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Analytical Method Development and Validation for the Estimation of Daclatasvir in API Form and Marketed Formulation by RP-HPLC

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Saleha Tabassum[1], A. Yasodha[2], K. Sujatha[3]

[1]Department of Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001 India.

[2]Professor & Principal, Department of Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001 India.

[3]Assistant Professor, Department of Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001 India.

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ABSTRACT

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Daclatasvir in bulk form and marketed formulation. Separation of Daclatasvir was successfully achieved on a Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. column in an isocratic mode of separation utilizing Methanol and Acetonitrile in the ratio of 80:20% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 271nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 6-16mcg/mL for Daclatasvir. The correlation coefficient was found to be 0.9989 for Daclatasvir. The LOD and LOQ for Daclatasvir were found to be 0.05µg/mL and 0.15µg/mL respectively. The proposed method was found to be good percentage recovery for Daclatasvir, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

INTRODCUTION

Daclatasvir is an orally available antiviral agent that inhibits the NS5A region of the hepatitis C virus (HCV) and was used in combination with other oral antiviral agents to treat chronic hepatitis C before its withdrawal in 2019. Elevations in serum enzyme levels during Daclatasvir therapy are uncommon, and it has yet to be convincingly implicated in cases of idiosyncratic liver injury with jaundice [1]. Nevertheless, successful all-oral regimens of antiviral therapy in patients with chronic hepatitis C and cirrhosis are occasionally complicated by hepatic decompensation and may cause reactivation of hepatitis B in susceptible patients coinfected with the hepatitis B virus (HBV). Daclatasvir is a member of the class of biphenyls that is a potent inhibitor of nonstructural protein 5A and is used (as its hydrochloride salt) for treatment of hepatitis C. It has a role as a nonstructural protein 5A inhibitor and an antiviral drug. It is a member of biphenyls, a member of imidazoles, a carbamate ester, a Carboxamide and a valine derivative [2]. It is a conjugate base of a Daclatasvir (2+). Daclatasvir is a Hepatitis C Virus NS5A Inhibitor. The mechanism of action of Daclatasvir is as a P-Glycoprotein Inhibitor, and Organic Anion Transporting Polypeptide 1B1 Inhibitor, and Organic Anion Transporting Polypeptide 1B3 Inhibitor, and Breast Cancer Resistance Protein Inhibitor [3]. The IUPAC name of Daclatasvir is methyl N-[(2S)-1-[(2S)-2-[5-[4-[4-[2-[(2S)-1-[(2S)-2-(methoxy carbonyl amino)-3-methyl butanoyl] pyrrolidin-2-yl]-1H-imidazol-5-yl] phenyl] phenyl]-1H-imidazol-2-yl] pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl] carbamate. The Chemical Structure of Daclatasvir is shown in follows:

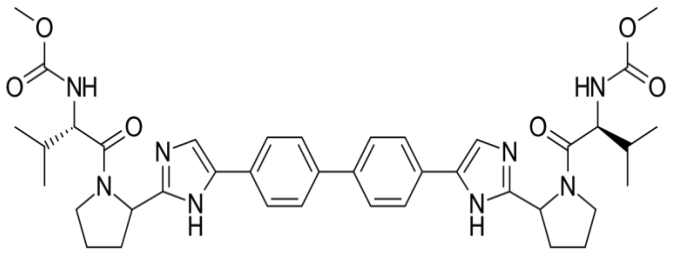

Fig-1: Chemical Structure of Daclatasvir

EXPERIMENTAL

Table-1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**ELICO SL-159** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.
7.	P^H Analyser **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

Table-2: List of Chemicals used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
4.	Dipotassium hydrogen orthophosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
5.	Potassium dihydrogen Orthophosphate	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
6.	Ortho Phosphoric acid	96%	A.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric Acid	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
8.	Sodium Hydroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
9.	Hydrogen Peroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai

Method Development:

Selection of Wavelength:

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm.

This has been performed to know the maxima of Daclatasvir, so that the same wave number can be utilized in HPLC UV detector for estimating the Daclatasvir. While scanning the Daclatasvir solution we observed the maxima at 226 nm.

