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Analytical Method Development, Validation and Stress Degradation Study of Daclactasvir by U V Spectroscopic Method

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ABSTRACT : To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for of stress degradation studies for quantification of Daclatasvir. The drug is freely soluble in organic solvents Methanol. The drug was identified in terms of solubility studies and on the basis of melting point done by capillary tube method. It showed absorption a maximum was determined in Methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The melting point of the pure drug Daclatasvir was determined by capillary tube method. It was found that in between 163^{o} -165^{o}C. It showed absorption maxima 317 nm in Methanol. On the basis of absorption spectrum the working concentration was set on 10µg/ml (PPM). The linearity was observed between 2-12 µg/ml (PPM). The values of LOD were found to be 5.82ug/ml for Daclatasvir and the calculated LOQ values were found to be 17.12ug/ml.The stability studies on the drug were carried out successfully. The drug which when subjected to thermal, photolytic, oxidative, and acidic stress degraded into many degradation products. In most of the cases, the degradation rate was seen to be directly proportional to the amount of stress applied. The thermal stress was increased by increasing the incubation temperature, the faster the degradation took place.

KEYWORDS Daclatasvir, Validation, force degradation, Methanol, U V Spectrophotomer, Method validation

I. INTRODUCTION

VALIDATION [1, 2, 3]

Validation is an integral part of quality assurance which helps to maintain current good manufacturing practices which results in safety, quality, purity and efficacy of product. According to WHO "Validation is establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce products which meet its predetermined specifications and quality characteristics."

The documented act of demonstrating that any procedure, process and activity will consistently lead to the expected results. It also includes the qualification of systems and equipment.

Manufacturers should plan validation in a manner that will ensure regulatory compliance and ensuring that product quality, safety and consistency are not compromised.

Analytical Method Validation [4, 5, 6, 7]

The validation of analytical method is the process in determining the suitability of a given methodology by laboratory studies; that the method can meet the requirements for intended use. Method validation is not simply a measure of procedure but method validation is a measure of performance of the total analytical system.

According to USP "Validation is the process of providing documented evidence that the method does what it is intended to do." In other words, the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible, and rugged for its intended use. Method validation is a regulatory requirement.

Various Guidelines describe typical analytical performance characteristics, how they are determined, and which subset of data is required to demonstrate validity, based on the methods intended use. These analytical performance characteristics are:

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1. Accuracy
2. Precision
3. Specificity
4. Limit of Detection (LOD)/ Detection Limit (DL)
5. Limit of Quantitation (LOQ)/ Quantitation Limit (QL)
6. Linearity and range
7. Ruggedness
8. Robustness

Development of Stability-Indicating Analytical Method [SIAM] [8]

The purpose of the stability study is to investigate how the quality of the drug product changes with time under the influence of environmental factors, to establish a shelf life for the product and to recommend storage conditions.

Forced Degradation

Since the conditions that cause instability and result in degradation products of the API cannot be predicted initially, one has to subject the API to a variety of stress conditions. Trial and error are needed to find the proper combination of stress agent concentration and time to effect degradation, preferably in the 5-15 % range. Depending on the API, not every stress agent may affect degradation, but each agent has to be evaluated to determine whether degradation occurs or not.

Typical degradative conditions involve hydrolysis, photolysis, acid/base reactions, and temperature. Achieving 100 % degradation would require too much effort and could be possibly cause secondary degradation. Secondary degradation products are the degradation products of the degradation products, which are not likely to be formed under normal storage conditions.

