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Research Article

Validation of a Cost-Effective RP-HPLC Method for Quantitative Investigation of Daclatasvir Dihydrochloride in Pharmaceutical Formulations

Sadaf Sarfraz,[1] Muhammad Saeed,[1] Mohsin Javed,[2] Nadia Hussain,[3,4] Shahid Iqbal,[5] Shahid Amin,[6] Ali Bahadur,[7] Ahmed Hussain Jawhari,[8] Randa A. Althobiti,[9] Eman Alzahrani,[10] Abd-ElAziem Farouk,[11] Samar O. Aljazzar,[12] and Eslam B. Elkaeed[13]

[1]*Department of Chemistry, Lahore Garrison University, Lahore, Pakistan*
[2]*Department of Chemistry, School of Science, University of Management and Technology, Lahore, Pakistan*
[3]*Department of Pharmaceutical Sciences, College of Pharmacy, Al Ain University, Al Ain, UAE*
[4]*AAU Health and Biomedical Research Center, Al Ain University, Abu Dhabi, UAE*
[5]*Department of Chemistry, School of Natural Sciences (SNS), National University of Science and Technology (NUST), H-12, Islamabad 46000, Pakistan*
[6]*PINSTECH, Nilore, Islamabad, Pakistan*
[7]*Department of Chemistry, College of Science and Technology, Wenzhou-Kean University, Wenzhou 325060, China*
[8]*Department of Chemistry, Faculty of Science, Jazan University, P.O. Box 2097, Jazan 45142, Saudi Arabia*
[9]*Department of Chemistry, College of Science, University of Bisha, P.O. Box 511, Bisha 61922, Saudi Arabia*
[10]*Department of Chemistry, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia*
[11]*Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia*
[12]*Department of Chemistry, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia*
[13]*Department of Pharmaceutical Sciences, College of Pharmacy, AlMaarefa University, Riyadh 13713, Saudi Arabia*

Correspondence should be addressed to Shahid Iqbal; shahidgcs10@yahoo.com, Ali Bahadur; abahadur@wku.edu.cn, and Eslam B. Elkaeed; ikaeed@mcst.edu.sa

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A well-known direct-acting antiviral (DAA) drug called daclatasvir may be used to treat chronic hepatitis C virus (HCV) infection. Herein, we reported a selective, precise, and a cost-effective analytical method for the measurement of an active pharmaceutical ingredient (API) of daclatasvir dihydrochloride in drug substances as well as drug products via the reversed-phase RP-HPLC technique. To obtain greater separation, the majority of the chromatographic conditions were improved. Best separation findings were achieved under chromatographic conditions with an HPLC column of USP L1 (150×4.6 mm, 5 μm) by utilizing a combination of acetonitrile and buffer solution of KH_2PO_4 (30 : 70, v/v) as a mobile phase at a stream rate of 1 mL.min^{-1} with a finding at 300 nm and a column temperature of 40°C. Linearity was examined in the range of 90–210 ppm ($R^2 = 0.999$) for daclatasvir dihydrochloride. The new technique has been verified using industry-recognized criteria, including applicability, system precision, accuracy, robustness, specificity, range, linearity, quantification limit, reagent stability, and detection limit. All the measured metrics were determined to be within acceptable limits using the criteria of the Worldwide Council for Harmonisation (ICH). In pharmaceutical labs, daclatasvir dihydrochloride may be analyzed qualitatively and quantitatively using the well-established RP-HPLC technique. Our study also highlights the need to evaluate the greenness of the method developed using a recognized tool ,i.e., Analytical Greenness Metrics (AGREE).

1. Introduction

A newly licenced direct-acting antiviral (DAA) medication called daclatasvir, sold under the brand name Daklinza®, is used to treat persistent HCV infection [1–3]. It is a novel inhibitor targeting the nonstructural protein 5A (NS5A) of HCV, developed by Bristol-Myers Squibb [4]. Daclatasvir binds to the N-terminus of NS5A to exert its inhibitory effects on viral RNA replication and virion assembly [5]. This interaction leads to structural changes in the protein, ultimately inhibiting its functions [6]. The chemical composition of daclatasvir dihydrochloride and the analytical methods employed to analyse it have been crucial in its development and clinical use.

Daclatasvir (generic name, INN) is a pharmaceutical drug having a molecular formula $C_{40}H_{52}Cl_2N_8O_6$ with a molecular weight of 811.8 g mol^{-1} [6]. The structural representation of the daclatasvir dihydrochloride drug is provided in Figure 1. This drug can be obtained by combining one mole of daclatasvir with two equivalent moles of hydrochloric acid. However, the qualitative and quantitative measurements of these types of expensive pharmaceutical drugs require a rapid, selective, precise, and cost-effective analytical method that needs to be developed and validated for their commercial applications [7].

