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METHOD DEVELOPMENT AND VALIDATION OF DACLATASVIR TABLET DOSAGE FROM

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ABSTRACT

A Novel simple, precise and economical high performance liquid chromatographic method has been developed and validated for the analysis of antiviral drug Daclatasvir in pure form and in tablet dosage form as well. The chromatographic analysis was performed on HPLC 30000 series analytical technologies ltd. Detector UV 3000 M. Column- cosmosil C18 (250mm*4.6ID, particle size: 5micron) Mobile phase methanol:water (60:40) detection wavelength 230nm, flow rate 1ml/min, Temperature – ambient, Sample size-5.0µg were selected to develop an accurate method. The flow rate of mobile phase was maintained at 1ml/min and the response was monitored at 230nm with a run time of 10 min. the volume. The volume of injection loop was20µl.The developed method was validated as per ICH guidelines; it was precise, accurate and robust. The calibration curve of Daclatasvir was linear in range og 10-50µg/ ml with a correlation coefficient> 0.997.

KEYWORD: Daclatasvir, HPLC, Method development., Antiviral drug.

INTRODUCTION

Daclatasvir is the first class direct acting antiviral agent which binds to and inhibits the function of the HCV protein NS5A.HCV prevalence varies greatly, but the highest prevalence (15-20%) has been reported from Egypt. The goal of antiviral therapy against HCV is to reach sustained virological response (SVR), which is traditionally defined as the absence of quantifiable virus in plasma at least 24 weeks after the end of therapy; however most replaces occur within 4 weeks of the treatment discontinuation, and a 98-99% concordance has been shown between absence of quantifiable virus 12 weeks after therapy and SVR24.

The chemical name of Daclatasvir hydrochloride is Methyl[(2S)-1-{(2S)-2-[4-(4'-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl) amino]-3-methylbutanoyl}-2-pyrrolidinyl]-1H-imidazol-4-yl}-4-biphenylyl)-1H-imidazol-2-yl]-1-pyrrolidinyl}-3-methyl-1-oxo-2-butanyl]carbamate is one of the highly potent and selective DAAs of HCV non- structural (NS) proteins few methods were reported for Daclatasvir determination such as LC-MS/MS.

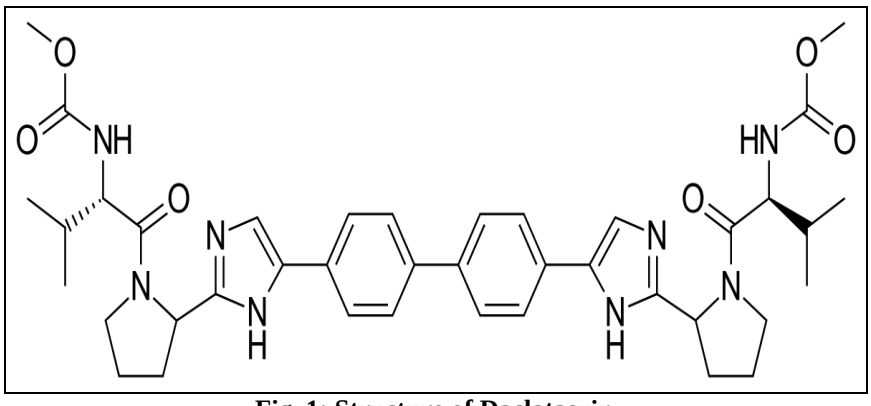

Fig. 1: Structure of Daclatasvir.

Experimental

Instrumentation

HPLC 30000 series analytical technologies ltd. Detector UV 3000 M. Column- cosmosil C18 (250mm*4.6ID, particle size: 5micron).

