Research Article

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

ISSN Print 2231 – 3648
Online 2231 – 3656

Analytical method development and validation for the estimation of DACLATASVIR in bulk and pharmaceutical dosage form using RP-HPLC

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ABSTRACT

A simple, precise, accurate and linear reverse phase isocratic HPLC was developed and validated for the determination of Daclatasvir in bulk and tablet dosage forms. Method development was carried out on Zorbax Eclipse XDB-C18 isocratic column, (250mm × 4.6mm, particle size 5µ, maintained at ambient temperature). The mobile phase was a mixture of 0.01M Potassium dihydrogen orthophosphate and Acetonitrile (15:85), with apparent pH of 2.5 and the flow rate was set at 1.0ml/min and UV detection at 284nm. The statistical analysis shows that the method was found to be accurate, reliable, simple and reproducible. The proposed method was successfully applied for the quantitative determination of Daclatasvir in bulk form and could be used for routine analysis with phenomenal accuracy and precisions.

Keywords: Daclatasvir, RP-HPLC, Reliable, Validation, Assay, Hepatitis, Isocratic.

INTRODUCTION

Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions of analytes with the surface of this porous media resulting in different migration times for a mixture of components. In the above definition, the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte

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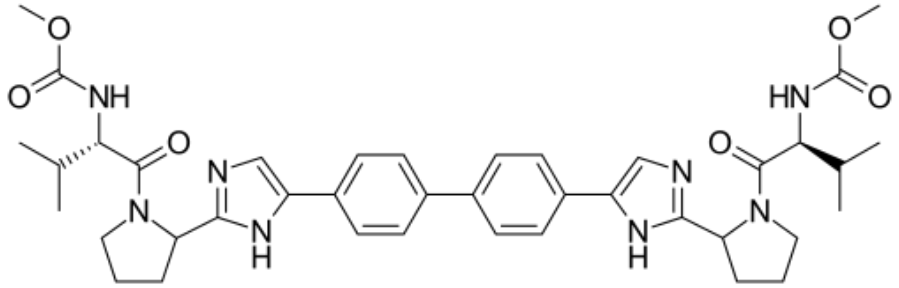


Figure 1: Chemical structure of Daclatasvir

Daclatasvir, Dimethyl N,N'-([1,1'-biphenyl]-4,4'-diylbis{1H-imidazole-5,2-diyl-[(2S)-pyrrolidine-2,1-diyl][(2S)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate, is a medication used in combination with other medications to treat hepatitis C genotype 1, 3, or 4 infections. The agents used in combination, which include Sofosbuvir, Ribavirin and Interferon varies based on the virus genotype and whether the person has cirrhosis. Daclatasvir stops HCV viral RNA replication and protein translation by directly inhibiting HCV protein NS5A. NS5A is critical for HCV viral transcription and translation. It is taken by mouth once a day. It is on the world health organization's list of essential medicines.

MATERIALS AND METHOD

Chemicals & drugs

The chemicals used were Acetonitrile HPLC grade (Qualigens) and Water HPLC grade (Milli-Q), potassium dihydrogen phosphate and ortho-phosphoric acid (Rankem). The drugs employed in the process were Daclatasvir standard powder, Daclatasvir tablets (Declahep ® 60mg) which were obtained as a fit sample from Hetero health care.

Instruments used in current study

The HPLC system used is Waters, HPLC 2695 system with auto injector. The detector used is a photo diode array (PDA) model 2996 with a wavelength range of 190-800 nm and sensitivity settings from 0.0001-2.0000 absorbance units. The software used is Empower 2. The UV/VIS spectrophotometer used is PG instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance of solutions. Thermo scientific pH meter, Sartorius micro balance and Ultrasonic sonicator are used.

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. For the analytical method validation the following parameters were considered.

- **Specificity:** Specificity is the ability to assess unequivocally the analyte in the presence of components like impurities, degradants, matrix, etc. which may be expected to be present.
- **Accuracy:** Accuracy refers to the closeness of a measured value to a standard or known value.
- **Precision:** The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.
- **Limit of detection:** It is the lowest amount of analyte in the sample which can be detected but not necessarily quantified as an exact value.
- **Limit of quantification:** It is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision & accuracy.
- **Linearity:** The linearity of an analytical procedure is to obtain test results which are directly proportional to the concentration of analyte in the sample (within a given range).
- **Robustness:** A method is robust if it is unaffected by small changes in operating conditions.

PREPARATION OF SOLUTIONS

- ✓ **Preparation of Standard drug solution:** A standard stock solution of the drug was

prepared by dissolving 250mg of Daclatasvir in 100ml volumetric flask containing 50ml of water, sonicated for about 15min and then made up to 100 ml with water to get approximately 2500µg/ml.

