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"Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Sofosbuvir and Daclatasvir from Pharmaceutical Dosage Form"

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ABSTRACT: The main aim of this study is to develop new, simple, sensitive, accurate, and economical analytical method for the simultaneous estimation of sofosbuvir and daclatasvir from pharmaceutical dosage form by RP-HPLC. Analytical chemistry is inherently quantitative Science. Whether determining the concentration of a species in a solution, evaluating equilibrium constant, measuring a reaction rate, or drawing a correlation between a compound's structure and its reactivity. Analytical chemistry is usually delineated because the space of chemistry accountable for characterizing the composition of matter each qualitatively (what is present) and quantitatively (how abundant is present).
The objective of the To Validate the proposed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.to perform forced degradation study of sofosbuvir and Daclatasvir drug by RP-HPLC. Spectroscopicchromatographic.technique incorporates UV-obvious, infrared, , NMR, Fluorescence and phosphorescencespectrophotometry and Electrochemical methods incorporatePotentiometry, Voltammetry,Voltametric techniques, Stripping techniques,amperometry techniques,Coulometry, Electrogravimetry,Conductancetechniques,while chromatographic methodsincorporateHigh performance liquid chromatography (HPLC), high- performance thin layer chromatography (HPTLC), gas chromatography (GC), Miscellaneous Techniques incorporate Thermal analysis,Mass spectrometry,Kinetic techniques. Hyphenated Methods incorporate GC-MS, ICP-MS, GC-IR, MS-MS.

Keywords:sofosbuvir and daclatasvir, Detection, RP HPLC, NP-HPLC, IEC, SEC,Chromatography

I. INTRODUCTION:

Analytical chemistry is inherently quantitative Science. Whether determining the concentration of a species in a solution, evaluating equilibrium constant, measuring a reaction rate, or drawing a correlation between a compound's structure and its reactivity. Analytical chemistry is usually delineated because the space of chemistry accountable for characterizing the composition of matter each qualitatively (what is present) and quantitatively (how abundant is present). Almost all chemists routinely make qualitative or quantitative measurements. The argument had made that the analytical chemistry is not a separate branch of chemistry but simply the application of chemical knowledge.

Selection of analytical method:
First stage within the choice or development of technique is to determine what's to be measured and how accurately it should measure. Unless one has series of methods at hand to assess quality of the product, validation programmed may have limited validity.

- The selected method must have the Subsequent parameters:
- As simple as possible,
- Most specific,
- Most productive, economical and convenient,
- As accurate and precise as required,

Multiple sources of key component (reagents, columns, TLC plates) should be avoided, to be fully Optimized before transfer for validation of its characteristics suchas accuracy, precision, sensitivity, ruggedness etc.

Instrumental Methods:

These methods are based upon the measurement of some physical properties as conductivity, Electrode potential, light absorption

International Journal of Pharmaceutical Research and Applications
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or emission, mass-to-charge ratio and fluorescence of substance. There are many techniques available for the analysis of analytes:

a) Spectroscopic Analysis:

- Ultraviolet and visible spectrophotometry,
- Fluorescence and phosphorescence spectrophotometry,
- Atomic spectrophotometry (emission & absorption),
- Infra-red spectrophotometry,
- Raman spectroscopy,
- X-ray spectroscopy,
- Radio chemical techniques including activation analysis,
- NMR spectroscopy,
- ESR spectroscopy.

b)Electrochemical Techniques:

- Potentiometry,
- Voltammetry,
- Voltametric techniques,
- Stripping techniques,
- amperometry techniques,
- Coulometry,
- Electrogravimetry,
- Conductance techniques.

c) Chromatographic Methods:

- Gas chromatography (GC),
- High performance liquid chromatography (HPLC),
- High-performance thin layer chromatography (HPTLC).

d)Miscellaneous Techniques:

- Thermal analysis,
- Mass spectrometry,
- Kinetic techniques.

e) Hyphenated Methods:

- GC-MS,
- ICP-MS,
- GC-IR,
- MS-MS.

Amongst all the techniques mentioned above UV-Visible spectrophotometry and High-performance liquid chromatography (HPLC) are the most widely used techniques for quantitative analysis of pharmaceutical substances**.**

COMPONENTSOF HPLC INSTRUMENT

1)Mobile phase reservoir:

The commonly used solvent reservoir is a glass bottle. Most of the manufacturers provide bottles with special caps. For connection of this mobile phase reservoir to the pump inlet Teflon tubing and filters are used.

2) Pump:

High pressure pumps are required to force solvents through packed stationary phase beds. Smaller bed particles needed higher pressure. There are several advantages to using smaller particles however they are not essential for all separations. The benefits are higher resolution, quicker analyses, and increased sample load capability.

