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# DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CLINDAMYCIN, ADAPALENE AND SOFOSBUVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC METHOD

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**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 $C_{18}$ BDS Hypersil (150 x 4.6mm, 5µ) with a mixture ofmixed 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 were2.84 and 3.999min for Clindamycin and Adapalene respectively. Calibration plots were linear ($r^2$=0.999) over the concentration range of 25-150µg/mL for Clindamycinand2.5-15µg/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.*

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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-arabinopyranosideAdapalene (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 spectrophotometricand chromatographic methodswere 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%ADAhave 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 with 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 $C_{18}$(150 x 4.6 mm, 5µ)analytical column. The mobile phase consisted of mixed phosphate buffer: acetonitrile (55:45%v/v). The flow rate was 1.0mL/min and UV detection was performed at 230nm. The mobile phase was shaken on an ultrasonic bath for 30min. The resulting transparent mobile phase was filtered through a 0.45µmembrane filter (Millipore, Ireland). The injection volume was 10µL and all the experiments were performed at ambient temperature.

### Preparation of standard solution:

Accurately Weighed and transferred 100mg of CLIN and 10mg of ADA working Standards into 100ml clean dry volumetric flasks, add 3/4$^{th}$ 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 10g was extracted with 50ml of 0.1% glacial acetic acid in methanol in centrifuge tube. The sample mixture was heated at 50$^0$C for 10 min and mixed occasionally during the heating process. After heating, the sample was allowed to cool for 10 min and add 2ml of water into the centrifuge tube and then cooled in an ice bath for 20min and centrifuged at 3000RPM for 10min. 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 ADAmixture at concentrations of 1000µg/mL and 100 µg/mL. The acceptance criteria are number of theoretical plates (N) atleast 2000 per each peak and tailing factor isnot more than 2.0.

### Linearity:

Standard calibration curves were plotted against the concentration ranging from 25-150µg/mL for CLIN and 2.5-15 µg/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 ofstandard. 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 3.3σ/s and 10σ/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 $60^0$c. For HPLC study, there sultant solution was diluted to obtain 100µg/ml&10µg/mlsolutionand10µlwereinjectedint othe system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies:**
To 1ml of stock solution of Adapalene and Clindamycin, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at $60^0$C.The resultant solutionwasdilutedtoobtain100µg/ml&10µg/soluti on and10µl solutions were injected into the system and the chromate grams 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 30mins at $60^0$C. The result ant solution was diluted to obtain 100µg/ml&10µg/ml solution and 10µl 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 placedinovenat$105^0$C for6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml&10µg/ml solution and10µl were injected into the system and the chromate grams were recorded to assess the stability of the sample**.**

**Photo Stability studies:**
The photochemical stability of the drug was also studied by exposing the 300µg/ml, 10µg/ml&25µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/$m^2$ in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/ml&10µg/ml solutions and 10µl 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 refluxingthedruginwaterfor6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 100µg/ml&10µg/ml solution and 10µl were injected into the system and the chromate grams 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;5µm) and(4.6×150 mm;5µm), 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 1.2mL/min were studied. A flow rate of 1.0mL/min gave an optimum signal to noise ratio with reasonable separation time using a $C_{18}$analytical column (4.6×250 mm; 5 µm), the retention times for CLIN & AD Awere observed to be 2.84 and 3.999 min respectively. Total run time was less than 7min. The chromatogram at 230nm 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 forCLIN & ADAin the range of 25-150µg/mL and2.5-15 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 ADAin 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 be0.13µg/mLand 0.4µg/mLfor CLIN, 0.08µg/mL and0.24µg/mL for ADA. In all deliberately varied conditions, the %RSD for replicate injections of CLIN & ADAwere 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 Table4. The validate 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 |

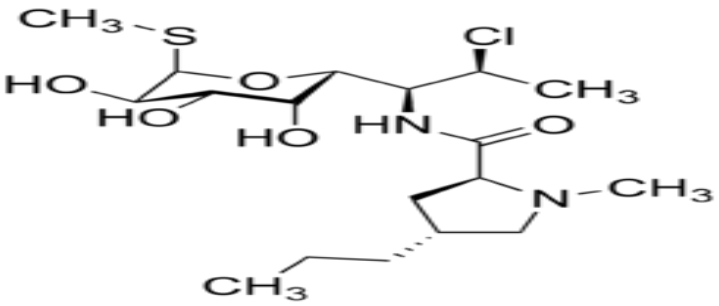

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**Fig. 1: Molecular structure of Clindamycin**

