531.1
%RSD	0.3	0.8

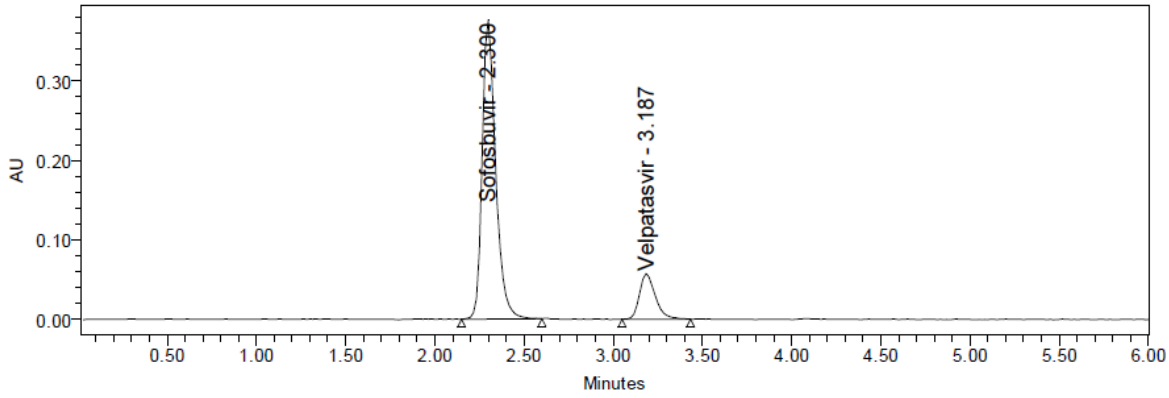


Fig. 2 System precision chromatogram

Discussion: From a solitary volumetric flask of working standard mixture six infusions were given and the gotten regions were made reference to above. Normal territory, standard deviation and % RSD were computed for two medications. % RSD got as 0.3% and 0.8% separately for Sofosbuvir and Velpatasvir .As the limit of Precision was < "2" the framework accuracy was passed in this strategy.

Repeatability:

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug.

Table.4 Repeatability table of Sofosbuvir and Velpatasvir

S. No	Area of Sofosbuvir	Area of Velpatasvir
1.	1879998	324046
2.	1864651	323049
3.	1870147	325133
4.	1864260	324094
5.	1866967	320595
6.	1857633	326062
Mean	1867276	323830
S.D	7472.1	1890.6
%RSD	0.4	0.6

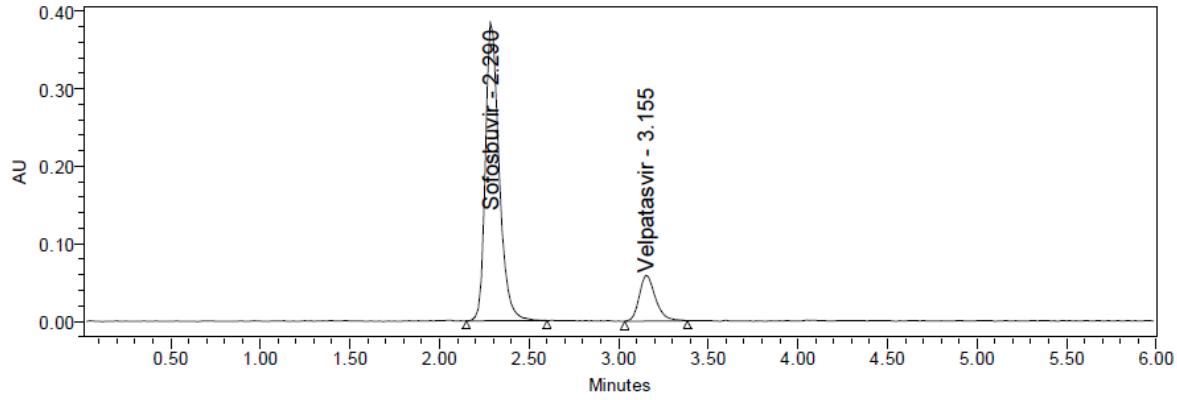


Fig.3 Repeatability chromatogram

Discussion: various sampling from a sample stock mixture was completed and 6 working sample mixer of equal concentrations were ready, every injection from every working sample mixer was injected and getting areas were declare in the above table. Average area, SD and % RSD were calculated for two drugs and obtained as 0.4% and 0.6% in that order. As the limit of Precision was < "2" the system precision was accepted in this technique.

