RJC

RASĀYAN ***J. Chem.***
Vol. 11 | No. 3 |1058 - 1066 | July - September | 2018
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

CONCURRENT ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN RAW AND TABLETS USING STABILITY INDICATING RP-HPLC METHOD

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ABSTRACT

A stability indicating RP-HPLC method is developed and evaluated to quantify sofosbuvir and velpatasvir in raw and tablets forms. The method involves separation and analysis of sofosbuvir, velpatasvir and their stress degradants using Spursil C18 (250 × 4.5 mm and i.d., 5 µm particle size) analytical column with a mixture of potassium dihydrogen phosphate (0.1 M) and methanol (pH 4.5, 60:40 *v/v*) as the mobile phase. The analysis was done using a photodiode array detector at 240 nm. Calibration curves showed linearity in concentration range 10–30 (velpatasvir) and 40–120 µg/mL (sofosbuvir). Good linearity ($R^2 \geq 0.9998$), good precision (RSD ≤ 0.138%, n = 5) and good accuracy (% recovery~100%) for velpatasvir and sofosbuvir were achieved. Stress degradation studies were performed on powdered tablet sample in athe cidic, basic, oxidative, thermal and photolytic atmosphere. The method showed no interference from the degradation products formed by applied stress conditions. Application of the proposed method to the commercially available tablets was carried out successfully. The performance of the proposed method was compared with those from previously published HPLC methods and they were satisfactory.

Key words: hepatitis C virus, sofosbuvir, velpatasvir, stability indicating, analysis, tablets

INTRODUCTION

Hepatitis C is a chronic infection by hepatitis C virus with nearly 130-150 million of the population infected globally[1,2]. Hepatitis C virus is a single-stranded ribonucleic acid virus belonging to the family *Flaviviridae*. The most important objective of hepatitis C virus therapy is to obtain a sustained virological response, resulting in complete eradication of Hepatitis C viral RNA levels, within 24 weeks following completion of treatment[3]. A tablet dosage combination of velpatasvir and sofosbuvir was approved in June 2016 by the United States Food and Drug Administration in treating hepatitis C virus-infected patients[4,5].

Sofosbuvir (a phosphoramidate prodrug) undergoes extensive intracellular metabolism to form deoxy-α-fluoro-β-C-methyluridine triphosphate (active antiviral agent). This active antiviral agent is a defective substrate for non-structural protein 5B which is required for transcription of viral RNA[6]. Velpatasvir inhibits hepatitis C virus non-structural protein 5A and possesses potent antiviral activity against all genotypes. Non-structural protein 5A is required for viral replication[7]. The chemical structures of the sofosbuvir and velpatasvir are given in Fig. -1 and -2.

The sofosbuvir and velpatasvir combination is not yet official in pharmacopoeia. Two articles on the concurrent estimation of sofosbuvir & velpatasvir in tablet dosage were recently reported by Sarath & Rao and Uppalapati & Parimi[8,9].

http://dx.doi.org/10.31788/RJC.2018.1132010

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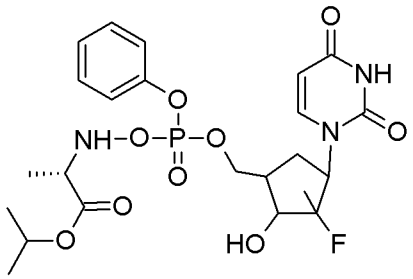
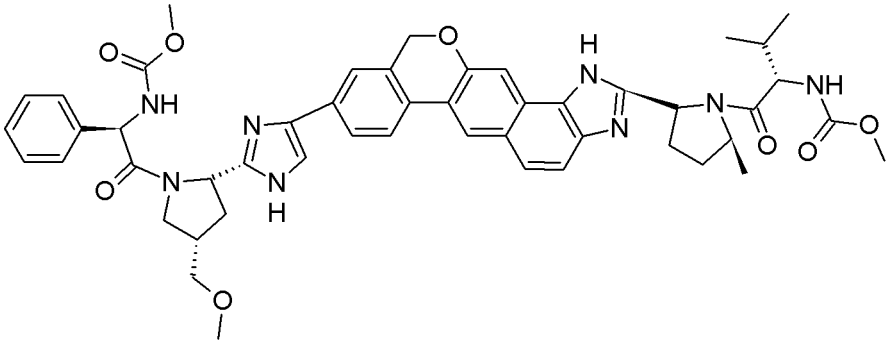
In Sarath & Rao method, separation and analysis were done using Discovery® C18 column with orthophosphoric acid plus acetonitrile (60:40 *v/v*) at a flow rate of 1.0 mL/min[8] as mobile phase. UV detection was performed at 240 nm. Uppalapati & Parimi achieved separation using mobile phase having 0.1% trifluoro acetic acid: methanol (42:58 *vol/vol*) on an XTerra RP18 column[9]. 1.0 mL/min is the flow rate and 269 nm is used for detecting analytes.

