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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD AND STRESS DEGRADATION STUDY OF DETERMINATION OF SOFOSBUVIR AND VELPATASVIR IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Velpatasvir and Sofosbuvir, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Hypersil C18 (4.6 x 250mm, 5µm) column using a mixture of Acetonitrile: Water (50:50% v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 235nm. The retention time of the Velpatasvir and Sofosbuvir was 2.079, 4.045 min respectively. The method produce linear responses in the concentration range of 5-25µg/ml for Velpatasvir, and 20-100µg/ml of Sofosbuvir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Velpatasvir, Sofosbuvir, RP-HPLC, PDA Detection, Validation.

INTRODUCTION

Sofosbuvir is a medication used for the treatment of hepatitis C. It is only recommended with some combination of ribavirin, peginterferon-alfa, simeprevir, ledipasvir, or daclatasvir. Cure rates are 30 to 97% depending on the type of hepatitis C virus involved. Safety during pregnancy is unclear; while, some of the medications used in combination may result in harm to the baby.It is taken by mouth and chemically it is Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate Molecular formula $C_{22}H_{29}FN_3O_9P$ Molecular Weight 529.453 g/mol and Soluble in Methanol, Acetonitrile and water [1-6].
Velpatasvir is an NS5A inhibitor which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes [7]

MATERIALS AND METHODS

Materials and Reagents

The reference standards of velpatasvir and sofosbuvir were procured from SuraPharma abs, Dilshuknagar, Hyderabad, India. The branded tablet formulation Epclusa (sofosbuvir 400 mg and velpatasvir 100 mg) was purchased from the local market. All the HPLC solvents and analytical reagent grade chemicals were purchased from S.D. Fine Chemicals, Hyderabad, India [8-11]

Instrumentation

A Waters HPLC system equipped with a 2695 binary pump, an auto sampler and a 2996 photo diode array detector was employed for the study. The output signal was monitored and processed with Empower software.

Chromatographic conditions

The separation of the drugs was achieved on a Discovery® C18 HPLC Column (250 x 4.6 mm; 5µ

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particle size) by running a mobile phase containing a 60:40 v/v mixture of 0.1% orthophosphoric acid in water and acetonitrile at a flow rate of 1.0 mL/min. The injection volume was 10 µL. The column temperature was maintained at 30°C and the analytes in the eluates were monitored at 240 nm. The run time was 6.0 min. A 50:50 v/v mixture of water and acetonitrile was used as the diluent to prepare drug solutions [12-14].

Preparation of standard solution

Accurately weighed and transferred 10 mg of Velpatasvir&Sofosbuvir working standard into a 10mL of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.15 & 0.6ml of the above Velpatasvir and Sofosbuvir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Tablet sample solution

Twenty tablets of "Epclusa" (velpatasvir 100 mg and sofosbuvir 400 mg) were accurately weighed and the average weight of the tablet was calculated. The tablets were finely powdered and a quantity of the powder equivalent to one tablet was transferred into a 100 mL volumetric flask. 70 mL of the diluent was added to it and sonicated for 5 minutes. Then the volume was made up with the diluent and mixed well to prepare the sample stock solution. This solution was filtered through a 0.45 µm nylon filter. 2.0 mL of the filtrate was transferred to a 20 mL volumetric flask and the volume made up to give final theoretical concentrations of 100µg/mL and 400 µg/mL of velpatasvir and sofosbuvir respectively

Method development and Validation

Different mobile phases were considered for simultaneous separation of the two drugs on a Hypersil C_{18} HPLC Column. Selection of the mobile phase was done on the basis of ideal resolution among velpatasvir and sofosbuvir and also their impurities formed during forced degradation studies. The required chromatographic conditions were optimized. The developed method was validated for precision, specificity, accuracy (recovery), linearity and robustness as per the ICH guidelines.

System Suitability

System suitability was established for initial evaluation of the method before running the sample for the validation parameters. The test was performed according to the USP. The standard solutions prepared as per the proposed method were analyzed. The results of the system suitability study are presented in Figure 3. The acceptance criterion is % RSD ≤ 2.0. A percent RSD of 0.8 indicates good system precision of the method. The tailing factor obtained from the standard injection is 1.49 & 1.35 and Theoretical plates obtained from the standard injection are 6231 & 7184 respectively.

Linearity

The linearity were observed for in the concentration rages from 5-25µg/mL forVelpatasvir and 20-100 µg/mL. The Linearity of the method was demonstrated by preparing different concentrations of drug substance and analyzing as per the proposed method. A plot of the area of the peak as a function of analyte concentration was prepared and its regression equation computed. The linearity data of the two drugs are given in Table 6.and Fig.

