RESEARCH ARTICLE

RP-HPLC Method for the Simultaneous Determination of Sofosbuvir and Daclatasvir in Pure and Pharmaceutical Dosage Forms

Amol Badkhal*, Prachi Verma, Sandip Umare, Vishal Patond, Dhirendra Sanghai, Priti Chincholkar

SGSPS Institute of Pharmacy, Akola, Maharshtra, India.

Received: 25th September, 2023; Revised: 18th October, 2023; Accepted: 10th November, 2023; Available Online: 25th December, 2023

ABSTRACT

Daclatasvir and sofosbuvir in pure and medicinal dosage forms can be determined quickly and easily using a reversed-phase high-performance liquid chromatography (RP-HPLC) technique. An accurate, reproducible method was developed and validated. The mobile phase contained a mixture of 90% methanol:10% water (0.05% OPA). UV estimation was done with a flow rate of 0.7 mL/min and wavelength of 275 nm. The temperature was 30°C. Sofosbuvir stays in the body for 3.361 minutes and daclatasvir for 5.745 minutes. A quantitative study of commercial dosage forms went well with this method, which was made and tested. Sofosbuvir and daclatasvir each had a %RSD of 0.43 and 0.28, respectively. Recoveries were 97.85 and 98.52% for sofosbuvir and daclatasvir, respectively. Three methods were checked for precision, accuracy, linearity, selectivity, specificity, limit of detection (LoD), limit of quantitation (LoQ), robustness, and ruggedness according to rules of the International Council for Harmonisation (ICH).

Keywords: Sofosbuvir, Daclatasvir, Reversed-phase high-performance liquid chromatography, Validation, Chromatography.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.4.51

How to cite this article: Badkhal A, Verma P, Umare S, Patond V, Sanghai D, Chincholkar P. RP-HPLC Method for the Simultaneous Determination of Sofosbuvir and Daclatasvir in Pure and Pharmaceutical Dosage Forms. International Journal of Pharmaceutical Quality Assurance. 2023;14(4):1158-1164.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Multi-ingredient products are becoming more important because they are easier for patients to take, work in more ways, have fewer side effects, and speed up treatment. A lot of research has been done on these kinds of formulations without separating them first. Most of the time, instruments like spectrophotometry, gas-liquid chromatography (GLC), high-performance thin layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and so on are used to figure out how many things are in a recipe. These methods are based on the size of the materials' homes, which are all different and not all the same.[1]

The present has a look at focuses on frequent step parameters concerned in HPLC circumstances. HPLC technique development is crucial in case of drug discovery, drug improvement and pharmaceutical products. It can be used for regular quality control studies in research and formula tests. It pays special attention to the optimization of HPLC conditions and other important factors during the process of system development and evaluation of drug substances.[2]

The analytical technique chosen must have all the best characteristics and the most crucial being that it must be less time-consuming. That is approximately the technique improvement relatively simple project but in the case of combined dosage shape the situation is one of a kind because the homes of the one drug may additionally bog down the residences of others. Some of these properties could be solubility, moving of λ_{max}, overlap of absorption, etc. But these problems are being solved by the many high-tech analysis tools that are now available.[3]

Similar separation principles to those used in traditional column chromatography provide the backbone of this technology. Cellular section is pumped from side to side packed column under high pressure, setting it apart from traditional chromatographic techniques. It's miles the maximum popular method today a few of the extraordinary chromatographic processes. Due to the tremendous evolution of liquid chromatography (LC) instruments presenting superior qualitative and quantitative results. While traditional chromatography relies on gravity to force the solvent through the column, the HPLC technique uses pressures (400 atmospheres) to force the solvent through the column and separate the sample into its component parts based on their differences in relative affinity.[4]

*Author for Correspondence: amolbadkhal@gmail.com

HPLC makes use of pumps to force a liquid solvent and the pattern aggregate through a column of highly adsorbent material. Different interactions across sample problems mean different drift costs for different parts, which in turn leads to the eventual separation of the column's two halves. HPLC uses pumps to force a mixture of pressurized fluid and an example through a section containing adsorbent, thereby separating the specimen into smaller and smaller pieces. Adsorbents, the dynamic component of the segment, are often a granular fabric composed of solid debris (such as Silica, polymers, and many more) from 2 to 50 µm in size. 'Cellular phase' refers to the pressurized fluid, which is typically a solution of solvents (such as water, acetonitrile, and/or methanol). The linkages between the pattern segments and the adsorbent are vital to the partitioning process, and these connections are in turn influenced by the commercial enterprise and temperature.[5]

MATERIAL AND METHODS

Materials

Sofosbuvir and dalactasvir (10 g each) was provided as gift sample by Hetero Pharmaceuticals Pvt. Ltd. having percentage purity 99.8 and 99.02% w/w, respectively. All reagents used are of HPLC grade.

