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New Analytical Method Development and Validation for Simultaneous Estimation of Sofosbuvir and Velpatasvir in Bulk and Pharmaceutical Dosage Form by RP-HPLC Method

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination Sofosbuvir and Velpatasvirin pharmaceutical dosage form. The column used was Kromosil C_{18}(150mm x 4.6 mm, 5µm)in isocratic mode, with mobile phase containing phosphate buffer andacetonitrile(70:30v/v). The buffer is prepared by adding 1.41gm of sodium dihyrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 3.5 with dil. orthophosphoric acid solution. The flow rate was 1.0ml/ min and effluents were monitored at 260 nm. The retention times of Sofosbuvir and Velpatasvirwere found to be 2.404min and 2.986 min, respectively. The linearity for Sofosbuvir and Velpatasvirwere in the range of 40-240µg/ml and 10-60 µg/ml respectively. The recoveries of Sofosbuvir and Velpatasvirwere found to be 99.64% and 99.25%, respectively. The proposed method was validated and successfully applied to the estimation of Sofosbuvir and Velpatasvirin combined tablet dosage forms.

Keywords: Sofosbuvir, Velpatasvir, Validation, Buffer and ICH Guidelines.

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INTRODUCTION

Chemically, Sofosbuvir is 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. The chemical formula is $C_{22}H_{29}FN_3O_9P$. The molecular formula is 529.458 g/mol. Sofosbuvir (tradename Sovaldi) is a direct acting antiviral medication[1] used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients .

Velpatasvir is chemically, (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. The chemical formula is $C_{49}H_{54}N_8O_8$. The molecular formula is 883.019 g/mol. Velpatasvir is a Direct-Acting Antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C [2], an infectious liver disease caused by infection with Hepatitis C Virus (HCV). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients . Velpatasvir acts as a defective substrate for NS5A (Non-Structural Protein 5A), a non-enzymatic viral protein that plays a key role in Hepatitis C Virus replication, assembly, and modulation of host immune responses. [3]. Different analytical methods have been reported in the literature for the assay of Sofosbuvir and Velpatasvirin pharmaceuticals and include spectrophotometry, HPLC, UPLC and HPTLC. The present study was to establish a simple, sensitive and

low cost RP-HPLC method for simultaneous estimation of Sofosbuvir and Velpatasvir in bulk as well as in other dosage forms. The developed method was validated as per ICH guidelines.

MATERIALS AND METHODS

Materials

Sofosbuvir and Velpatasvir were kindly supplied by Natco. Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A tablet VELPANAT(NATCO)containing 400mg of Sofosbuvir and 100mg of Velpatasvir were used.

Instrumentation

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Methods

Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of buffer (accurately weighed 1.41gm of sodium dihyrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 3.5 with dil. orthophosphoric acid solution) andacetonitrile (70:30 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A Kromosil C_{18} (150mm x 4.6 mm, 5µm) was used for determination. The flow rate was 1.0ml/min and the column was operated at ambient temperature (~30°C). The volume of sample injected was 10 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 260 nm.

Standard Preparation

Accurately weighed and transferred 40mg of Sofosbuvir and 10 mg of Velpatasvir working Standards into a 100 ml clean dry volumetric flask, add 70 ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 4ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent. The final concentrations of Sofosbuvir and Velpatasvir are 160 µg/ml and 40 µg/ml.

Sample Preparation

About
20 tablets were taken and their average weight was calculate d. The tablets were crushed to a fine powder and drug equivalent to 40mg and 10mg were transferred to a 100ml volumetric flask, dissolved in diluent. Transfer 4ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 160µg/ml and 40µg/ml for Sofosbuvir and Velpatasvir.

Method Validation

The developed method was validated as per ICH guidelines [4-6] for its accuracy, linearity, precision, specificity, robustness, limit of detection and limit of quantification by using the following procedures.

System suitability

System suitability and chromatographic parameters were va lidated such as asymmetry factor, tailing factor and number of theoretical plates were calculated.

