Egypt. J. Chem. **Vol. 65,** No. 2 pp. 81 - 91 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Development and Validation of HPLC Method for Quantification of Daclatasvir in Pure and Solid Dosage Form

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Abstract

Daclatasvir is a novel and highly selective NS5A replication inhibitor of HCV. It is stated to offer the potential for interferon free treatment when used in combination with another direct acting antivirals. The current project is aimed to develop and validate an easy to perform, precise, fast and accurate reverse phase HPLC method for Daclatasvir in the solid dosage form by wielding UV detector, in accordance with ICH Q2 (R1) guidelines. The process was carried out for daclatasvir, utilizing C-18 column (25 cm length, 4.6 mm internal diameter, and 5 µm particle size) in isocratic mode, with mobile phase comprising of acetonitrile and phosphate buffer (2:3 v/v). The flow rate was 1 mL/min and the detection wavelength examined out by UV detector was 304 nm. The retention time for daclatasvir was 3.0 ± 0.1 min. RSD (0.65 %), theoretical plates (1118117) and tailing factor (1) complied within acceptable certified limits. The developed method showed the linearity within a range (1 – 5 µg/mL). The accuracy of the developed method was figured out by recovery analysis and it was 99 %. The precision analysis i.e. interday (0.0.790937 %), intraday (0.364517 %) and repeatability (0.229167 %), were within the acceptance criteria. All the validation parameters were within the acceptable limits. The sample solutions of daclatasvir tablets (Dacriva, 60 mg) developed and correlated its chromatograms with working standard solution and perceived the percentage purity of daclatasvir in the tablet, found within recommended acceptable limit (90 – 110 %). It is concluded that the HPLC method is sensitive, stable, rapid and reliable. It can be deployed for the quantification of daclatasvir of standard and brands available in market in the solid dosage form in the pharmaceutical industry.

Keywords: NP HPLC; Daclatasvir; Assay; HPLC; Quantification; Validation

1. Introduction

Hepatitis C virus (HCV) is painstakingly a vivant culprit of liver disease worldwide with an ample diversity in genotype and a potential cause of substantial morbidity and mortality in the future [1,2,3]. The vagueness in the distribution pattern of HCV infection and chronic hepatitis C with the aspect of geography, comorbidities or associated perils and appraisal of associated cofactors which are responsible for hastening its progression [4,5,6], accentuating the lacunas in global prevention and control of HCV [7,8]. As there is a prolonged struggle to develop vaccine and post-exposure prophylactic treatment for HCV [9,10] so far there is no significant achievement. Hence the only utilized standard operating procedure for the disease is to prevent unsafe blood transfusions and injection treatments [11]. Antiviral medicines can cure approximately 90 % of persons with HCV (e.g. Interferon, DDA, Ribavirin, Sovaldi etc.). The PEG interferon was introduced as an effective combination therapy [12].

Daclatasvir is a novel, highly selective NS5A replication inhibitor of HCV infection with broad genotypic coverage, chemical structure shown in Figure 1. It is stated to offer the potential for either reduced or interferon free treatment when used with other direct acting antiviral agents [13,14]. Combination studies demonstrate additive to synergistic interaction when administered with other Anti- HCV agents such as inhibitors of NS3 protease and NS5B polymerase (asunaprevir or sofosbuvir) [15,16].

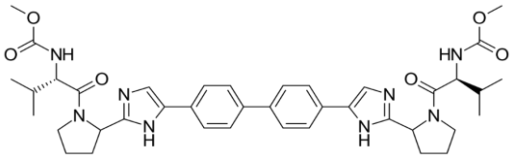

Figure 1. Chemical structure of Daclatasvir

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Receive Date: 14 June 2021, Revise Date: 14 July 2021, Accept Date: 07 August 2021

DOI: 10.21608/EJCHEM.2021.80600.3994

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There is a scarce availability of literature regarding the analytical determination of daclatasvir in bulk and pharmaceutical formulations, and lack of any official method in any standard Pharmacopoeia i.e. BP, USP, for chemical testing, quantification and validation of daclatasvir. Hence, there appears a dire need to produce a modest assay procedure for the determination of this drug [17]. The exploitation of HPLC in routine laboratory analysis is ubiquitous, the requirement of a sound validated HPLC method is calamitous for estimating daclatasvir [18].
Therefore, the prime objective of this study is the development of a simple, precise, fast and accurate reverse phase HPLC method for the estimation of Daclatasvir in pharmaceutical formulations in accordance with ICH guidelines, which can deliver improved sensitivity, less complex mobile phase, wide linear range and retention time less than 4 min. Special consideration was given to develop a method simply adaptable as a routine in quality control labs in pharmaceutical industries and academic institutions.