Determination of λ_{max} of Sofosbuvir and Daclatasvir

Dissolving 400 mg of sofosbuvir and 60 mg of daclatasvir, respectively, in 10 mL of methanol yields the standard stock solution. Concentrations of 40,000 µg/mL for sofosbuvir and 6,000 µg/mL for daclatasvir were achieved by further diluting stock standard solutions with methanol. Using methanol as a blank, we measured the maximum absorbance (λ_{max}) from 200 to 400 nm with a Shimadzu UV-visible spectrophotometer (model UV-730D). Maxima appeared in the Solution of combination around 275 nm (Figure 1).[6]

Selection of Mobile Phase

Preparation of standard solutions

Sofosbuvir and daclatasvir were dissolved in methanol at concentrations of 400 and 60 mg, respectively, using accurate weighing. The ultimate concentrations of sofosbuvir and daclatasvir were around 40,000 and 6,000 µg/mL, correspondingly, after the volume was raised up to the 10 mL level.

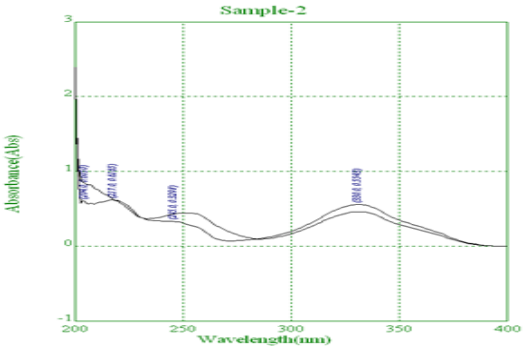

Figure 1: Overlain spectra of sofosbuvir and daclatasvir

Procedure

Method creation and optimization *via* HPLC involved a choice of an appropriate mobile phase. Different solvent solutions were used to inject and run pure pharmacological compounds. Here, we experiment with a range of mobile phase concentrations. Tests were conducted using methanol and water with varying concentrations of acidity (Table 1, Figures 2 and 3). The optimal mobile phase was determined after experimenting with various combinations of mobile phases.[7]

The retention times of sofosbuvir and daclatasvir, 3.361 and 5.745 minutes, respectively, were found to be most repeatable in a mobile phase consisting of Methanol: Water (90:10) (0.1% OPA) (Figure 4).

Calibration curves using HPLC

- *Standard stock solution*

Sofosbuvir 400 mg and daclatasvir 60 mg were precisely weighed and then mixed into 10 mL of methanol. This solution became the de facto industry norm. This means that there are 40000 µg/mL of sofosbuvir and 6000 µg/mL of daclatasvir in the stock solution.

Results from a test conducted with a linear analytical method are proportionate to the amount of analyte present in the sample. Analytical procedures are defined by the

