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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SOFOSBUVIR, VELPATASVIR AND VOXILAPREVIR IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

A simple reverse phase analytical technique was established for quantification of single and combinational drugs. A simple, economic, accurate reverse phase isocractic RP-HPLC method was established for the simultaneous quantification of Sofosbuvir, Velpatasvir and Voxilaprevir in bulk and tablet dosage forms. The estimation was done by using Discovery C18 (250×4.6mm,5µm) column with the flow rate of 1.0 ml/min, at λ_{max} 260 nm. Solution-A was 0.1% OPA (pH 1.8) in water and solution -B was Acetonitrile (mobile phase: solution A: solution B, 50:50 v/v). The Rt for Sofosbuvir, Velpatasvir and Voxilaprevir is 2.458 min., 3.282 min and 4.003 min. respectively. The system was established and validated as per ICH guidelines.

Keywords: Sofosbuvir, Velpatasvir, Voxilaprevir, RP-HPLC, Validation.

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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.

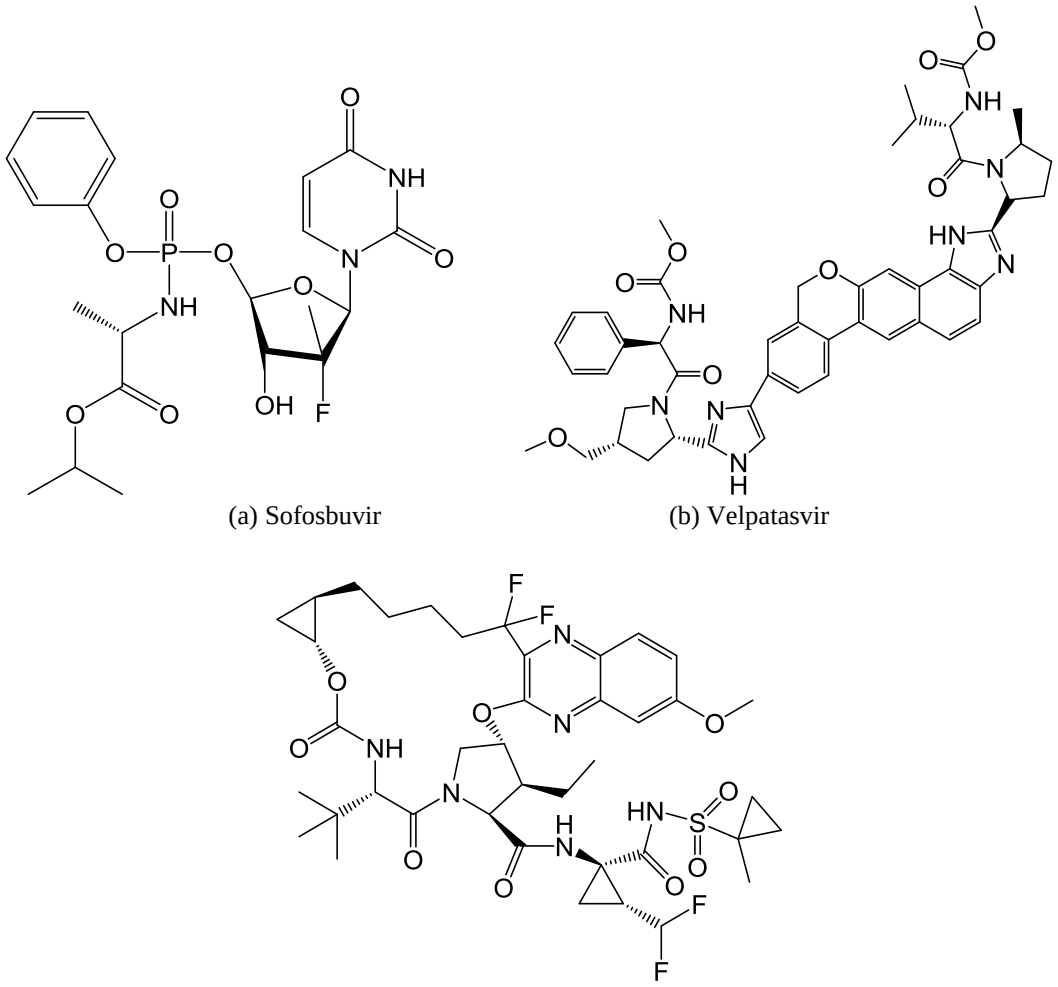
Sofosbuvir (Fig.-1a) is an antiviral drug in the treatment of chronic hepatitis C virus. It is chemically isopropyl (2s)-2[[[(2R,3R,4R,5R)-5-(2,4-dioxop[yrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl- tetra hydro furan-2-yl] methoxy- phenoxy-phosphoryl] amino] propanoate. Mainly Sofosbuvir is activated in the liver to the triphosphate by hydrolysis of the carboxylate ester[1].