II. MATERIALS

Table 1: Active Drug:

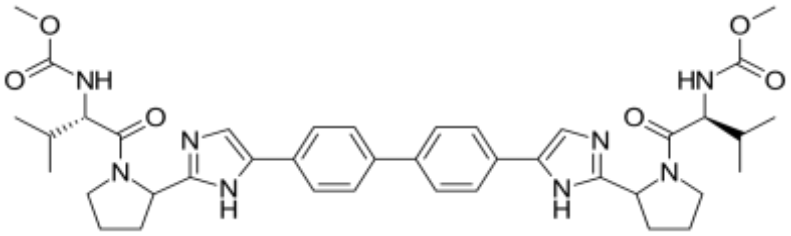
NAME OF DRUG	**DACLATASVIR**: It is a new oral, direct-acting antiviral with potent pan genotypic activity. It is an inhibitor of hepatitis C virus (HCV) NS5A protein. NS5A is involved in both viral RNA replication and virus particle assembly.
STRUCTURE	
MOLECULAR FORMULA	$C_{40}H_{50}N_8O_6$
CATEGORY	Antivirus
SOLUBILITY	DMSO (Slightly), Methanol (Slightly)
MELTING POINT	163^{o} - $165^{o}C$
DOSE	In adults, Daclatasvir is orally administered at a dosage of 30 mg twice daily.
USES	Daclatasvir is used with another antiviral medication (sofosbuvir) to treat chronic (long-lasting) hepatitis C, a viral infection of the liver.

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Table 2: Solvents and Chemicals:

Sr. No.	Solvents and Chemicals	Name of Company
1.	Methanol HPLC Grade	E. Merck Ltd.,Mumbai, India
2.	Water HPLC Grade	E. Merck Ltd.,Mumbai, India
3.	O – phosphoric acid AR Grade	E. Merck Ltd.,Mumbai, India

Table 3: Marketed Formulation:

Sr. No.	Marketed Formulation	Manufacturer
2.	Daclakem (Daclatasvir 60 mg tablet)	Alkem Laboratories Ltd., Mumbai, Maharashtra.

Table 4: List of Equipment:

Sr. No	Equipment	Make/ Model
1.	Precision balance	Mettler Tolledo
2.	pH meter	Labinda
3.	Grade 'A' certified Glassware	Borosil
4.	Ultrasonicator	FAST CLEAN Ultrasonic Cleaner
5.	Electronic balance	Electrolex New
6.	UV Spectrophotometer	Shimadzu UV 1800, Corporation Japan

III. RESULTS AND DISCUSSION

❖ **Development of UV-Spectrophotometric Method:**

➢ **Melting Point Determination:**

The melting point of the pure drug Daclatasvir was determined by capillary tube method. It was found that in between 163° - 165°C.

➢ **Preparation of Standard Stock Solution: (1000 µg/ml)**

A 10mg of standard Daclatasvir was weighed and transferred to 100ml volumetric flask and dissolved in 50ml methanol. The flask was shaken and volume was made up to the mark with methanol. From this stock solution further 10ml was transferred in 100ml volumetric flask and diluent was added up to mark to give a solution containing 100µg/ml Daclatasvir.

➢ **Preparation of Sample Solution:**

The accuracy of the analytical method for Daclatasvir was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 317 nm and results were expressed in terms of % recoveries. Standard deviation and % RSD was calculated. An accurately weighed tablet powder equivalent to 10 mg of Daclatasvir was transferred in series of 10.0 ml volumetric flasks and then known amount of Daclatasvir was added over the range of 80%, 100%, 120%. The content in the flask were shaken for 10-15 min. with methanol and volume was adjusted up to mark with same. The solutions were then filtered through Whatmann No.1 filter paper and were used as sample. Accurately measured 1.0 ml portion of filtrate was transferred to a 10.0 ml volumetric flask further diluted upto the mark with methanol to obtained final concentration of 10µg/ml.

➢ **Determination of Absorption Maxima:**

➢ **Selection of Wavelength:**

2, 4, 6, 8, 10, and 12 µg/ml solution of Daclatasvir was prepared in diluent and spectrum was recorded between 200-400nm. The overlain spectrum of Daclatasvir at different concentration was recorded and peak maxima of drug were found. The peak maximum of Daclatasvir was