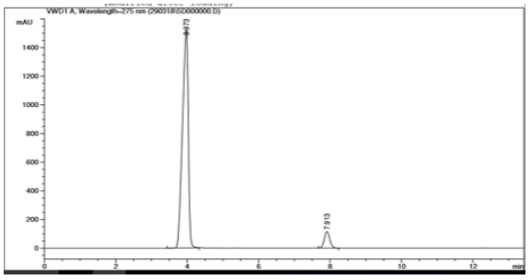

Figure 2: Chromatogram of trial 2

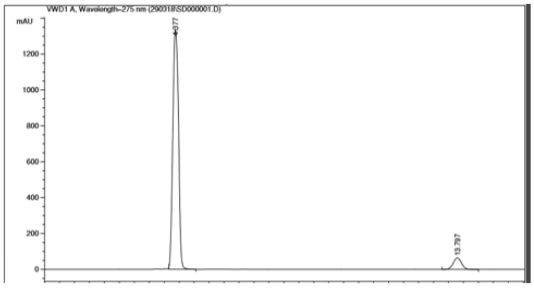

Figure 3: Chromatogram of trial 2

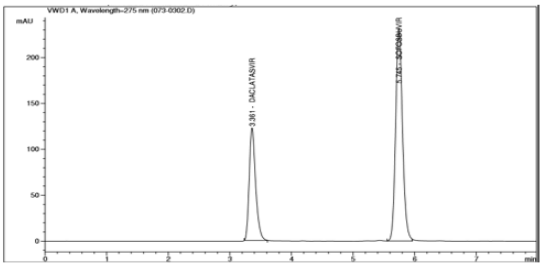

Figure 4: Chromatogram trial 3 Methanol: Water (90:10)

concentration range across which they are proved to have appropriate precision, accuracy, and linearity. Concentration ranges for both medications were obtained by serially diluting standard stock solutions of 400 mg/mL sofosbuvir and 60 mg/mL daclatasvir with mobile phase. Concentration versus absorbance was plotted as a calibration curve after measuring the absorbance of this medication at 275 nm.

- *Procedure*

When conditions were stable enough, the mobile phase was permitted to mix with the stationary one. Standard solutions of sofosbuvir and daclatasvir were injected at a range of concentrations, and the peak areas were measured[8] (Tables 2 and 3) (Figures 5 and 6).

- *System suitability test*

System suitability is a requirement of the Pharmacopoeia and is used to check if the precision and repeatability of the chromatographic system are good enough for analysis. Five separate injections of standards were used to get results for tests.[9]

Table 1: Different trials

Figure No.	*Column*	*Flow rate, mobile phase, and wavelength*	*Inj Vol.*	*Obser vation*	*Concl usion*
1	C18(COSMOSIL) (250 ×4.6 mm, 5.0µ)	Flow rate 0.7 mL.,80% methanol: 20% Water (0.1% OPA) 275 nm,	20 µL	Peaks could not be resolved well.	rejected
2		Flow rate 0.7 mL, 70% methanol:30% water (0.1% OPA) 275 nm,		peaks were not obtained	
3		Flow rate 0.7 mL 90% Methanol :10% Water (0.05% OPA) 275 nm,		Peaks could not be resolved well.	selected

Table 2: Standard calibration curve of sofosbuvir

Conc.	*Area I*	*Area II*	*Mean*	*SD*	*%RSD*
30	592.63	596.25	594.44	2.56	0.43
60	1178.47	1169.87	1174.17	6.08	0.52
90	1757.27	1789.08	1773.175	22.49	1.27
120	2404.53	2389.5	2397.015	10.63	0.44
150	2936.61	2932.76	2934.685	2.72	0.09

Table 3: Standard calibration curve of daclatasvir

Conc	*Area I*	*Area II*	*Mean*	*SD*	*%RSD*
5	315.39	316.64	316.01	0.88	0.28
10	586.38	578.86	582.62	5.32	0.91
15	854.39	854.12	854.255	0.19	0.02
20	1187.22	1186.01	1186.615	0.86	0.07
25	1438.47	1432.4	1435.435	4.29	0.30

Preparation of Standard Stock Solution

Sofosbuvir standard solution

Weigh sofosbuvir 400 mg and dissolve in the mobile phase to get a volume up to 10 mL using methanol. The mobile phase was added to the stock solution to bring the concentration of sofosbuvir to 40,000 µg/mL.

Daclatasvir standard solution

Daclatasvir 60 mg was accurately weighed and then dissolved in the mobile phase to bring the volume up to 10 mL methanol. To achieve a final concentration of approximately 6000 µg/mL of daclatasvir, stock solution was further diluted by the mobile phase.

Procedure

Mobile phase filtration was performed before allowing it to equilibrate with the stationary phase. Injecting a 20 µL std drug solution made in five replicates, and recording system suitable parameters (Table 4).

