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# Stability-Indicating HPLC Method for Simultaneous Estimation of Sofosbuvir and Ledipasvir

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**ABSTRACT:** The optimization of chromatographic conditions for the analysis of Ledipasvir and Sofosbuvir was successfully achieved using a Hibar C18(250×4.6mm)5µm column using 1% O-Phosphoric acid (pH 6.4): Acetonitrile (30:70 v/v) at a flow rate of 0.8 ml/min, ensuring excellent peak shape and resolution. System suitability tests confirmed compliance with essential parameters, including resolution, tailing factor, and theoretical plate count, ensuring reliability. The retention time of ledipasvir and sofosbuvir were found to be 7.1min and 3.7 min, respectively. During force degradation, drug product was exposed to hydrolysis (acid and base hydrolysis), oxidation, thermal degradation and photo degradation. Both the drugs were not degraded under thermal, oxidative, photolytic, acid and neutral hydrolytic conditions, but Sofosbuvir showed degradation under alkaine hydrolytic condition with a retention time 2.8 min and 4.1 min, respectively. The degraded products of Ledipasvir and Sofosbuvir were well resolved from the individual bulk drug response. The specificity of the method was confirmed by peak purity profile of the resolved peaks. In conclusion, the developed chromatographic method for Ledipasvir and Sofosbuvir is precise, accurate, linear, sensitive, and robust, making it suitable for routine pharmaceutical analysis and quality control. The validation results confirm compliance with regulatory standards, ensuring reliable identification and quantification of these antiviral drugs. Given its high stability and reproducibility, this method provides an efficient and dependable approach for analyzing Ledipasvir and Sofosbuvir in pharmaceutical formulations.

**KEYWORDS:** Accuracy, Chromatographic Optimization, Degradation, Ledipasvir, Retention Time, Linearity, Precision, Pharmaceutical Analysis, Robustness, Sofosbuvir.

## INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a powerful analytical tool used for separation, identification and quantification of components in a mixture, particularly valuable in pharmaceutical research and quality control. The technique operates on the principle of differential distribution of compounds between a stationary phase (solid adsorbent) and a mobile phase (liquid), with separation achieved based on compound solubility and molecular size. The system typically uses high pressure to force the mobile phase through a packed column, enabling efficient separation and analysis. HPLC is essential in developing and validating analytical methods for new drugs, especially when standard procedures are not available in pharmacopoeias. Method validation ensures precision, accuracy, specificity, and robustness of analytical techniques, covering parameters like system suitability, linearity, limit of detection (LOD), and limit of quantification (LOQ). Stability-indicating methods (SIMs) are critical for ensuring drug efficacy, safety, and quality throughout the shelf life, by detecting active ingredients apart from degradation products under stress conditions. In the context of Ledipasvir and Sofosbuvir two potent antiviral drugs used in Hepatitis C treatment there is a continued need for optimizing treatment strategies, understanding resistance mechanisms, improving outcomes in special populations, ensuring safety, evaluating cost-effectiveness, and exploring efficacy in HIV-HCV co-infected patients. Studying these drugs not only enhances treatment protocols and patient care but also contributes to a deeper understanding of HCV biology, resistance, and potential for broader therapeutic applications.

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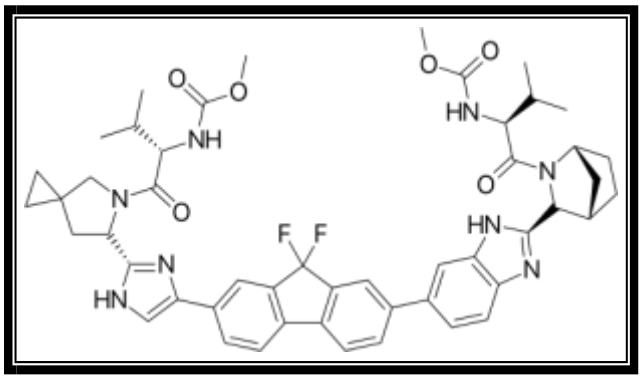

