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RESEARCH ARTICLE

Validated Stability Indicating HPLC Method for Estimation of Daclatasvir

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Abstract: For the determination of daclatasvir from formulation, a new, simple, specific, accurate and robust isocratic reversed-phase (RP) stability indicating high-performance liquid chromatographic (HPLC) method has been developed and validated. The liquid chromatographic separation isocratically accomplished by means of a portable acetonitrile, buffer (0.1% Octa sulphonic acid) (70:30). The analysis was carried out using at ambient temperature on Xterra C_{18} (4.6x150 mm, 5.0 µm) at flow rate of 1 mL/min and the UV detection at 237 nm. The method was validated for accuracy, precision, linearity, range, selectivity and robustness. The linearity of the proposed method was investigated in the range of 20 to 100 µg/mL (r^2 = 0.999). The drug was subjected to oxidation, hydrolysis, heat and photolysis to apply stress conditions. The method provided good peak parameters with retention time of 2.398 min. Degradation products resulting from stress studies did not interfere with the detection of daclatasvir and the assay can thus be considered as stability-indicating HPLC method.

Keywords: Assay, High performance liquid chromatography, Reverse phase, Daclatasvir validation

Introduction

Hepatitis C is a chronic infection with significant morbidity and mortality. Over the past few years, treatment methods have shifted with the discovery and approval of agents targeting sp ecific proteins vital for replication of hepatitis C virus (HCV). Daclatasvir (DCV) (Figure 1) is an inhibitor of HCV nonstructural protein NS5A. DCV is an oral, direct-acting antiviral with potent activity that has been recently approved in many countries worldwide. *In vitro* data shows that against several HCV genotypes, DCV exerts a very powerful antiviral effect. Clinical trials have shown that the use of DCV plus sofosbuvir with or without ribavirin in patients with chronic HCV genotype 1,3 or 4 infections, including patients with advanced liver disease, post-transplant recurrence and HIV-1 co-infection, is a significant option[1-5].

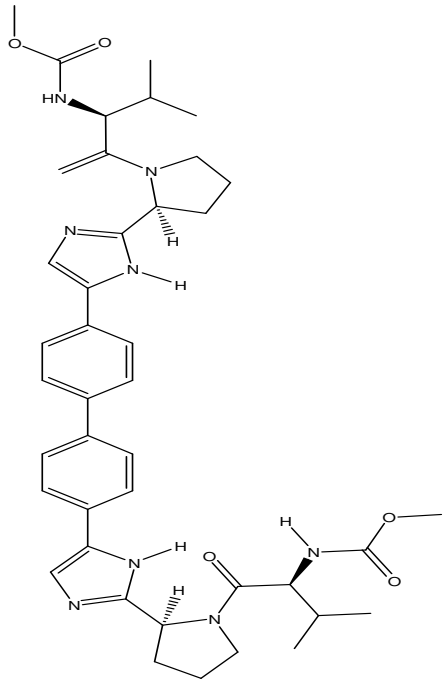

Figure 1. Chemical structure of daclatasvir

Experimental

Daclatasvir was a hetero laboratory gift sample. All other components used in the formulation were acquired from acetonitrile, which was deionized and then doubled with liquid chromatographic (HPLC) grade water of high performance. The ortho-phosphate potassium dihydrogen is a buffer.

Chromatographic parameters

High performance liquid chromatography equipped with auto sampler and PDA detector. The chromatographic separation was achieved at ambient temperature on an Xterra C_{18} (4.6x 150 mm, 5.0 µm). Mobile phase comprises of buffer (Octa sulphonic acid pH 3.5) 300 mL (30%) and 700 mL acetonitrile HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µm filter under vacuum filtration and the separations were achieved by isocratic elution with a flow rate of 1.0 mL/min. Optimized chromatographic conditions shown in Table 1. Optimized chromatogram shown in Figure 2.

