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Development and validation of stability indicating RP-HPLC method for the estimation of Daclatasvir in bulk and formulation

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ABSTRACT

A Novel simple, precise and economical reverse phase high performance liquid chromatographic method has been developed and validated for the estimation of Daclatasvir in single dosage form HPLC – WATERS Model NO.2690 series Compact System Consisting of Inertsil-C18 ODS column with a mobile phase constituting of Acetonitrile and Methanol (70:30) Flow rate 1ml/min and detection was carried out at 230nm. The selected chromatographic conditions were found to effectively separate Daclatasvir (Rt: 2.658 min). The developed method was validated for linearity, accuracy, precision, LOD, LOQ, robustness, ruggedness and for system suitability parameters as per ICH guidelines. Linearity for Daclatasvir was found in the range of 20-80µg/ml, respectively. The method was found to be robust. The proposed method could be used for routine analysis of Daclatasvir in single dosage forms. The bulk drug was subjected to forced degradation studies like acid, alkali, oxidative, thermal conditions.

Key words: Daclatasvir, RP-HPLC, Acetonitrile and methanol.

INTRODUCTION

Daclatasvir Chemically Methyl [(2S)-1-{(2S)-2-[4-(4-{2-[(2S)-1-{(2S)-2-[(methoxy carbonyl)amino]-3-methyl butanoyl}-2-pyrrolidinyl]-1H-imidazol-4-yl}-4-biphenyl yl)-1H-imidazol-2-yl]-1-pyrrolidinyl}-3-methyl-1-oxo - 2-butanyl] carbamate and It is an antiviral drug used to treat chronic (long-lasting) hepatitis C, a viral infection of the liver. Daclatasvir is an antiviral and acts directly against the hepatitis C virus.[1,2]

Literature survey reveals that there is no method development for single drug of Daclatasvir in combination only one method was developed. Therefore our commit to establish a new technique for analysis in pharmaceutical dosage forms, when an in depth study, set to develop a new RP-HPLC technique and its validation consistent with ICH guidelines. For the determination of this technique we tend to used Acetonitrile and methanol(70:30) Inertsil-C18 ODS column,was used, at a flow of 1.0 ml/min. and PDA detector done at 230nm[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]

4.1Specificity:
Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

4.2Linearity:
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

4.3Precision:
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%

4.4Limit 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.

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

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

4.5Robustness:
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

4.6System 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

4.7Ruggedness:
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]

5.1Acid 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

5.2Alkaline 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

5.3Thermal 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

5.4Oxidative 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 60^{o}C . 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