Present work describes validated rapid, precise, accurate stability indicating RP-HPLC method in the quantitation of sofosbuvir & velpatasvir combination in raw and tablets.



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

Fig. -1: Chemical Name and Structure of Sofosbuvir



Methyl{(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxycarbonyl)amino]-2-phenylacetyl}-4-(methoxymethyl)-2-pyrrolidinyl]-1H-imidazol-4-yl}-1,11-dihydroisochromeno[4',3':6,7]naphtho[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl}carbamate

Fig.-2: Chemical Name and Structure of Velpatasvir

EXPERIMENTAL

Instrumentation

HPLC system (Waters) with binary HPLC pump (model number 2695), PDA detector (model number 2998) and degasser with 10 µL injection loop were used. The chromatographic data was processed by Waters Empower2 software. Electronic balance ELB 300 and Digisun pH meter were used.

Materials

Lara Drugs Private Limited (Hyderabad, Telangana, India) was kind enough in providing sofosbuvir and velpatasvir reference substances. Sovihep V tablets having 400 mg of sofosbuvir & 100 mg of velpatasvir (Zydus Heptiza, Ahmedabad, India) were obtained from commercial sources. Methanol of HPLC grade was acquired from Merck Pvt Ltd. (Mumbai, Maharashtra, India). Potassium dihydrogen phosphate, orthophosphoric acid, hydrochloric acid, hydrogen peroxide and sodium hydroxide of analytical reagent

grade were acquired from Sd Fine Chemicals Ltd. (Mumbai, Maharashtra, India). Water purified via Milli-Q system was used in the study.

Chromatographic Conditions

The chromatographic separation and analysis of selected drug combination were worked out on a Spursil C18 (250 × 4.5 mm, i.d., 5 µm particle size). The mobile phase used is a mixture of 10^{-1} M potassium dihydrogen phosphate & and methanol (pH 4.5, 60:40 *v/v*). 1 mL/min is the flow rate. Mobile phase filtration using 0.45 µm membrane filter was done and 10 min sonication was performed. The column temperature is 25±2 ºC while analyzing. The analyte elution was monitored using a photodiode array detector set at 240 nm. 10 µL is the injection volume.

Solutions of Stock Standard and Tablet Sample

The stock solution was prepared by weighing 400 mg sofosbuvir & 100 mg velpatasvir, and dissolved in 30 mL of mobile phase in a volumetric flask having 100 ml capacity. A concentration of 4000 µg/mL and 1000 µg/mL of sofosbuvir and velpatasvir was achieved by the mobile phase to the final volume of the volumetric flask. The prepared solution was further diluted using mobile phase to attain working standards having concentration 10, 15, 20, 25 & 30 µg per mL for velpatasvir and 40, 60, 80, 100 & 120 µg per mL for sofosbuvir.