Proposed method for estimation of sofosbuvir and daclatasvir laboratory mixture

Preparation of laboratory mixture (standard)

After transferring 400 mg of sofosbuvir by weight to a 10 mL volumetric flask, the contents were vigorously shaken for 5 minutes, and the volume was adjusted with the mobile phase. The right volume was achieved by adding a mobile phase after shaking a 10 mL volumetric flask with 60 mg of daclatasvir for 5 minutes at room temperature. The laboratory combination was made by properly combining and diluting the standard solutions with the mobile phase. The concentration of sofosbuvir was 10 µg/mL and daclatasvir was 20 µg/mL.

- *Preparation of laboratory mixture (sample)*

Using the correct weighing of medication samples, five separate sofosbuvir and daclatasvir mixes are created in the lab to achieve a concentration of 400 µg/mL of sofosbuvir and 60 µg/mL of daclatasvir. By comparing the peak areas of the standard

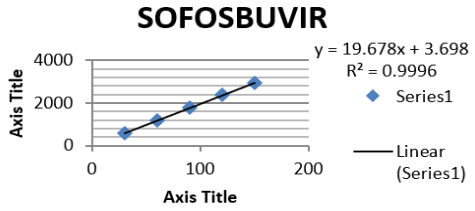

Figure 5: Various concentration and average area of sofosbuvir

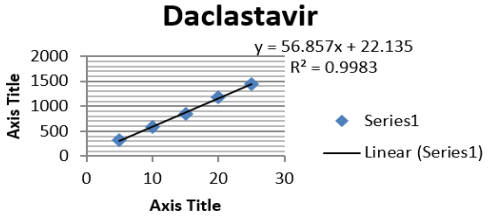

Figure 6: Various concentration and average area of daclatasvir

laboratory combination with the sample laboratory mixture, a concentration can be determined (Table 5).

Relevance of Proposed Method for Estimation of Sofosbuvir and Daclatasvir in Formulation

Standard stock solution

A 10 mL volumetric flask was used to transfer the 400 mg of sofosbuvir after they were precisely weighed. Define the volume using the mobile phase. After precisely measuring 60 mg of daclatasvir, the solution was transferred to a 10 mL volumetric flask. It was then agitated for five minutes at room temperature, and the volume was increased to the correct mark using the mobile phase. A laboratory mixture with a concentration of 400 µg/mL of sofosbuvir and 60 µg/mL of daclatasvir was prepared by mixing and diluting respective standard solutions with the appropriate mobile phase.

Sample solution preparation

Using the correct weighing of medication samples, five distinct tablet mixes of sofosbuvir and daclatasvir are created to achieve a concentration of 400 µg/mL of sofosbuvir and 60 µg/mL of daclatasvir.

Procedure

After the stationary phase had reached equilibrium, injections of both the standard and the sample solution (20 µg/mL) were made. Response was recorded as the area of the main peaks in the chromatograms. By comparing the peak of a sample to a standard, the sofosbuvir and Daclatasvir concentrations were determined (Table 6).

Validation Parameters

Accuracy

The effectiveness of the new approach was tested using recovery experiments. Standard medication was added to a pre-analyzed tablet solution at three different concentrations: 80, 100, and 120%. Statistical evidence supporting recovery studies is provided in Tables 7 and 8.

Precision

Sofosbuvir and daclatasvir standards were analyzed across several replicates to develop the methodology. In order to document any daily or weekly shifts in the solution's performance, it was analyzed three times. Results for intraday and interday fluctuations are provided in Tables 9, 10, 11 and 12.

Interday sofosbuvir

Inter-day precision study of sofosbuvir is shown in Table 9

Interday daclatasvir

Inter-day precision study of daclatasvir is shown in Table 10.

Table 4: Showing result of system suitability parameters

	Peak area		*Retention time*		*Asymmetry*		*No .of theoretical plates*		*Resolution*
	DAC	*SOFO*	*DAC*	*SOFO*	*DAC*	*SOFO*	*DAC*	*SOFO*	
1.	582.862	1169.969	3.392	5.597	0.71	0.84	5600	13531	12.49
2	582.46	1172.46	3.293	5.595	0.71	0.85	5603	13918	12.57
3	583.321	1170.63	3.293	5.595	0.71	0.85	5784	13918	12.66
MEAN	582.881	1171.01	3.326	5.59	0.71	0.84	5662.33	13789.0	12.57
+ S.D	0.43081	1.290	0.057	0.001	0.0	0.005	105.37	223.43	0.085
%RSD	0.7	0.11	1.72	0.02	0	0.68	1.86	1.62	0.68

