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Validated RP-HPLC method for estimation of daclatasvir in tablet dosage form

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Abstract
By considering the current regulatory requirement for an analytical method development, a reversed phase high performance liquid chromatographic method for routine analysis of daclatasvir in capsule dosage form has been developed and validated. A simple, precise, rapid and accurate reverse phase HPLC method was developed. A column of Inertsil ODS-3V C18, 250x4.6mm i.d with 5µ 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, system suitability, LOD and LOQ. 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.

Keywords: daclatasvir, retention time, system suitability, linearity, recovery studies, RP-HPLC

Introduction

Analytical chemistry is defined as the study of quantification and chemical components identification of natural and artificial materials constituted with one or more compounds or elements, separation. Analytical chemistry is separated into two main categories, qualitative analysis that is to say the identification with regard to the chemical components exist in the sample, whereas quantitative analysis estimates the amount of certain element or compound in the substance i.e., sample [1]. Pharmaceutical analysis plays a very outstanding role in the examination of pharmaceutical formulations and bulk drugs regarding the quality control and assurance. Rapid increase in pharmaceutical industries and production of drug in and around the world bring forward a rise in inevitable demand to seek novel and systematic analytical techniques in the pharmaceutical industries. As a consequence, analytical method development has become the basic activity of analysis [2].

Development in scientific and concrete analytical methods has been resulted from the advancements of analytical instruments. The improvements of the analytical method development and analytical instruments have reduced the time and cost of analysis and enhanced precision and accuracy. When there are no authoritative methods are available, new methods are being developed for analysis of novel products. To analyze the existing either pharmacopoeial or non-pharmacopoeial products novel methods are developed to reduce the cost besides time for better precision and ruggedness. These methods are optimized and validated through trial runs. Alternate methods are proposed and put into practice to replace the existing procedure in the comparative laboratory data with all available merits and demerits [3].

HPLC is one among must useful tools available for quantitative analysis. Reverse Phase Chromatography refers to the use of a polar mobile phase with non-polar stationary phase in contract to normal phase being employed with a non-polar mobile phase HPLC is always used in injection with another analytical tool for quantitative and qualitative analysis. The mode of operation of the system is isocratic i.e. one particular solvent or mixture is pumped throughout the analysis for some determination. The solvent composition may be attended gradually to give gradient elution. The rate of distribution between stationary and mobile phase is controlled by diffusion process. A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantification of Daclatasvir in bulk and tablet dosage form with greater precision and accuracy. The main principle involved in HPLC is adsorption. When a mixture of components is introduced into a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated [4, 5].

RP-HPLC (Reverse Phase High Pressure Liquid

Chromatography) separates molecules on the basis of differences in their hydrophobicity. The components of the analytic mixture pass over stationary-phase particles bearing pores large enough for them to enter, where interactions with the hydrophobic surface removes them from the flowing mobile-phase stream. The strength and nature of the interaction between the sample particles and the stationary phase depends on both hydrophobic interactions and polar interactions. As the concentration of organic solvent in the eluent increases, it reaches a critical value for each analyte which desorbs it from the hydrophobic stationary-phase surface and allows it to elute from the column in the flowing mobile phase. RP-HPLC method: For routine analytical purpose it is desirable to establish methods capable of analysing huge number of samples in a short time period with good robust, accuracy and precision without any prior separation step. HPLC method generate large amount of quality data which serve as highly powerful and convenient analytical tool [6, 7].

Liver failure and hepatocellular carcinoma due to chronic hepatitis C virus (HCV) infections are the leading indications for liver transplantation (LT) in the United States. However, the recurrence of HCV infections after LT is nearly universal and is characterized by high serum HCV RNA levels and allograft hepatitis of variable severity. The rate of fibrosis progression is greatly accelerated in these patients versus nontransplant HCV patients, with 10% to 30% developing cirrhosis within 5 years of transplantation. Daclatasvir (DCV), is an investigational oral nonstructural 5A replication complex inhibitor. In phase 2 clinical trials, DCV has generally been well tolerated in combination therapy, with no unique adverse events identified to date. Furthermore, in vitro and in vivo testing suggests that DCV should not lead to any clinically significant drug-drug interactions when it is co administered with other drugs. Daclatasvir is a new medication used to treat hepatitis C. It was approved in Europe in August 2014 for treatment of adults with chronic hepatitis C genotypes 1, 2, 3 or 4. Daclatasvir is a direct-acting antiviral agent against Hepatitis C Virus (HCV) used for the treatment of chronic HCV. It is marketed under the name DAKLINZA and is contained in daily oral tablets as the hydrochloride salt form. 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 NS5A replication complex inhibitor, meaning it interferes with a protein the virus uses to reproduce [8, 9].

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 relapses occur within 4 weeks of treatment discontinuation and a 98-99% concordance has been shown between absence of quantifiable virus 12 weeks after therapy and SVR24. Therefore the absence of measurable virus 12 weeks post end of treatment (SVR12) is presently accepted by European and US regulators as the primary endpoint in clinical trials.

