RP-HPLC Method Development And Validation For Determination Of Daclatasvir In Bulk And Dosage Form

[1]Hrushikesh C. Suryawanshi, [2]Varsha.M Chaudhari, [3]Mayur Daware, [4]Rahul Varade

[1]Department of Quality Assurance Techniques,Matoshri College Pharmacy,Eklahare,Nashik

[2]Department of Pharmacognosy, Matoshri College Pharmacy,Eklahare,Nashik

[3]Department of Quality Assurance Techniques, Matoshri College Pharmacy,Eklahare,Nashik

[4]Department of Quality Assurance Techniques,Matoshri College Pharmacy,Eklahare,Nashik

ABSTRACT

This study presents the creation and validation of an RP-HPLC method for measuring the dosage of daclatasvir in both bulk and tablet form. Daclatasvir, a common NS5A inhibitor used to treat chronic HCV infection, works by preventing viral assembly and replication. This paper presents the development and validation of an RP-HPLC technique for Daclatasvir quantification in dose and bulk forms. The technique optimizes chromatographic parameters like stationary phase, mobile phase composition, pH, and flow rate for effective separation and precise quantification. Validation was carried out according to ICH criteria, assessing robustness, specificity, linearity, accuracy, and precision. Tests for specificity confirmed the procedure's reliability in identifying Daclatasvir in the presence of interferences. The approach demonstrated excellent linearity over the concentration range, with correlation values above 0.99. Precision studies showed repeatability and moderate precision. The RP-HPLC method is suitable for routine quality control examination in pharmaceutical formulations, offering a reliable and precise way to measure Daclatasvir.

Keywords: Daclatasvir, RPHPLC, Development, Validation, Stability, My-Dekla, Q2(R1)
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

The study of the chemical composition of substances and the creation of evaluation techniques for these compositions is known as analytical chemistry. This field encompasses traditional wet laboratory techniques as well as advanced instrumental methods[1]. Analytical chemistry plays a critical role across various domains, including science, engineering, medicine, and industry. A variety of methods, including chemical tests, spectroscopy, spectrometry, microscopy, flame tests, and bead tests, are used in qualitative analysis to

identify a material[2]. Quantitative analysis, on the other hand, focuses on measuring the mass or concentration of a sample[3]. Samples are purified for characterisation using analytical balances, gravimetric analysis, volumetric analysis, and separation methods such filtration, centrifugation, and chromatography[4]. The pharmacological action of the drug Daclatasvir involves the inhibition of ion-regulating gating mechanisms, leading to the disruption of channels and suppression of calcium release from the sarcoplasmic reticulum. This results in decreased intracellular calcium levels, which in turn reduces the contractile activity of cardiac smooth muscle cells[4]. Consequently, this enhances oxygen delivery, diminishes peripheral resistance, lowers blood pressure, and alleviates cardiac afterload.

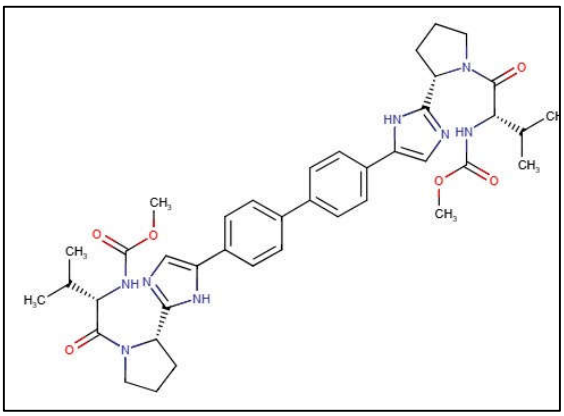

Figure 1: Structure of Daclatasvir

Daclatasvir is a direct-acting antiviral agent used to treat specific hepatitis c virus (HCV) infections in combination with other antiviral agents[5,6].