Sample & Standard Preparation for the UV Analysis

25 mg of Daclatasvir standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.4ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase [4].

Optimization of Chromatographic Conditions:

The chromatographic conditions were optimized by different means [5]. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table-3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry C_{18}, 5µm, 250mmx4.6mm i.d.	Methanol : Water = 75:25	0.8ml/min	271nm	Very Low response	Method rejected
Inertsil C_{18}, 5µm, 250mmx4.6mm i.d.	Methanol : Water = 50:50	0.9ml/min	271nm	Low response	Method rejected
Kromasil C_{18}, 5µm, 250mmx4.6mm i.d.	Acetonitrile: Water = 60:40	1.0ml/min	271nm	Tailing peaks	Method rejected
Develosil C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol :Acetonitrile=50:50	1.0ml/ min	271nm	Broad Peak	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol :Acetonitrile =80:20	1.0ml/ min	271nm	Tailing peaks	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Acetonitrile: Methanol =65:35	1.0ml/ min	271nm	Good Peak	Method accepted

Preparation of Mobile Phase:

650mL (65%) of Acetonitrile and 350mL of Methanol (35%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration [6].

Sample & Standard Preparation for the HPLC Analysis

25 mg of Daclatasvir standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.4ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Method of Validation

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, robustness, ruggedness, sensitivity and specificity according to ICH Q2 (R1) guideline and USP guidelines [7,8,18,22,30,35].

System Suitability Parameters

System suitability tests were performed to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters monitored for system suitability include retention time, theoretical plate number, peak area, tailing factor, and resolution. The repeatability of these parameters was checked by injecting 3 times the test solution of Daclatasvir 10 µg/mL. The results shown in Table 5 were within acceptable limits [9].

Method of Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of an analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity [10]. The linearity of the analytical method was demonstrated over the concentration range investigated by analysis (n=1) at a concentration range of 6-16 µg/ ml. The absorbance obtained at respective concentration was recorded, and the graph is plotted as concentration (µg/ml) versus absorbance. The linear regression equation and the coefficient correlation were obtained from the Calibration curve.

Method of Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and

the value found. This is sometimes termed trueness. The accuracy of proposed method was determined on the basis of recovery study. Recovery study was carried out by spiking standard working solution to sample solution (formulation) at three different levels 80%, 100% and 120%. The final concentration of Daclatasvir was determined at each levels of the amount; three determinations were performed [11]. The percentage recovery was calculated as mean ± standard deviation.

Method of Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day precision study, three different solutions of same concentration were prepared and analysed in the same day (morning, noon and evening), whereas in the inter-day precision study, the solutions of same concentration were prepared and analysed, for three consecutive days, and the absorbance were recorded [12]. The result was indicated by calculating percentage RSD.

Method of Robustness

The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [13].

Method of Ruggedness

The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days [14].

RESULTS AND DISCUSSION

Analytical Method Development:

Selection of Wavelength:

The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450. The scanned UV spectrum is shown below,