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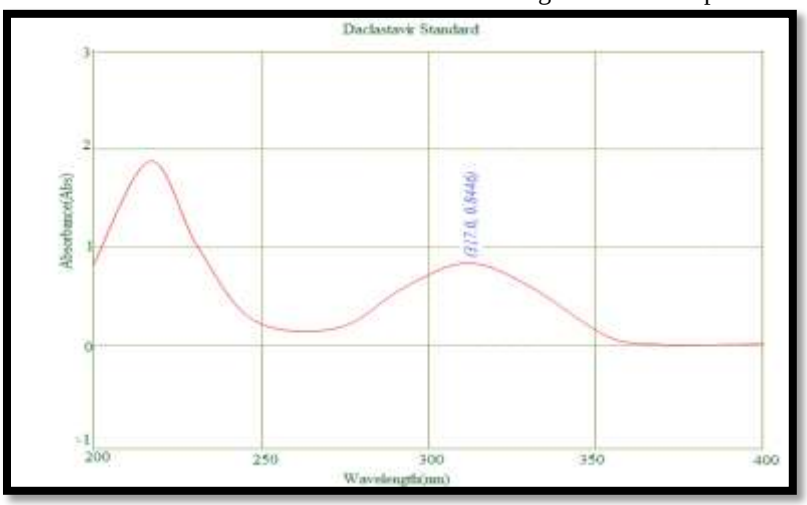
317nm. The spectrum of Daclatasvir at target concentration was recorded. The drug showed absorption maxima at 317.0 nm

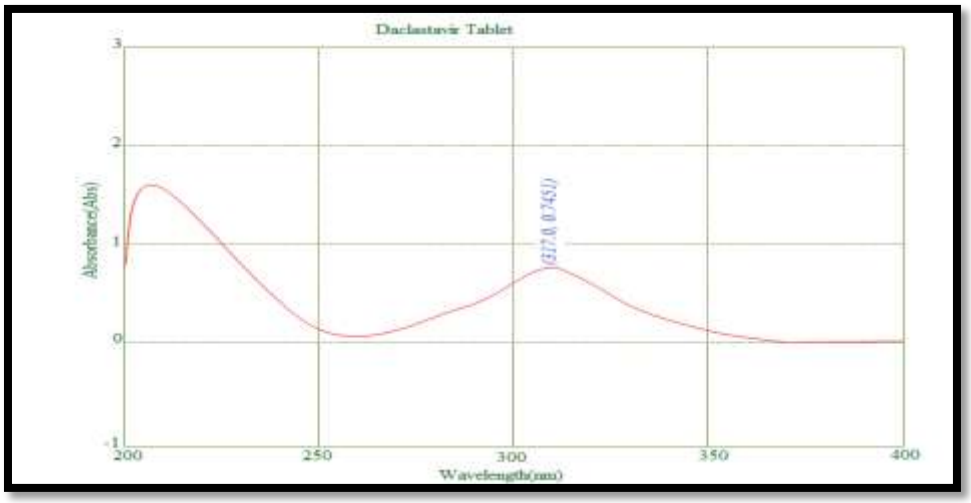


"Fig 1", Spectra of Standard Daclatasvir With Solvent (10µg/ml)

- **Calibration Curve for Daclatasvir:**

Appropriate volume of aliquots from standard Daclatasvir stock solution was transferred to different volumetric flasks. The volume was adjusted to the mark with the diluent to obtain the concentration of 2, 4, 6, 8, 10, and 12µg/ml.

Calibration curve of each solution against the diluent was recorded at 317 nm was measured and the plot of absorbance v/s concentration was plotted. The absorptivity of drug at wavelength 317 nm was found by straight-line equation. It shows straight line meaning the calibration curve obeys Beers law.



"Fig 2", Spectra of Daclatasvir Tablet With Solvent (10µg/ml)

