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Research Article

**DEVELOPMENT AND VALIDATION FOR ESTIMATION OF
CLINDAMYCIN, ADAPALENE AND SOFOSBUVIR IN BULK
AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC
METHOD****G. Swathi^{1*}, G. Deepthi², Deepthi Visakh³, J. Prathyusha⁴, Nafees Fatima⁵, M. Sai lakshmi⁶**

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Article Received: October 2021 **Accepted:** October 2021 **Published:** November 2021**Abstract:**

A simple, accurate, rapid and precise isocratic stability indicating reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous determination of Clindamycin and Adapalene in tablets. The chromatographic separation was carried out on C₁₈ BDS Hypersil (150 x 4.6mm, 5μ) with a mixture of mixed phosphate buffer : acetonitrile (55:45%v/v) as a mobile phase at a flow rate of 1.0mL/min. UV detection was performed at 230nm. The retention times were 2.84 and 3.999min for Clindamycin and Adapalene respectively. Calibration plots were linear (r²=0.999) over the concentration range of 25-150μg/mL for Clindamycin and 2.5-15μg/mL for Adapalene. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Clindamycin and Adapalene in bulk and tablet dosage form.

Sofosbuvir is used primarily to treat hepatitis C and viral hemorrhagic fevers. It is possible to select a sofosbuvir resistant mutant of HCV that can replicate to levels similar to wild type virus grown without sofosbuvir. Analysis of the mutations responsible for the sofosbuvir resistance may aid in understanding the mechanism of action of sofosbuvir.

Keywords: Clindamycin , Adapalene, RP-HPLC, Tablets, hepatitis C.**Corresponding author:****Gundeboina. Swathi,**

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INTRODUCTION:

Clindamycin (CLIN) (Fig-1) is a semisynthetic lincosamide antibiotic that has largely replaced lincomycin due to an improved side effect profile. Clindamycin is an antibiotic, similar to and a derivative of lincomycin. Clindamycin can be used in topical or systemic treatment. It is effective as an anti-anaerobic antibiotic and antiprotozoal. It is chemically Methyl (5R)-5-[(1S)-2-chloro-1-[[[(4R)-1-methyl-4-propyl-L-prolyl] amino] propyl]-1-thio- β -L-arabinopyranoside Adapalene (ADA) (Fig-2) is a chemically stable retinoid-like compound. Biochemical and pharmacological profile studies have demonstrated that adapalene is a modulator of cellular differentiation, keratinization, and inflammatory processes all of which represent important features in the pathology of acne vulgaris. Mechanistically, adapalene binds to specific retinoic acid nuclear receptors (gamma and beta) and retinoid X receptors but does not bind to the cytosolic receptor protein. [1-4] It is chemically 6-[3-(adamantan-1-yl)-4-methoxyphenyl] naphthalene-2-Carboxylic acid.

Literature survey [5-10] reveals that few spectrophotometric and chromatographic methods were reported for estimation of CLIN and ADA in single and combination with other drugs. In this study, an attempt has been made to develop an accurate, rapid and reproducible reverse phase HPLC method for simultaneous determination of CLIN and ADA in tablet dosage form and validate it, in accordance with International Conference on Harmonization (ICH) [11-12] guidelines.

MATERIALS AND METHODS:

Chemicals and reagents:

The reference samples of CLIN (API) and ADA (API) were obtained from Pulse Pharmaceuticals, Hyderabad. The branded formulations LACNEgel was procured from the local market. Gel claimed to contain 1% CLIN and 0.1% ADA have been utilized in the present work. All chemicals and reagents used were HPLC grade and purchased from Merck chemicals, India.

Chromatographic conditions:

Separation was performed on an isocratic waters HPLC 2965 system instrument equipped with a binary pump and variable wavelength PDA detector with auto injector. Data was analysed by using Empower2 software. Degassing of the mobile phase was done by using bath sonicator. A Shimadzu balance was used for weighing the materials. The separation was achieved on a BDS C₁₈(150 x 4.6 mm, 5 μ) analytical column. The mobile phase consisted of

mixed phosphate buffer: acetonitrile (55:45% v/v). The flow rate was 1.0 mL/min and UV detection was performed at 230 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 μ m membrane filter (Millipore, Ireland). The injection volume was 10 μ L and all the experiments were performed at ambient temperature.

Preparation of standard solution:

Accurately weighed and transferred 100 mg of CLIN and 10 mg of ADA working standards into 100 mL clean dry volumetric flasks, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents.

Preparation of sample preparation:

Lacne Gel with label claim of 1% CLIN and 0.1% ADA in 10 g was extracted with 50 mL of 0.1% glacial acetic acid in methanol in centrifuge tube. The sample mixture was heated at 50°C for 10 min and mixed occasionally during the heating process. After heating, the sample was allowed to cool for 10 min and add 2 mL of water into the centrifuge tube and then cooled in an ice bath for 20 min and centrifuged at 3000 RPM for 10 min. After centrifugation, the supernatant liquid layer, which contains the analytes of interest, was separated.

Method validation:

The developed method was validated according to ICH guidelines. The system suitability was evaluated by five replicate analyses of CLIN and ADA mixture at concentrations of 1000 μ g/mL and 100 μ g/mL. The acceptance criteria are number of theoretical plates (N) at least 2000 per each peak and tailing factor is not more than 2.0.

Linearity:

Standard calibration curves were plotted against the concentration ranging from 25-150 μ g/mL for CLIN and 2.5-15 μ g/mL for ADA. Different linearity levels were prepared and injected into the HPLC system keeping the injection volume constant.

Recovery:

To study the reliability and suitability of developed method, recovery experiments were carried out at three levels 50%, 100% and 150%. Known concentration of sample was spiked with known amount of standard. At each level, three determinations were performed with expected results. The %RSD of individual measurements was also determined.

Precision:

Precision of assay was determined by System and Method Precision. Every sample was injected six times. The repeatability of sample application and measurements for peak area were expressed in terms of %RSD.

Specificity:

All chromatograms were examined to determine whether compound of interest coeluted with each other or with any additional excipient peaks. Marketed formulation was analysed to determine the specificity of the optimized method in presence of common excipients.

