Method Development and Validation of Novel Analytical Methods for Daclatasvir in Bulk and Pharmaceutical Formulations Using UPLC Technique

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Abstract:

A simple accurate, precise rapid isocratic RP-UPLC method development for the estimation of Daclatasvir in tablet dosage form. The chromatographic system was carried on Acquity BEH C18 (50×3.0mm ×1.7µm id) using mobile phase consisting a mixture of 0.1% Orthophosphoric acid: Acetonitrile(60:40) v/v with detection of 248 nm. The retention time of Daclatasvir was found to be 1.190min. Calibration curve was linear over the concentration range of 50-150 µg/mL of Daclatasvir. The correlation coefficient for peak was found to be 0.9996. All the analytical validation parameters were determined and found in the limit as per ICH guidelines.

Key words: Daclatasvir, RP-UPLC, Acquity BEH column.

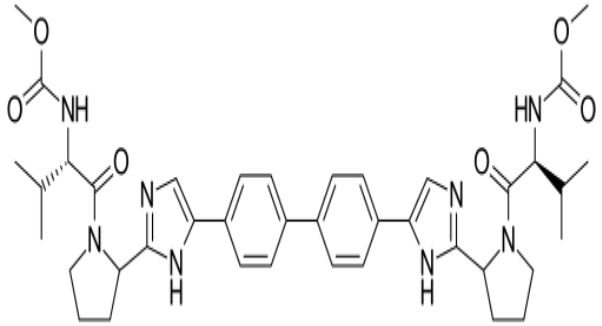
Introduction:

Ultra Performance Liquid Chromatography (UPLC) which is based upon small, porous particles (sub 2 micron particles). Van Deemter equation is the principle behind this evolution which correlates the connection between linear velocity and plate height. The small particles require a high pressure to work with UPLC i.e., 6000 psi which is typically the upper limit of conventional HPLCs. It was observed that when the particle size is decreased below 2.5 µm, there is a remarkable increase in the effectiveness and this effectiveness does not lessen on increasing the linear speed or rate of flow. This method reduces the mobile phase volume consumption by at least 80% compared to HPLC with a shorter runtime of about 1.5 min. The smaller sized particles increase the pressure up to 1000 bars or more which can alone increase the retention factor of the separation. Lower injection volume is required for UPLC which results in higher efficiency and increase in resolution. The higher column temperature reduces the mobile phase viscosity resulting in the high diffusion coefficient and flow rate without significant loss in efficiency and increase in column back pressure.

Drug profile:

Daclatasvir is a medication used in combination with other medications to treat hepatitis C (HCV). The other medications used in combination include Sofosbuvir, Ribavirin, and interferon. It varies depending on the virus type and whether the person has cirrhosis. It is taken by mouth once a day.

Structure



IUPAC Name: Dimethyl *N*,*N*′-([1,1'-biphenyl]-4,4'-diylbis{1*H*-imidazole-5,2-diyl-[(2*S*)-pyrrolidine-2,1-diyl][(2*S*)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate

Molecular Formula: $C_{40}H_{50}N_8O_6$

Mechanism of action:

NS5A is a viral non-structural phospoprotein that is part of a functional replication complex in charge of viral RNA genome amplification on endoplasmic reticulum membranes. It has the ability to bind to HCV RNA. It is shown to have two distinct functions in HCV RNA replication based on phosphorylated states.

Materials & Methods:

Instrumentation:

Instrument	Make
UV-Visible Spectrophotometer	Nicolet evolution 100
UV-Visible Spectrophotometer software	Vision Pro
UPLC software	Open lab EZ chrome
UPLC	Agilent Technologies
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Mettler Toledo

Syringe	Hamilton

Chemicals:

Chemical	**Grade**
Water	HPLC Grade
Orthophopshoric Acid	HPLC Grade
Methanol	HPLC Grade
Ethanol	AR Grade
Acetonitrile	HPLC Grade

Mobile phase preparation:

Prepare a mixture of 60 volumes of Buffer, 40 volumes of Acetonitrile. This mobile phase was sonicated for 10 min to remove gases.

Preparation of Standard solution:

Accurately Weighed about 100 mg of Daclatasvir & transferred in to a 100mL volumetric flask, then added 70mL of diluent, sonicated for 3min. Make final volume up to mark with the diluents & mix well. Take 5mL of standard stock solution and transferred in to 50mL volumetric flask then diluted up to mark with diluents & mix well.

