

Validated Stability-Indicating RP-HPLC Method for Daclatasvir in Tablets

Short Title: Validated Stability-Indicating Method Daclatasvir by RP-HPLC

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ABSTRACT

Objectives: The current study goal was to create a precise, sensitive and validated RP-HPLC method for assessing the direct-acting antiviral daclatasvir (DCV) as well as to evaluate the stability of the DCV in both drug and tablet formulations. The current investigation was to perform stability indicating method in different stress conditions, including hydrolysis such as (acidic, basic and neutral), oxidation and photolysis.

Materials and methods: All the experiment was performed on HPLC Agilent 1100 having stainless steel Hypersil C₁₈ column with a particle size of 5µm and a dimension of 4.6 X 250 mm. The mobile phase chosen was acetonitrile: 0.05% o-Phosphoric acid (50:50 v/v) in isocratic mode with 0.7 mL/min flow rate and wavelength 315 nm was selected for detection.

Results: This method was validated for linearity and range, accuracy, precision, LOD, LOQ and robustness in accordance with ICH requirements. The results were satisfactory. It was observed that retention time (t_R) was 3.760±0.01 min. In acidic condition, DCV degradant shows t_R at 3.863, 4.121, 4.783 min and MS/MS spectra scans having m/z 339.1, 561.2 fragment ion. In basic condition, DCV degradant shows t_R at 5.188, 5.469 min. and MS/MS spectra scans having m/z 294.1, 339.1, 505.2, 527.2 fragment ions observed. In oxidation conditions, DCV degradant shows t_R at 4.038 min and MS/MS spectra scans having m/z 301.1, 339.1 fragment ions were observed.

Conclusion: All the mass fragments shows the additional degradation observed for different stress conditions. This will help to identify the structure of degradant and its pathways. No degradation was observed in neutral and photolytic conditions.

Key words: Daclatasvir, RP-HPLC, tablets, validation, stability-indicating method.

INTRODUCTION

Millions of people suffer from hepatitis C virus (HCV) infections. DCV permits once-daily oral treatment, has been shown in in vitro experiments to have very potent antiviral activity against a number of HCV genotypes.^{1,2} Since, direct acting antivirals (DAAs) for HCV have become available, any possible medication interactions between antiretrovirals and DAAs must be assessed before co-therapy.³ Cirrhosis and hepatocellular cancer have been linked to chronic hepatitis C in various parts of the world.⁴ An effective, new, and non-selective structural protein inhibitor is DCV, formerly known as BMS-790052.⁵ The multifunctional protein NS5A, which is a crucial part of the HCV replication complex, is inhibited by DCV. It inhibits viral RNA replication and the development of virion. Data from computer models and in vitro investigations point to an interaction between DCV and the protein's N-terminus in domain 1 that may result in structural alterations that reduce NS5A activity.⁶⁻⁸ Chemically DCV is methyl((1S)-1-(((2S)-2-[5-(4'-(2-((2S)-1-((2S)-2-((methoxycarbonyl)amino)-3-methylbutanoyl)-2-pyrrolidiny)-1H-imidazol-5-yl)-4-biphenyl)-1H-imidazol-2-yl)-1-pyrrolidiny)carbonyl)-2-methylpropyl) carbamate. The molecular formula of DCV is C₄₀H₅₀N₈O₆ and the molecular weight is 738.89 g/mol.⁹ Nimje et. al developed the stability indicating method for DCV by HPTLC and LC-MS/MS.^{10,11} Some bioanalytical methods such as UPLC-MS/MS for DCV in human plasma and HPLC with UV detector were