Limit of detection and limit of quantification:

Limit of detection (LOD) and limit of quantification (LOQ) were estimated from signal-to-noise ratio. LOD and LOQ were calculated using $3.3\sigma/s$ and $10\sigma/s$ formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve.

Robustness:

To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included are variation of flow rate and Detection Wavelength.

Force Degradation studies:**Oxidation:**

To 1 ml of stock solution, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at $60^\circ C$. For HPLC study, the resultant solution was diluted to obtain $100\mu g/ml$ & $10\mu g/ml$ solution and $10\mu l$ were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution of Adapalene and Clindamycin, 1 ml of 2N Hydrochloric acid was added and refluxed for 30 mins at $60^\circ C$. The resultant solution was diluted to obtain $100\mu g/ml$ & $10\mu g/ml$ solution and $10\mu l$ solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution of Adapalene and Clindamycin, 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins at $60^\circ C$. The resultant solution was diluted to obtain $100\mu g/ml$ & $10\mu g/ml$ solution and $10\mu l$ were injected into the system and the chromatograms were

recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at $105^\circ C$ for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to $100\mu g/ml$ & $10\mu g/ml$ solution and $10\mu l$ were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the $300\mu g/ml$, $10\mu g/ml$ & $25\mu g/ml$ solution to UV Light by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/ m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain $100\mu g/ml$ & $10\mu g/ml$ solutions and $10\mu l$ were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of $60^\circ C$. For HPLC study, the resultant solution was diluted to $100\mu g/ml$ & $10\mu g/ml$ solution and $10\mu l$ were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION:

During the optimization of HPLC method, two columns symmetry C-18 and C-8 analytical column (4.6×250 mm; $5\mu m$) and (4.6×150 mm; $5\mu m$), organic solvent (acetonitrile), one buffer (phosphate) were tested. Initially Water: Acetonitrile and Phosphate buffer, were tried in different ratios. Finally mobile phase consisting of mixture of acetonitrile: Phosphate buffer in ratio 45:55 (v/v) was selected as mobile phase to achieve clear separation and sensitivity. Flow rates between 0.8 to 1.2 mL/min were studied. A flow rate of 1.0 mL/min gave an optimum signal to noise ratio with reasonable separation time using a C_{18} analytical column (4.6×250 mm; $5\mu m$), the retention times for CLIN & AD were observed to be 2.84 and 3.999 min respectively. Total run time was less than 7 min. The chromatogram at 230 nm showed a complete resolution for all peaks (Fig. 3). Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability tests. All critical parameters tested meet the acceptance criteria on all days. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks.

Linearity was obtained for CLIN & ADA in the range of 25-150 µg/mL and 2.5-15 µg/mL. The correlation coefficient (r^2) was found to be greater than 0.999 in all instances. The results of calibration studies are summarized in Table 1. The proposed method afforded high recoveries for CLIN and ADA in dosage form. Results obtained from recovery studies presented in Table 2 indicate that this assay procedure can be used for routine quality control analysis of binary mixture in sample. Precision of the analytical method was found to be reliable based on %RSD (<2%) corresponding to peak areas and retention times. As can be seen in Table 3 the %RSD values were less than 2 for System & Method precision. Hence, the method was found to be precise for these two drugs.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common excipients and impurities. Also the peak areas were compared with standard and percentage purity calculated was found to be within limits. LOD and LOQ were found to be 0.13 µg/mL and 0.4 µg/mL for CLIN, 0.08 µg/mL and 0.24 µg/mL for ADA. In all deliberately varied conditions, the %RSD for replicate injections of CLIN & ADA were found to be within the acceptable limit. The tailing factors for two peaks were found to be less than 1.5 and the results are shown in Table 4. The validated method was used in analysis of marketed tablet dosage form. The results for the drugs assay showed good agreement with label claims and the results are shown in Table 5. Degradation studies results were shown in Table 6 and 7.

Table 1: System suitability parameters of proposed method

Parameters	Clindamycin	Adapalene	Acceptance Criteria
Retention time (min)	2.840	3.999	-
No. of theoretical plates	3994	7159	NLT 2000
Tailing factor	1.37	1.36	NMT 2.0
Resolution	-	6.13	NLT 2.0

Table 2: Accuracy data for proposed method

Sample	Fixed concentration (µg/ml)	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Clindamycin	50	25	24.91	99.64	0.52
	50	50	50.29	100.58	0.52
	50	75	75.09	100.12	0.5
Adapalene	5	2.5	2.52	100.8	0.7
	5	5	4.98	99.6	0.3
	5	7.5	7.49	99.8	0.3

Table 3: precision data of proposed method

Sr. No.	System Precision		Method Precision	
	Clindamycin*	Adapalene*	Clindamycin*	Adapalene*
1	7946937	706817	8335304	736764
2	7969334	707122	8102945	730901
3	7963883	712549	8008503	715439
4	7969023	703841	8085368	725787
5	7914425	714817	8059329	721034
6	8021271	709253	8128062	728438
Mean	7964146	709067	8119919	730006
Std. Dev.	34878.0	4034.3	113158	7610.5
%RSD	0.4	0.6	1.39	1.04

Table 4: Robustness for flow rate variation of CLIN & ADA

S.NO	Robustness condition	Clindamycin Area %RSD	Adapalene Area %RSD
1	Flow rate- 0.8	0.8	1.2
2	Flow rate-1.2	0.1	0.3
3	Mobile Phase(53:47)	0.3	0.9
4	Mobile Phase(57:43)	0.5	0.1
5	Temperature-28°C	0.0	0.0
6	Temperature-32°C	0.8	0.9

Table 5: Analysis of marketed formulation by proposed method

Brand Name	Drug	Labelled claim	Amount found*	% Assay*
LACNE	Clindamycin	1%	9.98 mg	99.89
	Adapalene	0.1%	4.99 mg	99.89

Table 6: Degradation Data of Clindamycin

S.No.	Degradation Condition	% Drug found	% Drug Degraded
1	Acid	99.5	0.5
2	Alkali	99.3	0.7
3	Oxidation	99.1	0.9
4	Thermal	99.2	0.8
5	UV	99.9	0.1
6	Neutral	99.9	0.1