Velpatasvir (Fig.-1b) is an NS5A inhibitor which acts on hepatitis C virus. Velpatasvir is chemically Methyl {(2 S) - 1 -[(2S, 5S) – 2 - (9 - { 2 -[(2S ,4S) – 1 - {(2R) - 2 -[(methoxycarbonyl) amino] -2 - phenylacetyl} - 4- (methoxymethyl) -2 - pyrrolidinyl] -1 H - imidazol-4-yl} -1, 11 – dihydroisochromeno [4', 3': 6,7] naphtha [1,2-d] imidazol-2-yl) - 5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate used as an anti-cholinergic and anti-spasmodic[2].

Voxilaprevir (Fig.-1c) it is also a protease inhibitor and acts as a transporter of polypeptide. Voxilaprevir is chemically (1R,18R,20R,24S,27S,28S)-N-[(1R,2R)-2-(Difluoromethyl)-1-{[(1-methylcyclopropyl) sulfonyl] carbamoyl} cyclopropyl]-28-ethyl-13,13-difluoro-7-methoxy-24-(2-methyl-2-propanyl)-22, 25-dioxo-2,21-dioxa-4,11,23,26-tetra aza penta cyclo nonacosa-3(12),4,6,8,10-pentaene-27-carboxamide[3].

Some of the literature was available for the combination of Sofosbuvir and Velpatasvir.[4-8] No literature available for the estimation of Sofosbuvir, Velpatasvir and Voxilaprevir in a combined dosage form. Sofosbuvir(400mg), Velpatasvir(100mg) and Voxilaprevir(100mg) were available in a mixed dose form of VOSEVI. The US FDA was approved in 2017. The HPLC technique was used for the development and validation of combinational drugs were reported [4-9]. But no method was found for the estimation of

Sofosbuvir(Sof), Velpatasvir(Vel) and Voxilaprevir(Vox) in pharmaceutical dosage forms in the literature. As per ICH guidelines the method was developed and validated[10].



(a) Sofosbuvir (b) Velpatasvir

(c) Voxilaprevir

Fig.-1: Chemical Structures of the Three Drugs

EXPERIMENTAL

Reagents and Chemicals

The three drug samples were gifted from Spectrum labs Hyderabad. HPLC grade solvents and chemicals were used for the study. The marketed form of Vosevi was purchased from Ankur pharmacy Bangalore. The OPA and Acetonitrile were supplied by Merck [India] Pvt., Ltd., Mumbai.

Instrumentation

The HPLC system module 2965, Empower2 software and 2996 module of PDA detector, a quaternary gradient delivery pump, automatic sample injector and a column Discovery C 18(250×4.6mm,2.5µm) respectively. Electronic balance, Sonicator (ultra-sonic cleaner power sonic 420), PH meter, water bath and other glassware were used for the present investigation.

Chromatographic Conditions

Discovery C18(250×4.6mm,2.5µm) column was operated at 30^0 C temperature with a flow rate of 1.0 ml/min., at fixed λ_{max} 260 nm. Solution-A was 0.1% OPA (pH 1.8) in water and solution -B was Acetonitrile (mobile phase: solution A: solution B, 50:50 v/v). 20µl was injected into HPLC system.