Preparation of Sample solution: (Sample name: Daklinza 30 mg)

Weigh 20 capsules by removing the shell then crush with mortar and pestle then weigh a quantity of powder equivalent to 100mg of Daclatasvir and transferred in to a 100 mL volumetric flask, then add 70mL of diluent, sonicated for 30min. Make final volume up to mark with the diluent & mix well. Taken 5mL of standard stock solution and transferred in to 50mL volumetric flask then diluted up to mark with diluent & mixed well, filter this final solution through 0.45µm PVDF Syringe filter.

Optimized Chromatographic conditions:

Mobile phase	0.1% Orthophosphoric acid: Acetonitrile (60:40) v/v
Column	Acquity BEH C18 (50*3.0mm. 1.7µm)
Flow rate	0.5mL/min
Column temperature	30°C

Sample temperature	10°C
Wavelength	248 nm
Injection volume	10 µL
Run time	5 min
Retention time	1.190min

Results & Discussion:

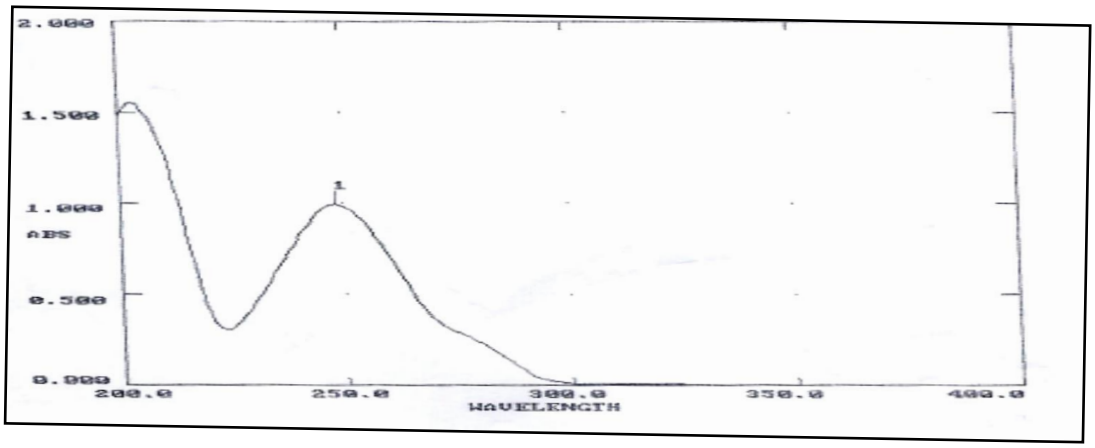


Fig. 1: UV-VIS Spectrum of Daclatasvir (248 nm)

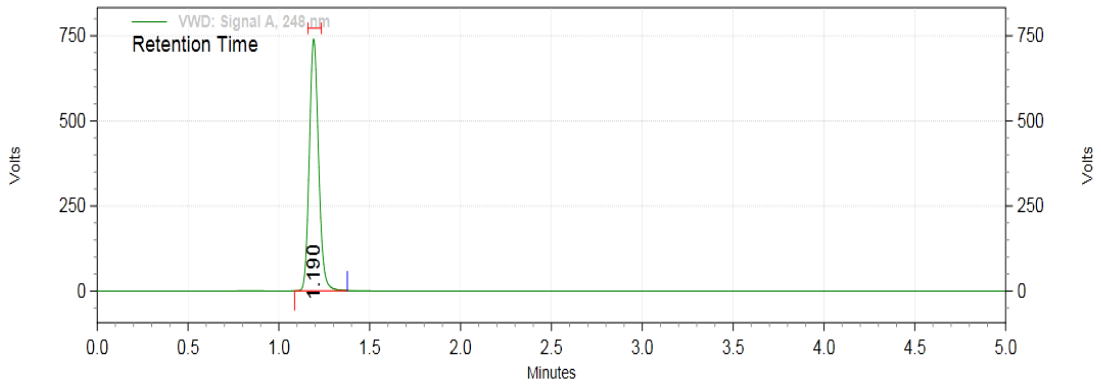


Fig. 2: Chromatogram of Optimized trial

Results for Optimized Trial:

S.NO	Name	RT	Area	TP	TF
1	Daclatasvir	1.190	44113817	2652	1.2

Observation:

From the above trial Daclatasvir eluted with good peak shape. The Theoretical plates & tailing factor ware found to be within limits. So, this trail was considered and validated according to ICH guidelines.