- ✓ **Working Standard Solution**: 5ml of the primary standard stock solution of 2500µg/ml was taken in 50ml volumetric flask and thereafter made up to 50ml with mobile phase to get a concentration of 250µg/ml.
- ✓ **Preparation of Sample solution**: 20 Tablets of Daclatasvir (Declahep ® 60 mg, Hetero Healthcare, Tablets) were weighed and powdered. A sample of the blended tablet powder, equivalent to 250mg of the active ingredient, was mixed with 70ml of mobile phase in 100ml volumetric flask. The mixture was allowed to stand for 1 hour with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45µm membrane filter, followed by addition of mobile phase up to 100ml to obtain a stock solution of 2500µg/ml. The resultant solution was further diluted by taking 5ml of the stock solution with 50ml of mobile phase to get the concentration of 250µg/ml.

Composition of Mobile Phase

3.48gms of Potassium dihydrogen phosphate (0.03M) is dissolved in 1000ml of water and the pH is adjusted to 2.5 with dilute ortho-phosphoric acid (mobile phase solvent-A) and then acetonitrile (mobile phase solvent-B) is added in an isocratic mode in the ratio of 15:85 (v/v). They were filtered before use through a 0.45 µm membrane filter and degassed by sonication.

RESULTS AND DISCUSSIONS

- ✓ **Method Validation:** The following parameters were used to validate the method for the estimation of Daclatasvir in bulk sample and in Tablets.
- ✓ **System Suitability:** The system suitability tests were carried out on freshly prepared standard stock solution of Daclatasvir. The system was suitable for use, the tailing factors for Daclatasvir were 1.23 and USP theoretical plates were found to be significantly high around 16305.

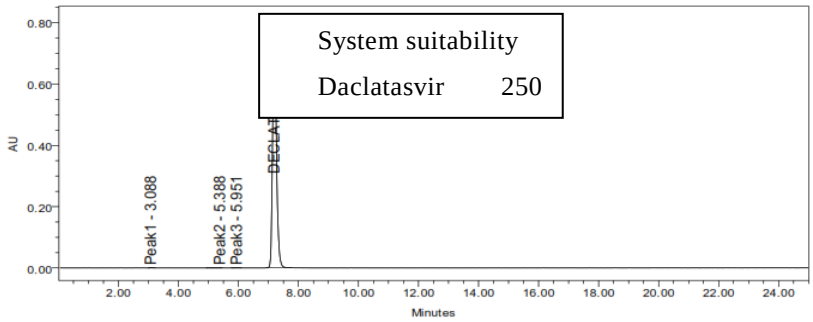


Figure: Typical System suitability Chromatogram of Daclatasvir Working standard solution

- ✓ **Precision:** The precision of the method was ascertained separately from the peak area obtained by actual determination of 6 replicas of a fixed amount of drug and formulation. The HPLC system was set up, equilibrated and then injected the 250µg/ml Daclatasvir standard 6 times and recorded the response (peak area). The proposed method was extended to the pharmaceutical dosage forms by injecting the 250µg/ml of Daclatasvir sample with the formulated sample from (Declahep®-60mg, Tablets Hetero Healthcae) and recorded the response (peak area). The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated.

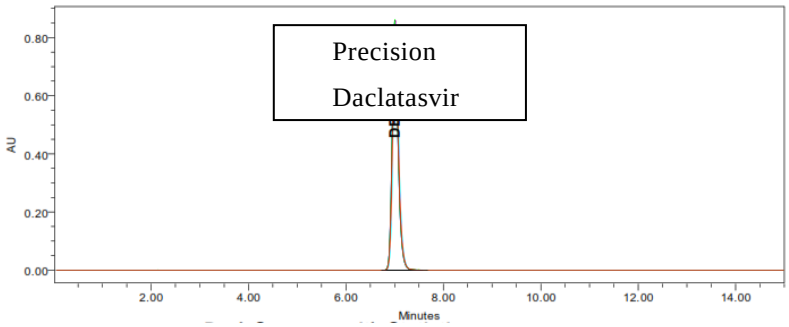


Fig: Chromatogram to illustrate Precision of Standard Daclatasvir standard

Table: Precision of Standard drug with statistics

Injection No.	Name of the drug & conc. (250 µg/ml)	Retention time in min.	Peak Area
1	Daclatasvir injection-1	7.008	7239863
2	Daclatasvir injection-2	7.002	7365271
3	Daclatasvir injection-3	7.001	7313593
4	Daclatasvir injection-4	7.011	7217132
5	Daclatasvir injection-5	7.013	7396742
6	Daclatasvir injection-6	7.022	7225713
Mean			7293052.3
% RSD.			76833.1
Std. Deviation			1.1