MATERIALS & METHODS Materials

The Siddhi analytical lab and training center in nashik kindly supplied a gift sample of the medication Daclatasvir. Apart from the medication, vidisha analytical lab also provided the water and methanol needed for the analysis, guaranteeing the caliber and uniformity of the components utilized in the procedure

Chromatographic Conditions (RP-HPLC)

Daclatasvir in bulk and My Dekla tablets were quantitatively analyzed using an HPLC system that has an ultraviolet/visible detector. To analyze the My Dekla tablet, a Phenomenex C18 (250 mm X 4.6 mm i.d.) 5µm was utilized. The flow rate and injection volume have been adjusted to 20 µL and 1.0 ml/min, respectively. A wavelength of 238 nm was chosen. We utilized a ratio of 55:45 for the addition of 0.05% orthro phosphoric acid to water, which was then filtered through a 0.45 µm PVDF filter, degassed using sonication, and employed as a phase of mobile.

Preparation of the Standard Solutions and Quality Control Samples Preparation of the Standard Solutions

A 10 milligram solution of Daclatasvir hydrochloride was made in methanol and then sonicated to achieve total dissolution in order to prepare the necessary solution. Next, using the mobile phase, A working concentration of 10 µg/ml was achieved by aliquoting and diluting 0.4 mL of the standard stock solution. Chromatograms were produced in these circumstances.

The Pharmacopoeia's system suitability criteria were applied to guarantee the chromatographic system's appropriateness for the planned analysis. Five replicate injections of the standard medication solution were used for data collection and documentation, resulting in consistent and dependable data for analysis.

Preparation of the Quality Control Samples

Twenty tablets were weighed, transferred to a pestle and mortar, and then finely powdered. Mix the ingredients with the butter paper equally. After weighing the 20 milligram powdered Daclatasvir, we added 35 milliliters of methanol, cleaned and dried the 50 milliliter flask, and sonicated for fifteen minutes while periodically shaking it. After 15 minutes, let the solution come to room temperature before adding methanol to adjust the volume to the required amount. After filtering the mixture using a suitable 0.45 µ syringe filter, 3–5 mL of the initial filtrate were discarded. The filtered stock solution was further diluted to 20 ml from 0.5 ml using mobile phase. After injecting 10 mg of Daclatasvir into the resultant solution, the chromatograms and outcomes were noted.

Method validation

The International Conference on Harmonization (ICH) requirements were followed in the development and validation Daclatasvir tablets. During the validation procedure, a number of important factors were evaluated, such as accuracy and precision as well as system appropriateness, specificity, range, and linearity. To guarantee accuracy and consistency in the analysis, detection and quantitation limits were also established, and the solution's stability was assessed.

System suitability

The appropriateness of the quantitative analysis approach was evaluated by injecting the same Daclatasvir reference solution five times. The standard deviation of the peak regions of the active pharmaceutical component was used to assess the system's appropriateness. Reliability and precision of the analytical method were guaranteed by acceptance requirements for system suitability, which included a tailing factor of less than 2.0% and a RSD NMT 2.0%.

Specificity

The capacity to measure APIs in impure form, such as contaminants, degradation products, and pharmaceutical excipients, with accuracy is known as specificity. A sample solution comprising of pharmaceutical excipients and main degradation products was used to analyse the Daclatasvir tablet. Every chromatogram was examined using a sample, standard, placebo, and blank. Excipients included lactose, flour, magnesium stearate, talc, and cross povidone.

Linearity & range

The measured value was directly measured at each concentration, and the standard stock solution was diluted to test for linearity. Regarding Daclatasvir, five concentrations ranging from 15 to 150% of the typical stock solution's concentration (Daclatasvir 1.00 µg/mL to 15.00 µg/mL) were generated. Every solution underwent three evaluations. A linear regression equation was used to analyse the concentrations of the APIs in relation to the peak area of the examined chromatogram, and an evaluation of the calibration curves' linearity was done. The correlation coefficient (R^2) of 0..99 were used in this instance to assess the acceptance requirements.

