Gollapalli Nagaraju et al /J. Pharm. Sci. & Res. Vol. 11(8), 2019, 3035-3042

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Simultaneous Estimation of Daclatasvir and Sofosbuvir in Tablet Dosage form by Reverse Phase High-Performance Liquid Chromatography

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Abstract

A simple, rapid reversed-phase high performance liquid chromatographic method had been developed and validated for estimation of Daclatasvir and Sofosbuvir in tablet dosage form. The estimation was carried out on Inertsil ODS-C_{18} column (250 x 4.6 mm, 5µ) column with a mixture of Acetonitrile: Methanol: 0.1% Triethylamine buffer (pH-3.0) 25:35:40 (v/v/v) as mobile phase. UV detection was performed at 250 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time was 2.09 and 3.50 min for Daclatasvir and Sofosbuvir respectively and total run time was 6.0min at a flow rate of 1.0 mL/ min. The calibration curve was linear over the concentration range of 5.0-25.0 µg/ mL for Daclatasvir and 2.0-10.0 µg/ mL for Sofosbuvir. The LOD and LOQ values were found to be 0.313 and 0.948 µg/ mL for Daclatasvir and 0.021 and 0.065 µg/mL for Sofosbuvir, respectively. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for the simultaneous estimation of Daclatasvir and Sofosbuvir in tablet dosage form.

Keywords: Sofosbuvir, Daclatasvir; RP- HPLC; Validation, Chromatography

INTRODUCTION

Hepatitis C is a comprehensive liver disease produced by the hepatitis C virus (HCV) and can increase liver cirrhosis, liver failure, liver cancer and liver transplantation. The standard treatment for HCV is pegylated-interferon (Peg-IFN) and ribavirin (RBV) whoever these agents caused side effects such as bacterial infections, anemia, hematological toxicity, and neutropenia and anorectal symptoms.

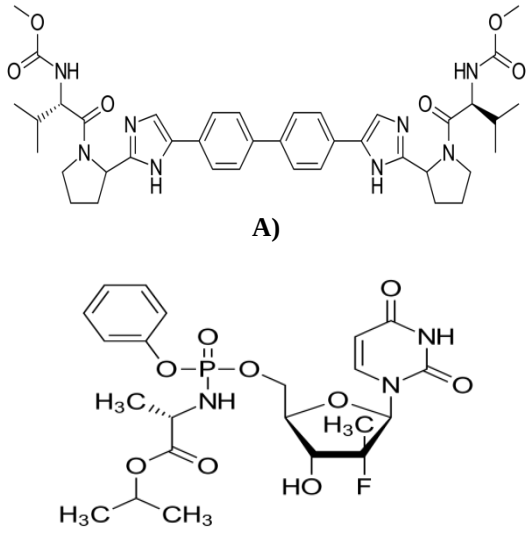
Telaprevir and boceprevir were the first generation direct-acting protease inhibitors that developed and approved for the treatment of genotype I chronic hepatitis C However, they have to be co-administered with interferon and ribavirin therefore they were associated with their common side effects so their effectiveness were limited [1-2].

Second-generation direct-acting antiviral drugs were developed and aimed to have a high pangenotypic activity with fewer undesirable side effects. These drugs include daclatasvir and sofosbuvir. Both medicines have effective antiviral activity and genotypic coverage [3-5].

Daclatasvir, Methyl [(2S)-1-{(2S)-2-[4-(4'-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl) amino]-3-methylbutanoyl}-2-pyrrolidinyl]-1H-imidazol-4-yl}-4-biphenylyl)-1Himidazol-2-yl]-1-pyrrolidinyl}-3-methyl-1-oxo-2-butanyl] carbamate, is a nucleotide analogue NS5A polymerase inhibitor [6].

Sofosbuvir,(S)-Isopropyl2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy) (phenoxy) phosphorylamino) propanoate, is a nucleotide analogue HCV NS5B polymerase inhibitor that is used in the treatment of chronic hepatitis C genotypes 1,2,3 or 4 [21].