Preparation of 0.1% OPA Buffer Solution
Transferred 1.0 ml of OPA into 1000 ml of milli-Q water.

Preparation of Mobile Phase
Mixture of 500ml (50%) OPA buffer (pH 1.8) and 500ml of (50%) ACN were mixed and degassed to sonicate it. The filtration was done under vacuum with 0.45 µ filter paper.

Preparation of Diluent
Water and ACN were taken as a diluent. (50:50).

Preparation of Standard Solution
Working standards of (40mg) Sofosbuvir, (10mg) Velpatasvir and (10mg) Voxilaprevir were weighed and taken into three-separate 50ml dry volumetric flasks. 5ml of the diluent was added and made up to the mark. From the prepared solutions, 1ml of each solution was added to a 10ml flask to obtain a mixed standard solution. (This is a 100% for various experimental procedures)

Preparation of Sample Solution
Five tablets were crushed homogenously to prepare a powder equivalent to one tablet and taken in a 100ml volumetric flask. 60ml of diluent was added to the powder, diluted, made up to the mark and sonicated for 25min.Then 1ml was taken in 10ml flask diluted up to the mark, filter through the 0.45µ filter.

RESULTS AND DISCUSSION

Method Development
Initially, the method trails were performed with acidic and basic phosphate buffer, acetonitrile and methanol by using gradient and isocratic mode buffer, which was analyzed with C4, C8, C18, cyano and Amino columns. Finally, the separation was arrived on a Discovery C18 (250×4.6mm, 5µ) column with the flow rate of 1.0 ml/min, at 260 nm wavelength. Solution-A was 0.1% OPA (pH 1.8) in water and solution -B was Acetonitrile (mobile phase: solution A: solution B, 50:50 v/v).

Method Validation
Specificity
Drug -Drug interaction (or) drug-excipient interaction was known through this parameter. The mixture of sample and standard drugs were separated. No interference of the diluent at the Rt values of three drugs. The HPLC chromatograms are shown in Fig.-2 and 3.

Linearity
The linearity study was done by injecting the different concentrations (50% - 150%) of the drugs. From the linearity curve the correlation coefficient was found to be less than 1.0. The results were incorporated in Table-1.The linearity plots were shown in Fig.-4.

Table-1: Linearity Studies

Linearity level	Sofosbuvir		Velpatasvir		Voxilprevir	
	Conc (ppm)	Area	Conc (ppm)	Area	Conc (ppm)	Area
20%	100	131914	25	67843	25	89404
50%	200	260891	50	143741	50	167390
75%	300	390813	75	214067	75	257196
100%	400	506095	100	282278	100	328684
125%	500	632125	125	353686	125	423322
150%	600	747118	150	415507	150	502028
Correlation	0.9995		0.9995		0.9994	