Ten tablets of Sovihep V (400 mg sofosbuvir and 100 mg velpatasvir /tablet) were powdered. Tablet powder equal to 400 mg sofosbuvir & 100 mg velpatasvir is accurately weighed into a volumetric flask having 100 ml capacity and mixed with 30 milli liters mobile phase. Sonication of solution performed for 20 minutes and filled by mobile phase for obtaining a final concentration of 1000 µg/mL velpatasvir and 4000 µg/mL sofosbuvir. Filtration of the solution was through 0.45 µm membrane. For analyzing the analytes, the above-prepared tablet sample solution was diluted further to reach a concentration of 80 µg/mL and 20 µg/mL sofosbuvir and velpatasvir, respectively with the mobile phase.

Degradation Study

In this study, acidic, basic, oxidative, thermal, and photolytic degradation of tablet solution (20 µg/mL velpatasvir & 80 µg/mL sofosbuvir) were studied[10]. Acid and base degradation studies were performed using tablet powder equivalent to 400 mg sofosbuvir and 100 mg velpatasvir in HCl (0.1 N, 10 mL) and NaOH (0.1 N, 10 mL), respectively and sonicated at room temperature for 30 min. For oxidative degradation, the same concentration of tablet sample was used in 30% hydrogen peroxide (10 mL) followed by sonication at room temperature for 30 min. Thermal and photolytic degradation studies were performed on tablet powder (equivalent to 400 mg sofosbuvir and 100 mg velpatasvir). The tablet powder was exposed to 105 ºC for 30 min in oven (thermal degradation) or to sun light for 1 day (photolytic degradation). After degrading, tablet solution was prepared as explained in "stock standard and tablet sample solutions" section.

After the application of stress on the tablet sample, the stressed samples dilution was done by mobile phase to a concentration of 20 µg per mL velpatasvir & 80 µg per mL sofosbuvir for analysis. The degraded samples are filtered and then injected into the HPLC system. Calculation of peak area for velpatasvir & sofosbuvir were noted from the respective chromatogram. Working standard solution at the same concentration level was used to calculate the percentage of the drug remained in each degradation condition. The peak purity of velpatasvir and sofosbuvir was also determined in all the degradation conditions.

RESULTS AND DISCUSSION

Method Optimization

Parameters of chromatography parameters – a time of retention, peak tailing, theoretical plates count, and resolution were determined to optimize the method. For that, trials are carried out - different mobile phase ratios and different stationary phase types, with temperature difference, values of pH and flow rate. On this basis method is employed to separate velpatasvir and sofosbuvir from themselves and also from stress degradants, Spursil C18 (250 × 4.6 mm, 5 µm) column having temperature 25±2 ºC was selected which gave good symmetric and sharp peaks. Based on less analysis time, peak response, peak symmetry and

column efficiency, a mixture of methanol and 10^{-1} M potassium dihydrogen orthophosphate and methanol (40:60 *v/v*) was selected as mobile phase with adjustment pH to 4.5 units and 1.0 millilitre/minute flow rate, using photodiode array detector, a wavelength of 240 nm was selected as detection wavelength. The chromatographic parameters optimized exhibit a good peak shape, resolution and a good number of theoretical plates. The typical chromatogram of velpatasvir and sofosbuvir by the developed method is presented in Fig. -3.