Table 1. Optimized chromatographic conditions

S.No	Parameter	Optimized condition
1	Mobile phase composition	70:30 Methanol: Buffer of pH 3.5
2	Stationary phase	Xterra C_{18} (4.6x150 mm, 5.0 µm)
3	Flow rate	1 mL/min
4	Run time	7 min
5	Column temperature	Ambient
6	Volume of injection	10 µL
7	Detection wavelength	237 nm
8	Retention time of the drug	2.3 min

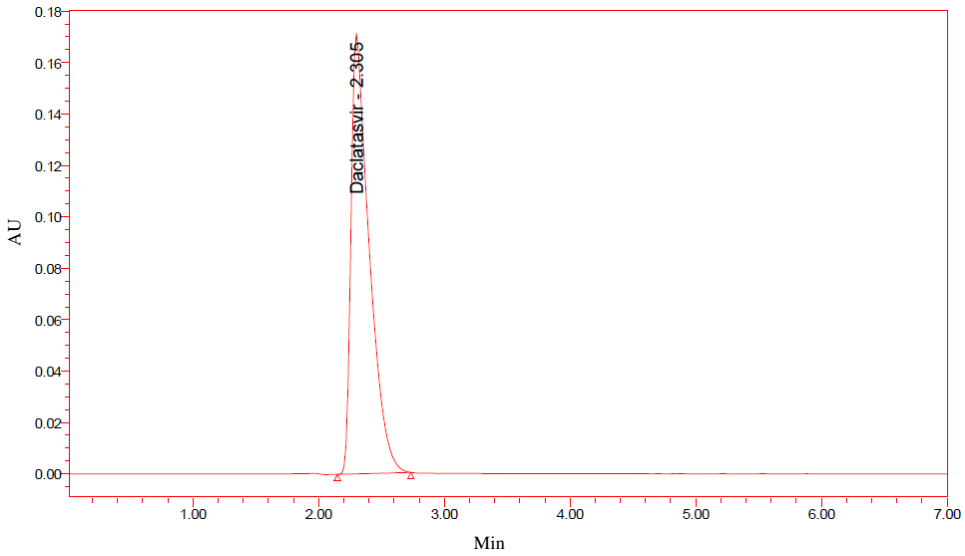

Figure 2. Optimized chromatogram for daclatasvir

Preparation of the daclatasvir standard and sample solution

Standard solution preparation

A stock solution of daclatasvir was prepared by weighing precisely 10 mg of daclatasvir in 10 mL of smooth dry volumetric flask added diluents, sonicated to fully dissolve it and iluents up to the mark. Additional daclatasvir iluent out (0.6 mL) from the above stock solution into a 10 mL volumetric flask and diluted to the mark (60 ppm).

Sample solution preparation

A inventory solution of daclatasvir was prepared by properly weighing 10 mg of daclatasvir sample (about 70 mg of Tablet powder) into a 10 mL soft dry volumetric flask added diluents, sonicated to completely dissolve it and generated volume up to the mark with the iluents. Further iluent 0.6 mL of daclatasvir of the above inventory solution into a 10 mL volumetric flask and diluted to the mark with iluents.

Procedure

Injected 10 µL of the standard, sample into the chromatographic system and measured the areas for the daclatasvir peaks and calculated the % assay by using the formula.

System suitability

Tailing factor should not be more than 2.0 for the peaks due to daclatasvir in standard solution. Theoretical plates should not be less than 2000 for the daclatasvir peaks in standard solution.

Calculation: (For daclatasvir)

$$\text{Assay } \% = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label claim}} \times 100$$

Where, AT= Average area counts of test preparation. As = Average area counts of standard preparation. WS = Weight of working standard taken in mg, P= Percentage purity of working standard, **LC**= Label claim of daclatasvir mg/mL (60 mg)**.**

Results and Discussion

Method development parameters[6-17]

1) Tailing factor - 1.65.
2) Theoretical plates obtained - 2559.08.

Precision

Preparation of stock solution

10 mg of Daclatasvir working standard appropriately transferred into a 10 mL smooth dry volumetric flask added diluents, sonicated to dissolve it entirely and created volume up to the mark with the diluent (Stock solution). Additional daclatasvir pipetted 0.6 mL of the stock solution above into a 10 mL volumetric flask and dilute to the mark with diluent.