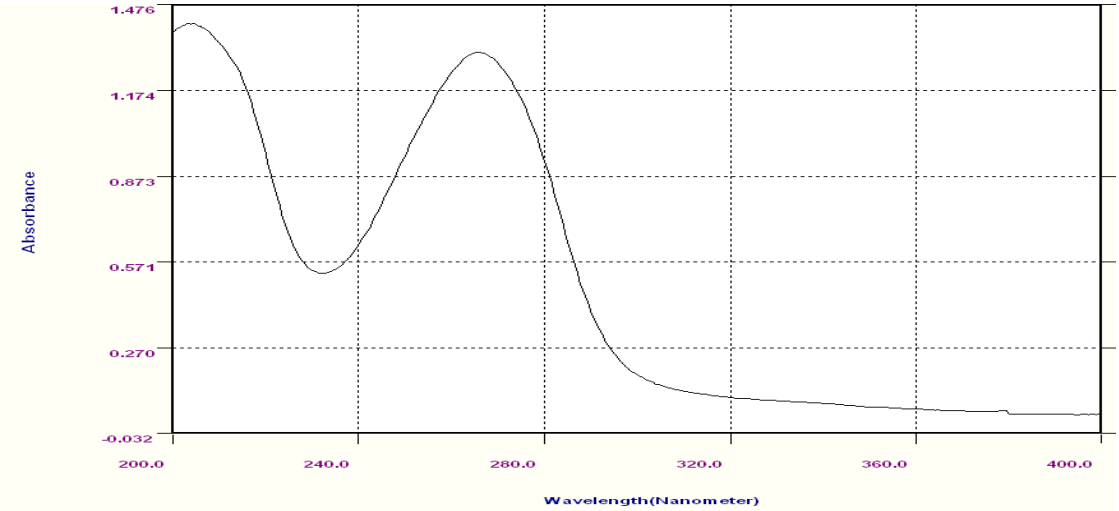

Fig-2: UV Spectrum for Daclatasvir (271 nm)

Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-4: Summary of Optimized Chromatographic Conditions

Mobile phase	Methanol :Acetonitrile = 80:20% v/v
Column	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.
Flow rate	1.0 ml/ min.
Wavelength	271nm
Sampling System	Automatic
Temp. of Auto sampler	Ambient
Volume of injection	10µl
Run time	08 min.
Mode of Separation	Isocratic