**Fig 01: Structure of Ledipasvir**

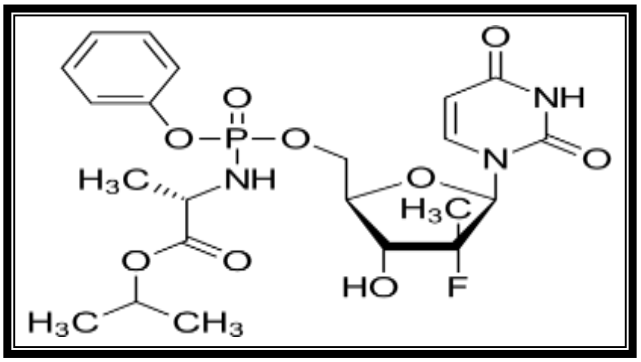

**Fig 02: Structure of Sofosbuvir**

Chronic Hepatitis C affects a large number of people worldwide. It is a viral infection that attacks the liver and leads to inflammation. Chronic Hepatitis C treatment has continued to evolve and interferon-free, oral treatment with a combination of Sofosbuvir and Ledipasvir, which are two direct-acting anti-viral agents. The Oral administration of Sofosbuvir and Ledipasvir combination was well tolerated and suppressed the effect of predictive factors of Chronic Hepatitis. Ledipasvir is anti-viral drug chemically (2S)-1-[(6S)-6-[5-(9, 9-difluoro-7-{2-[1R, 3S, 4S)-2-[(2S)-2-{[hydroxyl (methoxy) methydene] amino}-3-methyl-butanoyl]-2-azabicyclo [2.2.1] heptan-3-yl]-1H-1, 3-benzodiazol-6-yl}-9H-Fluoren-2-yl)-1H-imidazole-2- yl]- 5- azaspirol [2.4] heptan- 5- yl]-2 {[hydroxyl (methoxy) methylidene]amino}- 3- methyl butan- 1-one having formula $C_{49}H_{54}F_2N_8O_6$ and relative molecular mass of 889.00 g/mol. It acts by inhibiting NS5A protein which is mainly responsible for viral RNA Replication. The chemical structure of Ledipasvir is exhibited in **Fig. 1**. Sofosbuvir is [1-4] isopropyl (2S)-2-[[[(2R, 3R, 4R, 5R)- 5- (2, 4-dioxoprrimidin- 1- yl )- 4-fluoro-3-hydroxy- 4- methyl-tetrahydrofuran-2-yl] methoxy-phenoxy phosphoryl] amino] propanoate having formula $C_{22}H_{29}FN_3O_9P$ and relative molecular mass of 529.45 g/mol. It acts by inhibiting NS5B polymerase used in the treatment of hepatitis C. The chemical structure of Sofosbuvir is exhibited in **Fig. 2**. The pharmaceutical dosage form having a combination of Ledipasvir and Sofosbuvir provides a new method of treatment effectively for several people suffering from chronic hepatitis C Virus Infection.

## MATERIALS AND METHODS

Ledipasvir and Sofosbuvir were procured from sequent Labs Pvt. Ltd., Mangalore, India. Cimivir-L tablet, Ledipasvir 90 sofosbuvir 400 from Biocon Pharmaceuticals, Bangalore. HPLC grade solvents were used in chromatographic separation of Ledipasvir and Sofosbuvir, and a 0.45 µ membrane filter was obtained from Millipore.

**Instruments used in the study:**

The equipment used in the estimation of drugs includes an Electronic Balance (Shimadzu digital electronics balance) for precise weighing, and an Ultra-Sonicator (Model SE60US, Labman Scientific India) to ensure proper dissolution of samples. A Thermal

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Oven (Model i-THERM A17782, Dwaraka Scientific) is used for controlled heating purposes, while a pH Meter (Model ORION STAR A111, Thermo Scientific) is employed to measure the pH of Paper from Millipore to remove particulates prior to HPLC analysis. The main analytical instrument used is the HPLC System (Shimadzu HPLC prominence *i* LC-*2030* liquid chromatography system with UV-VISIBLE detector and auto sampler injector. Chromatogram were recorded and integrated on PC installed with lab solutions chromatographic software), which enables accurate and efficient separation and quantification of the drugs.

**Method Development**

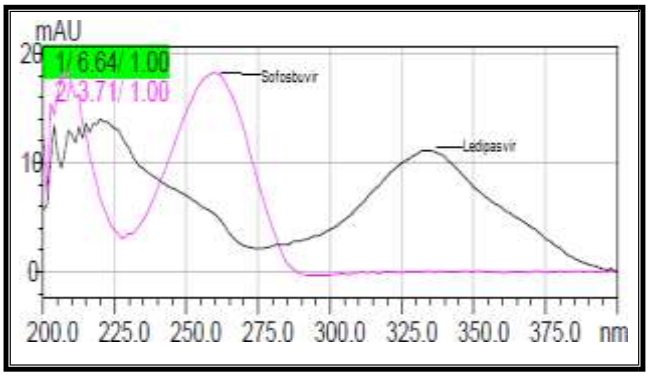
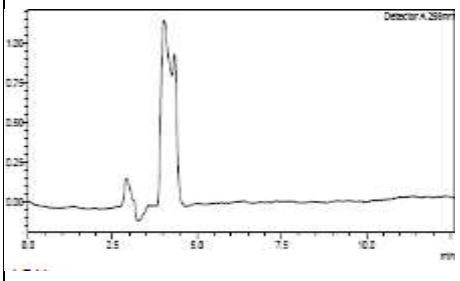
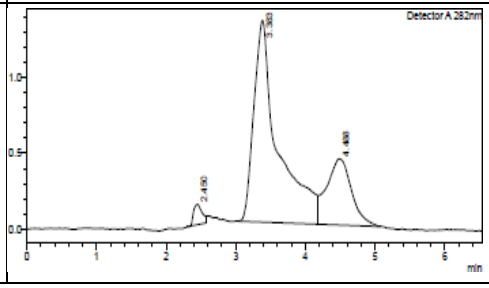



**Fig.3: Overlay Spectrum of Ledipasvir and Sofosbuvir**

**SELECTION OF THE MOBILE PHASE**

Based on the solubility and polarity, different mobile phases were tried and the chromatograms were recorded and results are displayed in table:1