Several analytical (chromatographic and spectroscopic) methods have been reported in the literature to determine the amount of daclatasvir dihydrochloride drug in bulk and dosage forms [8, 9]. These reported chromatographic methods have several drawbacks which make them unsuitable for their commercial application in pharmaceutical industries. In addition, previously reported methods to determine daclatasvir dihydrochloride entail low sensitivity. Mostly, the complications are associated with column dimensions [10] and retention times [11, 12]. In the case of spectroscopic methods, impurities, degradants, and other excipient components can interfere with the absorbance of the desired analyte in the sample, and consequently, the observed results may differ from the true value [13, 14]. However, in the presence of contaminants or excipients that elute at various intervals, chromatographic procedures give an accurate estimate of the desired analyte, making quantification achievable [15, 16]. The comparison indicates that the quantification by HPLC is better than that carried out via UV-Visible spectroscopy [17]. Hence, there is a strong need to develop a standard chromatographic method to measure the actual amount of daclatasvir dihydrochloride in drug substances as well as the product of daclatasvir tablets.

Using RP-HPLC, we developed an effective analytical approach for the detection of daclatasvir dihydrochloride medications to fill this research gap. To achieve better separation, all the parameters, including (i) column temperature, (ii) flow rate, (iii) mobile phase ratio, and (iv) HPLC column length, were tuned. The proposed technique is approved for use in terms of reagent stability, detection limit, precision, accuracy, specificity, robustness, linearity, range, and system appropriateness. Its simplicity, dependability, selectivity, and speed are used to define the originality of this RP-HPLC technology. The results of this research suggest that the suggested approach has a number of benefits over the previously published method because it makes it possible to determine the examined medication in a straightforward and sensitive manner with reliable Greeness results. A short description of the suggested strategy for comparing the results of the present investigation with those previously published is given in Table 1.

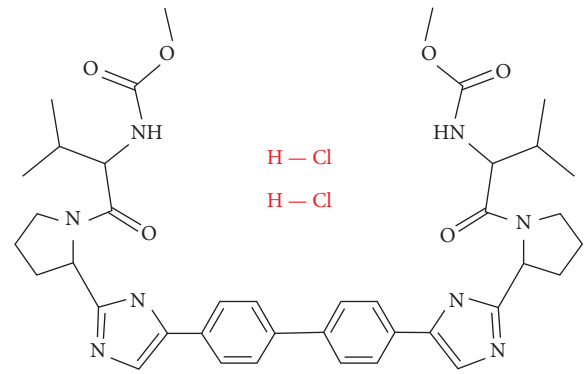

Figure 1: Structural formula of daclatasvir dihydrochloride drug.

2. Materials and Methods

2.1. Chemicals and Reagents. Acetonitrile (CH_3CN, 99.8%), potassium dihydrogen phosphate (KH_2PO_4, >99%), phosphoric acid (H_3PO_4, 99.99%), and deionized water for chromatography (HPLC grade) were procured from Merck. Reference samples of daclatasvir dihydrochloride and drug products were supplied by the pharmaceutical industry in Lahore.

2.2. Preparation of Solutions

2.2.1. Buffer Preparation. The pH of this solution was raised to 3.0 ± 0.05 using diluted phosphoric acid, and the volume was increased to one litre with deionized water after accurately weighing and dissolving 2.73 g of KH_2PO_4 in about 800 mL of deionized water. After being sonicated for ten minutes, a 0.45 m hydrophobic PTFE membrane filter was used to filter the buffer solution (Millipore, USA).

2.2.2. Mobile Phase Preparation. A 30 : 70 (v/v%) combination of acetonitrile and a buffer solution of potassium dihydrogen phosphate was made, and it was sonicated for 30 minutes to remove gas. In addition, acetonitrile and water were taken as diluents in a proportion of 20 : 80 (v/v%).

2.2.3. Preparation of the Typical Stock Solution. A 50 mL volumetric flask was filled with precisely weighed 41.25 mg of daclatasvir dihydrochloride, which is equivalent to 37.50 mg of daclatasvir. To achieve the desired concentration of 750 ppm, daclatasvir was dissolved into the diluent by sonicating the flask for 10 minutes. A 0.45 m hydrophobic PTFE membrane filter was also used to filter the standard stock solution.

Table 1: Comparison of performance features of the current work vs published work.

Analytical techniques	Linearity (ppm)	Remarks	Reference
HPLC-UV	0.6–6	Poor sensitivity acetonitrile–10 mM acetate buffer	[18]
HPLC–UV	0.5–101	Poor sensitivity phosphate buffer : acetonitrile (50 : 50) $t_R = 2.33$ min	[19]
HPLC–UV	2–199	Poor sensitivity phosphate buffer : acetonitrile (60 : 40) $t_R = 7.3$ min	[14]
HPLC–UV	10–151	Poor sensitivity water and methanol (20 : 80) $t_R = 2.0$ min	[20]
HPLC–UV	21–80	Poor sensitivity acetonitrile : methanol (70 : 30) $t_R = 2.7$ min	[11]
HPLC–UV	2–100	Poor sensitivity methanol : water : acetonitrile (25 : 30 : 45) $t_R = 6.9$ min	[21]
HPLC–DAD	0.6–60	Poor sensitivity phosphate : acetonitrile (75 : 25) $t_R = 5.4$ min	[5]
Derivatization	6–50	Very poor sensitivity and reagent use	[13]
Spectrophotometry	4–12	Low sensitivity	
Proposed HPLC method	90–210	Highly sensitive and simple method with agree (greenness calculator) score 0.68	—

2.2.4. Formation of the Standard Solution. To create the typical solutions of 90, 120, 150, 180, and 210 ppm, correspondingly, 3.0, 4.0, 5.0, 6.0, and 7.0 mL of the standard stock solution (750 ppm) were accurately transferred into each 25 mL volumetric flask, diluted to the proper concentration with dilutant, and thoroughly mixed by sonication.