Intermediate precision (Day- Day Precision):

Table.5 Intermediate precision table of Sofosbuvir and Velpatasvir

S. No	Area of Sofosbuvir	Area of Velpatasvir
1.	1739034	315041
2.	1727429	311014
3.	1753570	314211
4.	1732900	312788
5.	1746569	314417
6.	1721314	312294
Mean	1736803	313294
S.D	12037.4	1523.7
%RSD	0.7	0.5

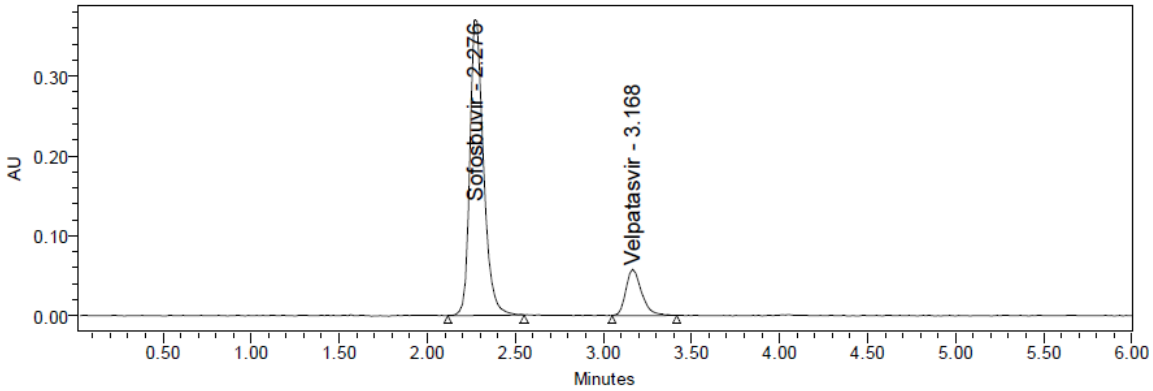


Fig.4 Inter Day precision Chromatogram

Linearity

Table.6 Sofosbuvir and Velpatasvir Linearity table

Sofosbuvir		Velpatasvir	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
100	471179	25	83118
200	889229	50	154439
300	1341160	75	234694
400	1815292	100	322364
500	2216339	125	393667
600	2635713	150	465378

