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DEVELOPMENT AND VALIDATION OF Q-ABSORBANCE RATIO METHOD FOR THE SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND DACLATASVIR DIHYDROCHLORIDE IN SOLID DOSAGE FORM.

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ABSTRACT

A simple and sensitive Spectrophotometric method based on Q-Absorbance Ratio Method was developed for the simultaneous estimation of Sofosbuvir and Daclatasvir dihydrochloride in solid dosage form. This method was based on the formation of Q-Absorbance equation at two wavelengths, one was Isoabsorptive Point and another one was λmax of one of them. So, λ_1 was 273 nm and λ_2 was 261 nm. and Daclatasvir dihydrochloride were comply with the Beer's Lambert's law over the linearity range 4-32 µg/ml. The method was validated as per International Conference on Harmonization (ICH) guidelines. The parameters of ICH guidelines are Linearity, Precision (Repeatability and Reproducibility), Limit Of Detection (LOD), Limit Of Quantitation (LOQ) and Accuracy. All parameters were found to be within the accepted limits. The method was found to be simple, rapid, sensitive, precise, accurate and cost effective for use in routine analysis of both drugs in pharmaceutical dosage form.

KEYWORDS: Sofosbuvir, Daclatasvir dihydrochloride, Q-Absorbance Ratio method, Methanol: Water (50:50).

INTRODUCTION[1-4]

- Sofosbuvir: It is prodrug nucleotide analog used as a part of combination therapy to treat HCV infection or to treat co-infection of HIV and HCV. It is chemically Propan-2-yl (2S)-2-{[(S)-{[(2R,3R,4R,5R)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-4-fluoro-3-hydroxy- 4-methyloxolan-2-yl]methoxy}(phenoxy)phosphoryl]amino}propanoate.
- Pharmacokinetic study: When given orally, Sofosbuvir reaches its maximum plasma concentration in about 0.5 to 2 hours with a maximal concentration (Cmax) of 567 ng/ml. Sofosbuvir is eliminated by three routes: urine (80%), feces (14%), and respiration (2.5%); however, elimination through the kidneys is the major route.
- Molecular Formula:$C_{22}H_{29}FN_3O_9P$
- Solubility: Soluble in water and freely soluble in Acetonitrile, Methanol, Ethanol, Acetone and DMSO.
- Mechanism of action: Sofosbuvir is nucleotide analog inhibitor, which specifically inhibits HCV NS5B (non-structural protein 5B) RNA-dependent RNA polymerase. More specifically, Sofosbuvir prevents HCV viral replication by binding to the two Mg^{2+} ions present in HCV NS5B polymerase's GDD active site motif and preventing further replication of HCV genetic material.
- Daclatasvir dihydrochloride: It is HCV NS5A inhibitor, which is Direct Acting Antiviral. It is chemically Methyl N-[(2S)-1-[(2S)-2-[5-(4'-{2-[(2S)-1-[(2S)-2- [(methoxycarbonyl)amino]-3-methy=lbutanoyl]pyrrolidin-2-yl]-1H-imidazol-5-yl}-[1,1'- biphenyl]-4-yl)-1H-imidazol-2-yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate dihydrochloride.
- Molecular Formula: $C_{40}H_{52}Cl_2N_8O_6$.
- Solubility: Soluble in Water. Freely soluble in Acetonitrile, Methanol, Ethanol, DMSO and Acetone.
- Pharmacokinetic study: Peak plasma concentrations typically occurred within 2 hours after administration of doses ranging from 1 - 100 mg once daily. Approximately 88% of Daclatasvir is eliminated in the feces (53% is eliminated unchanged), while 6.6% is eliminated primarily unchanged in the urine.
- Mechanism of action: It has the ability to bind to HCV RNA. It is shown to have two distinct functions in HCV RNA replication based on phosphorylated states. Maintaining the HCV replication complex is mediated by the cis-acting function of basally phosphorylated NS5A and the trans-acting function of hyperphosphorylated NS5A modulates HCV assembly and infectious particle formation. Daclatasvir is shown to

disrupt hyperphosphorylated NS5A proteins thus interfere with the function of new HCV replication complexes and also blocks both intracellular viral RNA synthesis and virion assembly/secretion in vivo.

- Aim: Very few methods have been reported for the individual estimation of Sofosbuvir and Daclatasvir and their combination with other drugs. Literature review does not found stability indicating RP-HPLC method for Sofosbuvir and Daclatasvir in tablet.
- So Aim of present work is to develop new simple, sensitive, economic and less time consuming Stability indicating RP-HPLC method development and validation for estimation of Sofosbuvir and Daclatasvir in tablet.