2. Experimental

2.1. Chemicals and Reagents

All the chemicals used were of HPLC grade. Daclatasvir dihydrochloride (99.8% pure) was procured from CCL Pharmaceuticals, Lahore Pakistan. Acetonitrile (RCI Labscan Ltd. Thailand), Water (Fisher Scientific Inc. USA), Anhydrous Potassium Dihydrogen Phosphate (Samchun, South Korea), Sodium Perchlorate Monohydrate and Phosphoric acid (Uni-Chem, South Korea) were procured and used.

2.2. Instrumentation

Analytical weighing balance (XS 205 from Mettler Toledo, USA), Sonicator E60H (Elmasonic E60 H, Greece), pH meter (744 from Metrohm, Switzerland), Syringe filter (Agilent PTFE type 0.45 µm, USA), Nylon 0.45 µm Millipore (Merck Filters, USA), RO plant (ThermoScientific, USA), Filtration assembly ME1 (1L) with Scott Vacuum pump Vacuum brand and Glass part (Sartorius, Germany), UV-Visible Spectrophotometer (Shimadzu, Japan) with Shimadzu HPLC 20A including HPLC Pump (PerkinElmer 200LC, USA), Column C 18 (L1 Packing 5µm, 4.6 mm x 25 cm), Column compartment CHC, UV detector 87E and Injector SM4, Separation module Alliance e2695 and PDA Detector 998, was used.

2.3. Software

ChemDraw *ver*. 20.0 was used for the drawing of chemical structures.

2.4. Preparation of Solutions

Preparation of buffer: The buffer was prepared by dissolving 2.72 g of anhydrous potassium dihydrogen phosphate and 14.04 g of sodium perchlorate monohydrate in 1000 mL of HPLC grade water. The pH of the buffer was adjusted at 2.1 with the aid of phosphoric acid. The resultant solution was then subjected to the filtration process.
Preparation of Diluent: The solution of acetonitrile and HPLC grade water in the ratio of 1:1 was used as diluent.
Preparation of mobile phase: The mobile phase was prepared in a graduated cylinder by mixing 600 mL of the acetonitrile and 400 mL of the Buffer solution (3:2). The final solution was then subjected to vacuum filtration by the filtration assembly with 0.5 µm Millipore filter. The filtrate is then relocated to the solvent reservoir and sonicated to eliminate the air bubbles [19].
Preparation of standard stock solution: The standard stock solution was prepared by the following procedure; Weigh 100 mg of Daclatasvir API and transferred into a 100 mL volumetric flask. The standard stock solution was prepared by dissolving the Daclatasvir in the mobile phase and made the volume up to the mark (100 mL). The resultant concentration of the standard stock solution is 1 mg/ mL or 1000 µg/ mL (Stock solution). Take 3 mL of the aliquot and dilute it with the 100 mL of the mobile phase in the volumetric flask. The resultant strength of the solution will be 3 µg/mL (Standard solution).
Preparation of sample solution: Weigh 10 Daclatasvir tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 100 mg of Daclatasvir into a 100 mL volumetric flask, make volume up to the mark with the mobile phase. Sonicate the solution to mix it thoroughly and filter through a 0.45 µm filter. Take 3 mL of the aliquot and dilute it with the 100 mL of the mobile phase in the volumetric flask. The resultant strength of the solution will be 3 µg/mL.

2.5. Determination of λmax

The standard solution (3 µg/mL) of Daclatasvir was then subjected to scanning by UV-Visible spectrophotometer in the range of 200 – 800 nm against mobile phase as blank. The wavelength corresponding to the maximum absorbance was recorded which was λmax.

2.6. Chromatographic conditions

HPLC was subjected to setting;
Mode: Liquid Chromatography, **Detector:** UV 304 nm, **Flow Rate:** 1.00 mL/min, **Mobile Phase:**

Buffer:Acetonitrile (2:3), **Injection volume:** 10 µL, **Column temperature:** 30 °C.
Initially blank was run. Then the column was subjected to 50 % acetonitrile flushing for 30 min to remove all molecules those expected to be previously retained within the column. The column was then stabilized at Mobile Phase. Daclatasvir standard solution of 3 µg/mL was used to develop the method. The data was acquired, stored and analyzed with Shimadzu Chromatographic Software.