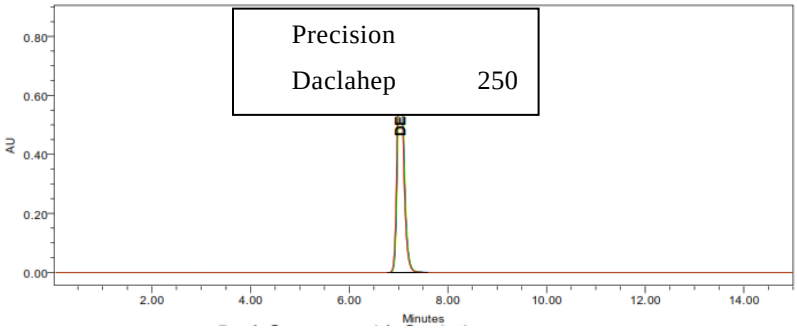


Fig: Chromatogram to illustrate Precision of Daclatasvir Sample solution

Table: Precision study of Sample Solution (Declahep® 60 mg, Tablets) **with statistics**:

Injection No.	Name of the drug & conc. (250 µg/ml)	Retention time in min.	Peak Area
1	Declahep® injection-1	7.023	7338308
2	Declahep® injection-2	7.037	7325813
3	Declahep® injection-3	7.041	7326778
4	Declahep® injection-4	7.053	7371503
5	Declahep® injection-5	7.040	7305680
6	Declahep® injection-6	7.047	7362341
Mean		7.023	7338403.8
Std. Deviation			24629.6
% RSD			0.3

✓ **Linearity:** Aliquots of standard Daclatasvir stock solution were taken in different 10ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Daclatasvir are in the range of 100-300µg/ml. Each of these drug solutions (10µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 284nm and a Calibration graph was obtained by plotting peak area versus concentration of Daclatasvir.

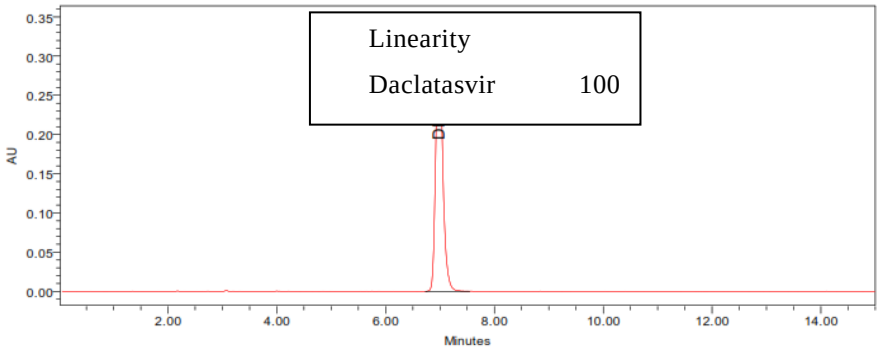
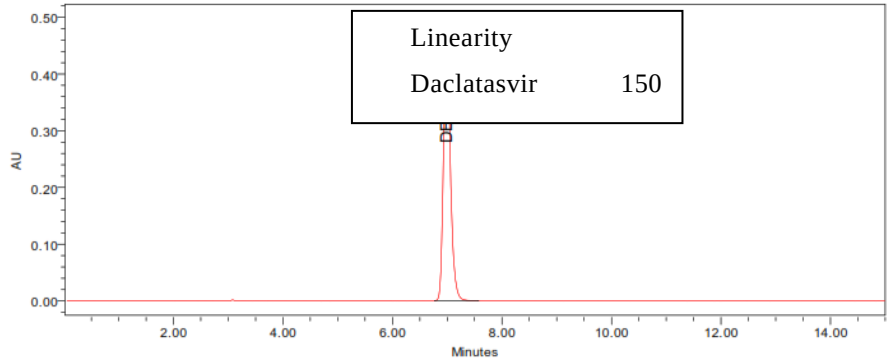
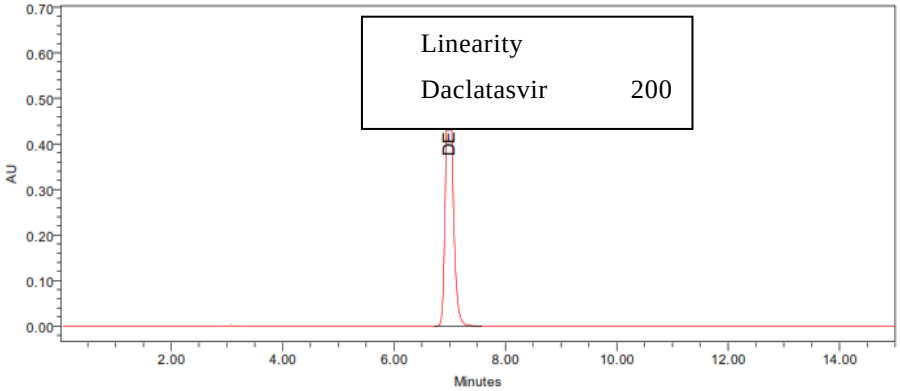


