RESEARCH ARTICLE

Development and Validation for Simultaneous Estimation of Sofosbuvir and Daclatasvir Dihydrochloride in Pharmaceutical Dosage form by Ratio Derivative and Dual Wavelength Methods

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ABSTRACT

Development and validation of new simple, sensitive, accurate and precise spectrophotometric method involving ratio derivative and dual-wavelength method, was done as per ICH Q2 (R1) for simultaneous estimation of Sofosbuvir (SOFO) and daclatasvir dihydrochloride (DACLA) in a combined dosage form. The overlapping in the spectra of both drugs was the reason for the selection of both methods. The absorbance difference (ΔA) value between 235.8 nm and 270.6 nm was selected for the quantitative determination of SOFO, where DACLA gives equal absorbance at the selected wavelength in the dual-wavelength method (Method A). The determination of DACLA is done quantitatively by measuring the difference in absorbance value at 249 nm and 268.6 nm where SOFO gives equal absorbance at a selected wavelength. Ratio spectra method (Method B) was based on dividing the spectra of a mixture with standard spectra of one of the analytes, and the first derivative spectra was recorded with $\Delta\lambda = 8$ nm and scaling factor 1. The amount of SOFO and DACLA was estimated in the binary mixtures by computing the first derivative signal at 247.0 nm and 341.0 nm, respectively. The calibration curve was linear in the concentration range of 10–90 µg/mL for SOFO and 4–20 µg/mL for DACLA for both the methods. The methods were successfully applied for the simultaneous determination of these drugs in combined dosage form with acceptable recoveries.

Keywords: Daclatasvir dihydrochloride, Dual Wavelength method, Ratio Derivative method, Simultaneous estimation, Sofosbuvir.

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INTRODUCTION

95% of cases with hepatitis C infection can be cured by antiviral medicines, by reducing the mortality rate from liver cancer and cirrhosis, but the reach to therapeutics is low.[1] World Health Organizatio (WHO) preferred Sofosbuvir, daclatasvir, and the sofosbuvir/ledipasvir combination regimens to cure hepatitis C rates above 95%. These medicines are much more effective, safer, and better-tolerated than older therapies. The novel combination of SOFO and DACLA is directly acting antiviral that shows potent activity against the hepatitis C virus (HCV). This combination can be used in the treatment of hepatitis C with HIV in liver fibrosis and have a greater margin of safety and efficacy. Chemically, Sofosbuvir is a propan-2-yl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2, 4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxyphenoxyphosphoryl] amino] propanoate, (Figure 1) hepatitis C virus (HCV) nucleotide analog NS5B polymerase inhibitor.[2] It is a prodrug, undergoes phosphorylation in vitro to the active phosphorylated form. The drug is freely soluble in acetone, ethanol, and slightly soluble in water. Sofosbuvir is a nucleotide analog used in combination with other drugs for the treatment of hepatatis C infection.[3] In 2015, FDA approved the combination of Sofosbuvir with Ledipasvir, in 2017 it has been approved with Velpatasvir and in 2018 it has been approved with DACLA. Daclatasvir

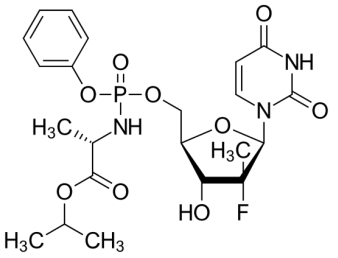

Figure 1: Sofosbuvir Structure

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dihydrochloride is methyl N-[(2S)-1-[(2S)-2-[5-[4-[4-[2-[(2S)-1-[(2S)-(methoxycarbonylamino)-3-methylbutanoyl]pyrrolidin-2-yl]-1H-imidazol-5-yl]phenyl]phenyl]-1H-imidazol-2-yl] pyrrolidin-1-yl]-3-methyl-1-oxobutan-2- yl] carbamate, (Figure 2) which is Hepatitis C Virus NS5A inhibitor. The drug is easily soluble in water, methanol, and ethanol. A literature survey reveals that there are few methods have been reported for the determination of Daclatasvir alone[4-20] and in combination with other drugs.[21] Various HPLC, HPTLC and Spectrophotometric method was reported and for quantitative estimation of Sofosbuvir alone[22-30] and in combination with other drugs.[31-38] Various methods were reported for the estimation of Sofosbuvir and DACLA in a combined dosage form. DACLA is official in Indian Pharmacopeia 2018.[39] The official method for the DACLA is liquid chromatography. UV-visible spectrophotometry is an appropriate method for dosage control of pharmaceutical preparations as it is a rapid, sensitive, and inexpensive analytical tool. So an attempt was made to develop and validate a simple, sensitive, precise, and accurate UV-spectrophotometric method for simultaneous estimation of SOFO and DACLA in a combined dosage form. The developed method is suitable for the assay of combined dosage form available in the market. In our study, for dual-wavelength, the absorbance difference value between 235.8 nm and 270.6 nm used for SOFO estimation where DACLA has same absorbance and ΔA between 249 nm and 268.6 nm used for estimation of DACLA where SOFO has same absorbance. For ratio derivative method, SOFO estimation was carried out at maxima 247.0 nm and DACLA determination at minima 341.0 nm.