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Sofosbuvir and Velpatasvirat different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug. The response was found to be linear in the range 40-240µg/ml &10-60µg/ml for Sofosbuvir and Velpatasvir.

Accuracy

Accuracy was performed in triplicatefor various concentrations of Sofosbuvir and Velpatasvir equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. The average % recovery was calculated.

Precision

A) Method Repeatability

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

B) Intermediate Precision (Day to Day variability)

Two days as per test method conducted the study. For Day-1 and Day-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. As per test method two instruments were checked. The relative standard deviation of the results obtained from different and instruments was less than 2.0.

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. The LOD and LOQ of Sofosbuvirwere found to be 0.59 µg/ml and 1.80 µg/ml respectively. The LOD and LOQ of Velpatasvir were found to be 0.29 µg/ml and 0.89 µg/ml respectively.

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

The assay and % purity were calculated for brand VELPANAT (Natco)with label claim 400 mg and 100mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form.

RESULTS AND DISCUSSION

A reverse-phase column procedure was proposed as a suitable method for the simultaneous estimation of Sofosbuvir and Velpatasvirdosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrilein the ratio 70:30v/v was used as mobile phase, which showed good resolution of Sofosbuvir and Velpatasvirpeak. The wavelength of detection selected was 260nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Sofosbuvir and Velpatasvir were about 2.404mins and

2.986mins and none of the impurities were interfering in its assay. The chromatogram of the drugs is shown in Fig. 1. Calibration curve of Sofosbuvir is shown in Fig. 2 and for Velpatasvir is in Fig.3. The observed peak area values for respective concentrations are shown in Table 1.

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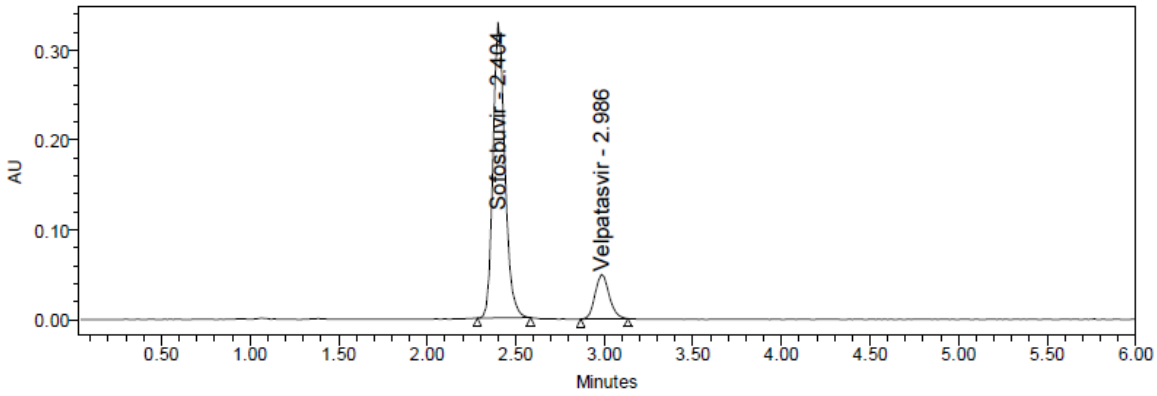

Figure 1: HPLC chromatogram of Sofosbuvir and Velpatasvirin optimized chromatographic conditions

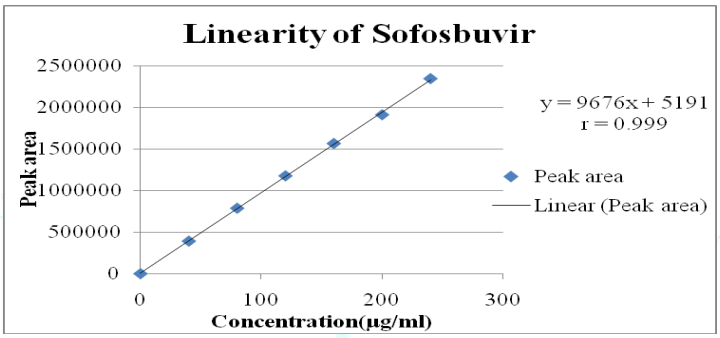

Figure2: Calibration curve of Sofosbuvir in the range 40 to 240µg/ml.