2.2.5. Preparation of a Sample Solution. Ten daclatasvir tablets were roughly weighed, ground, and added to the sample in a 50 mL volumetric flask. Each tablet contained around 37.50 mg of the medication. The mixture was then sonicated for 25 minutes to thoroughly saturate the active ingredient in the diluent after 30 mL or so of diluent was inserted. After being diluted to the right concentration, the solution was filtered and kept at room temperature. A 25 mL flask was filled to the mark with a diluent after 5.0 mL of the sample solution's supernatant was transferred there. This created the sample solution, which had an estimated concentration of 150 ppm.

2.3. Characterization Techniques. A Shimadzu HPLC system (20AT, Kyoto, Japan) coupled with a UV-visible detector having dual-wavelength was used in this study. The system also has gradient elution capability and an autosampler associated with the Agilent HPLC column of USP L1 (150×4.6 mm, 5 μm) containing octadecylsilyl silica gel as a stationary phase [22]. For this investigation, a pH metre (S220, Mettler Toledo), an ultrasonic water bath (8510, Branson), and an analytical balance (PB210S, Sartorius) were also used.

2.4. Chromatographic Conditions. By using chromatographic conditions on reversed-phase octadecylsilyl silica gel (stationary phase) with a pore size of 5 m and a stainless steel HPLC column with dimensions (150×4.6 mm) coupled with a UV-visible detector set at 300 nm, analytical separation was achieved. The mobile phase was isocratically eluted at a flow rate of 1 mL.min^{-1}. The system was equilibrated for 30 minutes before the solution was injected into the column.

3. Results and Discussion

3.1. Method Validation. To verify adherence to ICH standards and regulatory compliance Q2(R1) [23, 24], most of the analytical parameters were investigated which define the system's suitability by measuring relative standard deviation (RSD), standard deviation, accuracy in terms of average recovery for various spiked levels and tailing factor. Moreover, the precision, limit of detection (LOD), specificity, limit of quantification (LOQ), robustness, and correlation coefficient (R^2) in linearity were the basis of method validation.

3.1.1. System Suitability. A standard solution of daclatasvir dihydrochloride (150 ppm) was prepared as described above at 100% level and six replicate samples into the HPLC system were injected. The observed chromatogram representing system suitability for the standard solution of daclatasvir dihydrochloride is provided in Figure 2. The system suitability parameters were evaluated in terms of RSD value for retention time (0.06%), peak response (0.07%), tailing factor (~1.33), and theoretical plates (~2793) which were found within the acceptance criteria, as expressed in Table 2.

3.1.2. Linearity. Five concentrations, 90, 120, 150, 180, and 210 ppm, were produced (Table 3) and inserted into the HPLC system under chromatographic conditions for reference solutions that were 60 to 140 percent spiked. By creating a graph between different daclatasvir concentrations and their peak response, the linearity was examined. The correlation coefficient (R^2) was then calculated, as shown in Figure 3. The observed linear equation ($Y = 72744\ X + 385027$) shows the values of slope (72744), intercept (385027), and $R^2 = 0.9992$. The value of R^2 indicates excellent linearity [25].

3.1.3. Accuracy. A placebo of daclatasvir tablets was spiked with the standard solution in triplicate at 60%, 100%, and 140% concentration spiked levels. Figure 4 represents chromatogram showing no interference for the placebo sample of daclatasvir tablets. The estimate of the mean recovery of three levels for those three levels is shown in Table 4 because of the injection of these solutions into the HPLC device under predefined chromatographic conditions. The average recovery was calculated as 100.60 percent, which is within the acceptable range of accuracy (98.0 to 102.0 percent), with the mean recovery being determined to be 100.79, 101.01, and 99.99 percent, respectively.

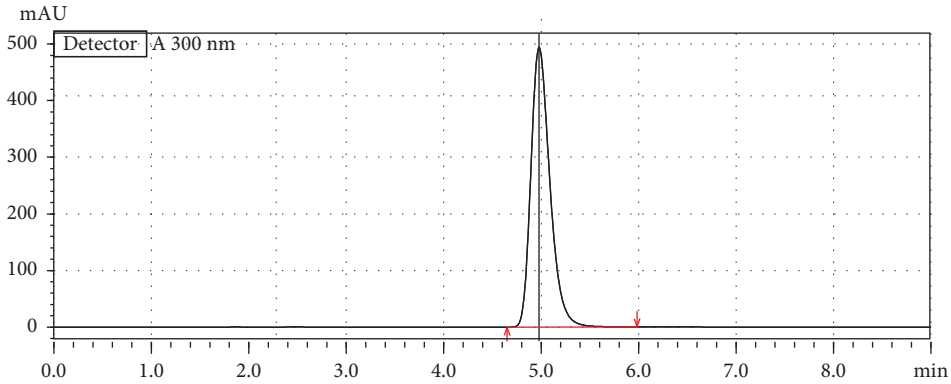

Figure 2: Chromatogram representing the system suitability for standard solution of daclatasvir dihydrochloride.