The sofosbuvir and daclatasvir combination is associated with a high rate of SVR4 in difficult-to-treat patients infected with genotype 1 or 4. Combination with ribavirin increases the SVR rate in cirrhotic and treatment experienced patients with no additive effect of extension of treatment from 12 to 24 weeks. Since patient compliance is an important point in the treatment so taking the two drugs in one tablet will be a better choice. On another hand, the combined therapy is economically reduced the cost of the treatment and this will give a chance for many companies to formulate the three drugs in one tablet sooner. Additionally, the co-administered drugs might affect each other and there is no sufficient information about drug-drug interaction and thus the establishment of separation method is of great importance [31].



A)

B)

Fig.1: Chemical Structures of A) Daclatasvir B) Sofosbuvir

Literature survey reveals that there are few reported HPLC [6-11] and UV [12], UHPLC [13] and LC-MS/MS [14-19] for sofosbuvir and HPLC methods [21-27], UV [28-30] for daclatasvir individually and simultaneous estimation with different drugs like ledipasvir [31-35], velpatasvir [36-37] and simeprevir [38].

The reported methods have some drawbacks in terms of sensitivity, ruggedness and robustness. This study describes a validated RP-HPLC method for the simultaneous quantitative detection of sofosbuvir and daclatasvir in its pure form and which is commercially available in tablet form. This method was more sensitive than the previously reported HPLC methods. The study was analytically validated according to the ICH guidelines [39-40]. The purpose of this study was to develop simple, rapid, precise and accurate RP-HPLC method for the simultaneous estimation of daclatasvir and sofosbuvir in combined tablet dosage form.

MATERIALS AND METHODS

Instrumentation

Chromatographic separation was performed on a Agilent chromatographic system equipped with 1200 series isocratic pump; Rheodyne injector with 20 µL fixed volume loop, variable wavelength programmable UV detector and the output signal was monitored and integrated by EZICHROME ELITE Chromatographic Software. Double beam UV-Visible spectrophotometer (Labindia-3120) was used to carry out spectral analysis and the data was recorded by UVWIN-5 software. Ultrasonicator (1.5L) was used for degasification of mobile phase and samples. Standard and sample drugs were weighed by using shimadzu electronic analytical balance (AX-220) and pH of the mobile phase was adjusted by using systronics digital pH meter.

Chemicals and solvents

All chemicals and reagents used were of HPLC grade. Pure standards of Daclatasvir and (DCV) Sofosbuvir (SFV) employed in the study were obtained as gift sample from Micro labs, Bangalore. The other reagents used were methanol and acetonitrile from qualigens ltd. Mumbai, India, triethylamine from Hi-media, Mumbai, India, and HPLC grade water from Merck chemicals, Mumbai, India.

Preparation of standard stock solution

Standard stock solution of Daclatasvir and Sofosbuvir (1mg/mL) were prepared by accurately weighing about 100 mg pure drug and transferring in to 100 mL volumetric flask and dissolved in methanol.

The standard stock solution was further diluted with mobile phase to get calibration curve standard concentrations of 5, 10, 15, 20 and 25 µg/mL of Daclatasvir and 2, 4, 6, 8 and 10 µg/mL of Sofosbuvir.

Preparation of 0.1% triethylamine buffer (pH - 3.0)

Buffer solution was prepared by taking accurately a quantity of 0.1 mL of triethylamine dissolved in 100 mL HPLC grade water. The pH of the solution was adjusted to 3.0 with ortho- phosphoric acid and degassed for about 30 min in a ultra bath sonicator.

Preparation of mobile phase

The mobile phase used was acetonitrile, methanol and freshly prepared 0.1% triethylamine buffer solution (pH-3.0) in the ratio of 25:35:40 (v/v/v) and the mobile phase was filtered through 0.45 µ membrane filter and sonicated before use.

Method Development

For developing the method, a systematic study of the effect of various factors were undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

Detection wavelength

The spectrum of diluted solutions of the Daclatasvirin and Sofosbuvir and methanol was recorded. The absorption spectrum of Daclatasvirin and Sofosbuvir obtained by scanning the sample separately on UV spectrophotometer in UV region (200-400 nm) in spectrum mode showed that the drug has maximum absorbance at isobestic point 250 nm. Analysis was carried out by adjusting the UV detector of the HPLC system at 250 nm.