2.7. System Suitability

The system suitability was estimated by the following procedure;
Five replicate Injections of Daclatasvir standard solution (3 µg/mL) were administered into the system to check the system suitability. System suitability parameters in terms of retention time, peak area, percentage assay, tailing factor and plate count on the datasheet were recorded, also the mean, standard deviation and % RSD was calculated.
Acceptance Criteria:
Retention time: % RSD should not be more than 2%.
Peak Area: % RSD should not be more than 2%.
Percentage assay: % RSD should not be more than 2%.
Tailing factor: Not more than 2.
Plate count: Not less than 30000

2.8. Method Validation

The method validation was done in terms of specificity, detection limit (LOD) and quantitation limit (LOQ), linearity, accuracy, repeatability and reproducibility, the robustness of Daclatasvir in accordance with ICH Q2 R1 guidelines (ICH, 2005).

2.8.1. Linearity
The linearity of the method was determined by the following procedure;
1 mL from the stock solution (100 μg/mL) was taken in a test tube. It was diluted with mobile phase and the volume was made up to 100 mL to produce 1 μg/mL solution. Similarly, a series of dilutions were prepared by taking 2 mL, 3 mL, 4 mL and 5 mL were diluted. The volume was made up to 100 mL using mobile phase to produce 1 μg/mL, 2 μg/mL, 3 μg/mL, 4 μg/mL and 5 μg/mL. Each dilution was injected in three replicates, and the average peak area of each concentration was utilized to develop linearity curve.
Performance criteria: Linearity study based upon ICH guidelines, required minimum of 5 developed concentrations. The graph between concentration and response must increase linearly.

2.8.2. Accuracy
The accuracy of the method was determined by the following procedure;
The accuracy of the developed method was evaluated by the recovery studies at three concentrations 2, 3 and 4 µg/mL in triplicates. Daclatasvir standard solution of 3 µg/mL was deployed to observe the percent recovery of Daclatasvir in sample solutions. The concentration of Daclatasvir was observed in the recovered aliquot of each sample solution. The average percentage recovery was calculated to evaluate the method's accuracy.
Performance criteria:
Percentage recovery: lies within 95 – 105%.
Precision of Bias: % RSD lies within intermediate precision.

2.8.3. Specificity/ Selectivity
The specificity of the developed method was estimated by the following procedure;
The specificity was executed by inserting injections of Daclatasvir standard solution 3 µg/mL and Daclatasvir sample (Daclatasvir standard along with powdered excipients) solution. The chromatograms were recorded and observed for deviations from the acceptance criteria (retention time at same wavelength).
Acceptance Criteria: the developed method does not show any effect by the presence of excipients.

2.8.4. Precision
The precision was estimated in terms of repeatability, interday and intraday. The precision of the method was analyzed in terms of Avg. peak area, Standard deviation and relative standard deviation.
Acceptance Criteria:
Retention time: % RSD should not be more than 2%.
Peak Area: % RSD should be within performance criteria.
Percentage Assay: % RSD should not be more than 2%.

2.8.5. Limit of Detection and Limit of Quantitation
The limit of detection (LOD) of a compound is defined as the lowest concentration that can be detected. The limit of quantification (LOQ) is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. The value of the linearity test was manipulated defining that below lowest concentration level in linearity curve response decreases. Signal to noise ratio limits at this concentration was utilized for estimation of LOQ. While concentration below this was prepared to determine LOD, as per ICH Guidelines. 5 replicate injections of each concentration 3 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.25 µg/mL, 0.1 µg/mL were investigated to confirm reproducibility.

Performance Criteria: LOD is characteristically 3 times of the noise level and LOQ stretches signal to noise ratio of 10 times.

Acceptance Criteria

LOD: Signal- to- noise (SN) between 3 or 2:1 is usually acceptable.

LOQ: Signal-to-noise (SN) ratio 10:1 is usually acceptable.

