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NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN BULK AND PHARMACEUTICALDOSAGE FORMBYRP-UPLCMETHOD

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ABSTRACT:

A simple, specific and accurate reverse phase ultra-performance liquid chromatographic method was developed for the simultaneousdetermination Sofosbuvir and Velpatasvirin pharmaceutical dosage form. The column used was Kromosil C18(150mm x 4.6 mm, 5µm)inisocratic mode, with mobile phase containing phosphate buffer andacetonitrile(75:25v/v). The flow rate was 1.0ml/ min and effluents weremonitored at 260 nm. The retention times of Sofosbuvir and Velpatasvirwere found to be 2.404min and 2.986 min, respectively.The linearityfor Sofosbuvir and Velpatasvirwere in the range of 35-210µg/ml and 8-48 µg/ml respectively. The recoveries of Sofosbuvir andVelpatasvirwere found to be 99.64% and 99.25%, respectively. The proposed method was validated and successfully applied to the estimationofSofosbuvirandVelpatasvirincombinedtabletdosageforms.

KEYWORDS: Sofosbuvir,Velpatasvir,Validation,BufferandICHGuidelines.

INTRODUCTION

Sofosbuvir[1] is an oral nucleoside analogue and powerful inhibitor of hepatitis C virus (HCV) RNA polymerase that is used to treat chronic hepatitis C in combination with other antiviral drugs. Nonetheless, successful antiviral therapy for hepatitis C with sofosbuvir and other direct acting agents in patients with cirrhosis is occasionally complicated by hepatic decompensation; additionally, treatment can cause reactivation of hepatitis B in susceptible patients coinfected with the hepatitis B virus for unknown reasons (HBV), IUPAC propan-2-yl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy-phenoxyphosphoryl]amino]propanoate.

Having the molecular formula $C_{22}H_{29}FN_3O_9P$ with molecular weight 529.5.

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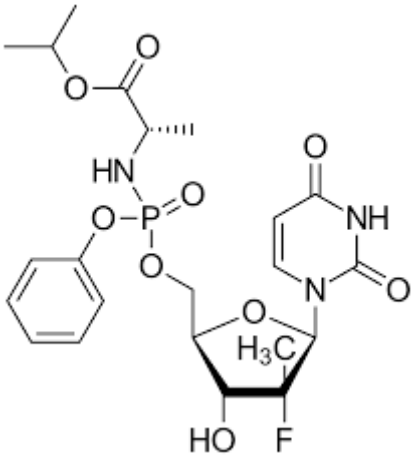

Figure1: Chemical Structure of Sofosbuvir

Velpatasvir[2]is a Direct-Acting Antiviral (DAA) drug used in combination therapy to treat chronic Hepatitis C, an infectious liver condition caused by Hepatitis C Virus infection (HCV). HCV is a single-stranded RNA virus with nine genotypes, the most prevalent of which is genotype 1, which affects 72 percent of all chronic HCV patients in the United States. Velpatasvir functions as a faulty substrate for NS5A (Non-Structural Protein 5A), a non-enzymatic viral protein involved in Hepatitis C Virus proliferation, assembly, and immune response control. IUPAC name is (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 having molecular formula $C_{49}H_{54}N_8O_8$ with molecular weight 880.

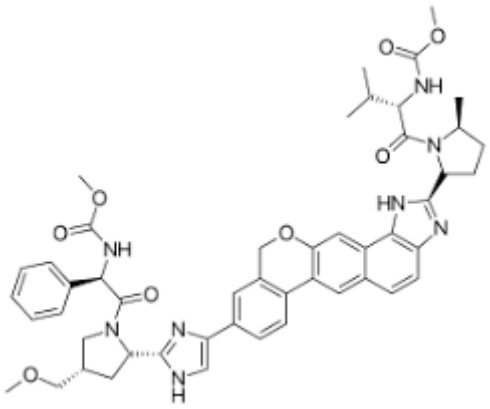

Figure2: Chemical Structure of Velpatasvir

The low cost RP-UPLC method forsimultaneous estimationofSofosbuvir and Velpatasvirin bulk and dosageforms.The analytical method was developed and validated as per the ICH guidelines

MATERIALSANDMETHODS

Materials

Sofosbuvir and Velpatasvir were kindly supplied by spectrum lab Pvt Ltd..Acetonitrile, water (UPLC grade, Merck)and all theotherreagents of AR grade were purchased from M R Enterprisers.A tablet EPCLUSA (Gilead Sciences, In)containing 400mg of Sofosbuvirand100mgofVelpatasvirwereused.