3) Injector:

Sample can be introduced in various ways. The normal method is to use an injection valve. In additional sophisticated LC systems, the auto sampling devices are incorporated wherever the sample is introduced with the help of auto samplers and microprocessors.

4) Column:

HPLC columns are 5, 10, 15 and 25 cm in long and are filled with small diameter (3, 5 or 10 μm) particles. The internal diameter of the columns is normally 4.6 mm. This is considered the effective compromise for sample capability, mobile phase consumption, speed and resolution.

5) Detector:

When a substance has passed through the column there are several ways to detect it. A common method which is easy to explain is ultra-violet absorption. The amount of light absorbed will depend on the amount of a particular compound that passes through the beam at the time.

6) Data system:

Modern data collection techniques can aid the signal analysis since the detector signal is electronic. In additionally, some systems will store data in a retrievable form for highly sophisticated computer analysis at a later time. The main goal in exploitation electronic data systems is to increase analysis accuracy and precision, whereas reducing operator attention.

Advantages of HPLC

1. Wide variety of packing materials allows the separation of most chemical species. The phases that are most extensively used for drug substances of low molecular weight (less than 1000) and their

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decomposition products or metabolites are the adsorption systems is based on silica and the reversed – phase systems is based on octyl silyl or octadecyl silyl bonded on silica.
2. The different types of detectors available permit the sensitive detection of most chemical type, and the accuracy and precision with which eluted substances may be quantified.
3. Micro particulate packing materials give excellent separation of similar substances.
4. The number of theoretical plates given by a standard analytical column is of the order of 5000-10000 and this gives adequate resolution of the vast majority of mixtures which are likely to require separation.
5. A combination of HPLC and spectrometric techniques allows the simultaneous quantization and identification of solutes as they elute from the column.
6. In the pharmaceutical industry, it is essential to produce pure drug substances, suitable for human consumption for in a cost-effective manner. The purity of drug substance can be checked by separation techniques such a GC, TLC, HPLC. These techniques tend to be more sensitive and specific than spectroscopic method.
7. HPLC has an advantage over GC as an analytical technique, since analytes need be neither volatile nor extremely stable to eleven temperatures. Highly accurate, almost universal detectors, makes quantification easier than with TLC.
8. Standardization either by external or internal standard techniques made it possible to convert for structurally depended differences in detector response.

Method Development

Various new analytical methods are required for controlling the quality of constantly growing new drugs. Alternate methods for existing (non-Pharmacopoeia) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. Once alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit / demerits are made available.

Method Validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH guidelines, typical analytical performance characteristics that should be considered in the validation of the types of methods are:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Reproducibility
- Linearity Range
- Limit of Detection
- Limit of Quantitation
- Robustness
- Ruggedness

1) Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy was tested (% Recovery and % RSD of individual measurements) by analysing samples at least in triplicate at each level (50, 75, 100, 125 and 150 % of label claim) is recommended. For each determination recent samples were prepared and assay value is calculated. Recovery was calculated from regression equation obtained in linearity study. Accuracy was determined from the mean relative error for a set of replicate analysis (i.e. the difference between measured and nominal concentration) for spiked samples.

2) Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision could be considered at three levels; repeatability, intermediate precision and reproducibility.

2.1) Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed as intra - assay precision. Intermediate precision expresses with in laboratories variations: different days, different analyst and different equipment.

2.2.) IntermediatePrecision

Intermediate precision expresses within-laboratories variations: totally different days, different analysts, different equipment, etc.

2.3). Reproducibility

International Journal of Pharmaceutical Research and Applications
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The procedure is carried out by different analyst in different laboratories using different equipment, regents and laboratories setting. Reproducibility was determined by measuring repeatability and intermediate precision. Reproducibility is assessed by means of an inter - laboratory trial.

3) Linearity Range

Most analytical methods are based up on process where the method produces a response that is linear and which increases or decreases linearity with analyte concentration. The equation of a straight line takes the form.

$Y = a + b x$

Where „a‟ is the intercept of the straight with the y axis and „b' is the slope of the line.

The range of an analytical procedure is that the interval between the higher and lower concentration (amounts) of analyte within the sample (including these concentrations) for which it's been incontestable that the analytical procedure contains a appropriate level of precision, accuracy and linearity.

4) Limit Of Detection

This is the smallest amount of an analyte which can be detected by a particular method. It is formally defined as follows.

$X - Xb = 3sb$

Where „X‟ is the signal from the sample. „Xb‟ is that the signal from the analytical blank and Sb is that the SD of the reading for the analytical blank. The detection limit is typically

Expressed because the concentration of analyte (percentage parts per million) in the sample. We can calculate LOD by using the following formula.

LOD = 3 * SD / slope of calibration curve
SD = Standard deviation of intercepts.

5) Limit Of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analytic in a sample which can be quantitatively determined with suitable precision and accuracy. Quantification limit is expressed as the concentration of analyte (e.g. - % ppm) in the sample. The quantitation limit is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision.