➢ **Rationale**

- This combination is safe and effective for the treatment in patient for β-Thalassemia Major in Genotype 3a, in patient with Hepatitis C Genotype 3 who is undergoing hemodialysis, in treatment of HCV in kidney transplant patient.
- Daclatasvir in combination with Sofosbuvir reduces the risk of long term complication of Hepatitis C such as Liver cancer or needing liver transplant.

MATERIAL AND METHODS

Instruments and Apparatus

A double beam UV/Visible spectrophotometer (Shimadzu 1600, Japan) having spectral width of 2 nm, wavelength accuracy of ± 0.5 nm and a pair of 10 mm matched quart cell was used to carry out Spectrophotometric measurements. UV was connected with computer and spectra were automatically recorded by UV-Probe 2.0 system software. CP224S, Sartorius, Germany analytical balance was used for all weighing process. Frontline FS-4 Ultra Sonicator used for sonication purpose.

MATERIALS

Pure sample of Sofosbuvir and Daclatasvir dihydrochloride obtained from Zydus Healthcare Ltd., Ahmedabad, Gujarat. Distilled water was used. Methanol of AR grade used as solvent from Central Drug House (P) Ltd., New Delhi, India.

5.3 Preparation of solutions

Preparation of Diluent

Prepare mixture of Water and Methanol in the ratio of (50:50) %v/v.

Preparation of Stock solution

Accurately weigh 10 mg of Sofosbuvir and Daclatasvir dihydrochloride in 100 ml calibrated volumetric flask. Add Methanol in flask and shake to dissolve and make volume up to mark with methanol. (Sofosbuvir 100 µg/ml and Daclatasvir dihydrochloride 100 µg/ml).

Preparation of Working Standard solution

Take 1 ml from above solution in 10 ml calibrated volumetric flask and make volume up to mark with diluent.

Method Development

Selection of Analytical wavelength

The Working Standard solution of 10 µg of Sofosbuvir and Daclatasvir dihydrochloride were properly scanned individually in the UV range of 200 nm to 400 nm. Best maximum response of Sofosbuvir was gain at 261 nm and Daclatasvir dihydrochloride was gain at 316 nm. The Isoabsorptive Point was found at 273 nm. So, for this Q-Absorbance Ratio Method, λ_1 was 273 nm and λ_2 was 261 nm.

Preparation of Calibration Curve

The calibration curves were graph over a concentration range of 4-32 µg/ml for Sofosbuvir and Daclatasvir dihydrochloride. Accurately measured standard solution of Sofosbuvir and Daclatasvir dihydrochloride (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2 ml) were added in to 10 ml calibrated volumetric flask and make volume up to mark with diluent. The absorbances of solutions were measured between 200 to 400 nm against diluent as blank. The calibration curves were assembled by plotting absorbance versus concentrations and the regression equations were calculated.

Method Validation[5]

Linearity and Range

Linearity of method was assessed by analyzing series of eight individual concentrations of both drugs and repeated for three times. The calibration graph obtained by plot of Absorbance versus Concentration showed good linear relationship.

Precision

Method Precision (Repeatability)

Repeatability expresses the precision under the same operating conditions over a short

interval of time. It is also termed as Intra-Assay Precision.

The precision of instrument was checked by repeated proper scanning of absorbance of solutions (n=6) of Sofosbuvir and Daclatasvir dihydrochloride (12 µg/ml) same day except changing the parameter of the Q-Absorbance Ratio method and % RSD was calculated.

Intermediate Precision

Intermediate Precision expresses within-laboratories variations different day, different analyst and different equipment.

Intermediate Precision of the Q-Absorbance Ratio was determined by analyzing the corresponding answer three times on the two individual days (Interday Assay) for three individual concentrations (12, 16 and 20 µg/ml). The result is expressed as % RSD.

Limit of Detection and Limit of Quantitation:

The Limit of Detection and Limit of Quantitation of the method were calculated by apply the below equations.

$$LOD = 3.3 * \frac{\sigma}{S}$$

$$LOQ = 10 * \frac{\sigma}{S}$$

Where, σ = the standard deviation of the response.

S = slope of the calibration curve.