Procedure

Five times the standard solution was introduced and all five HPLC injections were evaluated for the region. The percentage of RSD was found to be within the specified times for five replicate injections.

Acceptance criteria

The % RSD for the area of five standard injections results should not be more than 2%.

Intermediate precision / Ruggedness

Precision was carried out on distinct days using distinct columns of the same size to evaluate the technique's intermediate precision (also known as Ruggedness).

Preparation of stock solution

10 mg of Daclatasvir working standard taken into a 10 mL smooth dry volumetric flask and diluted sonicated to dissolve it completely and created volume with the diluent up to the mark (Stock solution). Additional stock solutions daclatasvir pipetted 0.6 mL in a volumetric flask of 10 mL and dilute with diluent to the mark.

Procedure

Five times the standard solution was injected and the region for all six HPLC injections was evaluated. The percentage of RSD was found to be within the specified limits for the area of five replicate injections.

Acceptance criteria

The % RSD for the area of six standard injections results should not be more than 2%.

Accuracy

Preparation of standard stock solution

10 mg of Daclatasvir working standard dissolved into a 10 mL smooth dry volumetric flask containing diluent and sonicated to dissolve it entirely and created volume up to the mark with the diluent.

Preparation sample solutions

For preparation of 50, 100 and 150% solution (With respect to target assay concentration) 5, 10 and 15 mg of daclatasvir working standard mixed with diluents into a 10 mL smooth dry volumetric flask sonicated to dissolve it fully and created volume up to the mark with the diluent (Stock solution). Pipetted 0.6 mL from the above stock solutions into a 10 mL volumetric flask and diluted to the diluent mark (30 ppm) to prepare respective concentrations.

Procedure

Precision solutions of 50, 100 and 150% injected and calculated the amount discovered for daclatasvir and the amount added calculates D the recovery values of the person and the mean recovery.

Acceptance criteria

The % recovery for each level should be between 98.0 to 102.0%

Linearity

Preparation of stock solution

Working standard of 10 mg of daclatasvir was weighed precisely and transferred into a 10 mL smooth dry volumetric flask, diluent was added and sonicated to fully dissolve it and create delivery with the diluent up to the mark (Stock solution). 0.2, 0.4, 0.6, 0.8 and 0.10 mL stock solutions diluted to 10 mL to prepare 20, 40, 60, 80 and 100 ppm of daclatasvir solutions.

Procedure

Injected each level into the system of chromatography and assessed the peak region. Peak region graph *versus* concentration (on the peak region of x-axis and y-axis) made and calculated the correlation coefficient linearity plot shown in Figure 3.

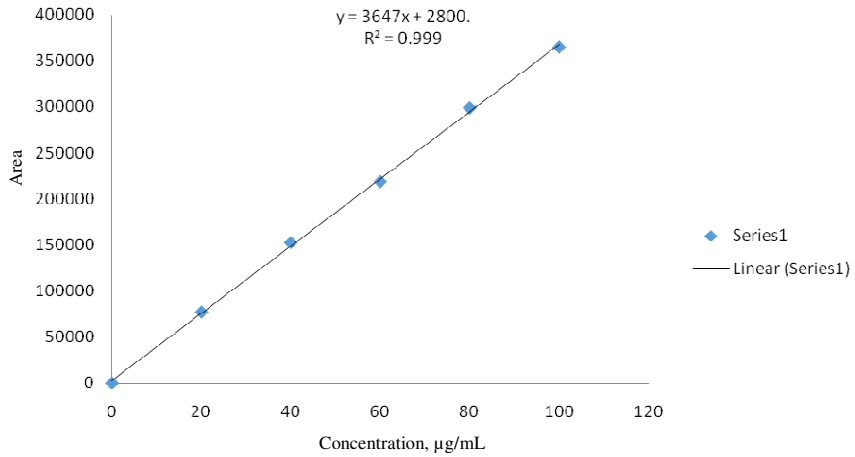

Figure 3. Linearity plot for daclatasvir

Acceptance criteria

Correlation coefficient should be not less than 0.999.

