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METHOD DEVELOPMENT AND VALIDATION OF DACLATASVIR IN BULK & PHARMACEUTICAL DOSAGE FORM BY UV-VISIBLE SPECTROPHOTOMETRY

CH. V. S Gautam*, N. Harika, V. Balaji, V. Srinivas Prasad

*Asst. Professor, Department of Pharmaceutical analysis& Quality assurance,
SSJ College of Pharmacy, V.N Pally, Gandipet, Hyderabad, Telangana,India.

Abstract:

Objective: The objective of the present work is to develop a simple, efficient, and reproducible spectrophotometric method for the quantitative estimation of hepatitis-C drug - Daclatasvir in active pharmaceutical ingredient (API) form and in pharmaceutical dosage form

Methods: The developed ultraviolet spectrophotometric method for the quantitative estimation of hepatitis-C drugs - Daclatasvir based on measurement of absorption at a wavelength maximum (λ_{max}) of 317 nm using methanol as solvent.

Results: The method was validated in terms of, precision, linearity, accuracy, and robustness, LOD, LOQ as per the ICH guidelines. The method was found to be linear in the range of 50-150% for Daclatasvir. The percentage recovery values were in the range of 99.9-100.9% for Daclatasvir at different concentration levels. Relative standard deviation for precision and intermediate precision results were found to be <2%. The correlation coefficient value observed for Daclatasvir drug substances was not <0.99, 0.99, respectively. Results obtained from the validation experiments prove that the developed method is quantified for the estimation of Daclatasvir drug substances.

Conclusion: The developed method can be successfully applied for routine analysis, quality control analysis, and also suitable for stability analysis of Daclatasvir in API & its pharmaceutical dosage form as per the regulatory requirements.

Keywords: *Daclatasvir, Method development, Validation, Ultraviolet-visible spectrophotometry.*

Corresponding author:

CH. V. S Gautam,
Asst. Professor,
Department of Pharmaceutical analysis& Quality assurance,
SSJ College of Pharmacy,
V.N Pally, Gandipet, Hyderabad, Telangana, India.
Email of author: chvsgautam@gmail.com

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INTRODUCTION:

The chemical name of Daclatasvir dihydrochloride[1] is methyl ((1S)- 1-(((2S) – 2 - (5- (4'- (2- ((2S) - 1- ((2S) - 2-((methoxycarbonyl)amino) -3- methylbutanoyl) -2-pyrrolidinyl) -1H-imidazol-5-yl) -4- biphenylyl) -1H-imidazol-2-yl) -1-pyrrolidinyl)carbonyl) -2 -methylpropyl) carbamate dihydrochloride, and this drug is used for the treatment of hepatitis-C virus (HCV) infection. Daclatasvir is a chiral molecule with four stereocenters (1, 1', 2, 2) in the S configuration. DACLATASVIR is a white to yellow crystalline non-hygroscopic powder. It is freely soluble in water, dimethyl sulfoxide, and methanol; soluble in ethanol (95%); practically insoluble in dichloromethane, tetrahydrofuran, acetonitrile, acetone, and ethyl acetate. Daclatasvir structure is shown in Fig. 1.

Daclatasvir is a first in class direct acting antiviral agent which binds to and inhibits the function of the HCV protein NS5A. NS5A is involved in both viral RNA replication and virus particle assembly. A putative inhibitor-binding region spanning amino acids 21-30 of NS5A was identified.