Limit of detection and Limit of Quantification

From the above the LOD values of Velpatasvir and Sofosbuvir were found to be 0.56 and 3.6µg/ml respectively. The LOQ values of Velpatasvir and Sofosbuvir were found to be 1.7 and 11.1µg/ml respectively. Thus the method developed was found to be sensitive.

Precision

In the precision study,% RSD was found to be less than 2 % for Velpatasvir 0.6% and Sofosbuvir 0.2 which indicates the system has a good reproducibility for precision studies 5 replicate studies of Velpatasvir and Sofosbuvir formulation(method precision) was performed.% RSD was determined for peak areas of Velpatasvir and Sofosbuvir and the acceptance limits should be NMT 2% and the results were found to be with in the acceptance limits The chromatograms of precision were showed in Figs:7.22-7.26. results were reported in Table:7.25

Accuracy

The accuracy studies were shown as % recovery for Velpatasvir and Sofosbuvir at 50%, 100%,150% ,the limits of recovery should be in range of 98-102% the limits obtained for Velpatasvir and Sofosbuvir were found to be within the limits. Hence the method was found to be accurate. The accuracy studies shows % recovery of the Velpatasvir 100% and Sofosbuvir and the limits of % recovery of drugs were 98-102% and from the above results its indicates that the method was accurate and also revealed that the commonly used exciepients present in the pharmaceutical information do not interfere in the proposed method. the chromatograms of shown in results were shown Tables:7 and 8.

Robustness

FORCED DEGRADATION STUDIES

Acid degradation

Degradation was observed by the additon of 0.5 N HCl

Alkaline degradation

Degradation was observed by the additon of 0.5N NaoH

Thermal degradation
Degradation was observed when the sample solution was kept under heat at 60-80^0 C for 3 hours.

Peroxide degradation
Degradation was observed by the additon of 3% H_2O_2

Photolytic degradation
Degradation was observed by sunlight exposre

Table 1. Optimized Chromatographic condition

Parameters	Chromatographic conditions
Mobile phase ratio	Acetonitrile: Water(50:50% v/v)
Column	Hypersil C18 (4.6×250mm) 5µ
Detector	PDA Detector
Column temperature	40ºC
Wavelength	235 nm
Flow rate	0.9 ml/min
Injection volume	10 µl
Run time	8minutes

Table 2. Result of system suitability parameters

S.no	Parameter	Velpatasvir	Sofosbuvir
1	Retention time	1.933	3.396
2	Theoretical plates	4242	6515
3	Tailing factor	1.35	1.43
4	Area	49607	423559

Table 3. Linearity Results of Velpatasvir

S.no	LinearityLevel	Concentration	Peak Area
1	**1**	5ppm	15065
2	**2**	10ppm	31009
3	**3**	15ppm	46166
4	**4**	20ppm	60569
5	**5**	25ppm	76862
	Correlationcoefficient	0.999	

Table 4. Linearity Results of Sofosbuvir

LinearityLevel	Concentration	Area
1	20ppm	131289
2	40ppm	284775
3	60ppm	427559
4	80ppm	555861
5	100ppm	712514
Correlation coefficient	0.999	

Table 5. Data of LOD and LOQ

Drug	LOD	LOQ
Velpatasvir	0.56	1.7
Sofosbuvir	3.6	11.1

Table 6. Data of precision

No. Injections	VelpatasvirPeak Area	Sofosbuvir Peak Area
Injection1	46054	427962
Injection2	46803	429623
Injection3	46150	427826
Injection4	46056	427829
Injection5	46247	429559

Average	46262	428559.8
S.D	312.7099	943.2246
% RSD	0.675954	0.220092

Table 7. Accuracy Results of Velpatasvir

%Concentration (atspecification Level)	Peak Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Mean Recovery
50%	22938.33	7.5	7.4	99.9	99.6%
100%	45426	15	14.8	98.9	
150%	70093.33	22.5	22.0	100.0	

Table 8. Accuracy Results of Sofosbuvir

%Concentration (atspecification Level)	Peak Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	209357	112.5	112.3	99.8%	99.4%
100%	420697.66	225	223.8	99.4%	
150%	631550.66	337.5	335	99.2%	

Robustness

Table 9. System suitability data of Results for Velpatasvir

Flow Rate (ml/min)	System Suitability Results		
	USP Plate count	USP Tailing	Retention time(min)
Low	4242	1.63	2.291
Actual	4251	1.33	2.079
High	5365	1.56	1.890