**Table 1: Selection of Mobile Phase**

| Mobile phase | Ratio (%) | Chromatograms | Observation |
|---|---|---|---|
| ACN | 100 |  | Both drugs not separate |
| Water : Methanol | 80:20 |  | Broad peak and Less resolution |

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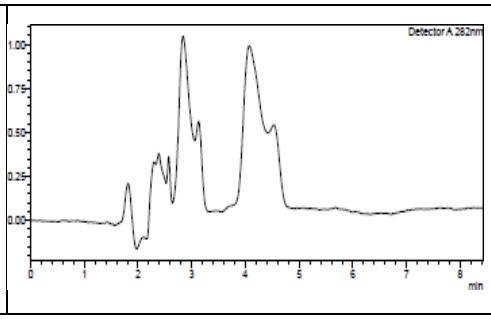
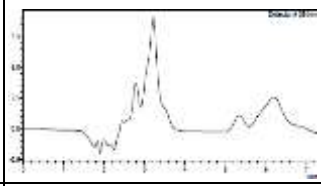
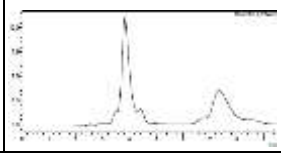
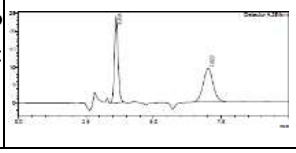
| | | | |
|---|---|---|---|
| Water: ACN | 80:20 |  | Good resolution |

**SELECTION OF BUFFER**

In the mobile phase system consisting of 1% ortho phosphoric acid: acetonitrile, different buffers like phosphate and acetate. The chromatograms were recorded and results are given in table 2.

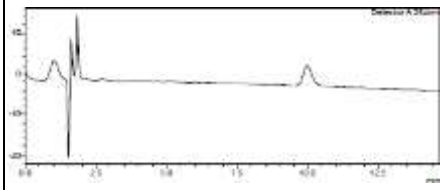
**Table 2: Selection of Buffer**

| S. no | Buffer | Chromatograms | Observation |
|---|---|---|---|
| 1. | Photassium dihydrogen Phosphate: ACN |  | Split peak |
| 2. | Ammonium acetate: ACN |  | Peak tailing |
| 3. | 1% ortho phosphoric acid: ACN |  | Good peak shape &good resolution |

**Selection of flow rate:**

Keeping the ratio of mobile phase constant (30;70 v/v), the chromatogram were recorded with different pH like 3.4, 6.0, 6.4 and 6.9 were tried. For pH 6.4, good resolution and symmetrical peak was obtained and hence selected for further studies table 3.

**Table 3: Selection of Flow Rate**

| S.no | Flow rate (ml/min) | Chromatogram | Observation |
|---|---|---|---|
| 1 | 0.5ml/min |  | Ledipasvir Rt>10min |

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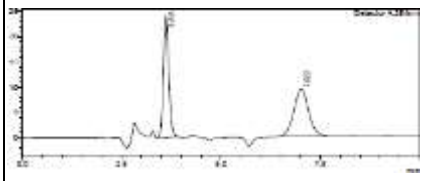
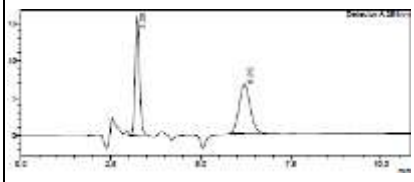
| | | | |
|---|---|---|---|
| 2 | 0.8ml/min |  | Symmetric peak & Good resolution |
| 3 | 0.9ml/min |  | Medium resolution |

**FIXED CHROMATOGRAPHIC CONDITIONS**

| | | |
|---|---|---|
| Chromatographic method | : | RP-HPLC |
| Column (stationary phase) | : | Hibar, C18 column (250mmx 4.0mm, 5µm) |
| Mobile phase | : | 1% ortho phosphoric acid (pH6.4):Acetonitrile |
| Ratio of mobile phase | : | 30:70 v/v |
| Detection of wavelength | : | 254nm |
| Flow rate | : | 0.8ml/min |
| Retention time | : | Sofosbuvir 3.7±0.02 Ledipasvir 7.1±0.02 |
| Temperature | : | Room temperature |

**Linearity and range:**

A calibration graph was plotted with measured peak areas against concentration. From the graph it was found that ledipasvir and sofosbuvir shows good linearity in the concentration range1-10µg/mland1-10µg/ml. The peak area of these solutions was measured at 254nm. The slope, intercept, and correlation coefficient values were calculated respectively (table 4). The linear graph and standard chromatogram obtained are shown in fig 4 to . The linearity table is shown in table 33 and 34.