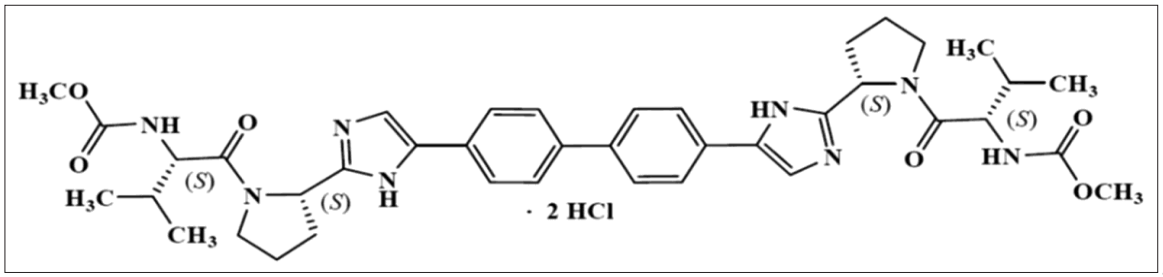


Fig. 1: Structure of DACLATASVIR dihydrochloride

Table 1: Characteristic profile of drug

S.No	Parameters	Characteristics
1	Drug name	Daclatasvir dihydrochloride
2	Molecular formula	$C_{40}H_{52}Cl_2N_8O_6$
3	Molecular Weight:	811.806g/mol
4	CAS number	1009119-65-6
5	Colour	white to yellow crystalline colour
6	Odor	Odorless
7	Taste	Bitter
8	Appearance	non-hygroscopic powder
9	Melting range	260℃-267.5℃
10	Solubility	freely soluble in water, dimethyl sulfoxide, and methanol; soluble in ethanol (95%); practically insoluble in dichloromethane, tetrahydrofuran, acetonitrile, acetone, and ethyl acetate
11	Therapeutic category	antiviral (Hepatitis c)

LITERATURE REVIEW

From the literature survey, it is evident that very few research articles are available for Daclatasvir Vikas Kekan, Sachin Gholve, Omprakash Bhusnure [2] in 2017 developed a simple, specific and economic UV spectrophotometric method has been developed using as a solvent methanol: water (8:2) to determine the Daclatasvir content in bulk and pharmaceutical dosage formulations. The quantitative determination of the drug has been carried out at a predetermined λmax of 317nm, it was proved linier in the range 2-12 µg/mL and exhibited good correlation coefficient (R 2 =0.998) and excellent mean recovery (98-100.09%). The method was validated statically and by recovery studies for linearity, precision, repeatability and reproducibility as per ICH guideline. The obtained results proved that the method can be employed for the routine analysis of daclatasvir in bulk as well as in the commercial formulations

Ashok Chakravarthi V, Sailaja BBV, Praveen Kumar A in august 2016 develop a simple, efficient, and reproducible spectrophotometric method for the quantitative estimation of hepatitis-C drugs - Daclatasvir and Sofosbuvir in its active pharmaceutical ingredient (API) form. Methods: The developed ultraviolet spectrophotometric method for

the quantitative estimation of hepatitis-C drugs - Daclatasvir and Sofosbuvir is based on measurement of absorption at a wavelength maximum (λmax) of 317 and 261 nm using methanol as solvent. The method was validated in terms of specificity, precision, linearity, accuracy, and robustness as per the ICH guidelines. The method was found to be linear in the range of 50-150% for Daclatasvir and in the range of 43-143% for Sofosbuvir. The percentage recovery values were in the range of 99.4-100.6% for Daclatasvir and in the range of 99.7-100.6% for Sofosbuvir at different concentration levels. Relative standard deviation for precision and intermediate precision results were found to be <2%. The correlation coefficient value observed for Daclatasvir and Sofosbuvir drug substances was not <0.99, 0.99, respectively. Results obtained from the validation experiments prove that the developed method is quantified for the estimation of daclatasvir and Sofosbuvir [3,4].

Jeevana Jyothi B, Padmaja G in august 2016 developed Daclatasvir di hydrochloride (DCH) is a new drug gained its FDA approval on July 24, 2015 for treatment of hepatitis C. As there are no reported UV spectrophotometric methods for estimation of Daclatasvir dihydrochloride, the present work was aimed at development of accurate and precise spectrophotometric method for its estimation by absorbance maxima method. The working standard solution of 10 µg/ml was scanned in the wavelength range of 400-200 nm. Absorption maximum, λmax was found at 214 nm. Calibration curve was obtained with good correlation coefficient value of 0.986. Linearity was observed i n concentration range of 2-12 µg/ml. Method accuracy was revealed by recovery studies obtained in between 99.95 and 100.09.

EXPERIMENTAL:

Materials and methods

Daclatasvir dihydrochloride tablets (label claim 60mg, brand name daclinza) were obtained from local pharmacy. HPLC grade methanol (MeOH purity ~99.8%) was obtained from Rankem (Mumbai, India).

The API was obtained as a gift sample from Dr.Reddy's Laboratories, Hyderabad.

Instrumentation

A double beam UV-vis spectrophotometer (Lasa) having two matched quartz cells with 1 cm light path length and loaded with UV probe software was used for recording of spectra and measuring absorbance for method development and validation study.