Table 7: Degradation Data of Adapalene

S.No.	Degradation Condition	% Drug found	% Drug Degraded
1	Acid	98.1	1.9
2	Alkali	98.2	1.8
3	Oxidation	98.4	1.6
4	Thermal	98.5	1.5
5	UV	99.9	0.1
6	Neutral	99.9	0.1

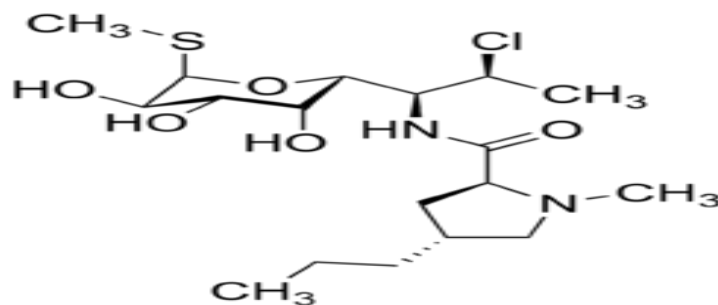


Fig. 1: Molecular structure of Clindamycin

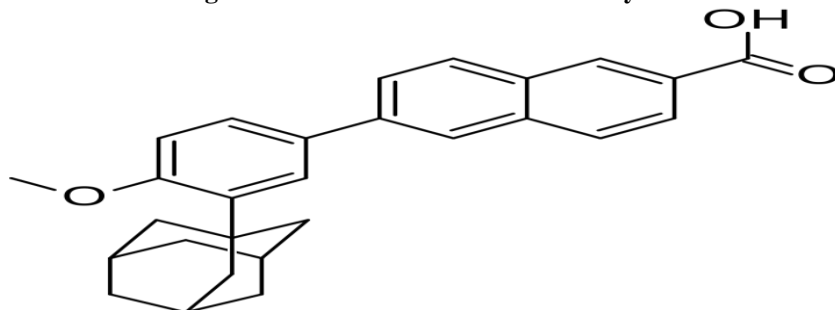


Fig. 2: Molecular structure of Adapalene

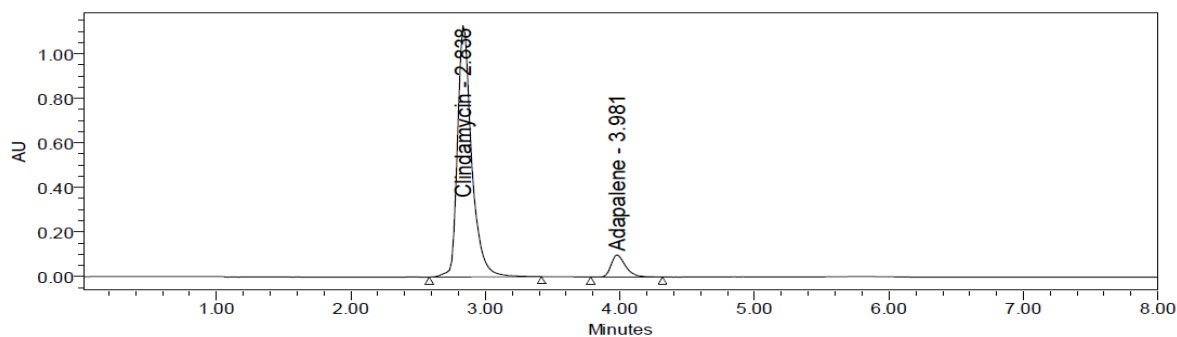


Fig. 3: Typical chromatogram of standard for CLIN & ADA

Development of RP-HPLC Method For Sofosbuvir:

Trails

Trail 1:

Column	: ODS C18 (4.6 × 250mm) 5μ
Column temperature	: Ambient
Wavelength	: 260nm
Mobile phase ratio	: Water (100%) V/V
Flow rate	: 0.5ml/min
Injection volume	: 20μl
Run time	: 7minute

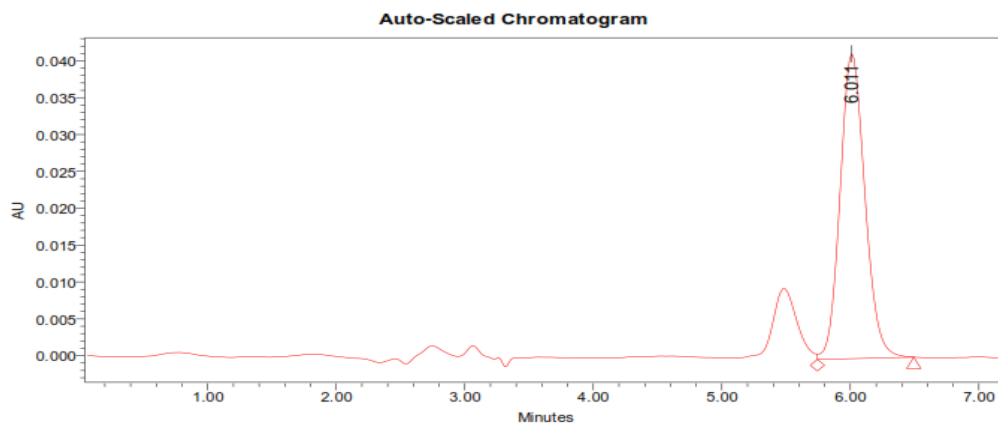


Figure No 4. : Chromatogram for trail 1

Table No. 8: Peak results for trail 1

S. No	Peak Name	R _t (Min)	Area	Height	USP Tailing	USP Plate count
1	Sofosbuvir	6.011	553927	41393	1.6	849

Observation:

From the above trail it was observed that it shows less plate count and improper baseline in the chromatogram. So go for further trails to get good peak

Trail 2:

Column : ODSC18 (4.6 × 250mm) 5μ
 Column temperature : Ambient
 Wavelength : 260nm
 Mobile phase ratio : water : Acetonitrile (50:50) V/V
 Flow rate : 0.8ml/min
 Injection volume : 20μl
 Run time : 8minutes