**Table 4: Regression data**

| Linear regression | Ledipasvir | Sofosbuvir |
|---|---|---|
| Slope | 5976.13 | 0.13807.7 |
| Intercept | 14699.1 | 24637.8 |
| Correlation coefficient | 0.9987 | 0.9995 |

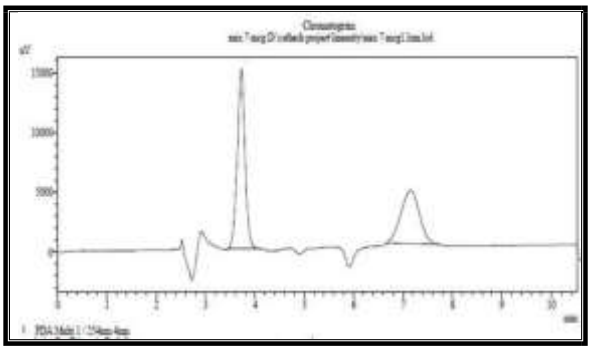


**Fig 4: Chromatogram of Ledipasvir and Sofosbuvir**

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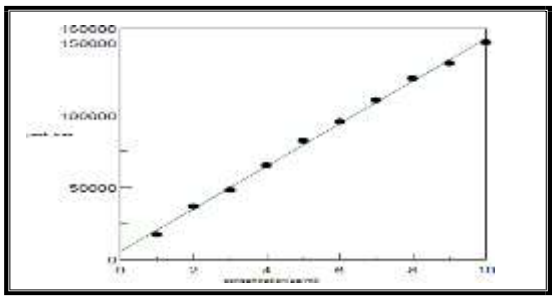


**Fig 5: Calibration graph of Ledipasvir (1-10µg/ml)**

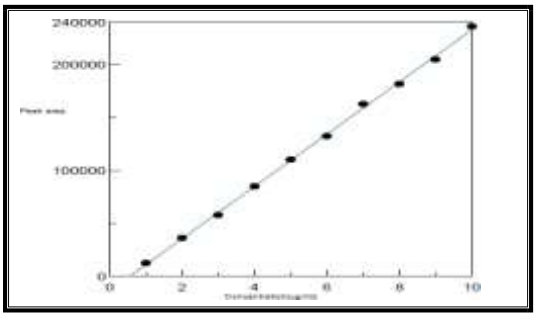


**Fig 6: Calibration graph of Sofosbuvir (1-10µg/ml)**

**Recovery study:**

Recovery studies were carried out at 50, 100, and 150% levels. The percentage recovery and its %RSD were calculated, shown in table 5 & 6.

**Table 5: Recovery study for Ledipasvir**

| Level | % Recovery | %RSD* |
|---|---|---|
| 50% | 98.34% | 0.05 |
| 100% | 98.01% | 0.84 |
| 150% | 99.27% | 0.94 |

*RSD of six determinations

**Table 6: Recovery study for Sofosbuvir**

| Level | % Recovery | %RSD* |
|---|---|---|
| 50% | 99.73% | 0.54 |
| 100% | 100.36% | 0.25 |
| 150% | 101.48% | 0.14 |

*RSD of six determinations

**Precision:**

**Intra-day and inter-day precision:**

Intra-day and inter-day precision was determined by injecting standard solutions in between linearity range (4 and 8µg/ml for ledipasvir and 4and 8µg/ml for sofosbuvir) were injected for three times and % RSD was calculated (table 7 and 8).

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**Table 7: Intra-day precision**

| Concentration (ng/spot) | Peak area | | %RSD* | |
|---|---|---|---|---|
| | Ledipasvir | Sofosbuvir | Ledipasvir | Sofosbuvir |
| Ledipasvir (4µg/ml) & Sofosbuvir (4µg/ml) | 65221 | 84645 | 0.0123 | 0.0270 |
| | 65531 | 84660 | | |
| | 65537 | 84690 | | |
| Ledipasvir(8µg/ml) & Sofosbuvir(8µg/ml) | 125378 | 181499 | 0.0300 | 0.0185 |
| | 125319 | 181491 | | |
| | 125308 | 181437 | | |

*RSD of three determinations

**Table 8: Inter-day precision**

| Concentration (ng/spot) | Peak area | | % RSD* | |
|---|---|---|---|---|
| | Ledipasvir | Sofosbuvir | Ledipasvir | Sofosbuvir |
| Ledipasvir (4µg/ml) & Sofosbuvir (4µg/ml) | 65310 | 84650 | 0.0177 | 0.0343 |
| | 65319 | 84666 | | |
| | 65333 | 84609 | | |
| Ledipasvir (8µg/ml) & Sofosbuvir (8µg/ml) | 124219 | 181409 | 0.0065 | 0.0128 |
| | 124225 | 181410 | | |
| | 124009 | 181450 | | |

*RSD of three determinations

**Repeatability:**

Repeatability of injection was determined by injecting standard solutions (4µg/ml) of ledipasvir and (4µg/ml) of sofosbuvir for six times, noted peak areas and % RSD was calculated table 9 & 10.

**Table 9: Repeatability injection of Ledipasvir**

| Concentration (µg/ml) | Repeatability | %RSD* |
|---|---|---|
| 4 | 65251 | 0.0215 |
| | 65242 | |
| | 65255 | |
| | 65235 | |
| | 65260 | |
| | 65275 | |

*RSD of six determinations

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**Table 10: Repeatability injection of Sofosbuvir**

| Concentration (µg/ml) | Repeatability | %RSD* |
|---|---|---|
| 4 | 84734 | 0.0102 |
| | 84712 | |
| | 84722 | |
| | 84730 | |
| | 84725 | |
| | 84735 | |

*RSD of six determinations

**Limit of detection and limit of quantification (LOD and LOQ):**

Limit of detection and limit of quantification that were calculated from the equation. The result was shown in table 11 & 12, that proved the sensitivity of the method.