Table 2: System suitability studies for daclatasvir dihydrochloride.

Sr. no.	Parameters	Observed value	Acceptance criteria
1	RSD (retention time)	0.06%	<2.0%
2	RSD (peak response)	0.07%	<2.0%
3	Tailing factor	~1.33	0.8–2.0
4	Theoretical plates	~2793	>2000

Table 3: Various concentration of standard solution for linearity measurements.

Sample name	Volume of stock solution (mL)	Total volume (mL)	Final concentration (μg.mL^{-1}, ppm)
Linearity–S1	3.0	25	90
Linearity–S2	4.0	25	120
Linearity–S3	5.0	25	150
Linearity–S4	6.0	25	180
Linearity–S5	7.0	25	210

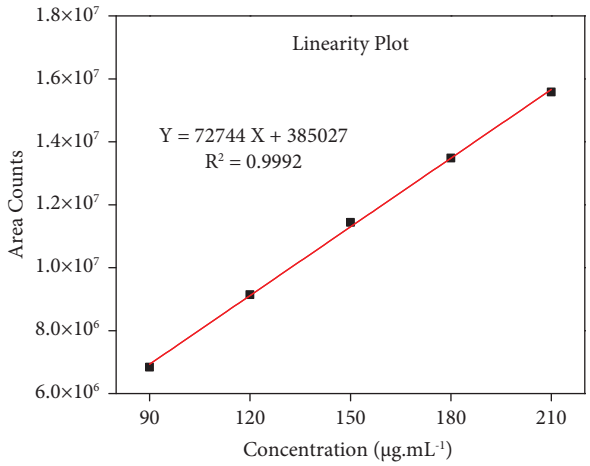

Figure 3: Linearity plot between various concentrations of daclatasvir and their peak response.

3.1.4. Range. The lowest and highest concentrations (amounts) of analyte in the sample at which the analytical technique may be used with an acceptable degree of accuracy, precision, and linearity are referred to as the limits of a method. Each of these fundamental qualities has had the validity of the developed analytical approach evaluated, and the results produced satisfy the criteria for approval, as displayed in Table 5.

3.1.5. Specificity. The specificity parameter was also estimated under the optimized chromatographic conditions by injecting various solutions of placebo material (without active substance) for both products of daclatasvir (30 mg, 60 mg) tablets in triplicate preparations against the standard solution [25–27]. It was found that there is no interference in the sample solutions for daclatasvir at the retention period of 5 min when compared to the reference solution, demonstrating the proposed method's outstanding specificity.

3.1.6. Precision. Using the standard method under normal operating conditions, six determinations of the assay were carried out on both products (30 mg, 60 mg) on the same day (intraday) by a single operator (precision-repeatability) as well as on a different day (interday) by another operator on different equipment (precision-intermediate) against the standard solution. The obtained results of the mean assay and % RSD are presented in Table 6. The calculated precision has average RSD values of 0.51% and 0.44% for 30 mg and 60 mg daclatasvir tablets, respectively. It was observed that the % RSD values for both products (30 mg and 60 mg) lie within the specified limit, i.e., <2%, which showed excellent precision of the developed method [28].

3.1.7. Robustness. Intentionally changing a single aspect of the method's operational circumstances also served to verify it. Six identical samples of a standard daclatasvir solution (150 ppm) were injected into the HPLC system in accordance with usual practise, with the flow rate, wavelength, and column temperature being adjusted. The peak responses were noted in order to evaluate the robustness of the suggested technique in terms of standard deviation and percent RSD. Figures 5–9 indicate chromatograms representing the robustness of

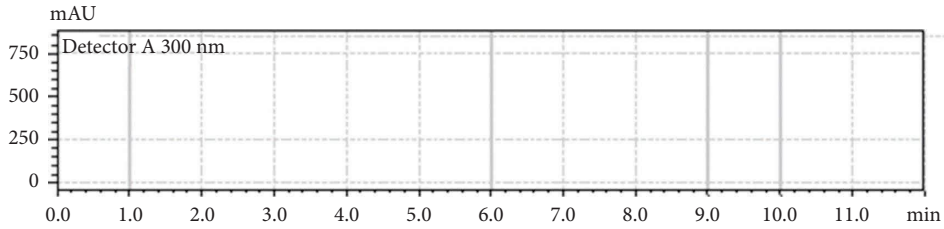

Figure 4: Chromatogram showing no interference for placebo sample of daclatasvir tablets.

Table 4: Various concentrations of daclatasvir and % average recovery data.

Analyte level	Theoretical concentration (ppm)	Measured concentration (ppm)	Individual recovery (%)	Mean recovery (%)
Level-1	90	90.22	100.25	100.79
	90	92.07	102.30	
	90	89.85	99.83	
Level-2	150	151.42	100.95	101.01
	150	151.35	100.90	
	150	151.77	101.18	
Level-3	210	211.43	100.68	99.99
	210	208.42	99.25	
	210	210.11	100.05	

Table 5: Precision, accuracy, and linearity data of RP-HPLC method of daclatasvir.

