



Original Article

The Estimation of Daclatasvir in Tablet Dosage Form by RP-HPLC

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A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of daclatasvir in capsule dosage form. A column of Inertsil ODS-3V C18, 250x4.6mm.i.d with 5micron particle size was used. The mobile phase comprises of 0.01M Ammonium acetate with pH adjusted to 3.5 (mobile phase solvent-A) and methanol (mobile phase solvent-B) in the ratio of 20: 80 (v/v).The flow rate was 1.0 ml/min and the effluents were monitored at 284 nm. The retention time was 7.79 min. The detector response was linear in the concentration of 100-300µg/ml. The respective linear regression equation being $Y= 28817.742X-14741.2$. The limit of detection (LOD) and limit of quantification (LOQ) for were found to be 0.05µg/ml and 0.15µg/ml respectively. The assay was found to be 99.85%.The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of daclatasvir in bulk drug and in its pharmaceutical dosage form.

Key words: Daclatasvir, RP-HPLC, system suitability, linearity, recovery studies.

1. INTRODUCTION

Daclatasvir (brand name Daklinza) is a new medication used to treat hepatitis C¹. It was approved in Europe in August 2014 for treatment of adults with chronic hepatitis C genotypes 1, 2, 3 or 4^{2, 3}. Daclatasvir is one of the new direct-acting antiviral drugs that target different steps of the hepatitis C virus (HCV) lifecycle. It is the first-ever approved HCV NS5A⁴ replication complex inhibitor, meaning it interferes with a protein the virus uses to reproduce. The chemical formula of daclatasvir is methyl N-[(2S)-1-[(2S)-2-[5-[4-[4-[2-[(2S)-1-[(2S)-2-(methoxycarbonylamino)-3-methylbutanoyl]pyrrolidin-2-yl]-1H-imidazol-yl]phenyl]phenyl]-1H-imidazol-2-

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yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate.^{5,6}
Its molecular formula is C₄₀H₅₀N₈O₆ and molecular weight
738.89 g/mol^{7,8}.

Literature survey reveals no chromatographic methods for the estimation of daclatasvir from pharmaceutical dosage forms. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of daclatasvir in bulk drug samples and in pharmaceutical dosage form.

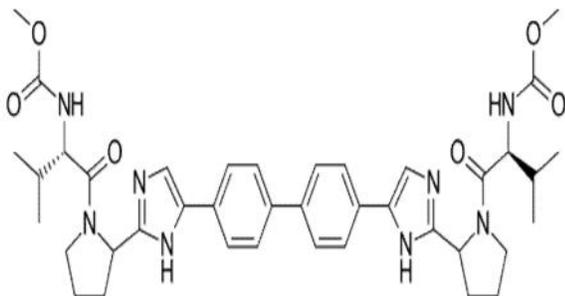


Fig 1: Structure of daclatasvir

2. MATERIALS AND METHODS

Daclatasvir was obtained as a gift sample from Hetero Drugs Ltd Hyderabad. Methanol and water used were of HPLC grade (Qualigens), Ammonium acetate was procured from Rankem. Commercially available daclatasvir tablet (Declahep®-60 mg) was procured from local market.

Instrument:

Quantitative HPLC was performed on Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing powered with-2 Empower Software. A Inertsil ODS 3V C18, 250x4.6mm.i.d of particle size 5micron column was used. The detector used is a photodiode array (model 2996) with a wavelength range of 190-800 nm.

HPLC Conditions:

The contents of the mobile phase were 3.35 gms of Ammonium acetate (0.1M) in 1000 ml of water and by adjusting the pH to 3.5 (mobile phase solvent-A) and methanol (mobile phase solvent-B) in a isocratic mode in the ratio of 20: 80 (v/v) of separation was used. They were filtered before use through a 0.45 µm membrane filter and degassed by sonication. The run time was set at 15min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 284 nm.

Preparation of Standard Stock solution:

A standard stock solution of the drug was prepared by dissolving 250 mg of Daclastavir working standard in 100ml of the diluent. The contents were sonicated for 15 minutes to obtain 2500µg/mL.

Working Standard solution:

5ml of the primary standard stock solution of 2500µg/mL was taken in 50 ml volumetric flask and thereafter made up to 50 ml with mobile phase to get a concentration of 250µg/ml.