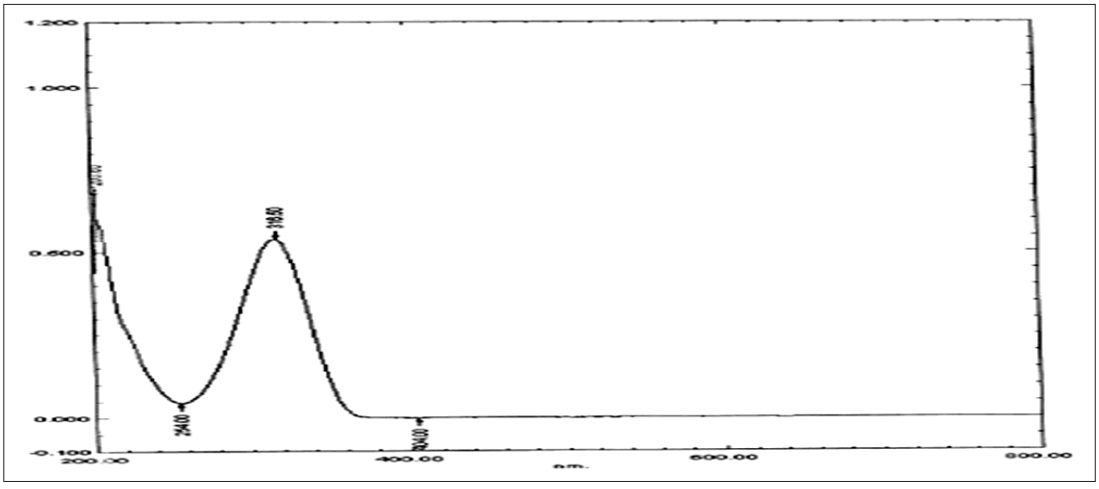
Method development

Selection of diluent

Methanol was used as diluent for the preparation of standards for Daclatasvir Formulation based on the solubility characteristics of the drug substances.

Selection of suitable wavelength detection

A spectrum for Daclatasvir was measured from 200 to 800 nm for wavelength maxima by recording UV-vis spectrum of standard solution. The corresponding spectrum of Daclatasvir is shown in Fig. 3.8. Maximum absorbance (λmax) was shown at 317 nm for standard solution of Daclatasvir . Based on the spectra maxima, 317 nm were selected for identification and quantification of Daclatasvir drug.



Preparation of Standard stock solution

A standard stock solution of Daclatasvir [5] was prepared by dissolving 10 mg of daclatasvir in a 10ml clean dry volumetric flask, 7mL of Methanol was added and sonicated for about 10min and then made up to 10mL with Methanol to get a 1 µg/mL standard stock solution.

Calibration curve were prepared by dilution of above stock solution in the range of 25µg/mL- 150 µg/mL.

Table 2: Preparation of Standard stock solution

S.No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm
1	0.25	10	25
2	0.5	10	50
3	0.75	10	75
4	1.0	10	100
5	1.25	10	125

Preparation of Sample solutions

Daclatasvir dihydrochloride [6] (label claim 60 mg)

Five tablets containing 60 mg of daclasatavir were weighed and then Powdered. An amount of powder equivalent to 1 mg of daclasatavir and was transferred in a 100 mL volumetric flask, with 70mL of methanol and sonicated for 25min, to ensure complete solubility of the drug, and volume made up with the diluent (Methanol) and filtered through 0.45µm membrane filter. From this 1ml was pipetted out and transferred to 10 mL volumetric flask and made up to 10mL with Methanol to get the concentration of 1µg/mL of Daclatasvir

METHOD VALIDATION

The method was validated for the Parameters like linearity, precision, limit of detection (LOD), limit of quantification (LOQ), Accuracy, , robustness based on ICH/CPMP guidelines(14-16).

PRECISION

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of same standard concentration. Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marked sample [7]. The results of Precision studies are expressed in **Table 3.**

RESULTS& DISCUSSION:

Table 3: Method precision of daclatasvir

Sample preparation	**% Assay Daclatasvir**
1	100.72
2	100.81
3	100.41
4	100.19
5	101.20
6	103.20
Mean	101.08
± SD	0.995987
% RSD	0.89

The % RSD for repeatability of sample preparation is 0.89%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%

Accuracy

Accuracy of the method was determined by analysis of standard at three different levels. Values were found to be within the limit given in (Table 3). The mean recovery was in the range of 99.43-100.54%

which shows there is no interference from excipients [8].