Validation of Developed RP-HPLC Method:**SYSTEM SUITABILITY:**

Figure No. 5: Chromatogram showing injection -1 for System Suitability

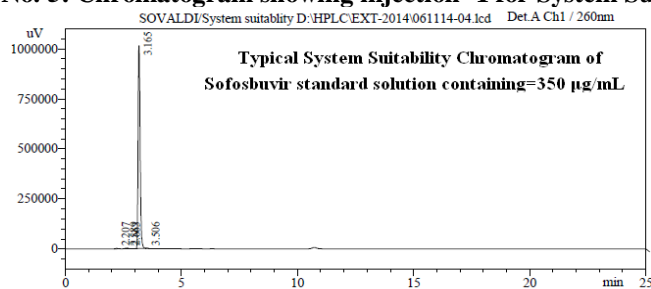


Figure No. 6: Chromatogram showing injection -2 for System Suitability

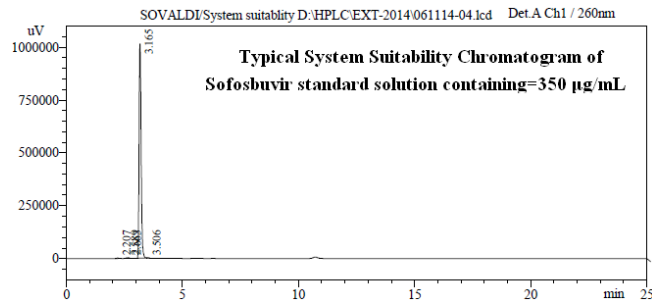


Figure No. 7: Chromatogram showing injection -3 for System Suitability

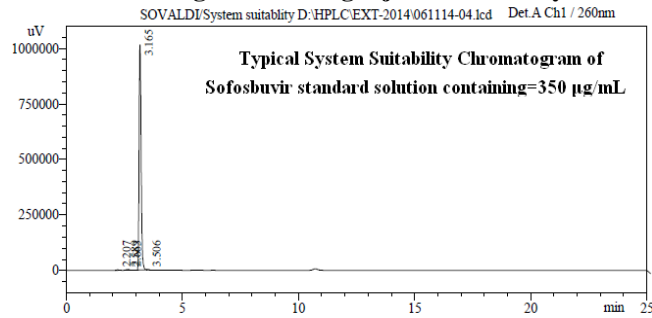


Figure No. 8: Chromatogram showing injection -4 for System Suitability

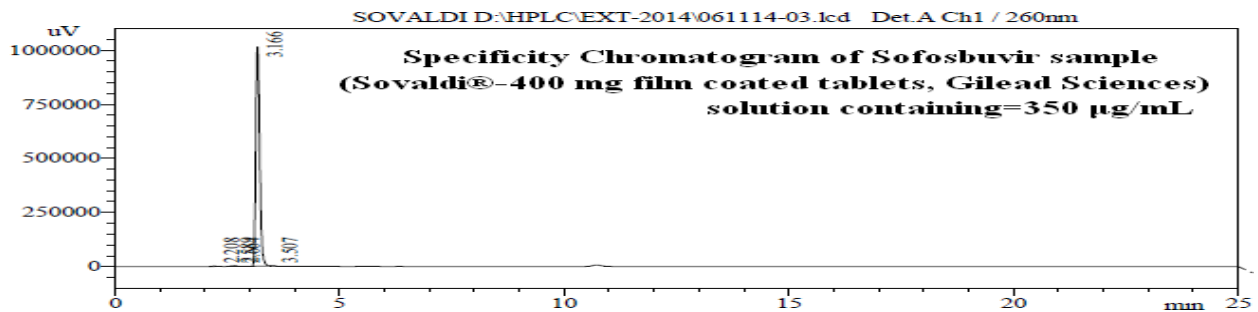
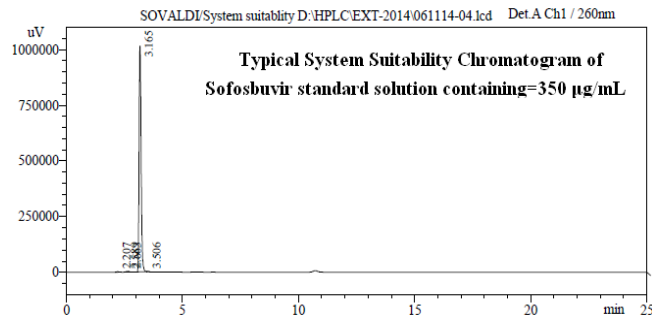


Figure No. 9: Chromatogram showing injection -5 for System Suitability

Table No. 9:

S. No	Peak Name	RT (min)	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	USP Hite Count	USP Tailing
1	Sofosbuvir	3.166	6241725	92251	9917	1.36
2	Sofosbuvir	3.168	6235465	92274	9644	1.36
3	Sofosbuvir	3.164	6233791	92291	9816	1.36
4	Sofosbuvir	3.167	6236755	92183	9017	1.36
5	Sofosbuvir	3.165	6232983	92291	9374	1.36
Mean		3.166	6237240			
Std. Dev.		0.001	4089			
%RSD		0.043	0.066			

Acceptance criteria:

%RSD of five different sample solutions should not more than 2 The %RSD obtained is within the limit, hence the method is suitable.

