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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE ESTIMATION OF DACLATASVIR DIHYDROCHLORIDE IN TABLET DOSAGE FORM

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ABSTRACT

Simple, precise and accurate area under curve spectroscopic method has been developed and validated for the estimation of Daclatasvir dihydrochloride in bulk and Pharmaceutical dosage form. The drug shows maximum absorption (λ max) at 302nm in 0.1N HCl solution and Area under Curve [AUC] in absorption spectra were measured between the wavelength range 297 to 307 nm which obeys Beer's law in the concentration range of 3-15µg/ml. The linearity study carried and regression coefficient was found to be 0.9993 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 100.61-101.04. The LOD and LOQ were found to be 0.0165 and 0.0495µg/ml. The % relative standard deviation were found less than 2. According to ICH guidelines the method has been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for reliable quantification of Daclatassvir dihydrochloride in bulk form and pharmaceutical dosage form.

KEYWORDS: Daclatassvir dihydrochloride, Area under curve spectroscopy, validation, pharmaceutical formulations.

INTRODUCTION

Daclatasvir is an inhibitor of hepatitis C virus (HCV) NS5A protein. It is a new, oral, direct-acting antiviral with potent pangenotypic activity. It is a first in class direct acting antiviral agent which binds to and inhibits the function of the HCV protein NS5A.[1] Chemically Daclatasvir Dihydrochloride 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) carbamatedidi hydrochloride (Fig.1).The synthesis involves an alkylation and formation of the imidazole ring, a coupling reaction and the formation of the dihydrochloride salt. Daclatasvir is a white to yellow crystalline nonhygroscopic powder.[2]

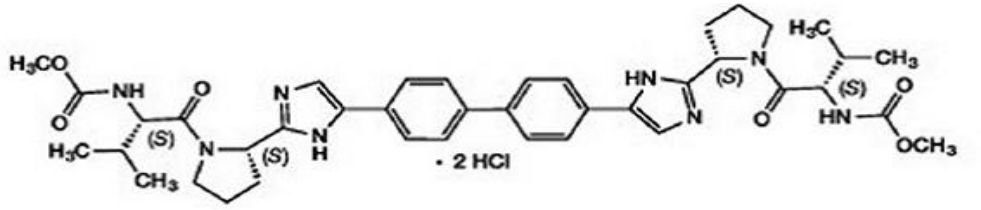

Fig. 1: Chemical structure of daclatasvir dihydrochloride.

Literature survey revealed that there were few analytical methods have been reported for the determination of Daclatasvir Dihydrochloride in pure drug and pharmaceutical dosage forms by using UV spectrophotometric,[1-5] HPLC[4-13] and HPTLC [4-15] so far.

The aim of present work is to develop and validate a novel, rapid, simple, precise and specific Area Under Curve Spectrophotometric method for estimation of Daclatasvir Dihydrochloride in bulk and tablet dosage form.

MATERIAL AND METHOD INSTRUMENT

UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken on analytical balance.

Chemicals

Daclatassvir dihydrochloride pure drug was obtained as a gift sample from MSN laboratories Hyderabad and its pharmaceutical dosage form Daclatassvir dihydrochloride 20 tablet labelled claim 60mg from local pharmacy manufactured by Zydus Heptiza Pharma India Ltd.

Solvent

0.1N HCl is used as a solvent.

Selection of analytical wavelength

Appropriate dilutions of Daclatassvir dihydrochloride were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400nm. Area under Curve [AUC] in absorption spectra were measured between the wavelength range 297to 307nm as the wavelength for detection (Fig-2).

Preparation of standard stock solution

100mg of Daclatasvir Dihydrochloride was weighed accurately and transferred in to 100ml volumetric flask and dilute in 0.1N HCl up to mark. From this, the solution was further diluted into 100µg/ml and pipette out 0.3, 0.6, 0.9, 1.2 and 1.5ml into 10ml individual volumetric flask and dilute in 0.1N HCl up to mark, this gives 3, 6, 9, 12, and 15µg/ml concentration.