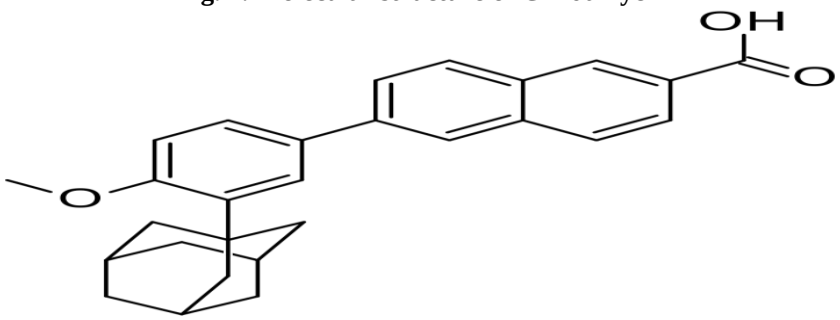


**Fig. 2: Molecular structure of Adapalene**

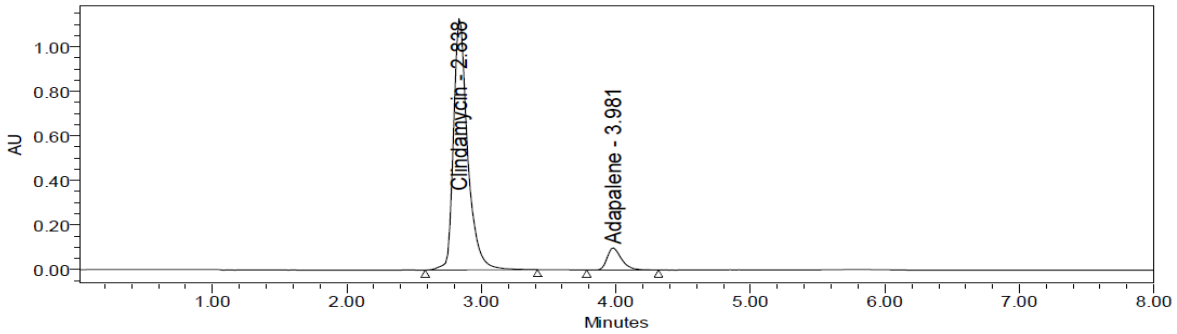



**Fig. 3: Typical chromatogram of standard for CLIN & ADA**

**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 |

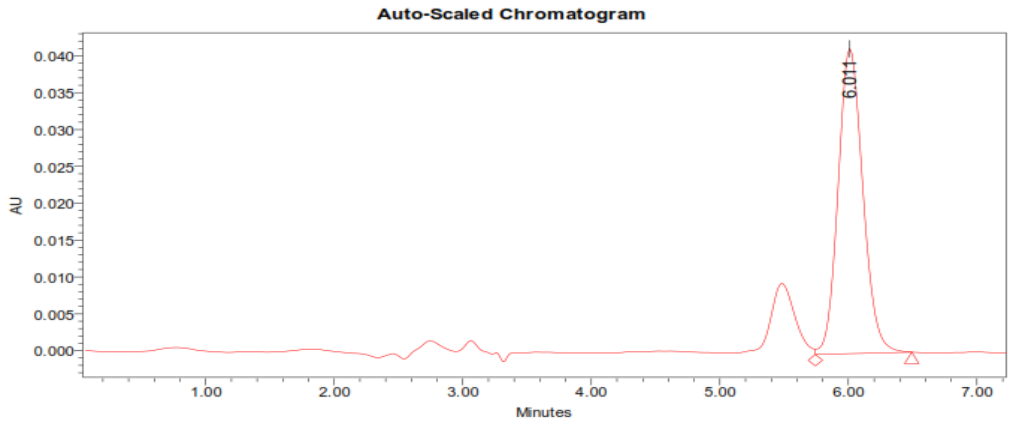



**Figure No 4. : Chromatogram for trail 1**

**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**

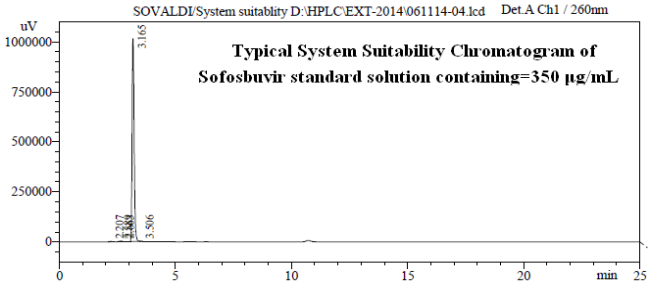


**Figure No. 6: Chromatogram showing injection -2 for System Suitability**

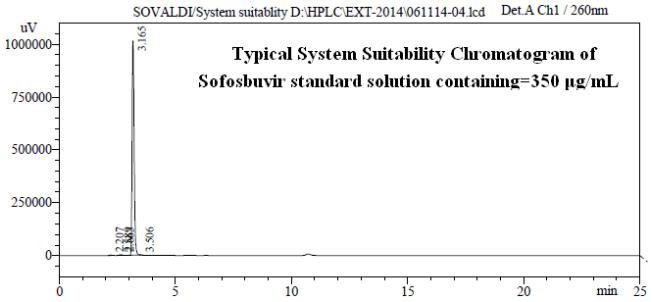