Instrumentation

TheLCsystemconsistedofaWatersmodel515,PDAdetector 2998 with 20 µL sample loop. The output signalsweremonitored.

Methods

Chromatographicconditions

The elution was isocratic, and the mobile phase was a mixture of buffer and acetonitrile (75:25 v/v) (accurately weighed 1.41gm sodium dihydrogen ortho phosphate in a 1000ml volumetric flask, add about

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900ml milli-Q water, degas to sonicate, and finally make up the volume with water, then pH adjusted to 3.5 with dil. orthophosphoric acid solution Prior to use, the mobile phase was filtered using a 0.45-m membrane filter (HVLP, Germany). For the measurement, a Kromosil C18 (150mm x 4.6mm, 5m) was employed. The flow rate was 1.0 ml/min, and the column was kept at 30 degrees Celsius. The amount of sample injected was 10 litres. The column was equilibrated with mobile phase for at least 30 minutes prior to injection of the solutions. TheUVdetector wassetatwavelengthof260nm.

StandardPreparation

In a 100 ml clean dry volumetric flask, accurately weigh and transfer 40 mg of Sofosbuvir and 10 mg of Velpatasvir working Standards, add 70 ml of diluent, sonicate for 30 minutes, and make up to the final volume with diluent. 4 mL of the aforementioned stock solution was pipetted into a 10 mL volumetric flask, and the remaining volume was made up with diluent. Sofosbuvir and Velpatasvir have ultimate concentrations of 160 g/ml and 40 g/ml, respectively.

SamplePreparation

We took about 20 tablets and estimated their average weight. The tablets were crushed to a fine powder, and 40mg and 10mg of the medication were transferred to a 100ml volumetric flask, where they were dissolved in diluent. 4mL of the aforementioned solution was transferred to a 10mL volumetric flask and filtered through a 0.45 membrane filter to get a concentration of 160g/mL for Sofosbuvir and 40g/mL for Velpatasvir.

MethodValidation

The developed analytical method was validated as per ICH guidelines **4-6** for its accuracy, linearity, precision, specificity, robustness,limit ofdetection and limitofquantification by using thefollowingprocedures.

Systemsuitability

System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and numberof theoreticalplateswerecalculated.

Linearity

The linearity was determined using linear regression analysis and the least square method, and it was tested by making standard solutions of Sofosbuvir and Velpatasvir at various concentration levels. The absorbance of the resultant solutions was measured, and a calibration curve between absorbance and drug concentration was constructed. For Sofosbuvir and Velpatasvir, the response was shown to be linear in the ranges of 35-210 µg/ml and 8-48 µg/ml.

Accuracy

Accuracywasperformedintriplicateforvariousconcentrations of Sofosbuvir and Velpatasvir equivalent to50%, 100% and 150% of the standard amount were injectedinto the UPLC system per the test procedure. The average %recoverywascalculated.

Precision

MethodRepeatability

As part of the test method, six sample solutions of the same concentration (100%) were produced and injected into the UPLC system.

Intermediate Accuracy (Day to Day variability)

The study took two days to complete according to the test technique. Six sample solutions of the same concentration (100%) were produced and injected into the UPLC system as per the test procedure on Days 1 and 2. Two instruments were tested according to the test technique. The data collected from several instruments have a relative standard deviation of less than 2.0.

Limits of Quantification and Limits of Detection

According to ICH rules, LOD and LOQ were derived from the calibration curve's average slope and standard deviation. Sofosbuvir's LOD and LOQ were found to be 0.57 g/ml and 1.72 g/ml, respectively. The LOD and LOQ of Velpatasvir were found to be 0.27 µg/ml and 0.81 µg/ml respectively.

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Robustness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Sofosbuvir and Velpatasvir were noted. The factors selected were flow rate and variation in the mobile phase composition.

Assay

Theassayand%puritywerecalculatedforbrandEPCLUSA (Gilead Sciences, In)with label claim 400 mg and 100mg. Theobserved value was compared with that of standard valuewithout interference from the excipients used in the tabletdosageform.