LOQ = 10 * SD / slope of calibration curve
SD = Standard deviation of intercepts.

6) Robustness

This term refers to how resistant the precision and accuracy of an assay is to small Variation in the method, e.g. changes of instrumentation, slight variation in extraction procedure, sensitivity to minor impurities in reagents, etc. Robust assays may not be capable of the highest precision or specificity but they are regarded as fit for the purpose for which they are designed

7) **Ruggedness**

Degree of reproducibility of test results obtained by the analysis of the same samples under a variety of condition such as different laboratories, different analysts, different instruments etc, normally expressed as the lack of influence on test results of operational and environmental variable of the analytical method. Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst. Degree of representative of test results is then determined as a function of the assay variable.

II. REVIEW OF LITERATURE:

BenzilDudekula, Dr. C. Ramachandraiah, Dr. N. Devanna,(2017) Analytical method was developed for the estimation of Sofosbuvir and Daclatasvir drug substance by liquid chromatography. The chromatographic separation was achieved on C18 column (XTerra RP18 150 x 4.6, 5um) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Trifluoro acetic acid in water: Acetonitrile (60:40). The flow rate was 1.0 ml/ minute and ultra violet detector at 275nm. The average retention time for Sofosbuvir and Daclatasvir found to be 2.09 and 3.50 min. The proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 80-240 µg/ml for Sofosbuvir and12-36µg/ml for Daclatasvir

Dr. M. Manoranjani, 2020 The chromatographic separation was achieved on Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5um) at ambient temperature. The separation achieved employing a mobile phase consists of

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 844-858 www.ijprajournal.com ISSN: 2456-4494

0.1%v/v Trifluoroacetic acid in water: Acetonitrile (50:50). The flow rate was 1.0ml/ minute and ultraviolet detector at 275nm. The average retention time for Daclatasvir and Sofosbuvir found to be 2.805 min and 3.734 min. The proposed method was validated for selectivity, precision, linearity, and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 12.0 - 36.0µg/mL for Daclatasvir and 80.0 -240.0µg/mL of Sofosbuvir.

I. PLAN OF WORK

The experimental work for development a stability indicating RP-HPLC method for the simultaneous estimation of sofosbuvir and Daclatasvir was planned as follows.

Plan of work for completion of objectives was as follows:

- Literature survey.
- Selection of drug.
- Drug-theoretical study,
- Aspects ofHPLC.
- Procurement of drug and chemicals.
- Development and validation of sofosbuvir and daclatasvir-HPLC.
- Stability indicating study.

Method for Estimation

- Selection of column
- Selection and optimization of Mobile phase
- Selection of chromatographic conditions
- Preparation of calibration curve
- System suitability parameter study
- To adopt selected method on bulk and tablet.
- Recovery studies and combination of data.
- Validationofproposedmethods as per ICHguidelines.
- Stability indicating study, it involves,
- Acid degradation
- Alkali/Base degradation
- Oxidative degradation
- Photolytic degradation
- Thermal degradation
- Collection of data, calculation etc.
- Thesis submission and communication of paper

II. DRUG PROFILE OF SOFOSBUVIR

IUPAC NAME	propan-2-yl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl] methoxy-phenoxy phosphoryl] amino] propanoate
MOLECULAR FORMULA	C22H29FN3O9P
MOLECULAR WEIGHT	529.5 g/mol
APPEARANCE	White to off-white crystals or powder
MELTING POINT	94–125 ºC.
STORAGE	Store at room temperature below 30 °C (86 °F).
SOLUBILITY	In water, 105 mg/L at 25 °C

Mechanism of Action:

Sofosbuvir is a direct-acting antiviral agent (pan-genotypic polymerase inhibitor) against the hepatitis C virus. HCV RNA replication is mediated by a membrane-associated multiprotein replication complex. The HCV polymerase (NS5B protein) is an RNA-dependent RNA polymerase (RdRp). It is the essential initiating and catalytic subunit of this replication complex and is critical for the viral replication cycle. There is no human homolog for HCV NS5B RdRp. Sofosbuvir is a monophosphorylated pyrimidine nucleotide prodrug that undergoes intracellular metabolism to form the pharmacologically active uridine analogue triphosphate (GS-461203). GS-461203 competes with natural nucleotides for incorporation (by HCV NS5B) into the nascent RNA strand during replication of the viral genome. GS-461203 differs from endogenous pyrimidine nucleotides in that it has been modified at the 2' position with the addition of a methyl and a fluor functional group. Incorporation of GS-461203 into nascent RNA strongly reduces the efficiency of further RNA elongation by RdRp, resulting in premature

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 844-858 www.ijprajournal.com ISSN: 2456-4494

termination of RNA synthesis. The stopping of viral replication leads to a rapid decline of HCV viral load and clearing of HCV levels in the body.