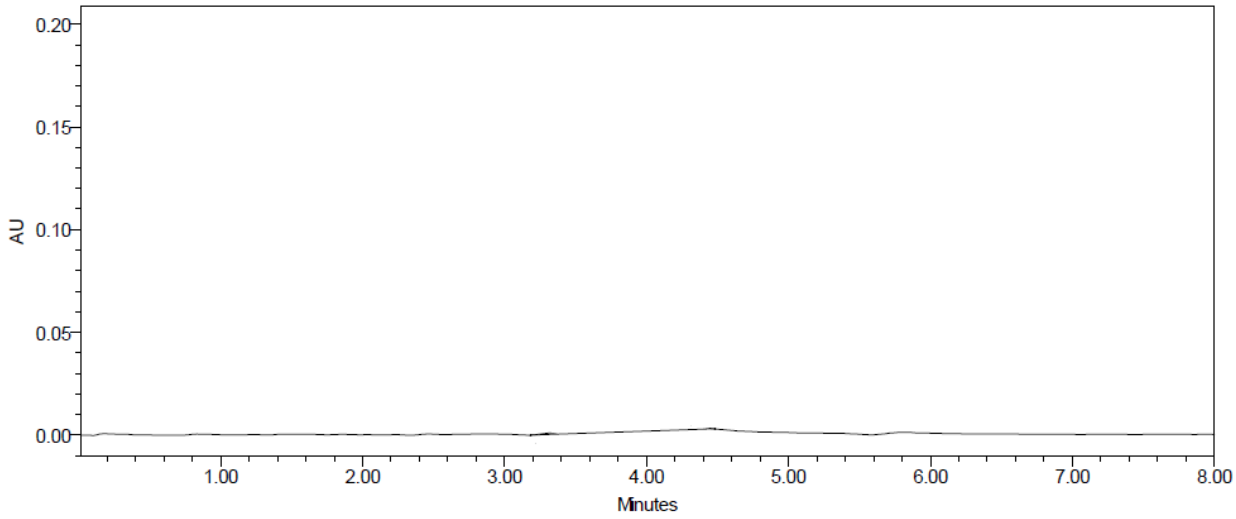

Fig-3: Chromatogram for Blank Solution

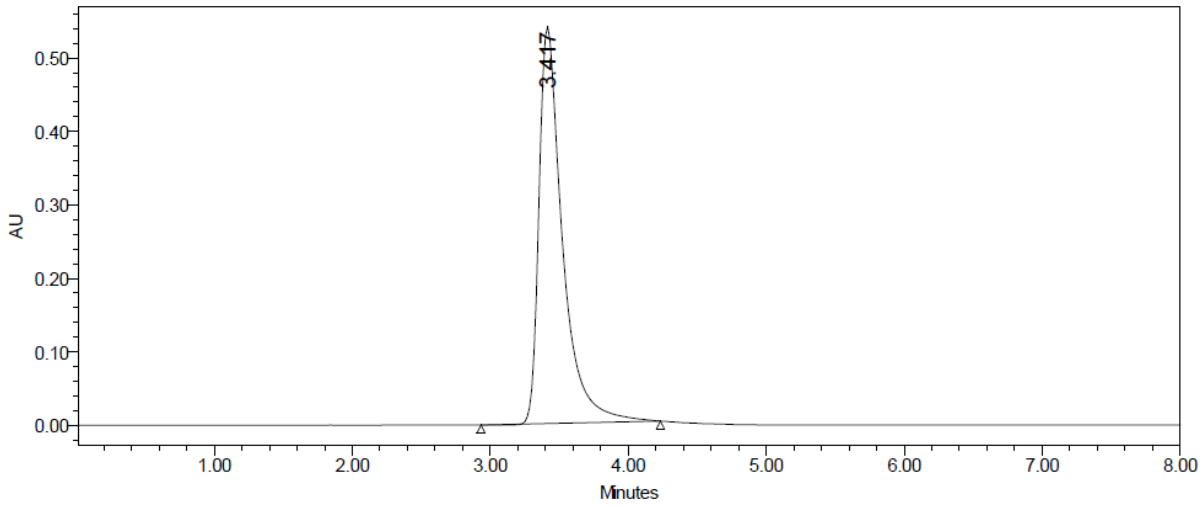

Fig-4: Chromatogram of Daclatasvir in Optimized Condition

Analytical Method Validation

System Suitability Parameter

This includes the type of equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be examined [15]. The following system suitability test parameters were determined. The obtained data are shown in Table-5.

Table-5: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	2.81
2	Asymmetry	T ≤ 2	Daclatasvir =0.26
3	Theoretical plate	N > 2000	Daclatasvir =2986

Accuracy

Accuracy: Recovery Study of Daclatasvir

The accuracy of the proposed developed method the % recovery studies were carried out by adding different quantities (80%, 100%, and 120%) of pure drug of Daclatasvir was taken and added to the prepared pre-analyzed formulation of concentration 10µg/ml. From that % recovery values were measured [16]. The results were shown in Table-6.

Table-6: Data of Recovery Studies for Daclatasvir

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.105	93435	101.312	Mean= 100.0163% S.D. = 1.293505 % R.S.D.= 1.293294
S_2 : 80 %	8	7.898	91287	98.725	
S_3 : 80 %	8	8.001	92356	100.012	
S_4 : 100 %	10	10.195	115135	101.95	Mean= 101.4033% S.D. = 0.613379 % R.S.D.= 0.60489
S_5 : 100 %	10	10.152	114687	101.52	
S_6 : 100 %	10	10.074	113879	100.74	
S_7 : 120 %	12	12.171	135647	101.425	Mean= 100.6053% S.D. = 0.730041 % R.S.D. = 0.725649
S_8 : 120 %	12	12.044	134324	100.366	
S_9 : 120 %	12	12.003	133897	100.025	

Precision

Repeatability

Repeatability was assessed using six time repetition of working concentration [17].

The results are shown in Table-7.

Table-7: **Data Showing Repeatability Analysis for Daclatasvir**

HPLC Injection Replicates of Daclatasvir	**Area Under the Curve**
Replicate – 1	112546
Replicate – 2	113824
Replicate – 3	111351
Replicate – 4	111584
Replicate – 5	112419
Replicate – 6	112572
Average	**112382**
Standard Deviation	**876.7543**
% RSD	**0.7801**

Result & Discussion: The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml for Daclatasvir (n =6) showed a RSD of 0.7801% for Daclatasvir [19]. It was concluded that the analytical technique showed good repeatability.

Intermediate Precision:

Intra-Assay & Inter-Assay:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Daclatasvir revealed that the proposed method is precise [20,21].