2.8.6. Robustness/Ruggedness

The robustness of the method was estimated by the following procedure;

The HPLC parameters were deliberately varied from normal procedural conditions including;

- Column Temperature was changed from 25 °C to 30 °C and 35 °C.
- Flow rate was changed from 1.0 mL/min to 0.5 mL/min and 1.5 mL/min.
- Ratio of Acetonitrile to Buffer in the mobile phase was changed the ratio from 60:40 to 61:39 and 59:41.
- Carrying out the analysis by two different analysts.

Acceptance Criteria: Method should respond specifically to the given analyte of interest.

Retention time: % RSD should not be more than 2%.

Peak Area: % RSD should not be more than 2%.

Tailing factor: Not more than 2.

Plate count: Not less than 3000

2.9. Application of HPLC Method for Daclatasvir Commercial Tablet

Daclatasvir tablets are available in seven brands in Pakistan, we picked out one brand to check the efficacy of newly developed non-compendial, unofficial method. The brand was Dakriva Tablet, 60 mg manufactured by CCL Pharmaceuticals Lahore Pakistan (national).

HPLC was subjected to setting;

Mode: Liquid Chromatography, **Detector:** UV 304 nm, **Flow Rate:** 1.00 mL/min, **Mobile Phase:** Buffer:Acetonitrile (2:3), **Injection volume:** 10 µL, **Column temperature:** 30 °C.

Initially blank was run. Then the column was subjected to 50 % acetonitrile flushing for 30 min to remove all the previously retained molecules within the column. The column was then stabilized at Mobile Phase.

The method was tested by the following procedure;

The working standard solution as well as Sample solution of Daclatasvir (with Dakriva Tablet) was prepared with the same procedure as was prepared for the Analytical method protocol. 3 µg/mL Daclatasvir Standard Solution was injected in 3 replicate injections of a working standard solution. The same was executed with 3 injections of sample solution and calculated the assay percentage.

Acceptance Criteria:

Retention time: % RSD should not be more than 2%.

Peak Area: % RSD should not be more than 2%.

Percentage assay: % RSD should not be more than 2%.

Tailing factor: Not more than 2.

Plate count: Not less than 3000.

3. Results

3.1. Determination of λmax

The solution was scanned with the aid of UV-Visible spectrophotometer in the range of 200 to 800 nm. The maximum absorption of the electromagnetic radiation was found at 304 nm wavelength. It was denoted as λ_{max} (Figure 2).

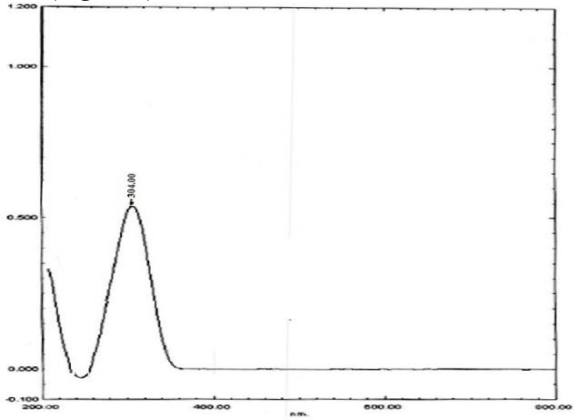

Figure 2. UV-Visible spectrophotometric scan of Daclatasvir

3.2. System Suitability

For the evaluation of chromatographic parameters, a system suitability test was performed before each validation run. The system suitability parameters in term of percentage relative standard, tailing factor and no. of theoretical plates are noticed (Figure 3; Table 1).

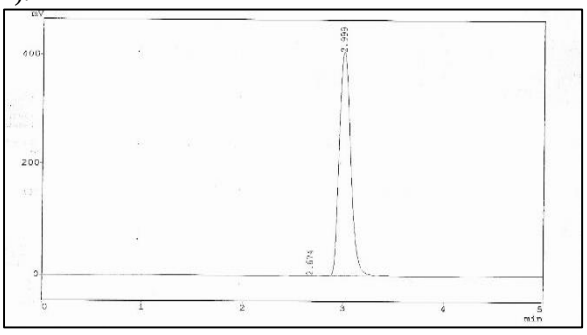

Figure 3. Chromatogram of system suitability

Table 1. System suitability parameters

Sr. No.	Retention time	Peak Area
1	2.99	3246461
2	3.00	3248224
3	2.99	3226940
4	2.99	3234550
5	3.00	3289792
Relative standard deviation (% RSD)		0.65%
Tailing factor (Average)		1
No. of theoretical plates (Average)		1118117

3.3. Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of the analyte in the sample.
In the first step of validation, linearity was figured out. Incremental or replicate analysis was employed for the purpose viz. a number of solutions were prepared in gradually increasing concentrations. Presently, five concentrations (1 – 5 μg/mL) were made and run on the HPLC system (Table 2).