III DRUG PROFILE OF DACLATASVIR

IUPAC NAME	methyl N-[(2S)-1-[(2S)-2-[5-[4-[4-[2-[(2S)-1-[(2S)-2-(methoxycarbonylamino)-3-methylbutanoyl] pyrrolidin-2-yl]-1H-imidazol-5-yl] phenyl] phenyl]-1H-imidazol-2-yl] pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl] carbamate
MOLECULAR FORMULA	C40H50N8O6
MOLECULAR WEIGHT	738.9 g/mol
CAS NO	1009119-64-5
APPEARANCE	White to off-white crystals or powder
BOILING POINT	1071.2±65.0 °C
STORAGE	Store at room temperature below 30 °C (86 °F).
SOLUBILITY	Freely soluble (>700 mg/mL)

Mechanism of Action: -

NS5A is a viral non-structural phosphoprotein that is part of a functional replication complex in charge of viral RNA genome amplification on endoplasmic reticulum membranes. 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 hyper phosphorylated 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. It is also reported that daclatasvir also blocks both intracellular viral RNA synthesis and virion assembly/secretion in vivo.

III. MATERIALS AND METHODS USED

1. MATERIALS

a) INSTRUMENTS

i) HPLC:

Make	Analytical Technologies LTD (3000 Series)
Pump	P-3000-M Reciprocating (40MPa)
Detector	UV-3000-M
Software	HPLC- Workstation
Column	CosmosilC18(250MM×4.6ID Particle size 5µ

ii) Analytical Balance: Digital Analytical balance

Make	Wesner High Precision Balance
Model	PGB-100

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 844-858 www.ijprajournal.com ISSN: 2456-4494

iii) Sonicator:

Make	Wesner Ultra Sonicator
Model	WUC-4L

b) Drug:

Drug and drug product samples suppliers and manufacturers

Name of drug and drug product	Supplier and manufacturer by
Sofosbuvir Drug	Mylan laboratories Ltd.
Daclatasvir Drug	Arene life sciences Ltd
Sofosbuvir and Daclatasvir combination tablet.	Hercynite plus, Nalco pharma. Pvt Ltd.

c) Reagents:

Sr. No	Chemical	Make
1.	Water	Rankem
2.	Methanol	Merck life science

Method:

1 Chromatographic Procedure:

The method was carried out on CosmosilC18 (250mm x 4.6 i.d., particle size: 5 micron) mobile phase using Methanol: water in the ratio of 75:25 v/v flow rate 0.8 mL/min. The wavelength of Sofosbuvir and Daclatasvir at 280 nm was found to be appropriate. The linearity range was obtained in the concentration of 20-100µg/mL for Sofosbuvir and3-15µg/mL for Daclatasvir. The retention time of Sofosbuvir and Daclatasvir was found to be 6.08 min and 3.92 min respectively. The developedmethod was found be accurate, robust and sensitive which can be used for estimation of combination of Sofosbuvir and Daclatasvir From pharmaceutical dosage form.

2 Selection of Stationary phases:

On base of reversed phase HPLC manner and the number of carbon present in molecule (analyte), stationary phase with CosmosilC18 bonded phase i.e. 250 x 4.6 mm, 5µ was selected.

3 Selection of Mobile Phase:

The choice of mobile phase was done after considering the solubility of drug in different solvent, as well as based on literature review and finally mobile phase was selected for this as Methanol and Water in the ratio of 75:25 v/v respectively.

4 Preparation of Mobile Phase:

Take a mixture of Methanol: Water in the ratio of 75:25 v/v. Mix it well, sonicate it to degas.

5 Preparation of Diluent:

Preparation of a mixture of Methanol: Water in the ratio of 75:25 v/v.

6 Preparation of Blank:

Diluent is used as blank.

7 Preparation of sofosbuvir Standard stock solution:

weigh accurately 10mg of pure drug dissolved in 100ml of solvent (solvent was used as our mobile phase only); this gives 100ppm standardstock solution.

8 Preparation of sofosbuvir Sample solution:

Take 2ml, 4ml, 6ml, 8ml and 10ml of standard stock solution and dilute up to 10 ml it gives a 20, 40, 60, 80 and 100 ppm sample solution respectively.

9Preparation of Daclatasvir Standard stock solution:

weigh accurately 10mg of pure drug dissolved in 100ml of solvent (solvent was used as our mobile phase only) this gives 100 ppm solution.

10 Preparation of Daclatasvir Sample solution:

Similarly, for the Daclatasvir, the dilutions of 3, 6, 9, 12 and 15 ppmwereprepared by diluting the standard stock solution of 0.3, 0.6, 0.9, 1.2and 1.5mlrespectively.