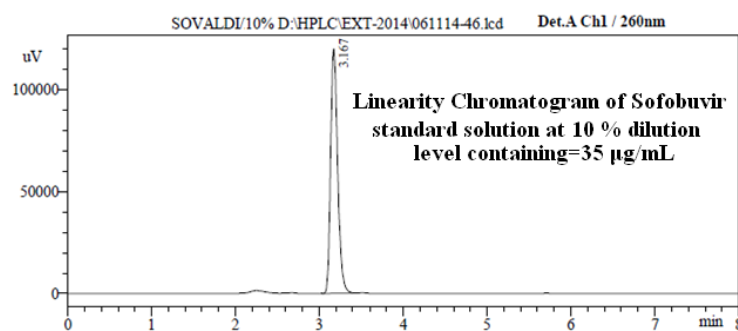
LINEARITY:

Figure No. 10: Chromatogram showing Linearity level-1

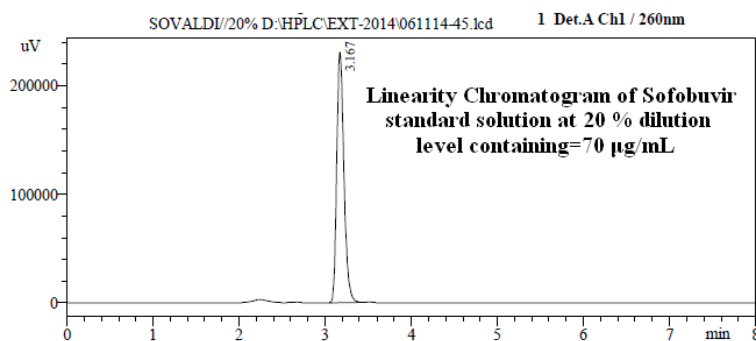


Figure No. 11: Chromatogram showing Linearity level-2

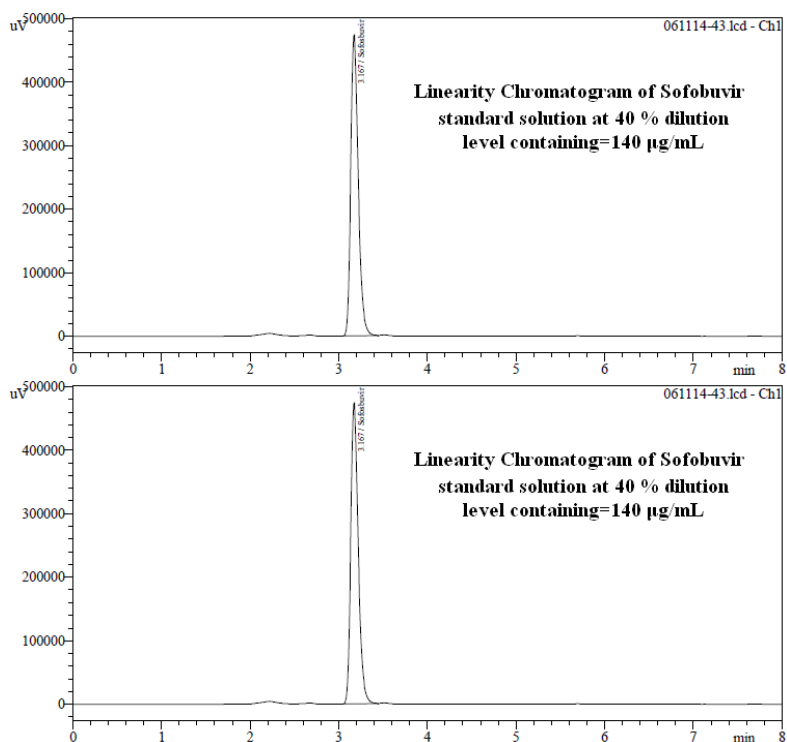


Figure No. 12: Chromatogram showing linearity level-3

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Table No. 10: Calibration of Sofobuvir

Concentration (µg/ml)	Average Peak Area
35	698762
70	1534217
140	2791236
210	4089902
280	5180679
350	6315827
420	7486081

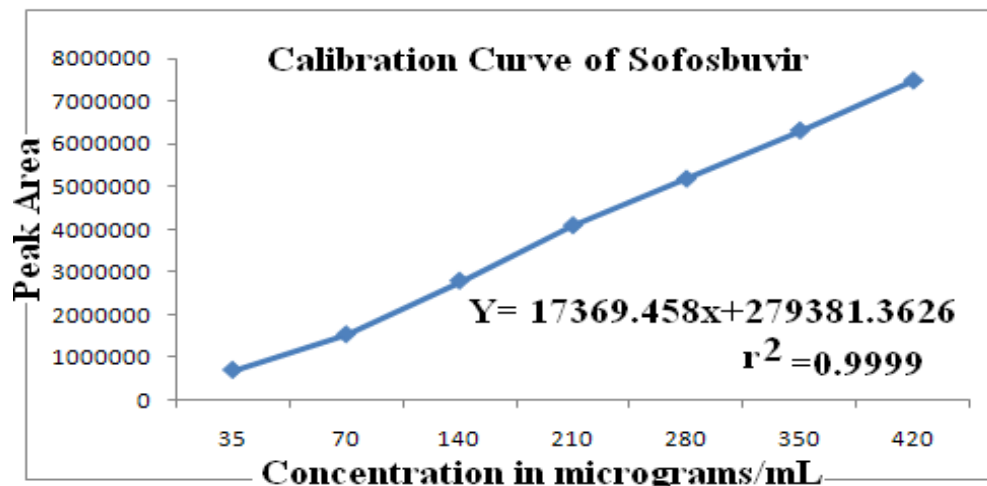


Figure No. 13: Linearity Plot

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Sofosbuvir is a straight line.

$$Y = mx + c$$

$$\text{Slope (m)} = 17369.458$$

$$\text{Intercept (c)} = 279381.3626$$

$$\text{Correlation Coefficient (R}^2\text{)} = 0.999$$

VALIDATION CRITERIA:

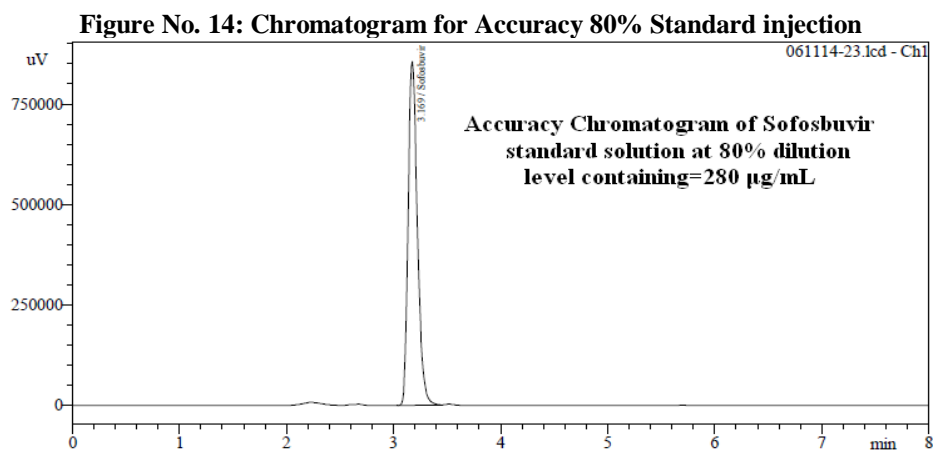
The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION:

Correlation Coefficient (R²) is 0.99, and the intercept is 17369. These values meet the validation criteria.

