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METHOD DEVELOPMENT AND VALIDATION OF DACLATASVIR BY UPLC

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ABSTRACT

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 A simple and selective UPLC method is described for the determination of Daclatasvir Chromatographic separation was achieved on a Acquity BEH C18 (50*3.0mm. 1.7µm) using mobile phase consisting 0.1% Orthophosphoric acid:Acetonitrile (60:40) v/v with detection of 248 nm. Linearity was observed in the range 30-70 µg /ml for Daclatasvir (r^2 =0.9987) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

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KEY WORDS: Daclatasvir, UPLC, pharmaceutical dosage form

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INTRODUCTION

Chromatography is a non-destructive procedure for resolving a multi-component mixture of traces, minor or constituents in to individual fractions. It is a method of separating a mixture of components in to individual components through a porous medium under the influence of solvent. For many years, researchers have looked at “fast LC” as a way to speed up analyses. The need for speed, the availability of affordable and easy to use mass spectrometers. Smaller columns and faster flow rates (amongst other increasing mass transfer by increasing the diffusivity of the analytes, has also been investigated. However, using conventional particle sizes and pressures, limitations are soon reached and compromises must be made, sacrificing resolution. HPLC technology simply doesn’t have the capability to take full advantages of sub-2µm particles. UPLC can be regarded as new invention for liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography. UPLC brings dramatic improvements in sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that operates at high pressure than that used in HPLC & in this system uses fine particles(less than 2.5µm) & mobile phases at high linear velocities decreases the length of column, reduces solvent consumption & saves time. According to the van Deemter equation, as the particle size decreases to less than2.5 µm, there

is a significant gain in efficiency, while the efficiency does not diminish at increased flow rates or linearvelocitiesTherefore by using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC
The technology takes full advantage of chromatographic principles to run separations Using columns packed with smaller particles(less than2.5µm) and/or higher flow rates for increased speed, this gives superior resolution and sensitivity.
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, vary depending on the virus type and whether the person has cirrhosis. It is taken by mouth once a day. NS5A is a viral nonstructural 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. Maintaining the HCV replication complex is mediated by the cis-acting function of basally phosphorylated NS5A and the trans-acting function of hyperphosphorylated NS5A modulates HCV assembly and infectious particle formation. Daclatasvir is shown to disrupt hyperphosphorylated NS5A proteins thus interfere with the function of new HCV replication complexes. It is also reported that daclatasvir also blocks both intracellular viral RNA synthesis and virion assembly/secretion in vivo (1-4).
The scope of developing and validating a method is to ensure a suitable strategy for evaluation of a particular analyte which is more specific, accurate and precise. The main focus is drawn to achieve improvement in the manufacturing and analytical conditions and making proper amendments in the standard operating procedures being followed. The above review indicates that there are fewer methods for the simultaneous estimation of *Daclatasvir* but that a method was found to be cost effective and time consuming. So my aim was to develop a new method with minimum run time and less solvent consumption

for the estimation of *Daclatasvir* in a formulation. Hence the present study aims to develop simple, precise and accurate methods for the determination of *Daclatasvir b*y UPLC in tablet dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λ_{max})

Preparation of Standard solution

About 50mg of Daclatasvir was weighed into a 50 mL volumetric flask, to this 50 mL of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

Dilutions

Necessary dilutions are made from standard stock solutions to get the concentration range of 10µg/mL of Daclatasvir**.** The wavelength of maximum absorption (λ_{max}) of the solution of the drug in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 248 nm for Daclatasvir), 248 nm was selected as detector wavelength for the UPLC chromatographic method.

Preparation of Standard solution (6-8)

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

Preparation of Sample solution

Dalsiclear 60mg- Weigh 20 tablets 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 added 70mL of diluent, sonicated for 30min. Made final volume up to mark with the diluent & mixed 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

RESULTS AND DISCUSSION

The % assay found to be within the limitsthat is 95.0-105.0% (Fig-1 and table-1).