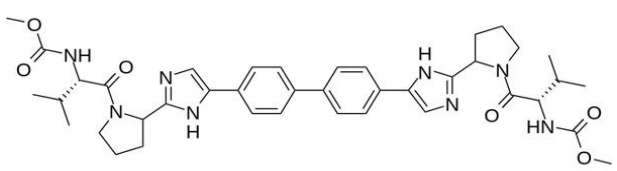

Fig 1: Structure of daclatasvir

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-yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate. Its molecular formula is $C_{40}H_{50}N_8O_6$ and molecular weight is 738.89 g/mol.

Literature survey reveals many chromatographic methods for the estimation of Daclatasvir in pharmaceutical dosage forms. But the availability of a HPLC method with high sensitivity and selectivity will be very useful for the determination of Daclatasvir in pharmaceutical formulations. 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.

Experimental section

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 Inetsil ODS 3V C18, 250x4.6mmi.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.

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.

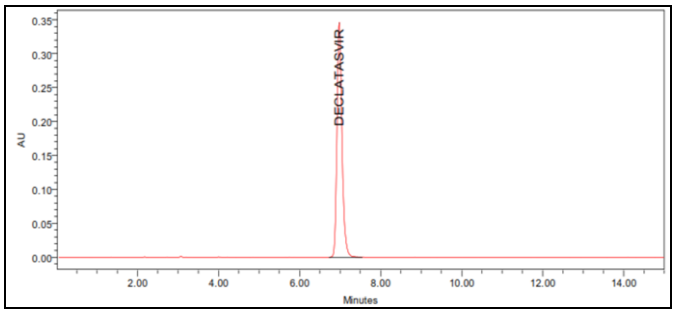

Fig 2: Typical System suitability Chromatogram of Daclatasvir Working standard solution

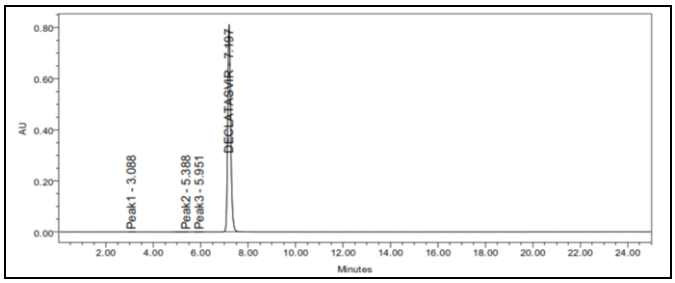

Fig 3: Typical Chromatogram of Daclatasvir Working sample (Declahep®-60 mg tablets) solution

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.

Table 1: Linearity data for Daclastavir

Sl. No.	Conc. (µg/ml)	Peak Area
1	100	300150
2	150	430241
3	200	582416
4	250	746895
5	300	886294

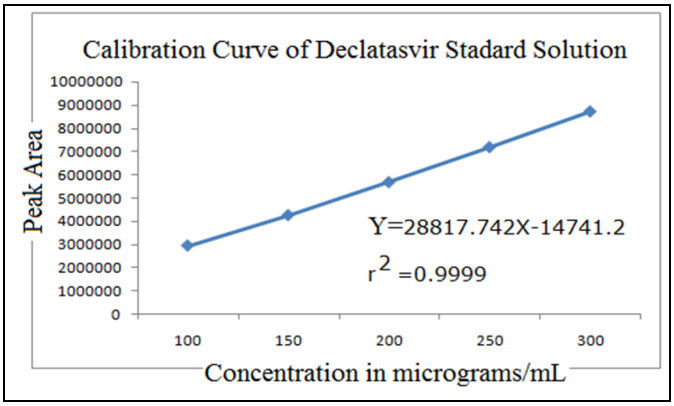

Fig 4: Calibration curve of Daclastavir

Table 2: 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

Accuracy and recovery studies

Recovery studies were conducted by analyzing pharmaceutical formulation in the first instance for the active ingredient in the concentration of 200 µg/mL, 250 µg/mL and 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 [25 µg/mL of Daclatasvir] 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.

Table 3: Recovery study data for Daclatasvir

Recovery study data for Daclatasvir (n=3)				
Added Conc.(µg/mL)	**Area**	**Measured Conc.(µg/mL)**	**% Recovery**	**Mean % Recovery**
200	582416	200.12	100.06	100.11
200	582425	200.31	100.15	
200	582415	200.24	100.12	
250	746898	250.18	100.07	100.07
250	746886	250.13	100.05	
250	746895	250.23	100.09	
300	886294	300.37	100.12	100.10
300	886287	300.32	100.10	
300	886291	300.29	100.09	

Precision

Method precision was determined by injecting 100% test solution of six determinations for assay and the observed values of % RSD were shown in Table 3. % RSD for Daclatasvir in test solution for six determinations was not more than 2.0% for assay. Intermediate precision of the method was studied by injecting the test solution of six determinations and the values were shown in Table 3. The %RSD difference between the two analysts is 0.0% for assay. Less difference between the two analysts shows that the developed method was precised and has good intermediate precision.

Table 4: Precision and Intermediate precision data

Determination	**Method Precision (% Assay)**	**Intermediate Precision (% Assay)**
Determination-1	100.46	100.19
Determination-2	100.99	100.15
Determination-3	100.26	99.86
Determination-4	100.34	100.92
Determination-5	100.87	100.84
Determination-6	101.29	100.36
Average	100.70	100.38
SD	0.41	0.41
%RSD	0.40	0.41

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.

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 g 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 Daclatasvir in 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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Conflicts of interest
There are no conflicts of interest.

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