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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANIOUS ESTIMATION OF SOFOSBUVIR AND DACLATASVIR IN BULK AND PHARMACEUTICAL FORMULATION BY RP- HPLC

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ABSTRACT

A simple, Accurate, precise technique was developed for the simultaneous estimation of the Sofosbuvir and Daclatasvir in Tablet dosage form. Chromatogram was run through Std Ascentis C18 150 x 4.6 mm, 5µ. Mobile phase containing Acetonitrile: Water taken in the proportions 60:40 was pumped through column at a flow rate of 0.7ml/min. Temperature was kept up at 30°C. Optimized wavelength selected was 279nm. Retention time of Sofosbuvir and Daclatasvir were observed to be 2.198 min and 2.765 min. %RSD of the Sofosbuvir and Daclatasvir were and observed to be 0.4 and 0.3 respectively. %Recovery was obtained as 99.88% and 99.80% for Sofosbuvir and Daclatasvir respectively. LOD, LOQ values obtained from regression equations of Sofosbuvir and Daclatasvir were 1.73, 5.23 and 0.12, 0.36 respectively. Regression equation of Sofosbuvir is y = 9010x + 21702, and y = 10136x + 1757 of Daclatasvir Retention times were decreased and that run time was decreased, so the technique developed was simple and conservative that can be embraced in regular Quality control test in Industries.

INTRODUCTION

Sofosbuvir and daclatasvir is a direct acting antiviral medication used as a part of combination therapy to treat chronic hepatitis C. It is an infectious liver disease caused by infection with hepatisis C virus. HCV is a single standard RNA virus that is chategorised into nine distinict genotypes. Sofosbuvir and daclatasvir inhibits the HCV viral RNA replication and protein translation. Sosbuvir and other direct acting antiviral are therefore very potent options for the treatment of hepatitis C, as they exhibit high barrier to the development of resistance. Daclatasvir antiviral action by preventing RNA replication and virion assembly via binding to NS5A, a non structural phosphoprotein encoded by HCV.

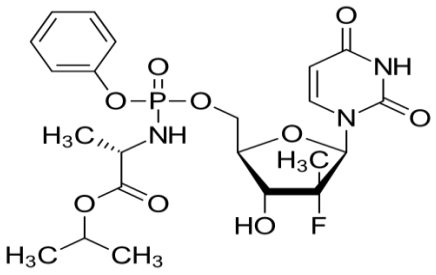

Fig 1: Structure of sofosbuvir

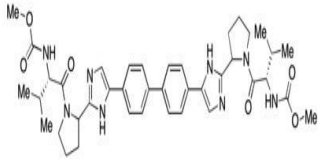

Fig 2: Structure of daclatasvir

MAREIALS AND METHODS:

Preparation of buffer:

0.1%OPA Buffer: Take 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Preparation of mobile phase: Mix a mixture of above buffer 40ml (40%) and acetonitrile 60ml (HPLC grade 60%)and degassed in ultrasonic water bath in 5min.

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Stock solution:

Preparation of Standard stock solutions: Accurately weighed 40 mg of Sofosbuvir, 6mg of Daclatasvir and transferred to 25ml volumetric flask and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1600µg/ml of Sofosbuvir and 240µg/ml of Daclatasvir)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (4000µg/ml of Sofosbuvir and 600µg/ml of Daclatasvir)

Working solution:

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (160µg/ml Sofosbuvir of and 24µg/ml of Daclatasvir)

Preparation of Sample working solutions (100% solution): 0.4ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (160µg/ml of Sofosbuvir and 24µg/ml of Daclatasvir)

Procedure: 10µL of the blank, standard and sample were injected into the chromatographic system and areas for the sofosbuvir and dalatasvir the peaks were used for calculating the % assay by using the for the formula.

Results and discussion: method validation: specificity, linearity range, accuracy, precision, repeatability, intermediate precision, limit of detection, limit of quatification, rubustness.

Specificity: The system suitability for specificity was carried out to determine whether there is an interference of any impurities in retention time of analytical peak. the specificity study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak.

LINEARITY: To establish the linearity of the method, serial dilution were prepared to obtain the mixture of sofosbuvir and daclatasvir ranging from 40ppm to 240ppm and 6ppm to 36ppm level all the solutions were filtered through a 0.45µm Millipore filters.the final solution were injected in duplicate manner keeping the injection volume 10µl.Calibration curve was plotted between mean peak area and concentration. The correlation coefficient and slope were determined from the calibration curve. The linearity chats of sofosbuvir and daclatasvir was shown in figure no. 5&6. The correlation coefficient was found to be 0.999 for both drugs and hence the method was set to be linear .The were tabulated in table 1.

ACCURACY: Accuracy was evaluated by standard addition method of three known concentration of the drug and the spiked solution were analysed. The recovery of the added drug was determined by calculating the pre-analysed drug concentration with concentration of spiked drug. The % recovery was calculated and the result was reported in table no. 2 &3.

PRECISION: The precision of the analytical method was studied by injecting six replicates of standard and sample concentration on the same day and another day. The concentration sofosbuvir and daclatasvir ranging from 25µg/ml to 125µg/ml at two levels intra and inter day precision. The %RSD was calculated and results were reported and table no. 4&5.