reported by Mamdouh, Rezka and Nannetti et al. respectively.^{12,13} By using UHPLC-MS/MS and LC-MS, Alessandra and Jiang et al. demonstrated the measurement of several direct antiviral drugs in human blood plasma.^{14,15} All of these techniques are effective for detecting DCV in human blood plasma, however they don't appropriate for routine assays to detect DCV in medication and tablet dosage forms. Several HPLC methods are reported for determining DCV single and its combination with other drugs under different conditions, according to a review of the literature.¹⁶⁻²¹ As per regulatory guidelines it is essential to ascertain the ability of assay method to quantitate the drug component in presence of its degradation products. The above mentioned reported HPLC methods have not ascertained the ability of the assay method to quantitate DCV in presence of its degradation products. Hassib et al. demonstrated the RP-HPLC Method for determining DCV and the behaviour of its degradation under diverse circumstances. This study used acetonitrile and potassium dihydrogen phosphate buffer as the mobile phase, which is driven through column C₁₈ at a rate of 2 mL/min.²² The flow rate in the above reported method is on higher side which can adversely affect the quality of the chromatography not giving the analyte sufficient time to interact with the stationary phase. Moreover, the ability of the method to quantitate DCV in presence of its degradation product is not evaluated. The stability indicating HPLC-UV was developed by Zaman et al. for characterizing forced degradation products and quantifying DCV.²³ In the above method, the retention time of DCV is 16.4 min. making it a time consuming method for assay of formulation containing DCV. The method can be preferred for quantitation of known impurities in DCV formulation. The preferred retention time in HPLC assay method is 3 to 6 min. Baker et al. presented a stability-indicating HPLC-DAD method utilising a waters C₈ column with isocratic elution of the mobile phase made up of a 75:25 v/v combination of acetonitrile and phosphate buffer with a pH of 2.5.²⁴ While a novel QDa mass detector for DCV was developed by Jagadabi et al. to determine the mass of possible contaminants²⁵, stability-indicating UPLC method was reported. However, the above method is primarily used for estimation of process related and known impurities in DCV. Considering the limitations of the reported methods like high flow rate, high retention time, stability indicating property of assay method being not evaluated, it was thought worthwhile to develop and validate stability indicating RP-HPLC method for DCV formulations which is economical and less time consuming without affecting the quality of the method as per parameters given in ICH guidelines. In the present research work, economical solvents and simple procedure were used for estimation of DCV by RP-HPLC. In order to evaluate the stability of DCV in drugs and their drug derivatives, a force degradation research was conducted. This information helps to determine the shelf life of the DCV under various settings. The quantitative examination of the DCV following exposure to various stability indicating experiments is described in the current piece of work. The approach was validated method in accordance with ICH standards, demonstrating accuracy, precision and reliability. Therefore, this method can be applied to the routine study of DCV stability under various conditions.

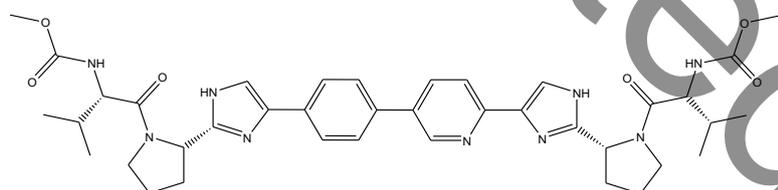


Figure 1: Structure of DCV
MATERIALS AND METHODS

Chemicals and reagents

Mylan Laboratories Ltd, Hyderabad, India kindly provided DCV pure drug sample to us. All HPLC grade solvents were used throughout the analysis, including water (Fisher Scientific, India), methanol (Fisher Scientific, India) and acetonitrile (Fisher Scientific, India). Analytical Grade reagents such hydrogen peroxide, hydrochloric acid, sodium hydroxide and o-phosphoric acid were used throughout the analysis. The DCV tablets "MyDeklaTM60" and "DACLACURE 60" were purchased from a local market drug store. They were manufactured by Mylan Laboratories Limited and Emcure Pharmaceutical Industry, respectively. Each tablet contained 60 mg of DCV.

Instrumentation and Chromatographic conditions

The HPLC Agilent 1100 with variable detector (G1314A) and (G13104) pump was used for all of the studies. The solutions were degassed using the DGU-20A3 Prominence degasser. The data was gathered and processed using the Chemstation software. The material employed was a stainless steel Hypersil C₁₈ that was 4.6 X 250 mm in size, 5 µm particle size, filled with octadecyl silane stationary phase and had ligands bound to the silica surface and the column temperature was set at 40 °C. For this research, a humidity chamber from Newtronic and a Rolex ultrasonicator were employed. An examination into photodegradation was conducted using a UV lamp (6w, Vilber Lourmat, France) and photostability chamber. The light output was 200Wh/m² and its intensity was 1.2 million lux h. A weighing balance (AX200, Shimadzu Corporation Japan) with a minimum 0.1 mg and maximum 200 g capacity was employed throughout the analytical procedure. Use was made of the isocratic mobile phase with acetonitrile and 0.05 % o-phosphoric acid (50:50 v/v). Before being used in the study, the

mobile phase was filtered through membrane filter of size 0.45 μm and using ultrasonic sonicator for degassing the solvents for 10 min. The flow rate of mobile phase was 0.7 mL/min and the injection had a 10 μL volume. A variable wavelength detector was used to perform a wavelength scan and a wavelength of 315 nm was chosen for investigation.