**Figure No. 7: Chromatogram showing injection -3 for System Suitability**

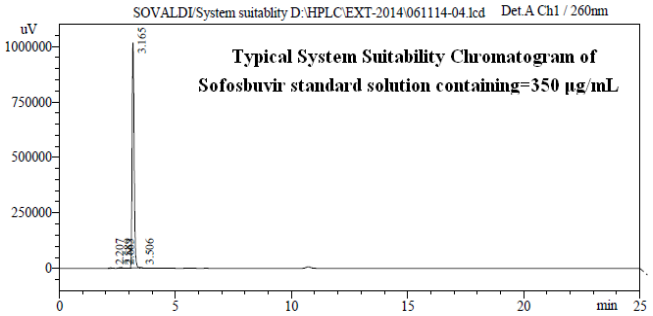
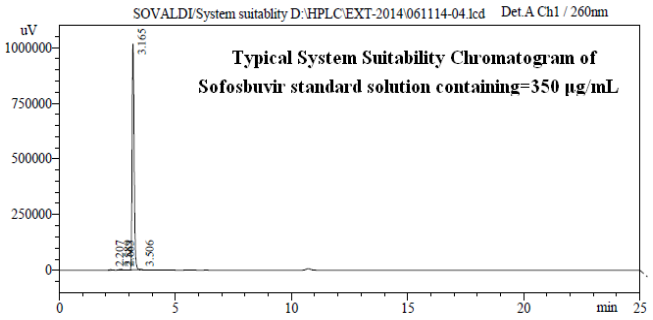



**Figure No. 8: Chromatogram showing injection -4 for System Suitability**



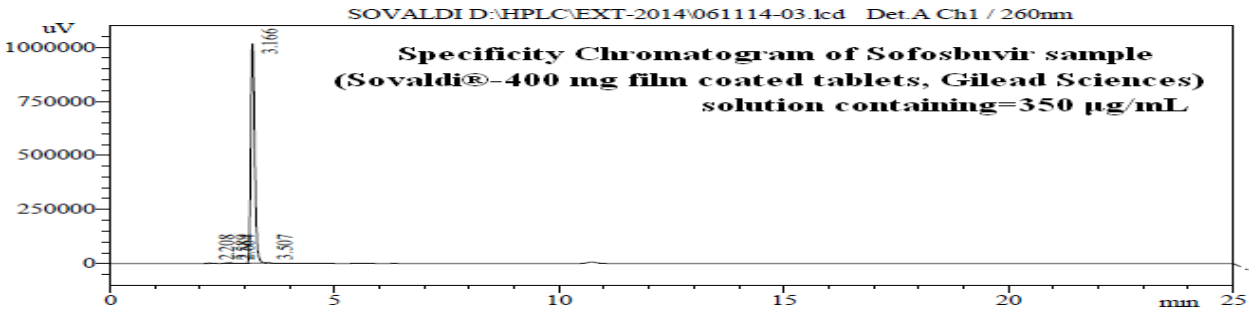




**Figure No. 9: Chromatogram showing injection -5 for System Suitability**

**Table No. 9:**

| S. No | Peak Name | RT (min) | Area (µV*sec) | Height (µV) | USP Plate 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:**