Choice of stationary phase

Preliminary development trials have performed with octadecyl columns with different types, configurations and from different manufacturers. Finally the expected separation and shapes of peak was succeeded analytical column Inertsil ODS-C_{18} column (250 x 4.6 mm, 5µ).

Selection of the mobile phase

Several systematic trials were performed to optimize the mobile phase. Different solvents like methanol, water and acetonitrile in different ratios and different pH values of the mobile phase ratios, by using different buffer solutions in order to get sharp peak and base line separation of the components and without interference of the excipients. Satisfactory peak symmetry, resolved and free from tailing was obtained in mobile phase acetonitrile: methanol: 0.1% triethylamine buffer (pH-3.0); 25:35:40 (v/v/v) in isocratic condition.

Selection of the mobile phase flow rate

Flow rates of the mobile phase were changed from 0.5- 1.2 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 mL/min flow rate was ideal for the successful elution of the analyte.

After completion of several systematic trials to optimize the chromatographic conditions, a sensitive, precise and accurate RP-HPLC method was developed for the analysis of Daclatasvirin and Sofosbuvir in pharmaceutical dosage forms.

Method Validation

The proposed method was validated as per ICH guidelines. The parameters studied for validation were system suitability, specificity, linearity, precision, accuracy (recovery), ruggedness and robustness, limit of detection and limit of quantification, filter validation and solution stability [39-40].

Specificity and Selectivity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there were no peaks of diluents and placebo at main peaks. Hence, the chromatographic system used for the estimation of Daclatasvirin and Sofosbuvir was very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. The standard solution shown symmetric peak with retention times of 2.0±0.05 min for Daclatasvirin and 3.5±0.05 min for Sofosbuvir. The results were depicted in Fig. 2 to 4.

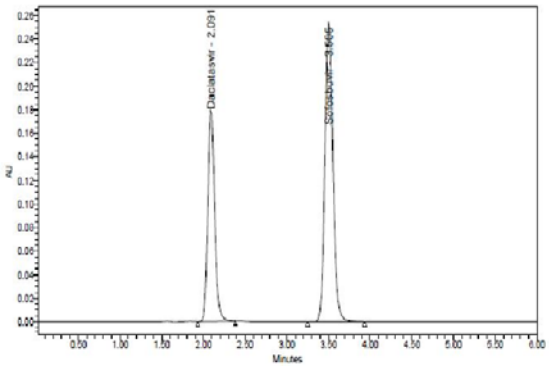


Fig.2: Chromatogram representing specificity of standard solution

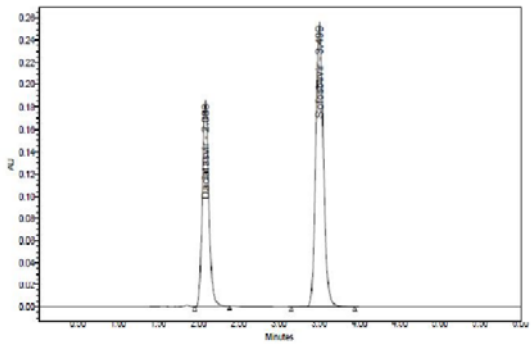


Fig.3: Chromatogram representing specificity of test sample solution

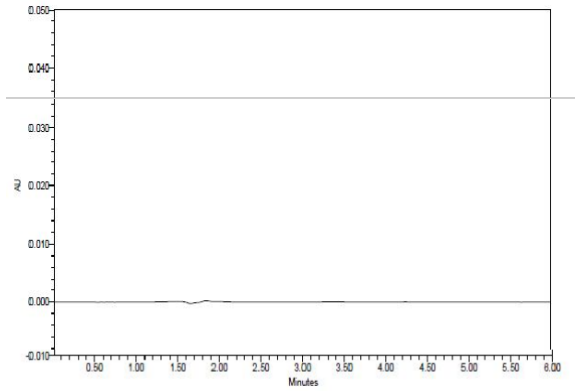


Fig.4: Typical chromatogram of the Placebo

System suitability

Standard solution (15 µg/mL of DCV and 6 µg/mL of SFV) was prepared as per the proposed method and injected into the HPLC system in five replicates and system suitability parameters were evaluated.