Preparation of standard stock and tablet solution

The standard stock solution was made by placing a quantity of 10 mg of DCV standard in a 10 mL volumetric flask with mobile phase, shaking well and adding mobile phase to achieve a concentration of 1000 $\mu\text{g/mL}$. The stock solution was diluted, mixed well and used to test all DCV validation parameters with required concentrations. After 15 min of sonication, the mixture was filtered through membrane filter paper of size 0.45 μm . Twenty DCV tablets (MyDeklaTM60 and DACLACURE 60) were weighed and the average weight was calculated. The tablet was triturated in a dry, spotless mortar to create a fine powder. A quantity of tablet fine powder containing 10 mg DCV was accurately taken, dissolved in minimum mobile phase, thoroughly mixed and stirred for 5 min. in a 10 mL volumetric flask. To create a volume of 1000 $\mu\text{g/mL}$ concentrated solution, up to 10 mL of mobile phase solvent was added. Sample solutions from the above solution were adequately diluted for future research examination. All of the solutions were filtered using the membrane filter paper with a pore size of 0.45 μm .

Validation of Proposed Method

Linearity and range

Different series of DCV solutions were made from standard stock solutions and diluted with diluent for the linearity test. The concentration range of 10.00-50.00 $\mu\text{g/mL}$ was identified, and five separate concentrations (10, 20, 30, 40, and 50 $\mu\text{g/mL}$) were chosen. In order to evaluate linearity, peak area and peak height were subjected to least square regression analysis to produce a calibration equation with slope, Y-intercepts and correlation coefficient (r^2).

Accuracy

To confirm the methods accuracy for quantifying DCV, standard addition and recovery tests were carried out. Recovery studies employ a typical addition procedure to a synthetic mixture created in a lab to assess the accuracy of the method in triplicate. Three different concentration levels of known amounts of DCV samples were put to a pure drug with a consistent weight of 10 $\mu\text{g/mL}$ (80%, 100%, and 120% of label claim) in triplicates. To make up the volume of diluted samples, mobile phase was used. The recovery was calculated using the peak area.

Precision

For the determination of DCV, the repeatability (Intraday) and intermediate precision (Interday) of the procedure were used to establish its precision for each six samples. The intraday precision was conducted on the same day with the same sample at a certain time. The same sample solution was used on one consecutive day to accomplish the interday precision. To determine the methods accuracy, the results were obtained and the RSD% was computed. For accuracy, the RSD% of the DCV peak areas was measured.

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) estimates were used to determine the measurement of DCV sensitivity. DCV was determined at signal-to-noise ratios of 3:1 and 10:1 for LOD and LOQ, respectively.²⁶

Robustness

A robustness analysis was used to examine the impact of small deliberate adjustments on the ideal chromatographic settings created for DCV. A 10% change in flow rate, a 2 nm shift in wavelength, and a 2% change in the composition of the mobile phase were among the other modifications. Three samples of DCV (30 $\mu\text{g/mL}$) were evaluated using it in all of the aforementioned circumstances. All system suitability traits and conditional changes were compared under all altered settings.

Stability-indicating analytical method [ICH Q1A]

A crucial step in the process of developing a drug medicine is stability testing. The purpose of stability testing is to demonstrate how product's quantity fluctuates over time and in different environmental contexts. Recommendations can be made for the shelf life period based on factors including temperature, humidity and light. The assay of the active ingredient and the degradation products produced during stability tests are the two key aspects of the drug product that are crucial in determining the shelf life duration. The conditions like 0.1N HCl, 0.1N NaOH, 30% H₂O₂, humidity, hydrolysis, UV light and temperature were used in the forced degradation investigation of DCV. The quantity 20 mL of 0.1N HCl, 0.1N NaOH and water were used to treat 20 mg of DCV, which is the comparable amount. For four hours, the solutions were refluxed at 60°C. A 30% H₂O₂ solution was employed for the oxidation investigation, which was refluxed at 60°C for 6 hours. After filtering the solution, the appropriate amount of dilution was added. For ten days, a layer of dry, solid DCV powder was placed in direct sunlight in order to perform photolytic testing. On the platform, a petridish held the medication. For ten days, visible light at 1.2 million lux h and UV light at 200Wh/m² were used for the degradation experiment. All of these conditions were applied continually until a significant deterioration was attained. All of

the degradation research samples were gathered and diluted to the proper concentration prior to analysis. This solution was put into the HPLC instrument for testing. The standard untreated drug was compared to the DCV assay of the stressed samples. The conclusion drawn from the RP-HPLC analysis of all stressed samples is that the suggested analytical strategy can identify all degraded products, demonstrating the methods potential to indicate stability.