**Table 11: Results of LOD & LOQ of Ledipasvir**

| | |
|---|---|
| Correlation coefficient ±SD* | 0.9987±0.0001342 |
| Slope ±SD* | 3.123±2.608 |
| Intercept ±SD* | 1112.65±2.436 |
| LOD(ng/spot) | 0.008µg/ml |
| LOQ(ng/spot) | 0.27µg/ml |

*RSD of six determinations.

**Table 12: Results of LOD & LOQ of Sofosbuvir**

| | |
|---|---|
| Correlation coefficient ±SD* | 0.9995±0.4080 |
| Slope ±SD* | 3.123±2.611 |
| Intercept ±SD* | 1112.65±2.822 |
| LOD(ng/spot) | 0.0006µg/ml |
| LOQ(ng/spot) | 0.0020µg/ml |

*RSD of six determinations.

**Specificity:**

There were no additional peaks observed while injecting solvents or mobile phase alone. The peak purity index of standard ledipasvir 0.9998 and sofosbuvir 0.9995.

**Robustness:** Inorder to demonstrate the robustness of the method, the following optimized conditions were slightly changed.

±0.1mlflow rate
±2% organic solvent
±0.5$P^H$

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The responses for these changed chromatographic parameters were almost same for the fixed chromatographic parameters and hence the developed method was said to be robustness

**Stability of solution:**

The solution under room temperature was stable for 24 hours. (Table 138)

**Table 13: Stability of solution**

| Hours | Ledipasvir | | Sofosbuvir | |
|---|---|---|---|---|
| | Concentration (µg/ml) | Peak area | Concentration (µg/ml) | Peak area |
| 0hrs | 5 | 82453 | 5 | 110043 |
| 6hrs | | 82412 | | 110012 |
| 24hrs | | 82219 | | 109702 |

**System suitability studies:**

The system suitability parameters like peak area, tailing factor, theoretical plate count, resolution, and retention time were calculated from the standard chromatogram (table 14).

**Table 14: System suitability studies**

| Drug | Theoretical plate (N) | Retention time (min) | Tailing factor (10%) | Resolution |
|---|---|---|---|---|
| Ledipasvir | 2703 | 3.7 | 1.062 | - |
| Sofosbuvir | 1762 | 7.1 | 0.994 | 6.90 |

**FORCED DEGRADATION STUDIES: LEDIPASVIR**

**Acid hydrolysis:**

Ledipasvir was subjected to acid hydrolysis, no additional peak was observed along with standard, which is shown in figure 15.

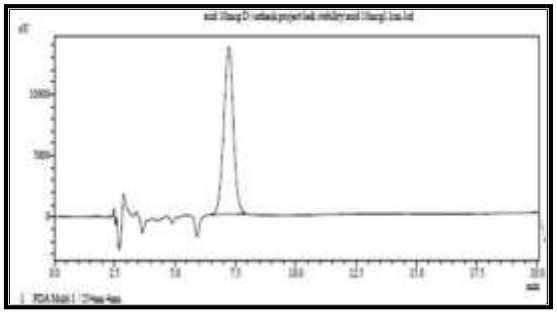

**Figure 15: Chromatogram of acid hydrolysis of Ledipasvir**

**Alkaline hydrolysis:**

The drug was subjected to alkaline hydrolysis no additional peaks were observed, which was shown in figure 16.

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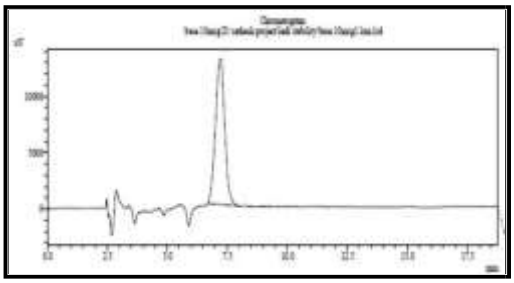

**Figure 16: Chromatogram of Alkaline hydrolysis of Ledipasvir**

**Neutral hydrolysis:**

The drug was subjected to neutral hydrolysis no additional peaks were observed, which was shown in figure 17.

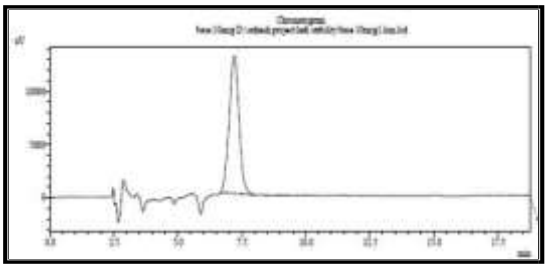

**Figure 17: Chromatogram of neutral hydrolysis of Ledipasvir**

**Oxidative degradation:**

The drug was subjected to oxidation no additional peaks were observed, which was shown in figure 18.

