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Quantitative Estimation of Daclatasvir In Drug Substances and Formulated Drug Product By UPLC

Sreekanth Nadig* and Jane T Jacob

Department of Pharmaceutical Chemistry. Nitte Gulbishetty Institue of Pharmaceutical Sciences, Mangalore, India

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ABSTRACT

A simple, faster reverse phase UPLC method has been developed for quantitative estimation of Daclatasvir in pharmaceutical dosage form . Daclatasvir is one of recently developed drug for the tretatement of Hepatitis C virus.Daclstavir is a NS5A inhibitor developed by Bristol-Myers squibb. Chromatographic separation was achieved on Kinetex C18, 50x 4.6 mm ,2.6µm column. Detection wavelength was set at 318 nm. Drug product was subjected for stress conditions of Acid, Alkali ,Peroxide and Thermal degradation. Daclatasvir was found to have degraded significantly in alakline condition. Peak purity results of Daclatasvir indicated that all degradents are separated from the analyte peak. The developed method was validated as per ICH guidelines with respect to Specificity, Linearity, Accuracy, Precision and Robustness.

Key Words: Daclatasvir,UPLC, Forced degradation,Kinetex core shell column

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INTRODUCTION

Hepatitis C viral infection (HCV) infection is increasing through out the globe.Daclatasvir is a drug used for tretatment of Hepatitis C virus that inhibits the HCV nonstructural protein NS5A [1]. Daclatasvir is chemically Methyl [(2S)-1-{(2S)-2-[4-(4'-{2-[(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}-2-pyrrolidinyl]-1H-imidazol-4-yl}-4-biphenylyl)-1H-imidazol-2-yl]-1-pyrrolidinyl}-3-methyl-1-oxo-2-butanyl]carbamate [Fig 1]. Literature survey reveals that there are no sufficient information available on analytical methods for the estimation of Daclatasvir [1-7]. And also , to the best of our knowledge there are no methods reported to determine of Daclatasvir by Ultra Performance Liquid Chromatography (UPLC) in formulated drug products. Hence an attempt was made to develop and validate a UPLC method for the estimation of Daclatasvir in formulated drug product. Ultra Performance Liquid chromatography is a relatively new technique in the field of Liquid chromatography. UPLC helps in improving speed , sensitivity , resolution and reduction in solvent consumption. UPLC makes use of stationary phase with particle size less than 2µm in general.Instrumentation has been designed to accommodate high pressure and high temperatures as low particle size phases are used [8].However in this method new technology in the filed of chromatographic columns ie Kinetix core shell technology with 2.6µm was used .

Forced degradation studies in Acid, Alkali , Peroxide and Thermal conditions by using PDA detector were performed to ensure that degradents were separated from analyte peak.

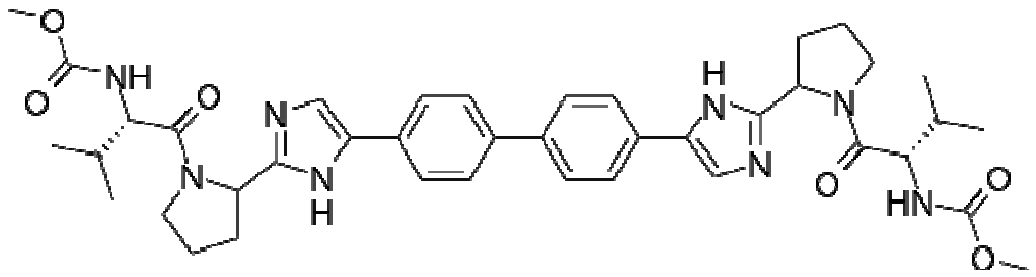

Fig 1.Chemical structure of Daclatasvir

MATERIALS AND METHODS

2.1 Materials : Chemicals and reagents:
Potassium dihydrogen phosphate and potassium hydroxide used was of analytical reagent grade from Merck chemicals. HPLC grade Methanol and Acetonitrile was from Rankem chemicals and HPLC grade water from Millipore Milli Q Water purification system were used throughout the experiment.

2.2 Equipment:
Ultra performance Liquid chromatography system (from Waters) with auto sampler was used for the study. Data was acquired and processed by using Waters Empower software.

2.3 Chromatographic Conditions:
The analysis was carried out on Kinetex C18 , 50x 4.6mm ,2.6 µm was used which is one of the rugged column in core shell technology.The column oven temperature was maintained at 35°C. The mobile phase consists of mixture of 0.01 M potassium dihydrogen phosphate buffer with pH 7.0 and Acetonitrile in the ratio of 55:45 was used. The flow rate was set to 1.0 mL/minute in isocratic mode. The injection volume was 5µL and the detection was performed at 318 nm. The typical retention time of Daclatasvir is about 1.96 minutes in the final optimized conditions. The novelty of this of this method is combination of UPLC with 2.6 µm core shell technology column.