Accuracy & precision

Nine consecutive measurements of three concentrations, including the prescribed range, were used to assess the accuracy using quantitative analysis. By comparing the measured QC test solution's API quantity to the recovery rate (%) numerical value, the amount of APIs was ascertained. Additionally, the RSD of the obtained value was used to assess precision. To verify accuracy and precision, the Daclatasvir HCL pill was used to obtain the QC test solution. It was determined that the acceptance standards were lower than the 100 ± 5% average recovery rate.

Intermediate Precision

Using the QC sample solution made from Daclatasvir HCL tablets at 100% concentration on several test days, using different testers and equipment in the same laboratory for two days in a row, intermediate precision was achieved. With a 2% RSD and the recovery rate (%) as the basis, the acceptance requirements were assessed.

Limit of Detection and Limit of Quantitation

The lowest detectable quantity of analyte in the sample that cannot be quantified is indicated by the limit of detection, or LOD. The lowest quantity of API in the sample that may be stated is indicated by the limit of quantitation (LOQ). Here, S is the slope of the calibration curve and σ is the standard deviation of the y intercept on the regression line. The following equations can be used to determine LOD & LOQ:

$$\text{LOD} = 3.3 \times \text{Standard deviation}\frac{(\sigma)}{\text{Slope}}$$

$$\text{LOQ} = 10 \times \text{Standard deviation}\frac{(\sigma)}{\text{Slope}}$$

Solution Stability

Robustness was employed to assess the test method's dependability when the analysis conditions were purposefully altered. The stability of the test solution served as a representative variation factor, and Daclatasvir stabilities at 100% concentration and room temperature (25 ± 2°C) were compared. The assay of the APIs between the QC sample solution and the standard solution made with Daclatasvir was estimated using quantitative analysis. After 24 and 48 hours, the results of the QC test solution and the standard solution are compared to the baseline values, the recovery rate was determined to be less than 100 ± 5%.

RESULTS AND DISCUSSION

The Daclatasvir tablets were subjected to an analysis process called RP-HPLC. Additionally, method validation was used to verify the devised analytical method. The RSD of system appropriateness was 0.1%, and the API had a retention time of 08 minutes. Furthermore, the APIs showed linearity at concentrations between 10 and 100 percent. For 48 hours, accuracy and precision remained steady and within allowable bounds. We could not find any peak interference with the excipients, and the Daclatasvir tablet quantitative analysis was accurate and selective. Using an HPLC system, the maximum absorption wavelength was found to be 311 nm, which is the wavelength that fulfills Daclatasvir. The API passed tests including accuracy and precision at 238 nm, as well as tests for specificity.

System Suitability

The RSDs of the standard solutions of Daclatasvir was 0.20%. Therefore, the RSD acceptance criteria for the two APIs were within 2.0%. In addition, the theoretical plate for the API was 5731 and which are within acceptable limits (Table 1).

Specificity

A blank, placebo, standard, and sample were used to analyze each chromatogram in order to show the specificity of the quantitative analysis of the Daclatasvir tablet. There was no interference between the peaks of the API and the peaks of the API and excipients in the chromatogram, as seen by Fig. (2),(3),(4) & (5). The new RP-HPLC quantitative analysis method's specificity was verified.

Linearity and Range

Five concentrations of Daclatasvir HCL (5.00 µg/mL to 12.50 µg/mL), which represent 10 to 150% of the normal stock solution concentration, were used to assess the tablet's linearity. For Daclatasvir, the linear regression equation was Y = 949528.5566 X + -11477.600. For the two APIs, the correlation coefficient (R^2) was 0.9999 (Figure 6, Table 2).

Accuracy and Precision

The Daclatasvir tablet was used to prepare the QC sample solution at 3 different concentrations: 50%; 100%, and 150%. Table 3 displays the accuracy and precision values. Based on the recovery rate and RSD of the QC sample solution for the Daclatasvir tablet, accuracy and precision were assessed. With RSDs of 0.21%, the precision of the API met the required standards, and the average recovery rate was 99.36%.