Linearity & Range

A series of standard concentrations were prepared from 50 % to 150 % of the target concentration of DCV and SFV. Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 5, 10, 15, 20 and 25 µg/mL of DCV and 2, 4, 6, 8 and 10 µg/mL of SFV. Injection was made at intervals of 10.0 min. Linearity of DCV was found to be exist between 5-25 µg/mL and for SFV was 2-10 µg/mL. The chromatograms were recorded and linearity graph was plotted by using peak area of drug against respective concentrations to obtain the linearity range. The results were depicted in Table.1 and Fig.5 to 6.

Table.1.0: Linearity and range of DCV and SFV

%Level	Concentration µg/mL	Area of Daclatasvirin	Concentration µg/mL	Area of Sofosbuvir
50%	5	358575	2	289124
75%	10	691556	4	591568
100%	15	1081521	6	890541
125%	20	1451468	8	1205846
150%	25	1842135	10	1510214
Concentration range	5-25µg/mL		2-10 µg/mL	
Slope (m)	73596		151432	
Correlation coefficient (r^2)	0.9992		0.9997	

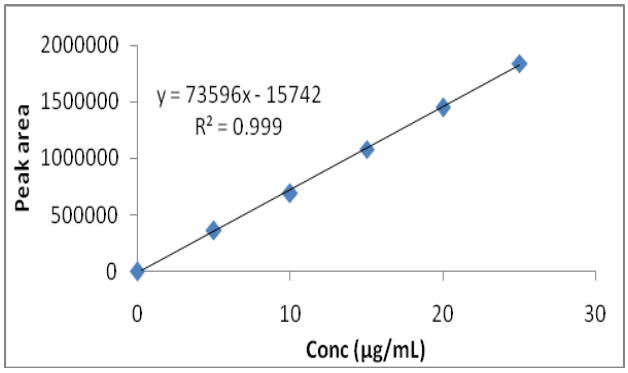


Fig.5: Linearity of Daclatasvirin

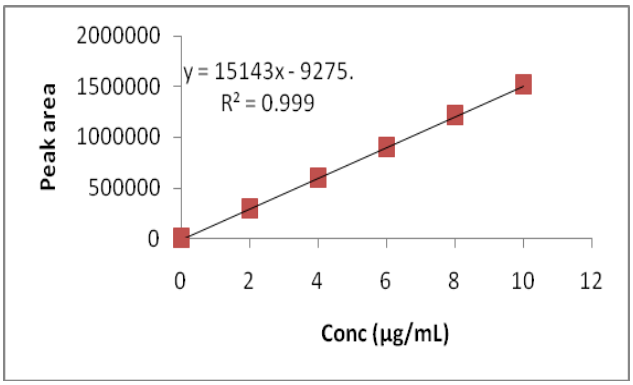


Fig.6: Linearity of Sofosbuvir

Precision

The intra-day and inter-day precision studies were carried out using a test sample assay method with six replicates on the same day and different days. The results were depicted in Table. 2 to 3.

Ruggedness

This is to prove the lack of influence of operational and environmental variables of the test results by using the method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from system to system and from analyst to analyst. It was carried out by using a test sample assay method with six replicates using different analyst, column and HPLC system. The results were depicted in Table.4.

Table. 2: Intraday precision data for Daclatasvirin and Sofosbuvir

Sample. No	Area of DCV	%Assay	Area of SFV	%Assay
1	1091521	98.88	894541	98.45
2	1091063	98.83	892265	98.20
3	1099852	99.63	893215	98.31
4	1082413	98.05	893426	98.33
5	1086315	98.40	891757	99.33
6	1099514	99.60	893475	98.34
Mean	1091780	98.90	893113	98.49
SD	6973.67	0.63	983.51	0.42
% RSD	0.64	0.64	0.11	0.43

Table.3: Interday precision data for Daclatasvirin and Sofosbuvir

Sample. No	Area of DCV	%Assay	Area of SFV	%Assay
1	1098259	99.48	898126	98.85
2	1083695	98.17	893421	98.33
3	1084237	98.21	893825	98.37
4	1091595	98.88	891618	98.13
5	1093572	99.06	896481	98.67
6	1095285	99.21	897523	98.78
Mean	1091107	98.84	895166	98.52
SD	5950.73	0.54	2587.58	0.28
% RSD	0.55	0.55	0.29	0.29