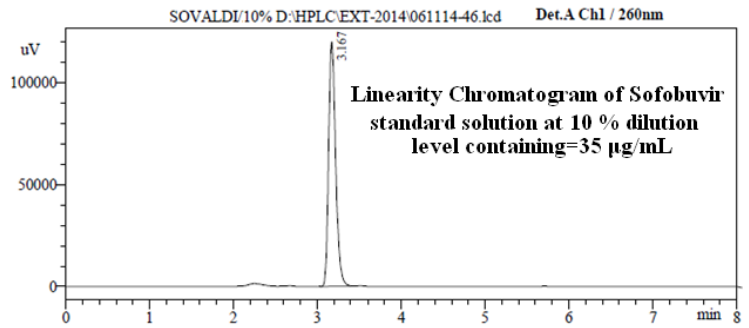



**Figure No. 10: Chromatogram showing Linearity level-1**

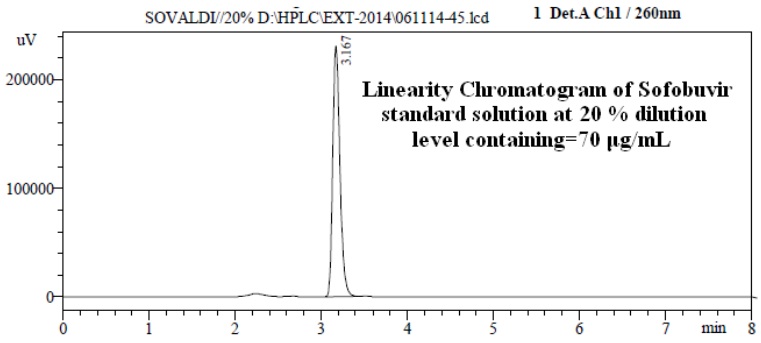



**Figure No. 11: Chromatogram showing Linearity level-2**

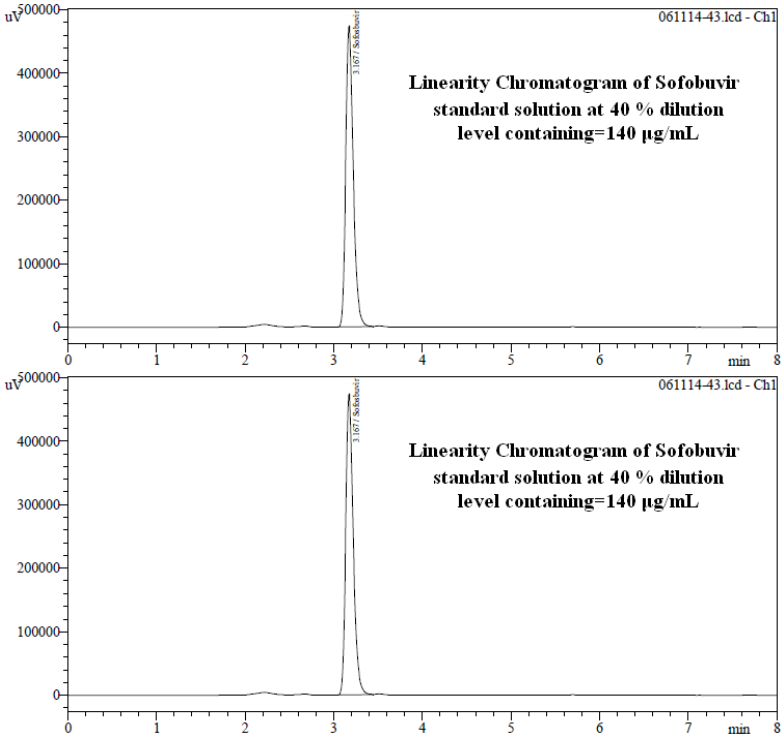



**Figure No. 12: Chromatogram showing linearity level-3**

## CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

**Table No. 10: Calibration of Sofosbuvir**

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