ACCURACY:

Accuracy at different concentrations (80%, 100%, and 120%) was prepared and the % recovery was calculated.

Accuracy 80% (Standard):**Accuracy 100% (Standard):**

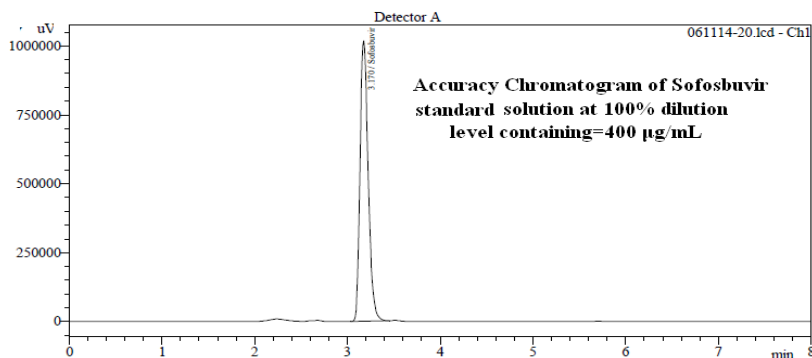


Figure No. 15: Chromatogram for Accuracy 100% Standard injection

Accuracy 120% (Standard):

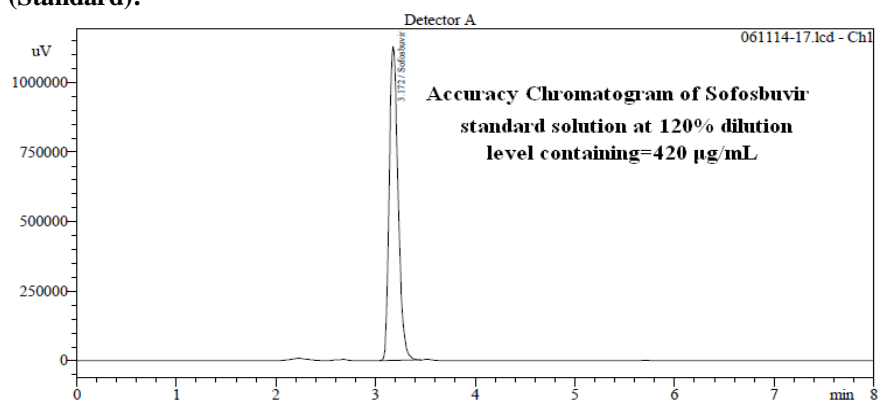


Figure No. 16: Chromatogram for Accuracy 120% Standard injection

Table No. 11: Results of Accuracy for Standard injections

S. No.	Name	% Concentration (at specification Level)	Rt (Min)	Area	Height	USP Tailing	USP Plate Count
1	sofosbuvir	80%	3.166	5127921	71944	1.36	9772
2	sofosbuvir	100%	3.167	6224163	92210	1.36	9184
3	sofosbuvir	120%	3.168	7012659	170368	1.36	9754

Accuracy 80% (Sample):

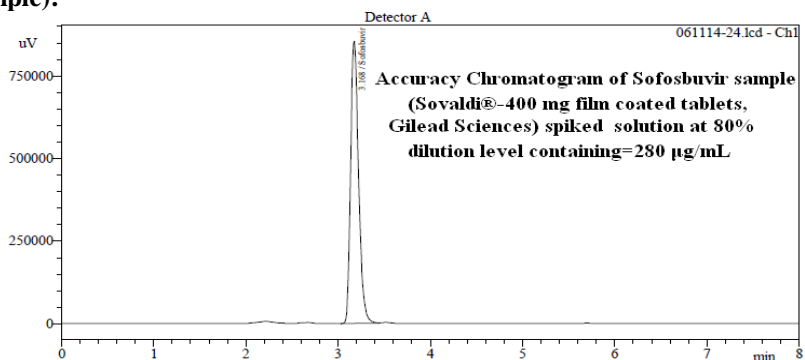
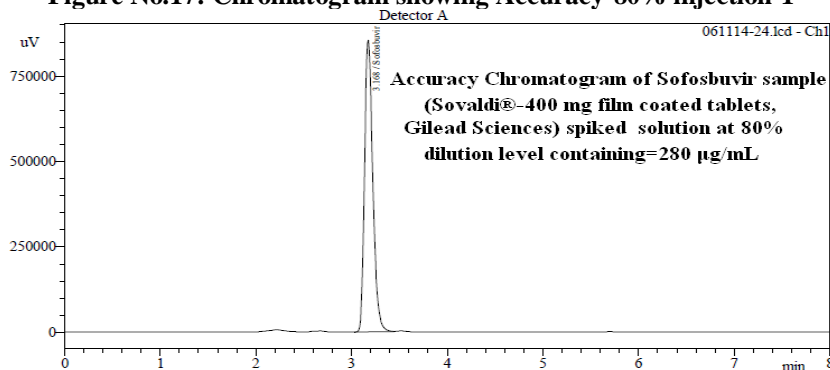
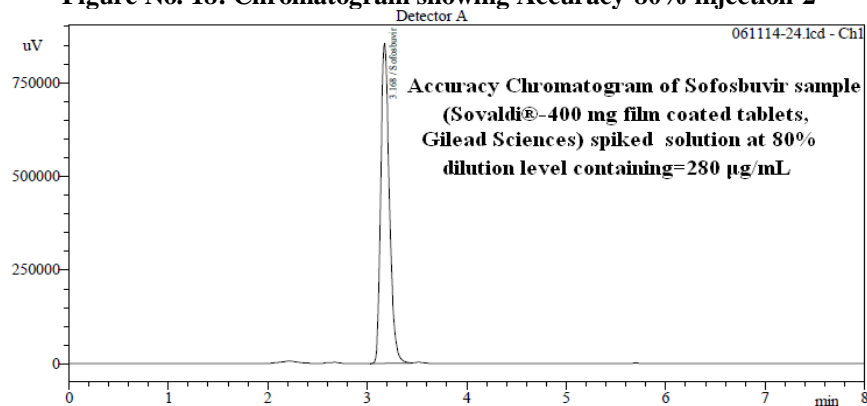
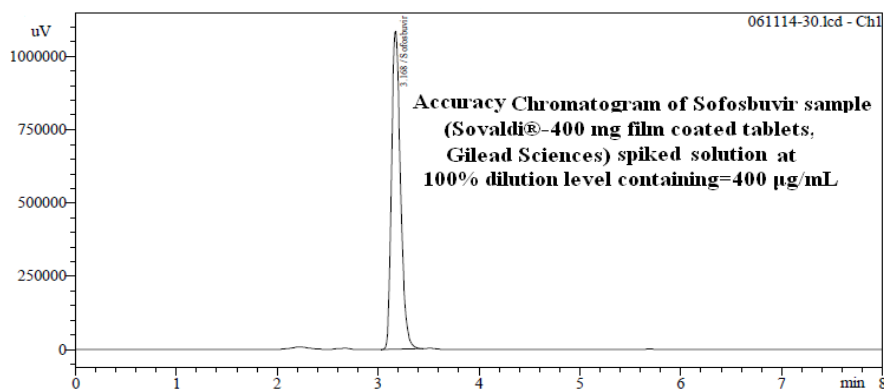