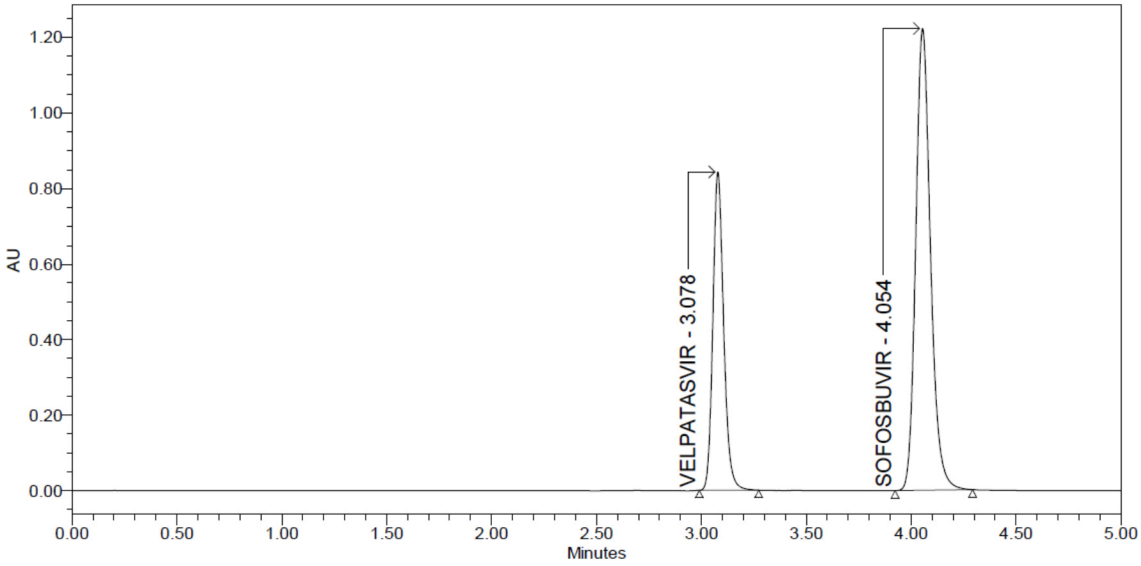


Fig.-2: Typical Chromatogram of Velpatasvir and Sofosbuvir by the Developed Method

Method Validation

Validation was performed by referring ICH guidelines[11]. HPLC Suitability, linearity, selectivity, accuracy, sensitivity, precision, specificity and robustness were determined.

System Suitability Study

System suitability established by five consecutive injections with the same working standard solutions. Parameters considered were: USP plate count, USP tailing, USP resolution, peak areas' and retention times' relative standard deviation of velpatasvir and sofosbuvir. The values for the system suitability parameters of the method, as presented in Table- 1, are within acceptance limits.

Table-1: System suitability parameters for velpatasvir and sofosbuvir

Sample Name.	Retention time	Peak Area	Plate count	Tailing factor	Resolution
Velpatasvir (20 µg/mL)					
A	3.089	2443324	17609	1.16	-
B	3.088	2446997	17643	1.13	-
C	3.090	2431515	17668	1.13	-
D	3.088	2446115	17338	1.13	-
E	3.090	2426416	17340	1.14	-
Average	3.089	2438873	17520	1.138	-
RSD	0.032	0.382	0.949	1.146	-
Recommended limit	RSD ≤ 2	RSD ≤ 2	> 2000	≤ 2	-
Sofosbuvir (80 µg/mL)					
A	4.061	5979429	16412	1.2	8.66
B	4.059	5943909	16363	1.2	8.59

C	4.061	5962666	16396	1.2	8.59
D	4.061	5930444	16458	1.2	8.59
E	4.062	5947656	16444	1.21	8.58
Average	4.061	5952821	16415	1.202	8.602
RSD	0.027	0.316	0.231	0.372	0.380
Recommended limit	RSD ≤ 2	RSD ≤ 2	> 2000	≤ 2	> 1.5

Selectivity

The selectivity of the optimized HPLC method was examined with standard working solution of velpatasvir (20 µg per mL) & sofosbuvir (80 µg per mL) relative to the placebo blank, blank mobile phase and tablet sample solution (velpatasvir -21 µg/mL and sofosbuvir -80 µg/mL) (Fig. -4). No interference was observed by coelution of the excipients in placebo blank, components of the mobile phase at the same retention time of velpatasvir and sofosbuvir at 240 nm, demonstrating the selectivity of this method. The time of velpatasvir & sofosbuvir retention in working solution and tablet solution are alike.