Table 5: Statistical data for estimation of sofosbuvir and daclatasvir in laboratory mixture

S. No	*Conc of sample*		*Peak area of sample*		*Amount found*		*% label claim*	
	SOFO	DACL	SOFO	DACL	SOFO	DACL	SOFO	DAC
1	120	20	2356.69	1184.82	119.62	20.45	99.68	102.25
2	120	20	2332.71	1171.39	118.40	20.21	98.67	101.05
Mean					119.01	20.33	99.17	101.65
Standard deviation					0.862	0.169	0.71	0.84
% RSD					0.02	0.83	0.72	0.83

Table 6: Statistical data for estimation of sofosbuvir and daclatasvir in marketed formulation

S. No	*Conc. of sample*		*Peak area of sample*		*Amount found*		*% label claim*	
	SOFO	DACL	SOFO	DACL	SOFO	DACL	SOFO	DACL
1	30	5	592.632	315.399	29.94	5.15	99.8	103.16
2	30	5	596.254	316.645	30.12	5.180	100.4	103.60
Mean					30.03	5.165	100.1	103.38
Standard deviation					0.127	0.021	0.424	0.31
%Relative Standard deviation					0.42	0.41	0.42	0.30

Table 7: Recovery studies of sofosbuvir and daclatasvir

Level of recovery (%)	*80*		*100*		*120*	
	SOFO	*DACL*	*SOFO*	*DACL*	*SOFO*	*DACL*
Amount present (mg)	60 60	5 5	60 60	5 5	60 60	5 5
Amount of Std. Added(mg)	48 48	4 4	60 60	5 5	72 72	6 6
Amount Recovered(mg)	46.75 46.61	3.97 4.02	58.71 58.23	4.92 5.02	71.26 70.41	5.83 5.98
% Recovery	97.39 97.11	99.42 100.54	97.85 97.05	98.52 100.47	98.97 97.80	97.16 99.73

Table 8: Statistical validation of recovery studies

Level of recovery (%)	*Drug*	*Mean Recovery*	*Standard Deviation*	*% RSD*
80	Sofosbuvir	97.39	0.20	0.20
	Daclatasvir	99.98	0.79	0.79
100	Sofosbuvir	97.45	0.57	0.58
	Daclatasvir	99.50	1.38	1.39
120	Sofosbuvir	101.58	0.83	0.81
	Daclatasvir	101.58	1.82	1.79

Table 9: Inter-day precision study of sofosbuvir

Conc	*Area I*	*Area II*	*Mean*	*Amt Found*	*% Amt Found*	*SD*	*%RSD*
30	609.4 679	607.2 564	60 8.36	30.74	102.47	0.96	0.16
90	1750.7 894	1751.2 145	175 1.00	88.83	98.70	0.30	0.02
150	293 0.66	293 1.21	293 0.94	148.81	99.21	0.39	0.01

Table 10: Inter-day precision study of daclatasvir

Conc.	*Area I*	*Area II*	*Mean*	*Amt Found*	*% Amt Found*	*SD*	*%RSD*
5	308.9 612	317.1 236	31 2.04	5.09	101.80	5.77	1.85
15	854.2 653	855.4 621	85 4.86	14.64	97.60	0.85	0.10
25	144 0.42	143 2.56	143 6.49	24.87	99.48	5.56	0.39

Intraday sofosbuvir

Intra-day precision study of sofosbuvir is shown in Table 11.

Intraday daclatasvir

Intra-day precision study of daclatasvir is shown in Table 12.

Specificity

To determine the method's level of specificity, we looked at how well it separated the peaks of sofosbuvir and daclatasvir from those of the matrix.

