INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND DACLATASVIR DRUG PRODUCT BY RP-HPLC METHOD

Benzil Dudekula[1*], Dr.C.Ramachandraiah[2], Dr.N.Devanna[3]

[1]*S.V.Engineering college for women, Tirupati-517507, Chittor (Dt).*
[2]*SKIT, Srikalahasthi-517640.*
[3]*Jawaharlal Nehru Technological University Anantapur, College of Engineering, Anantapur-515002, Andhra Pradesh.*

ARTICLE INFO

Article history
Received 19/06/2017
Available online
30/08/2017

Keywords
Sofosbuvir And Daclatasvir,
Isocratic,
HPLC,
C18,
Trifluoro Acetic Acid,
Methanol,
Acetonitrile And Validation.

ABSTRACT

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*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 and 12-36µg/ml for Daclatasvir.

Corresponding author
Dr. N. Devanna
Department of Chemistry,
Jawaharlal Nehru Technological University Anantapur,
College of Engineering, Anantapur,
Andhra Pradesh, India-515002.
devanna.chemistry@jntua.ac.in
+91-9440580507

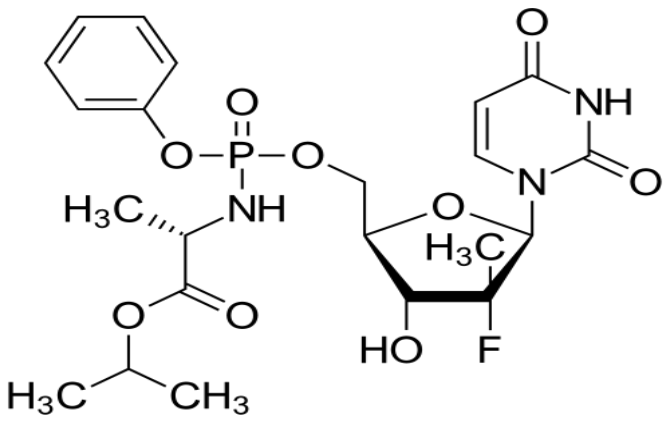
*Please cite this article in press as **Dr. N. Devanna** et al. Analytical Method Development and Validation for The Simultaneous Estimation of Sofosbuvir and Daclatasvir Drug Product by RP-HPLC Method. Indo American Journal of Pharmaceutical Research.2017:7(07).*

INTRODUCTION

SOFOSBUVIR

Sofosbuvir is a medication used for the treatment of hepatitis C. It is only recommended with some combination of ribavirin, peginterferon-alfa, simeprevir, ledipasvir, or daclatasvir. Cure rates are 30 to 97% depending on the type of hepatitis C virus involved. Safety during pregnancy is unclear; while, some of the medications used in combination may result in harm to the baby.It is taken by mouth.

Structure:



Structure of Sofosbuvir.

IUPAC Name: Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate
Molecular formula : C22H29FN3O9P
Molecular Weight : 529.453 g/mol
Solubility: Soluble in Methanol, Acetonitrile and water.
Pka: 9.3

Mechanism of action:

Sofosbuvir inhibits the hepatitis C NS5B protein.[7] Sofosbuvir appears to have a high barrier to the development of resistance.[21]

Sofosbuvir is a prodrug. It is metabolized to the active antiviral agent GS-461203 (2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate). GS-461203 serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis.[22] Although sofosbuvir has a 3' hydroxyl group to act as a nucleophile for an incoming NTP, a similar nucleotide analogue, 2'-deoxy-2'-α-fluoro-β-C-methylcytidine, is proposed to act as a chain terminator because the 2' methyl group of the nucleotide analogue causes a steric clash with an incoming NTP.[23] Sobosbuvir would act in a similar way.

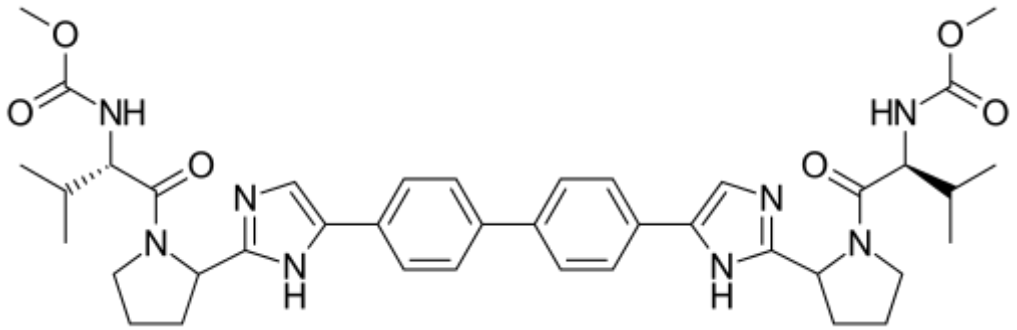
Half Life: Sofosbuvir has a terminal half life of 0.4 hours.