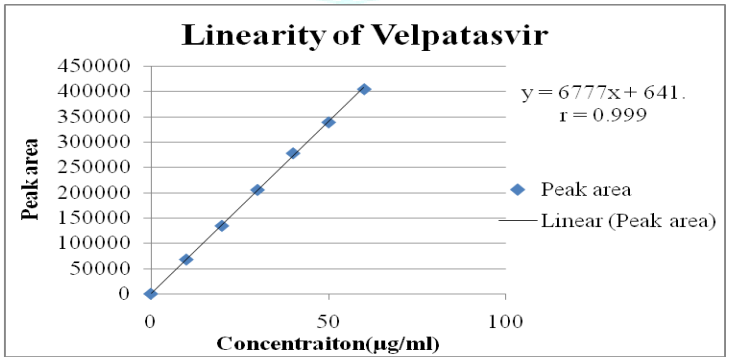

Figure 3: Calibration curve of Velpatasvir in the range 10 to 60µg/ml.

Table 1: Calibration curve of Sofosbuvir and Velpatasvir

S.No	Sofosbuvir			Velpatasvir		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	40	2.405	390764	10	2.987	119279
2	80	2.406	786093	20	2.982	235126
3	120	2.402	1174300	30	2.978	349782
4	160	2.401	1563383	40	2.964	467871
5	200	2.407	1907925	50	2.992	574112
6	240	2.409	2341934	60	2.995	692155

The accuracy data, method precision data, intermediate precision data for day and intermediate precision data relating to change of instrument are shown in Table 2, Table 3, table 4, and Table 5 respectively. Robustness data relating to change in flow rate and robustness data relating to change in mobile phase composition are shown in Table 6 and Table 7 respectively. Results of analysis of laboratory samples are shown in Table 8. Table 9 shows system suitability parameters.

Table 2: Accuracy data

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	40.95	98.97 ± 0.43	10.00	10.04	100.07 ± 0.46
2(n=6)	100%	80.00	80.03	100.34 ± 0.28	20.00	20.96	99.56 ± 0.55
3(n=6)	150%	120.00	119.06	100.42 ± 0.40	30.00	29.97	99.98 ± 0.59

*n=6 (Average of 6 determinations)

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	160	2.403	1562412	40	2.982	272468
2	160	2.405	1565061	40	2.989	277211
3	160	2.405	1568363	40	2.987	271649
4	160	2.406	1566157	40	2.989	270677
5	160	2.408	1566158	40	2.991	273575
6	160	2.401	1561519	40	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	160	1566934	1569202	40	275638	275929
2	160	1567283	1560292	40	272833	274633
3	160	1569474	1563848	40	279383	274182
4	160	1565849	1564122	40	271132	272901
5	160	1563938	1565393	40	270293	270322
6	160	1562384	1563033	40	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	160	1564847	1564283	40	276282	273832
2	160	1565838	1568822	40	272837	272922
3	160	1561934	1562838	40	277927	271973
4	160	1562931	1563848	40	271983	270283
5	160	1562482	1561344	40	277282	272833
6	160	1563013	1563939	40	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)

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::69:31)	1566072	3048	0.75	277789	1720	0.37
2	M.P-2-(BUFFER:ACN::70:30)	1566995	1237	0.30	277045	1356	0.29
3	M.P-3-(BUFFER:ACN::71:29)	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 (VELPANAT)	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)	40 – 240	10 – 60
Regression equation	y = 9676x + 5191	y = 6777x +641
Correlation Coefficient(r)	0.9994	0.9999
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(30:60:10)	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 statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in simultaneous estimation of Sofosbuvir and Velpatasvirin marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of Sofosbuvir and Velpatasvirin bulk and its pharmaceutical dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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