Table 2. Linearity analysis of Daclatasvir

Sr. No.	Concentration (μg/mL)	Retention time (min)	Average peak area
1	1	3.152	1090661
2	2	3.077	1884866
3	3	2.999	3252349
4	4	3.017	4388316
5	5	3.074	5022034

The linear relationship between concentration and average peak areas of the samples was noticed and a calibration curve was obtained by plotting a graph. The trend line was drawn and, the slope was calculated by the linear regression equation (Figure 4).

3.4. Accuracy

It is a portrayal of precise mistakes, a proportion of factual predisposition; as this reason a contrast between an outcome and "genuine" esteem, ISO calls this certainty. Similarly, ISO characterizes exactness as portraying a mix of the two kinds of observational mistakes above (arbitrary and methodical), so high exactness requires both high accuracy and high genuineness.

It is executed by comparing the results of non-standard developed method to a standard method devouring defined accuracy i.e. British Pharmacopoeia method 2015. It is also referred to as the closeness of test results to the true value by that procedure. The average peak area of Daclatasvir standard solution was found to be 3258726 (Table 3).

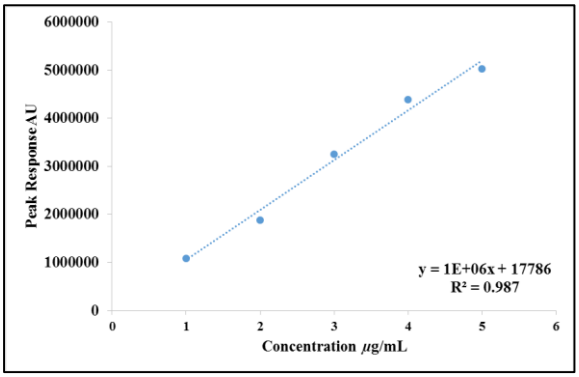

Figure 4. Linearity curve of Daclatasvir

The straight line depicted the linearity in the following concentration range 1 – 5 μg/mL. The correlation coefficient (R2) was found to be 0.987.
Linearity equation from the curve;
y = 1E+06x + 17786
R2 = 0.987
Where,
Regression equation = 1E+06x + 17786
Slope (b) = 1E+06
Y-intercept = 17786
Pearson *coefficient of correlation R2* = 0.987

Table 3. Accuracy analysis of Daclatasvir

Concentration μg/mL	Peak Area	Average peak area	Concentration recovered (μg/mL)	Percentage Recovery	Mean
2	1188970	1188969.67	1.99	99	
	1188970		1.99		
	1188969		1.97		
3	3246461	3240541.67	2.97	99	
	3248224		2.98		99
	3226940		2.97		
4	4396613	4391082	3.96	99	
	4380020		3.97		
	4396613		3.98		

3.5. Specificity/ Selectivity

Specificity is the ability of an analytical procedure to respond only to the target analyte. Specificity is the exclusive determination of the analyte despite the presence of excipients, degradation products, and matrix components.

The specificity results by inserting injections of Standard and Sample (Daclatasvir standard along with powdered excipients) lay in the acceptance criteria as they show the same retention time at 304 nm, in the chromatograms.

3.6. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple sampling of the same homogeneous sample under prescribed conditions. Precision may be considered as repeatability and reproducibility.

Despite the two words precision and accuracy depicted be tantamount in informal use, they are calculatingly analogized in the context of the scientific method. Precision is the degree of agreement amongst individual test results.

The intraday study was conducted with a 5 hours gap and statistical parameters were noticed. The outcomes fall inside the approved criteria i.e. the % RSD is less than or equal to 2%. On the other hand, the interday study was done by conducting the analysis on two consecutive days and statistical parameters were noticed. The outcomes followed the acceptance criteria i.e. the % RSD is less than or equal to 2 %. Similarly, the repeatability studies were carried out by performing the analysis in two different labs The outcomes fall inside the approved criteria i.e. the % RSD is less than 2 % (Table 4).