2.4 Sample Preparation:
2.4.1 Diluent Preparation:
Methanol is used as first diluent for extraction and a mixture of water and methanol in the ratio of 50:50 %v/v was used as second diluent for the better peak shape.

2.4.2 Standard Preparation :
A standard solution was prepared to get a concentration of 60µg/mL Daclatasvir. Accurately weigh and transfer 30 mg of Daclatasvir standard into a 50 mL volumetric flask added 35 mL of methanol, dissolved and diluted to volume with methanol. Transferred 5.0 mL of above solution into a 50 mL volumetric flask, diluted to volume with second diluent .

2.4.3 Test Preparation:
Test solution was prepared by taking homogenous mixture of formulated powder equivalent to 180 mg of Daclatasvir into a 500 mL volumetric flask. Added about 400 mL of methanol, sonicated for about 30 minutes with inetrmittent shaking and made up to the volume with methanol.A portion of this solution was centrifuged at 4000 rpm for about 5 minutes.3 mL of the clear supernatent solution was further diluted to 20 mL with second diluent.

2.5 EXPERIMENTAL DESIGN:
2.5.1 Method Validation:
The principal purpose of analytical method validation is to ensure that selected analytical procedure will give reproducible and reliable results that are adequate for the intended purpose as described in ICH guidelines [9]. The described method has been validated in terms of specificity, precision, linearity, accuracy and robusteness. Specificity of the method was evaluated by performing placebo inetrference study and by subjecting the drug product into forced stress conditions. Linearity of the method was statistically proved by correlation. The precision of the method was expressed in term of coefficient of variation (RSD) for % of Assay. The accuracy was expressed in terms of percent recovery of the known amount of analyte added to the sample preparation.

RESULTS AND DISCUSSION

3.1 System suitability:

As integral part of chromatographic analysis, checking suitability of the system before any analysis is important. System suitability parameters like USP Tailing, Theoretical plates and Relative standard deviation (RSD) for six replicate injections of standard (Fig 2) were evaluated and found to be satisfactory as per common chromatographic practices. Results are shown in Table No 1.

Table 1: Results of System Suitability Test

Name of Drug substances	%RSD for replicate injections	USP Tailing factor	Theoretical plates
Daclatasvir	0.4	1.0	2143

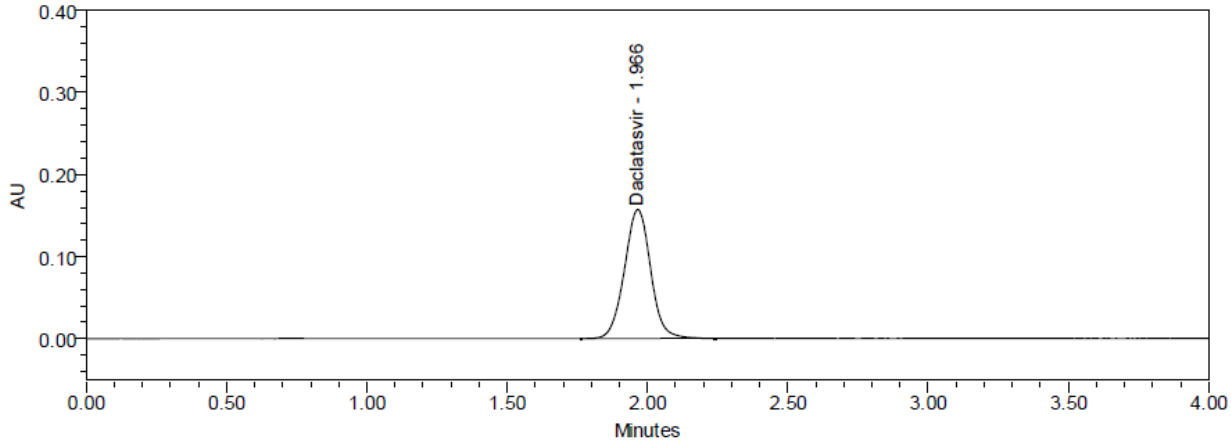

Fig. 2 Typical Chromatogram of Daclatasvir Standard.

