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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Sofosbuvir and Velpatasvir in Tablet dosage form. Chromatogram was run through StandardChromosol C18 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1%OPA: Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 260.0 nm. Retention time of Sofosbuvir and Velpatasvir were found to 2.298 min and 2.906 %RSD of the Sofosbuvir and Velpatasvir were and found to be 0.2 and 0.6 respectively. %Recovery was obtained as 100.15% and 99.65% for Sofosbuvir and Velpatasvir respectively. LOD, LOQ values obtained from regression equations of Sofosbuvir and Velpatasvir were 0.53, 1.61 and 0.22, 0.67 respectively. Regression equation of Sofosbuvir is y = 1917.x+1750, y = 1283x+2441of Velpatasvir. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION

Pharmaceutical Analysis is that centre branch of drug store training and research, which is advancing speedy. It can be arranged as union of new medications particles and pharmaceutical investigation.The liquid chromatographic techniques, the pivoted arrange systems in perspective of balanced silica offers the most imperative probability of triumphs. In any case, an extensive number of (structure) factors (parameters) impact the selectivity and the assurance. Trade legitimate methods are made for the prescription thing to diminish the cost and time[1]. Then evaluate the best division condition from trial runs. In the wake of improving the separation condition, favour the procedure for release to routine research focus.Sofosbuvir and Velpatasvir are white and light tan crystalline powder, both the medicine are freely soluble in methanol and practically insoluble in water[2]. It inhibits genotype one to six hepatitis c virus (HCV) RNA replicons in-vitro and has high sustained virologic response (SVR) rates. The literature survey on HPLC and spectrophotometric methodology for Velpatasvir and Sofosbuvir in together with alternative medicine has been extensively studied[4].From the literature survey, it has been noted that High performance liquid chromatographic activity ways were reportable within the estimation of Sofosbuvir and Velpatasvir[5]. Thus this analysis paper describes the assessment of Sofosbuvir and Velpatasvir tablet dosage form adopting High

performance liquid chromatographic (HPLC) methodology.

MATERIALS AND METHOD:

Instrumentation: Electronics Balance-Denver, p^H meter -BVK enterprises, Ultrasonicate-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Sofosbuvir and Velpatasvir solutions.

Materials and Reagents: Sofosbuvir and Velpatasvir pure drugs (API) Received from lab.Combination Sofosbuvir and Velpatasvir tabletsNatco Pharma, Received from pharmacy.Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid (OPA). Alltheabovechemicals andsolvents are obtained from Rankem chemicals pvt. Ltd.

Method:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 200 mg of Sofosbuvir, 50 mg of Velpatasvirand transferred to 50ml volumetric flasks and3/4 thof diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labelled as Standard stock solution. (4000µg/ml of Sofosbuvir and 1000µg/ml Velpatasvir)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (400µg/mlof Sofosbuvir and 100µg/ml of Velpatasvir)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated,then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (4000µg/ml of Sofosbuvir and 1000µg/ml of Velpatasvir)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(400µg/ml of Sofosbuvir and 100µg/ml of Velpatasvir)

Preparation of buffer:

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

RESULTS AND DISCUSSION:

20µL of the blank, stock and sample was administered into the chromatographic system and areas for the peak were used for computation with flow rate 1ml/min, column employed chromosol C18 (4.6 x 150mm, 5µm), detector wavelength 260nm, column temperature 30°C, run time 5 min. diluent used were water and acetonitrile in the ratio 50:50 hence both peaks have good resolution, tailing Factor, theoretical plate count and resolution. Sofosbuvir and Velpatasvir was eluted at 2.298min and 2.906 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.The optimized chromatogram given fig.1

Validation Parameters:

System suitability:

All the system suitability parameters were within the range and satisfactory as per ICH guidelines[3]. According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. The results were given in Table 1 and Fig. 2.

Specificity:

Retention times of Sofosbuvir and Velpatasvir were 2.309min and 2.917 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific and the result were given in fig. 2.