Preparation of sample solution

20 tablets of Daclatassvir dihydrochloride marketed formulations were weighed and powdered. A quantity of tablet powder equivalent to 100mg of Daclatassvir dihydrochloride was transferred into a 100ml of volumetric flask then it was diluted with 0.1N HCl and made up to the mark.

METHOD AND VALIDATION

The method was validated according to ICH guidelines.

RESULTS AND DISCUSSION

Method: Area under curve spectroscopy linearity

The linearity of an analytical method is its capacity to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 3-15µg/ml and Area under Curve [AUC] in absorption spectra were measured between the wavelength of 297 to 307nm as absorbance values are shown in table-1 (Fig-3). The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in (Fig-4). Statistical parameter like slope, intercept, regression equation, correlation coefficient and Sandell's sensitivity were determined. (table-2).

Precision

The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the same sample. Precision was determined by intra-day and inter-day study. Intra-day precision was determined by analysing the same concentration for three times in a same day. Inter-day precision was determined by analysing the same concentration daily for three days. (table-3).

Accuracy

The accuracy of an analytical method says that closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels as 80%, 100% and 120%. In which the formulation concentration kept constant and varied pure drug concentration. (table-4).

Ruggedness

The ruggedness is defined as the reproducibility of results when the method is performed under the variation in conditions. This includes different analyst, laboratories, instruments, temperature etc. Ruggedness was determined between different analyst, the value of %RSD was found to be less than 2. (table-5).

Limit of Detection and Limit of Quantitation

The limit of detection is an individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ were calculated using formula.

LOD = 3.3(SD)/S and LOQ = 3(LOD)

LOD and LOQ value of Daclatasvir Dihydrochloride were found be 0.0165 and 0.0495µg/ml respectively.

Table 1: Results of calibration curve at 297-307nm by Area under curve spectroscopy.

Sl. No.	Concentration in µg/ml	Absorbance ±Standard deviation*
1	0	0
2	3	0.227±0.000816
3	6	0.421±0.000894
4	9	0.611±0.000753
5	12	0.803±0.001049
6	15	0.994±0.001414

*Average of six determinations.

Table 2: Regression parameter for daclatasvir dihydrochloride by area under curve spectroscopy.

Regression parameter	Results
Range(µg/ml)	3-15
λ_{max}(nm)	302
Regression Equation	Y= 0.0656x+0.0105
Slope(b)	0.0656
Intercept(a)	0.0105
Correlation coefficient(r^2)	0.9993
Sandell's equation	0.0151
Limit of detection(µg/ml)	0.0165
Limit of quantitation(µg/ml)	0.0495

Table 3: Determination of precision results for daclatasvir dihydrochloride by area under curve spectroscopy.

ıcentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
3	0.224±0.000577	0.257	0.225±0.001	0.444
6	0.397±0.001528	0.384	0.396±0.00057	0.145
9	0.595±0.001	0.168	0.596±0.001	0.167
12	0.797±0.0011	0.125	0.795±0.001528	0.192
15	0.987±0.001158	0.117	0.988±0.001528	0.154

*****Average of six determinations, **percentage relative standard deviation.

Table 4: Determination of accuracy results for daclatasvir dihydrochloride by area under curve spectroscopy.

Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
80	6	4.8	10.86	100.61±0.7	0.695
100	6	6	12.13	101.04±0.811	0.802
120	6	7.2	13.28	100.67±0.459	0.456

*****Average of six determinations, **percentage relative standard deviation.

Table 5: Determination of Ruggedness results for daclatasvir dihydrochloride by area under curve spectroscopy.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.225	0.224
±Standard deviation*	0.001	0.0005
%RSD	0.444	0.257