RESULTS

Method development of DCV

The aim of this study was to develop and validate RP-HPLC method for degradant separation using RP-HPLC data to clarify the structure. The chromatographic method was created to look into a number of important contaminants detected in DCV. For the method development, DCV was injected into HPLC simultaneously with all stress samples. To accomplish effective separation, different ratios of various solvent combinations were explored. Using a C₁₈ column as the stationary phase and an acetonitrile: 0.05 % OPA in water (50:50 v/v) mobile phase with a flow rate of 0.7 mL/min, a wavelength of 315 nm and a column temperature of 40°C with a total run time of 10 min, the best separation of DCV from its related substance was observed. The measured DCV retention time (t_R) was 3.760 ± 0.01 min. The chromatogram of DCV is depicted in Figure 2.

Method validation

The HPLC instrument met the analysis criteria for system suitability test for the DCV chromatogram is shown in table 1.

Linearity and range

By injecting five various level concentrations of pure DCV ranging from 10 to 50 $\mu\text{g/mL}$, the analytical methods linearity was examined. Plotting peak area versus concentration yielded the slope, Y-intercepts and correlation coefficient of each DCV concentration. Data generated from linearity studies statistically show a correlation between peak area and concentration. The DCV method was found to be linear (10 to 50 $\mu\text{g/mL}$) concentration ranges with regression coefficients of 0.9998. The DCV linearity curve is depicted in figure 3.

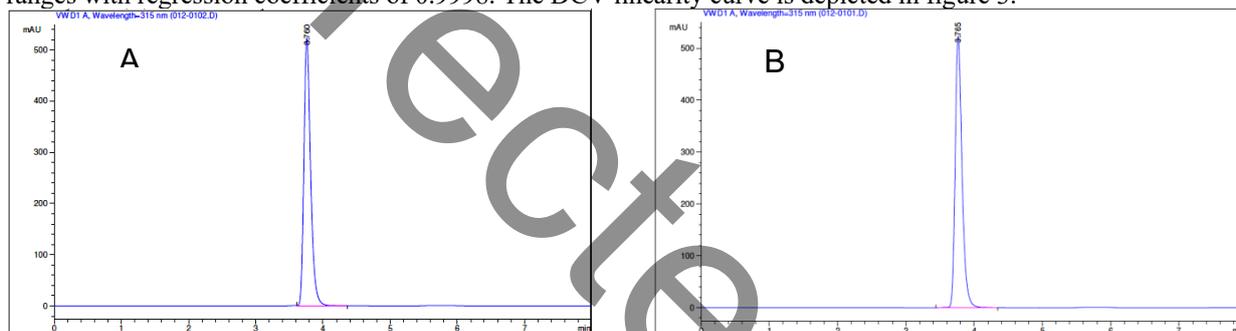


Figure 2. Chromatogram of DCV (A) and tablet (B) showing $t_R 3.760 \pm 0.01$ min.

Accuracy

The correctness of an analytical procedure is determined by the agreement between the true value and the experimental value. To ascertain the accuracy, three concentrations (18, 20 and 22 $\mu\text{g/mL}$) from the various ranges of the DCV standard curves were chosen. DCV recovery was examined in triplicate for concentrations of the drug sample at 80%, 100% and 120%. Recovery experiments were used to examine the effects of excipients, which are frequently used in pharmaceutical formulations of drugs. A good level of quantitative skill was demonstrated by the recovery of DCV in the sample. In table 1, the accuracy result is displayed.

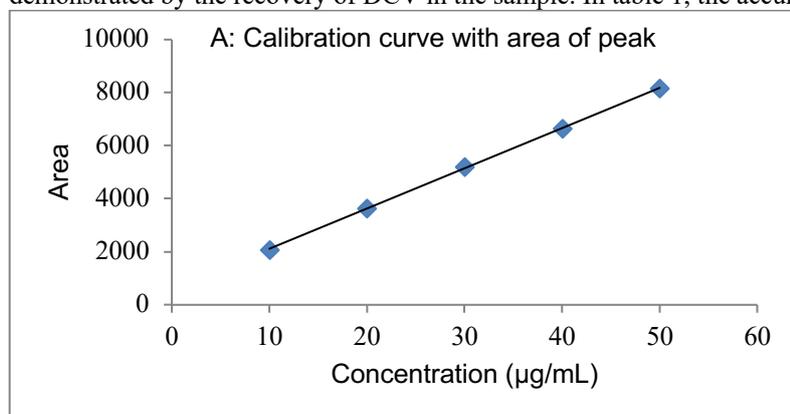


Figure 3. Calibration curve for DCV with (A) area of peak and (B) height of peak (10 to 50 $\mu\text{g/mL}$).