Fig.1: Optimized Chromatogram

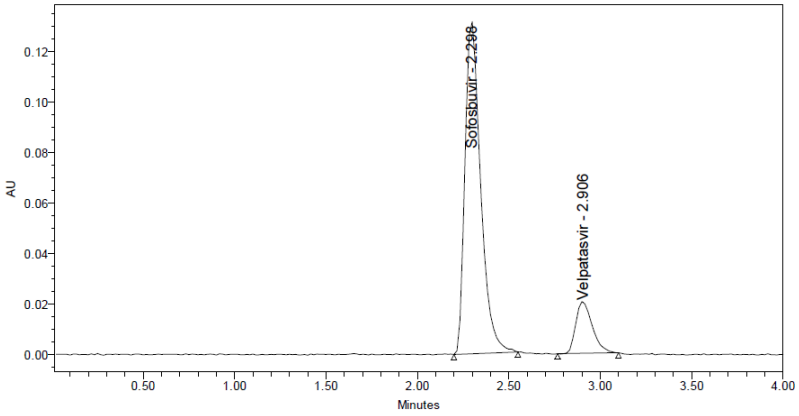


Table 1:Systemsuitability parameters forSofosbuvir and Velpatasvir

S. No	Sofosbuvir			Velpatasvir			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.285	3408	1.42	2.890	4747	1.27	3.6
2	2.290	3470	1.43	2.901	5112	1.28	3.7
3	2.300	3496	1.38	2.907	4640	1.35	3.7
4	2.306	3532	1.46	2.909	4696	1.33	3.6
5	2.306	3517	1.44	2.916	4969	1.36	3.7
6	2.309	3664	1.45	2.917	5591	1.26	3.8

Fig. 2:SystemsuitabilityChromatogram

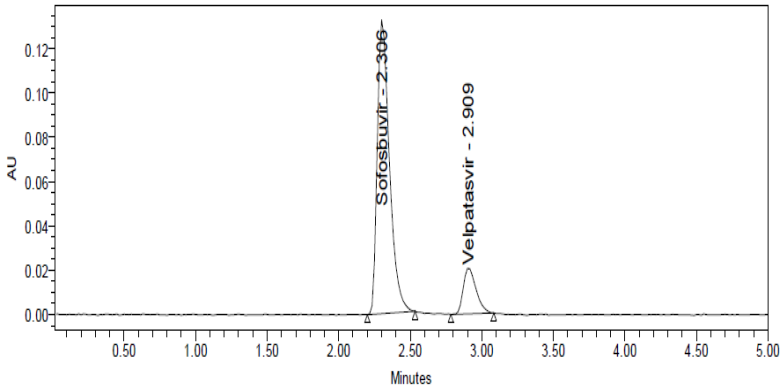


Table 3: Linearity table for Sofosbuvir and Velpatasvir

Sofosbuvir		**Velpatasvir**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
100	192016	25	36417
200	382703	50	68094
300	584004	75	96949
400	781101	100	132630
500	943583	125	163811
600	1156003	150	193051