System suitability:

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.192	44125114	2560	1.2
2	1.193	44176891	2421	1.1
3	1.193	44165637	2676	1.3
4	1.190	44147346	2706	1.2
5	1.187	44105682	2704	1.1
6	1.190	44102411	2786	1.2
Mean	1.191	44137180	-	-
SD	0.023	31102.11	-	-
%RSD	0.2	0.1	-	-

Method precision:

Injection	DACLATASVIR	
	Area	% Assay
1	44113817	100.1
2	44176366	100.2
3	44078346	100.0
4	44150181	100.0
5	44008775	99.7
6	44025521	99.8
Average		99.9
SD		0.19
%RSD		0.19

Linearity:

S.No	Concentration (µg/mL)	Area
1	50	21720461
2	80	34167231
3	100	44035624
4	120	52943892
5	150	67035271

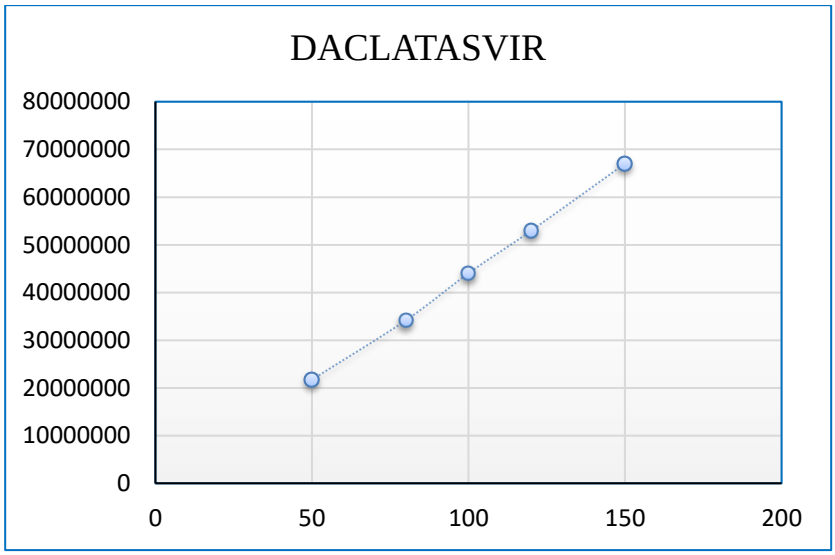


***Fig 3* :** Graph for Linearity data of DACLATASVIR.

Linearity results

S.No	Parameter	DACLATASVIR
1	Correlation coefficient	0.9996
2	Slope	455392.02
3	Intercept	1558706.27

Accuracy:

Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%.

Recovery:

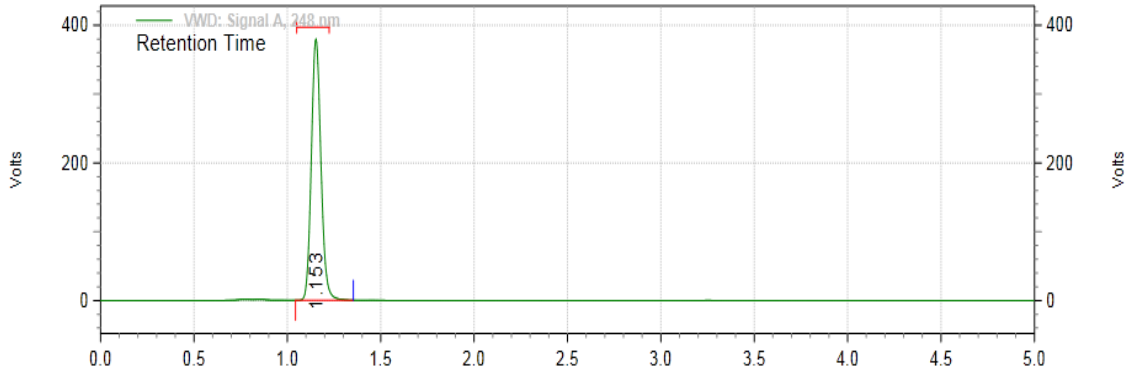


Fig. 4: Chromatogram of 50% recovery-1

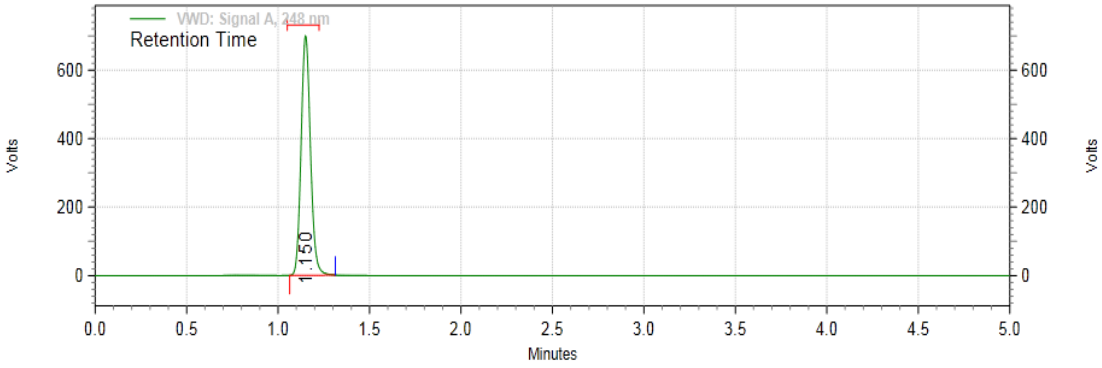


***Fig.5*:** Chromatogram of 100% recovery-1

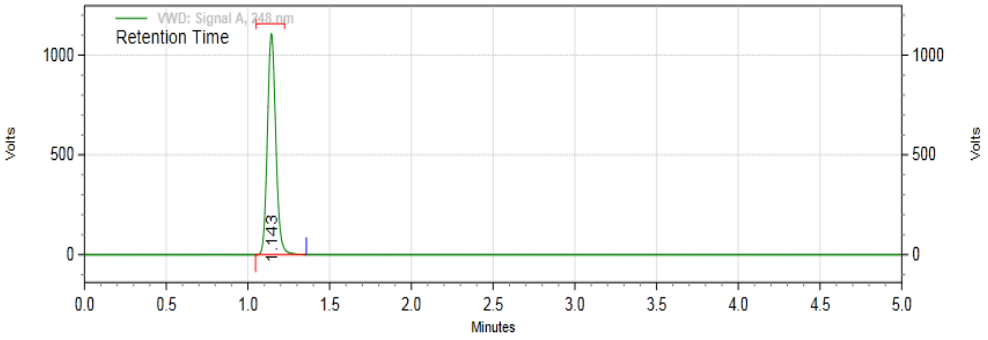


Fig.6*:* Chromatogram of 150% Recovery-1

Results of Recovery

% Recovered	Area	Concentration Added	Concentration Recovered	%Recovery	Average
50% _01	7004575	250	252.18	100.9	100.5
50% _02	7020900	250	252.77	101.1	
50% _03	7002470	250	252.11	100.8	
100% _01	13910853	500	500.83	100.2	
100% _02	13902676	500	500.53	100.1	

100% _03	13701006	500	493.27	98.7	
150% _01	21010188	750	756.42	100.9	
150% _02	21026894	750	757.02	100.9	
150% _03	21021825	750	756.84	100.9	

Robustness:

Chromatographic changes		**Retention time(min)**	**Tailing Factor**	**Theoretical Plates**
Flow rate (mL/min)	0.4	1.473	1.1	2942
	0.6	0.933	1.2	2047
Temperature (°C)	25	1.143	1.2	2467
	35	1.137	1.1	2496

Ruggedness:

Name of the Analyst	**%Assay**
Analyst 01	98.8
Analyst 02	98.9
%RSD	0.18

Discussion:

System suitability:

The plate count and tailing factor results were found to be within the limits and The % RSD was found to be 0.1 so system is suitable and giving precise results

Method precision:

The %RSD of Assay for 6 Samples determinations of DACLATASVIR found to be within the acceptance criteria (less than 2.0%). %Assay Also within the limits (95.0 to 105.0) hence method is precise.

Linearity:

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparation 0.9996.

Accuracy:

The percentage mean recovery of Daclatasvir was found between 98.0 to 102.0%.

Robustness:

The tailing factor was found to be within the limits on small variation of flow rate and wavelength.

Ruggedness:

From the results of two analysts % Assay and %RSD obtained acceptance criteria 2% so method is rugged.

Conclusion:

A new precise, accurate, rapid method has been developed for the estimation of Daclatasvir pharmaceutical dosage form by UPLC.

From results the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of Daclatasvir Educational institutions and Quality control laboratories.

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