*****Average of six determinations, **percentage relative standard deviation.

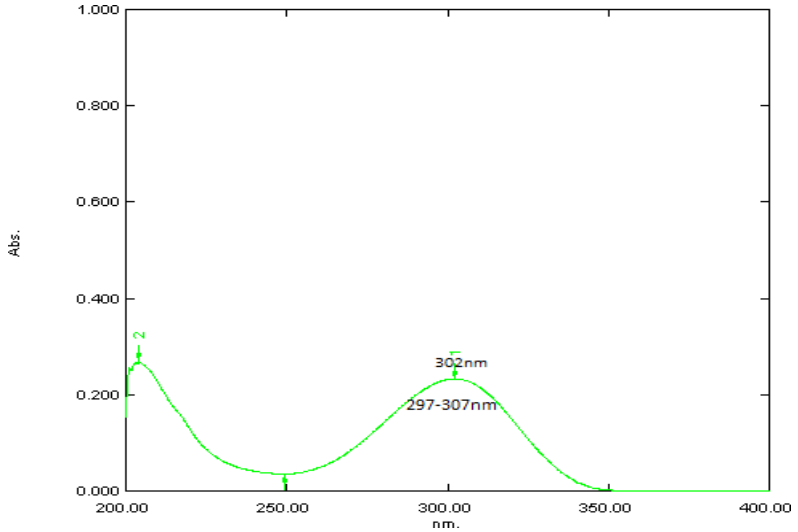

Fig. 2: Area under curve spectrum of daclatasvir dihydrochloride at 297-307nm.

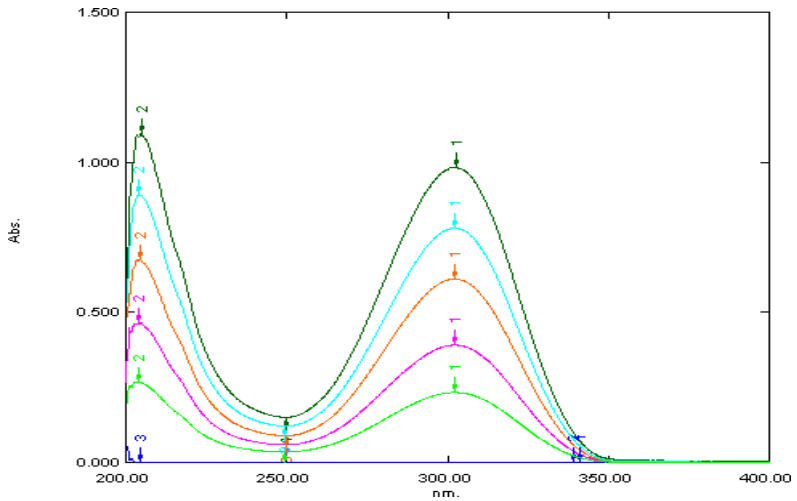

Fig. 3: Area under curve overlain spectra of daclatasvir dihydrochloride showing absorbance at 297-307nm.

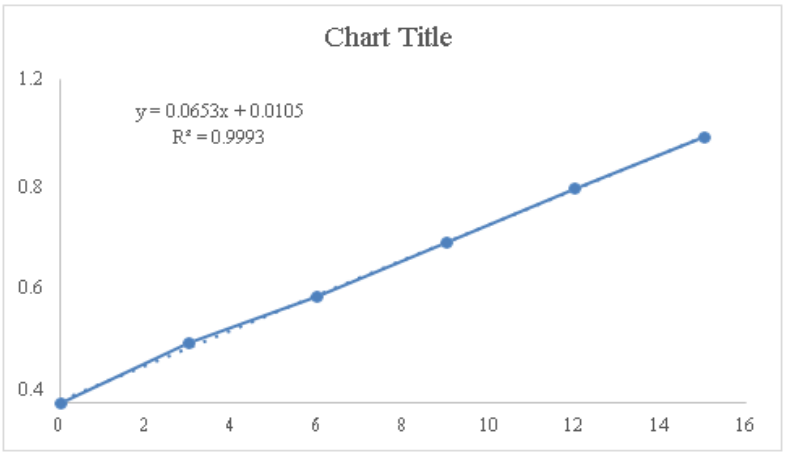

Fig. 4: Calibration curve of daclatasvir dihydrochloride by area under curve method.

CONCLUSION

The present analytical method was validated as per ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, specific, accurate, economical and sensitive and can be used for routine analysis of Daclatasvir Dihydrochloride in bulk drug and in pharmaceutical dosage forms.

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