Material and chemical reagent
Material: HPLC
List of chemical

Sr. No.	Chemicals	Source
1.	Daclatasvir	**Natco pharmaceuticals**
2	Unhydrous lactose	Sd fine lab Mumbai
3	MCC	Sd fine lab Mumbai
4	Croscarmelose sodium	Ozone chemicals mumbai
5	Silicon dioxide	Sd fine lab Mumbai
6	Magnessium stearate	Sd fine lab Mumbai

Method Development
Preparation of Standard Stock Solution
Daclatasvir HCL standard stock solution: (100 µg/ml) A 100 mg of Daclatasvir HCL standard was weighed and transferred to a 100 ml volumetric flask. 50 ml of methanol was transferred to this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml Daclatasvir HCL. From this solution 10 ml was transfer to 100 ml volumetric flask. The volume was adjusted to the mark with the Ethanol to give a solution containing 100µg/ml Daclatasvir HCL.

Preparation of formulated tablet sample solution
Twenty tablets were weighed and finely powered. Powder equivalent to 40 mg Daclatasvir HCL was accurately weighed and transferred to volumetric flask of 100 ml capacity. 50 ml of methanol was transferred to this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol. The solution was filtered through whatmann filter paper (0.45µ). From this solution 1ml was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark to give a solution 400 µg/ml Daclatasvir HCL (Solution A). From the solution A 2.5 ml was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark to give a solution containing 10 µg/ml Daclatasvir HCL (Solution 1).

Chromatographic Conditions
The mobile phase consisting of methanol: Water in the ratio of 60:40 *v/v*, was filtered through 0.45µ membrane filter, sonicated and was pumped from the solvent reservoir. The flow rate of mobile phase was maintained at 1ml/min and the response was monitored at 230 nm with a run time of 10min. The volume of injection loop was 20µl.The column and the HPLC systems were kept at ambient temperature.

Method Validation
Specificity
The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.997, indicating the non interference of any other peak of degradation product, formulation excipients or impurity.

Linearity and Range
Linearity was tested for the range of concentrations 10-50µg/ml. Each sample in five replicates was analyzed and peak areas were recorded. Response factor was calculated by taking the ratio of mean peak area of Daclatasvir HCL. Table No. 15 represents the response factor of Daclatasvir HCL. The response factors were plotted against the corresponding concentrations to obtain the calibration curve. Figure No: 10 represents the chromatogram of linearity and calibration curve for Daclatasvir HCL respectively.

Accuracy
To check accuracy of the method, recovery studies were carried out by preparing sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 10µg/ml of Daclatasvir HCL standard solution. These solutions were injected to obtain the chromatogram. The drug concentrations were calculated by using linearity equation of Daclatasvir HCL. The results obtained are shown in Table No:16

Precision
The intra-day precision study of Daclatasvir HCL was carried out by estimating the peak responses six times on the same day with15µg/ml concentration and inter-day precision study of Daclatasvir HCL was carried out by estimating the peak responses six times on different days with 10 µg/ml concentration and % RSD value obtained was calculated to determine method precision. The results obtained are shown in Table No:17 and 18

Robustness
The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, mobile phase composition and pH and its impact on peak area were studied.

Limit of detection and quantification (LOD and LOQ)
From the linearity data the limit of detection and Quantitation was calculated, using the following formula.
LOD= 3.3 σ/ S and
LOQ = 10 σ/ S
Where,
σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

RESULT AND DISCUSSION

Validation of daclatasvir

Chromatographic Parameters

Sample name	Daclatasvir
Mobile phase	Methanol:Water (60:40) pH3
Flow rate	0.8ml/min
wavelength	316nm
Samle volume	20µl
Pressure	10-11MPa
Run time	6.19min