Coefficient			

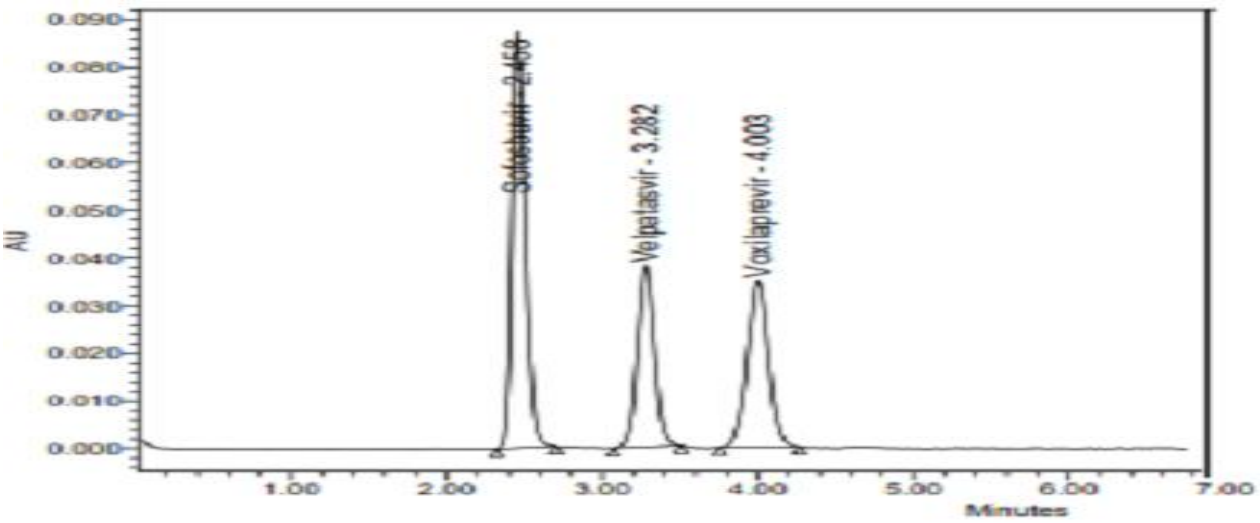


Fig.-2: Drug Mixture

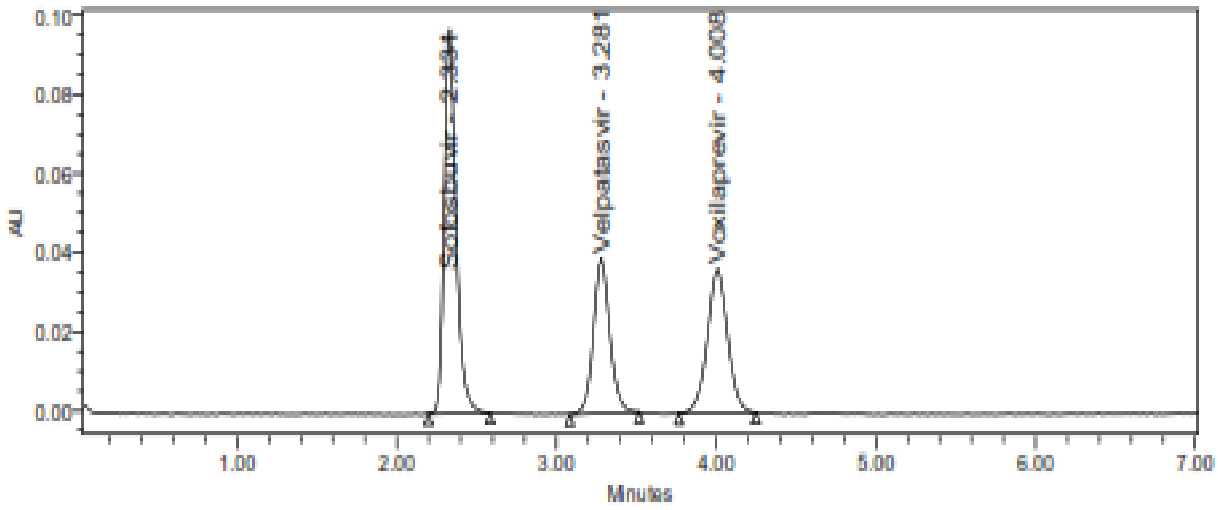


Fig.-3: Dosage for

Accuracy

The recovery study was done by standard addition method for three drugs at 50%,75% and 150% level. The results were given in Table-2.