RESULTSANDDISCUSSION

For the simultaneous estimate of Sofosbuvir and Velpatasvir dose forms, a reverse-phase column process was presented as a suitable method. By altering the mobile phase composition, the chromatographic conditions were improved. To optimize the mobile phase, several ratios were tested. Finally, the mobile phase was made up of buffer and acetonitrile in a 75:25v/v ratio, which resulted in good resolution of the Sofosbuvir and Velpatasvir peaks. The drug's absorbance was optimized at 260nm;Sofosbuvir and Velpatasvir had retention times of 2.404 and 2.986 minutes, respectively, using our suggested approach, and none of the contaminants interfered with the assay. The medicines' chromatogram is given in Fig. 1. Figure 2 depicts the Sofosbuvir calibration curve, while Figure 3 depicts the Velpatasvir calibration curve. The observed peak area values for respective concentrationsare shown in Table-1.

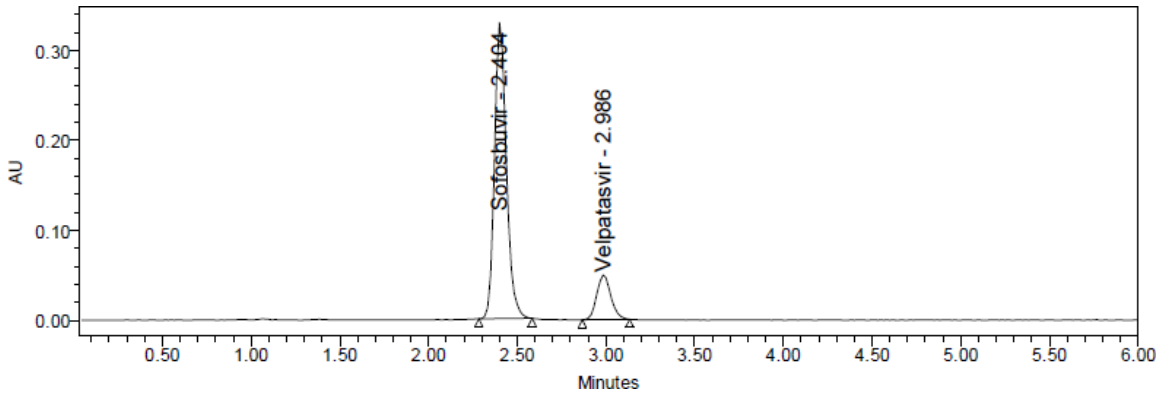

Figure1:chromatogramofSofosbuvirandVelpatasvirinoptimizedchromatographic conditions

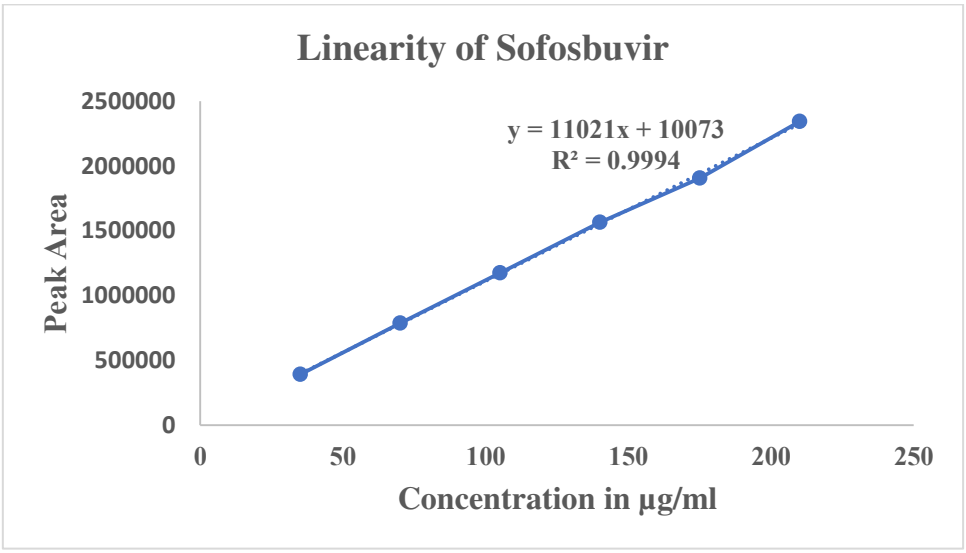

Figure2:Calibrationcurve of Sofosbuvir in therange35to 210 µg/ml.