III. RESULT AND DISCUSSION:

1 Selection of Analytical Wavelength

From the standard stock solution further dilutions were done using methanol and water scanned over the range of 200 – 400 nm. The spectrum was obtained. It was observed that the both the drugs showed considerable absorbance at 280 nm so it was selected as detection wavelength.

International Journal of Pharmaceutical Research and Applications
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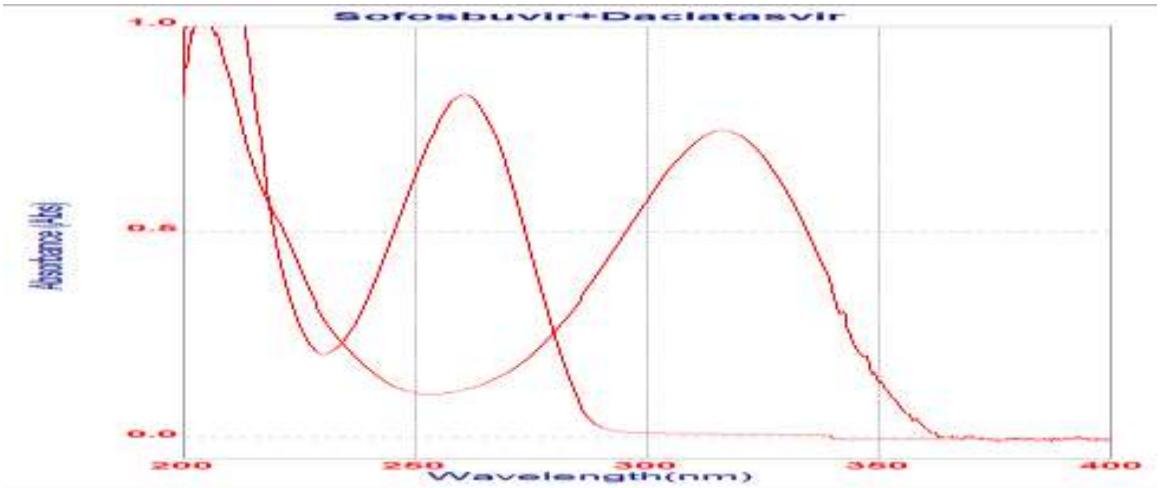

Fig: Wavelength detection for sofosbuvir And Daclatasvir

Conclusion-

From the above data, the Sofosbuvir and Daclatasvir showed good absorbance at 280nm. Hence, this wavelength was selected for further study and analysis of Sofosbuvir and Daclatasvir.

Trials for RP-HPLC MethodDevelopment:

Reverse Phase High Performance Liquid Chromatography Method Development Different trials taken were as follows

Trial 1: -Daclatasvir

Chromatographic Conditions: -

Column	:	CosmosilC18, 250 x 4.6 mm, 5µ
Mobile Phase	:	Methanol: Water (80: 20)
Flow Rate	:	0.8 mL/min
Injection Volume	:	20 µL
Wavelength	:	280 nm
Run Time	:	10.95 minute

Fig-: Chromatogram of Daclatasvir Trial-1

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 844-858 www.ijprajournal.com ISSN: 2456-4494

Conclusion-

From the above information of Daclatasvir the peak shape is not proper observed so needs to be optimized

Trial 2: - Daclatasvir

Chromatographic Conditions: -

Column	:	CosmosilC18, 250 x 4.6 mm, 5µ
Mobile Phase	:	Methanol: Water (85: 15)
Flow Rate	:	0.8 mL/min
Injection Volume	:	20 µl
Wavelength	:	280 nm
Run Time	:	7.26 min

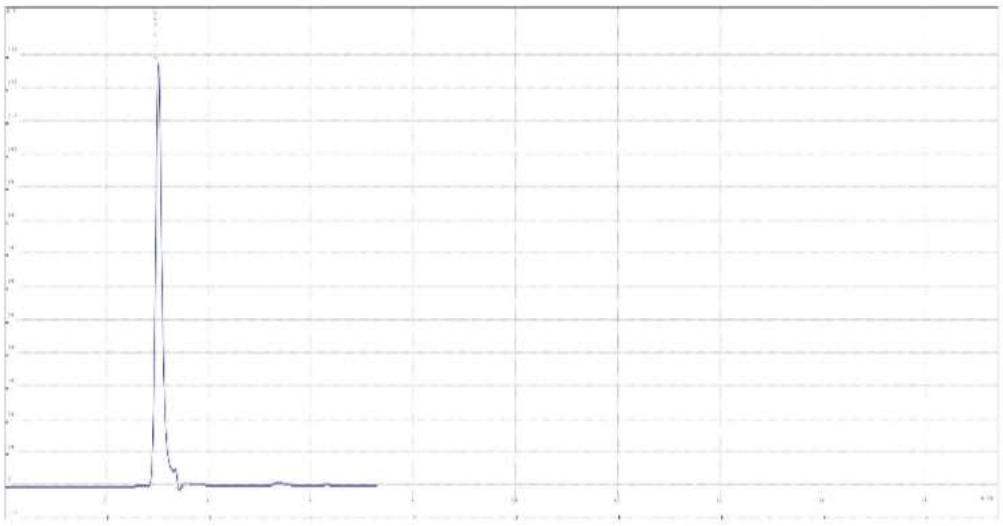

Fig -: Chromatogram of Daclatasvir Trial-2

Conclusion-

From the above information of Daclatasvir the peak shape is poor and not proper observed so needs to be optimized