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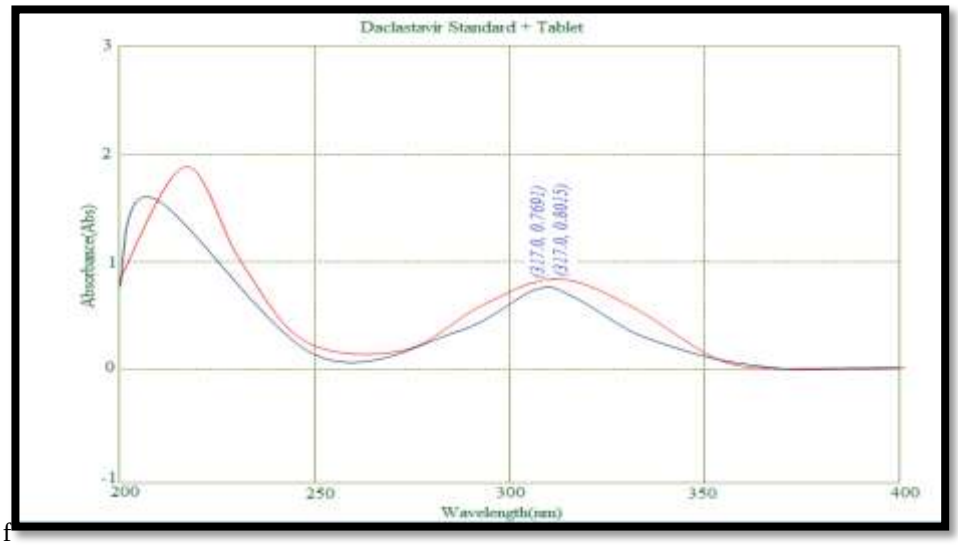

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"Fig 3", Overlay Spectra of Standard Daclatasvir and Tablet With Solvent (10µg/ml)

❖ **Method Validation Parameter of UV:**

The developed method was validated as per ICH guidelines for following parameters:

➢ **Accuracy :** Accuracy was reported as % recovery which was calculated from the expression as equation given below,

$$\% \text{ Recovery} = \frac{B - A}{C}$$

Where,

B = Total amount of drug estimated

A = Amount of drug found on pre-analyzed basis

Table 5.The Result of Accuracy studies

Level (%)	Formulation (µg/ml)	Added pure drug (µg/ml)	Amount recovery (µg/ml)	% Recovery	Mean	%R.S.D.
80%	3	5	7.90	98.75	99.06	0.44
80%	3	5	7.95	99.37		
100%	5	5	9.93	99.30	98.65	0.93
100%	5	5	9.80	98.00		
120%	7	5	11.92	99.33	99.49	0.23
120%	7	5	11.96	99.66		

➢ **Precision:**

The precision of the method was checked by carrying out repeatability, intraday and interday precision. Result of precision studies expressed in % RSD according to ICH guidelines acceptable limit (< 2) which indicates good repeatability and low variability in inter-day. For intraday and interday study the six solutions at different concentrations (2, 4, 6, 8, 10, and 12µg/ml) were prepared using six different aliquots of stock solution. The absorbance of the resulting solutions were recorded at 317 nm and the obtained data were used to calculate S.D. and %R.S.D.

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Intraday Precision Study:

Table 6. The Result of Intraday Precision Study

Sr. no.	Conc. (µg/ml)	Absorbance			Mean	S.D.	%R.S.D.
		Morning	Afternoon	Evening			
1.	2	0.125	0.127	0.128	0.127	0.0015	1.205
2.	4	0.225	0.223	0.226	0.224	0.0015	0.679
3.	6	0.329	0.329	0.33	0.329	0.0005	0.175
4.	8	0.447	0.445	0.448	0.446	0.0015	0.341
5.	10	0.549	0.551	0.548	0.550	0.0015	0.278
6.	12	0.662	0.661	0.659	0.660	0.0015	0.231
Average					0.389	0.0013	0.485

***(n=6) number of determination**

Interday Precision Study:

Table 7.The Result of Interday Precision Study

Sr. no.	Conc. (µg/ml)	Absorbance			Mean	S.D.	%R.S.D.
		Day 1	Day 2	Day 3			
1.	2	0.189	0.187	0.190	0.189	0.0015	0.809
2.	4	0.229	0.231	0.232	0.231	0.0015	0.662
3.	6	0.329	0.334	0.332	0.332	0.0025	0.758
4.	8	0.447	0.452	0.450	0.450	0.0025	0.559
5.	10	0.549	0.556	0.557	0.554	0.0043	0.786
6.	12	0.662	0.668	0.659	0.663	0.0045	0.691
Average					0.403	0.0028	0.711

***(n=6) number of determination**

- **Linearity and Range:**

For linearity study, six solutions at different concentrations (2, 4, 6, 8, 10, and 12µg/ml) were prepared using six different aliquots of stock solution. The absorbances of the resulting solutions were recorded at 317 nm and the obtained data were used to plot the graph of linearity.

