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Stability-Indicating RP-HPLC Method for the Estimation of Antiviral Drugs in Bulk and Formulation

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ABSTRACT

For the estimation of Daclatasvir in single dosage form HPLC, a novel, straightforward, accurate, and cost-effective reverse phase high performance liquid chromatographic method has been developed and validated. The WATERS Model NO.2690 series Compact System consists of an inertsil-C18 ODS column with a mobile phase consisting of acetonitrile and methanol (70:30) at a flow rate of 1 ml/min. Detection was done at 230 nm. Daclatasvir was successfully separated under the chosen chromatographic conditions (Rt: 2.658 min). ICH recommendations were followed in the validation of the proposed technique for linearity, accuracy, precision, LOD, LOQ, robustness, ruggedness, and system appropriateness characteristics. Daclatasvir's linearity was determined to be between 20 and 80 µg/ml. It was discovered that the approach was reliable. Daclatasvir in single dose forms might be routinely analyzed using the suggested approach. The bulk medication was put through forced degradation tests under thermal, oxidative, acidic, and alkaline settings.

Key words: methanol, acetonitrile, RP-HPLC, and daclatasvir.

INTRODUCTION

Daclatasvir via Chemical Means Methyl [(2S) -1-{(2S) -2-[4-(4-{2-[(2S)] -1-{(2S) -2-[amino (methoxy carbonyl)] 3-methyl butanoyl [2-pyrrolidinyl] (4-yl~-4-biphenyl yl) -1H-imidazol -1H-imidazol-2-yl -1-pyrrolidinyl 2-butanyl -3-methyl-1-oxo] carbamate as well as This antiviral medication is used to treat chronic (long-lasting) hepatitis C, a liver virus infection. As an antiviral, daclatasvir targets the hepatitis C virus directly. [1,2]

According to a review of the literature, only one approach has been devised for the combination of Daclatasvir and no other drugs. As a result, we pledge to build a new method for pharmaceutical dosage form analysis after conducting a thorough investigation to create a new RP-HPLC methodology and validate it in accordance with ICH recommendations. Acetonitrile and methanol (70:30) were employed in an Inertsil-C18 ODS column at a flow rate of 1.0 ml/min, and a PDA detector was set up at 230 nm to determine this approach. 3.

MATERIALS AND METHODS

2.1. Chromatographic parameters and Apparatus:

A Waters HPLC with Auto sampler, Empower 2.0 software, Inertsil-C18 ODS column and PDA detector was used in the study. Digital pH meter(POLOMAN), Electronic balance (SARTORIOUS) used for this study.

2.2. Drug samples:

The Daclatasvir drug used for estimation for this study was procured from tablet. The label claim was 60mg.

2.3. Reagents and solutions:

HPLC grade Acetonitrile HPLC grade, Methanol HPLC grade and Daclatasvir drug was utilized in the study. The quantitative relation of 70:30%v/v was used as a mobile phase. The mobile phase was degassed in ultrasonic water bath for 5 minutes and filtered under vacuum filtration.

2.4. Preparation of the Daclatasvir standard & sample solution:

2.4.2. Mobile phase:

Acetonitrile and methanol taken in the ratio 70:30

Standard solution preparation: (Daclatasvir 60µg/ml)
Accurately Weighed and transferred 6mg Daclatasvir working Standard into a 10 ml clean dry volumetric flask, add 7ml of mobile phase, sonicated for 20 minutes and make up to the final volume with mobile phase Acetonitrile and methanol(70:30).From the above stock solution, 1 ml was pipetted out in to a 10ml Volumetric flask and then make up to the final volume with mobile phase.

2.4.4. Sample solution preparation:

Five tablets were weighed, and caluculated the average weight of five tablets, finely powdered then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask,80 ml of mobile phase acetonitrile and methanol(70:30) was added to the same. The flask was sonicated for 10min and volume was made up to the mark with mobile phase acetonitrile and methanol(70:30).The above solution was filtered using whatman1filter.From the above solution1ml was transferred into a 10ml volumetric flask and the volume was made up to the mark with mobile phase acetonitrile and methanol(70:30) to obtain 6mcg/ml of Daclatasvir. The solution was sonicated for 10min and injected under above chromatographic conditions and peak area was measured.

Label Claim: 60mg of Daclatasvir in tablet dosage form.

3. METHOD DEVLOPMENT:

Trials were performed for the method development and the best peak with least fronting factor was found to be with RT= 2.658min.