Robustness

Temperature variation was generated as part of the robustness, deliberate change in flow rate, mobile phase structure to evaluate the impact on the technique.

a) The flow rate was varied at 0.9 mL/min to 1.1 mL/min.
Standard solution 60 ppm of daclatasvir prepared and analysed using the varied flow rates along with method flow rate. When evaluating the above results, it can be concluded that the variation in the flow rate had a significant influence on the method. It thus suggests that even if the flow rate shifts to ±10 percent, the technique is robust. Only in less flow condition is the technique robust. The flow rate information is shown in Table 3.

b) The organic composition in the mobile phase was varied ±10%.
The normal 60 µg/mL solution for daclatasvir was prepared and evaluated using the diverse mobile phase structure along with the real mobile phase structure.

From the assessment of the above outcomes, it can be concluded that the technique was considerably influenced in the mobile stage by the variation in 10% organic composition. It thus demonstrates that the method is robust even if the mobile phase ±10 is changed. Table 4 shows the data regarding mobile phase composition.

Table 2. Degradation data of daclatasvir

Condition	Daclatasvir	
	Area	% Degraded
Standard	1720758	
Acid	1626465	5.479736
Base	1649322	4.151426
Peroxide	1651577	4.020379
Thermal	1668536	3.034825

Table 3. System suitability results for daclatasvir

S.No	Flow rate, mL/min	System suitability results		
		Retention time	USP Plate count	USP Tailing
1	0.9	2.473	3140	1.9
2	1.0	2.305	2997	1.94
3	1.1	1.835	3115	1.8

Table 4. System suitability results for daclatasvir

S.No	Change in organic composition in the mobile phase	System suitability results		
		Retention time	USP Plate count	USP Tailing
1	10% less	2.398	3321	1.9
2	*Actual	2.305	2997	1.94
3	10% more	1.828	3315	1.8

Limit of detection

Based on the slope of the normal reaction deviation (SD) and the calibration curve (S), the LOD's can be calculated at ages approximating the LOD by formula. Based on the standard deviation of the regression line *y*-intercepts, the normal reaction deviation can be determined.

Limit of quantification

LOQ's can be calculated based on the formula based standard response deviation (SD) and calibration curve slope (S). Again, it is possible to determine the normal reaction value based on the standard deviation of regression line *y*-intercepts.

Forced degradation studies[18-22]

Stock solutions prepared by dissolving 10 mg of daclatasvir working standard into a 10 mL diluents. Table 2 shows degradation data of daclatasvir.

Acid degradation

0.6 mL of Daclatasvir from the above stock solution pipetted into a 10 mL volumetric flask, added about 3 mL of 0.1 N HCl and sonicated for 10 minutes and kept it in darkness for

12 hours, then refluxed for 1 hour in a heating mantle at 60 °C. The sample solution was neutralized using 0.1 N NaOH and diluted with diluents to the mark. The final sample was filtered through 0.44-micron injection filters and injected into HPLC system. Chromatogram representing acid degradation is shown in Figure 4.