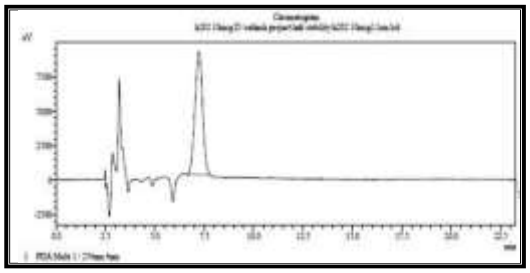

**Figure 18: Chromatogram of oxidative degradation of Ledipasvir**

**Photolytic degradation:**

The drug was subjected to photolytic degradation no additional peaks were observed, which was shown in figure 19.

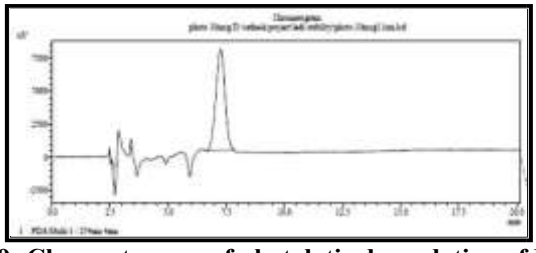

**Figure 19: Chromatogram of photolytic degradation of Ledipasvir**

**Thermal degradation**

The drug was subjected to thermal degradation no additional peaks were observed at Rf value 0.34, which was shown in figure 20.

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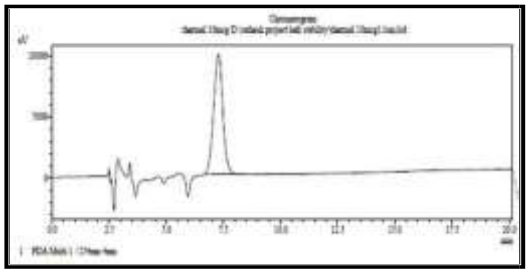

**Figure 20: Chromatogram of thermal degradation of Ledipasvir**

**Table 15: Summary of forced degradation studies for Ledipasvir:**

| Type of stress | Stress condition | Retention time(min) | |
|---|---|---|---|
| | | Drug | Degraded Product |
| Acid hydrolysis | Drug refluxed with 0.1M HCl for about 5 hours | 7.1 | No additional peak |
| Basic hydrolysis | Drug refluxed with 0.1M NaoH for about 5 hours | 7.1 | No additional peak |
| Neutral hydrolysis | Drug treated with water and refluxed for about 5 hours | 7.1 | No additional peak |
| Oxidative degradation | Drug treated with 6% hydrogen peroxide at normal room temperature | 7.1 | No additional peak |
| Photo degradation | Drug exposed to Sunlight for 5 hours | 7.1 | No additional peak |
| Thermal degradation | Drug introduced in Hot air oven for 5 hours | 7.1 | No additional peak |

**FORCED DEGRADATION STUDIES: SOFOSBUVIR**

**Acid hydrolysis:**

Sofosbuvir was subjected to acid hydrolysis, no additional peak was observed, that is shown in figure 21.

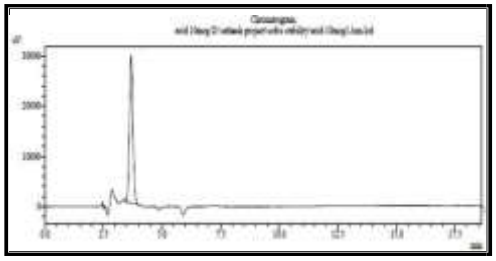

**Figure 21: Chromatogram of acid hydrolysis of Sofosbuvir**

**Alkaline hydrolysis:**

The drug was subjected to alkaline hydrolysis three additional peaks were observed at Rt 2.8 and 4.1 value which was shown in figure 22.

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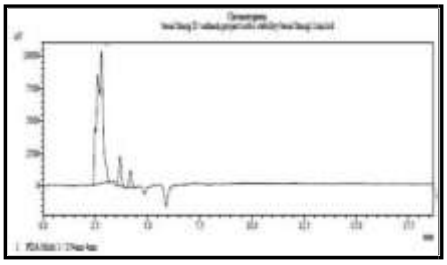

**Figure 22: Chromatogram of Alkaline hydrolysis of Sofosbuvir**

**Neutral hydrolysis:**

The drug was subjected to neutral hydrolysis no additional peaks were observed, which was shown in figure 23.

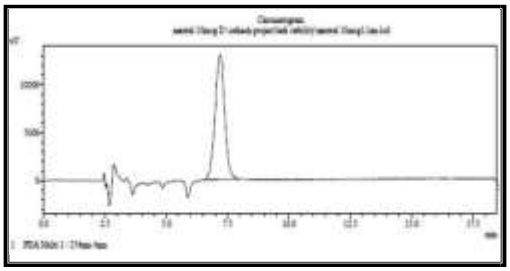

**Figure 23: Chromatogram of neutral hydrolysis of Sofosbuvir**

**Oxidative degradation:**

The drug was subjected to oxidation no additional peaks were observed, which was shown in figure 24.