MATERIALS AND METHODS

Apparatus

A UV/Visible spectrophotometer (Shimadzu UV- 1700) with 2 nm spectral width, 0.5 nm wavelength accuracy, and two matched quartz cell was used to measure the absorbance of all the solutions. UV probe software (version 2.33) was used to obtained spectra automatically. Toshcon ultrasonic bath (Toshniwal process instrument pvt ltd.) and Reptech analytical balance based on electromagnetic force compensation technology were utilized in work**.**

Chemicals and Reagents

SOFO and DACLA bulk powder was procured as gift sample from Cadila Pharmaceuticals Ltd. (Gujarat, India). SOVIHEP D, a commercial fixed-dose combination, was purchased from the local market. Methanol (S. D. Fine Chemicals Ltd., Mumbai, India) & Distilled water used was of high-purity.

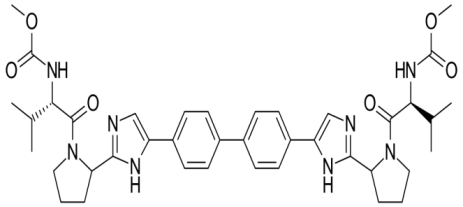

Figure 2: Daclatasvir dihydrochloride Structure

Preparation of Standard Solution

SOFO and DACLA standard Stock solutions were prepared by transferring 50 mg of the respective drug to 50 ml separate volumetric flasks and dissolved in methanol to represent 1 mg/mL of each drug. Aliquot 10.0 ml from the above solution in separate 100 ml volumetric flasks and make up the volume with diluent.

Preparation of Diluent

Prepare a mixture of Distilled Water and Methanol in the ratio of (30:70) %v/v.

METHODOLOGY

Selection of Analytical Wavelength

Method A - Dual Wavelength

SOFO (50 µg/mL) and DACLA (8 µg/ml) solutions were prepared distinctly from working standards using diluents and scanned in the wavelength range of 200 – 400 nm (Figure 3). The quantitative determination of SOFO in the binary mixture was done by computing the absorbance difference between 235.8 nm and 270.6 nm. The ΔA value of DACLA at this wavelength shows zero value. The quantitative determination of DACLA in the binary mixture was done by measuring the ΔA between 249 nm and 268.6 nm. The ΔA value of SOFO at this wavelength shows zero value.

Method B - Ratio Derivative Method

Various solutions of SOFO and DACLA were used as a divisor for optimization of divisor concentration and selection of optimized wavelength. An accurate choice of both standard divisors and working wavelengths is fundamental for various reasons. Stored spectra of standard SOFO solutions were divided wavelength by wavelength by a standard spectrum of DACLA ranging from 4–20 µg/mL as shown in Table 1. DACLA solutions of different concentrations underwent same procedure when concentrations ranging from 10–90 µg/mL of SOFO were used as the divisor in the same way as shown in Table 2. Thus, a concentration of 16 µg/mL of DACLA and 70 µg/ml of SOFO as divisor were selected as they gave the