Sr. no.	Parameter	Results
1	% RSD (area)	0.07
2	% RSD (assay)	0.48
3	% RSD assay (cumulative)	0.53
4	Individual recovery	99.25–102.30%
5	Average recovery	100.6%
6	Correlation coefficient (R^2)	0.9992
7	Regression equation	$Y = 72744\ X + 385027$

Table 6: Precision data for 30 mg and 60 mg daclatasvir tablets.

Parameter	Operator-1 (day 1)	Operator-2 (day 2)	Average value
	30 mg daclatasvir tablets		
Mean assay (%)	99.35	99.30	99.33
RSD (%)	0.53	0.48	0.51
	60 mg daclatasvir tablets		
Mean assay (%)	99.20	99.46	99.33
RSD (%)	0.52	0.36	0.44

daclatasvir dihydrochloride standard solution at different values of flow rates (0.8 mL.min^{-1} and 1.2 mL.min^{-1}), wavelengths (298 nm and 302 nm), and column temperature (38°C), respectively. As shown in Table 7, the observed findings varied within the predetermined bounds, suggesting that the analytical procedure was unaffected by purposefully adjusting the wavelength, column temperature, and flow rate parameters.

3.1.8. Detection Limit. The LOD is a characteristic of a limit test and refers to the smallest quantity of analyte in a sample that can be detected but not always quantitated under certain experimental circumstances. The assessed value (1.40 ppm) of this parameter, which was calculated using the following formula under linearity, is shown in Table 7.

$$\text{LOD} = 3.3\,\text{x}\,\frac{\text{Standard deviation of area}}{\text{Slope}}. \quad (1)$$

3.1.9. Quantification Limit. The lowest quantity of the analyte in the sample that can be accurately and precisely identified under the experimental circumstances is referred to as the LOQ, and it is used to describe low levels of the substance in sample matrices. This parameter was also evaluated under linearity by the following formula, and the obtained value (4.25 ppm) is specified in Table 8.

$$\text{LOQ} = 10\,\text{x}\,\frac{\text{Standard deviation of area}}{\text{Slope}}. \quad (2)$$

3.1.10. Solution Reagent Stability. As per the standard analytical method, the sample as well as standard solutions of both products, i.e., daclatasvir 30 mg and 60 mg tablets, were analyzed under optimized chromatographic conditions. Then, these solutions were kept at ambient conditions for 12 hours and tested again under the same chromatographic conditions by recording their peak responses. Finally, the calculated % RSD values of standard and sample solutions are expressed in Table 9. It was observed that the % RSD values vary within the specified limits for both daclatasvir 30 and 60 mg tablets. Therefore, the solution reagent stability test has been found acceptable because the cumulative RSD values for both standard and sample solutions are less than 2%.

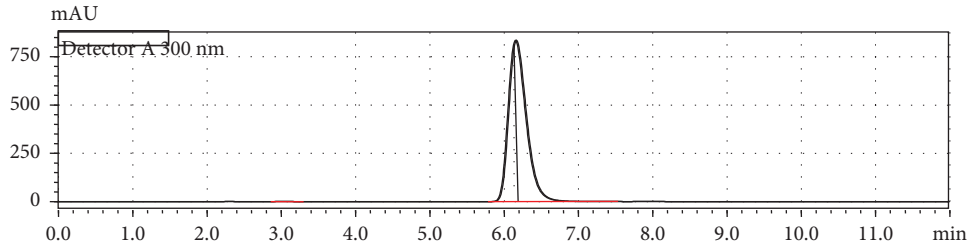

Figure 5: Chromatogram representing the robustness of daclatasvir dihydrochloride standard solution at flow rate of 0.8 mL.min^{-1}.

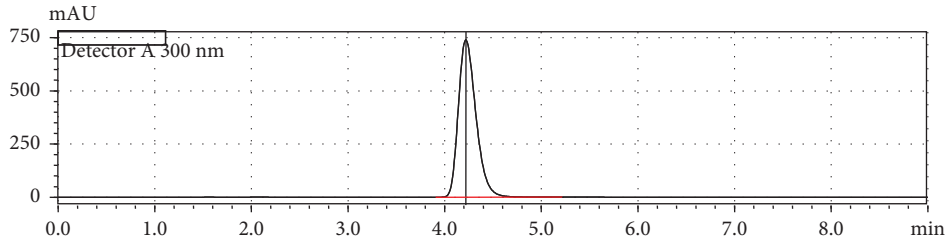

Figure 6: Chromatogram representing the robustness of daclatasvir dihydrochloride standard solution at flow rate of 1.2 mL.min^{-1}.

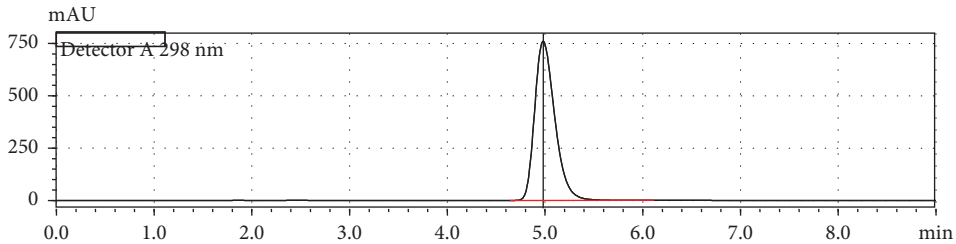

Figure 7: Chromatogram representing the robustness of daclatasvir dihydrochloride standard solution at wavelength of 298 nm.