Trial 3: -Sofosbuvir

Chromatographic Conditions: -

Column	:	CosmosilC18, 250 x 4.6 mm, 5µ
Mobile Phase	:	Methanol: Water (85: 15)
Flow Rate	:	0.8 mL/min
Injection Volume	:	20 µl
Wavelength	:	280 nm
Run Time	:	9.83 minute

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 844-858 www.ijprajournal.com ISSN: 2456-4494

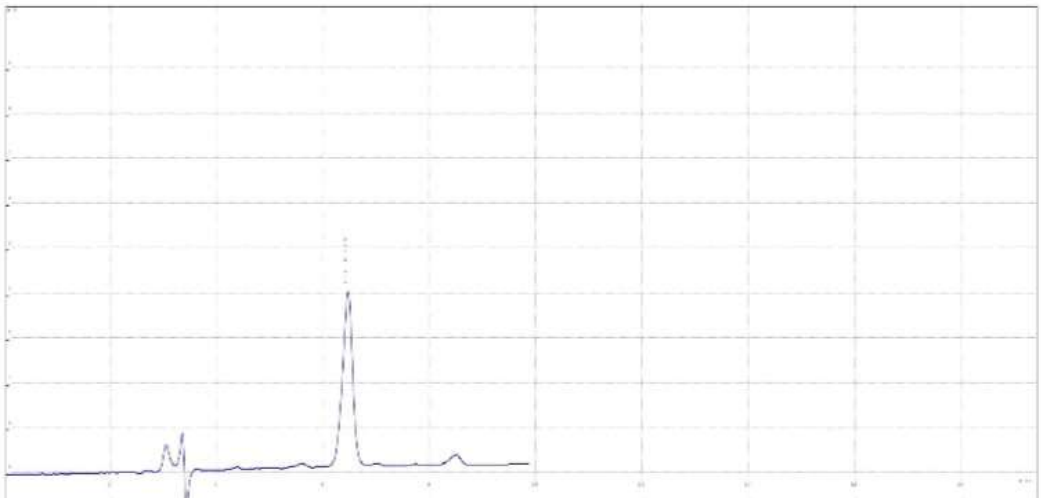

Fig -: Chromatogram of Sofosbuvir Trial-3

Conclusion-
From the above information of sofosbuvir, the peak shape is not proper observed so needs to be optimized.

Trial 4: -Sofosbuvir

Chromatographic Conditions: -

Column	:	CosmosilC18, 250 x 4.6 mm, 5µ
Mobile Phase	:	Methanol: Water (80: 20)
Flow Rate	:	0.8 mL/min
Injection Volume	:	20 µl
Wavelength	:	280 nm
Run Time	:	10.32 minute
Retention Time	:	7.06

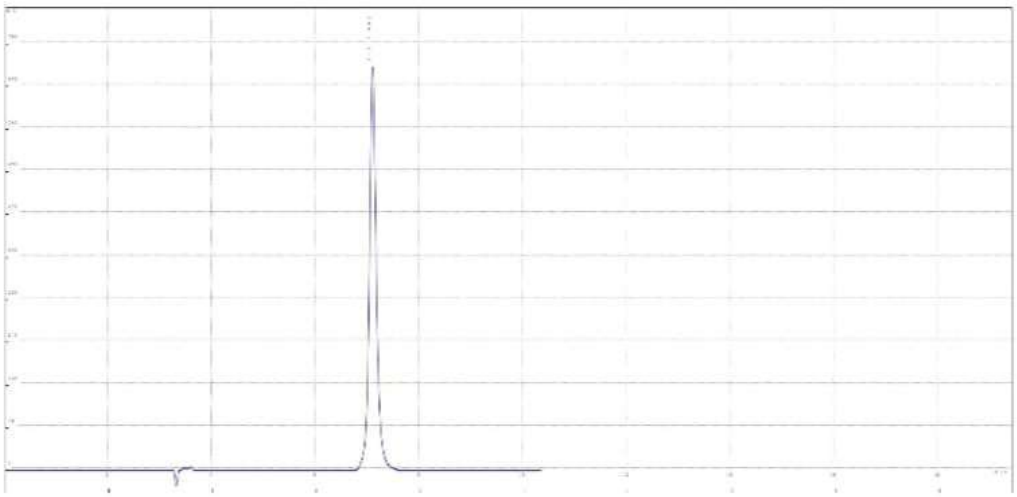

Fig-: Chromatogram of Sofosbuvir Trial-4

Conclusion-
From the above information of sofosbuvir the peak observed at 7.06 min, but reduction of retention time is required method so needs to be optimized.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 844-858 www.ijprajournal.com ISSN: 2456-4494