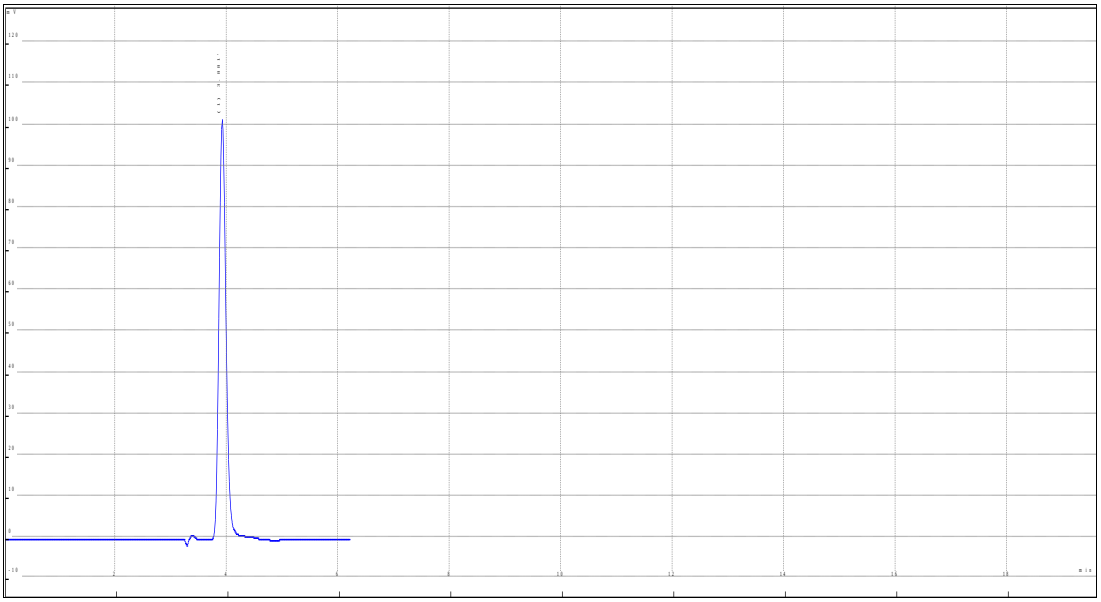

Figure 1: Chromatogram of Daclatasvir using MeOH 60%:40%v/v (pH3).

OBSERVATION

By using above method, It is found that Daclatasvir is having good peaks, minimum tailing, good retention time, resolution time between peak were sufficient. Hence this method was suitable

System Suitability Test

Table 1: System Suitability Test for Daclatasvir.

Sr. No	Concentration (µg/ml)	Peak area	Amount found	% Amount found
1	30	1525196	29.86	99.53
2	30	1520122	29.97	99.9
Mean			29.915	99.715
SD			0.077781746	0.26163
%RSD			0.260009179	0.26238

Observation

All the parameters of system suitability were observed within the limits for Daclatasvir.

Table 2: Standard Calibration curves of Daclatasvir.

Sr. No.	Conc.	Peak Area
1	10.00	549697
2	20.0	1019638

3	30.0	1525196
4	40.0	2019341
5	50.0	2467705
Slope	48357	
Intercept	65600	
Correlative Coefficient (r2)	0.999	

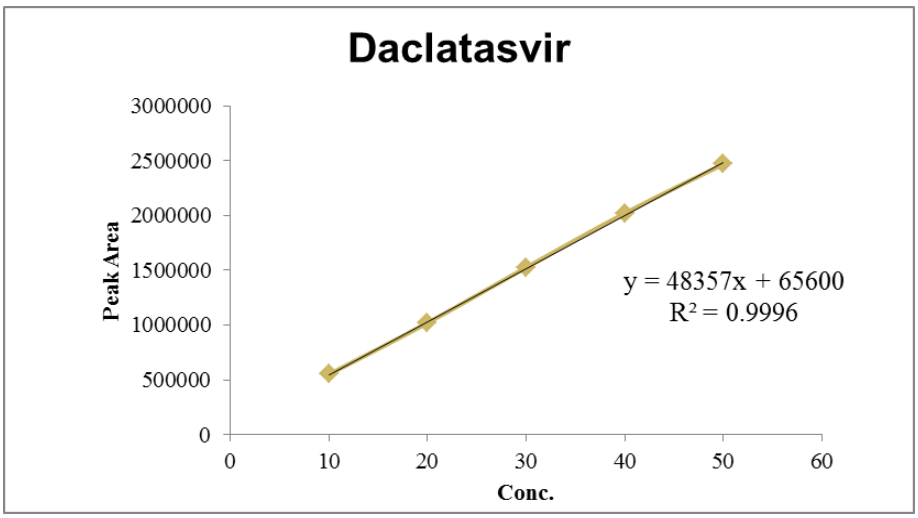

Figure 2: Standard calibration Curve of Daclatasvir.