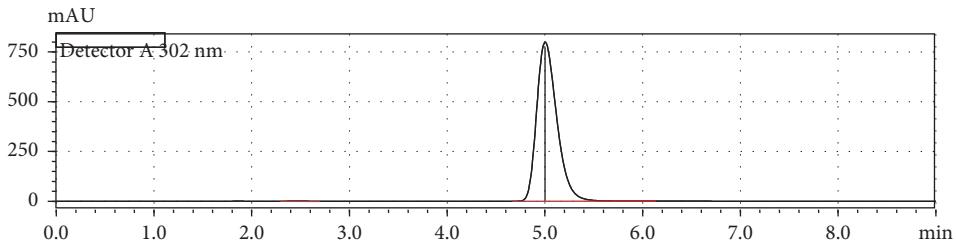

Figure 8: Chromatogram representing the robustness of daclatasvir dihydrochloride standard solution at wavelength of 302 nm.

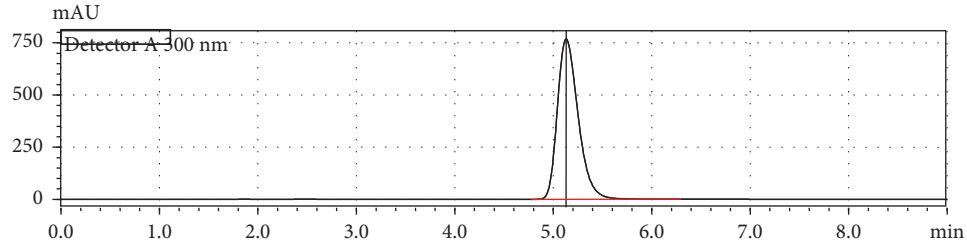

Figure 9: Chromatogram representing the robustness of daclatasvir dihydrochloride standard solution at column temperature of 38°C.

Table 7: By adjusting the wavelength, flow velocity, and column temperature, robustness data may be calculated in terms of standard deviation and percent RSD.

Parameter	Flow rate		Wavelength (λ_{max})		Temperature	
	0.8 mL.min^{-1}	1.2 mL.min^{-1}	298 nm	302 nm	@ 38°C	@ 42°C
Standard deviation	0.02	0.01	0.01	0.00	0.05	0.03
% RSD	0.26	0.29	0.16	0.02	1.02	0.62

Table 8: LOD and LOQ values for daclatasvir dihydrochloride.

Drug	Detection limit (ppm)	Quantification limit (ppm)
Daclatasvir dihydrochloride	1.40	4.25

Table 9: Solution stability data for 30 and 60 mg daclatasvir tablets.

Parameter	30 and 60 mg daclatasvir tablets		
	Standard	30 mg tablets	60 mg tablets
% RSD (initial)	0.05	0.57	0.46
% RSD (after 12 hrs)	0.90	1.24	0.79

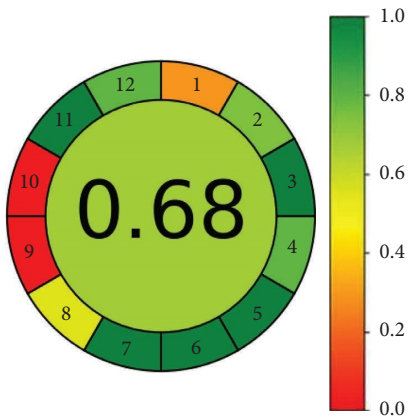

Figure 10: Agree analysis of the proposed method (reference colour scale is on the right, and the circle representing the outcome of the evaluation is on the left).

The findings of the present study were evaluated with those reported in the literature using chromatographic manner [19]. There was no discernible difference between the findings of the suggested approach and the stated HPLC method, according to statistical analysis of the acquired values done using Student's *t*-test (0.46) and variance *F*-test (1.81) [19].

3.2. Greenness of the Method Developed. In a range of settings, including government policy, industrial management, educational practise, and technological development, the ideas of green chemistry are increasingly extensively employed. A brand-new system for assessing greenness, the Analytical Greenness Metric (AGREE), is based on the 12 green analytical chemistry principles. AGREE is a thorough, adaptable, and simple evaluation approach that yields an understandable and instructive result. The factors that are taken into consideration in AGREE are drawn from the 12 GAC principles and converted into a common 0–1 scale. The availability of freeware software available at (git.pg.edu.pl/p174235/AGREE), which simplifies the applications for this metric, is one of its benefits. The final assessment result is the total of the scores for each of the 12 input variables, which are translated into scores on a 0–1 scale [18, 29–31]. The end result is a clock-like circle with the overall score and colour image in the centre, as shown in Figure 2 [21, 32, 33]. According to findings given in Figure 10, the AGREE score is 0.62. Based on the findings, our proposed approach is found to be greener and is thus recommended for the analysis of daclatasvir dihydrogen chloride in pharmaceutical products.