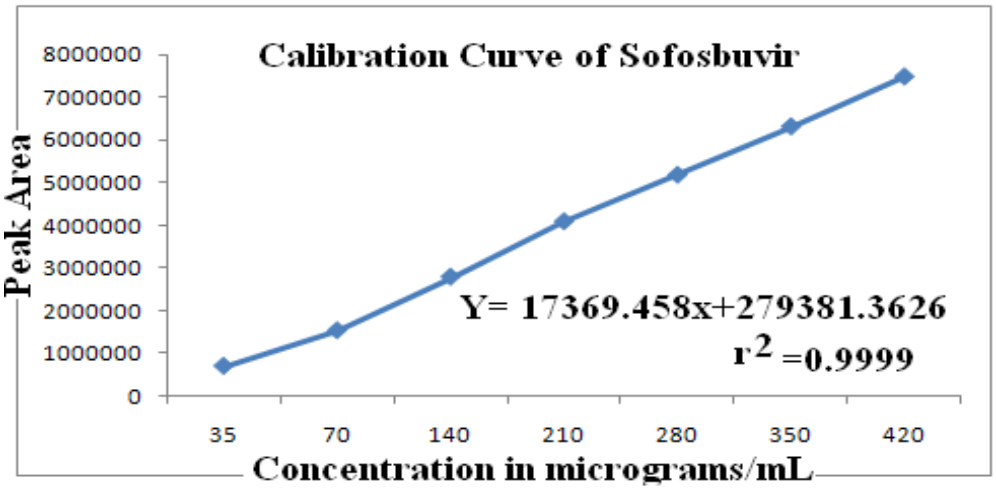



**Figure No. 13: Linearity Plot**

**LINEARITY PLOT:**

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

Y = mx + c
Slope (m) = 17369.458
Intercept (c) = 279381.3626
Correlation Coefficient ($R^2$) = 0.999

**VALIDATION CRITERIA:**

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

**CONCLUSION:**

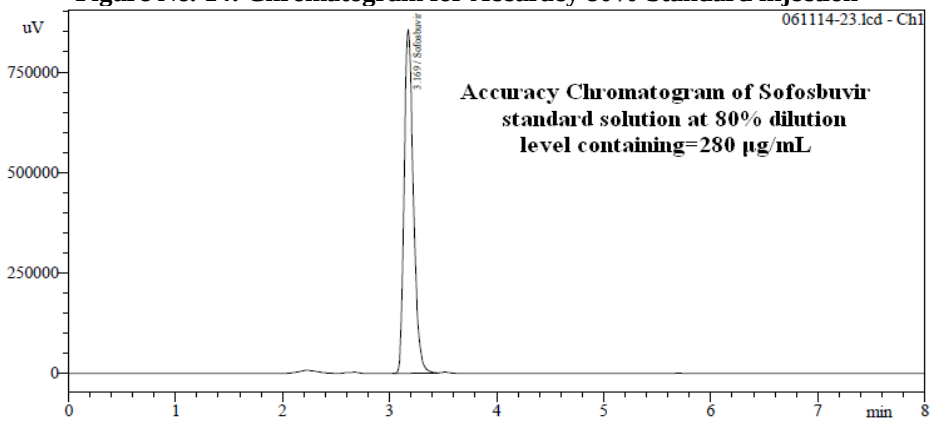
Correlation Coefficient (R2) is 0.99, and the intercept is 17369. These values meet the validation criteria.