Mean retention time for-

Sofosbuvir: 5.749

Daclatasvir: 3.349

Table 11: Intra-day precision study of sofosbuvir

Conc	*Area I*	*Area II*	*Mean*	*Amt Found*	*% Amt Found*	*SD*	*% RSD*
30	608.74	600.12	604.43	30.54	101.80	6.10	1.01
90	1752.8	1747.98	1750.39	89.17	99.07	3.41	0.19
150	2922.73	2926.98	2924.86	148.50	99.00	3.01	0.10

Table 12: Intra-day precision study of daclatasvir

Conc	*Area I*	*Area II*	*Mean*	*Amt Found*	*% Amt Found*	*SD*	*% RSD*
5	309.25	309.22	309.24	5.05	101.00	0.02	0.01
15	861.41	860.4	860.90	14.75	98.36	0.71	0.08
25	1439.52	1444.13	1441.83	24.97	99.88	3.26	0.23

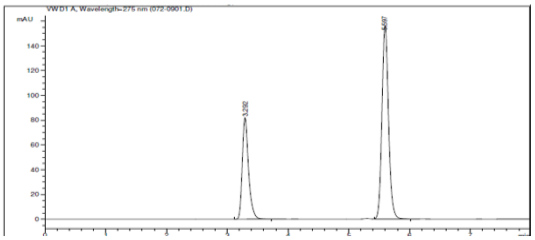

Figure 7: Estimation of sofosbuvir and Daclatasvir in Laboratory mixture

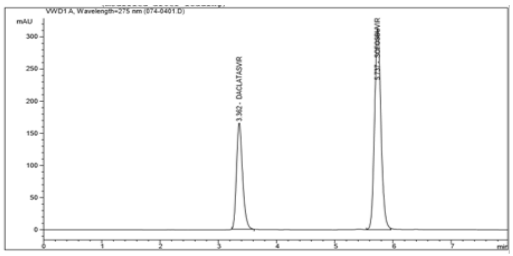

Figure 8: The chromatogram obtained by tablet for trial 1 formulation of sofosbuvir and daclatasvir

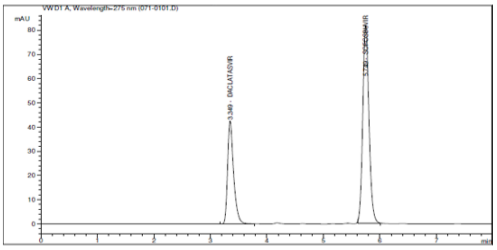

Figure 9: Chromatogram obtained by tablet for trial 2 formulation of sofosbuvir and daclatasvir

The results were extremely similar to those from a laboratory mixture, which suggests there was no interference from the matrix.

Linearity and range

Final concentrations in the range of 30 to 150 µg/mL for SOFO and 5 to 25 µg/mL for DAC were obtained by transferring aliquot portions from the stock standard solution into a succession of 10.0 mL volumetric flasks and diluting them to mark with the mobile phase.

Calibration curves were generated by graphing peak area versus drug concentration and injecting a fixed volume of 20.0 µL of each sample. Data is presented in Tables 13, 14, 15 and 16.

Robustness

A method's robustness lies in its resistance to breakdown under controlled experimental conditions. Optimal method parameters were tweaked somewhat in order to assess the proposed approach's stability. Researchers looked at how altering the mobile phase's make-up and flow rate affected the drug peak's retention time and tailing factor.

To achieve optimum chromatographic conditions, we altered the flow rate by ± 0.1 mL min^{-1} and the mobile phase composition by ± 1 mL. Tables 17, and 18 displays outcomes of robustness analyses. Because the parameters of the system were likewise found to be satisfactory, it was decided to end the analysis.

RESULT AND DISCUSSION

Create and validate an RP-HPLC technique for the simultaneous quantification of sofosbuvir and daclatasvir in bulk and in combo tablets.

The RP HPLC method was used to concurrently quantify the sofosbuvir and daclatasvir in the tablets, Propane: 0.1%. The separation was achieved by using a C18 (COSMOSIL) column of (4.6 ×250 mm) with a particle size packing of 5 µm in conjunction with a water (90:10) mobile phase flowing at 0.7 mL/min. The detection was carried out at a wavelength of 275 nm. Both and have been measured to have a retention time of 3.697 and 6.089 minutes, respectively. The examination of the tablet formulation was performed after the chromatographic conditions were established.[6]