4. Conclusion

The antiviral compound daclatasvir dihydrochloride has been tested in both drug substance and drug product (tablet dose) forms using a straightforward, dependable, affordable, and quick RP-HPLC approach. The developed method has been found to be linear, selective, precise, accurate, robust, and reproducible, as per ICH guidelines. The acquired findings were found to be well within the predetermined limits after statistical analysis of all the parameters using standard deviation and RSD revealed little variance. The developed RP-HPLC method has been found more applicable compared to other analytical methods reported in the literature [8, 31]. Therefore, this RP-HPLC technique can be applied for qualitative and quantitative evaluation of daclatasvir dihydrochloride in pharmaceutical laboratories for routine quality control measurements in a very short time interval. In addition, the results of the greenness evaluation of the proposed method showcase an exceptional commitment to environmental sustainability and a promotion of a greener future.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

The manuscript was written with the contributions of all authors. All authors have approved the final version of the manuscript.

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References

[1] K. Chayama, S. Takahashi, J. Toyota et al., "Dual therapy with the nonstructural protein 5A inhibitor, daclatasvir, and the nonstructural protein 3 protease inhibitor, asunaprevir, in hepatitis C virus genotype 1b–infected null responders," *Hepatology*, vol. 55, pp. 742–748, 2012.

[2] Y. Fujii, Y. Uchida, and S. Mochida, "Drug-induced immunoallergic hepatitis during combination therapy with daclatasvir and asunaprevir," *Hepatology*, vol. 61, no. 1, pp. 400-401, 2015.

[3] A. Poudel, K. Bhuvan, S. Shrestha, and L. J. J. Nissen, "Access to palliative care: discrepancy among low-income and high-income countries," *Journal of global health*, vol. 9, 2019.

[4] O. Belda and P. Targett-Adams, "Small molecule inhibitors of the hepatitis C virus-encoded NS5A protein," *Virus Research*, vol. 170, no. 1-2, pp. 1–14, 2012.

[5] M. Baker, D. El-Kafrawy, M. Mahrous, and T. Belal, "Validated stability-indicating HPLC-DAD method for determination of the recently approved hepatitis C antiviral agent daclatasvir," *Annales Pharmaceutiques Françaises*, vol. 10, pp. 176–184, 2017.

[6] G. A. Shabir, "Validation of high-performance liquid chromatography methods for pharmaceutical analysis: understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization," *Journal of Chromatography A*, vol. 987, no. 1-2, pp. 57–66, 2003.

[7] M. Gad, S. Hassan, H. Zaazaa, and S. Amer, "Multivariate development and optimization of stability indicating method for determination of daclatasvir in presence of potential degradation products," *Chromatographia*, vol. 82, 2020.

[8] S. P. Dandamudi, J. K. Battineni, V. Bakshi, H. Peddapalli, and N. Boggula, "Validated RP-HPLC method for estimation of daclatasvir in tablet dosage form," *International Journal of Advanced Research and Development*, vol. 3, pp. 1170–1174, 2018.

[9] V. Kekan, S. Gholve, and O. Bhusnure, "Development, validation and stability study of UV spectrophotometric method for determination of daclatasvirin bulk and pharmaceutical dosage forms," *International Journal of ChemTech Research*, vol. 10, pp. 281–287, 2017.

[10] K. Sumathi, K. Thamizhvanan, and S. Vijayraj, "Development and validation of stability indicating RP-HPLC method for the estimation of daclatasvir in bulk and formulation," *Der Pharmacia Lettre*, vol. 8, pp. 107–113, 2016.

[11] S. T. Hassib, E. A. Taha, E. F. Elkady, and G. H. J. C. Barakat, "Reversed-phase liquid chromatographic method for determination of daclatasvir dihydrochloride and study of its degradation behavior," *Chromatographia*, vol. 80, no. 7, pp. 1101–1107, 2017.

[12] M. Gad, S. A. Hassan, H. E. Zaazaa, and S. M. J. C. Amer, "Multivariate development and optimization of stability indicating method for determination of daclatasvir in presence of potential degradation products," *Chromatographia*, vol. 82, no. 11, pp. 1641–1652, 2019.

[13] A. S. Eldin, S. M. Azab, A. Shalaby, M. J. J. O. P. El-Maamly, and P. Research, "The development of a new validated HPLC and spectrophotometric methods for the simultaneous determination of daclatasvir and sofosbuvir," *Antiviral Drugs*, vol. 1, pp. 28–42, 2017.

[14] K. Keyvan, M. R. Sohrabi, F. J. S. A. P. A. M. Motiee, and B. Spectroscopy, "Improved spectral resolution for the rapid simultaneous spectrophotometric determination of sofosbuvir and daclatasvir as anti hepatitis C virus drugs in pharmaceutical formulation and biological fluid using continuous wavelet and derivative transform," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 251, 2021.

[15] S. Yasien, M. M. Iqbal, M. Javed et al., "Quantification of multi-class pesticides in stomach contents and milk by gas chromatography–mass spectrometry with liquid extraction method," *Arabian Journal of Chemistry*, vol. 15, 2022.