Accuracy (Recovery)

The accuracy of the method was determined by calculating recoveries of DCV and SFV by method of standard additions. Known amount of DCV and SFV were added to a pre quantified sample solution (containing DCV and SFV in 10 and 4 µg/ mL proportion, respectively), and the amount of DCV and SFV were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve. The results were depicted in Table. No- 5 to 6.

$$\% \text{ Recovery } = \frac{\text{Drug Recovered}}{\text{Nominal Concentration}} \text{X } 100$$

Table.4: Ruggedness of Daclatasvirin and Sofosbuvir

Sr. No.	DCV (%Assay)			SFV (%Assay)		
	SET I	SET II	SET III	SET I	SET II	SET III
1	99.89	99.45	99.40	99.50	101.60	102.60
2	98.77	99.20	99.70	101.90	101.40	99.60
3	98.43	99.67	99.88	99.60	99.50	101.90
4	99.81	99.54	99.60	102.00	101.60	101.40
5	98.20	98.98	98.20	99.40	99.90	101.60
6	96.60	98.20	99.56	100.60	101.00	99.50
Average	98.62	99.17	99.39	100.50	100.83	101.10
SD	1.21	0.54	0.60	1.20	0.91	1.27
% RSD	1.23	0.54	0.61	1.20	0.91	1.25
Overall Average	99.06			100.81		
Overall % RSD	1.23			1.20		

SET – I : Variability due to HPLC system
SET – II : Variability due to HPLC column
SET – III : Variability due to Analyst

Table.5: Accuracy of Daclatasvirin

Level of % recovery	Target Conc. (µg/mL)	Amount of drug spiked (µg/mL)	Nominal Conc. (µg/mL)	Drug recovered (µg/mL)	% Recovery	Mean	SD	% RSD
80	10	8	18.00	18.13	100.72	100.72	0.67	0.66
				18.01	100.06			
				18.25	101.39			
100	10	10	20.00	20.12	100.60	100.70	0.26	0.26
				20.20	101.00			
				20.10	100.50			
120	10	12	22.00	22.21	100.95	100.36	0.91	0.90
				22.18	100.82			
				21.85	99.32			

Table.6: Accuracy of Sofosbuvir

Level of % recovery	Target Conc. (µg/mL)	Amount of drug spiked (µg/mL)	Nominal Conc. (µg/mL)	Drug recovered (µg/mL)	% Recovery	Mean	SD	% RSD
80	4	3.20	7.20	7.25	100.69	100.37	0.29	0.29
				7.21	100.14			
				7.22	100.28			
100	4	4.00	8.00	8.12	101.50	101.08	0.38	0.38
				8.08	101.00			
				8.06	100.75			
120	4	4.80	8.80	8.82	100.23	100.30	0.24	0.24
				8.85	100.57			
				8.81	100.11			

Table. 7: Robustness of Daclatasvirin and Sofosbuvir

S.No.	Parameter	Condition	DCV		SFV	
			Area (n=3)	% change	Area (n=3)	% change
1	Standard	Standard conditions	1078259	0.00	90541	0.00
2	Mobile Phase composition (±2%)	Acetonitrile : Methanol: 0.1%Triethylamine buffer (pH-3.0); 23:33:44 (v/v/v)	1089257	-1.02	90244	0.33
		Acetonitrile : Methanol: 0.1%Triethylamine buffer(pH-3.0); 27:37:36 (v/v/v)	1073285	0.46	90549	-0.01
3	Mobile phase pH (±0.2units)	2.8	1078576	-0.03	90243	0.33
		3.2	1072254	0.56	90939	-0.77
4	Wavelength (±2nm)	248	1088259	-1.49	90140	0.44
		252	1079257	-0.09	90596	-0.06
5	Flow rate (mL) ±0.2mL	1.2	1078296	0.09	90141	0.44
		0.8	1078651	-0.04	90595	-0.06