Table 10. Data of degradation studies

Type of degradation	Area of sample		Assay content (% w/w)	
	Velpatasvir	Sofosbuvir	Velpatasvir	Sofosbuvir
Acid (0.5N HCl)	40883	400283	89.02	94.6
Base(0.5N NaOH)	41998	407536	91.4	96.3
Peroxide (3% H_20_2)	41927	401372	91.2	94.9
Thermal (at 60-80^0 c)	42838	403321	93.2	95.3
Photolytic (sunlight)	42692	404613	92.9	95.7

Table 11. Summary for RP-HPLC Method

S.No	Parameters	Acceptance criteria	Results obtained
1	System suitability	Theoretical Plates- NLT 2000	Velpa- 4242Sofos-6515
		Tailing factor - NMT 2	Velpa -1.15Sofos -1.78
		Retention time	Velpa -2.033Sofos -4.096
2	Precision	% RSD of Velpa -NLT 2 % RSD of Sofos -NLT 2	Velpa -0.2Sofos -0.8
3	ID Precision	% RSD of Velpa -NLT 2 % RSD of Sofos -NLT 2	**DAY-1** Velpa -0.1 Sofos -0.7 **DAY-2** Velpa -0.3 Sofos -1.0
4	Linearity	Correlation coefficient NLT 0.999	Velpa -0.999 Sofos -0.999
5	Accuracy	Percentage Recovery 98-102%	Velpa -99.6% Sofos -99.4%
6	Limit of Detection	1:3	Velpa -0.5µg/ml Sofos - 13.7 µg/ml
7	Limit of quantitation	1:10	Velpa -1.7µg/ml Sofos - 41.7µg/ml