Route of elimination: Sofosbuvir, as a single agent, has very mild toxicity. The most common adverse reactions are headache and fatigue.

DACLATASVIR

Daclatasvir is an NS5A inhibitor which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.

Structure:



Structure of Daclatasvir.

IUPAC Name: Methyl {(2S) – 1 - [(2S,5S) – 2 - (9 - {2- [(2S, 4S) -1 - {(2R) – 2 - [(methoxycarbonyl) amino] – 2 - phenylacetyl} – 4 - (methoxymethyl) – 2 - pyrrolidinyl] - 1H – imidazol – 4 - yl} -1, 11 – dihydroisochromeno [4', 3': 6, 7] naphtha [1, 2 - d] imidazol – 2 - yl) – 5 – methyl – 1 - pyrrolidinyl] – 3 – methyl – 1 – oxo – 2 - butanyl} carbamate
Molecular formula : C49H54N8O8
Molecular Weight : 883.02 g·mol−1
Solubility: Soluble in Water, Methanol and Acetonitrile.
Pka: 3.74
Indication: Used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.
Mechanism of action: The substance blocks NS5A, a protein necessary for hepatitis C virus replication and assembly

EXPERIMENTAL:
Equipments:

The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (XTerra RP18 150*40.6,5um) as stationary phase ,Ultrasonic cleaner, Scaletech analytical balance ,Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

MATERIALS:

Pharmaceutically pure sample of Sofosbuvir and Daclatasvir were obtained as gift samples from Fortune pharma training institute, sri sai nagar, KPHB and Hyderabad, India.
HPLC-grade Acetonitrile was from qualigens reagents pvt ltd. Trifluoro acetic acid (AR grade) was from sd fine chem.

Chromatographic conditions

The sample separation was achieved on a C18 (XTerra RP18 150*40.6, 5um) column, aided by mobile phase mixture 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, that was filtered and degassed prior to use, Injection volume is 20 µl and ambient temperatures.

Preparation of mobile phase:
Buffer Preparation: Take accurately 1ml of Trifluoro acetic acid in 1000mL of water
Mobile phase: Then add 60 volumes of buffer and 40 volumes of Acetonitrile mixed well and sonicated for 10 min.
Diluent: water: Acetonitrile: 50:50 v\v

Preparation of standard stock solution:

A 40mg of pure Sofosbuvir and 6mg of Daclatasvir were weighed and transferred into 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution containing 800µg/ml of Sofosbuvir and 120 µg/ml of Daclatasvir. From the above solution 2ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 160µg/ml of Sofosbuvir and 24 µg/ml of Daclatasvir

Preparation of sample solution:

Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 400 mg of Sofosbuvir and 60mg of Daclatasvir sample were weighed and transferred to 100 ml of volumetric flask and dissolved in diluents. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 4 ml of solution is pipette out into a 100 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 160µg/ml of Sofosbuvir and 24 µg/ml of Daclatasvir .

RESULTS AND DISCUSSIONS

Determination Of Working Wavelength (λ max): 10 mg of the Sofosbuvir and Daclatasvir standard drug is taken in a 10 ml volumetric flask and dissolved in Acetonitrile and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the Acetonitrile to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using Acetonitrile as blank. The λmax was found to be 275nm

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Trifluoro acetic acid in water: Acetonitrile (60:40). The flow rate was 1.0 ml/ minute brought sharp peaks. The chromatogram was shown in Figure-1.

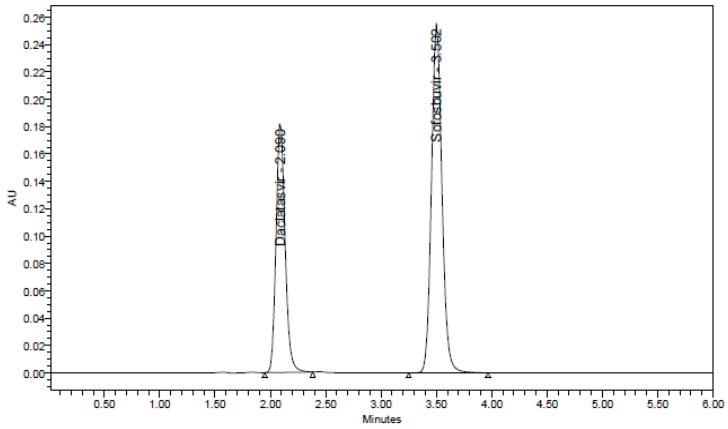


Figure: 1 Chromatogram of Daclatasvir and Sofosbuvir.