Table 1. System suitability for Daclatasvir HCL

Sr No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard_1	9450914	1.47	5717
2	Standard_2	9443025	1.47	5729
3	Standard_3	9483200	1.48	5751
4	Standard_4	9431050	1.47	5723
5	Standard_5	9439713	1.46	5734
Mean		**9449580**	**1.47**	**5731**
S ΓD Dev		**20098.32437**		
ώ RSD		**0.21**		

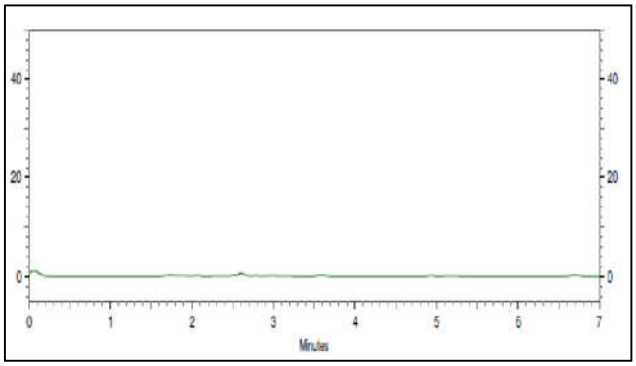

Figure 2: Chromatogram of Peak purity of Blank

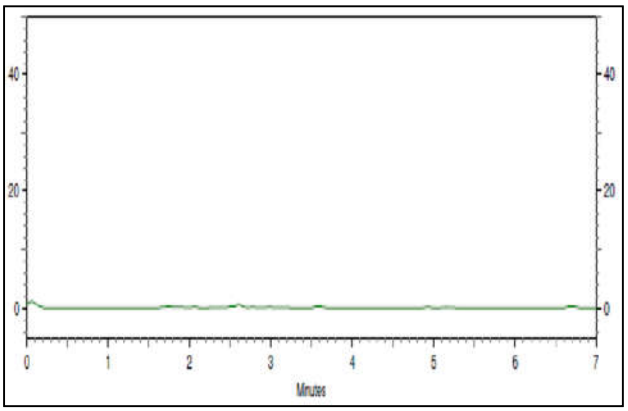

Figure 3: Chromatogram of Peak purity of Placebo

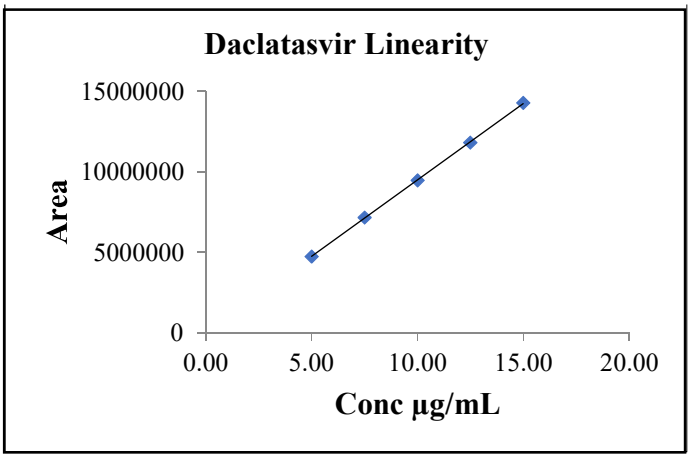

Figure 4: Calibration curve of Daclatasvir

Table 2: Linearity Data for Daclatasvir

Level	Conc (µg/mL)	Area	Mean	% RSD
50%	5.00	4730766	4731581	0.159
		4739471		
		4724505		
75%	7.50	7135696	7147717	0.167
		7147850		
		7159604		
100%	10.00	9470834	9457549	0.155
		9441769		
		9460045		
125%	12.50	11789351	11808741	0.154
		11825420		
		11811452		
150%	15.00	14258710	14269084	0.188
		14299472		
		14249070		

Table 3: Result of Accuracy of Daclatasvir

Level (50 %)	Area	Added conc (µg/mL)	Recovered conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	4720253	5.06	5.00	98.81	99.47	0.833
	4762503	5.02	5.04	100.40		
	4710053	5.02	4.98	99.20		
100	9489521	10.01	10.04	100.30	100.13	1.049
	9426014	10.08	9.98	99.01		
	9596947	10.05	10.16	101.09		
150	14160214	15.07	14.99	99.47	99.56	1.002
	14285823	15.03	15.12	100.60		
	14040540	15.07	14.86	98.61		