Precision

According to ICH regulations, the devised HPLC technology was evaluated for precision study. Intermediate precision (intraday precision) and the percent RSD from the six replicates of DCV samples were acquired. DCV samples were seen to have intraday precision on the same day. Three separate days were used to investigate the DCV for inter-day precision for repeatability study from the six replicates of DCV. The results are shown in table 1.

Parameter	HPLC assay
Retention time (t_R)	3.760±0.01
No. of theoretical plate (N)	8337
Tailing factor (A_s)	0.82
Range	10-50 µg/mL
Linearity (Regression equation)	$y = 151.68x + 598.86$
Intercept (SD)	1.5595
Slope (SD)	19.09
Correlation coefficient	$r^2 = 0.9998$
Accuracy at 80% level ^a (mean± SD)	100.78±0.6144
Accuracy at 100% level ^a (mean± SD)	99.79±0.7622
Accuracy at 120% level ^a (mean± SD)	97.95±0.1145
Intermediate precision ^b (mean± SD and RSD%)	101.73±0.3281 and 0.3237
Repeatability ^b (mean± SD and RSD%)	101.15±0.8914 and 0.8812
LOD (Limit of detection)	0.0416 µg/mL
LOQ (Limit of quantification)	0.1261 µg/mL

^a Replicates of three determinations.

^b Replicates of six determinations.

Standard deviation (SD) and Relative standard deviation (RSD)

Quantitative aspects

The signal-to-noise ratios (S/N) of 3 and 10 were used to assess the limits of detection (LOD) and limits of detection quantification (LOQ) for DCV.²⁶ LOD of 0.0416 µg/mL and LOQ of 0.1261 µg/mL were obtained after DCV injection, indicating enhanced sensitivity for detection and quantification.

Robustness

All the analytes were sufficiently resolved and the order of elution was unaltered when the chromatographic parameters, such as flow rate, mobile phase composition and wavelength were purposefully adjusted. Changes were made to the flow rate (0.2 mL/min), mobile phase composition (10 %), and wavelength (2 nm). Retention time, observed peak area, and RSD were reported in accordance with acceptable bounds. Table 2 displays the robustness results.

Robustness parameters	t_R Minute	Peak area*	% Assay ± SD	RSD%
Flow rate				
Flow rate (0.6 mL/min)	3.762	5237.72	100.63±1.0443	1.0377
Flow rate (0.7 mL/min)	3.788	5257.81	101.05±0.5272	0.5217
Flow rate (0.8 mL/min)	3.779	5263.78	101.13±1.1720	1.1588
Mobile phase composition				
Acetonitrile: 0.05% o-Phosphoric acid (49:51 v/v)	3.752	5207.84	100.05±1.1663	1.1657
Acetonitrile: 0.05% o-Phosphoric acid (50:50 v/v)	3.788	5238.12	100.63±1.0078	1.0014
Acetonitrile: 0.05% o-Phosphoric acid (51:49 v/v)	3.761	5220.25	100.29±1.1329	1.1296
Wavelength				
Wavelength (314 nm)	3.721	5256.88	100.99±0.5778	0.5721
Wavelength (315 nm)	3.742	5262.27	100.90±0.7735	0.7804
Wavelength (316 nm)	3.761	5236.80	100.79±0.8431	0.8498

*Results of three replicates, t_R Retention time, SD Standard deviation, RSD Relative standard deviation