METHOD VALIDATION:

Linearity:

Linearity was studied by analyzing five standard solutions covering the range of 80-240 µg/ml for Sofosbuvir and 12-36µg/ml for Daclatasvir. From the primary stock solution 1.0ml,1.5ml,2.0ml,2.5ml,3.0 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 80µg/mL , 120µg/mL ,160µg/mL ,200µg/mL and 240 µg/mL of Sofosbuvir and 12µg/mL , 18µg/mL ,24µg/mL ,30µg/mL and 36 µg/mL Daclatasvir. Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Table No: 1 Linearity data for Sofosbuvir.

S.No	level	Area
1.	50	832458
2.	75	1275312
3.	100	1668126
4.	125	2092030
5.	150	2534297
Correlation coefficient		0.9998

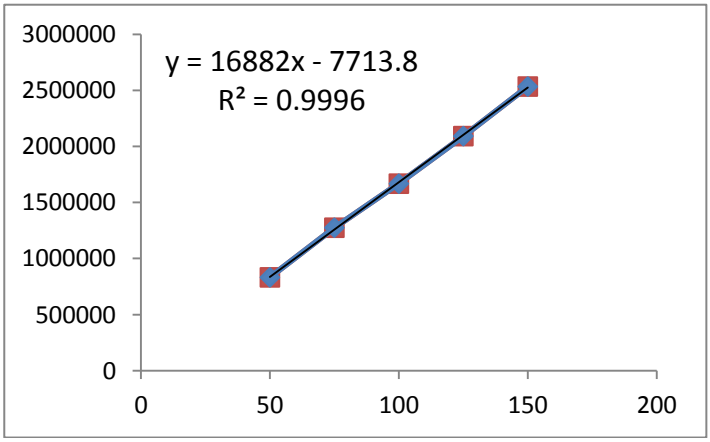


Figure -2: Linearity plot of Sofosbuvir.

Table No: 2: Linearity data for Daclatasvir.

S.No	level	Area
1.	50	481299
2.	75	773883
3.	100	1042877
4.	125	1315666
5.	150	1591168
Correlation coefficient		0.9999

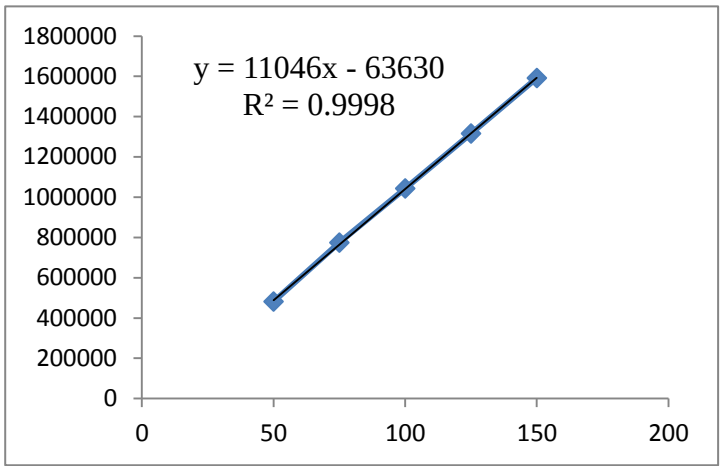


Figure No.3: Linearity plot of Daclatasvir.

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

LOD = 3.3 δ/S ……. (1)
LOQ =10 δ/S …….. (2)

Where,

σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Table no.3: LOD and LOQ values Calculated from calibration curve:

	SOFOSBUVIR (mg)	DACLATASVIR
LOD	0.005	0.001
LOQ	0.014	0.003

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 160 µg/ml of SOFOSBUVIR and 24 µg/ml DACLATASVIR without changing the parameter of the proposed chromatographic method.

Table.4: Summary of peak areas for method precision for Sofosbuvir.

Sample No	Retention time	Peak area	% Assay
1	3.499	1686766	99.9
2	3.499	1677924	99.2
3	3.501	1687828	99.2
4	3.501	1679039	99.7
5	3.499	1674492	99.2
6	3.499	1682158	99.3
Mean			99.4
%RSD			0.31

Table.5*:* Summary of peak areas for method precision for Daclatasvir.