In both calibration curves the $\mathbf{r^2}$ & the regression equation (**y**) for **Daclatasvir** were calculated and shown in above Figure. It indicates the capability of developed method to estimate both the drugs over the desired concentration ran.

Table 3: Linearity of Daclatasvir.

Sr. No.	Conc.	Area
1	10	549697
2	20	1019638
3	30	1525196
4	40	2019341
5	50	2467705

Table 4: Determination of Accuracy (% Recovery).

			Standard Deviation		Accuracy	Precision
Conc.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
	10	549697				
1	10	548053	550402.6	2770.73	0.5034	0.5034
	10	553458				
	30	1525196				
2	30	1528109	1528197	3045.95	0.1993	0.1993
	30	1531286				
	50	2467705				
3	50	2470877	2471569	4252.44	0.1720	0.1720
	50	2476125				

Table determination of recovery study.

Sr. No.	Composition	Area of standard	Area of sample	% Recovery
1	50	1525196	1524870	99.97%
2	100	2019341	2022637	100.16%
3	150	2467705	2458478	99.62%

Table 5: Precision Interday Data For Daclatasvir At 230 nm.

Conc.(µg/ml)	Interday		Mean	S.D.	%RSD
	Morning	Evening			
30	1525196	1521084	1523140	2907.623	0.190897
30	1528109	1530168	1529139	1455.933	0.095213
30	1531286	1529326	1530306	1385.929	0.090566

Table 6: Precision Intraday Data For Daclatasvir At 230 nm.

Conc.(µg/ml)	Intraday		Mean	S.D.	%RSD
	Day 1	Day 2			
30	1525196	1528714	1526955	2487.602	0.162913
30	1528109	1525298	1526704	1987.677	0.130194
30	1531286	1530203	1530745	765.7966	0.050028

Table 7: Ruggedness Data for Daclatasvir At 230 nm (30µg/ml).

Instrument 1	Instrument 2	Result of t test*	Inference
1525196	**1525220**	0.18960408	Not significant difference
1528409	1528714		
1521084	1525298		