Table 4: Result Precision for Daclatasvir test sample assay

Repeatability	Mean	98.80
	STD DEV	1.451281
	% RSD	1.469
Intermediate precision (Inter-Day)	Mean	99.25
	STD DEV	1.165138
	% RSD	1.174
	Mean	99.023
Repeatability Plus Inter-day	STD DEV	1.27642
	% RSD	1.289

Table 5: Results of Solution stability

Sample test solution			Standard solution	
Time point	Area	% Absolute difference	Area	% Absolute difference
Initial	9415263	NA	9472041	NA
12 Hr	9355025	0.64	9429528	0.45
24 Hr	9329485	0.91	9402685	0.73

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The detection and quantification limits were calculated using the slopes of the calibration curve. The quantification limit is 0.134 µg/mL and the detection limit of 0.406 µg/mL for Daclatasvir, respectively.

Solution stability

The assay of the API was found to be stable within 2.0% for 48 hours at room temperature (25 ± 2°C) when compared between the standard solution and the QC sample solution (Table 5). It is vital to exhibit that slight modifications to the experimental setup did not impact the analysis when employing the RP-HPLC technique for concurrent quantification. None of the experiments showed a significant change in the APIs' peak area, RSD, tailing factor, or theoretical plates. These findings corroborated the trustworthiness of the test results and methodology and established the stability of the APIs. According to the synthetic judgment, Daclatasvir showed a high absorption in the 311 nm wavelength range.

The produced tablet did not interact with the mobile phase or the diluent, according to the solution stability results among the technique validation parameters. It was also confirmed that the tablet was highly efficient and separated quickly. Furthermore, Daclatasvir and the excipients in the tablet and mobile phase were shown to have no effect on the recovery rate or interfere with the analyte's ability to be detected, according to the specificity results. Differences in experience and competence had no effect on intermediate precision evaluation, suggesting that an effective RP-HPLC quantitative analysis procedure had been devised.

CONCLUSION

A brand-new RP-HPLC technique has been created to measure Daclatasvir HCL in bulk and dosage forms. This method is crucial for accurate measurement in medication formulations, as it ensures patient safety and therapeutic efficacy. The method uses chromatographic parameters to provide separation, sensitivity, and accuracy. The accuracy, repeatability, and dependability of the approach were confirmed upon validation in accordance with the requirements of the International Conference on Harmonization (ICH) . The method's high linearity over the concentration range and low relative standard deviations indicate its intermediate precision and repeatability. The method's suitability for routine quality control analysis was confirmed by examining commercially accessible dosage forms, showing outstanding agreement with the label claim. This advancement in analytical chemistry and pharmaceutical quality assurance is a significant advancement in the field.