Accuracy (Recovery)

The accuracy study was performed by adding known amount of standard solution of Sofosbuvir and Daclatasvir dihydrochloride at 50%, 100% and 150% level to pre quantified sample solution of Sofosbuvir and Daclatasvir dihydrochloride (20 µg/ml and 6 µg/ml). The quantity of drug was determined by standard addition method. The analysis of recovery was repeated three times and calculates average recoveries.

RESULTS AND DISCUSSION

Method Development

The Working Standard solutions of 10 µg/ml Sofosbuvir and Daclatasvir dihydrochloride were properly scanned individually in the UV range of 200 nm to 400 nm. The λmax of Sofosbuvir was 261 nm and Daclatasvir dihydrochloride was 316 nm. The Isoabsorptive

point was found at 273 nm. For Q-Absorbance Ratio Method λ_1 was 273 nm and λ_2 was 261 nm. The diluent was used for this method was Methanol: Water (50:50) %v/v. Fig. - 1.

Validation of the Q-Absorbance Ratio Method: Linearity.

Daclatasvir dihydrochloride and Sofosbuvir were showed liner response in the range of 4-32 µg/ml. The calibration curve at wavelength 261 of Sofosbuvir and Daclatasvir dihydrochloride and at Isoabsorptive point 273 nm are shown in below Fig.- 2-4.

Precision

Method Precision (Repeatability)

The % RSD values of repeatability for Sofosbuvir and Daclatasvir dihydrochloride at 261 nm and 273 nm are mentioned in Table- 1.

Intermediate Precision (Reproducibility)

The Intermediate Precision (Intraday and Interday Precision) was performed for 3 standard concentrations (12, 16 and 20 µg/ml) of Daclatasvir dihydrochloride and Sofosbuvir. The % RSD values of both drugs for interday variations and intraday variations are stated in below Table- 2.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ measured at 273 nm (λ_1) and 261 (λ_2). Values are mention in below Table-3. Low values revealed that the developed method was sensitive.

Accuracy

The % RSD was found in the range of 99.8 ± 0.05 for Sofosbuvir and 100.9 ± 0.30 for Daclatasvir dihydrochloride at the 50 %, 100 % and 150 % level of standard additions. Detailed Recovery data of each level are mention in below Table-4. Fig. 5 and Fig. 6 showed the level of Accuracy.

Tables

Method Precision (Repeatability).

Table 1: Data of Method Precision.

Sample no.	Absorbance at 261 nm		Absorbance at Isoabsorptive point 273 nm
	Dac. 261 nm	Sof. 261 nm	
1	0.07483	0.49172	0.20886
2	0.07472	0.49158	0.20674
3	0.07494	0.49487	0.20874
4	0.07459	0.49224	0.20911
5	0.07449	0.49322	0.20747
6	0.07452	0.49346	0.20947
Average	0.07468	0.49284	0.20839
SD	0.00018	0.00125	0.00105
%RSD	0.24063	0.25425	0.50767

Intermediate Precision (Reproducibility)

Table 2: Data of Intermediate Precision.

Parameters	Daclatasvir dihydrochloride	Sofosbuvir	Isoabsorptive point
Wavelength (nm)	261	261	273
Intraday Precision (% RSD)(n=3)	0.01-0.51	0.01-0.14	0.01-0.51
Interday Precision (% RSD)(n=3)	0.24-0.26	0.24-0.25	0.50-0.60

LOD and LOQ.

Table 3: Values of LOD and LOQ.

Parameters	Daclatasvir dihydrochloride	Sofosbuvir	Isoabsorptive point
Wavelength (nm)	261	261	273
LOD (µg/ml)	0.36	0.33	0.34
LOQ (µg/ml)	1.10	1.0	1.05

Accuracy

Table 4: Values of LOD and LOQ.

Parameters	Daclatasvir dihydrochloride	Sofosbuvir	Isoabsorptive point
Wavelength (nm)	261	261	273
LOD (µg/ml)	0.36	0.33	0.34
LOQ (µg/ml)	1.10	1.0	1.05

Table 5: Regression Analysis data and summary of Validation Parameters.