Fig 1. Chemical Structure of Velpatasvir

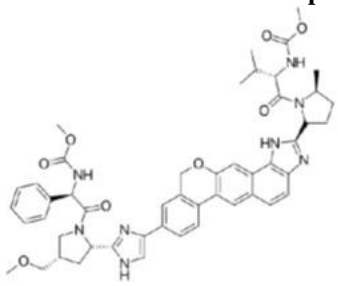


Fig 2. Chemical structure of Sofosbuvir

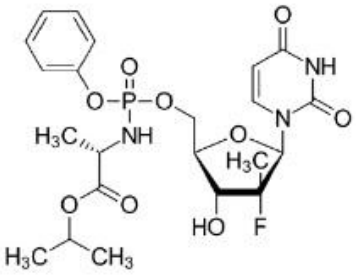


Fig 3. Typical chromatogram of Velpatasvir and Sofosbuvir

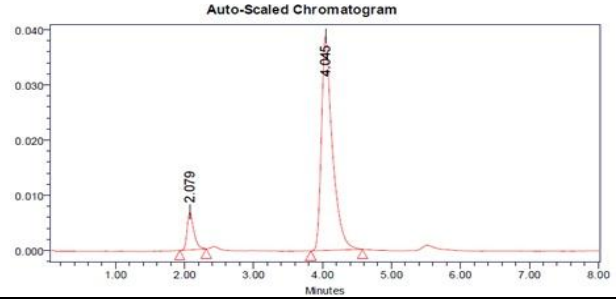


Fig 4. Calibration Curve for Velpatasvir

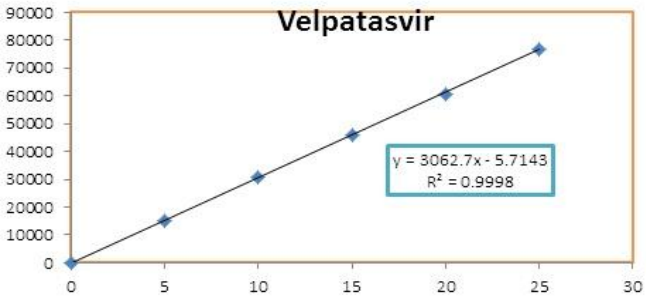


Fig 5. Calibration Curve for Sofosbuvir

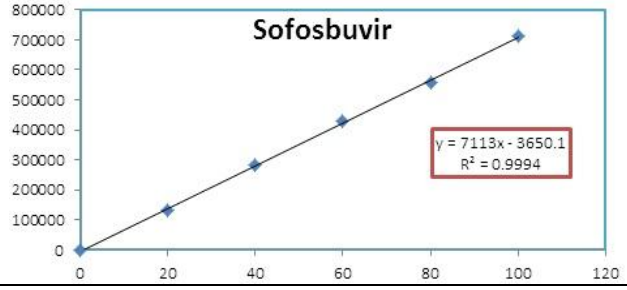


Fig 6. Showing acid degradation

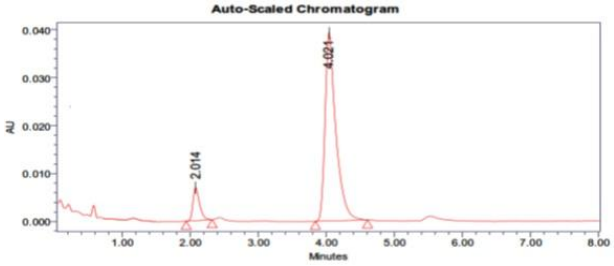


Fig 7. Showing Alkaline degradation

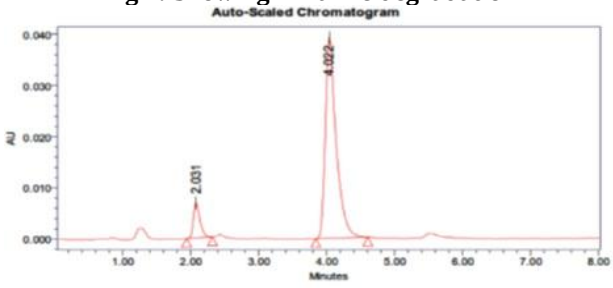


Fig 8. Showing Thermal degradation

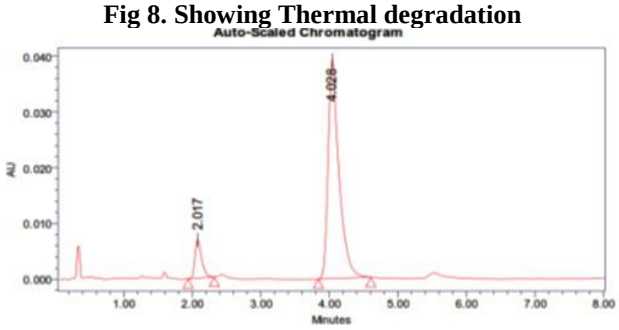


Fig 9. Showing peroxide degradation

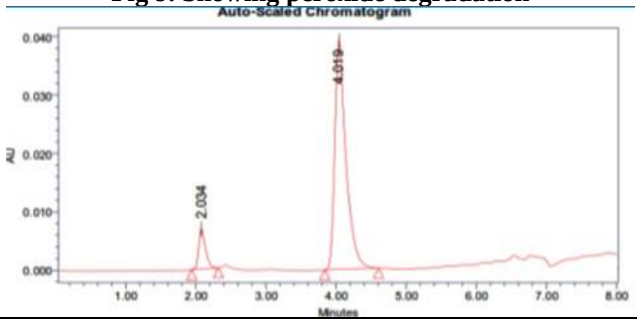
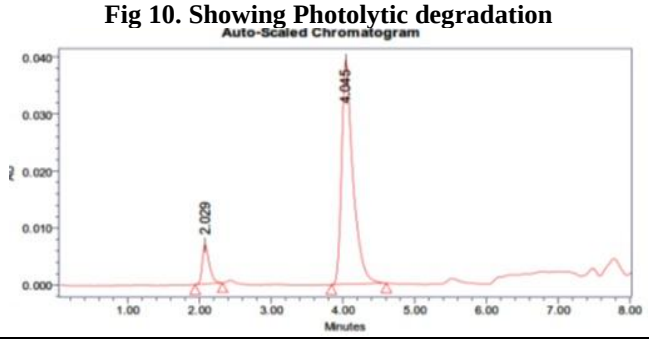


Fig 10. Showing Photolytic degradation



SUMMARY AND CONCLUSION

Summary

RP-HPLC method was developed for simultaneous estimation of Velpatasvir and Sofosbuvir in pharmaceutical dosage form. Chromatographic separation was performed on Hypersil C18 (4.6×250mm) 5µ column, with mobile phase comprising of mixture of Acetonitrile: Water in the ratio of 50:50% **(**v/v), at the flow rate 0.9ml/min. The detection was carried out at 235nm.

CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Velpatasvir and Sofosbuvir in tablet dosage form. It was also proved to be convenient and effective for the determination of Velpatasvir and Sofosbuvir in the bulk and combined dosage form. It inferred the method found to be simple, accurate, precise and linear. The method was found to be have a suitable application in routine laboratory analysis with high degree of accuracy and precision.

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