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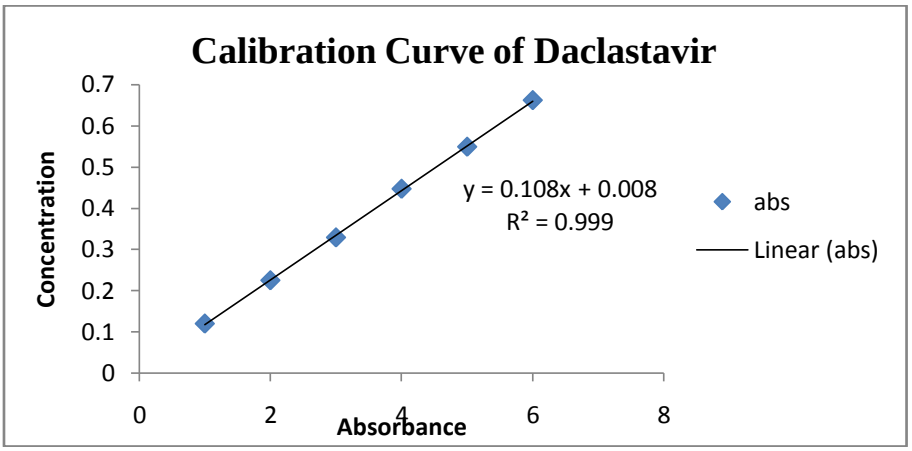

"Fig 4", Calibration Curve for Daclatasvir

➢ **Ruggedness :**

Sample solution of Daclatasvir were prepared from stock solution and analyzed by two different analysts using similar operational and environmental conditions.

Table 8.The Result of Ruggedness Study

Sr. no.	Analyst	Absorbance	Mean	%R.S.D.
1.	Analyst –I	0.618	0.620	0.57
		0.623		
		0.625		
2.	Analyst -II	0.595	0.598	0.70
		0.601		
		0.598		

➢ **Limit of Detection (LOD) and Limit of Quantification (LOQ) :**

Limit of detection (LOD) and limit of quantification (LOQ) of the development method were determined by dilution progressively low concentration of standard solution based on signal to noise ratio(LOD) and (LOQ) of Daclatasvir.

It is calculated by using slope and standard deviation from linearity and precision respectively:

Limit of detection (LOD):

LOD = 3.3 × SD / Slope

LOQ = 10 × SD / Slope

Where, SD – Standard deviation

The LOD and LOQ were calculated as per the equation given in section. The values of LOD were found to be 5.82ug/ml for Daclatasvir and the calculated LOQ values were found to be 17.12ug/ml. The low values of LOD and LOQ indicates the sensitivity of the method**.**

❖ **Degradation Study:**

Table 9. Optimized Stress Condition for Producing Stress Degradant Sample

Sr. No.	Stress condition	Optimum working condition	Time
1.	Acid Hydrolysis	1mg/ml in 1N HCL at room temp.	1hour
2.	Base Hydrolysis	1mg/ml in 1N NaOH at room temp.	1hour
3.	Oxidative Degradation	3% H_2O_2 at room temp. protected from light (dark solution)	12hour
4.	Photolytic Degradation	Bulk sample exposed to sunlight for 24 hrs(6 hrs per day in petridish)	1hour
5.	Thermal Degradation	Drug sample Placed in oven at 60°c	1 hour