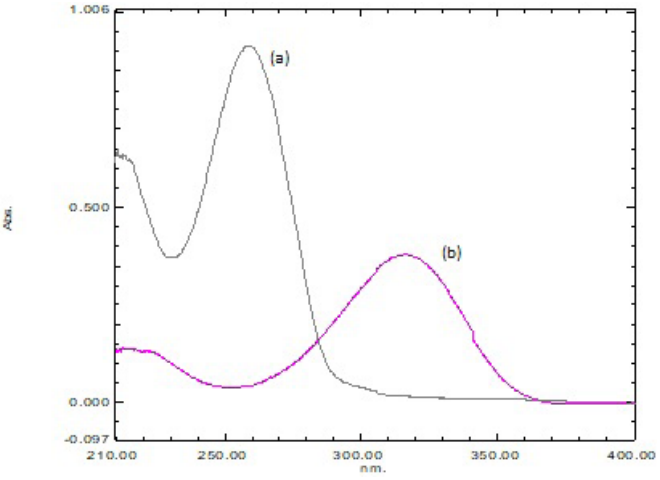

Figure 3: Overlay UV Spectra: a; SOFO (50 µg/mL), b; DACLA (8 µg/mL) in diluent

Table 1: Optimisation of divisor concentration for Sofosbuvir

DRUG	*DACLA divisor concentration (µg/mL)*	*Wavelength (nm) maxima*	*Wavelength (nm) minima*	*R^2 Maxima*	*R^2 Minima*
SOFO (10-90 µg/mL)	4	246	267	0.980	0.968
	8	247	265	0.978	0.972
	12	247	263	0.994	0.978
	16	247	263	0.999	0.978
	20	247	263	0.979	0.978

Table 2: Optimisation of divisor concentration for Daclatasvir dihydrochloride

DRUG	*SOFO divisor concentration (µg/mL)*	*Wavelength (nm) maxima*	*Wavelength (nm) minima*	*R^2 Maxima*	*R^2 Minima*
DACLA (4 - 20 µg/mL)	10	319	-	0.997	-
	30	307	341	0.997	0.997
	50	307	341	0.993	0.996
	70	307	341	0.999	0.999
	90	307	342	0.995	0.995

highest R^2 values and finest results in terms of S/N ratio, the absorbance values at 247.0 nm and 341.0 nm were used for the determination of SOFO and DACLA in prepared mixtures respectively. Figure 4 shows the overlain Spectra of various concentrations of SOFO and DACLA. The first derivative of ratio spectra of various concentration of DACLA is depicted in Figure 5 when 70 µg/mL of SOFO used as a divisor and the first derivative of ratio spectra of various concentration of SOFO is depicted in Figure 6 when 20 µg/mL of DACLA used as the divisor.

Optimization of Derivative Intervals and Scaling Factor

Δλ was tested at different values (2, 4, 8, 16 nm) as well as a different scaling factor was also used. The value of Δλ = 8nm and scaling factor of 1 was found optimal with respect to both slit width and wavelength interval.

Assay of Marketed Formulation

Weigh 10 tablets and determine the average net content of blend. Accurately weigh and transfer a quantity of tablet contents equivalent to about 100 mg of SOFO and 15 mg of DACLA into 50 mL volumetric flask followed by the addition of 30 mL methanol and kept for 20 minutes in sonication. The resultant solution was filtered through whatmann filter paper

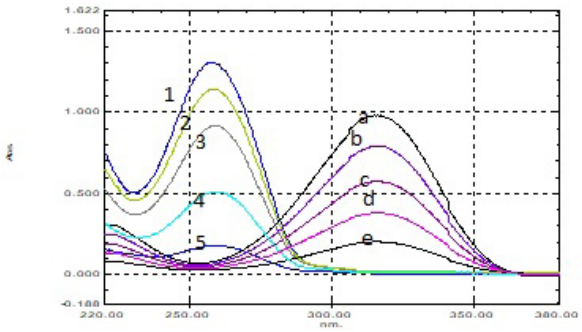

Figure 4: Overlay UV Spectra: a; SOFO (50 µg/mL), b; DACLA (8 µg/mL) in diluent

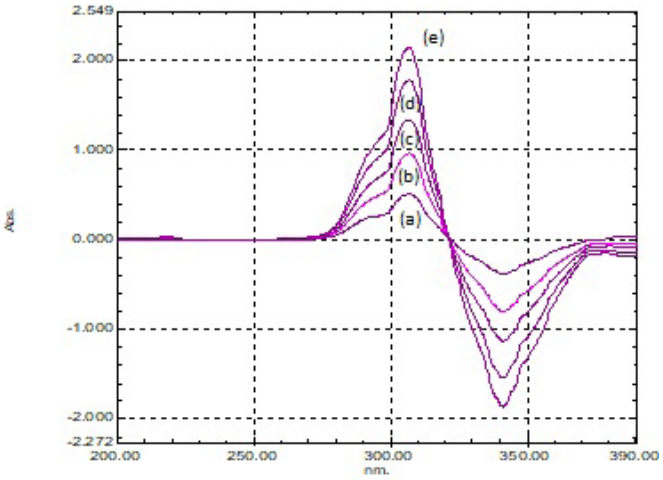

Figure 5: First Derivative of ratio spectra of a,4; b, 8; c, 12 d, 16; e, 20 µg/mL of a solution of DACLA when 70 µg/ml solution of SOFO used as the divisor.(Δλ = 8nm and scaling factor = 1)