Table-2: Results for Accuracy Method

Drug Name	% of conc	Amount added (ppm)	Amount found (ppm)	% Recovery	Mean % Recovery
Sofosbuvir	50%	200	199.4	99.4%	99.2%
		200	198.8		
		200	198.0		
	100%	400	395.9	98.8%	
		400	394.8		
		400	396.1		
	150%	600	605.1	99.5%	
		600	589.4		
		600	587.2		

Velpatasvir	50%	50	49.6	99.6%	100%
		50	50.0		
		50	49.8		
	100%	100	101.1	99.9%	
		100	99.3		
		100	99.5		
	150%	150	150.9	100.6%	
		150	150.5		
		150	151.4		
Voxilaprevir	50%	50	49.3	99.3%	99.2%
		50	49.7		
		50	50.0		
	100%	100	98.7	98.5%	
		100	98.5		
		100	98.3		
	150%	150	148.6	99.9%	
		150	151.0		
		150	150.4		

Method Precision

Six different sample solutions were prepared from the commercial tablets and injected into HPLC. The %RSD was below 1% for all drugs. The values were incorporated in Tables-3 to 5.

Table-3: Method Precision results for Sofosbuvir

Sample No	Retention Time(min)	Peak Area(au)	% Assay
1	2.331	502185	99.52
2	2.331	503811	99.84
3	2.331	500040	99.10
4	2.331	502033	99.49
5	2.331	502114	99.51
6	2.332	502291	99.54
Mean	2.331	502079	99.50
Std dev	0.0003	1201.4	0.238
% RSD	0.015	0.2	0.24

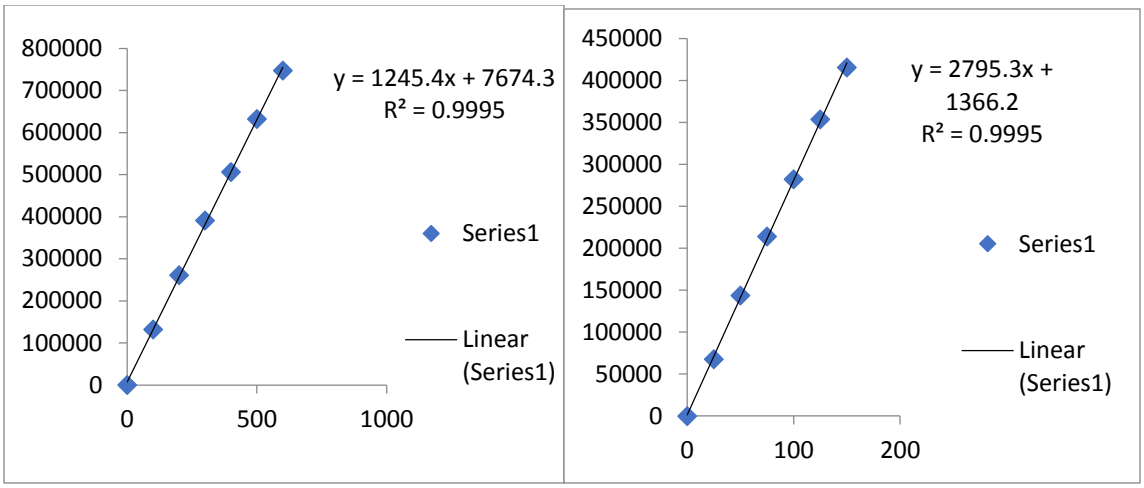
Table-4: Method Precision results for Velpatasvir

Sample No	Retention Time(min)	Peak Area (au)	% Assay
1	3.281	283583	99.82
2	3.281	282997	99.61
3	3.282	284718	100.22
4	3.282	285100	100.35
5	3.284	280187	98.62
6	3.285	283661	99.85
Mean	3.282	283374	99.74
Std dev	0.001	1744.2	0.614
% RSD	0.045	0.6	0.62

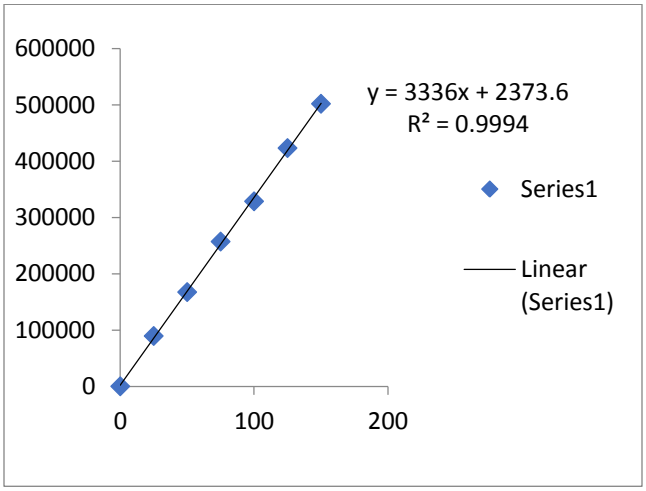
Table-5: Method Precision results for Voxilaprevir