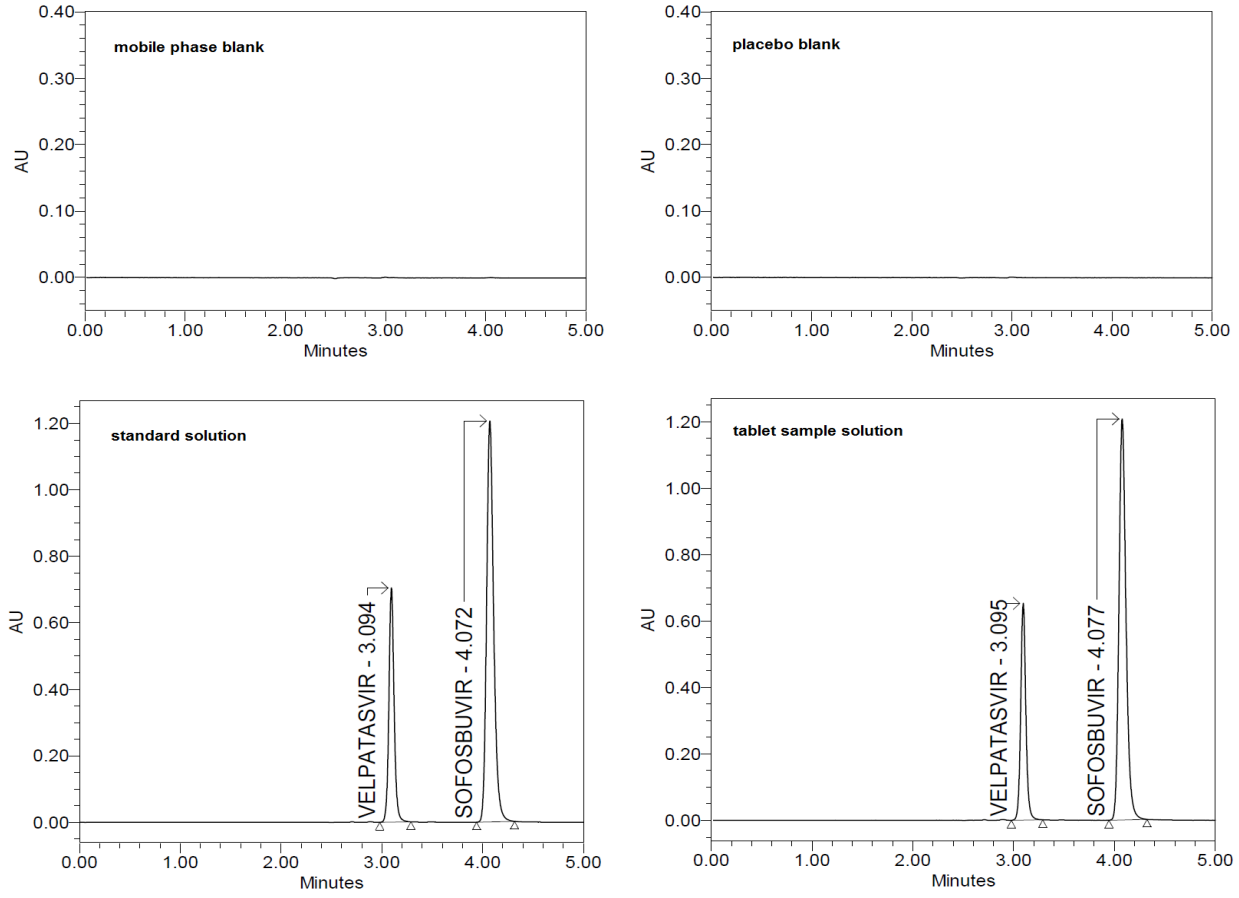


Fig.-4: Chromatograms of Selectivity Study

Linearity and Sensitivity

Linearity was done by preparing standard solutions of velpatasvir and sofosbuvir at five concentration levels. The linearity of detector response for velpatasvir and sofosbuvir was verified by prepared solutions in concentration range 10-30 and 40-120 µg per mL, respectively. Peak area of each sample against respective concentration of analytes was found to be linear. Correlation coefficient of velpatasvir & sofosbuvir are 0.9998 & 0.9999. Linearity results were presented in Table-2.

This method's sensitivity was represented as limits of quantification (LOQ) and detection (LOD). Determining detection and quantitation limits is based on the signal-to-noise ratio with 3:1 & 10:1. Determined LOD values and LOQ values for velpatasvir and sofosbuvir were shown in Table-2.