[16] S. Iqbal, M. M. Iqbal, M. Javed et al., "Modified QuEChERS extraction method followed by simultaneous quantitation of nine multi-class pesticides in human blood and urine by using GC-MS," *Journal of Chromatography B*, vol. 1152, Article ID 122227, 2020.

[17] S. S. Hussain Shah, M. I. Nasiri, H. Sarwar et al., "RP-HPLC method development and validation for quantification of daclatasvir dihydrochloride and its application to pharmaceutical dosage form," *Pakistan Journal of Pharmaceutical Sciences*, vol. 34, no. 3, pp. 951–956, 2021.

[18] N. N. Atia, S. R. El-Shaboury, S. M. El-Gizawy, M. N. J. J. O. P. Abo-Zeid, and B. Analysis, "Simultaneous quantitation of two direct acting hepatitis C antivirals (sofosbuvir and daclatasvir) by an HPLC-UV method designated for their pharmacokinetic study in rabbits," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 158, pp. 88–93, 2018.

[19] A. E. Ibrahim, M. Elhenawee, H. Saleh, and M. M. Sebaiy, "Overview on Hepatitis C, treatment strategy, instrumental analysis of anti-HCV drugs," *Journal of Pharmacy and Drug Innovations*, vol. 2, no. 2, 2021.

[20] V. A. Chakravarthy and B. Sailaja, "Method development and validation of assay and dissolution methods for the estimation of daclatasvir in tablet dosage forms by reverse phase HPLC," *European Journal of Medical Research*, vol. 3, pp. 356–364, 2016.

[21] S. Bbv and P. Kumar A, "Stability indicating reverse phase high-performance liquid chromatographic method for simultaneous estimation of labetalol and its degradation products in tablet dosage forms," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 9, pp. 242–249, 2016.

[22] S. Renet, M. C. Chaumais, T. Antonini et al., "Extreme bradycardia after first doses of sofosbuvir and daclatasvir in patients receiving amiodarone: 2 cases including a rechallenge," *Gastroenterology*, vol. 149, no. 6, pp. 1378–1380.e1, 2015.

[23] S. Shrivastava, P. Deshpande, and S. J. J. O.R. U. Daharwal, "Key aspects of analytical method development and validation," *Journal of Ravishankar University (PART-B)*, vol. 31, no. 1, pp. 32–39, 2019.

[24] P. Borman and D. J. I. Elder, *Q2 (R1) Validation of Analytical Procedures: Text and Methodology*, Wiley Online Library, Hoboken, NJ, USA, 2017.

[25] P. K. Sahu, N. R. Ramisetti, T. Cecchi, S. Swain, C. S. Patro, and J. J. J. O. P. Panda, "An overview of experimental designs in HPLC method development and validation," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 147, pp. 590–611, 2018.

[26] R. Waghmode, V. Tegeli, and A. J. J. O.A. S. R. Dalal, "Analytical method development and validation of rp-hplc method for the estimation of sofosbuvir in bulk and formulation," *Journal of Advanced Scientific Research*, vol. 13, no. 7, pp. 40–45, 2022.

[27] R. Ahmed, M. N. Mostafa, and M. J. W. J. P. P. S. Rahmatullah, "Oral glucose tolerance test (OGTT) with a combination of Colocasia esculenta stems and Eichhornia crassipes aerial parts," *World Journal of Pharmaceutical Sciences*, vol. 7, pp. 207–214, 2018.

[28] R. Singh, M. Kumar, A. Mittal, and P. K. J. B. Mehta, "Microbial enzymes: industrial progress in 21st century," *3 Biotech*, vol. 6, pp. 1–15, 2016.

[29] M. G. Fawzy, E. B. J. S. A. P. A. M. Kamel, and B. Spectroscopy, "Sustainable Stability-Indicating spectra manipulations for the concurrent quantification of a novel Anti-COVID-19 drug and its active Metabolite," *Green profile assessment*, vol. 300, 2023.

[30] L. A. Al-Khateeb, M. El-Maghrabey, and R. El-Shaheny, "Sensitive determination of naftazone using carbon quantum dots nanoprobe by fluorimetry and smartphone-based techniques," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 302, Article ID 123109, 2023.

[31] R. Elgamal and F. Belal, "Stability indicating 1st derivative synchronous spectrofluorimetric method for the determination of the newly approved antiviral drug daclatasvir in presence of its oxidative and photolytic degradation products: application to tablet dosage form," *Pharmaceutica Analytica Acta*, vol. 9, no. 10, 2018.

[32] V. A. Chakravarthy and S. Bbv, "Method development and validation of ultraviolet-visible spectroscopic method for the estimation of hepatitis-C drugs- daclatasvir and sofosbuvir in active pharmaceutical ingredient form," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 9, pp. 61–66, 2016.

[33] A. E. Ibrahim, R. E. Saraya, H. Saleh, and M. J. H. Elhenawee, "Development and validation of eco-friendly micellar-hplc and hptlc-densitometry methods for the simultaneous determination of paritaprevir, ritonavir and ombitasvir in pharmaceutical dosage forms," *Heliyon*, vol. 5, 2019.