Table 4. Precision analysis of Daclatasvir

Intraday Precision								
Sr. No.	**Concentration (μg/mL)**	**Retention time**		**Peak area**		**Mean**	**Standard deviation σ**	**% RSD**
		Day 1	**Day 2**	**Day 1**	**Day 2**			
1	3	2.999	2998	3234623	3246461	3240542	11812.33	0.364517
2		2.995	2.998	3232860	3248224			
3		2.996	2.996	3254144	3226940			
Interday Precision								
		0 h	**5 h**	**0 h**	**5 h**			
1	3	3.000	2.999	3276152	3243952	3260052	25784.95	0.790937
2		3.054	3.011	3230312	3289792			
3		2.998	3.000	3273692	3246412			
Repeatability Studies								
		Lab I*	**Lab II****	**Lab I***	**Lab II****			
1	3	2.995	2.998	3221450	3238324	3229887	7401.84	0.229167
2		2.998	2.996	3235290	3224484			
3		3.001	3.010	3232922	3226854			

*Post-graduate lab of Pharmaceutical Chemistry, **Post-graduate lab of Pharmaceutics

3.7. LOD & LOQ

The limit of detection (LOD) of a compound is defined as the lowest concentration that can be detected by using the method, but not essentially quantitated, above the baseline noise. The limit of quantification (LOQ) is the lowest concentration of a compound that can be quantified above the baseline noise with acceptable precision and accuracy. S/N = SD; the LOD = 3SD and LOQ = 10SD.

The Linearity test exposed that the curve was linear at the lowest concentration level 1 μg/mL, below this concentration linear response decreases. Signal to noise ratio at this concentration was 10:1 which is acceptable for estimation of LOQ. Therefore, LOQ of this method was set to 1 μg/mL. When concentration below 1 μg/mL was prepared to determine LOD, lowest concentration of Daclatasvir detected by this method was 0.25 μg/mL and Signal to noise ratio at this concentration was 2.5:1 which is acceptable for estimation of LOD, as per ICH Guidelines. 5 replicate injections of each concentration 3 μg/mL (Figure 5), 1 μg/mL (Figure 6), 0.5 μg/mL, 0.25 μg/mL, 0.1 μg/mL was investigated to confirm reproducibility. Below 0.25 μg/ml a flat signal was appeared i.e. at 0.1 μg/mL and shows S/N Ratio of 1:1. Here the value of h = 1 cm (Table 5).

3.8. Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage [20]. The method appeared to be robust with reference to column temperature, flow rate and ratio of solvents in mobile phase. The relative standard deviation (%CV) after altering these parameters were less than 0.5 %. The analysis achieved were within the acceptable recovery range (± 10%) and % CV (less than 5%). Therefore, this method proved to be robust (Table 6-8).

3.9. Ruggedness

The reproducibility in terms of ruggedness is estimated under the conditions such as different evaluators, using the same instrument but by different manufacturer and different days [21]. In the present study the ruggedness of the procedure was tested by two different analysts and statistical parameters were tabulated. The results i.e. % RSD was less than 2 % (Table 9).

Application of HPLC Method for Daclatasvir Commercial Tablet

The column was then stabilized at "Mobile Phase". 3 μg/mL. Daclatasvir Standard Solution was injected in 3 replicate injections of working standard solution (Figure 7). Relative standard deviation (RSD) was calculated and originated to be not more than 2.0 %. Tailing factor and number of theoretical plates were also within tolerable ranges (Table 10; Figure 8).

Table 5. Determination of LOD & LOQ

Sr. No.	Concentration (μg/mL)	Peak Area	Peak Height (cm)	S/N Ratio
1	3	3248224	10	20:1
2	1	1188970	5	10:1
3	0.5	15683	2.5	5:1
4	0.25	5677	1.25	2.5:1
5	0.1	No Peak observed	1	1:1

Table 6. Robustness analysis of Daclatasvir at different flow rates

Sr. No.	Retention time (min)	Peak Area	Mean	Standard deviation σ	% RSD
Flow rate 0.5 mL/min					
1	3.001	3238242	3242378	3615.786	0.111516
2	3.000	3244940			
3	3.001	3243952			
Flow rate 1.00 mL/min					
1	2.995	3248224	3246372	2191.909	0.067519
2	2.996	3226940			
3	2.999	3243952			
Flow rate 1.5 mL/min					
1	2.991	3245218	3246023	860.4867	0.026509
2	2.991	3246930			
3	2.990	3245922			