Preparation of Sample solution:

20 Tablets of Daclatasvir (Declahep® 60 mg, Hetero Health Care, Tablets,) were and then powdered. A sample of the blended tablet powder, equivalent to 250 mg of the active ingredient, was mixed with 70 ml of mobile phase in 100 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by addition of mobile phase up 100 ml to obtain a stock solution of 2500µg/mL. The resultant solution was further diluted by taking 5 ml of the stock solution with 50 ml of mobile phase to get the concentration of 250µg/mL

3. RESULTS AND DISCUSSION

Validation for the method was carried out as per ICH Q2(R1) guidelines. The validation parameters such as system suitability, linearity, recovery studies, robustness, detection limit, quantitation limit were studied.

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.

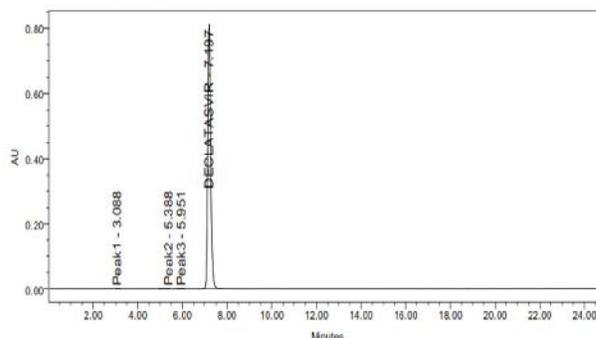


Fig 2: Typical System suitability Chromatogram of Daclatasvir Working standard solution

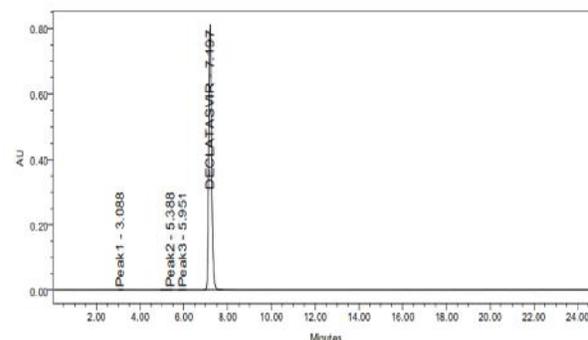


Fig 3: Typical Chromatogram of Daclatasvir Working sample (Declahep®-60 mg tablets) solution

Linearity:

Aliquots of standard Daclatasvir stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Daclatasvir are in the range of 100-300 µg/ml. Each of these drug solutions (10 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 284 nm and a Calibration graph was obtained by plotting peak area versus concentration of Daclatasvir Figure 4.

The plot of peak area of each sample was found to be linear in the range of 100-300µg/ml with correlation coefficient of 0.999.

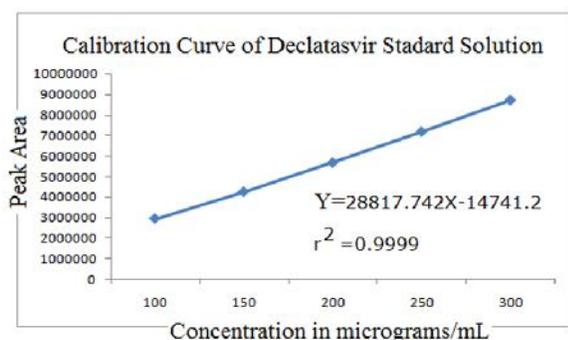


Fig 4: Calibration curve of daclastavir

Table 1: Optical & Regression Characteristics of HPLC method

Parameter	Results of HPLC Method
Detection wavelength (nm)	284
Linearity range (µg/mL)	100-300
Regression Equation (y=mx + c)	Y=28817.742X-14741.2
Slope (m)	28817.742
Intercept (c)	-147412
Correlation coefficient	0.9999
Relative Standard deviation*	1.1
% error in bulk samples	0.234

Assay and 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 (contains 200 µg/mL of Daclatasvir); 100% of the working standard solution (contains 250 µg/mL of Daclatasvir) and 120% of the working standard solution (contains 300 µg/mL of Daclatasvir) by the proposed method. Each concentration was injected 3 times and the peak area was recorded. Known amounts of pure drug [10% of the working standard solution contains 25 µg/mL of Daclatasvir for 80% of the working standard, for 100% of the working standard, for 120% of the working standard] 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.

Robustness:

A method is robust if it is unaffected by small changes in operating conditions. 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.

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.

4. CONCLUSION

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 2, it was it found that the retention time was 7.185 min. The contents of the mobile phase were Buffer: methanol 20: 80 (v/v). solvent-A (Buffer) is 3.35 gms of ammonium acetate (0.1M) in 1000 ml of water and by adjusting the pH to 3.5 and solvent-B is methanol in a isocratic mode of separation was used to resolute the Daclatasvir at 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 99.92%. From the recovery studies it was found that about

119.10 % 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.

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