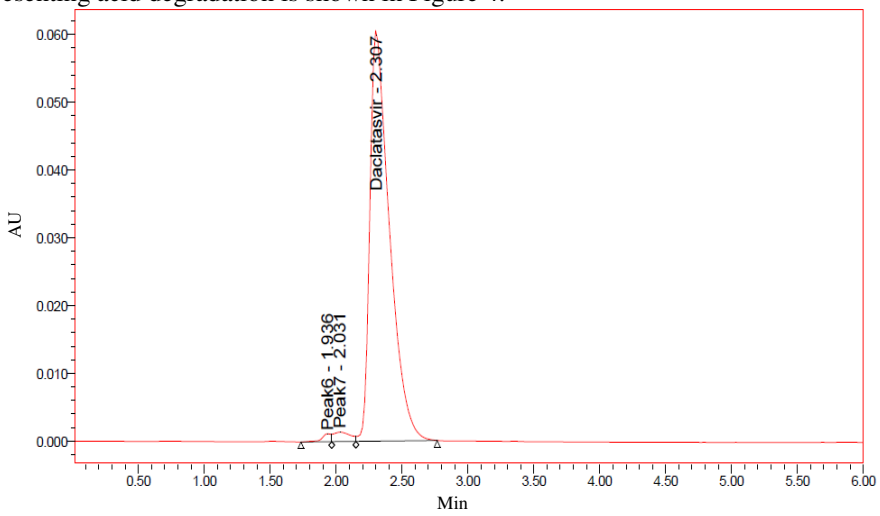

Figure 4. Chromatogram representing acid degradation

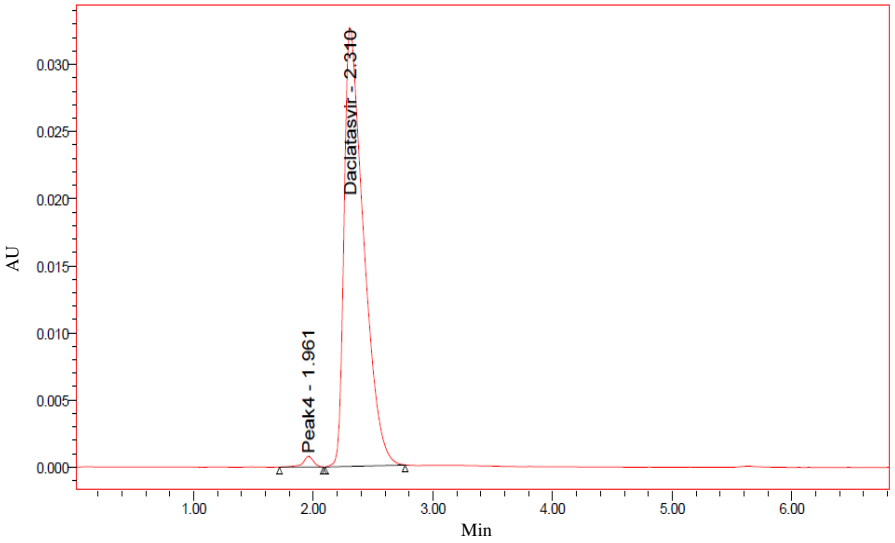

Figure 5. Chromatogram representing base degradation

Base degradation

0.6 mL of Daclatasvir from the above inventory solution was pipette out into a 10 mL volumetric flask and about 3 mL of 0.1 N NaOH was added and sonicated for 10 minutes and kept it in darkness for 12 hours, then refluxed for 1 hour in a heating cabinet at 60 °C. The sample solution was neutralized with 0.1 N HCl and diluted with diluents up to the mark. The final sample was filtered with 0.44 micron injection filters and injected into HPLC system. Chromatogram representing base degradation is shown in Figure 5.

Peroxide degradation

0.6 mL of Daclatasvir from the above stock solution pipetted into a 10 mL volumetric flask added approximately 3 mL of 3 percent hydrogen peroxide (H_2O_2) and sonicated for 10 minutes, kept in the dark for 12 hours and refluxed for 1 hour under heat at 60 degrees in a heating mantle. Chromatogram (Figure 6) showing the final sample was filtered through 0.44 micron injection filters and injected into the HPLC system.