Trial 5: Combination of Sofosbuvir and Daclatasvir

Chromatographic Conditions: -

Column	:	CosmosilC18, 250 x 4.6 mm, 5µ
Mobile Phase	:	Methanol: Water (85: 15)
Flow Rate	:	0.8 mL/min
Injection Volume	:	20 µL
Wavelength	:	280 nm
Run Time	:	8.53 minute
Retention Time	:	Not observe

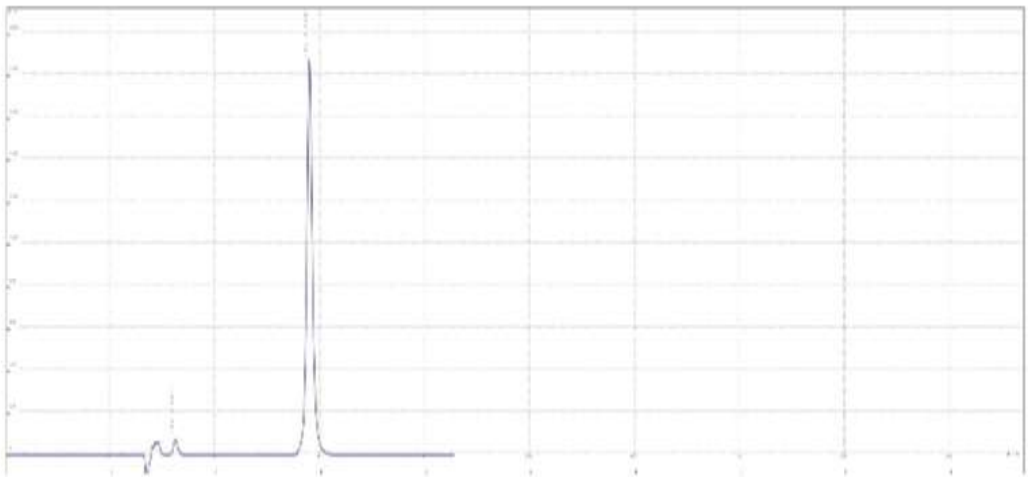

Fig-: Chromatogram of Sofosbuvir and Daclatasvir combination trial-1

Conclusion-

From the above information of sofosbuvir and Daclatasvir the peak shape not observed proper so needs to be optimized.

Trial 6: Combination of Sofosbuvir and Daclatasvir

Chromatographic Conditions: -

Column	:	CosmosilC18, 250 x 4.6 mm, 5µ
Mobile Phase	:	Methanol: Water (80: 20)
Flow Rate	:	0.8 mL/min
Injection Volume	:	20 µL
Wavelength	:	280 nm
Run Time	:	9.13 minute
Retention Time	:	Not observe

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 844-858 www.ijprajournal.com ISSN: 2456-4494

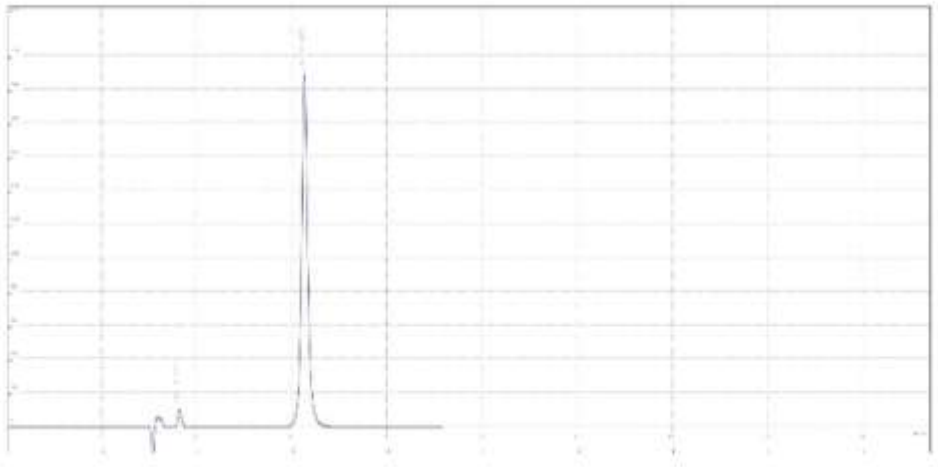

Fig-: Chromatogram of Sofosbuvir and Daclatasvir combination Trial-2

Conclusion-
From the above information of sofosbuvir and Daclatasvir the peak shape not observed proper so needs to be optimized.