Figure: Linearity Chromatograms of Daclatasvir standard dilutions

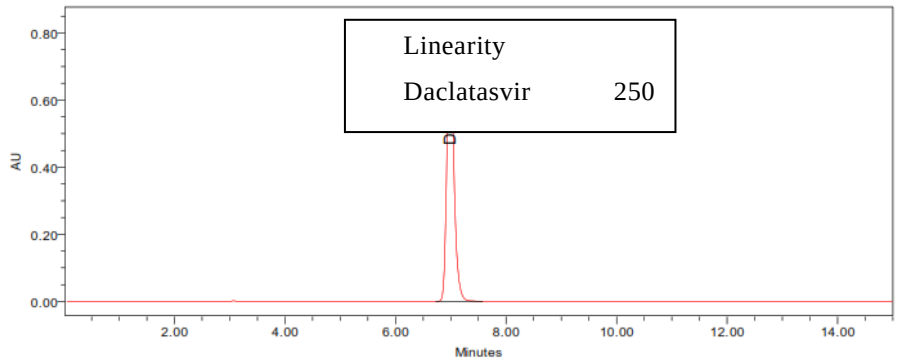
Chromatogram of Daclatasvir at 100mcg/ml (40%)



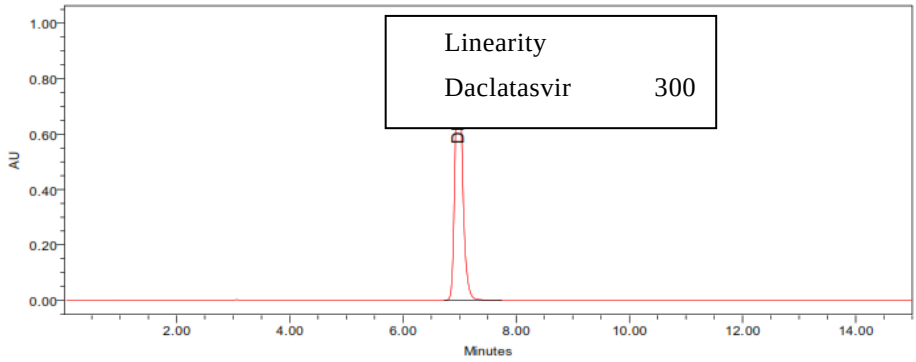
Chromatogram of Daclatasvir at 150mcg/ml (60%)



Chromatogram of Daclatasvir at 200mcg/ml (80%)



Chromatogram of Daclatasvir at 250mcg/ml (100%)



Chromatogram of Daclatasvir at 300mcg/ml (120%)

Table: Standard calibration values of Daclatasvir

Concentration of drug (µg/ml)	Peak Area
100	2951349
150	4256089
200	5667685
250	7170168
300	8698745

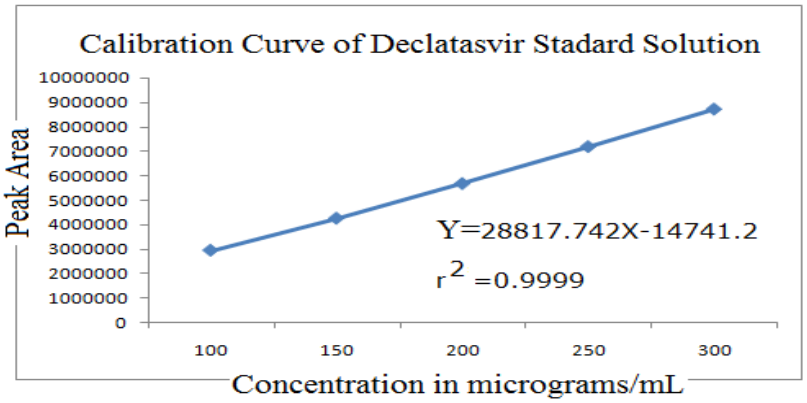


Fig: Standard Calibration Curve of Daclatasvir

✓ **Recovery Studies**: Recovery studies were conducted by analyzing pharmaceutical formulation in the first instance for the active ingredient in the concentration of 80% of the working standard (200µg/ml of Daclatasvir); 100% of the working standard solution (250µg/ml of Daclatasvir) and 120% of the working standard solution (300µg/ml of Daclatasvir) by the proposed method. Each concentration was injected 3 times and the peak area was recorded. Known amounts of pure drug was then added to each 3 previously analyzed formulation and the total amount of the drug was once again determined by the proposed method (each concentration was again injected 3 times) after keeping the active ingredient concentration within the linearity limits.