Table.8. Solution stability of Daclatasvirin at room temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	1078253	1078252	NA	1078252	1078243	NA
6h	1083695	1081634	0.190	1083634	1083695	-0.006
12h	1084234	1081256	0.275	1084256	1084214	0.004
20h	1081595	1081542	0.005	1081542	1081595	-0.005
26h	1073571	1083564	-0.931	1073564	1073501	0.006
30h	1075282	1065211	0.937	1075211	1075282	-0.007
36h	1078253	1079452	-0.111	1078252	1078053	0.018

Robustness

Robustness was performed by change in mobile phase ratio, mobile phase flow rate and wavelength of the detector. The test was carried out by small variation in the chromatographic conditions at a concentration equal to standard concentrations 15 µg/mL for DCV and 6µg/mL for SFV and % change was calculated. %change in the results was calculated. The results were depicted in Table. 7.0.

$$\% \text{ change } = \frac{\text{Peak area of standard - Peak area of test (parameter change)}}{\text{Peak area of standard}} \text{X } 100$$

Limit of detection and Limit of quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines. LOD = 3 .3 × σ / S; LOQ = 10 × σ / S;

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Solution Stability

Solution stability was assed using standard and test stock solutions. These stocks were prepared and stored at room temperature and refrigerated conditions (2-8°C) for 36 h and % differences was calculated. The results were depicted in Table. 8 to 11.

$$\% \text{ Difference } = \frac{\text{Fresh stock Peak area - Stability stock peak area}}{\text{Fresh stoock peak area}} \text{X } 100$$

Table. 9. Solution stability of Sofosbuvir at room temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	890127	890167	NA	890127	890167	NA
6h	890511	890012	0.056	890422	890412	0.001
12h	890821	890356	0.052	890821	890856	-0.004
20h	890618	890696	-0.009	890618	890696	-0.009
26h	890482	890043	0.049	890482	890413	0.008
30h	890528	890589	-0.007	890528	890589	-0.007
36h	890127	890421	-0.033	890127	890421	-0.033

Table. 10. Solution stability of Daclatasvirin at refrigerated temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	1078253	1078252	NA	1078252	1078253	NA
6h	1083695	1083634	0.006	1083634	1083695	-0.006
12h	1084234	1084256	-0.002	1084256	1084234	0.002
20h	1081595	1071542	0.929	1081542	1081595	-0.005
26h	1073571	1073564	0.001	1073564	1073571	-0.001
30h	1075282	1075211	0.007	1075211	1075282	-0.007
36h	1078253	1069452	0.816	1078252	1077253	0.093

Table. 11. Solution stability of Sofosbuvir at refrigerated temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	890127	890167	NA	890127	890167	NA
6h	890422	890412	0.001	890422	890412	0.001
12h	890821	890056	0.086	890821	890856	-0.004
20h	890618	890696	-0.009	890618	890696	-0.009
26h	890482	891443	-0.108	890482	890443	0.004
30h	890528	891589	-0.119	890528	890589	-0.007
36h	890127	890421	-0.033	890127	895421	-0.595

Table. 12. Filter interference results for Daclatasvirin and Sofosbuvir

DCV				
Filtration Method	**Centrifuged**	**Nylon**	**PTFE**	**PVDF**
Area (Inj. 1)	1078253	1073564	1073571	1073564
Area (Inj. 2)	1083695	1075211	1075282	1075211
Avg. Area	1080974	1074388	1074427	1074388
% Difference		0.609	-0.004	0.004
SFV				
Filtration Method	**Centrifuged**	**Nylon**	**PTFE**	**PVDF**
Area (Inj. 1)	890482	890127	890167	890443
Area (Inj. 2)	890528	890422	890412	890589
Avg. Area	890505	890275	890290	890516
% Difference		0.026	-0.002	-0.025

Filter validation

A study was conducted to determine the effect of filter on the assay, dissolution and impurities. Test solution was prepared as per the test method. Some portion of the above solution was filtered through three different filters namely 0.45 µ PVDF filter, 0.45 µ PTFE and 0.45µ Nylon filter and some portion was centrifuged and injected into the HPLC system. The % difference values between centrifuged and filtered sample were calculated. The results were depicted in Table. 12.