Table 13: Linearity study of sofosbuvir

Sr No.	*Conc.*	*Area-I*	*Area-II*	*Mean*	*SD*	*%RSD*
1	30	592.63	596.25	594.44	2.56	0.43
2	60	1178.47	1169.87	1174.17	6.08	0.52
3	90	1757.27	1789.08	1773.175	22.49	1.27
4	120	2404.53	2389.5	2397.015	10.63	0.44
5	150	2936.61	2932.76	2934.685	2.72	0.09

Table 14: Regression equation data for sofosbuvir

Regression Equation Data y = 19.67x + 3.698	
Slope(m)	19.67
Intercept(c)	3.698
Correlation Coefficient	0.999

Table 15: Linearity study of daclatasvir

Sr No.	*Conc*	*Area-I*	*Area-II*	*Mean*	*SD*	*%RSD*
1	5	315.39	316.64	316.01	0.88	0.28
2	10	586.38	578.86	582.62	5.32	0.91
3	15	854.39	854.12	854.255	0.19	0.02
4	20	1187.22	1186.01	1186.615	0.86	0.07
5	25	1438.47	1432.4	1435.435	4.29	0.30

Table 16: Regression data for daclatasvir

Regression Equation Data y = 56.857x +22.135	
Slope(m)	56.857
Intercept(c)	22.135
Correlation Coefficient	0.998

Validation

System suitability test

The chromatographic system's applicability was tested to make sure it had sufficient resolution and repeatability.

- *Accuracy*

Extensive recovery trials at 100, 100, and 120% concentration levels attest to the reliability of the approach. Recoveries were determined to be between 99 and 101%.

- *Precision*

The results of the precision investigations, which included measurements of both intra- and inter-day accuracy, were found to be within the threshold of acceptability. The reproducibility of the approach is shown by a %RSD less than 2.0.

Table 17: Robustness study of sofosbuvir

Parameters	*Conc.*	*Amount of detected (mean ± SD)*	*%RSD*
Mobile phase composition (89+11)	60	1040 ± 3.17	0.31
Mobile phase composition (91+09)	60	2297.18 ± 0.95	0.04
Wavelength change 274 nm	60	1284.1 ± 2.62	0.20
Wavelength Change 276 nm	60	1047.41 ± 4.00	0.38
Flow rate change (0.6 mL)	60	1372.84 ± 3.37	0.25
Flow rate change (0.8 mL)	60	1005.30 ± 7.10	0.71

Table 18: Robustness study of daclatasvir

Parameters	*Conc.*	*Amount of detected(mean ± SD)*	*%RSD*
Mobile phase composition (89+11)	10	625.4 ± 2.28	0.36
Mobile phase composition (91+09)	10	1112.28 ± 1.37	0.12
Wavelength change 274 nm	10	556.8 ± 2.69	0.48
Wavelength Change 276 nm	10	622.79 ± 3.71	0.59
Flow rate change (0.6 mL)	10	670.32 ± 0.71	0.11
Flow rate change (0.8 mL)	10	495.38 ± 4.26	0.86

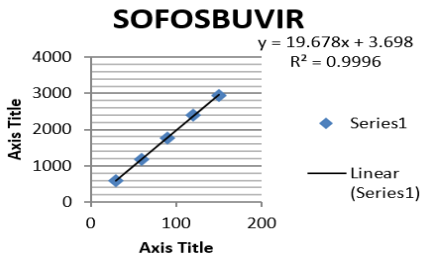

Figure 10: Observations of linearity study of sofosbuvir

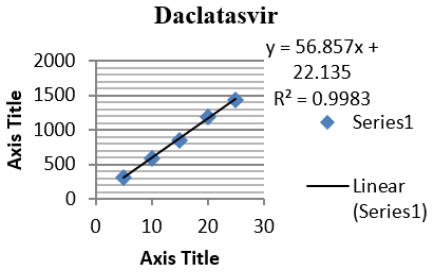

Figure 11: Observations of linearity study of daclatasvir

- *Specificity*

Ability to reliably evaluate analyte in the presence of matrix, matrix components, contaminants, degradation products, etc. The suggested approach is tested by injecting a blank and control sample solution to look for interference at the sofosbuvir and daclatasvir retention period. Thus, no significant interference was observed between the retention times of sofosbuvir (5.74) and daclatasvir (3.34).