Validation Parameters of Sofosbuvir and Daclatasvir System Suitability:
Six replicate injections of sofosbuvir and daclatasvir standard solution were injected, and the chromatograms were recorded. It produced theoretical plate >2000, tailing factor

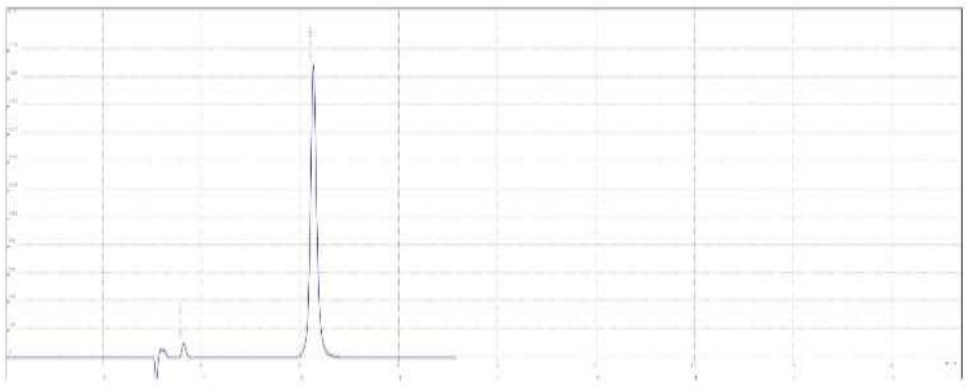

Fig-: Chromatogram of system suitability

Table No7: System suitability of developed method

Parameters	Sofosbuvir	Daclatasvir	Acceptance Criteria
Retention time (min)	6.089	3.925	
Theoretical plates	8884	8797	>2000
Tailing factor	1.10	1.11	<2
Resolution	0.00	5.85	>2

Conclusion: The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable

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Volume 7, Issue 4 July-Aug 2022, pp: 844-858 www.ijprajournal.com ISSN: 2456-4494

Sr.No.	Validation parameters	Validation parameters				Reported observation	
	Assay	Assay data for SOF				99.76%	
		Assay data for DAC				99.91	
1	System suitability	Retention tim				SOF	6.08 mi
						DAC	3.92 min
		Resolution				SOF	0.00
						DAC	5.85
		Theoretical plates NLT 2000				SOF	8364
						DAC	7890
		Tailing/Asymmetry factor NMT 2				SOF	1.26
						DAC	1.18
2	**Linearity**	Correlation coefficient				SOF	0.9993
						DAC	0.9994
		Retention time				SOF	4.32
						DAC	5.47
3	**Accuracy and % recovery**	Acceptable limit is 98-102% Mean sample recovered for that three-sample prepared at different level RSD-NMT 2%					
			50%			**100%**	**150%**
		SOF	99.85			99.90	99.94
		DAC	99.97			100.50	100.19
4	**Precision**						
	Interday precision	RSD-NMT 2%			SOF		0.02%
					DAC		0.0.1%
	Intraday precision	RSD-NMT 2%			SOF		0.02%
					DAC		0.09%

IV. CONCLUSION

- The developed RP-HPLC method was new, simple, sensitive, precise, accurate and robust, for the detection of SOF and DAC in pure and pharmaceutical tablet dosage form according to acceptance criteria.
- The method has wider linear dynamic range with good accuracy and precision.
- The coefficient of correlation was obtained in acceptable range. Hence the method is linear
- The % recovery was obtained in acceptable range.
- The percentage relative standard deviation of main peak area, tailing factor, and theoretical plate is inacceptable range. Hence, it is indicating that

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the proposed method was found to be providing good degree of precision.

- The change in flow rate and wavelength, correlation coefficient was found well within the acceptance criteria. Hence, it is indicating that the method is rugged.
- Variation in flow rate and wavelength composition does not have any effect on the % RSD of standard and assay value. Hence, it is indicating that the method is robust.
- There was no interference of any degraded product with peak of drug.
- Thus, from the above result it is conclude that the developed and validated RPHPLC method outlined is very obvious, affordable, dynamic, low cost, rapid easy to perform with small sample volume and good repeatability.
- It can be adopted for the routine quality control analysis and determination of SOF and DAC because of good resolution of the chromatographic peaks.
- There was no interference of any degraded product with peak of drug.
- Thus, from the above result it is conclude that the developed and validated RP- HPLC method outlined is very obvious, affordable, dynamic, low cost, rapid easy to perform with small sample volume and good repeatability.
- It can be adopted for the routine quality control analysis and determination of SOF and DAC because of good resolution of the chromatographic peaks.

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