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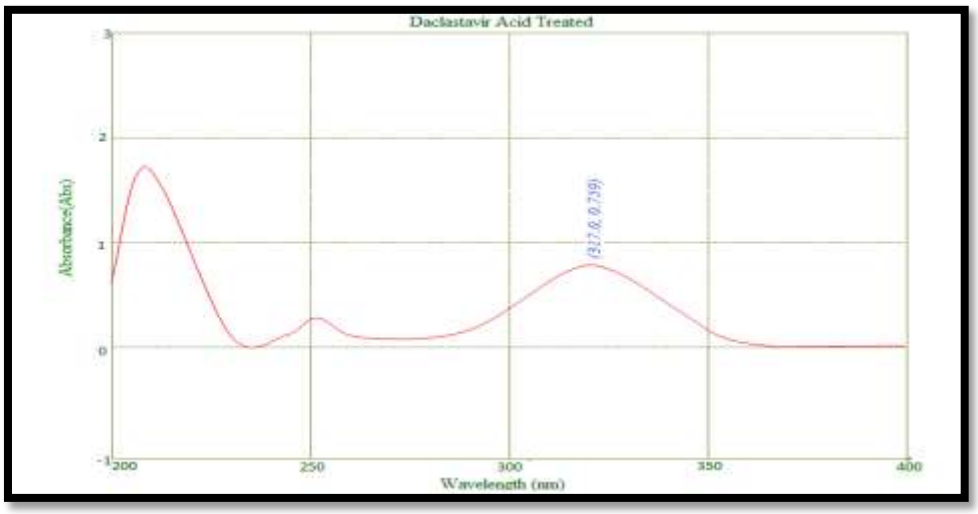
➢ **Acidic Hydrolytic Degradation :**

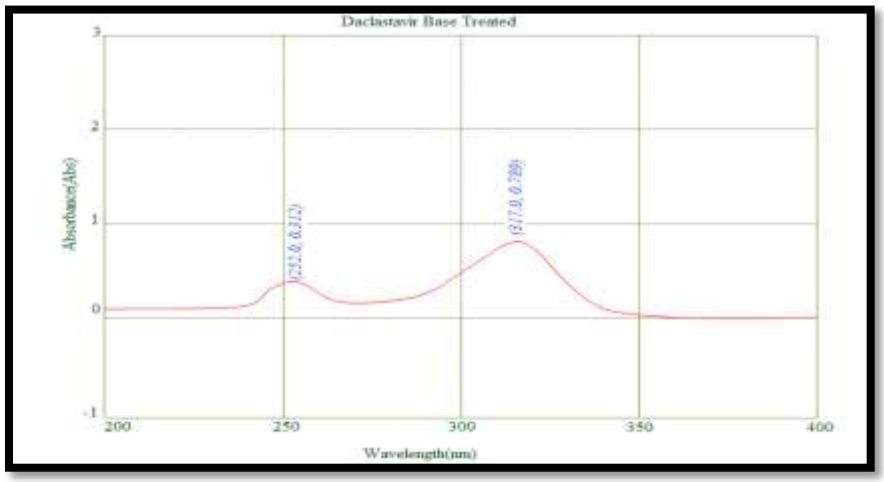
To 1 ml of stock solution Daclatasvir, 1 ml of 0.1N Hcl was added into separate 10ml std flask and refluxed for 30mins at 60^0 C .The resultant solutions was diluted to obtain 100µg/ml solution of Daclatasvir and then scan over a range of 400-200 nm by UV- Spectrophotometer.



"Fig 5", Acidic Hydrolytic Degradation Spectrum for UV of DCV

➢ **Basic Hydrolytic Degradation:**

To 1 ml of s tock solution of Daclatasvir, 1 ml of 0.1M NaOH was added into separate 10ml std flask and refluxed for 30mins at 60^0 C . The resultant solution was diluted to obtain 100µg/ml solution of Daclatasvir and then scan over a range of 400-200 nm by UV- Spectrophotometer.



"Fig 6", Basic Hydrolytic Degradation Spectrum for UV of DCV

➢ **Oxidative Degradation:**

To 1 ml of s tock solution Daclatasvir, 1 ml of 30% H_2O_2 was added into separate 10ml std flask and refluxed for 30mins at 60 ^{0}C. The resultant solution was diluted to obtain 100µg/ml solution of Daclatasvir and then scan over a range of 400-200 nm by UV- Spectrophotometer.