Degradation behavior of the drug and characterization of degradants

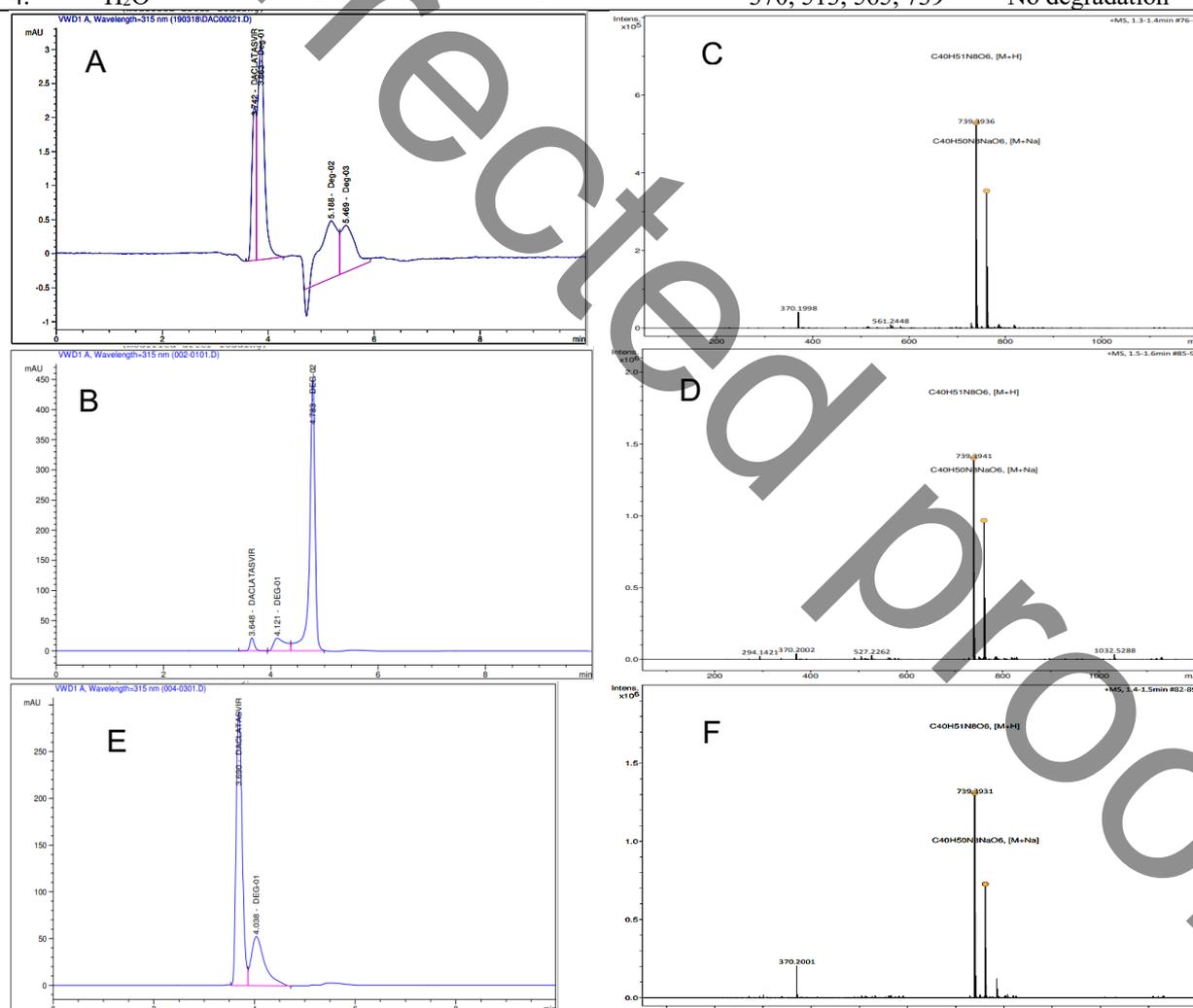
DCV dramatically degrades in alkaline, acidic and oxidative environments but is found to be stable in neutral and photolytic situations. Table 3 includes the degradation study observation table in all conditions. According to data from mass spectra and HPLC chromatograms seen in various stress settings, some drug product impurities or derivatives were observed as D1, D2, and D3 under various stress conditions including alkaline, acidic and

oxidative. All of the HPLC chromatograms, mass spectra, and ion fragmentation peaks are shown in Figure 4. To examine if any excipients were discovered, blank samples were first put through the same arduous testing as DCV samples. The HPLC chromatograms and Mass spectra of commercial tablets and blank samples were contrasted. In $[M+H]^+$ ESI positive mode, the peak of DCV, mass spectra was discovered to be at m/z 739.3, which was further broken down into m/z 370.1, m/z 513.2 and m/z 565.2. Considering that those are the m/z peaks that are most typical of pure DCV drug.