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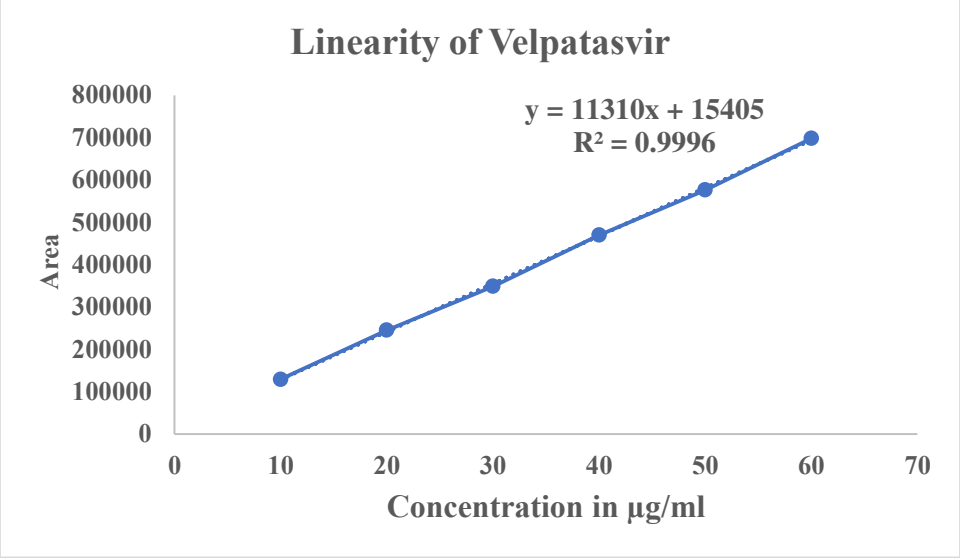

Figure3:Calibrationcurve ofVelpatasvirin therange8 to 48µg/ml.

Table1:CalibrationcurveofSofosbuvirandVelpatasvir

S.No	Sofosbuvir			Velpatasvir		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	35	2.401	390745	8	2.987	129279
2	70	2.404	786083	16	2.982	245126
3	105	2.406	1174240	24	2.978	348882
4	140	2.403	1563323	32	2.964	469871
5	175	2.405	1904525	40	2.992	576112
6	210	2.407	2342034	48	2.995	698155

Theaccuracydata,methodprecisiondata,intermediateprecisiondatafordayandintermediateprecisiondatarelating to change of instrument are shown in Table 2, Table3, table 4, and Table 5 respectively.Robustness data relatingtochange inflowrate androbustnessdatarelatingtochange in mobile phase composition are shown in Table 6 and Table7 respectively. Results of analysis of laboratory samples areshowninTable8.Table9showssystemsuitabilityparameters.

Table2:Accuracydata

S.No		Sofosbuvir			Velpatasvir		
	Spiked level	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD
1(n=6)	50%	40.00	40.75	98.98 ± 0.33	10.00	10.03	100.07 ± 0.46
2(n=6)	100%	80.00	80.23	100.44 ± 0.38	20.00	20.96	99.56 ± 0.55
3(n=6)	150%	120.00	119.16	100.32 ± 0.20	30.00	29.97	99.98 ± 0.59

*n=6(Averageof6determinations)

Table 3: Method Precision data of Sofosbuvir and Velpatasvir

S.No	Sofosbuvir			Velpatasvir		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	140	2.403	1562412	32	2.982	272468
2	140	2.405	1565061	32	2.989	277211
3	140	2.405	1568363	32	2.987	271649

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4	140	2.406	1566157	32	2.989	270677
5	140	2.408	1566158	32	2.991	273575
6	140	2.401	1561519	32	2.974	272713
Mean			1564945			273049
Std.dev			2560.9			2264.3
%RSD			0.2			0.8

Table 4: Intermediate Precision data relating to change of day

S.No	**Inter-day precision**					
	Sofosbuvir			**Velpatasvir**		
	Peak area			**Peak area**		
	Conc (µg/ml)	**Day-1**	**Day-2**	**Conc (µg/ml)**	**Day-1**	**Day-2**
1	140	1566934	1569202	32	275638	275929
2	140	1567283	1560292	32	272833	274633
3	140	1569474	1563848	32	279383	274182
4	140	1565849	1564122	32	271132	272901
5	140	1563938	1565393	32	270293	270322
6	140	1562384	1563033	32	274842	273932
Mean		1565977	1564315		274020.2	273649.8
SD		2527.214	2936.945		3337.527	1905.742
%RSD		0.16	0.18		1.21	0.69