Fig. 3: CalibrationcurveofSofosbuvirand Velpatasvir

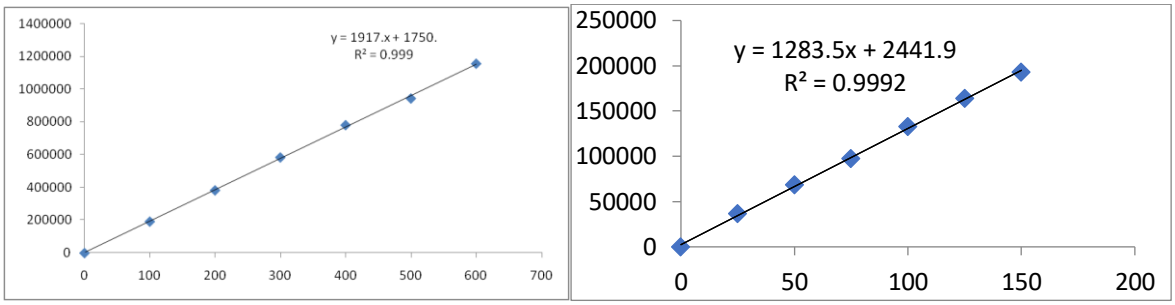
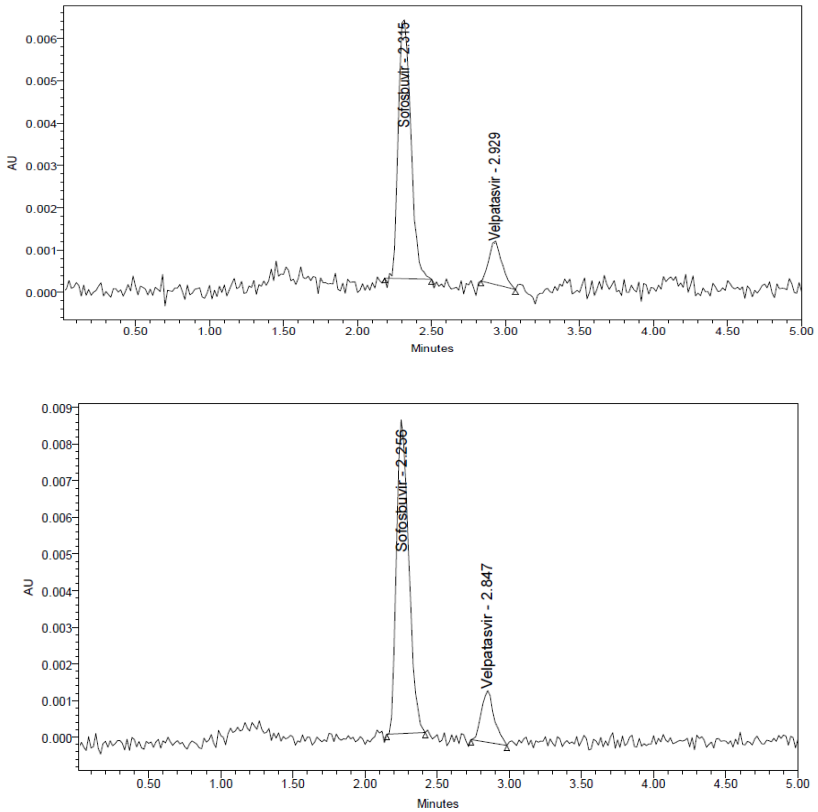


Table 4: System precision table of Sofosbuvir and Velpatasvir

S. No	Area of Sofosbuvir	Area of Velpatasvir
1.	780152	130171
2.	787867	132928
3.	788979	131381
4.	783907	132590
5.	782071	132172
6.	785353	130805
Mean	784722	131675
S.D	3376.0	1073.3
%RSD	0.4	0.8

Fig. 4:LOD& LOQ Chromatogram ofStandard drugs Sofosbuvir and Velpatasvir



Linearity: Six linear concentrations of Sofosbuvir (100-600µg/ml) and Velpatasvir (25-150µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Sofosbuvir was y = 1917.x+ 1750 and of Velpatasvirwas y = 1283x + 2441 Correlation coefficient obtained was 0.999 for the two drugs. The results are mentioned in table 3 and fig. 3.

Precision: From a single volumetric flask of working standard solution six injections was given and the average area, standard deviation and % RSD was calculated for two drugs.% RSDobtained as 0.4%and 0.8% respectively for Sofosbuvir and Velpatasvir. As the limit of Precision was less than "2" the system precision was passed in this method. The results was given in table 4.

Repeatability: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.2% and 0.6% respectively forSofosbuvir and Velpatasvir. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.15% and 99.65% for Sofosbuvirand Velpatasvirrespectively.

Sensitivity: LOD for Sofosbuvir and Velpatasvir were noted as 0.53µg/ml and 0.22µg/ml severally.LOQ for Sofosbuvir and Velpatasvir were noted as 1.61 µg/ml and 0.67 µg/ml severally.

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (45B:55A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

CONCLUSION:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Sofosbuvir and Velpatasvir in Tablet dosage form. Retention time of Sofosbuvir and Velpatasvir were found to be to 2.298 min and 2.906. The sample recoveries of said formulations were agreed with individual label claim amount as per ICH guidelines. Hence the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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