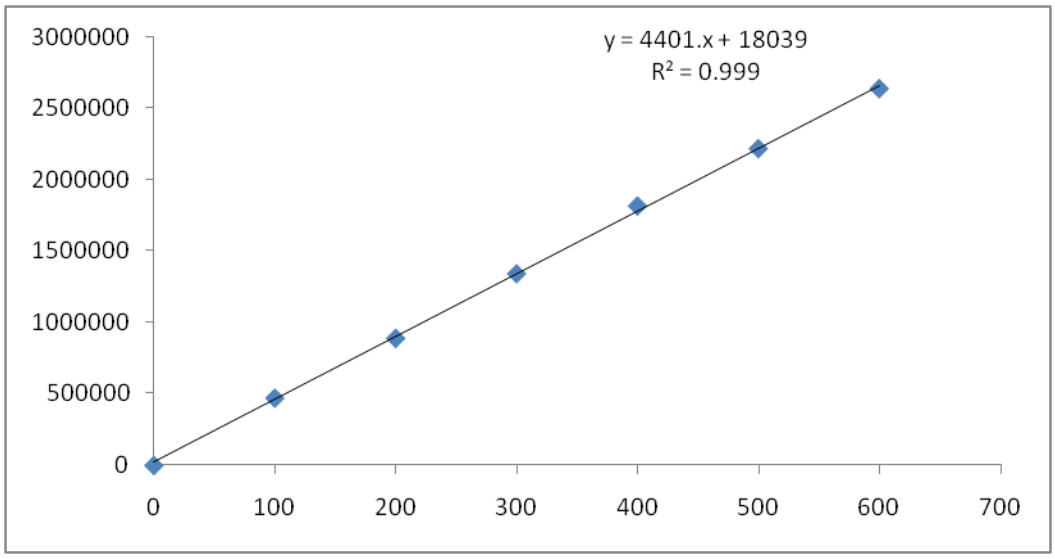


Fig.5 Sofosbuvir calibration curve

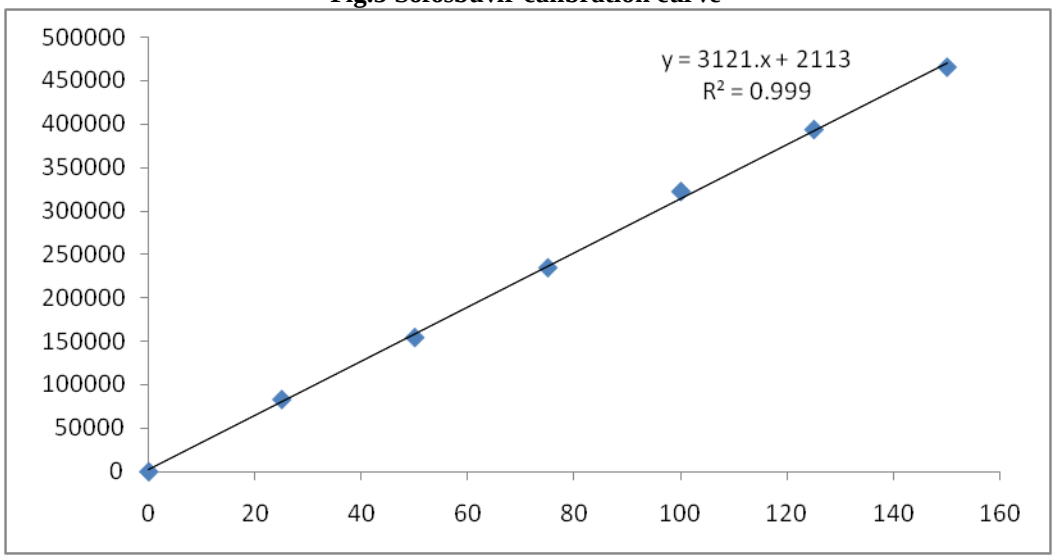


Fig. 6 Velpatasvir calibration curve

Discussion: 6 direct convergences of Sofosbuvir (100-600µg/ml) and Velpatasvir (25-150µg/ml) was infused in a copy way. Normal territories were made reference to above and linearity conditions acquired for Sofosbuvir was y = 4401.x + 18039 and of Velpatasvir was y = 3121x + 2113 Correlation coefficient got was 0.999 for the two medications

LOD & LOQ:

Table.7 LOD & LOQ table of Sofosbuvir and Velpatasvir

Molecule	LOD	LOQ
Sofosbuvir	0.33	0.99
Velpatasvir	0.43	1.29

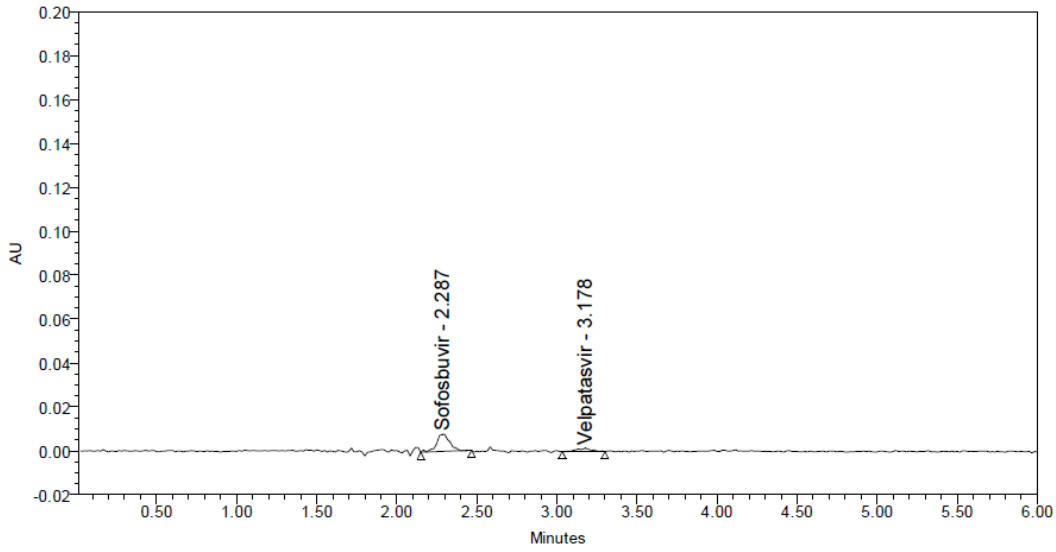


Fig.7 LOD Chromatogram of Standard

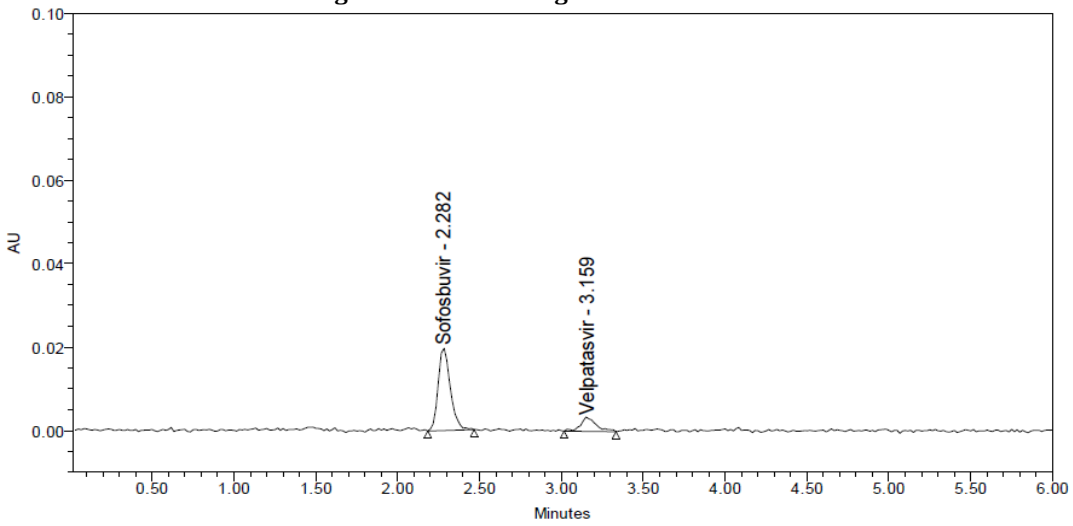


Fig.8 LOQ Chromatogram of of Standard

System Suitability Parameter

All the system suitability variables were within the range and acceptable as per ICH guidelines.

Table.8 System suitability variables for Sofosbuvir and Velpatasvir

S No	Sofosbuvir			Velpatasvir			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	USP Resolution
1	2.286	4321	1.30	3.150	6208	1.31	5.7
2	2.286	4418	1.28	3.173	6041	1.27	5.7
3	2.287	4488	1.32	3.173	6058	1.23	5.8
4	2.290	4586	1.31	3.175	6079	1.26	5.7
5	2.296	4592	1.27	3.179	6071	1.28	5.9
6	2.300	4372	1.27	3.187	6237	1.30	5.8

Discussion: As per ICH procedure plate count should be > 2000, tailing factor should be < 2 and resolution must be > 2. All the system suitable variables were accepted and were within the limits.

FORCED DEGRADATION STUDIES:

Degradation were performed with the detailing and the corrupted examples were infused. Examine of the infused tests was figured and every one of the examples passed the points of confinement of corruption.