$$\% \text{ Difference} = \frac{\text{Centrifuge Peak area - Filter peak area}}{\text{Centrifuge peak area}} X\,100$$

Analysis of Marketed Formulation

Preparation test solution

A total of 20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet (Containing 400 mg of SFV and 60 mg of DCV) was transferred to 25 mL volumetric flask, 10 mL of methanol was added and content of the flask were ultra sonicated for 10 min. The solution was filtered through whatmann filter

paper No. 41. The sample solution thus prepared was diluted with mobile phase to get the solution containing SFV and DCV in 15 & 6 µg/mL proportion, respectively. The test solution was injected in to HPLC and % assay was calculated.

RESULTS AND DISCUSSION

In this RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate analytes. The mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with acetonitrile: methanol: 0.1% triethylamine buffer (pH-3.0); 25:35:40 (v/v/v) at isocratic flow rate of 1.0 mL/min was found to be robust method.

The developed method was validated as per the ICH guidelines for the quantification of Daclatasvir and Sofosbuvir in pharmaceutical formulations.

A suitability test was applied to various system suitability parameters and the results obtained were within acceptable limits of tailing factor ≤ 2.0 and theoretical plates >2000.

The calibration curve was constructed with series of concentration in the range of 5-25 µg/mL and 2-10 µg/mL for Daclatasvir and Sofosbuvir. The correlation co-efficient of Daclatasvir and Sofosbuvir was found to be > 0.998. This concluded that the method was linear throughout the range selected.

Specificity was studied for the quantification of excipients in the tablet dosage form of Daclatasvir and Sofosbuvir. From the results it was indicated that none of excipients were interfere at analytes retention time. Hence the developed method was specific.

The precision of the method was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample with in the day (intraday) and next consequent three days for inter day precision.

For each cases % RSD was calculated and results were the acceptable limits. The low values of RSD indicate that the method was precise.

The % recovery for each case was calculated and was found to be 100.36 to 100.72 % for Daclatasvir and 100.30 to 101.08 % for Sofosbuvir and found to be results were within acceptance limits. Hence the developed method is accurate throughout the selected range.

Robustness test was carried out by small variation in the chromatographic conditions and % change was calculated. The % change in the results was calculated and it was found robust as % change was below 2.0 %.

A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 0.313µg/mL for Daclatasvir and 0.021µg/mL for Sofosbuvir and LOQ is found to be 0.948µg/mL for Daclatasvir and 0.065µg/mL for Sofosbuvir.

Sample and standard solution are stable at 5°C for 36 hrs as the % difference in the area was found to be less than 2.0 %. Filter interference was done on three types of 0.45µ filters (Nylon, PVDF, PTFE), and the % difference was found to be below 2.0 % for sample solutions and standard solutions calculated against centrifuged samples and standard.

The validated method was applied for the assay of commercial tablets of Daclatasvir and Sofosbuvir (HEPCINAT-PLUS Tablets: 400mg of Sofosbuvir and 60 mg of Daclatasvir). Peak area of the detector response was used to calculate % assay. The % assay was found to be 99.57 % for Daclatasvir and 99.38 % for Sofosbuvir. The results presented good agreement with the labelled content.

Thus the method developed in the present investigation is simple, sensitive, accurate, rugged, robust, rapid and precise. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. Hence, the developed method can be successfully applied for the estimation of Daclatasvir and Sofosbuvir in tablet dosage forms by RP-HPLC.

CONCLUSION

A new, reversed-phase HPLC method has been developed for simultaneous analysis of Daclatasvir and Sofosbuvir in a tablet formulation. It was shown that, the method was linear, accurate, reproducible, repeatable, precise, selective and specific proving the reliability of the method. The run time is relatively short (6.0 min), which enables rapid determination of many samples in routine and quality control analysis of tablet formulations. Hence, the proposed method was successfully applied to analyze preparation containing Daclatasvir and Sofosbuvir.

Acknowledgements:

The authors are thankful to Chalapathi Institute of Pharmaceutical sciences, Lam, Guntur, A.P, India for Providing technical assistance and necessary facilities.

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