Acidic condition

For the alkaline condition, DCV was refluxed with 0.1 N HCl for 4 hours (1 mg/mL). Then, this acidic solution (20 μ g/mL) was added to the HPLC instrument while maintaining the same chromatographic conditions. The measured DCV retention time (t_R) was 3.762 min. Other peaks named as D1, D2 and D3 were seen at t_R of 3.863, 4.121, and 4.783 min. The mass spectrometer $[M+H]^+$ ESI mode employed the same sample. The fragmentation of additional peaks, which were not visible in pure DCV, was noticed as m/z 339.1 and m/z 561.2 in the mass spectrum shown in figure 4. This shows that a degradant is present in an alkaline medium.

Sr. No.	Stress condition	Name of degradation Product	Retention time (min)	Major peak of fragmentation pattern (m/z)	Percentage of degradation
1.	0.1N HCl	D1, D2, D3	3.863, 4.121, 4.783	339.1, 561.2	72 %
2.	0.1N NaOH	D1, D2	5.188, 5.469	294.1, 339.1, 505.2, 527.2	97 %
3.	30% H ₂ O ₂	D3	4.038	301.1, 339.1	28 %
4.	H ₂ O	-	-	370, 513, 565, 739	No degradation



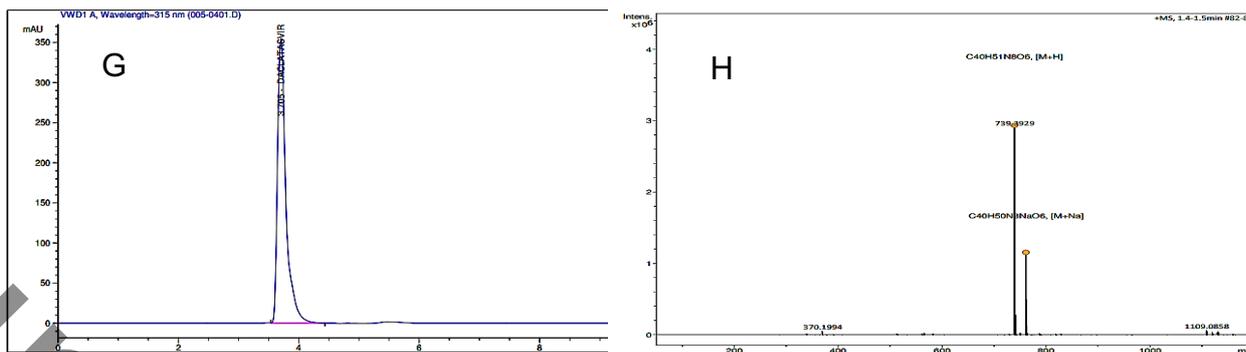


Figure 4. HPLC chromatograms and total ion MS/MS spectra scans relative to DCV stressed conditions. HPLC chromatogram of DCV exposed to 0.1N HCl, 60°C for 4 hr, degradant t_R at 3.863, 4.121, 4.783 min and MS/MS spectra scans having m/z 339.1, 561.2 fragment ion observed (A and B). HPLC chromatogram of DCV exposed to 0.1N NaOH, 60°C for 4 hr, degradant t_R at 5.188, 5.469 min. and MS/MS spectra scans having m/z 294.1, 339.1, 505.2, 527.2 fragment ions observed (C and D). HPLC chromatogram of DCV exposed to 30% H₂O₂, 60°C for 4 hr, degradant t_R at 4.038 min and MS/MS spectra scans having m/z 301.1, 339.1 fragment ions observed (E and F). HPLC chromatogram of DCV exposed to water, 60°C for 8 hr. (G and H)

Alkaline condition

In the alkaline condition, DCV was refluxed with 0.1 N NaOH for 4 hours at 60°C (1 mg/mL). After that, this alkaline solution was added to the HPLC instrument while still maintaining the same chromatographic conditions. The measured DCV retention time (t_R) was same as 3.648 min. While others chromatographic peaks observed at t_R 5.188 min and 5.469 min, another peak named as D1 and D2 respectively. The other mass fragmented ion such as m/z 294.1, m/z 339.1, m/z 505.2 and m/z 527.2 in the mass spectrum [M+H]⁺, which were not seen in pure DCV was shown in Figure 4. The same solution was utilized in the ESI mode of the mass spectrometer.

Oxidation condition

In the oxidation condition, DCV was refluxed with 3 percent hydrogen peroxide at 60°C for 4 hours (1 mg/mL). From this oxidized solution, a 20 µg/mL solution was made and put into the HPLC device with the same chromatographic settings. The measured DCV retention time (t_R) was 3.690 min. While one more additional peak named as D1 at t_R 4.038 min was observed. The mass spectrometer [M+H]⁺ ESI mode employed the same sample. The mass spectrum shown in figure 4 contains degradant fragments with m/z 301.1 and 339.1, which are absent in pure DCV drug sample.