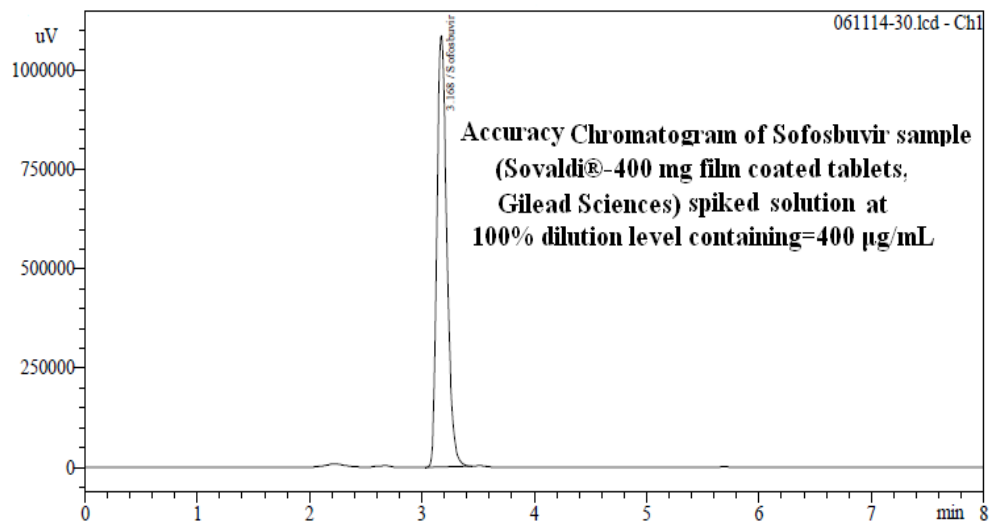
Figure No.17: Chromatogram showing Accuracy-80% injection-1**Figure No. 18: Chromatogram showing Accuracy-80% injection-2****Figure No. 19: Chromatogram showing Accuracy-80% injection-3****Table No. 12: Results of Accuracy for Sample injections (Conc.80%)**

S. No	Name	Rt (Min)	Area	Height	USP Tailing	Usp plate count
1	sofosbuvir	3.166	5677448	69943	1.36	9585
2	sofosbuvir	3.168	5680902	71944	1.36	9772
3	sofosbuvir	3.168	5686669	70928	1.36	9374

Figure No. 20: Chromatogram showing Accuracy-100% injection-1

Accuracy 100% (Sample):

Figure No. 21: Chromatogram showing Accuracy-100% injection-2



ASSAY (Standard):