Table 5: Intermediate Precision data relating to change of instrument

S.No	**Instrument to Instrument**					
	Sofosbuvir			**Velpatasvir**		
		Peak area			**Peak area**	
	Conc (µg/ml)	**Day-1**	**Day-2**	**Conc (µg/ml)**	**Day-1**	**Day-2**
1	140	1564847	1564283	32	276282	273832
2	140	1565838	1568822	32	272837	272922
3	140	1561934	1562838	32	277927	271973
4	140	1562931	1563848	32	271983	270283
5	140	1562482	1561344	32	277282	272833
6	140	1563013	1563939	32	270484	275752
Mean		1563508	1564179		274465.8	272932.5
Std.dev		1505.319	2512.804		3094.559	1828.069
%RSD		0.09	0.16		1.12	0.66

Table 6: Robustness data relating to change in flow rate (1.0ml/min)

S.No		**Sofosbuvir**			**Velpatasvir**		
	Flow rate (ml/min)	**Average Peak Area***	**Std.dev**	**%RSD**	**Average Peak Area***	**Std.dev**	**%RSD**
1	0.9ml/min	1566364	1453	0.36	276606	3411	0.73
2	1.0ml/min	1566108	1087	0.27	278575	1400	0.30
3	1.1ml/min	1566214	1233	0.30	276866	2723	0.58

*n=3 (Average of 3 determinations)

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Table 7: Robustness data relating to change in mobile phase composition

S.No		Sofosbuvir			Velpatasvir		
	Mobile phase variation (%)	Average peak area*	Std.dev	%RSD	Average peak area*	Std.dev	%RSD
1	M.P-1-(buffer:ACN::74:26)	1566072	3048	0.75	277789	1720	0.37
2	M.P-2-(buffer:ACN::75:25)	1566995	1237	0.30	277045	1356	0.29
3	M.P-3-(buffer:ACN:76:24)	1566451	1751	0.43	277058	3622	0.78

*n=3 (Average of 3 determinations)

Table 8: Results of analysis of laboratory samples (Assay)

S.No			Sofosbuvir		Velpatasvir	
	Sample	Label	Amount found	%Purity ± RSD*	Amount found	%Purity ± RSD*
1	Brand-1 (EPCLUSA)	400mg/100mg	399.99	99.48± 0.30	99.96	99.25± 0.73

*n=3 (Average of 3 determinations)

Table 9: System suitability parameters

Validation parameter	Results	
	Sofosbuvir	Velpatasvir
Linearity range (µg/ml)	35-210	8-48
Regression equation	y = 11021x + 10073	y = 11310x + 15405
Correlation Coefficient(r)	0.9994	0.9996
Accuracy	98.58% to 100.71%	98.94% to 100.58%
Precision (%RSD)	0.20	0.80
Robustness (%RSD)		
Flow rate: (1.0ml/min & 1.2ml/min)	NMT 0.36	NMT 0.73
Mobile phase: Buffer: ACN: MeOH (25:65:10)	NMT 0.75	NMT 0.78
Intermediate Precision (%RSD)		
Interday – (Day 1 & Day 2)	NMT 0.18	NMT 1.21
Instrument to Instrument (Inst-1 & Inst-2)	NMT 0.16	NMT 1.12

The procedure was simple, rapid, economical, sensitive, precise and accurate, according to statistical analysis of data and drug recovery data. As a result, it is simple to use for routine quality control analysis. The findings of this study revealed that the proposed method was suitable for determining drug concentration in pharmaceutical formulations with little additive influence. As a result, the suggested method can be used to estimate Sofosbuvir and Velpatasvir in marketed formulations simultaneously.

CONCLUSION

Theproposedmethodisrapid,accurateandsensitive.It uses less solvents and just takes a little time to change the set of conditions. This approach can be used to analyseSofosbuvir and Velpatasvir in bulk and pharmaceutical dose forms in a routine manner. It is free of influence from common excipients found in pharmaceutical preparations and can be used for quality control analysis with ease.

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