4. METHOD VALIDATION

Validation for the method was carried out as per ICH Q2(R1) guidelines. The validation parameters such as specificity, linearity, range, accuracy, precision, detection limit ,quantitation limit., system suitability studies.[4,5]

4.1 Specificity:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

4.2 Linearity:

From the stock solution, suitable dilutions were prepared using mobile phase Acetonitrile and methanol(70:30) as solvent at the range of 20, 30, 40, 50, 60, 70, 80(µg/ml) respectively. 20µl amount of every dilution was injected in to the column at a rate of 1.0ml/min. the drug within the rinse was monitored at 230nm and also the corresponding recordings were recorded. From these the mean peak areas were calculated and a plot of concentration Vs peak areas. The regression of the plot was computed by least square regression methodology. The slope and intercept worth for standardization curve was y= 31954x - 1625. (R2=0.999) table.1andfig.3

4.2. Accuracy:

Injected the standard solutions of Accuracy 50%, 100% and 150% and calculated the Amount found, Amount added for Daclatasvir and the individual recovery and mean recovery values. The % Recovery for each level should be between 98.0 to 102.0% table.2

4.3 Precision:

System precision : The standard solution was injected and measured the area for all injections in HPLC. The %RSD for the area of replicate injections was found to be within the specified limits table.4

Method precision: The sample solution was injected and measured the area for all injections in HPLC. The %RSD for the area of replicate injections was found to be within the specified limits table.5

Intermediate precision (analyst to analyst variability):

The stock solution (60ppm) was prepared and injected into the system by two different analysts and the precision study was performed table.6

Acceptance Criteria: The % RSD should not be more than 2%

4.4 Limit of detection and limit of Quantitation (LOD and LOQ):

From the linearity data the limit of detection and quantitation were calculated, using the following formula.

$$\text{LOD}=\frac{3.3\sigma}{S} \qquad \text{LOQ}=\frac{10\sigma}{s}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyte

4.5 Robustness:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.8ml/min,1.0ml/min and 1.2ml/min table.7

4.6 System suitability:

A Standard solution was prepared by using Daclatasvir working standard as per test method and was injected five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Daclatasvir, retention times and peak areas table.9

4.7 Ruggedness:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times in two different systems. Six samples were prepared and each was analyzed as per test method table.8

5. STABILITY INDICATING ANALYTICAL METHODS

Stability Indicating Studies are quantitative analytical procedure used to determine the amount of the Active Pharmaceutical Ingredients present in the degradation products. These methods can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference".[6,7]

Forced degradation studies were carried out as per ICH Q1A(R2) guidelines and the parameters such as acid hydrolysis, alkali hydrolysis, thermal degradation, oxidative degradation and photolytic degradation[8,9,10]

5.1 Acid hydrolysis:

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 60^0C .The resultant solutions was diluted to obtain 60µg/ml solution of Daclatasvir respectively with mobile phase and 10 µl solution was injected into the system and the chromatogram was recorded to assess the stability of sample table.10

5.2 Alkaline hydrolysis:

To 1 ml of stock solution of Daclatasvir, 1 ml of 0.1M NaOH was added into separate 10ml std flask and refluxed for 30mins at 60^0C . The resultant solutions was diluted to obtain 60µg/ml solution of daclatasvir respectively with mobile phase and 10 µl solution was injected into the system and the chromatogram was recorded to assess the stability of sample table.10

5.3 Thermal degradation:

To 1 ml of stock solution Daclatasvir was added into separate 10ml std flask and refluxed for 6hrs at 105 ^{0}C . The resultant solution was diluted to obtain 60µg/ml solution of daclatasvir respectively with mobile phase and 10 µl solution was injected into the system and the chromatogram was recorded to assess the stability of sample table.10

5.4 Oxidative degradation:

To 1 ml of stock solution Daclatasvir, 1 ml of 30% H_2O_2 was added into separate 10ml std flask and refluxed for 30mins at 60ºC . The resultant solutions was diluted to obtain 60µg/ml solution of daclatasvir respectively with mobile phase and 10 µl solution was injected into the system and the chromatogram was recorded to assess the stability of sample as table.10

Table1: **Linearity studies for the Daclatasvir**

Concentration (ppm)	Average Area	Statistical Analysis	
20	642546	Slope	31954
30	929286	y-Intercept	1625
40	1294490	Correlation Coefficient	0.999
50	1598308		
60	1942210		
70	2211312		
80	2554314		

Table2: Accuracy studies for the Daclatasvir

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm) (n=3)			% Recovery			Statistical Analysis	
		1	2	3	1	2	3	Mean	% RSD
50%	20	20.04	19.97	20.02	100.22	100.02	100.14	100.06	0.18
100%	40	40.01	40.05	39.98	99.85	100.14	99.96	100.04	0.091
150%	60	60.08	59.97	59.98	100.11	99.96	99.98	100.02	0.09