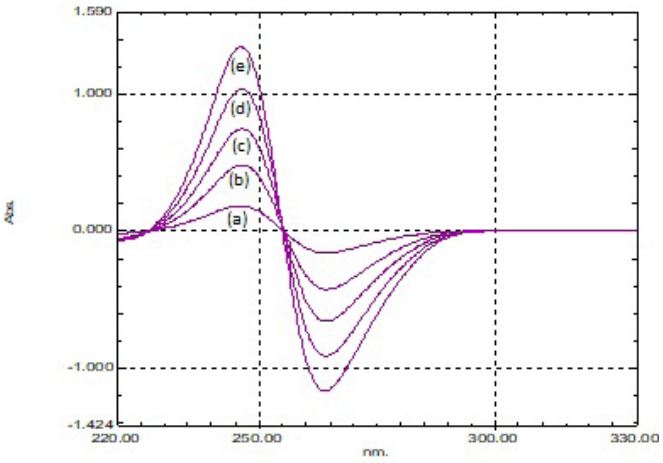

Figure 6: First Derivative of ratio spectra of a,10; b, 30; c, 50 d, 70; e, 90 µg/mL of solution of SOFO when 16 µg/mL solution of DACLA used as the divisor.

No. 41. Dilute and make up to mark with Methanol. Aliquot 5 mL of filtrate to get the solution containing 400 µg/mL of SOFO and 60 µg/mL of DACLA in a 25 mL volumetric flask and dilute it up to mark with diluent. Pipette out a suitable amount from the above solution to obtain 32 µg/mL of SOFO and 4.8 µg/mL of DACLA. For the quantitative determination of SOFO and DACLA, the absorbance (n = 3) were taken at selected wavelengths. The concentration of drugs in the tablet sample was determined by the respective regression line equation of both methods.

Validation of the Proposed Method

Validation was done according to ICH Q2 (R1)

Linearity

The series A, B, and C were prepared from the standard working solution of SOFO and DACLA. Series A was prepared by pipette out 1.0, 3.0, 5.0, 7.0 and 9.0 mL equivalent to 10, 30, 50, 70 and 90 µg/ml of SOFO and Series B was prepared by measuring 0.4, 0.8, 1.2, 1.6 and 2.0 ml equivalent to 4, 8, 12, 16 and 20 µg/ml of DACLA into a series of 10 mL volumetric flasks separately and make up the volume with diluent. Series C consisting of binary mixture containing 10–90 µg/mL of SOFO and 4–20 µg/mL of DACLA.