Table-8: Results of Intra-Assay & Inter-Assay Daclatasvir

Conc. of Daclatasvir (API) (µg/ml)	**Observed Conc. of Daclatasvir (µg/ml) by the Proposed Method**			
	Intra-Day		**Inter-Day**	
	Mean (n=6)	**% RSD**	**Mean (n=6)**	**% RSD**
8	8.07	0.27	8.95	0.34
10	10.39	0.39	10.56	0.65
12	11.19	0.23	12.01	0.34

Result and Discussion:

The intraday and interday studies results show that the mean % RSD was found to be within acceptance limit i.e. (≤2%). Hence it was concluded that there was no significant difference for the assay, which was tested within the day and between the days [23]. So, we concluded that the proposed method at selected wavelength was found to be precise.

Linearity and Range

Standard solutions of Daclatasvir in the concentration range of 0 µg/ml to 16 µg/ml were obtained by transferring (0.6, 0.8, 1.0, 1.2, 1.4, 1.6ml) of Daclatasvir stock solution (1000ppm) to the series of 10 ml volumetric flasks. The volumetric flasks were made up to the mark with mobile phase. The solutions were filtered through a 0.45 µm membrane filter and degassed under ultrasonic bath. The final resulted solutions were injected into HPLC the system [24]. The run time/stop time maintained was 8 min and the various types of peak areas were measured. The calibration data are shown in Table-9 and calibration curve data are shown in figure-5.

Table-9: Calibration Data for Daclatasvir

S. No.	Conc. (µg/ml)	Mean Peak Area
1	0	0
2	6	61233
3	8	84610
4	10	110247
5	12	130435
6	14	153354
7	16	172043

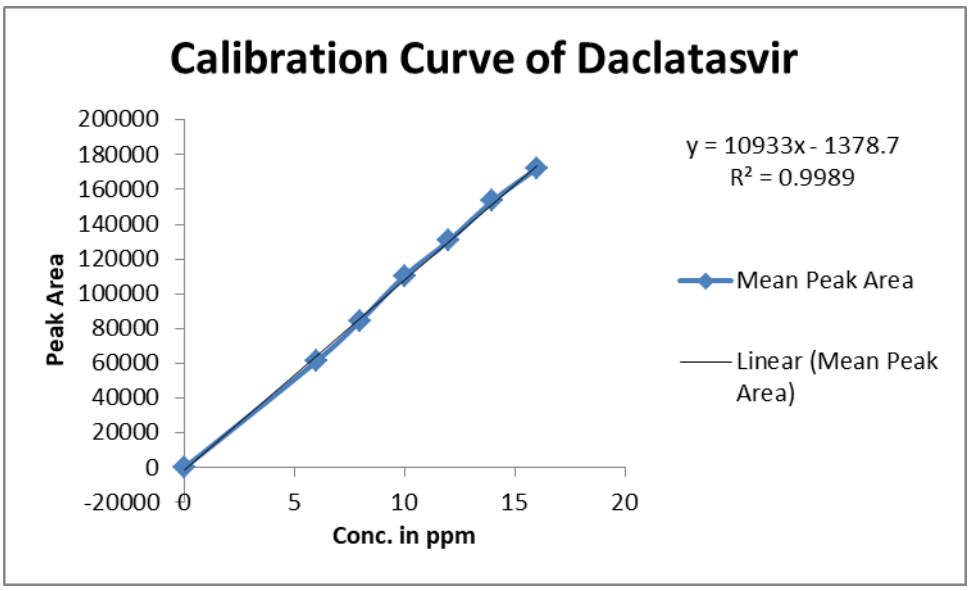

Fig-5: Calibration Curve for Daclatasvir

Result & Discussion

Linearity range was found to be 0-16 µg/ml for Daclatasvir. The correlation coefficient was found to be 0.998 and the slope was found to be 10933 and intercept were found to be 1378 for Daclatasvir [25].

Specificity:

Specificity of the pharmaceutical analysis is the ability to measure accurately and specifically the concentration of API, without interference from other active ingredients, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of samples and standard were compared [26].