Parameters	Sofosbuvir	Daclatasvir dihydrochloride	Sofosbuvir and Daclatasvir dihydrochloride
Wavelength (nm)	261	261	273
Beer's Law Linearity Range (µg/ml)	4 – 32	4 – 32	4 – 32
Regression Equation (y=mx+c)	Y = 0.04x - 0.005	Y = 0.007x – 0.011	Y = 0.016x- 0.002
Slope (m)	0.04	0.007	0.016
Intercept (c)	0.005	0.011	0.002
Correlation Coefficient (r^2)	0.999	0.999	0.999
LOD (µg/ml)	0.33	0.36	0.34
LOQ (µg/ml)	1.0	1.10	1.05
Repeatability (% RSD, n = 6)	0.25	0.24	0.50
Precision (% RSD, n = 3) Intraday Interday	 0.01 – 0.14 0.24 - 0.25	 0.01 - 0.51 0.24 – 0.26	 0.01 - 0.51 0.50 – 0.60
Accuracy ± SD (% Recovery, n = 3)	99.8 ± 0.05	100.9 ± 0.30	-
% Assay ± SD (n = 6)	99.99 ± 0.05	99.83 ± 0.65	-

Figures

Spectra of Sofosbuvir and Daclatasvir dihydrochloride

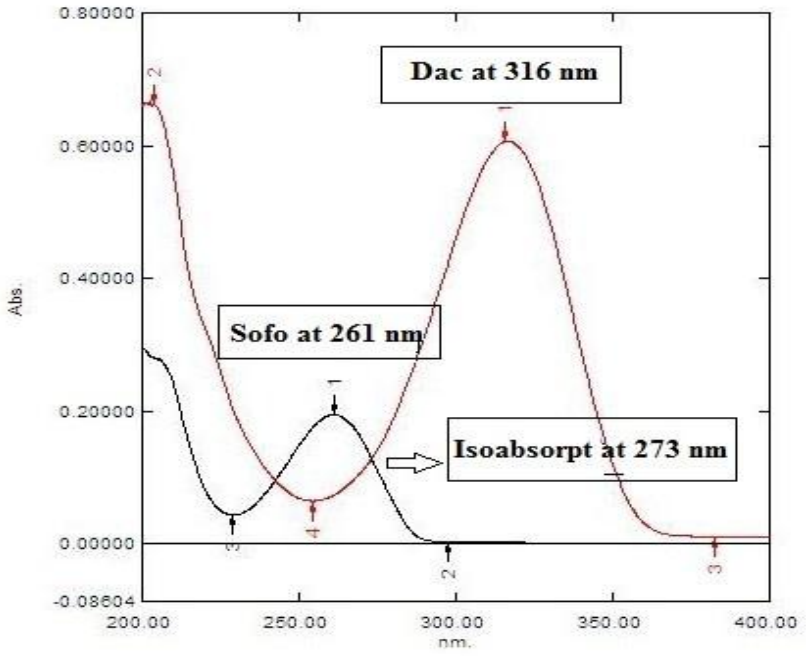

Fig. 1: Overlain spectra of sofosbuvir and daclatasvir dihydrochloride.

Linearity

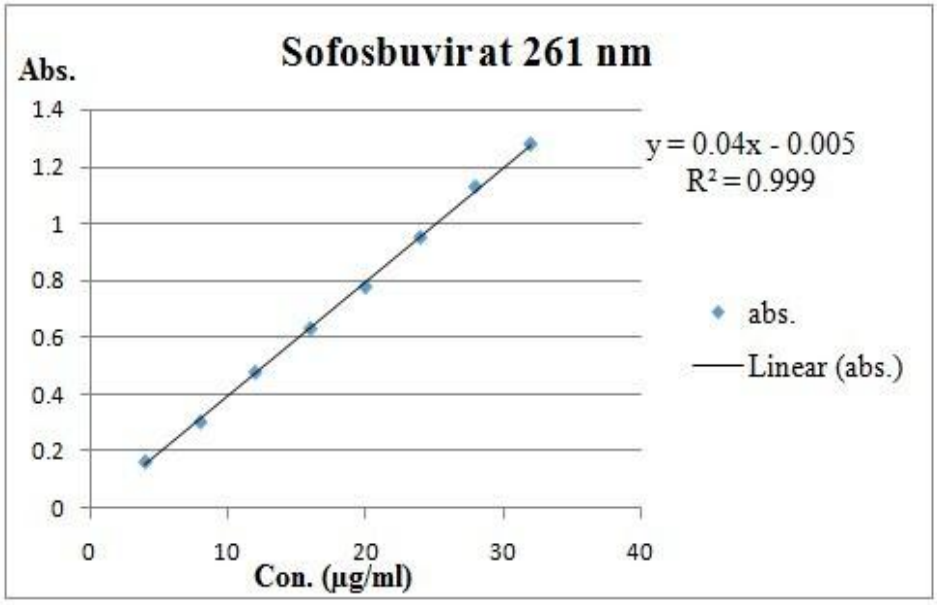

Fig 2: linearity of sofosbuvir at 261nm.