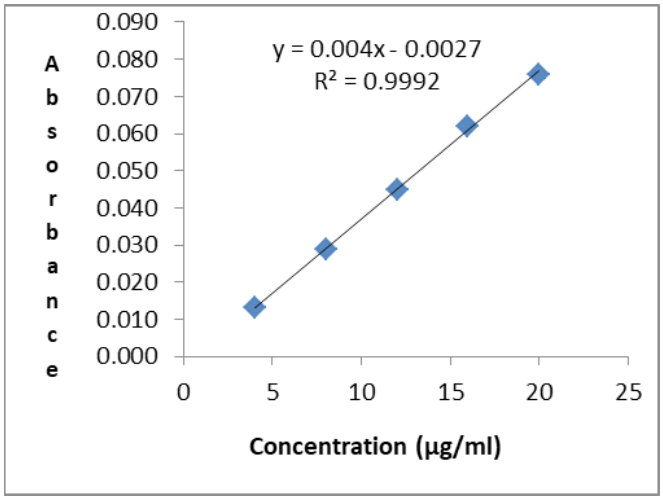

Figure 7: Linearity curve of DACLA

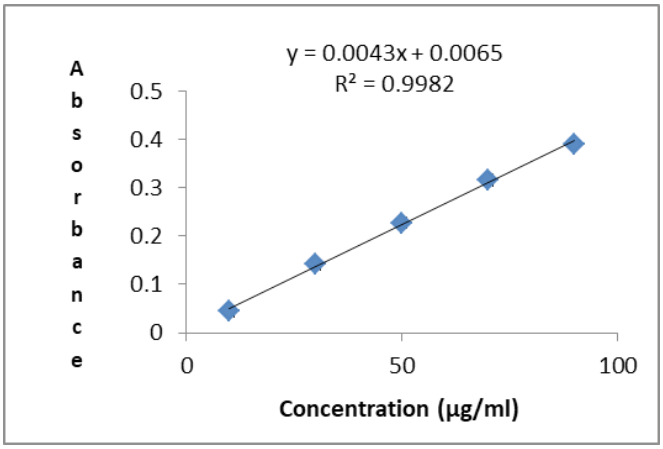

Figure 8: Linearity curve of SOFO

Method A-Dual-wavelength

The difference in absorbance between 235.8 nm and 270.6 nm and 249 nm and 268.6 nm was taken for the quantitative determination of SOFO and DACLA respectively in the binary mixture, and the regression analysis was performed and the graph was presented in Figures 7 and 8.

Method B – Ratio Derivative Method

The absorbance of the solutions of series C was measured at 247.0 nm using 16 µg/mL of DACLA as a divisor and was measured at 341.0 nm using 70 µg/mL of SOFO as a divisor. Regression analysis using the method of least squares was made for the slope, intercept and correlation coefficient values Figures 9 and 10.

Method Precision (Repeatability)

The precision of the instrument was checked by repeated (n = 6) scanning and measurement of the absorbance of binary mixture containing SOFO (30 µg/ml) and DACLA (8 µg/mL) without altering the parameter of the proposed method.

Intermediate Precision (Reproducibility)

It was demonstrated by interday and intraday variation study and performed by measuring the absorbance value at respective wavelengths for the respective method for three concentrations on three different days, thrice and three times on the same

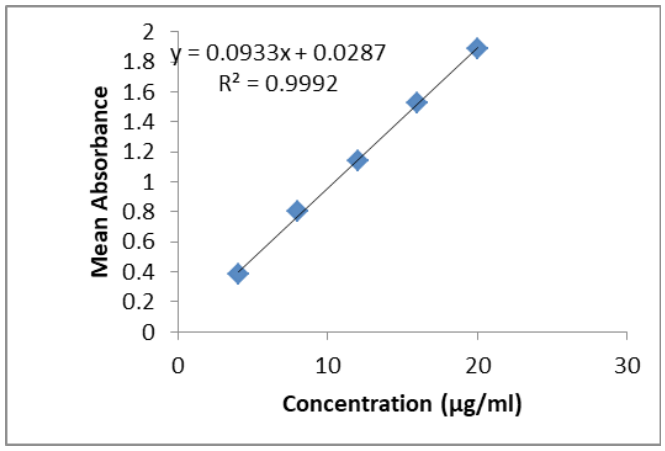

Figure 9: Linearity curve of DACLA

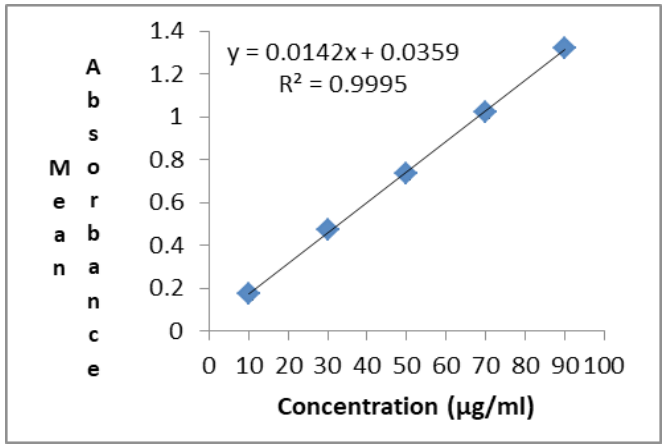

Figure 10: Linearity curve of SOFO

Table 3: Summary of Validation Parameters by Developed Method

Parameters	*Method A*		*Method B*	
	SOFO	*DACLA*	*SOFO*	*DACLA*
SLOPE	0.004	0.004	0.014	0.093
INTERCEPT	0.006	- 0.002	0.035	0.028
Correlation coefficient	0.998	0.999	0.999	0.999
L				inearity range (µg/mL) 10–90 4-20 10–90 4-20
LOD (µg/mL)	5.09	0.66	2.58	0.68
LOQ (µg/mL)	15.42	2.00	7.82	2.08
PRECISION				
Intra–day (n = 3)	0.29 – 0.60	0.75 - 1.23	0.55–0.89	0.69–0.59
Intra–day (n = 9)	0.98 - 1.32	1.59 – 1.51	0.81–1.17	0.89–0.79
Reputability (%RSD)	0.53	1.76	0.28	0.18