Method Robustness:

The influence of small changes in optimized chromatographic conditions such as changes in Flow Rate (± 0.1ml/min), Temperature (±5^{0}C), Wavelength of detection (±2nm) & Mobile Phase (±2%) studied to determine the robustness of the method are also in favour of (Table-10, RSD (%) < 2%) the proposed RP-HPLC method was used for the analysis of Daclatasvir (API) [27-28].

Table-10: Result of Method Robustness Test

Change in Parameter	**% RSD**
Flow (1.1 ml/min)	1.13
Flow (0.9 ml/min)	0.09
More Organic	0.78
Less Organic	0.72
Wavelength of Detection (273 nm)	0.77
Wavelength of detection (269 nm)	0.59

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantization (LOD and LOQ) can be determined by the following equations [29]. These equations are based on the signal to noise ratio. These two equations are useful for the determination of LOD and LOQ.

L.O.D. = 3.3 (SD/S).

L.O.Q. = 10 (SD/S)

Where,

SD = Standard deviation Response

S = Slope of the Calibration curve

The slope S and standard deviation response values are obtained from the calibration curve of the analyte (Drug) [31].

Result & Discussion

The LOD was found to be 0.05 µg/ml and LOQ was found to be 0.15 µg/ml for Daclatasvir which represents that sensitivity of the method is high.

Assay of Daclatasvir in Pharmaceutical Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system [32].

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded [33].

ASSAY:

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

Where:

AT = Peak Area of Test obtained with test preparation

AS = Peak Area of Standard obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Assay was performed as described in previous chapter. Results obtained are tabulated below:

Table-11: Assay of Daclatasvir Tablets/Capsules

Brand Name of Tablets	**Labelled amount of Drug (mg)**	**Mean (±SD) Amount (mg) found by the Proposed Method (n=6)**	**Mean (± SD) Assay (n = 6)**
Dacla Hep Tablets (Hetero Pharmaceuticals)	60mg	59.236 (± 0.452)	99.596 (± 0.369)

Results and Discussion: The assay of Dacla Hep Tablets containing Daclatasvir was found to be 99.596%.

Forced Degradation Studies

The API (Daclatasvir) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after a long time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation [34-35].

Results of Stability Studies:

The results of the stress studies indicated the specificity of the method that has been developed. Daclatasvir was stable in photolytic and acidic stress conditions. The result of forced degradation studies are given in the following table-12.

Table-12: Results of Forced Degradation Studies of Daclatasvir API

Stress Condition	Time	Assay of Active Substance	Assay of Degraded Products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	85.69	14.31	100.0
Basic Hydrolysis (0.1M NaOH)	24Hrs.	83.47	16.53	100.0
Wet Heat	24Hrs.	79.86	20.14	100.0
UV (254nm)	24Hrs.	87.92	12.08	100.0
3 % Hydrogen Peroxide	24Hrs.	80.81	19.19	100.0

SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for simultaneous analysis of Daclatasvir different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Phenomenex Luna C_{18}, 100A, 5µm, 250mm x 4.6mm i.d. column was

preferred because using this column peak shape, resolution and absorbance were good. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Daclatasvir it is evident that most of the HPLC work can be accomplished in the wavelength range of 271 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 10 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay & stability which can help in the simultaneous analysis of Daclatasvir in different formulations.

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Daclatasvir. Further the planned RP-HPLC methodology has glorious sensitivity, exactness and dependableness. The result shows the developed methodology is one more appropriate methodology for assay, purity & stability which may facilitate within the analysis of Daclatasvir in several formulations.