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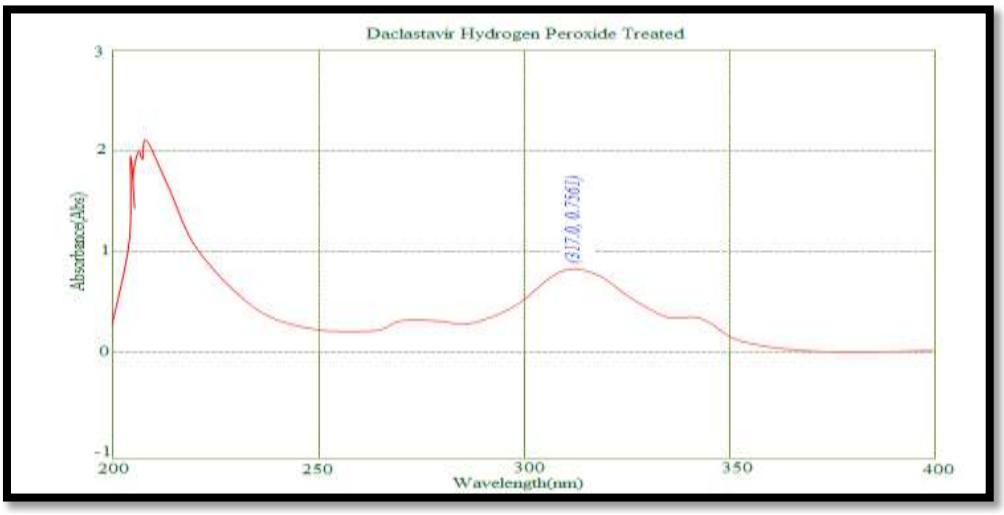
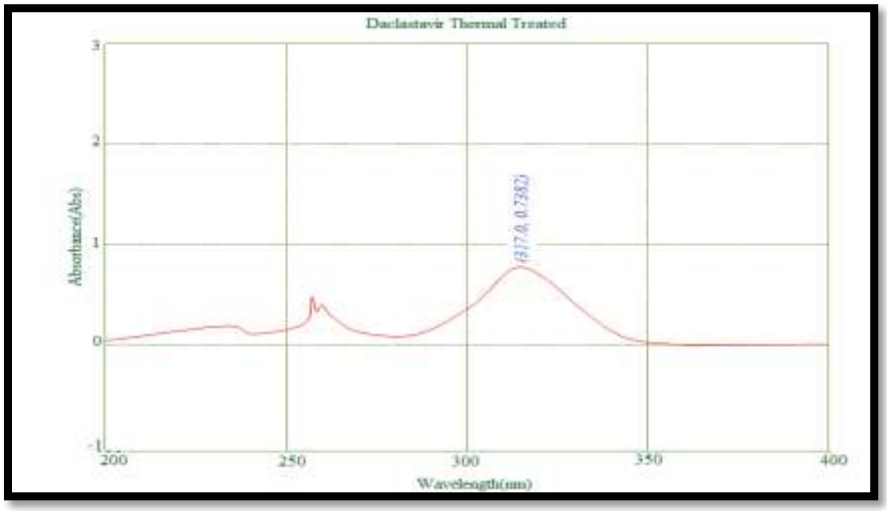


"Fig 7", Photolytic Degradation Spectrum for UV of DCV

➢ **Thermal Degradation:**

To 1 ml of s tock solution Daclatasvir was added into separate 10ml std flask and refluxed for 6hrs at 80 ^{0}C . The resultant solution was diluted to obtain 100µg/ml solution of Daclatasvir and then scan over a range of 400-200 nm by UV-Spectrophotometer



"Fig 8", Thermal Degradation Spectrum for UV of DCV

➢ **Photolytic Degradation :**

A sample powder of Daclatasvir (1 mg) was placed in Petri plate and exposed to sunlight for 30 min. Then the drug sample was transferred to 10 ml volumetric flask and diluted upto distilled water to get a conc.100µg/mL and then scan over a range of 400-200 nm.