Table-2: Regression Analysis and Sensitivity of Velpatasvir and Sofosbuvir

Parameter	Velpatasvir	Sofosbuvir
Linearity (µg/mL)	10-30	40-120
Equation of regression (A= SC + I)	y = 12198 x - 2111	y = 74403 x + 1087
Slope (S)	12198	74403
Intercept on Y-axis (I)	-2111	1087
Regression coefficient (R^2)	0.9998	0.9999
LOD (µg/mL)	0.186	0.428
LOQ (µg/mL)	0.620	1.609

A = Peak area of analyte; I = analytes' concentration (µg/mL)

Precision and Accuracy

For precision studies, the same standard solutions of velpatasvir and sofosbuvir were injected 6 times into the HPLC system on the same day. The percentage RSD values calculated for peak areas of velpatasvir and sofosbuvir were less than 0.2 % (Table-3) indicating the precise assay with the developed HPLC method. For accuracy, the percentage recovery was calculated for both active ingredients. The results (Table-3) are acceptable with good percent recovery.

Table-3: Precision and Accuracy Evaluation for the Developed Method

Injection Name.	Velpatasvir (40 µg/mL)		Sofosbuvir (80 µg/mL)	
	Area of peak	Recover %	Area of peak	Recover %
A	2432746	99.35	5953930	99.72
B	2438324	99.58	5954860	99.73
C	2438753	99.6	5957679	99.78
D	2431867	99.31	5956388	99.76
E	2433053	99.36	5956731	99.77
F	2438217	99.57	5955089	99.74
Mean	2436043	99.48	5956149	99.76
RSD	0.136	0.138	0.020	0.201

Recovery Test

Further evaluation of the accuracy of the method was carried through recovery test. Recovery test was determined by means of the standard addition method. The recovery experiments were performed by adding velpatasvir and sofosbuvir standards at three concentration levels to the placebo for three times. The recovery test results are summarized in Table 4. Hence, the obtained results indicated that the developed HPLC method was accurate enough for simultaneous quantitative evaluation of aspirin and pravastatin. There was no interference noticed from the common excipients of the tablet.

Table-4: Recovery of Velpatasvir and Sofosbuvir by the Developed Method

Concentration (µg/mL)		Recover %	Average %	Concentration (µg/mL)		Recover %	Average %
added	found			added	found		
Velpatasvir				Sofosbuvir			
9.90	9.95	100.48	100.40	40.00	39.92	99.81	99.63
9.90	9.96	100.65		40.00	39.80	99.50	
9.90	9.91	100.06		40.00	39.83	99.58	
19.80	19.90	100.51	100.36	80.00	79.73	99.67	99.71
19.80	19.85	100.25		80.00	79.81	99.76	

19.80	19.86	100.30		80.00	79.76	99.70	
29.70	29.83	100.45	100.47	120.00	119.61	99.68	99.68
29.70	29.87	100.58		120.00	119.64	99.70	
29.70	29.81	100.38		120.00	119.60	99.67	

Robustness

Under the slightly varied chromatographic conditions (mobile phase's flow rate -±0.1 mL/min & temperature in the column - ±2 ºC), velpatasvir and sofosbuvir peaks were well separated and there was no significant change in the system suitability parameters (Table-5), which illustrated the robustness of the method.

Table-5: System Suitability Values for Velpatasvir and Sofosbuvir under Slightly Varied Chromatographic Conditions

Investigated factors	Values	Plate Count	Tailing in peak	Resolution
Velpatasvir (40 µg/mL)				
Flow rate (mL/min)	1.0 - 0.1	21895	1.14	-
	1.0 + 0.1	19510	1.16	-
Temperature (ºC)	25 - 2	20992	1.18	-
	25 + 2	20742	1.16	-
Sofosbuvir (80 µg/mL)				
Flow rate (mL/min)	1.0 - 0.1	17480	1.20	9.23
	1.0 + 0.1	15889	1.19	8.92
Temperature (ºC)	25 - 2	16511	1.20	8.91
	25 + 2	16891	1.20	9.06

Degradation Studies

Solutions of velpatasvir and sofosbuvir tablet are stressed under acidic, oxidative, alkali, thermal & photolytic conditions. Method's ability of stability indication and specificity are tested pertaining to the above-said conditions. Results of degradation studies are provided in Table-6. Degraded sample's chromatograms shown in Fig. -5. The degradation percentage was estimated from the peak area obtained in degradation conditions and it was compared with the assay of the nondegraded condition.