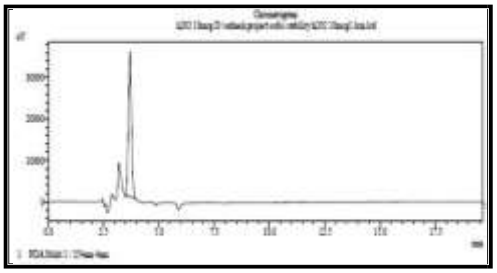

**Figure 24: Chromatogram of oxidative degradation of Sofosbuvir**

**Photolytic degradation:**

The drug was subjected to photolytic degradation no additional peaks were observed, which was shown in figure 25.

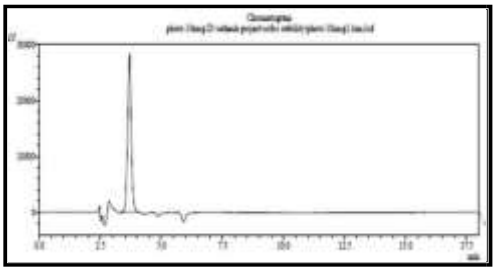

**Figure 25: Chromatogram of photolytic degradation of Sofosbuvir**

**Thermal degradation**

The drug was subjected to thermal degradation no additional peaks were observed, which was shown in figure 26.

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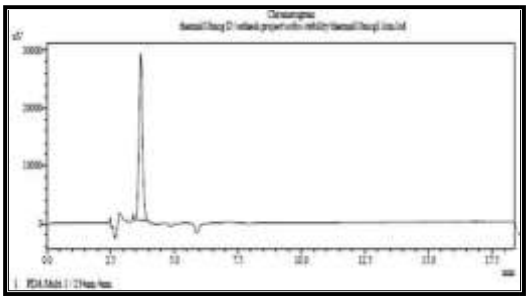

**Figure 26: Chromatogram of thermal degradation of Sofosbuvir**

Summary of forced degradation was shown in the table 16.

**Table 16 Summary of forced degradation studies for Sofosbuvir:**

| Type of stress | Stress condition | Retention time(min) | |
|---|---|---|---|
| | | Drug | Degraded Product |
| Acid hydrolysis | Drug refluxed with 0.1M HCl for about 5 hours | 3.7 | No additional peak |
| Basic hydrolysis | Drug refluxed with 0.1m NaoH forabout5hours | 3.7 | 2.8±0.02<br>4.1±0.02 |
| Neutral hydrolysis | Drug treated with water and refluxed for about 5 hours | 3.7 | No additional peak |
| Oxidative degradation | Drug treated with 6% hydrogen peroxide at normal room temperature | 3.7 | No additional peak |
| Photo degradation | Drug exposed to Sunlight for 5 hours | 3.7 | No additional peak |
| Thermal degradation | Drug introduced in Hot air oven for 5 hours | 3.7 | No additional peak |

**FORCED DEGRADATION STUDIES: LEDIPASVIR AND SOFOSBUVIR**

**Acid hydrolysis:**

Ledipasvir and sofosbuvir were subjected to acid hydrolysis, no additional peak was observed, that is shown in figure 27.

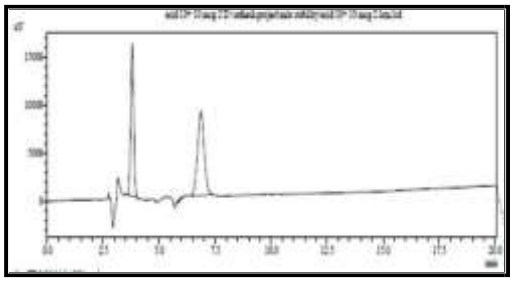

**Figure 27: Chromatogram of acid hydrolysis of Ledipasvir and Sofosbuvir**

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**Alkaline hydrolysis:**

The drug was subjected to alkaline hydrolysis two additional peaks were observed at Rt 3.1 and 4.2 value which was shown in figure 28.

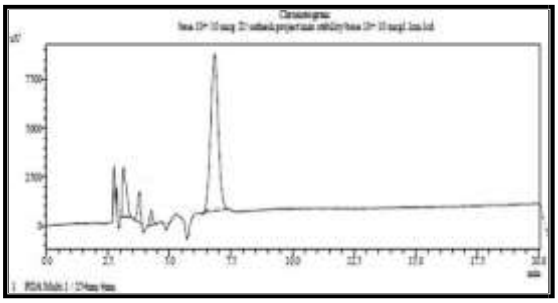

**Figure 28: Chromatogram of alkaline hydrolysis of Ledipasvir and Sofosbuvir**

**Neutral hydrolysis:**

Ledipasvir and Sofosbuvir were subjected to neutral hydrolysis no additional peaks were observed, which was shown in figure 29.

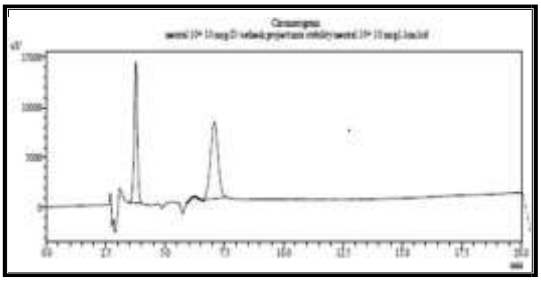

**Figure 29: Chromatogram of neutral hydrolysis of ledipasvir and sofosbuvir**

**Oxidative degradation:**

Ledipasvir and sofosbuvir was subjected to oxidation no additional peaks were observed, which was shown in figure 30.