**ACCURACY:**

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

**Accuracy 80% (Standard):**

**Figure No. 14: Chromatogram for Accuracy 80% Standard injection**




**Accuracy 100% (Standard):**

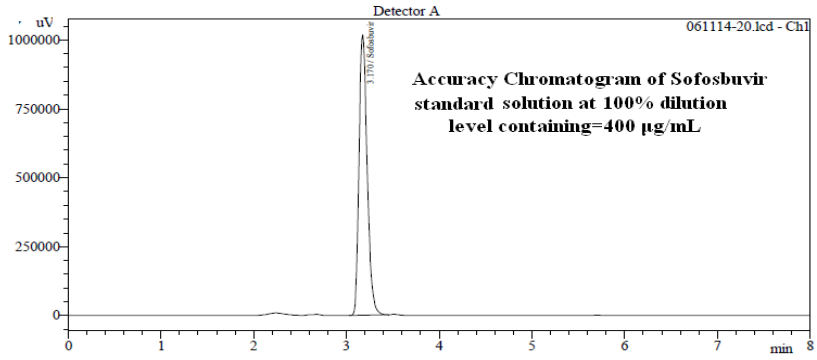



**Figure No. 15: Chromatogram for Accuracy 100% Standard injection**

**Accuracy 120% (Standard):**

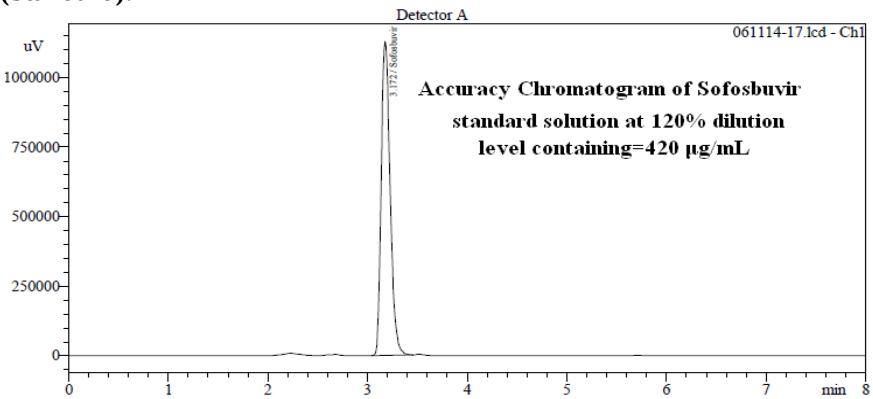



**Figure No. 16: Chromatogram for Accuracy 120% Standard injection**

**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 |

**Accuracy80% (Sample):**

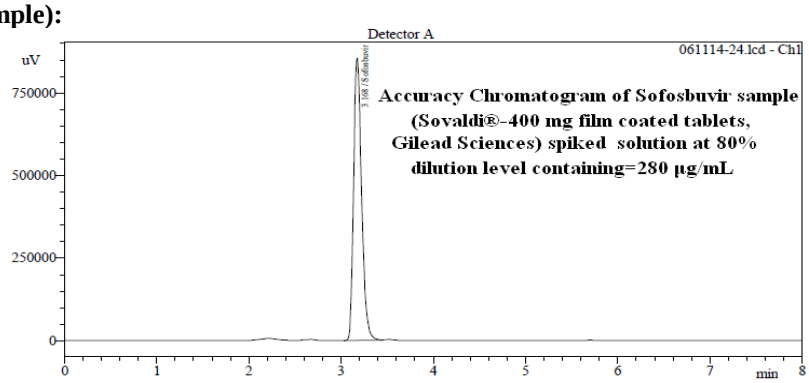


**Figure No.17: Chromatogram showing Accuracy-80% injection-1**

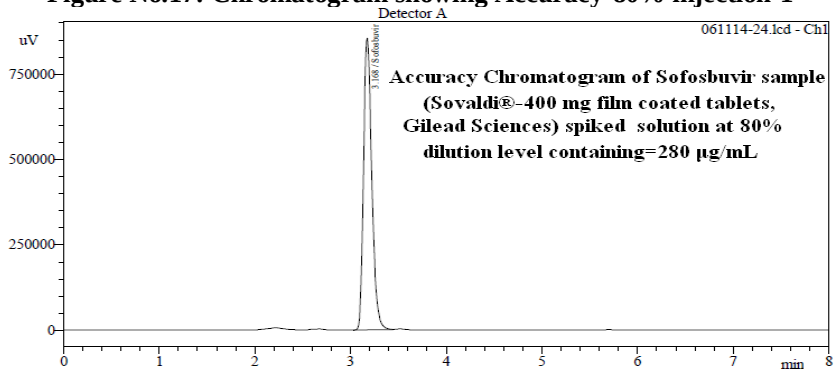



**Figure No. 18: Chromatogram showing Accuracy-80% injection-2**

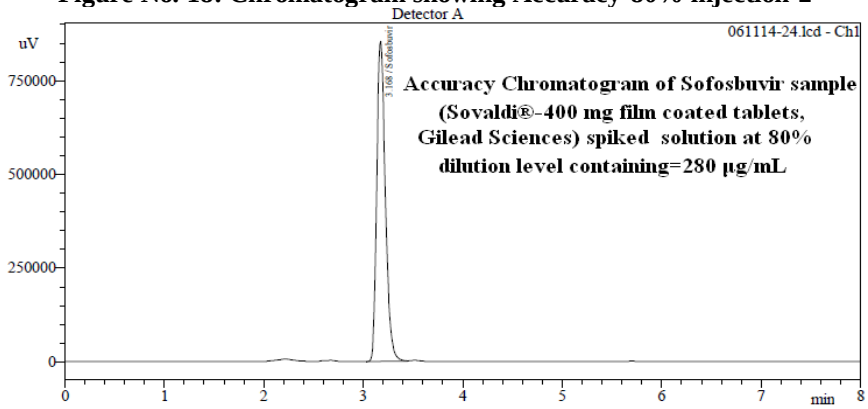
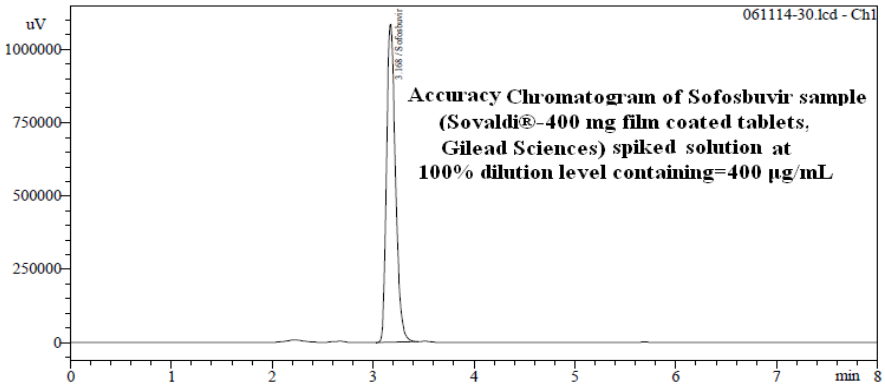