Table 3: Assay studies for the Daclatasvir

S.NO	Formulation Daclahep	Label claim (mg)	Peak area Mean±S.D (n=5)	%Assay Mean ± S.D (n=5)	% RSD
1	Sample	60	671154.8±3434.08	100.54±0.97	0.97
2	Standard		640347± 6710.04	100.18±0.95	0.95

Table4:System precision studies for the Daclatasvir

S.no	Injection	Peak Areas of Daclatasvir	Statistical Analysis	
1	1	674753	**Mean**	678433.8
2	2	674261	**SD**	6031.135
3	3	675298	**% RSD**	0.888979
4	4	679221		
5	5	688636		

Table5: Method precision studies for the Daclatasvir

S.no	Injection	Peak Areas of Daclatasvir	Statistical Analysis	
1	1	633495	**Mean**	638004
2	2	635992	**SD**	5988.87
3	3	639828	**% RSD**	0.9356
4	4	639098		
5	5	648289		
6	6	631322		

Table6: Intermediate precision studies (Analyst 1) and (Analyst 2) for Daclatasvir

S.no	Injection	Peak Areas of Daclatasvir (Analyst 1)	Peak Areas of Daclatasvir (Analyst 2)
1	1	634950	635291
2	2	635120	634282
3	3	638052	639294
4	4	647289	644147
5	5	635822	634024
6	6	636942	638692
Statistical Analysis	**Mean peak area**	638029	637621
	Mean	637825.4	
	SD	4115.2	
	%RSD	0.645	

Limit of detection and limit of Quantitation (LOD and LOQ):

From the linearity plot the LOD and LOQ are calculated:
LOD = 0.71µg/ml
LOQ= 2.16µg/ml

Table7: Robustness studies for the Daclatasvir

S.no	Flow 0.8ml		Flow 1.0ml		Flow 1.2ml	
	Std area	Tailing factor	Std area	Tailing Factor	Std area	Tailing Factor
1	620286	1.322089	621322	1.604878	602077	1.285372
2	619282	1.331920	611792	1.584354	601854	1.319385
3	621337	1.296438	622360	1.543805	602403	1.292055
4	620456	1.315454	611696	1.568590	603421	1.304561
5	620765	1.326551	613147	1.559986	602465	1.294621
Avg	623360	1.31849	616063.4	1.572323	602444	1.299199
	Flow rate between 0.8ml/min and 1.0ml/min			Flow rate between1.0ml/min and 1.2 ml/min		
Mean	4255.216			607806		
S.D	618244.3			8015.846		
%RSD	0.6882			1.3188		

Table8: System to system variability (sample) studies for the Daclatasvir

S.no	Injection	Peak Area of Daclatasvir System-1	Peak area of Daclatasvir System-2
1	1	638529	635442
2	2	635662	632986
3	3	632448	633562
4	4	645629	639280
5	5	639219	638694
6	6	638620	633282
Statistical Analysis	**Mean peak area**	638351.2	635541
	Mean	636946.1	
	SD	3802.78	
	% RSD	0.597	

Table9: System Suitability studies for the Daclatasvir

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.654	674753	10953.60	1.153
2	2.650	674261	10951.01	1.155
3	2.653	675298	10003.27	1.157
4	2.653	679221	10986.90	1.159
5	2.655	688636	10946.87	1.152
Mean	2.65327	678433.8	10768.34	1.155
SD	0.001817	6031.135		
% RSD	0.05221	0.888979		

Table10: Forced Degradation for the Daclatasvir

Mode of Degradation	Condition	Peak Area	% Degradation as compared with Control
Control sample	No treatment	634360	-
Acid	0.1 M HCl	457153	27.93
Base	0.1 M NaOH	432155	31.87
Thermal	105°C	423846	33.18
Oxidative	30% H_2O_2	444404	29.94