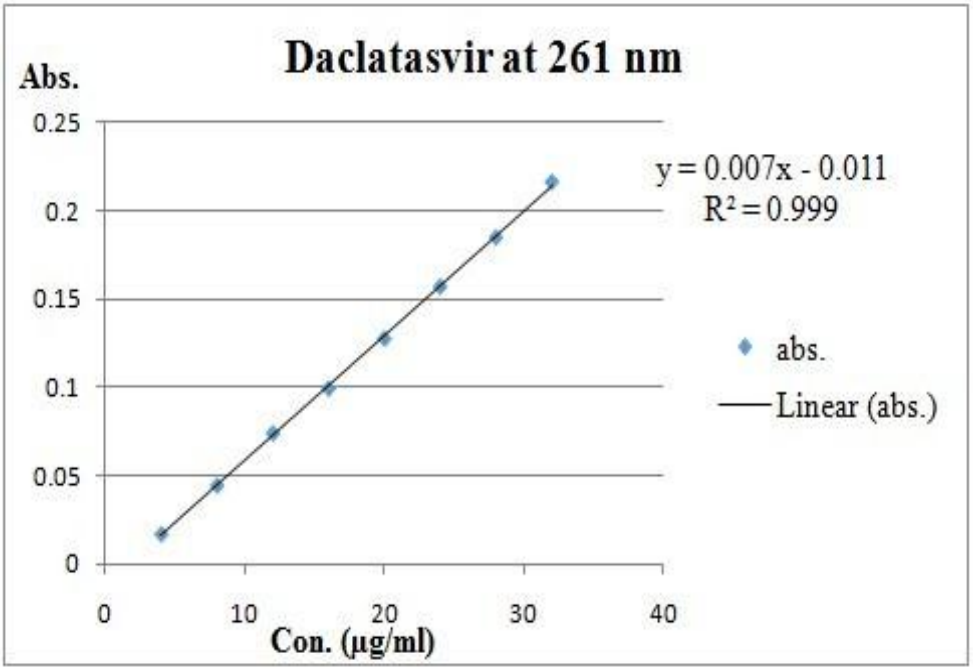

Fig 3: Linearity of daclatasvir at 261nm.

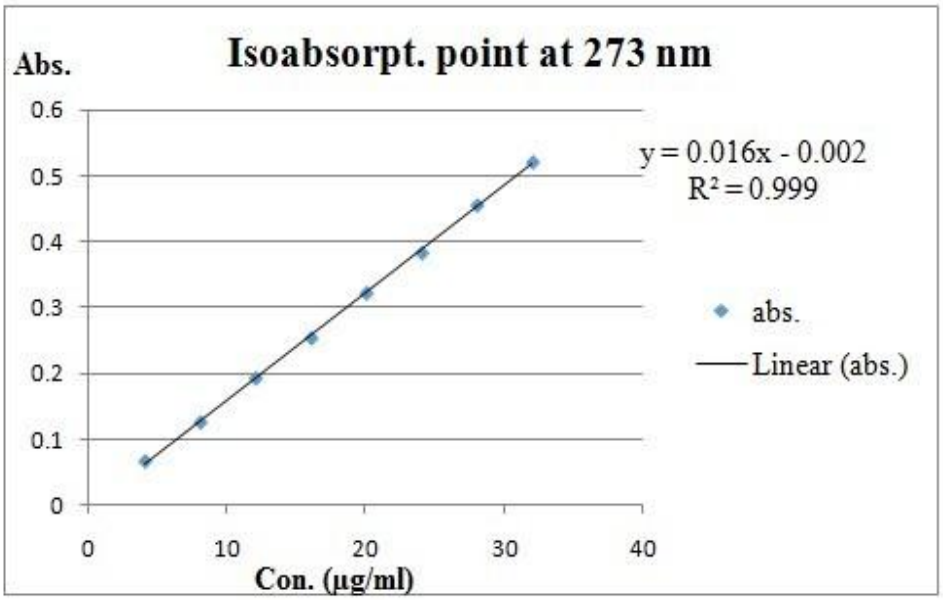

Fig 4: linearity of isoabsorptive point at 273 nm Accuracy.