- *Linearity*

The linear range for sofosbuvir was 30 to 150 µg/mL, and the linear range for daclatasvir was 5 to 25 µg/mL. The wavelength of 275 nm was utilized for detection. The calibration curve showed that the r^2 values for sofosbuvir and daclatasvir were 0.999 and 0.998, respectively (Figures 10 and 11).

- *Robustness*

The method's stability was measured by using three distinct sets of values for the chosen parameters. Retention times and tailing factors showed less variation, as expected.

Within acceptable parameters, the tablet formulation analysis was performed. Results from the validation research showed that the approach was linear and precise, falling within the tolerances set by the ICH recommendations. The recovery research results were consistent with the ICH guidelines, proving their reliability.[10]

CONCLUSION

In particular, sofosbuvir and daclatasvir can be measured in a selective manner using this approach. An accurate, precise, and robust RP-HPLC method has been established for the quantification of sofosbuvir and daclatasvir. This method has been shown to be better than others because it has a shorter retention time, a gradient mode, uses cheap, easy-to-find mobile phase, a column, UV sensing, and a higher peak sharpness. Because of the quick run time, several samples can be quickly quantified for regular and quality-controlled examination of different sofosbuvir and daclatasvir formulations. Because of these considerations, the method is well suited to the interference-free measurement of medicinal dose forms of sofosbuvir and daclatasvir. All of the method's validation parameters were successfully tested, and the results were all positive. Sofosbuvir and daclatasvir may now be routinely determined using this approach.

REFERENCES

1. Ahmad S, Ashwin B, Rahman BA, Gorad RU, Tare H. Quality by design (QBD) approach to develop stability indicating RP-HPLC method development for naproxen and pantoprazole. International journal of health sciences. 2022;6:11450-65.
2. Mujawar T, Ahmad S, Tajane P, Sable V, Gaikwad A, Tare H. Development and validation of ultraviolet spectrophotometric method for Saquinavir estimation in bulk and dosage form. Multidisciplinary Science Journal. 2023 Apr 9;5(2):2023020.
3. Ahmad S, Khairnar M, Bakhshi AR, Tare M, Baheti D, Tare H. QBD approach to develop stability indicating RP-HPLC method development for Levosulpiride and Ilaprazole. International Journal of Health Sciences. 2022;6:7413-29.
4. Mujawar T, Ahmad S, Gadilohar H, Deshmukh N, Gaikwad A, Sable V. Analytical method development and validation for the simultaneous estimation of Metoprolol and Benidipine by RP-HPLC in bulk and tablet dosage forms. Journal of Health Sciences.;6(S6):8188-202.
5. Kharate V, Kuchekar M, Harde M, Pimple B, Patole V, Salunkhe M, Wadgave P, Bhise M, Gaikwad A, Tare H. Development of Validated Stability Indicating HPTLC Method for Estimation of Febuxostat in Bulk and Tablet Dosage Form by Using QBD Approach. International Journal of Drug Delivery Technology. 2023;13(2):542-550.
6. Gaikwad AV, Rajurkar VG, Shivakumar T, Dama GY, Tare HL. Simultaneous estimation of ramipril & valsartan in tablets by HPTLC. Indo-Global Journal of Pharmaceutical Sciences. 2011;1(1):99-112.
7. Huang C, Song S, Liu W, Parganiha P, Zhuang P, Lohani S, Alwedi E, Ren H, Bishara D, Chang HY. Development of a derivatization RP-HPLC method for determination of sulfuryl chloride in chlorosulfonic acid for gefapixant citrate manufacture. Journal of Pharmaceutical and Biomedical Analysis. 2022 Jun 5;215:114752.
8. Ahmad S, Aakanksha D, Patil M, Bhise M, Barde L, Tare H. Development and Validation of HPLC And HPTLC for Simultaneous Analysis of E and Z Guggulsterone, A-11–KBA And 11–KBA from Herbal Formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):393-396.
9. Pund S, Changediya V, Rajurkar V. Design and Development of Analytical Method for Deflazacort Estimation: A Robust HPLC Approach. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):363-368.
10. Patil K, Narkhede S, Nemade M, Rane S, Chaudhari R, Dhobale G, Tare H. A Validated Sensitive Stability Indicating HPLC Method for the Determination of Etoricoxib in Bulk and Formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):352-357.