REFERENCES

1. https://go.drugbank.com/drugs/DB09102
2. https://pubchem.ncbi.nlm.nih.gov/compound/Daclatasvir
3. https://en.wikipedia.org/wiki/Daclatasvir
4. Quantitative Analysis of drugs in Pharmaceutical formulation, IIIrd Ed., by P.D. Sethi, pp.1-21, 51-56.
5. Quality assurance, worth the effort, Inforum, october2003 volume 7; Number.4.
6. Kasture et al, Hand book of Pharmaceutical Analysis, Volume-1.Shetti.P.D, High Performance Liquid Chromatography, 2001, P.11.
7. Validation of Analytical Procedures, Methodology, and ICH Harmonized Tripartite Guidelines, 1996.
8. Text on Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines, 1994.
9. Ravi Shankar, a Text book of Pharmaceutical Analysis, Third edition, page 2.2.
10. Practical HPLC method development" by Snyder, Glajch and Kirkland, A. Wiley Interscience Publication, p.4-10, 92-102.
11. Ahuja S. In: High Pressure Liquid Chromatography of Comprehensive Analytical Chemistry. Elsevier Publications. 2006.
12. Principles and Methods. In: Amesham Biosciences of Reversed Phase Chromatography. 6-8.
13. Synder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development, 2nd Ed, John Wiley and Sons Inc. Canada. 1997.
14. Mohammad T et al., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. International Pharmaceutica Sciencia. 2012, 2(3), 14.
15. Snyder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development. 2nd ed, 2001.
16. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4), 22-23.
17. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.
18. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
19. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.
20. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC Chromacademy.

21. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
22. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
23. British Pharmacopoeia. (International Ed.) 1993; Vol. 1:429, 483. Published on the Recommendation of the Medicines Commissions Pursuant to Medicines Act 1968, 1993.
24. United States Pharmacopoeia 29 NF 24, Published on the Recommendation of the Medicines Commissions Pursuant to Medicines, page no. 587.
25. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), P1-7.
26. Skoog, West, Holler, Crouch, "Fundamentals of analytical chemistry", eighth edition, 2009 (Indian edition), Cengage learning India Pvt ltd, New Delhi, Page no. 271-280.
27. A.V Kasture, K.R Mahadik, S.G Wadodkar, H.N. More, "A textbook of pharmaceutical analysis, Instrumental methods", Nirali Prakashan, vol.2, 9th edition, page no. 5-7, 28-30.
28. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4), 22-23.
29. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.
30. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
31. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.
32. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
33. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC Chromacademy.
34. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
35. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
36. Syed Suffdar Hussain Shah 1, Muhammad Iqbal Nasiri 2, Humera Sarwar 1, Aatka Ali 1, Syed Baqir S Naqvi 1, Sohail Anwer 1, Muhammad Kashif 1, RP-HPLC method development and validation for quantification of Daclatasvir dihydrochloride and its application to pharmaceutical dosage form, Pak J Pharm Sci. 2021 May; 34(3):951-956.
37. Pavithra v. *1, Jose Gnana Babu C. *2, Sowmya H G.*3, A Validated Rp-Hplc Method for the Estimation of Daclatasvir Dihydrochloride in Bulk and Pharmaceutical Formulation, International Journal of Pharmaceutical Research and Applications, Volume 6, Issue 3 May - June 2021, pp: 1291-1299.
38. Makupalli Gayathri Devi*, Kundavaram Raju, Archana Terala, RP-HPLC Method for Estimation of Daclatasvir in Bulk and Tablet Dosage Form, RGUHS Journal of Pharmaceutical Sciences, Year: 2018, Volume: 8, Issue: 4, Page no. 103-119, DOI: 10.5530/rjps.2018.4.1.
39. 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, Scholars Research Library Der Pharmacia Lettre, 2016, 8 (15):107-113.
40. Sadaf Sarfraz, Muhammad Saeed, Mohsin Javed, Nadia Hussain, Shahid Iqbal, Shahid Amin, Ali Bahadur, Ahmed Hussain Jawhari, Randa A. Althobiti, Eman Alzahrani, Abd-ElAziem Farouk, Samar O. Aljazzar, Eslam B. Elkaeed, "Validation of a Cost-Effective RP-HPLC Method for Quantitative Investigation of Daclatasvir Dihydrochloride in Pharmaceutical Formulations", Journal of Chemistry, vol. 2023, Article ID 4908091, 9 pages, 2023. https://doi.org/10.1155/2023/4908091.

 **Author -1**	Saleha Tabassum – Corresponding Author Department of Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001
Image **Author -2**	A. Yasodha Professor & Principal, Department of Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001
Image **Author -3**	K. Sujatha Assistant Professor, Department of Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001