Both the drugs were degraded in all the stress conditions applied. In the entire degradation conditions, one degradant peak is observed. From the results, it was observed that the velpatasvir is degraded more in thermal & less in oxidative degradations. Sofosbuvir degradation is more in acid condition but less in thermal degradation. The peak purity profile of velpatasvir and sofosbuvir was determined. The less value of purity angle than purity threshold value showed that velpatasvir & sofosbuvir peaks are homogeneous in every condition of degradation. Method's nature of stability indication & specificity is demonstrated because degradation products of applied stress have no any effect on the detection and quantitation of velpatasvir and sofosbuvir.

Comparison of the Proposed Method with Reported Methods

Two HPLC methods are done on simultaneous evaluation of velpatasvir & sofosbuvir in tablet forms[8,9]. Performance of reported and proposed HPLC methods is summarized in Table-7. From the values in the Table-7, it was observed that the proposed method have advantages of being more quick, precise & accurate than the reported HPLC methods. The less runtime in the proposed method not only reduces the single analysis time but also decreases the utilization of solvents. Thus the proposed method is economical than the reported HPLC methods. Both the reported methods were not fully validated. The LOD and LOQ are not presented in the Sarath & Rao[6] method. The specificity and selectivity are not reported in Uppalapati & Parimi[7] method.