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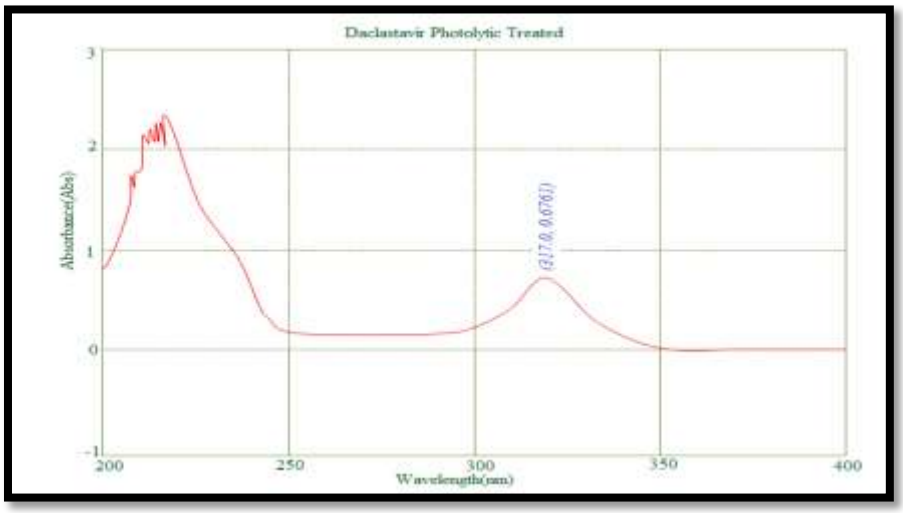

"Fig 9", Photolytic Degradation Spectrum for UV of DCV

IV. CONCLUSION

A validity stability indicating method was achieved HPLC. The validation of the system carried out effectively indicating method to be linear, precise, accurate, and specific and ruggedness .The stability studies on the drug were carried out successfully. The drug which when subjected to thermal, photolytic, oxidative, and acidic stress degraded into many degradation products. In most of the cases, the degradation rate was seen to be directly proportional to the amount of stress applied. The thermal stress was increased by increasing the incubation temperature, the faster the degradation took place. The more the concentration of H_2O_2 faster the drug degraded. Displayed a uniform rate of degradation when acidic stress applied. It showed a very high degradation rate when photolysis using UV radiations. The areas of degraded peaks were found to be lesser than area of standard drug concentration indicating that DCV undergo degradation under all condition.

ABBREVARTIONS

PPM -Parts per Million
UV -Ultra violet
WHO-World Health Organization
USP-United State Pharmacopoeia
RSD -Relative Standard Deviation
SD -Standard Deviation
°C -Degree Celsius
Fig. –Figure
% -Percentage
DCV-Daclatasvir
LOD - Limit of detection
LOQ - limit of quantification
ml – Milli liter
H_2O_2 –Hydrogen peroxide

REFERENCE

[1]. Potdar, M. A., **Pharmaceutical Quality Assurance,** Niraliprakashan, Pune, 2006; 8.1, 8.28-8.30.
[2]. Kasture A. V., Wadodkar S. G., Mahadik K. R., and More H. N., **Textbook of Pharmaceutical Analysis – II**, 11th Edition, Published By Nirali Prakashan 1996, Page no: 1, 156-165.
[3]. Beckett A. H., Stenlake J. B.; **Practical Pharmaceutical Chemistry**, 4th Edition, Part II, CBS Publications and Distributors, New Delhi, 1997;1, 275-300.
[4]. **Remington's Pharmaceutical Sciences**; 18th Edition, Mack Publishing Company, Pennsylvania, 1990; 1513-1519.
[5]. Sharma B. K.; **Instrumental Method of chemical Analysis**, 21th Edition, Goel Publishing Housing, Krishna Prakashan Ltd., 2002; 3.
[6]. Skoog D. A., Leqary J. J.; **Principle of Instrumental Analysis**, 54th Edition, Thomson Asia Ptd. Ltd; Singapore, 1992; 580.
[7]. Skoog D. A., Leqary J. J.; **Principle of Instrumental Analysis**, 54th Edition, Thomson Asia Ptd. Ltd; Singapore, 1992; 580.
[8]. ICH/CPMP Guidelines Q2B**, Validation of Analytical Procedures-Methodology**, 1996.