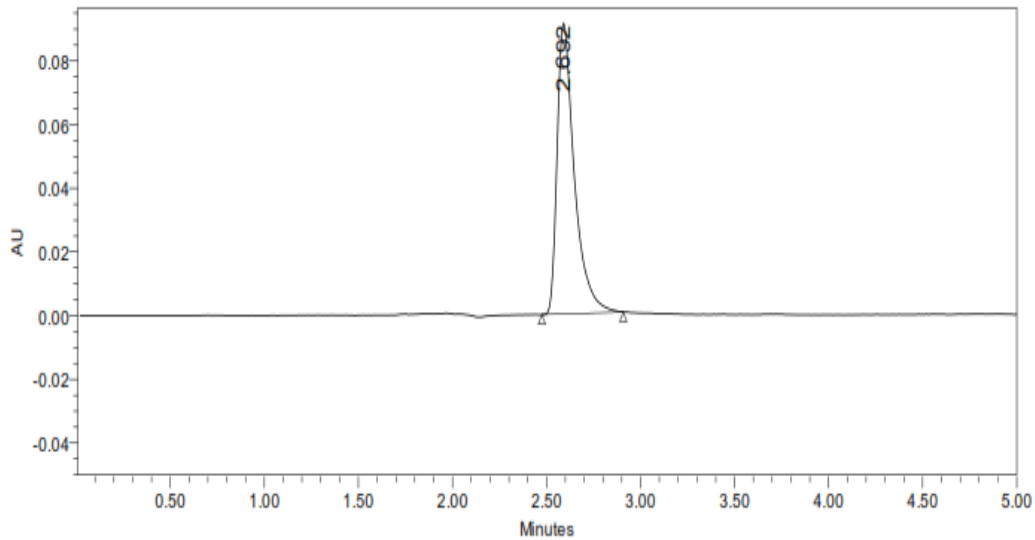


Figure No. 22: Chromatogram showing Assay of Standard injection -1

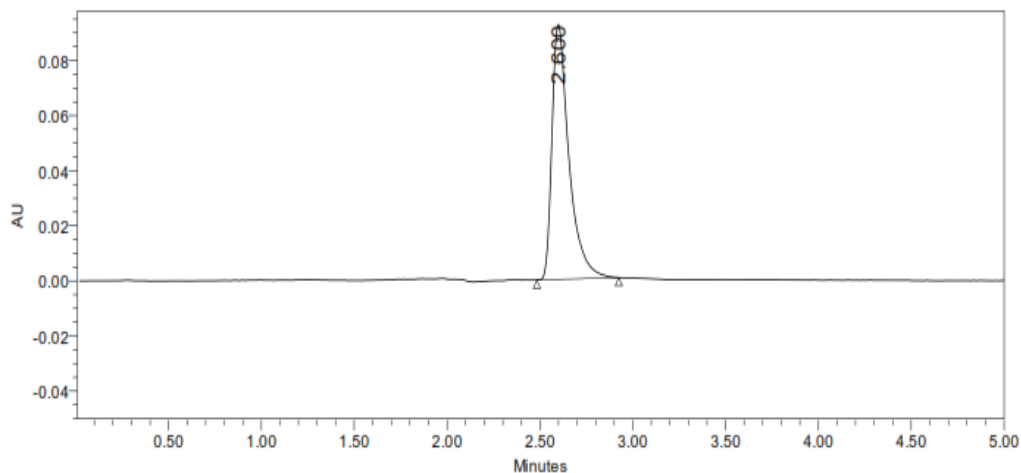


Figure No. 23: Chromatogram showing Assay of Standard injection-2

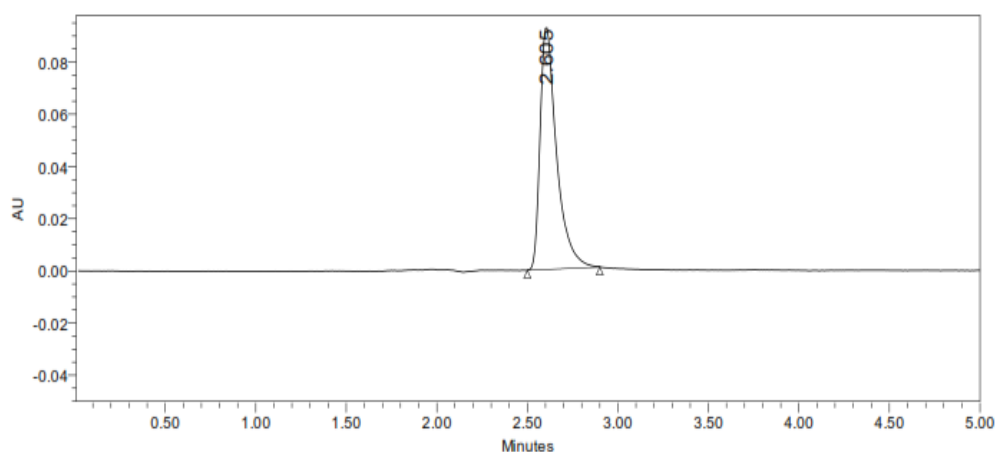


Figure No.24: Chromatogram showing Assay of Standard injection -3

Table No. 13: Peak results for Assay Standard:

S. No	Name	RT	Area	Height	USP tailing	USP plate count
1	Ribavirin	2.692	631544	92857	1.2	9847
2	Ribavirin	2.600	631022	92122	1.2	9028
3	Ribavirin	2.605	631933	92113	1.2	9664

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

$$= 631278.3 / 631426.4 \times 10/60 \times 60/0.0198 \times 99.8/100 \times 0.3966/200 \times 100$$

$$= 99.9\%$$

The % purity of Sofosbuvir in pharmaceutical dosage form was found to be 99.9%.

Table No. 14: Performance and Detection Characteristics of Ribavirin

PARAMETERS	SOFOSBUVIR
Calibration Range ($\mu\text{g/ml}$)	35-350 $\mu\text{g/ml}$
Optimized Wavelength	260nm
Mobile Phase	Acetonitrile : Water
Column	Zodiac C18 (250 \times 4.6 \times 5 μ)
Retention Time	3.166min
Regression Equation	$y = 17369.45x + 2729381.36$
Correlation Coefficient (R ²)	0.999
Precision (% RSD) i) Repeatability	0.066
ii) Intermediate Precision (Day 1)	0.042
Intermediate Precision (Day 2)	0.066
% Recovery	99.6 %
LOD($\mu\text{g/ml}$)	0.07 $\mu\text{g/ml}$
LOQ($\mu\text{g/ml}$)	0.21 $\mu\text{g/ml}$

CONCLUSION:

The developed stability indicating RP-HPLC method is simple, specific, accurate and precise for the simultaneous determination of CLIN & ADA in dosage form. The developed method provides good resolution between CLIN & ADA. It was successfully validated in terms of system suitability, linearity, precision, accuracy, specificity, LOD, LOQ and robustness in accordance with ICH guidelines. Thus the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Sofosbuvir in bulk drug and pharmaceutical dosage forms. In RP-HPLC method, optimization of chromatographic parameters was done. Parameters optimized were wave length, effect of nature of mobile phase, ratio of mobile phase and effect of flow rate. A Wave length 260nm was selected and the mobile phase consists of Acetonitrile and Water in 50:50 v/v ratios at a flow rate of 1.0ml/min were found to be optimum conditions for analysis.

Injection volume was selected to be 10 μl which gave a good peak area. Retention time of Sofosbuvir found to be 3.166 min. Percentage RSD of the Sofosbuvir found to be 0.066. Percentage Recovery was found to be 110-112.12% was linear and Precise over the same range. Retention time and run time was shown in different conditions which are simpler so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

The analytical method passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory. The column used for study was Apollo C₁₈ because it was giving good peak.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of Accuracy and Precision.

Finally, the RP-HPLC method developed could be used for estimation of Sofosbuvir in Pharmaceutical dosage forms.

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