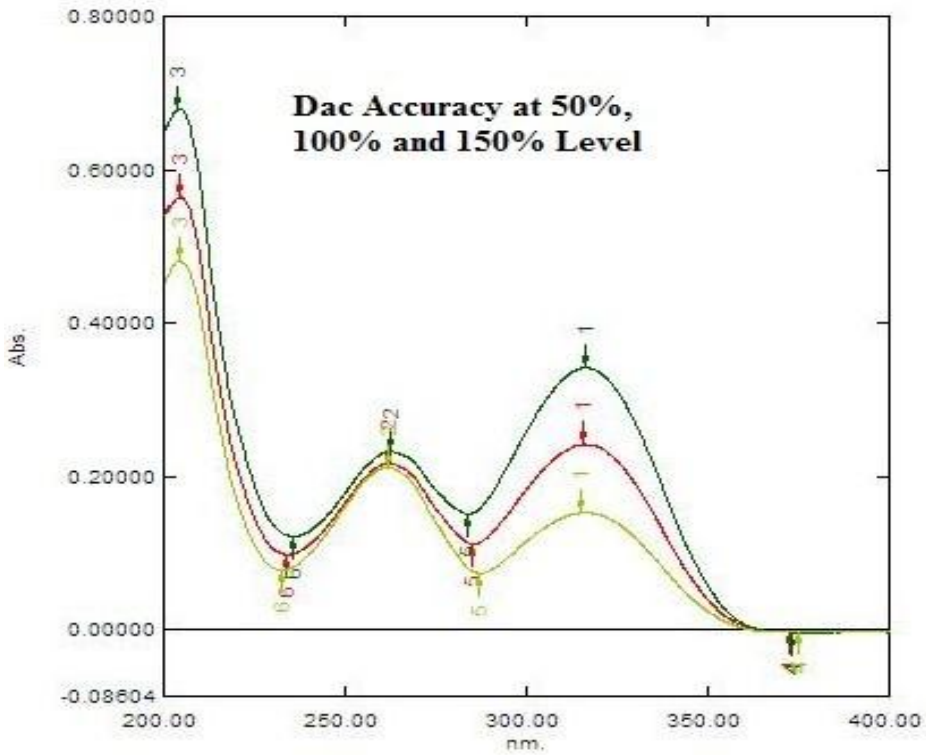

Fig 5: accuracy level of daclatasvir at 50%, 100% and 150%.

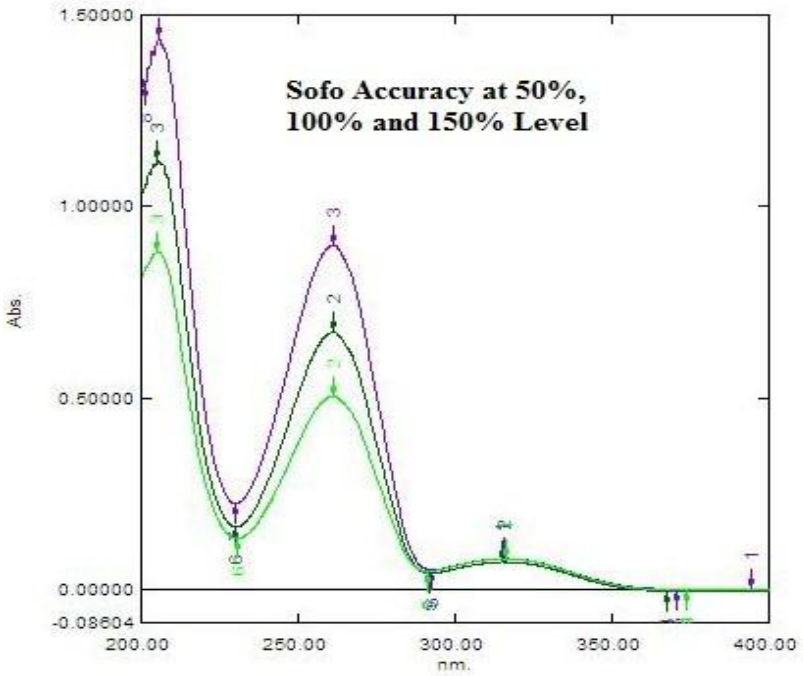

Fig 6: accuracy level of sofosbuvir at 50%, 100% and 150%.

CONCLUSION

The Q- Absorbance Ratio Method for simultaneous estimation of Sofosbuvir and Daclatasvir dihydrochloride shows linear response in 4 – 32 (µg/ml). Method was observed to be precise and accurate as can be reflected from calibration parameters data. Developed method was efficiently applied for determination of both drugs Sofosbuvir and Daclatasvir dihydrochloride in pharmaceutical tablet dosage form.

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