Sample No	Retention Time (min)	Peak Area (au)	% Assay
1	4.009	322180	98.66

2	4.010	323302	99.00
3	4.010	323076	98.93
4	4.010	326905	100.1
5	4.011	322501	98.76
6	4.012	321081	98.32
Mean	4.010	323174	98.96
Std dev	0.001	1988.4	0.61
% RSD	0.025	0.6	0.62



(A) Linearity for Sofosbuvir (B) Linearity for Velpatasvir



(C)Linearity for Voxilaprevir

Fig.-4: Calibration Plot for Drugs (A, B, C)

Intermediate Precision

The %RSD of the area and Rt values were analyzed for two different analysts and results were mentioned in Table-6. It is evident that the method is rugged from the above data.

Table-6: Percentage RSD for a different day and different analyst

S. No.	Day-1 – Analyst-1			Day-2 – Analyst-2		
	SOF	VEL	VOX	SOF	VEL	VOX
1	502185	283583	322180	506002	283342	320445
2	503811	282997	323302	505616	283680	325732

3	500040	284718	323076	500127	285401	322560
4	502033	285100	326905	508749	283176	325145
5	502114	280187	322501	508905	281746	324711
6	502291	283661	321081	509618	282593	328739
Average	502079	283374	323174	506503	283323	324555
Std Dev	1201.4	1744.2	1988.4	3527.3	1223.7	2833.2
%RSD	0.2	0.6	0.6	0.7	0.4	0.9

LOD and LOQ

The LOD and LOQ were calculated from the linearity curve method and presented in Table-7.

Table-7: LOD and LOQ values

S. No.	Sofosbuvir(µg/ml)	Velpatasvir (µg/ml)	Voxilaprevir (µg/ml)
LOD	0.21	0.46	0.20
LOQ	0.63	1.39	0.61

Robustness

No changes were observed in chromatograms when changing the flow rate, the concentration of mobile phase and temperature. Hence the method was robust. The results were shown in Table-8.

Table-8: Robustness studies of Sof, Vel and Vox

S. No.	Parameter	Sofosbuvir		Velpatasvir		Voxilaprevir	
		RT	Area	RT	Area	RT	Area
1	Decreased in flow rate(0.8ml/min)	2.549	557710	3.569	310450	4.369	360450
2	Increased in flow rate(1.2ml/min)	2.338	517598	3.267	289492	3.999	339060
3	Less organic composition	2.338	503839	3.188	286622	3.768	331225
4	More organic composition	2.342	521689	3.355	283046	4.279	342785
5	Change in temp(28^0C)	2.339	502022	3.266	277916	3.997	326746
6	Change in temp(30^0C)	2.338	502802	3.265	277107	3.997	328020

System Suitability

The system suitability checked for the developed method were tailing factor, retention time and theoretical plates were observed within the acceptance criteria, presented in Table-9.

Table-9: System suitability data of Sof, Vel and Vox

Parameters	Sofosbuvir	Velpatasvir	Voxilaprevir
Retention time (min)	2.331	3.281	4.009
Theoretical Plates (N)	4848	4996	4575
Tailing Factor (tF)	1.27	1.04	0.99

CONCLUSION

The present study concludes that the developed method was simple, selective and reproducible with shorter time. Hence this investigation was suitable for the estimation of Sofosbuvir, Velpatasvir and

Voxilaprevir simultaneously. The present method was accurate, precise and Robust. This method can apply for releasing routine, quality control and stability indicating studies.

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