Table 7. Robustness analysis of Daclatasvir at different column temperature

Sr. No.	Retention time (min)	Peak Area	Mean	Standard deviation σ	% RSD
Temperature 25 °C					
1	3.001	3246461	3240542	11812.33	0.364517
2	2.999	3248224			
3	2.997	3226940			
Temperature 30 °C					
1	2.997	3226461	3230542	6657.405	0.206077
2	2.997	3238224			
3	3.000	3226940			
Temperature 35 °C					

1	2.999	3246471	3240535	5118.282	0.15989
2	2.998	3238214			
3	2.998	3236920			

Table 8. Robustness analysis of Daclatasvir at different concentrations of mobile phase

Sr. No.	Retention time (min)	Peak Area	Mean	Standard deviation σ	% RSD
Acetonitrile 59 %					
1	2.998	3342462	3338210	8936.32	0.267698
2	2.997	3344227			
3	3.002	3327942			
Acetonitrile 60 %					
1	3.000	3246461	3245875	2690.299	0.082884
2	3.001	3248224			
3	3.054	3242940			
Acetonitrile 61 %					
1	2.989	3446872	3411073	54847.19	1.607916
2	2.997	3438417			
3	2.999	3347929			

Table 9. Ruggedness analysis of Daclatasvir.

Intraday Precision								
Sr. No.	**Concentration (μg/mL)**	**Retention time**		**Peak area**		**Mean**	**Standard deviation σ**	**% RSD**
		Analyst 1	**Analyst 2**	**Analyst 1**	**Analyst 2**			
1	3 μg/mL	3.001	3.000	3244319	3249471	3246895	2619.535	0.080678
2		3.054	3.001	3249556	3244234			
3		2.999	3.042	3246810	3246980			

Table 10. Standard readings of Daclatasvir

Sr. No.	Retention time (min)	Peak Area
1	3	3248224
2	2.99	3226940
3	2.99	3243952
	Average Peak Area	3239705.33
	Standard Deviation	11259.546
	% Relative standard deviation	0.35 %

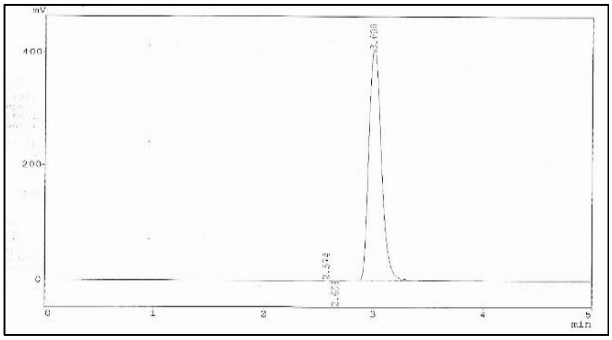

Figure 5. Chromatogram of LOD study of Daclatasvir sample solution of 3 μg/mL

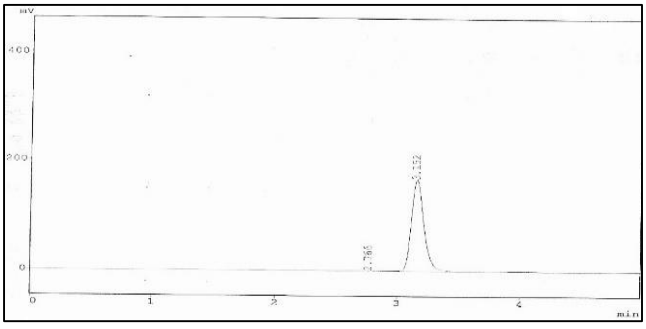

Figure 6. Chromatogram of LOD study of Daclatasvir sample solution of 1 μg/mL

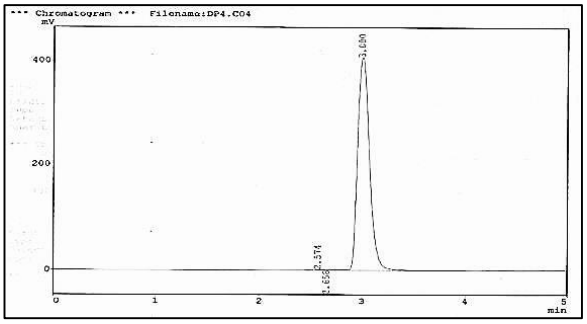

Figure 7. Chromatogram of Daclatasvir standard solution of 3 μg/mL

Now executed 3 injections of sample solution and calculated the assay percentage (Table 11; Figure 13).