ROBUSTNESS

Condition 1. Change in flow rate

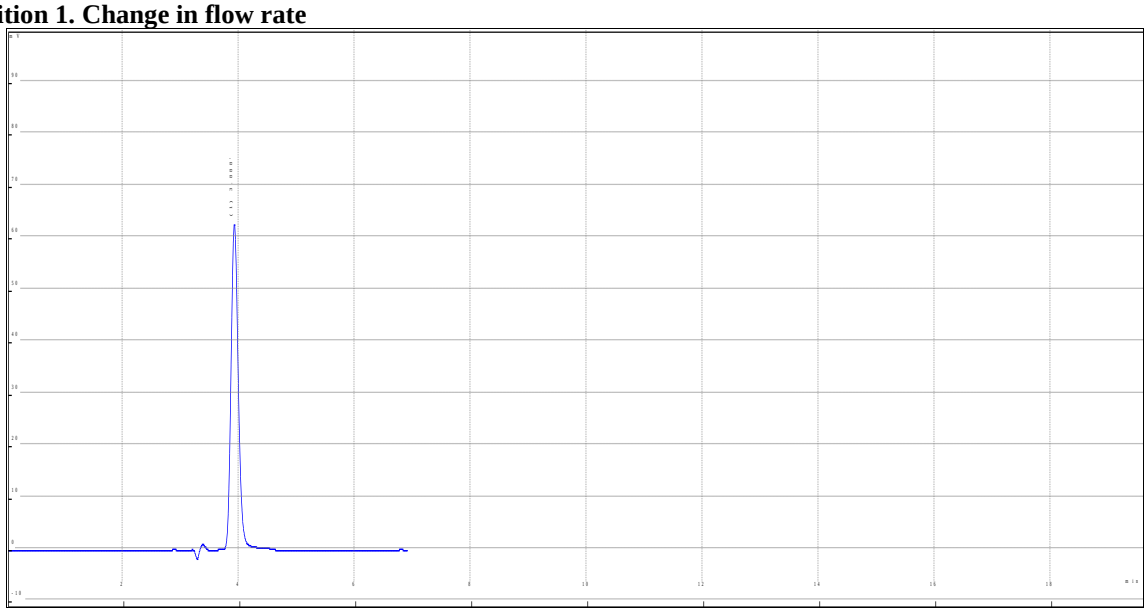

Figure 3: Chromatogram of Robustness flow change for 0.7ml.

Table 8: Results of robustness (For 0.7 mL).

Sr. no	Conc (µg/ml)	Peak Area
1	20	1012596
2	20	1012565
Mean		1012580.5
SD		21.9203102
% RSD		0.0021648

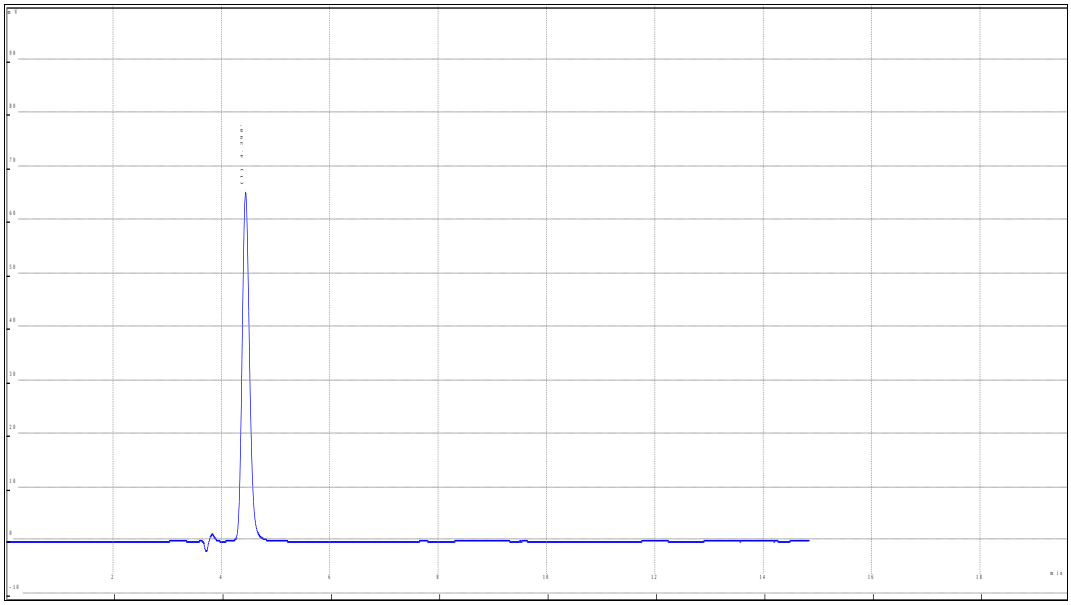

Figure 4: Chromatogram of Robustness flow change for 0.8ml.

Table 9: Results of robustness (For 0.8 mL).