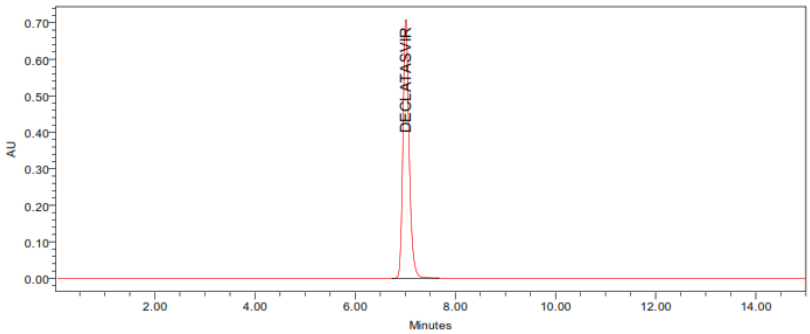
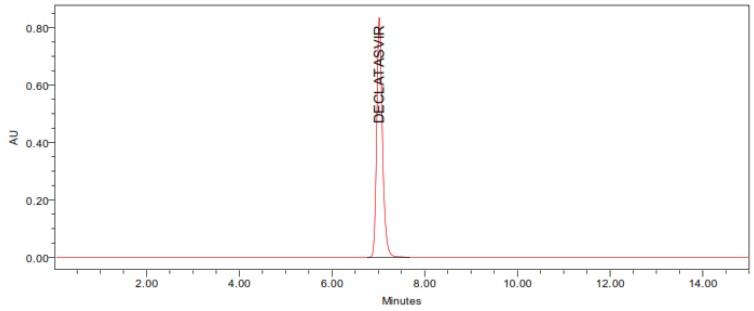
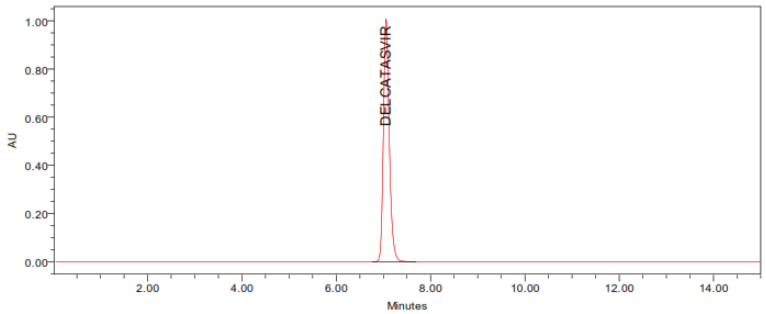


Figure: Recovery Chromatograms of Daclatasvir by accuracy studies

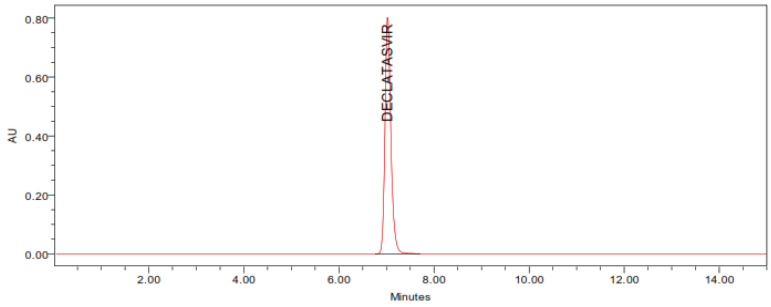
Chromatogram of accuracy standard (80%)



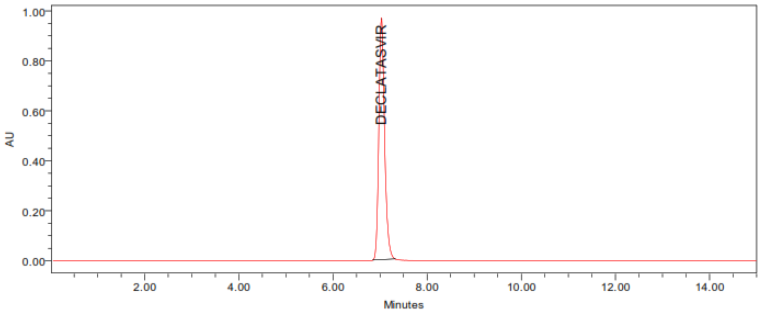
Chromatogram of accuracy standard (100%)