3.2 Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradents, matrix (placebo) etc. Specificity was tested by injecting placebo preparation and forced degradation samples.Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Forced degradation was attempted to stress conditions like acid hydrolysis, base hydrolysis,peroxide oxidation and thermal degradation. To check and ensure the homogeneity (peak purity) of peak in the stressed sample solutions, photo diode array detector was employed. In forced degradation study it was observed that Daclatasvir is susceptible to degradation in alkali stress conditions. Peak purity in all the degradation conditions has been proven for Daclatasvir peak. Results are tabulated in Table No 2.

Table 2: Results of Forced degradation Studies with Peak purity details

Stress Conditions	PA	PT	% Degradation
Acid Degradation	0.132	0.323	Nil
Base Degradation	0.332	8.625	5%
Peroxide Degradation	0.120	0.341	Nil
Thermal Degradation	0.157	0.467	Nil

PA = Purity Angle, PT= Purity Threshold

Note: Purity Angle should be less than Purity Threshold to meet Peak purity criteria acceptance criteria

3.3 Linearity:

The linearity of the analytical procedure was demonstrated to prove the proportional relationship of response versus concentration over the range. This gives confidence that the response and concentration are proportional and consequently ensures that calculations can be performed in the specified range. The linearity of detector response to different concentrations of Daclatasvir was studied by preparing a series of solutions. The data were subjected to statistical analysis using a linear-regression model. The results have indicated good linearity. Results are shown in Table No 3.

Table 3: Results of Linearity Studies (Response Vs Concentration)

Concentartion levels	Response (Area)
3%	29099
10%	105802
50%	540606
75%	765361
100%	1023180
125%	1267479
160%	1642777
Correlation : 0.999 Slope : 17020.2455 Intercept : 7682.1803	

3.4 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 same homogeneous sample under the prescribed conditions.Six sample solutions were prepared analyzed as per the method.The % RSD was calculated for results and it indicates that proposed method has got acceptable level of repeatability.Results are tabulated in TableNo 4.

Table 4. Method Precision data

Sample No	% Assay of Daclatasvir
Sample 1	98.5
Sample 2	98.4
Sample 3	98.1
Sample 4	98.8
Sample 5	98.2
Sample 6	99.0
Mean	98.5
SD	0.34641
%RSD	0.4

3.5 Accuracy:

Accuracy of an analytical method is the closeness of the test results obtained by the method to that of true value. Accuracy of the proposed method was established by recovery experiments. This study was conducted by preparing and anlyzing samples at 50%, 100% and 150% of targeted concentration, in triplicate and injected into the chromatographic system.Results obtained from recovery studies are given in Table No 5.

Table 5: Results of Recovery Study at Different Levels

Recovery levels	'mg' added	'mg' found	%Recovery	Mean	%RSD
50%-Prep-1	100.5	99.56	99.1		
50%-Prep-2	100.2	101.13	100.9	100.1	0.9
50%-Prep-3	100.8	101.25	100.4		
100%-Prep-1	200.1	196.95	98.4		
100%-Prep-2	199.5	196.67	98.9	98.8	0.4
100%-Prep-3	199.2	197.67	99.2		
150%-Prep-1	300.3	297.09	98.9		
150%-Prep-2	299.2	294.34	98.4	98.6	0.3
150%-Prep-3	298.6	293.94	98.4		

Table 6: Results of Robustness Study

Parameter	Deliberate change	%RSD	Tailing factor	Theoretical plates
Flow rate	0.8 mL/min	0.1	1.1	1980
	1.2mL/min	0.9	1.1	1463
Temperature	30°C	1.3	1.1	1368
	40°C	0.6	1.1	2001
pH of buffer	6.8	0.1	1.1	2400
	7.2	0.1	1.1	2386

3.6 Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters, and provides an indication of its reliability during normal usage. In the present study, an experimental design was planned for robustness testing varying some conditions, e.g. Flow rate, column

temperature and variation of buffer pH in the mobile phase. It can be seen that, with every employed condition, there were no major changes in the chromatographic behavior. All parameters have been observed within the limits required for system suitability tests. The results are shown in Table No 6.

From all the above validation parameters performed ,it indicates that method is specific and selective, precision was found be less than 1% RSD, correlation was found to be 0.999, recovery results were within the acceptable limits and robustness results were found to be satisfactory. This proves that method is specific, precise ,linear , accurate and robust.

CONCLUSION

A simple, faster and economical UPLC method could be developed for the quantitative estimation of Daclatasvir in formulated product. Method requires all commonly available materials for analysis. Analytical method was validated as per ICH guideline and proved that it is suitable for its intended purpose. The novelty of this method ie is combination of UPLC with 2.6 µm core shell technology column was found to be suitable for the analysis.

The above validated UPLC method can be used by government agencies, government laboratories, research institutions and manufacturing companies to analyze the drug product to check the quality of it.

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