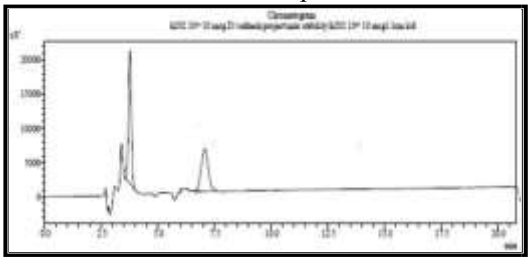

**Figure 30: Chromatogram of Oxidative degradation of Ledipasvir and Sofosbuvir**

**Photolytic degradation:**

Ledipasvir and Sofosbuvir were subjected to photolytic degradation, no additional peaks were observed at, which was shown in figure 31.

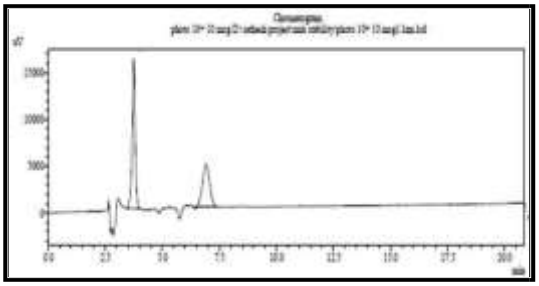

**Figure 31: Chromatogram of Photolytic degradation of Ledipasvir and Sofosbuvir**

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**Thermal degradation**

The drug was subjected to thermal degradation no additional peaks were observed, which was shown in figure 32.

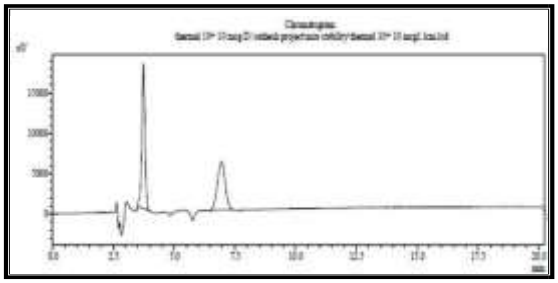

**Figure 32: Chromatogram of thermal degradation of Ledipasvir and sofosbuvir**

**Table 17: Summary of forced degradation studies for Ledipasvir and Sofosbuvir:**

| Type of stress | Stress condition | Retention time(min) | | | |
|---|---|---|---|---|---|
| | | Ledipasvir | Sofosbuvir | Ledipasvir Degradant | Sofosbuvir Degradant |
| Acid hydrolysis | Drugs refluxed with0.1M HCl for about 5 hours | 7.1 | 3.7 | No additional peak | No additional peak |
| Basic hydrolysis | Drugs refluxed with0.1m NaoH for about 5 hours | 7.1 | 3.7 | No additional peak | 2.8±0.02 4.1±0.02 |
| Neutral hydrolysis | Drugs treated with water and refluxed for about 5 hours | 7.1 | 3.7 | No additional peak | No additional peak |
| Oxidative degradation | Drugs treated with 6% hydrogen peroxide at normal room temperature | 7.1 | 3.7 | No additional peak | No additional peak |
| Photo degradation | Drugs exposed to Sunlight for 5 hours | 7.1 | 3.7 | No additional peak | No additional peak |
| Thermal degradation | Drugs introduced in Hot air oven for 5 hours | 7.1 | 3.7 | No additional peak | No additional peak |

**SUMMARY AND CONCLUSION**

Each year 3,99,000 people were died for hepatitis C. Ledipasvir and Sofosbuvir are the two anti-viral life saving drugs commonly used in combination for treating hepatitis C. In the stability-indicating reverse phase high performance liquid chromatography method, the wavelength selected for quantitation was 254 nm. (The method has been validated for linearity, accuracy, precision, robustness, limit of detection and limit of quantification. Linearity was observed in the concentration range of 1-10µg/ml for both the drugs). In this method, the separation was achieved by Hibar C18(250×4.6mm)5µm column using 1% O-Phosphoric acid (pH 6.4): Acetonitrile (30:70 v/v) as mobile phase with flow rate 0.8 ml/min. The retention time of ledipasvir and sofosbuvir were found to be 7.1min and 3.7 min, respectively. During force degradation, drug product was exposed to hydrolysis (acid and base hydrolysis), oxidation, thermal degradation and photo degradation. Both the drugs were not degraded under thermal, oxidative, photolytic, acid and neutral hydrolytic conditions, but Sofosbuvir showed degradation under alkaine hydrolytic condition with a

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retention time 2.8 min and 4.1 min, respectively. The degraded products of Ledipasvir and Sofosbuvir were well resolved from the individual bulk drug response. The specificity of the method was confirmed by peak purity profile of the resolved peaks. All the developed methods were validated according to ICH guidelines and were found to be simple, specific, sensitive, accurate, precise and economic, hence the proposed methods could be used for simultaneous estimation of Ledipasvir and Sofosbuvir in tablet dosage form. Among these methods UV spectroscopic methods are simple, reliable and less time consuming which could be an alternative for chromatographic analysis.

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