Neutral condition

In the neutral state, DCV was refluxed with water for 8 hours at 60°C (1 mg/mL). Under the same chromatographic conditions, 20 µg/mL solution made from this was fed into the HPLC instrument. The measured DCV retention time (t_R) was 3.705 min. No other peaks were seen in the chromatogram. The mass spectrometer [M+H]⁺ ESI mode employed the same sample. In the mass spectrum shown in figure 4, fragmented peaks are matched with pure DCV.

Photochemical degradation

After 15 days of exposure to direct sunlight, DCV exhibited no symptoms of deterioration. The UV chamber use was another stressor. For 15 days, the DCV was exposed to 200Wh/m² of UV radiation and 1.2 million lux/h of visible light. There was no change in the DCV chromatogram. As a result, it was confirmed that there was no degradation. This suggests that the drug is durable to photochemical stress.

DISCUSSION

In the present work, stability indicating RP-HPLC assay method was developed and validated for routine quality control of tablet formulation containing daclatasvir. In most of the reported RP-HPLC methods for DCV, the ability of the method to quantitate specifically DCV in presence of its degradation products was not evaluated. Though some RP-HPLC methods have reported estimation of DCV in presence of process impurities and know impurities, the method is primarily for quantitation of impurities and is time consuming. The proposed HPLC method is primarily for estimation of DCV in tablet formulation in presence of its degradation products. The chromatographic parameters selected such as mixture of acetonitrile and 0.05% o-phosphoric acid (50:50 v/v) as mobile phase in isocratic mode and Hypersil C₁₈ column with 0.7 mL/min flow rate was found to give acceptable peak shape as indicated by the system suitability parameters. The analyte DCV was eluted with acceptable retention time of t_R 3.760 ± 0.01 min and hence the analysis is not time consuming. With linearity in the range of 10-50 µg/mL, the method is well suited for DCV formulation. The method was validated as per ICH guidelines. The percent recovery, evaluated by standard addition method, was found to be in range of 97.95 to 100.78% indicating that the method is accurate and can selectively quantitate DCV in presence of the excipients present in the formulation. The standard deviation for intra-day and inter-day precision was found in the range of ±0.3281

and ± 0.8914 indicating the repeatability and reproducibility of the method for quantification of DCV. The DCV was treated in various conditions and the degradation was observed. The data obtained in ruggedness and robustness studies expressed the lack of influence of operational and environmental variables on the test results obtained by proposed method. To ascertain the ability of the proposed method to selectively quantitate DCV in presence of its degradation products forced degradation studies were performed under acid, alkaline, oxidative, neutral and photochemical conditions. The treated samples were analyzed by proposed method. It was found that DCV degraded under acid, base and oxidative stress conditions whereas it was found stable under neutral hydrolysis and photochemical stress conditions applied. The method was able to separate DCV peak from its degradation product peak and selectively quantitate DCV indicating the specificity of the method. The identity of the degradation products was confirmed by high-resolution ESI-MS/MS techniques.

CONCLUSION

The current investigation illustrated the need of developing and validating a DCV stability-indicating RP-HPLC technique under varied stress levels in compliance with ICH requirements. The RP-HPLC strategy demonstrated to be a highly potent tool for separating and identifying degradation products of DCV medication in three different stress states discovered by high-resolution ESI-MS/MS techniques. As part of the validation process, the reported methodologies linearity, accuracy, LOQ, LOD, precision and ruggedness were all put to the test. Using this method, a commercial DCV tablet formulation was tested. The suggested method for regular analysis is relatively more straightforward and rapid because it avoids more tedious chemical procedures. It is also simple, quick and cost-effective method. The finding of DCV degradation study opens up new directions for drug stability research and understanding, resulting in more tools for quality control and safer treatments.

Ethics

Ethics Committee Approval: The ethics committee approval not required for the proposed research. We were not used any kind of human being and animal matrices.

Informed Consent: Not applicable

Authorship Contributions Concept: H.M.N., Design: H.M.N., Data Collection or Processing: M.N.D., Analysis or Interpretation: H.M.N., M.N.D., S.J.P Literature Search: H.M.N., S.J.P., Writing: H.M.N., S.J.P., M.N.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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