**Figure No. 19: Chromatogram showing Accuracy-80% injection-3**

**Table No. 12: Results of Accuracy for Sample injections (Conc.80%)**

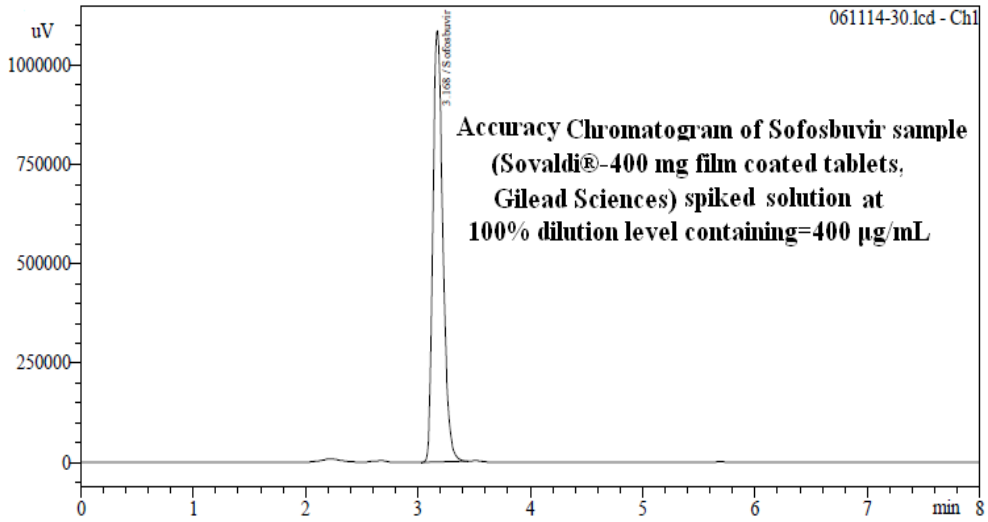
| S. No | Name | Rt (Min) | Area | Height | USP Tailing | Usp plate count |
|---|---|---|---|---|---|---|
| 1 | sofosbuvir | 3.166 | 5677448 | 69943 | 1.36 | 9585 |
| 2 | sofosbuvir | 3.168 | 5680902 | 71944 | 1.36 | 9772 |
| 3 | sofosbuvir | 3.168 | 5686669 | 70928 | 1.36 | 9374 |