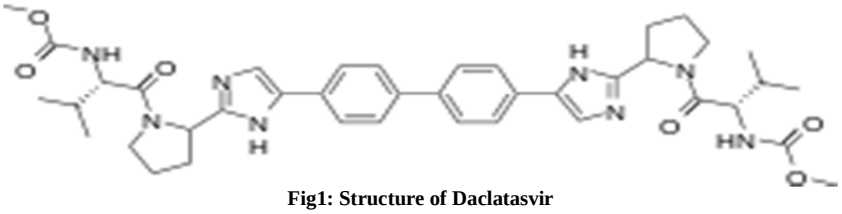

Fig1: Structure of Daclatasvir

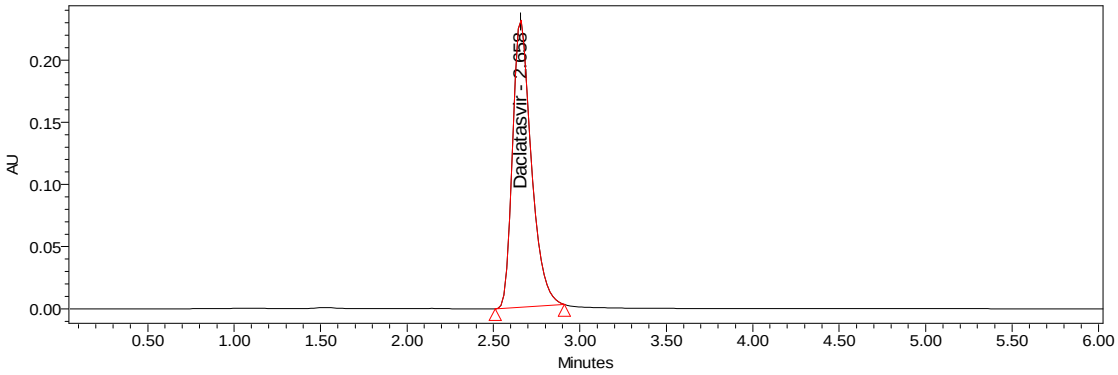

Fig2: Method Development Chromatogram

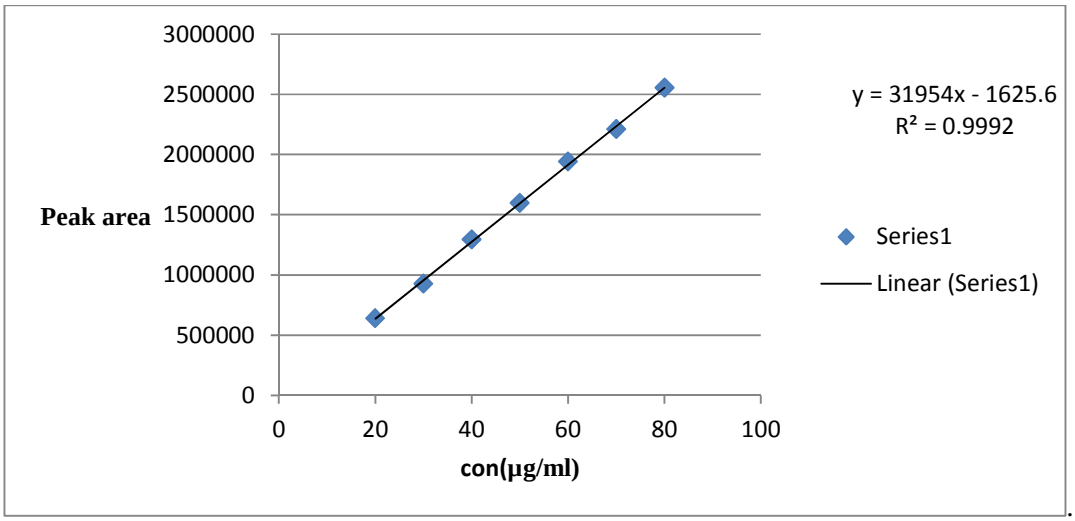

Fig3: Linearity graph

RESULTS AND DISCUSSION

A simple, fast and precise methodology has been developed and valid for the drug Daclatasvir. The estimation was carried out with a mix of Acetonitrile and methanol within the quantitative relation of 70:30%v/v. were studied by creating recurrent tablets of the samples were determined. The retention time was 2.658min. The standardization

curve was linear over the concentration range of 20-80ppm. The LOD and LOQ values were found to be 0.71µg/ml and 2.16µg/ml. Stress degradation studies were established for Daclatasvir by subjecting it to acid, base, oxidation, and thermal stress. The stress samples were assayed and results shown were within the range when compared against a reference standard. The high share of recovery and low share constant of variance make sure the quality of the strategy. Therefore it had been all over that the RP-HPLC methodology developed was considerably suit for routine analysis.

CONCLUSION

The developed RP-HPLC method for the estimation of Daclatasvir in bulk and formulation was found to be simple, precise, accurate and reproducible. The developed method was validated as per the ICH guidelines and the results obtained were well within the limits. The Statistical analysis of the developed method confirms minimal deviation and all the validation parameter was well within the specified range. Hence the proposed method can be successfully applied for analysis of Daclatasvir in bulk and formulation. Forced degradation studies of daclatasvir in bulk shown that the drug was mostly degraded in thermal medium compared to the other stress conditions.

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Conflict of interest statement
The authors herby by declare there are no conflicts of interest in the proposed article.

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