Sr. no	Conc (µg/ml)	Peak Area
1	20	1019638
2	20	1019620
Mean		1019629
SD		12.7279221
% RSD		0.00124829

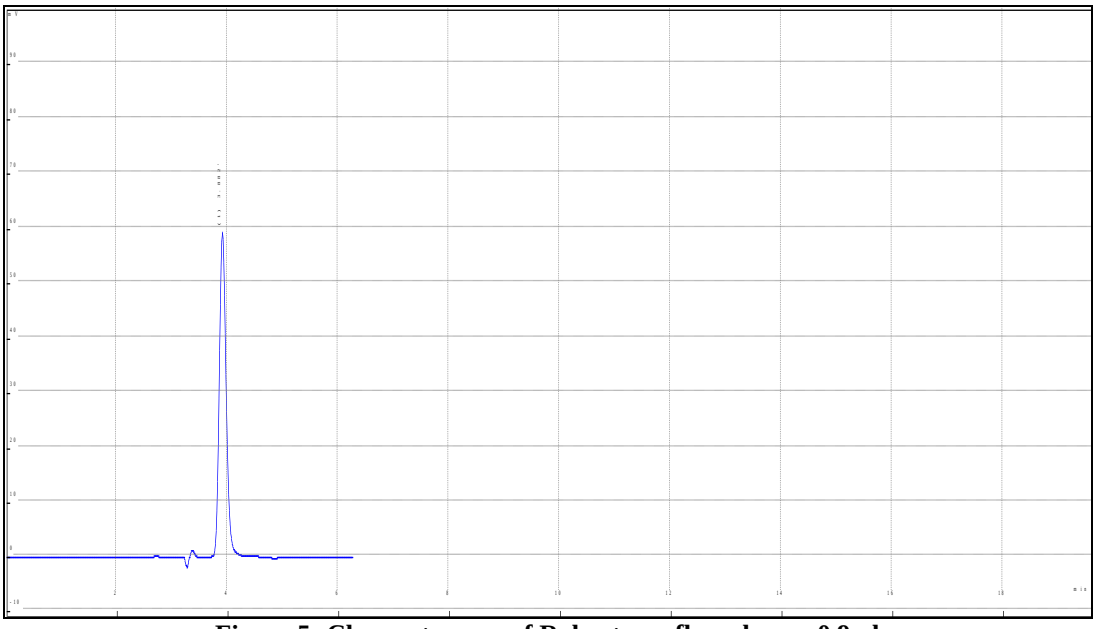

Figure 5: Chromatogram of Robustness flow change 0.9ml

Table 10: Result of Robustness (for 0.9 ml).

Sr. no	Conc (µg/ml)	Peak Area
1	20	1019638
2	20	1019665
Mean		1019651.5
SD		19.0918831
% RSD		0.00187239

Change in wavelength

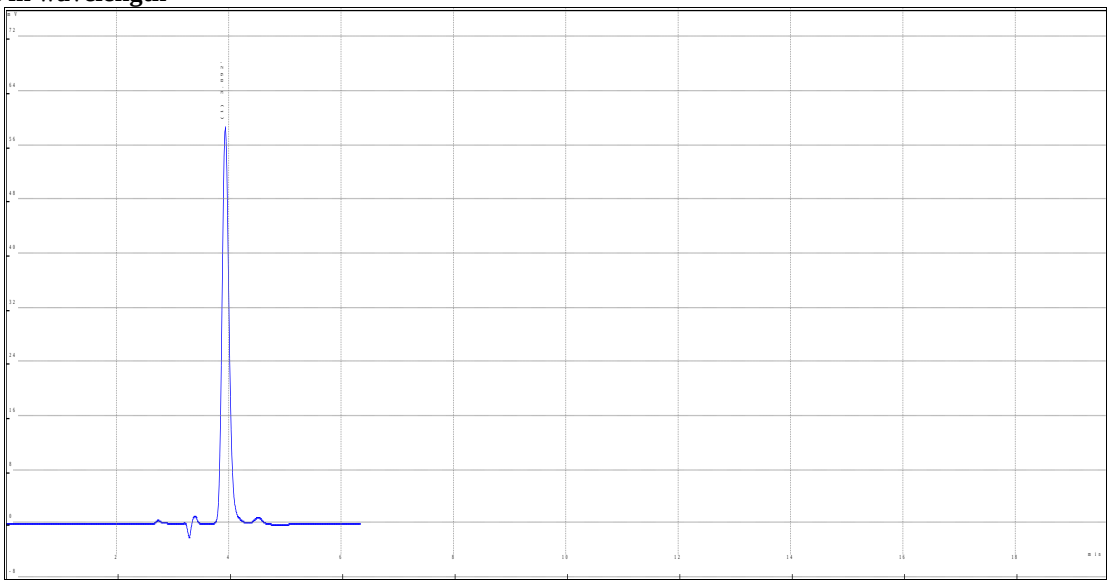

Figure 6: Chromatogram of Robustness change in wavelength at 314nm.