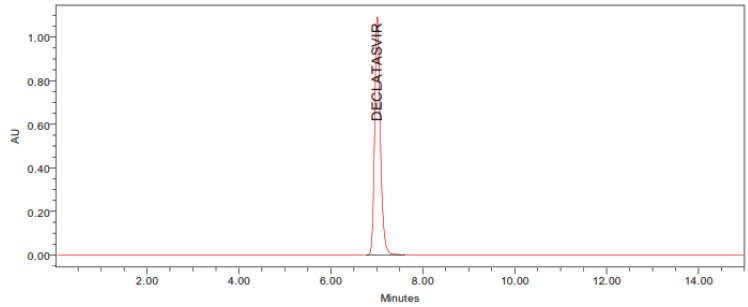
Chromatogram of accuracy standard (120%)



Chromatogram of accuracy spiked (80%)



Chromatogram of accuracy spiked (100%)



Chromatogram of accuracy spiked (120%)

Table: Recovery Peak areas of Daclatasvir by Accuracy studies

S.No	Recovery at 80% dilution level Peak areas		Recovery at 100% dilution level Peak areas		Recovery at 120% dilution level Peak areas	
	Standard	**Spiked**	**Standard**	**Spiked**	**Standard**	**Spiked**
1	6078549	6883692	7137688	8171910	8693037	9402205
2	6094909	6936077	7254913	8123507	8737102	9487382
3	6117299	6939025	7199150	8025701	8581219	9334452
Avg	**6096919.0**	**6919598.0**	**7197250.3**	**8107039.3**	**8670452.7**	**9408013.0**
Std.Dev	19453.0	31130.4	58635.6	74482.6	80358.1	76630.3
%RSD	**0.3**	**0.4**	**0.8**	**0.9**	**0.9**	**0.8**
% Recovery	102.0%		119.10%		93.50%	

✓ **Robustness:** To determine the robustness of this method, the experimental conditions were deliberately altered at two different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the mobile phase flow rate was varied by ±10%) and different column had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. When the chromatographic conditions were deliberately altered, system suitability results remained within acceptance limits and selectivity for individual substance was not affected. The results of the study prove the robust nature of the method.

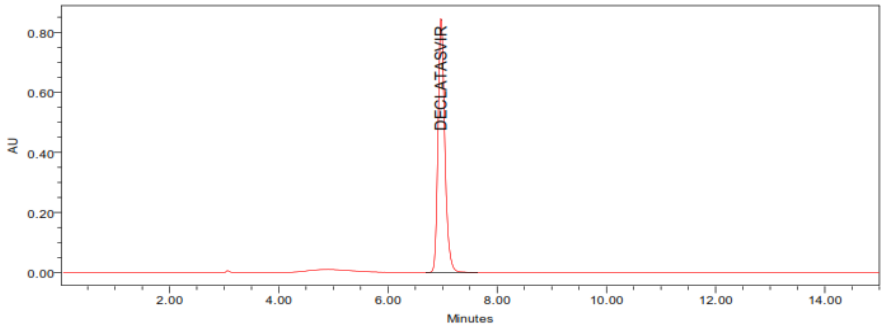
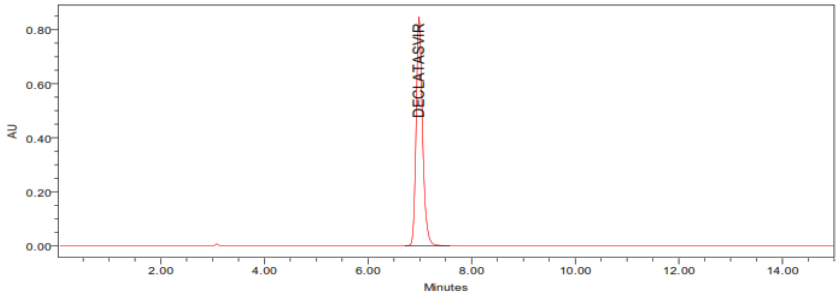
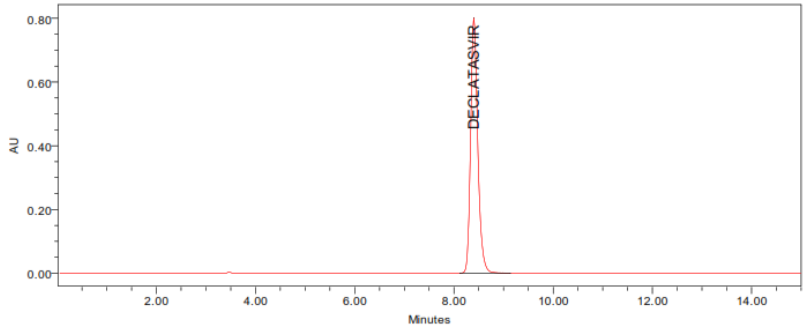