Table 4: Results of Recovery Studies of Both Methods

Method	*Spiked (µg/mL)*		*% Recovery ± SD*	
	SOFO	*DACLA*	*SOFO*	*DACLA*
A	16	2.4	99.68 ± 0.275	99.58 ± 0.486
B	16	2.4	99.87 ± 0.359	99.17 ± 0.529
A	32	4.8	100.06 ± 0.798	99.16 ± 0.679
B	32	4.8	100.28 ± 0.769	100.42 ± 0.785
A	48	7.2	100.25 ± 0.968	100.42 ± 0.957
B	48	7.2	100.10 ± 0.968	100.14 ± 0.959

Table 5: Results of assay in formulation

Tablet	*Method*	*% Recovery ± SD*	
		SOFO	DACLA
SOVIHEP D (400 mg SOFO + 60 mg DACLA)	A	100.38 ± 1.43	98.71 ± 1.29
	B	100.52 ± 1.58	100.35 ± 1.34

day, respectively. The analysis was carried out on a binary mixture containing three concentration range of SOFO (30, 50, 70 µg/mL) and DACLA (8, 12, 16 µg/mL), respectively and %CV were calculated.

Accuracy (Recovery Study)

The standard addition method was employed for a recovery study. Prequantified sample containing 20.0 µg/mL SOFO and 3.0 µg/mL DACLA were spiked at 50, 100, and 150 % with known amount of standard solution of SOFO and DACLA. The quantity of drug was estimated using regression line equations at respective wavelength for both the methods. A result was represented in Table 4.

Limit of Detection and Limit of Quantification

The limit of detection (LoD) and the limit of quantification (LoQ) of the drug were calculated using equations given in ICH Q2 (R1) guidelines.

RESULTS AND DISCUSSION

The dual-wavelength developed based on the absorbance difference at a wavelength where one drug gives the same absorbance so that the other drug can be estimated at that wavelength in a binary mixture. Likewise, SOFO estimation done by taking absorbance difference at a wavelength ($A_{235.8}$–$A_{270.6}$) where DACLA gives the same absorbance and DACLA estimation by taking absorbance difference at (A_{249}–$A_{268.6}$) where SOFO gives the same absorbance. The various parameters were optimized for the development of ratio Derivative spectroscopy method like Divisor concentration, wavelength selection, scaling factor, smoothing factor. SOFO in the concentration of 70 µg/mL used as a divisor for the estimation of DACLA in the binary mixture and DACLA in a concentration of 16 µg/mL used as a divisor for estimation of SOFO in the binary mixture. SOFO and DACLA were estimated at 247 nm and 341 nm, respectively. A scaling factor of 1 and a smoothing factor of 8 nm were selected. The linearity for both methods was found for SOFO and DACLA at 10 – 90 µg/ml and 4–20 µg/mL, respectively with a correlation coefficient of 0.999. The %RSD was found for both the methods less than 2%, so it complies with ICH Q2 (R1).The results of various validation parameters of both the methods were represented in Table 3. Good Recovery was obtained at each spiked level of both drugs to prequantified formulation and presented in Table 4. The assay results represented in Table 5 showed that the values obtained by these methods were in close agreement with a label claim of the marketed formulation.

CONCLUSION

Sofosbuvir and DACLA combination was used in the treatment of hepatatis C infection. Simple, Precise, accurate, and rapid method was developed for the estimation of both drugs in pharmaceutical formulation and validated as per ICH guidelines. Both methods can resolve the Spectral overlapping without prior separation, and both drugs can be estimated without interfering with each other. The results of the proposed method were found to be in close agreement of the label claim of marketed formulation. The proposed method can be used for the simultaneous estimation of both drugs in bulk as well as a pharmaceutical formulation.

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