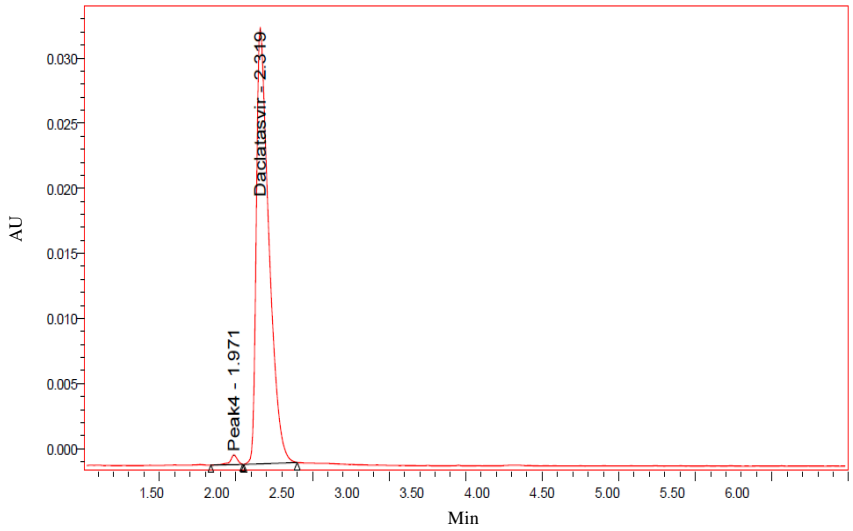

Figure 6. Chromatogram representing oxidative degradation

Thermal degradation

0.6 mL of the stock solution transferred into a 10 mL volumetric flask and kept in the oven for 12 hours under heat at 105 degrees and diluted with diluents up to the mark. The final sample was filtered and injected into the HPLC system through 0.44 micron injection filters. The thermal degradation chromatogram is shown in Figure 7.

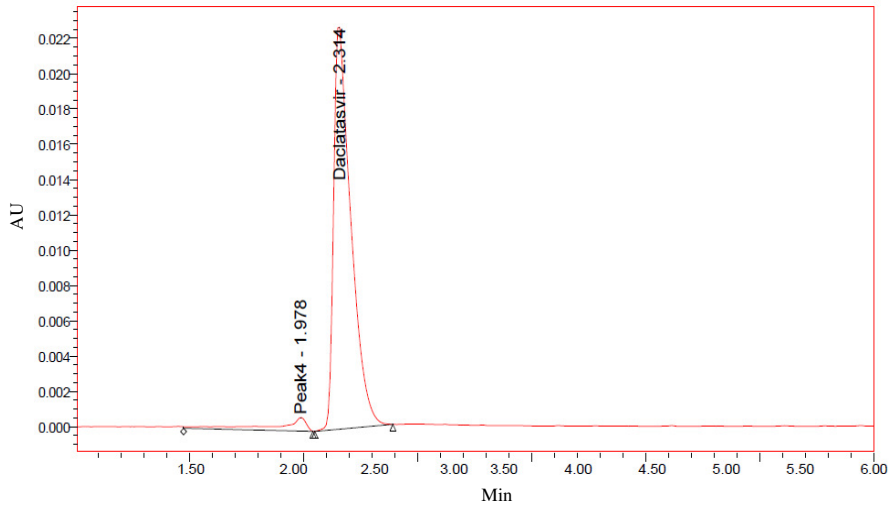

Figure 7. Chromatogram representing thermal degradation

Stability indicating analytical method for daclastavir dihydrochloride provided the suitable method for the quantification with no interference by other degraded products. Mobile phase optimization provided the suitable chromatographic conditions and increased peak symmetry about 1.29 along with peak parameters, tailing factor and others. Moreover, acceptable resolution of daclastavir dihydrochloride and the degradation products was obtained, confirming the stability idicating capability of the proposed method. For selection of the best wavelength of detection, a PDA detector was used. A satisfactory separation with good peak symmetry and steady baseline was achieved with Xterra C_{18} (4.6x150 mm, 5.0 µm) column and methanol-buffer of pH 3.5 (70:30 v/v) as the mobile phase at a flow rate of 1.0 mL/min. The quantitation of daclastavir dihydrochloride was achieved at 237 nm. The optimized conditions of the HPLC method were validated for the analysis of daclastavir dihydrochloride in tablet formulations and application for QC.

Conclusion

An isocratic, simple, specific stability indicating RP-HPLC method was developed and validated in accordance with ICH regulations for the assessment of daclatasvir dihydrochloride API. The results of the bulk drug stress test performed under ICH guidelines revealed that degradation was extremely susceptible to light under acidic, alkaline, oxidative, heat and photolytic circumstances by liable to acid, base, thermal and oxidative conditions.

Conflict of interest

Authors expressed no conflict of interest

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