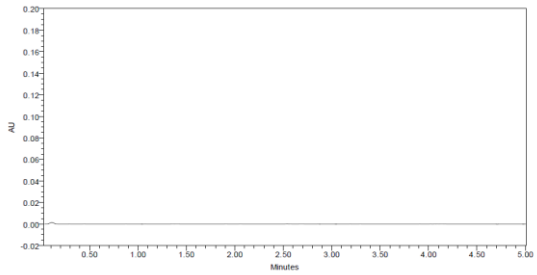

Fig. No. 3: Chromatogram showing Blank

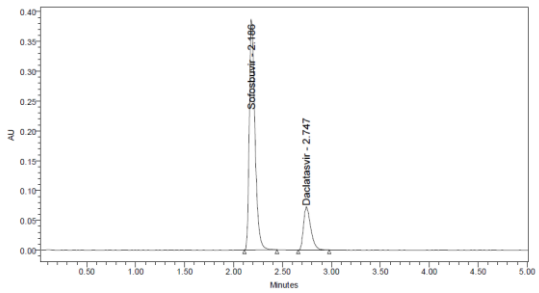

Fig. No. 4: Chromatogram showing optimized condition

Table 1: Linearity results for Sofosbuvir and Daclatasvir

Sofosbuvir		Daclatasvir	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
40	383540	6	61890
80	758279	12	123758
120	1112232	18	185457
160	1474488	24	247506
200	1833235	30	309513
240	2158526	36	361259

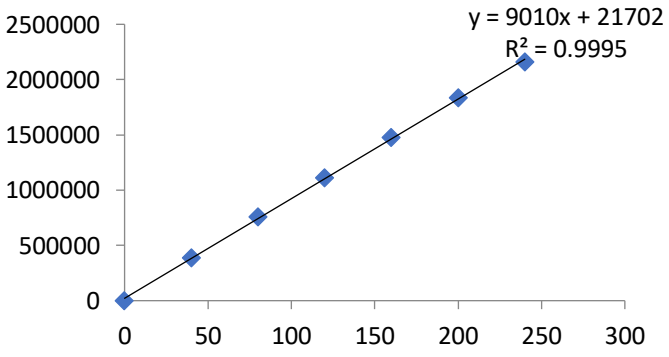

Fig. No. 5: Showing Calibration curve of Sofosbuvir

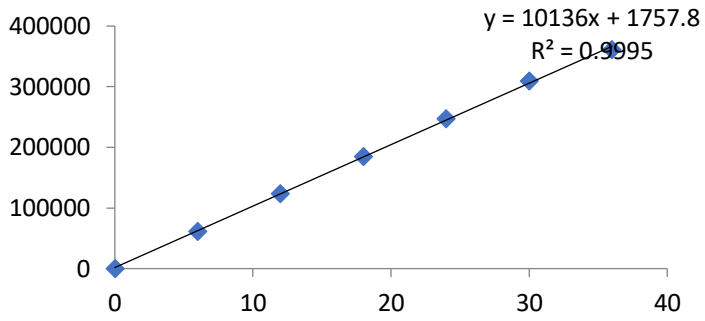

Fig. No. 6: Showing Calibration curve of Daclatasvir

Table 2 : Accuracy results for Sofosbuvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	80	79.91554	99.89	99.88%
100%	160	159.7218	99.82	
150%	240	239.787	99.91	

Table 3: Accuracy results for Daclatasvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12	11.99332	99.94	99.80%
100%	24	23.91667	99.65	
150%	36	35.92847	99.80	

Table 4: System precision results for Sofosbuvir and Daclatasvir

S. No	Area of Sofosbuvir	Area of Daclatasvir
1.	1042454	247496
2.	1061619	248687
3.	1050784	251651
4.	1062308	247323
5.	1059490	244217
6.	1049332	245659
Mean	1054331	247506
S.D	8024.9	2562.7
%RSD	0.8	1.0

Table 5: Intermediate precision results for Sofosbuvir and Daclatasvir

S. No	Area of Sofosbuvir	Area of Daclatasvir
1.	1033258	245258
2.	1042166	248698
3.	1043353	247744
4.	1022053	244606
5.	1049003	247757
6.	1026202	248146
Mean	1036006	247035
S.D	10575.5	1678.2
%RSD	1.0	0.7

Table 6: LOD & LOQ results for Sofosbuvir and Daclatasvir

Molecule	LOD	LOQ
Sofosbuvir	1.73	5.23
Daclatasvir	0.12	0.36

Table 7: Robustness data for Sofosbuvir and Daclatasvir

S.no	Condition	%RSD of Sofosbuvir	%RSD of Daclatasvir
1	Flow rate (-) 0.55ml/min	0.8	0.8
2	Flow rate (+) 0.65ml/min	1.0	1.6
3	Mobile phase (-) 55B:45A	0.7	1.7
4	Mobile phase (+) 45B:55A	0.9	0.9
5	Temperature (-) 25°C	0.8	1.8
6	Temperature (+) 35°C	0.2	0.7

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentration of the drug. The LOD was defined as the concentration which yields a signal-to-noise ratio 3:1 while the LOQ was calculated to be the lowest concentration that could be measured with signal-to-noise ratio 10:1. The LOD & LOQ were calculated by measuring the standard deviation of the response and slope. The result of LOD &LOQ was tabulated in table no. 6.

ROBUSTNESS: The small deliberate changes in method like flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within the range as per ICH guide lines. Robustness condition like flow minus (0.8ml/min), flow plus (1.3ml/min), mobile phase minus, mobile phase plus, temperature minus (25^{o}c), temperature plus (35^{o}c) was maintain and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed %RSD was found to be within the limits and results were tabulated in table no. 7.

CONCLUSION:

The proposed stability indicating RP-HPLC method was found to be simple, accurate, precise, robust, and rapid. This method gives good resolution between two compounds with a short analysis time. Hence this method can be used in quality control departments with respect to routine analysis for the assay of the tablets containing sofosbuvir and daclatasvir.

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