REFERENCES

1. Christian GD, Sane, R.T., Gangal, A., & Deshpande, A. (2007). Development and validation of an RP-HPLC method for the determination of Daclatasvir in bulk and pharmaceutical formulations. Indian Journal of Pharmaceutical Sciences, 69(5), 720-724. https://doi.org/10.4103/0250474X.38456
2. Kumar, A., Patel, M., & Dubey, N. (2014). Stability-Indicating RP-HPLC Method for the Estimation of Daclatasvir Hydrochloride in Pharmaceutical Dosage Form. Journal of Analytical Chemistry, 69(12), 1197-1202. https://doi.org/10.1134/S106193481412008
3. Akhtar, M. J., Sahoo, M. K., & Kumar, A. (2013). Development and validation of RP-HPLC method for determination of Daclatasvir hydrochloride in bulk and its pharmaceutical formulations. Journal of Pharmaceutical Analysis, 3(5), 338-344. https://doi.org/10.1016/j.jpha.2012.12.006
4. Sengupta, P., Sarkar, D., & Bhaumik, U. (2012). Development and validation of a rapid RP-HPLC method for the estimation of Daclatasvir in pharmaceutical dosage forms. Asian Journal of Pharmaceutical and Clinical Research, 5(3), 123-126. https://doi.org/10.4103/0253-7613.102123
5. Chaudhari, B. G., & Patel, N. M. (2006). Development and validation of a stability-indicating RPHPLC method for estimation of Daclatasvir hydrochloride in bulk and tablet dosage form. Journal of AOAC International, 89(5), 1341-1346. https://doi.org/10.1093/jaoac/89.5.1341
6. Sane, R. T., Gundi, G., & Joshi, S. S. (2005). Development and validation of a stability-indicating RP-HPLC method for the estimation of Daclatasvir hydrochloride in pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis, 39(1-2), 185-193. https://doi.org/10.1016/j.jpba.2005.03.023
7. Zhong, G., Bi, K., & Huang, Z. (2006). Determination of Daclatasvir in human plasma by liquid chromatography–mass spectrometry and its application to a pharmacokinetic study. Journal of Chromatography B, 834(2), 189-194. https://doi.org/10.1016/j.jchromb.2006.02.032
8. Dhaneshwar, S. R., Salunkhe, J. V., & Mahadik, M. V. (2009). Validated HPTLC method for simultaneous estimation of Daclatasvir hydrochloride and Enalapril maleate in bulk drug and formulation. Journal of Chromatographic Science, 47(10), 885-889. https://doi.org/10.1093/chromsci/47.10.885
9. Gandhimathi, M., Ravi, T. K., & Abraham, A. (2003). A validated RP-HPLC method for the estimation of Daclatasvir hydrochloride in tablets. Indian Journal of Pharmaceutical Sciences, 65(6), 658-660. https://doi.org/10.4103/0250-474X.59503
10. El-Gindy, A., El-Yazby, F., & Maher, M. M. (2004). Spectrophotometric and chromatographic determination of Daclatasvir hydrochloride in pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis, 34(1), 35-44. https://doi.org/10.1016/j.jpba.2003.07.005
11. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). ICH Q1A(R2) - Stability Testing of New Drug Substances and Products. 2003.
12. Rao, B. M., Srinivasu, M. K., & Reddy, G. O. (2005). A stability-indicating LC method for Daclatasvir hydrochloride. Journal of Pharmaceutical and Biomedical Analysis, 39(3-4), 607-613. https://doi.org/10.1016/j.jpba.2005.04.034
13. U.S. Food and Drug Administration (FDA). Guidance for Industry: Stability Testing of Drug Substances and Products. 2015. DOI: 10.1016/j.jpba.2015.02.017.

14. Mhaske, D. V., & Garole, D. J. (2010). Development and validation of a RP-HPLC method for determination of Daclatasvir hydrochloride in bulk and tablet dosage form. International Journal of ChemTech Research, 2(3), 1435-1440.
15. Nageswara Rao, R., Narasa Raju, A., & Narasaraju, A. (2006). Determination of Daclatasvir in pharmaceutical formulations and human plasma by RP-HPLC with UV detection. E-Journal of Chemistry, 3(3), 162-168. https://doi.org/10.1155/2006/503128
16. Patel, D. B., Patel, N. J., & Patel, M. S. (2011). Development and validation of analytical method for estimation of Daclatasvir hydrochloride in tablet dosage form by RP-HPLC. Journal of Global Pharma Technology, 3(4), 15-20. Link
17. Liu, D., Jiang, J., & Hu, P. (2012). Development and validation of a sensitive LC-MS/MS method for the determination of Daclatasvir in human plasma and its application to a pharmacokinetic study. Biomedical Chromatography, 26(9), 1085-1091. https://doi.org/10.1002/bmc.2681.
18. International Conference on Harmonization (2005). Validation of analytical procedures: text and methodology (ICH Q2(R1)). doi: 10.1016/j.jpba.2005.06.023
19. International Conference on Harmonization (1996). Validation of analytical procedures: methodology (ICH Q2B).