Table 11: Results of Robutness for chane in wavelength at 314nm.

Sr. no	Conc (µg/ml)	Peak Area
1	20	1024738
2	20	1024701
Mean		1024719.5
SD		26.1629509
% RSD		0.00255318

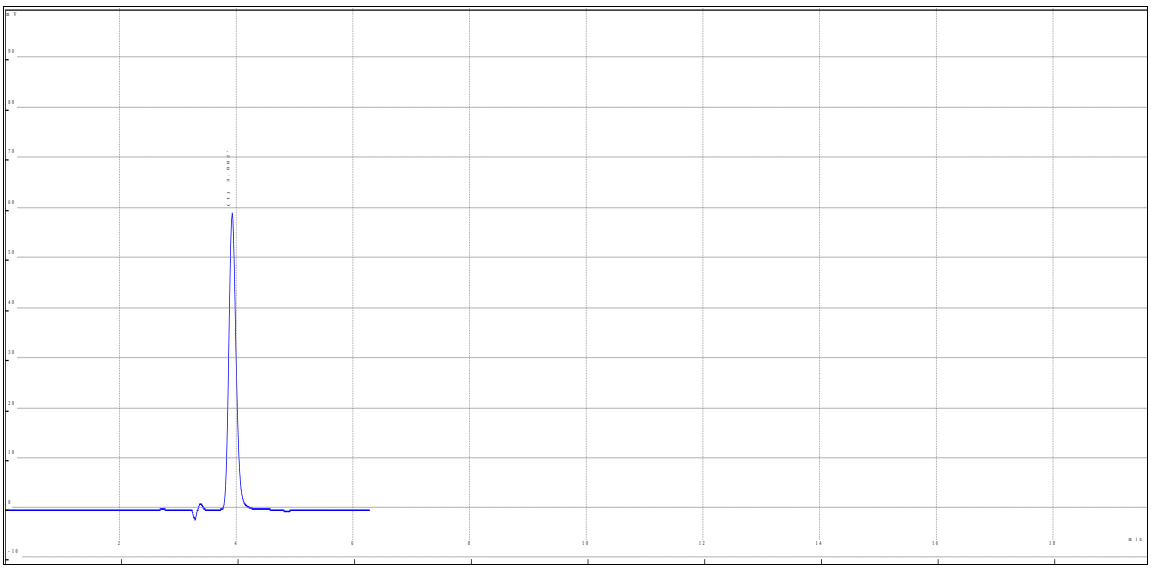

Figure 7: Chromatogram of Robustness change in wavelength 316.

Table 12: Result of Robustness for change in wavelength at 316nm.

Sr. no	Conc (µg/ml)	Peak Area
1	20	1019638
2	20	1019669
Mean		1019653.5
SD		21.9203102
% RSD		0.00214978

Limit of Detection
LOD=3.3 x slope/SD
=0.238

Limit of Quantitation
LOQ=10 x slope/ SD
=0.723

Table 13: Assay Results Of Formulated Tablet.

Formulation	Daclatasvir		Daclatasvir (%)
	Actual conc. µg/ml	Amount obtained µg/ml	
Tablet	10	9.83	98.30%

CONCLUSION

Sensitive, simple, selective and accurate HPLC method was developed foe analysis of antiviral drug (Daclatasvir) in tablet dosage form. The method is simple, rapid and helpful routine work for quick analysis of a large number of samples in short time.

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