Fig-: Chromatograms to illustrate robustness study of Daclatasvir Standard solution

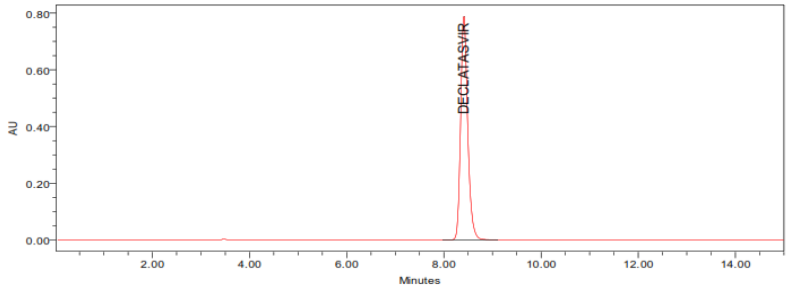
Chromatogram of Daclatasvir standard (Flow increased)



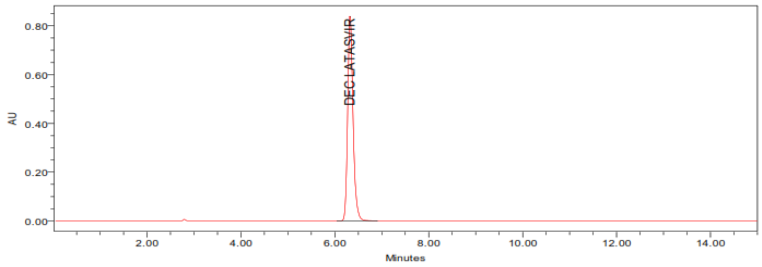
Chromatogram of Daclatasvir sample (Flow increased)



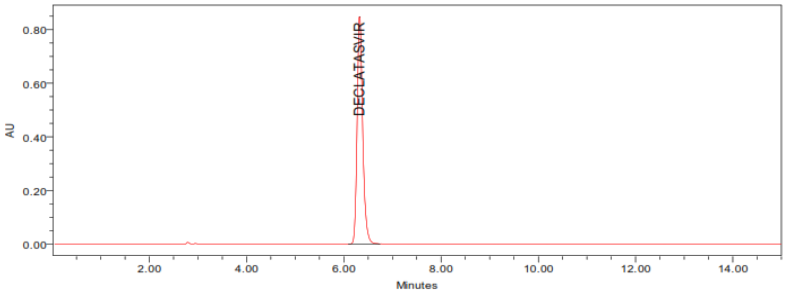
Chromatogram of Daclatasvir standard (Flow decreased)



Chromatogram of Daclatasvir sample (Flow decreased)



Chromatogram of Daclatasvir standard (different column)



Chromatogram of Daclatasvir sample (different column)

Table: Robustness study of Daclatasvir Standard solution at 100 % level (250 µg/mL):

Parameter	Peak areas of Daclatasvir in Flow increase	Peak areas of Daclatasvir in Flow decrease study	Peak areas of Daclatasvir in Variable column
Injection-1	6666481	8007918	7281195
Injection-2	6630941	7931643	7281896
Injection-3	6667925	7947609	7252862
Mean	**6655115.7**	**7962390.0**	**7271984.3**
% RSD	20948.3	40228.4	16564.1
Std. Dev	**0.3**	**0.5**	**0.2**

Table: Robustness study of Declahep®-60 mg tablets solution at 100 % level (250 µg/mL):

Parameter	Peak areas of Daclatasvir in Flow increase study	Peak areas of Daclatasvir in Flow decrease study	Peak areas of Daclatasvir in Variable column
Injection-1	6729479	7952639	7229027
Injection-2	6716886	7989223	7306596
Injection-3	6745916	7993609	7318377

Mean	**6730760.3**	**7978490.3**	**7284666.7**
% RSD	14557.4	22495.1	48544.1
Std. Dev	**0.2**	**0.3**	**0.7**

✓ **Limit of Detection [LOD] and Limit of Quantification [LOQ]:** The detection limit of the method was investigated by injecting standard solutions Daclatasvir into the HPLC column. By using the signal-to-noise method the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated. A signal-to-noise ratio (S/N) of 3 is generally accepted for estimating LOD and signal-to-noise ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise. The limit of detection (LOD) and limit of quantification (LOQ) for Daclatasvir were found to be 0.05µg/ml and 0.15µg/ml respectively.