Table 11: Commercial tablet sample readings

Sr. No.	Retention Time (min)	Peak Area
1	3.00	3289729
2	3.00	3258726
3	2.99	3246461
	Average Peak area	3264972
	Standard Deviation	22299.98
	% Relative standard deviation	0.68 %

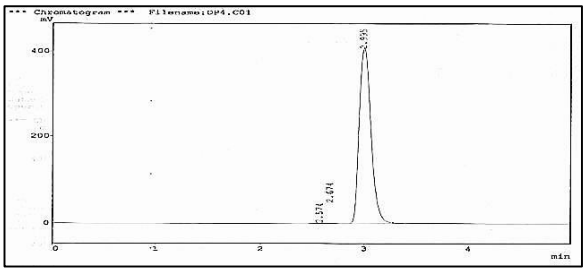

Figure 8. Chromatogram of Daclatasvir sample solution of Dakriva tablet

Assay Calculations:

$$Content/tablet = \frac{Area_{Sample}}{Area_{Std.}} \times \frac{Conc._{Std.\ solution}}{Conc._{sample\ solution}} \times labelled\ content\ /tablet$$

$$Percentage = \frac{Content\ per\ tablet}{Labelled\ content\ per\ tablet} \times 100$$

The assay calculation shows 100.78% of Daclatasvir in the sample solution, which clearly lies in the acceptable limit. The interpretations prove that above developed and validated method of quantification of Daclatasvir is easily applicable to any commercial solid dosage form.

4. Discussion

The HPLC analytical technique for the measurement of Daclatasvir is not available in compendial books like USP and BP, due to the recent discovery of the molecule [22] limited data is existing in the literature regarding its quantification. The achieved method so far, for the estimation of Daclatasvir in tablet dosage form are neither ample nor very sensitive or feasible in daily laboratory work, as in most cases UV spectroscopic method or a complex reverse phase LC was deployed [23,24]. Besides, as it is mostly employed in combination therapy for HCV therefore the previous methods established were for simultaneous determinations with other antivirals [25,26] also they have high detection and quantitation ranges. Furthermore, the robustness and ruggedness of the methods were not elaborated.

With recent development Daclatasvir is considered to be an individual molecule for treatment and mitigation of disease [27]. Therefore, the core objective of current research work was to develop an analytical method specifically for determination of Daclatasvir with HPLC which will be easy, less time consuming, sensitive and robust also it can be professionally applicable to solid dosage forms. Hence the method was developed for the assay of Daclatasvir in solid dosage form, using Reverse Phase C-18 column (25cm length, 4.6mm internal diameter and 5µm particle size) in isocratic mode, with mobile phase comprising of a Phosphate buffer and acetonitrile in the ratio of 2:3 v/v. The flow rate was 1 mL/min and the detection wavelength examined out by UV detector was 304 nm. The retention time for Daclatasvir was 3.0 ± 0.1 min. The method was statistically validated and the relative standard deviation was set up to be much less than 2.0 % representing high degree of accuracy and precision of the proposed HPLC method. Interday precision was established at 0.61 %. The detailed method had allowed the quantification of Daclatasvir over linearity in the concentration range of 1 – 5 µg/mL with average percentage recovery of 99 %. This method enumerates good accuracy, precision, sensitivity and wide linear range [28].

After above said successes the validated method was then efficaciously utilized to compute the content of Daclatasvir in a brand, available in Pakistan (Dacriva Tablets, 60 mg by CCL Pharmaceuticals). The achieved results confirm the authorized acceptable limit (95-105 %).

5. Conclusions

The developed method for Daclatasvir is reliable, reproducible, robust and suitable for finished solid pharmaceutical dosage forms (tablets) due to its validation data. In prospect, the method can be pragmatic to other dosage forms (currently only tablet dosage form of Daclatasvir is available), when and if accessible.

6. Conflicts of interest

The authors have declared that no conflicts to interest exist.

7. Formatting of funding sources
The project was funded by Punjab University College of Pharmacy University of the Punjab Allama Iqbal Campus Lahore Pakistan.

8. Acknowledgments
The authors are grateful for the technical support provided by Professor Dr. Khalid Hussain during the study. The authors also thankful to Director Quality Control Dr. Rizwan at CCL Laboratories for the sample donation.

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