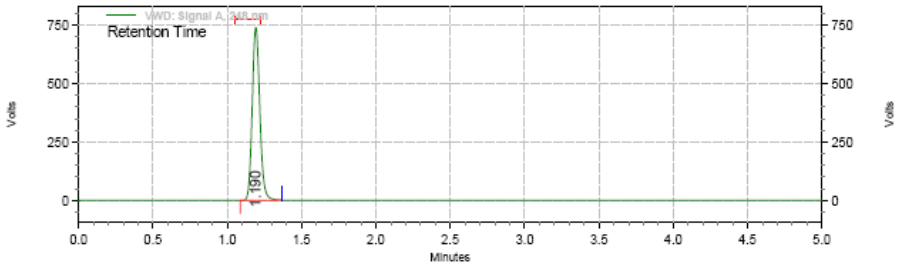

Fig-1 Chromatogram of Assay

Table-1 Results for Daclatasvir

Daclatasvir		
	Standard Area	**Sample Area**
Injection-1	44049957	43312224
Injection-2	44176366	43309599
Injection-3	43965547	43398270
Injection-4	44027772	44329833
Injection-5	43915825	44358634
Average Area	44027093.4	43741712
Standard deviatuion	551274.21	
%RSD	0.22	
Assay(%purity)	99.35	

The plate count and tailing factor results were found to be within the limits andThe % RSD was found to be 0.1 so system is suitable and giving precise results (Table-2).

Table-2 Results for system suitability of daclatasvir

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	-	-

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. The relationship between the concentration (in %) of daclatasvir and area of daclatasvir should be linear in the specified range and the correlation should not be less than 0.99 (Fig-2 and table-3).

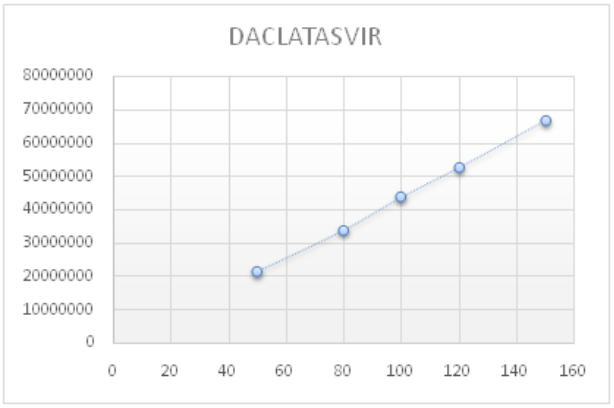

Fig-2 Graph for Linearity data of daclatasvir.

Table-3 Linearity results of daclatasvir

S.No	Parameter	DACLATASVIR
1	Correlation coefficient	1.000
2	Slope	455392.02
3	Intercept	1558706.27

The tailing factor was found to be within the limits on small variation of flow rate and wavelength (Table-4).

Table-4 Results for Robustness of daclatasvir

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

CONCLUSION

A simple 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.

REFERENCES

1. Chatwal**,** R. G.**;** Anand**,** K. S. High performance liquid chromatography**.** *Instrumental methods of chemical*

analysis, 5thed.; Himalaya publishers: Mumbai, 2010; 2.570-2.629.

2. Sharma, B. K. High performance liquid chromatography. *Instrumental methods of chemical analysis*, 24th ed.; Goelpublishers: Meerut, 2005; 295 - 300.
3. Dong,W. M. HPLC instrumentation and trends. *Modern HPLC for practicing scientists*, USA, 2006; 5-10, 78-110.
4. Swartz, M. E.; Ira Krull, S, Analytical method development. *Analytical method development and validation*, 1st ed.; Marcel Dekker, Inc: New York, 2009; 17-80.
5. ICH, *Text on Validation of Analytical Procedures*, ICH – Q2A, International Conference on Harmonisation, IFPMA, Geneva, 1995, 2-3, A–1 to A–3.
6. ICH, *Validation of Analytical Procedures: Methodology*, ICH – Q2B, International Conference on Harmonisation, 1996, 1-3.
7. ICH Guidelines, Q2 (R1) - Validation of Analytical Procedures: Text and Methodology,2005, 1-6.