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



**Accuracy 100% (Sample):**

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




**ASSAY (Standard):**

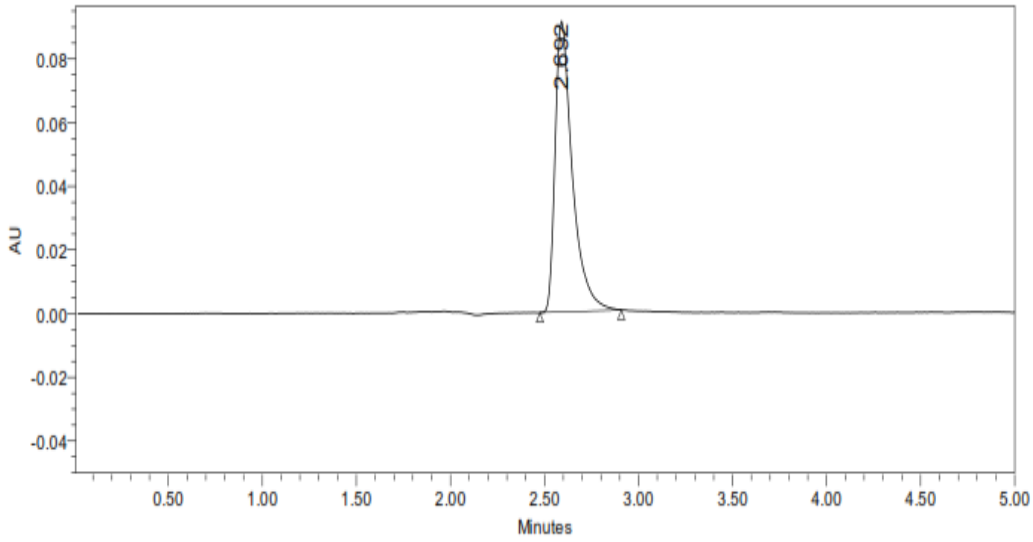



**Figure No. 22: Chromatogram showing Assay of Standard injection -1**

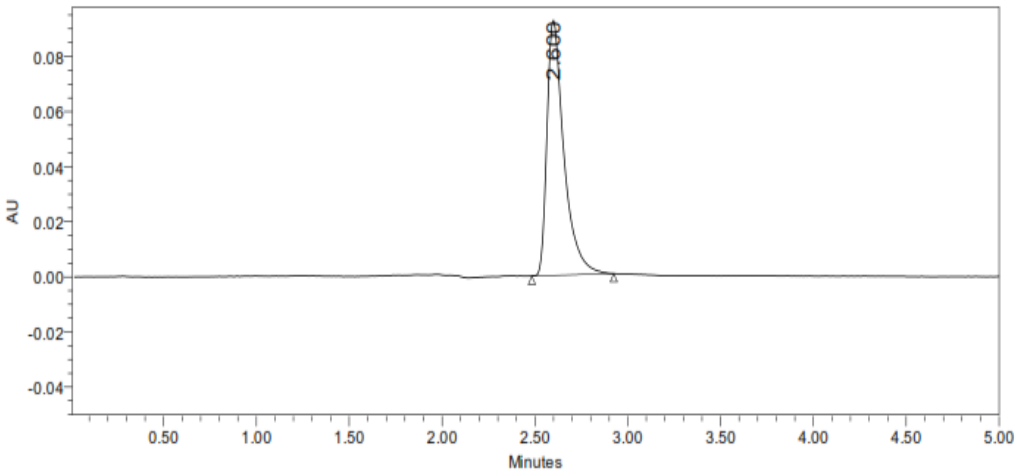



**Figure No. 23: Chromatogram showing Assay of Standard injection-2**

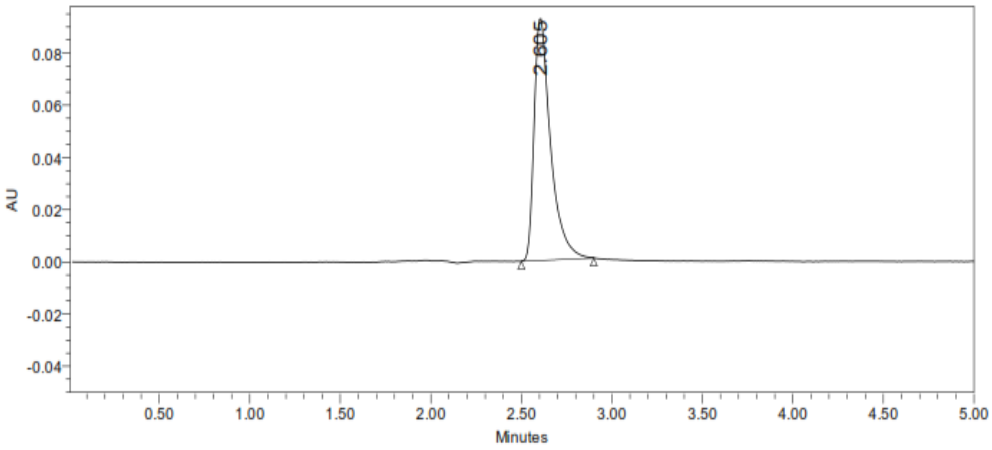



**Figure No.24: Chromatogram showing Assay of Standard injection -3**

**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 / 631426.4×10/60×60/0.0198×99.8/100×0.3966/200×100**

**= 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 (µg/ml) | 35-350µg/ml |
| Optimized Wavelength | 260nm |
| Mobile Phase | Acetonitrile : Water |
| Column | Zodiac C18 (250×4.6×5µ) |
| Retention Time | 3.166min |
| Regression Equation | y = 17369.45x + 2729381.36 |
| Correlation Coefficient (R2) | 0.999 |
| Precision (% RSD)<br>i) Repeatability | 0.066 |
| ii) Intermediate Precision (Day 1) | 0.042 |
| Intermediate Precision (Day 2) | 0.066 |
| % Recovery | 99.6 % |
| LOD(µg/ml) | 0.07µg/ml |
| LOQ(µg/ml) | 0.21µg/ml |

## CONCLUSION:

The developed stability indicating RP-HPLC method is simple, specific, accurate and precise for the simultaneous determination of CLIN & ADAin 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 10µl 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 $C_{18}$ 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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