Table. 9 Degradation Data of Sofosbuvir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.59	0.571	0.774
2	Alkali	2.80	0.537	0.755
3	Oxidation	1.92	0.630	0.718
4	Thermal	0.61	0.632	0.698
5	UV	0.65	0.608	0.677
6	Water	0.65	0.606	0.648

Table .10 Degradation Data of Velpatasvir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.90	1.916	2.321
2	Alkali	5.54	1.009	2.746
3	Oxidation	4.13	0.525	0.699
4	Thermal	2.56	0.508	0.712
5	UV	1.28	0.459	0.683
6	Water	0.48	0.476	0.667

Discussion: Concerning the pH alteration in mobile phase for the acid and base degradation examine have action in retention time of analytes. However neutralized of acid sample with 2N Base and base sample with 2N Acid there will be no alter in retention time.

Table.11 Summary Table

Parameters		**Sofosbuvir**	**Velpatasvir**	**LIMIT**
Linearity Range (µg/ml)		100-600 µg/ml	25-150µg/ml	R< 1
Regression coefficient		0.999	0.999	
Slope(m)		4401	3121	
Intercept(c)		18039	2113	
Regression equation (Y=mx+c)		y = 4401.x+18039	y = 3121x+2113	
Assay (% mean assay)		99.42%	99.86 %	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.3	0.8	NMT 2.0%
Method precision %RSD		0.4	0.6	NMT 2.0%
Accuracy %recovery		99.85%	99.76%	98-102%
LOD		0.33	0.43	NMT 3
LOQ		0.99	1.29	NMT 10
Robustness	**FM**	0.5	0.4	%RSD NMT 2.0
	FP	0.3	0.6	
	MM	0.8	1.1	
	MP	0.4	0.4	
	TM	1.1	0.6	
	TP	0.7	1.4	

CONCLUSION:

A simple, Accurate, precise technique was developed for the concurrent evaluation of the Sofosbuvir and Velpatasvir in Tablet dosage form. Retention times of these drugs were found to be 2.300 min and 3.187 min %RSD of the Sofosbuvir and Velpatasvir were and found to be 0.4 and 0.6 correspondingly. %Recovery was obtained as 99.85% and 99.76% for Sofosbuvir and Velpatasvir respectively. LOD, LOQ values obtained from regression equations of Sofosbuvir and Velpatasvir were 0.33, 0.99 and 0.43, 1.29 correspondingly. Regression equation of Sofosbuvir is y = 4401.x+18039, y = 3121x+2113 of Velpatasvir. Retention times were reduced and that run time was decline, so the developed technique was easy and cost-effective that can be adopted in regular Quality control test in Industries.

REFERENCES:

1. B.k Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication , Meerut, (2007)
2. Lindholm.J, Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis, pg . 13-14, (2004).
3. Rashmin, An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences , Vol.2 , Issue 2, Pg 191-196 (2012).
4. Malvia R, Bansal V , Pal O.P and Sharma P.K. A Review of High Performance Liquid Chromatography. Journal of Global Pharma technology (2010)
5. Douglas A Skoog, F. James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg 725-760.
6. Dr.S. Ravi Shankar, Text book of Pharmaceutical analysis, Fourth edition, Pg 13.1-13.2
7. Gurdeep R.Chatwal , Sham K .Anand, Instrumental Methods of Chemical Analysis , Pg 2.566-2.638 (2007)
8. Ashok Kumar, Lalith Kishore, navpreet Kaur , Anroop Nair. Method Development and Validation for Pharmaceutical Analysis. International Pharmaceutica Sciencia, Vol 2, Issue 3, Jul-Sep (2012)
9. Kaushal.C, Srivatsava.B, A Process of Method Development: A Chromatographic Approach. J Chem Pharm Res, Vol.2, Issue 2, 519-545, (2010)
10. Vibha Gupta, Ajay Deep Kumar Jain, N.S.Gill, Kapil, Development and Validation of HPLC method. International Research Journal of Pharmaeutical and Applied Sciences, Vol 2, Issue 4, Jul-Aug (2012)
11. Hokanson GC. A life cycle approach to the validation of analytical methods during Pharmaceutical Product Development. Part 1: The Initial Validation Process. Pharm Tech (1994) 92-100
12. Green JM. A Practicle guide to analytical method validation, Anal Chem (1996) 305A-309A
13. ICH, Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA , Geneva , (1996)
14. Ewelina rutkowska, Karolina paj k and Krzysztof J"ewiak* Lipophilicity – Methods of determination and its role in medicinal chemistry Acta Poloniae Pharmaceutica n Drug Research, Vol. 70 No.1 pp. 3n18, (2013).
15. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2 (2010) 1657-1658.
16. British Pharmacopoeia, The British Pharmacopoeial Commission, the stationary office, UK, London, 1408-1409 2 (2011).
17. IUPAC. Compendium of Chemical Terminology, 2nd edn. (The Gold Book). PAC69, 1137 (1997). Glossary of terms used in computational drug design (IUPAC Recommendations.
18. K. D. Tripathi, Essentials of Medical Pharmacology, 6th Edition, Jaypee brother's medical publishers (P) LTD, p-254-255.
19. https://www.drugbank.ca/drugs/DB11613.
20. https://www.drugbank.ca/drugs/DB08934.
21. B. Raj kumar*[1], dr. K. V.subrahmanyam et al**.,** a new validated rp-hplc method for the simultaneous determination of simeprevir and sofosbuvir in pharmaceutical dosage form. Indo american journal of pharmaceutical research, 2016,6(2).
22. P. Mohan Vikas*, Dr. T. Satyanarayana, D. Vinod Kumar, E. Mounika, M. Sri Latha, R. Anusha and Y. Sathish ET AL., development and validation of new rp-hplc method for the determination of sofosbuvir in pure form.2016, 5(5); 775-781.
23. Bakht Zaman,Faisal Siddique,Waseem Hassan et al., rp-hplc method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form and its application to in vitro dissolution studies
24. Mohamed El-Kassem M Hassouna1*, Maha Mohammed Abdelrahman2 and Mahmoud Abdelfatah Mohamed Assay and Dissolution Methods Development and Validation for Simultaneous Determination of Sofosbuvir and Ledipasvir by RP-HPLC Method in Tablet Dosage Forms Journal of Forensic Sciences & Criminal Investigation Submission: December 05, 2016; Published: January 03, 2017
25. Ravikumar Vejendla, C.V.S. Subramanyam, G. Veerabhadram estimation and validation of sofosbuvir in bulk and tablet dosage form by rp-hplc International Journal of Pharmacy Int J Pharm 2016; 6(2): 121-127

26. Gandla. Kumara Swamy*1 , K. Pranay1 , M. Rajkumar1 , D. Sudheer Kumar1, novel stabilty indicating rp-hplc method simultaneous determination of sofosbuvir and velpatasvir in bulk and combined tablet dosage forms, Asian Journal of Research in Biological and Pharmaceutical Sciences. 5(4), 2017, 143-151.
27. Uppalapati.Jyothi*1, Dr.Parimi.Umadevi2 , analytical method development and validation for the simultaneous estimation of sofosbuvir and velpatasvir drug product by rp-hplc method, ndo American Journal of Pharmaceutical Research, 2017ISSN NO: 2231-6876.
28. B vanaja, N.M. Vageesh, C.Kistayya*, V. Urukundu. rp-hplc method development and validation for simultaneous estimation of sofosbuvir and velpatasvir in pure and pharmaceutical dosage form, Innovat International Journal Of Medical & Pharmaceutical Sciences, 2018; 3(1).
29. V. Swetha*1, P.Sowjanya1 , G.VIjaya Kumar1 , M.A.Haneef2 .method development and validation of rp-hplc method and stress degradation study of determination of sofosbuvir and velpatasvir in bulk and pharmaceutical formulation, Indian Journal of Pharmaceutical Science & Research, Vol8|Issue 1| 2018 |6-11.