Sample No	Retention time	Peak area	% Assay
1	2.083	1035922	99.2
2	2.081	1034144	100.0
3	2.083	1022382	98.9
4	2.082	1038818	99.9
5	2.079	1046773	100.0
6	2.080	1048278	99.6
Mean			99.6
%RSD			0.48

Accuracy (recovery study):

The accuracy of the method was determined by calculating the recoveries of Sofosbuvir and Daclatasvir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Sofosbuvir and Daclatasvir. The percentage recovery results obtained are listed in Table 6

Table No.6: Recovery data for Sofosbuvir.

S.NO	Accuracy level	injection	Sample area
1	50%	1	99.4
		2	99.2
		3	99.3
2	100%	1	99.9
		2	99.2
		3	99.2
		1	99.1
3	150%	2	99.3
		3	98.8

Table No.7: Recovery data for Daclatasvir.

S.NO	Accuracy level	injection	Sample area
1	50%	1	99.7
		2	99.5
		3	99.9
		1	99.2
2	100%	2	100.0
		3	98.9
		1	99.0
3	150%	2	98.9
		3	99.5

Robustness:

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table no.8).

Table No.8: Results of Robustness data for Sofosbuvir.

parameter	RT	Area
Decreased flow rate (0.9ml/min)	4.337	2098358
Increased flow rate (1.1ml/min)	2.923	1383875

Table No.9: Results of Robustness data for Daclatasvir.

parameter	**RT**	**Area**
Decreased flow rate(0.9ml/min)	2.563	1290171
Increased flow rate(1.1ml/min)	1.738	852316

Table No.10: Validation parameters of evaluated method SOFOSBUVIR:

S.NO	**PAAMETER**	**RESULT**	**ACCEPTENCE CRITERIA**
1	System suitability		
	Theoretical plates	6613	Not less than 2000
	Asymmetry	1.11	Not more than 2
	Retention time	3.502	
	%RSD	0.08	Not more than 2
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.31	Not more than 2.0%
4	Linearity parameter	80-240 mcg/ml	
	Slope		
	Intercept		
	Correlation coefficient(r^2)	0.9998	Not less than 0.990
5	Accuracy		
	(Mean % recovery)		97 - 103%
	50%	99.3	
	100%	99.4	
	150%	99.0	
6	Robustness	All the system suitability parameters are within the limits.	

*RSD = Relative standard deviation.

Table No.11: Validation parameters of evaluated method of DACLATASVIR:

S.NO	**PAAMETER**	**RESULT**	**ACCEPTENCE CRITERIA**
1	System suitability		
	Theoretical plates	3226	Not less than 1000
	Asymmetry	1.15	Not more than 2
	Retention time	2.089	
	%RSD	0.27	Not more than 2
2	Specificity	Specific	Specific
3	Method precision (%RSD)	0.48	Not more than 2.0%
4	Linearity parameter	12-36 mcg/ml	
	Slope		
	Intercept		
	Correlation coefficient(r^2)	0.9999	Not less than 0.990
5	Accuracy		97 - 103%
	(Mean % recovery)		
	50%	99.7	
	100%	99.4	
	150%	99.2	
6	Robustness	All the system suitability parameters are within the limits.	

CONCLUSION

The above experimental results and parameters values it was concluded that, this newly developed method for the simultaneous estimation of SOFOSBUVIR AND DACLATASVIR was found to be simple, reproduce, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

REFERENCES:

1. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
2. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
3. http://www.ich.org/
4. Development and validation of RP- HPLC method for determination of Sofosbuvir and Daclatasvir in pharmaceutical dosage formulation Eena Joshy, Anu Babu, Delma D'cruz and Aneesh T. P.
5. http://www.irjponline.com/admin/php/uploads/1029_pdf.pdf
6. http://www.ncbi.nlm.nih.gov/pubmed/20502563
7. http://en.wikipedia.org/wiki/Sofosbuvir and Daclatasvir
8. https://www.drugbank.ca/drugs/DB08934
9. RP-HPLC Method for Simultaneous Determination of Sofosbuvir and Ledipasvir in Tablet Dosage Form and Its Application to In Vitro Dissolution Studies
10. A NEW VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF SIMEPREVIR AND SOFOSBUVIR IN PHARMACEUTICAL DOSAGE FORM
11. http://www.ijpsonline.com/article.asp?issn=0250-474X;year=2009;volume=71;issue=4;spage=477;epage=480;aulast=Patel
12. METHOD DEVELOPMENT AND VALIDATION OF ULTRAVIOLET-VISIBLE SPECTROSCOPIC METHOD FOR THE ESTIMATION OF HEPATITIS-C DRUGS - DACLATASVIR AND SOFOSBUVIR IN ACTIVE PHARMACEUTICAL INGREDIENT FORM

5487847845**1170632**