% Recovery = b-a/c×100

Where,

a = The amount of drug found before the addition of standard drug
b = The amount of drug found after the addition of standard drug
c = The amount of standard drug added

Table 3: Recovery studies of Daclatasvir

concentration	Sample preparation	% Recovery of daclatasvir	AVG	±SD	%RSD
	1	99.54			
50%	2	99.15	99.43	0.245	0.246
	3	99.61			
	1	99.17			
100%	2	100.84	100.06	0.836	0.835
	3	100.16			
	1	100.98			
150%	2	99.88	100.54	0.58	0.57
	3	100.76			

Linearity

Aliquots of standard Daclatasvir stock solution were taken in different 10ml volumetric flasks and diluted up to the mark with the diluents such that the final concentrations of daclatasvir are in the range of 25-150 µg/mL.. Evaluation was performed with PDA detector at 248nm and a Calibration graph was obtained by plotting absorbance versus concentration of daclatasvir.

The plot of area of each sample against respective concentration of daclatasvir was found linear in the range of 25-150 µg/mL with correlation coefficient of 0.999.. The respective linear regression equation being Y= 26504x+152930. The regression characteristics were calculated for this method and given in Table 2.4.

Table 4: Linear regression data for calibration curves

Drug	Daclatasvir
Linearity Range	25 -150 µg/mL
Slope(m)	265040
Y Intercept(b)	152930
Correlation coefficient	0.9998

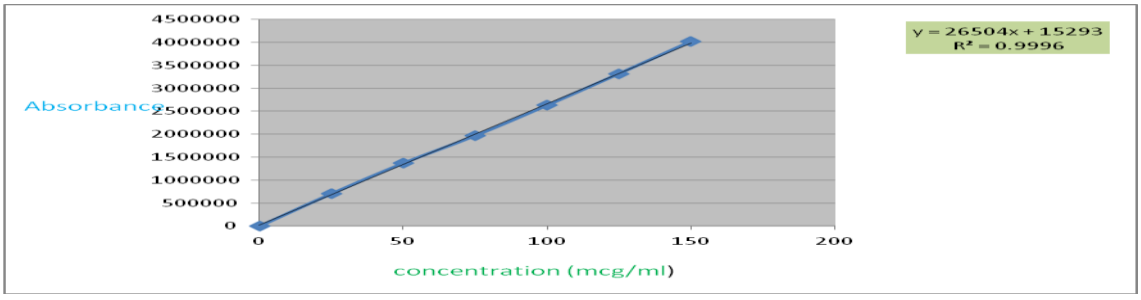


Fig 2: Calibration curve of Daclatasvir

Limit of Detection and Limit of Quantification

The sensitivity of measurement of Daclatasvir by use of proposed method was estimated in terms of the limit of quantification (LOQ) and the lowest concentration detected under the chromatographic condition as the limit of detection (LOD). The LOQ and LOD were calculated by the use of equations LOD=3×N/B and LOQ=10×N/B where N is the standard deviation of the absorbance of the drug and B is the slope of corresponding calibration plot. (LOD) limit of detection and (LOQ) limit of

quantification were found to be 1.9 µg/mL and 5.7 µg/mL respectively.

Robustness:

The robustness of the proposed method was performed by preparing the standard a change in wavelength for absorbance readings. The wavelength selected was ±2 nm to the λ_{max}, i.e315 and 319 nm for Daclatasvir drug, for standard solutions. In the robustness condition (wavelength variation of ±2 nm to the λ_{max}), the assay values of Daclatasvir were not <99%. % Assay results for robustness parameters were shown table.

Table 5: robustness parameters

Determination	**% Assay of** Daclatasvir **at 315 nm**	**% Assay of** Daclatasvir **at 319 nm**
Determination-1	99.5	99.5
Determination-2	99.3	99.3
Determination-3	99.4	99.4
Average	99.4	99.4
SD	0.11	0.11
%RSD	0.11	0.12

SUMMARY AND CONCLUSION:

A simple, rapid, accurate, precise, robust method was developed for estimation of daclatasvir dihydrochloride in bulk and its formulation by UV-Visible spectrophotometry.

A good linear relationship (r=0.999) was observed between concentration range of 25-150 µg/ml. The assay of Daclatasvir tablets was recovered which indicates high accuracy of the method.

The method was found to be robust by changing the wavelength.(±2 nm to the λ_{max}, i.e315 and 319 nm) In the robustness condition(wavelength variation of ±2 nm to the λ_{max}), the assay values of Daclatasvir were not <99%.

The method was found to be accurate and precise with % RSD of 0.55 &0.89%. Respectively.This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%.

(LOD) limit of detection and (LOQ) limit of quantification were found to be 1.9 µg/mL and 5.7 µg/mL respectively.

Thus the developed method can be easily used for the routine quality control of bulk and tablet dosage form of Daclasatavir within a short analysis is time.

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