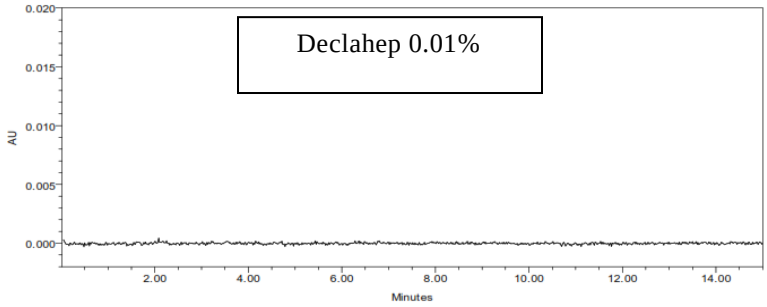
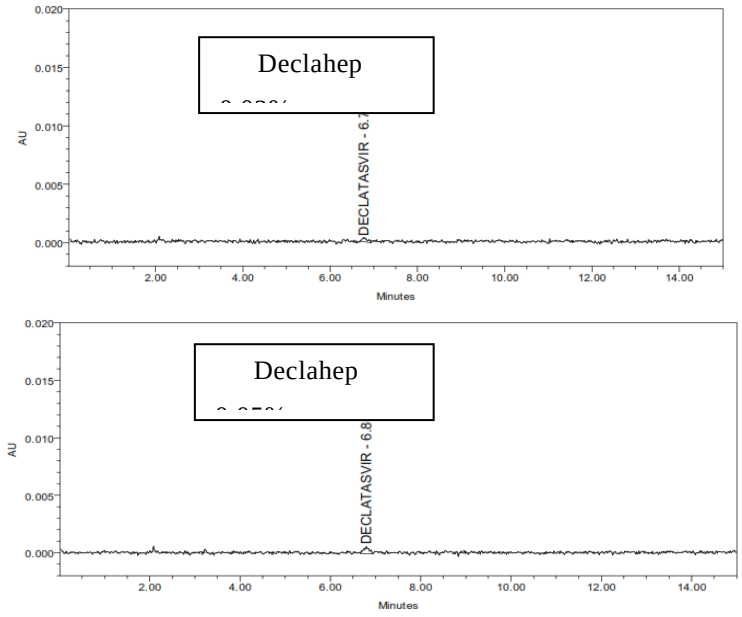
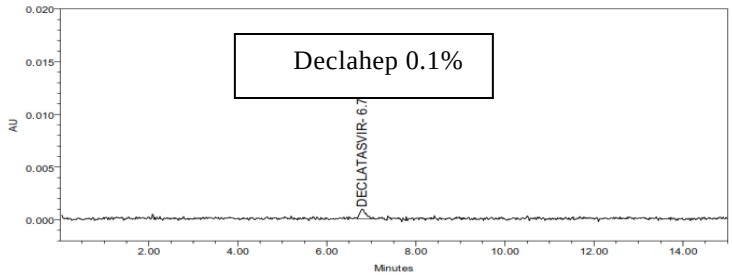


Figure: Limit of Detection and Limit of Quantification Chromatograms of Daclatasvir





CONCLUSIONS

There are no reports on the HPLC determination of Daclatasvir in pharmaceutical formulations in the literature prior to commencement of this work. The author has developed a sensitive, accurate and precise HPLC for the estimation of Daclatasvir in bulk drug and in tablet dosage form. From the typical chromatogram of Daclatasvir as shown in fig 3.1.2, it was it found that the retention time was 7.185 min. The contents of the mobile phase were Buffer: Acetonitrile 15: 85 (v/v). Solvent-A (Buffer) is 3.48 gms of Di Potassium hydrogen *ortho*-phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute orthophosphoric acid and Solvent-B is Acetonitrile in a isocratic mode of separation was used to resolute the Daclatasvirat a flow rate of 1.0 ml/min and eluents were monitored at 284 nm, was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r^2=0.9999) was observed between the concentration range of 100-300 µg/mL. The assay of Daclatasvirin bulk was found to be 99.85%. From the recovery studies it was found that about 191.10 % on average of Daclatasvir was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the Tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and sterile powder for injection dosage form of Daclatasvir within a short analysis time.

It can be seen from the results presented that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations.

The above proposed method obviates the need for any preliminary treatment and is the method could be of use for process development as well as quality assurance of Daclatasvir in bulk drugs.

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