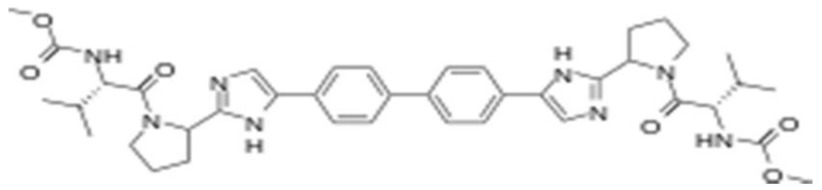

Fig1: Structure of Daclatasvir

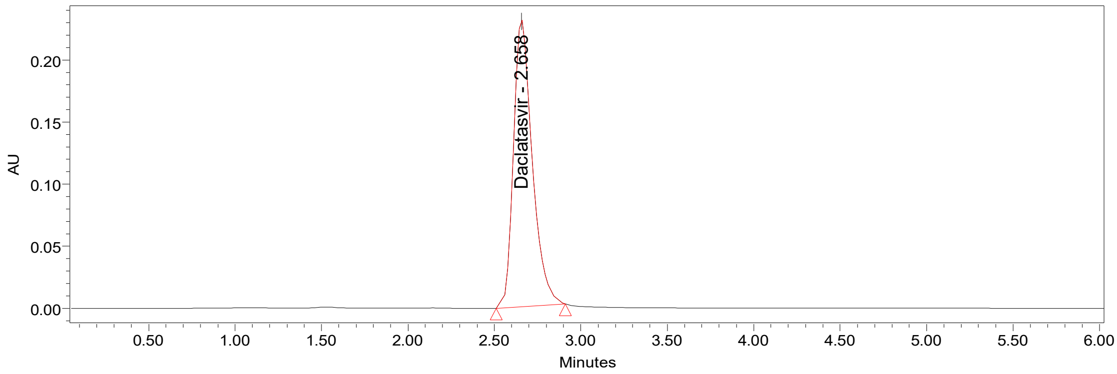

Fig2: Method Development Chromatogram

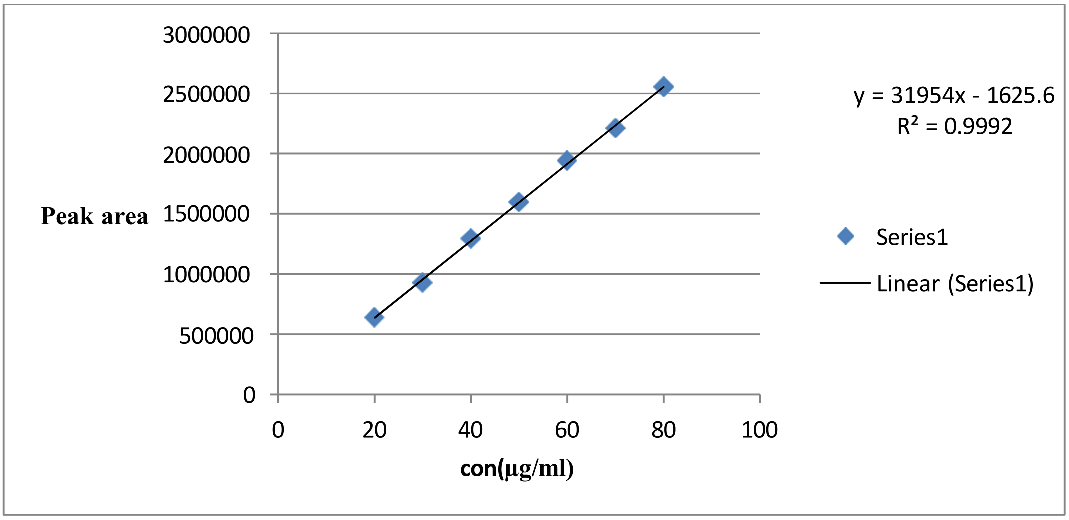

Fig3: Linearity graph

RESULTS AND DISCUSSION

For the medication daclatasvir, a straightforward, quick, and accurate technique has been created and shown to work. By making recurring tablets of the samples, the estimate was done using a mixture of methanol and acetonitrile within the quantitative relation of 70:30%v/v. 2.658 minutes was the retention time. The uniformity

Over the 20–80 ppm concentration range, the curve was linear. It was discovered that the LOD and LOQ values were 2.16µg/ml and 0.71µg/ml, respectively. Daclatasvir was subjected to acid, base, oxidation, and heat stress in order to create stress degradation studies. When compared to a reference standard, the findings of the analysis of the stress samples fell within the range. The strategy's quality is ensured by its low share constant of variation and high share of recovery. As a result, the RP-HPLC approach was widely accepted to be highly suitable for routine analysis.

CONCLUSION

It was discovered that the established RP-HPLC technique for the quantification of Daclatasvir in bulk and formulation was straightforward, accurate, exact, and repeatable. According to the ICH criteria, the devised technique was verified, and the findings were well within the acceptable range. All of the validation parameters were well within the intended range, and the statistical analysis of the established technique reveals low variance. Therefore, the suggested approach may be effectively used for formulation and bulk analysis of daclatasvir. In contrast to the other stress settings, forced degradation experiments of daclatasvir in bulk revealed that the medication was mostly broken down in a heated medium.

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