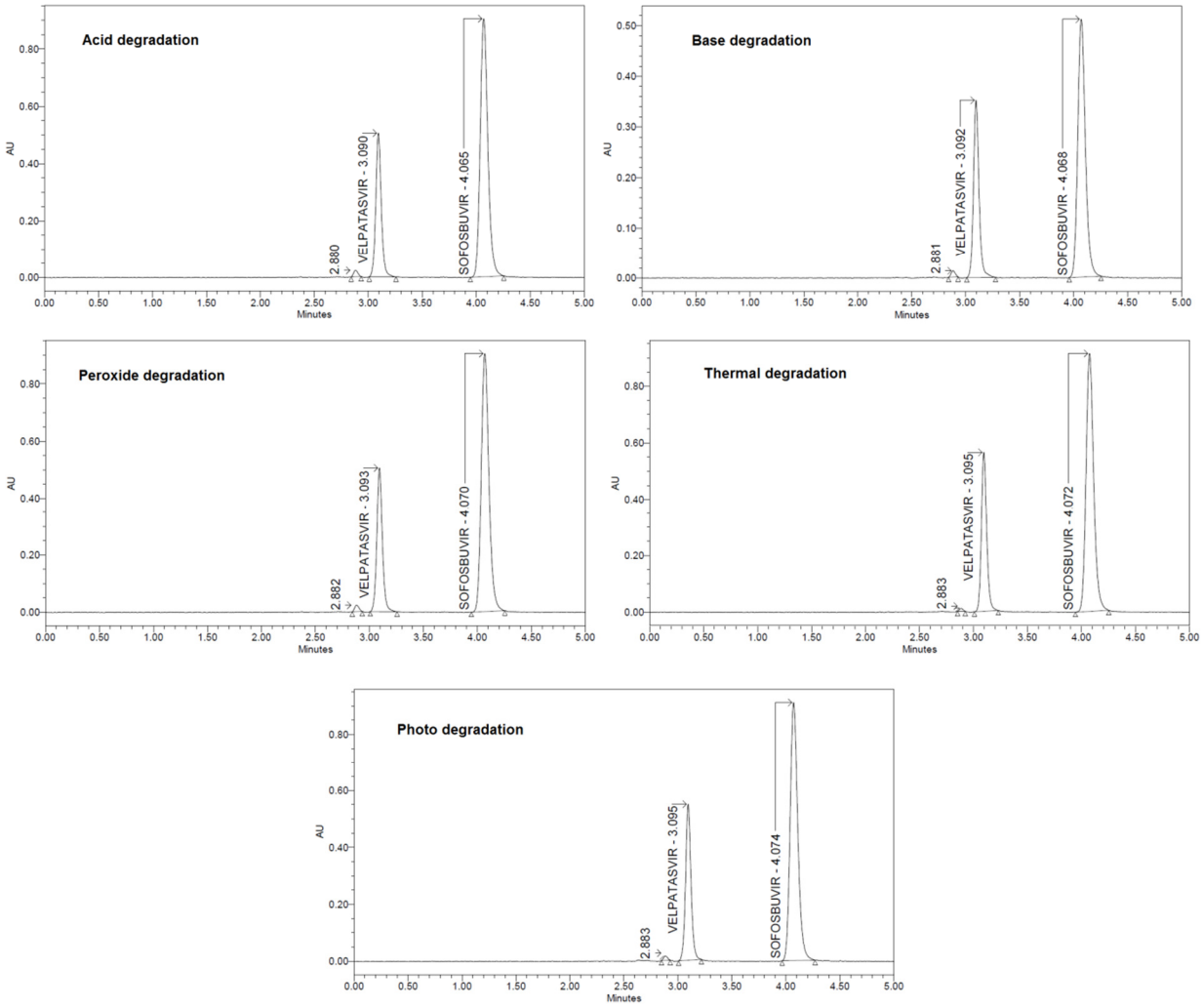


Fig.- 4: Chromatograms of stress testing

Table-6: Stress Testing of Velpatasvir and Sofosbuvir

Degrading type	Area of peak	Recover %	Degraded %	Purity	
				Angle	Threshold
Velpatasvir (40 µg/mL)					
Acid	2282112	93.20	6.80	0.420	0.908
Base	2297127	93.81	6.19	0.321	0.826
Oxidative	2304684	94.12	5.88	0.283	0.892
Heat	2272016	92.79	7.21	0.377	0.912
Sunlight	2295250	93.73	6.27	0.386	0.924
Sofosbuvir (80 µg/mL)					
Acid	5526135	92.55	7.45	0.354	0.525
Base	5543131	92.84	7.16	0.231	0.425
Oxidative	5529439	92.61	7.39	0.323	0.528
Heat	5585557	93.55	6.45	0.205	0.432
Sunlight	5568801	93.27	6.73	0.299	0.443

CONCLUSION

In this study, an RP-HPLC method which is stability indicating was optimized and validated for determining velpatasvir & sofosbuvir in raw and tablets. The method was rapid, cost-effective, accurate &

precise with good selectivity and specificity. Results of this method validation showed that the method was satisfactory and so this method can be applicable in regular quality control laboratories.

Table-7: Comparison of the Proposed Method with Reported HPLC Method

Drug	Run time (min)	Flow rate (mL/min)	Range µg/mL	LoD µg/mL	LoQ µg/mL	RSD %	Recover %	Reference
Vel	6	1.0	25.293-252.928	NR	NR	0.13-0.55	99.98-100.64	Sarath & Rao [8]
Sof			100.339-1003.391	NR	NR	0.37-0.98	98.96-100.15	
Vel	8	1.0	20-60	0.001	0.003	0.42	98.80-100.50	Uppalapati & Parimi [9]
Sof			80-240	0.005	0.02	0.25	99.30-100.50	
Vel	5	1.0	10-30	0.186	0.620	0.136-0.138	100.36-100.47	Proposed method
Sof			40-120	0.482	1.609	0.020-0.021	99.63-99.71	

Vel – velpatasvir; Sof – sofosbuvir; NR – not reported.

ACKNOWLEDGMENT

Authors thank M/s Lara Drug Private Limited, Hyderabad (Telangana, India) for providing gift samples, sofosbuvir and velpatasvir and also to CMR college of Pharmacy, Medchal for permitting to avail the facilities for research work.

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