JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 06, ISSUE 05, 2019

NOVEL STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF ANTI HEPATITIS-C VIRAL DRUG -DACLATASVIR

Pothula Raju[1] , A.Rajendiran[2] , R.Suneetha[3], Perli Krantikumar[4]

[1]Research Scholar , Institute of Pharmacy, CSJM University, Kanpur -208024.
[2]Research Guide , Institute of Pharmacy, CSJM University, Kanpur -208024.
[3]Associate Professor, Department of Pharmaceutical Chemistry, Sri Indu Institute of Pharmacy,Telangana-501510.
[4]Associate Professor & HOD, Department of Pharmaceutical Analysis, Sri Indu Institute of Pharmacy, Telangana-501510.

Abstract:

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of daclatasvir in capsule dosage form. A column of Zorbax Eclipse XDB-C18, 250x4.6mm i.d with 5 micron particle size was used. The mobile phase comprises of 0.03M di potassium hydrogen orthophosphate with pH adjusted to 2.5 using dilute ortho- phosphoric acid (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) in the ratio of 15: 85 (v/v). The flowrate was 1.0 ml/min and the effluents were monitored at 284 nm. The retention time was 7.79 min. The detector response was linear in the concentration of 100-300µg/ml. The respective linear regression equation being Y= 28817.742X-14741.2. The limit of detection (LOD) and limit of quantification (LOQ) for were found to be 0.05µg/ml and 0.15 µg/ml respectively. The assay was found to be 99.85%.The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of daclatasvir in bulk drug and in its pharmaceutical dosage form.

Keywords: Daclatasvir, RP-HPLC, system suitability, linearity, recovery studies.

Corresponding author:

[1]Pothula.Raju
Research Scholar
Institute of Pharmacy
CSJM University,Kanpur-208024
Mail.id : rnani36@gmail.com

ISSN- 2394-5125 VOL 06, ISSUE 05, 2019

INTRODUCTION:

Daclatasvir (brand name Daklinza) is a new medication used to treat hepatitis C [1]. It was approved in Europe in August 2014 for treatment of adults with chronic hepatitis C genotypes 1, 2, 3 or 4 [2,3]. Daclatasvir is one of the new direct-acting antiviral drugs that target different steps of the hepatitis C virus (HCV) lifecycle. It is the first-ever approved HCV NS5A[4] replication complex inhibitor, meaning it interferes with a protein the virus uses to reproduce.The chemical formula of daclatasvir is methyl N-[(2S)-1-[(2S)-2-[5-[4-[4-[2-[(2S)-1-[(2S)-2-(methoxycarbonylamino)-3-methylbutanoyl]pyrrolidin-2-yl]-1H-imidazol-5-yl]phenyl]phenyl]-1H-imidazol-2-yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate.[5,6] Its molecular formula is $C_{40}H_{50}N_8O_6$ and molecular weight 738.89 g/mol (Figure 1).[7,8]

Literature survey reveals that few Spectroscopic methods[9-11],LC-MS[12,13] and HPLC[14,15] have been developed for the estimation of daclatasvir from pharmaceutical dosage forms. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of daclatasvir in bulk drug samples and in pharmaceutical dosage form.

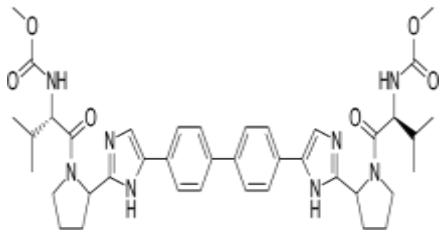

Fig 1: Structure of daclatasvir

EXPERIMENTAL:

Materials and methods:

Daclatasvir was obtained as a gift sample from Hetero Drugs Ltd Hyderabad. Acetonitrile and water used were of HPLC grade (Qualigens), potassium dihydrogen phosphate and ortho-phosphoric acid were procured from Rankem. Commercially available daclatasvir capsules (Declahep®-60 mg) were procured from local market.

Instrument:

Quantitative HPLC was performed on Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing powered with -2 Empower Software.A Zorbax Eclipse XDB-C18, 250x4.6mm i.d of particle size 5micron column was used.The detector used is a photodiode array (model 2996) with a wavelength range of 190-800 nm.

HPLC Conditions:

The contents of the mobile phase were 3.48 gms of di potassium hydrogen orthophosphate (0.03M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute ortho-phosphoric acid (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) in a isocratic mode in the ratio of 15: 85 (v/v) of separation was used. They were filtered before use through a 0.45 µm membrane filter and degassed by sonication.

The run time was set at 15min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 284 nm.

Preparation of Standard Stock solution:

A standard stock solution of the drug was prepared by dissolving 250 mg of Daclastavir working standard in 100ml of the diluent. The contents were sonicated for 15 minutes to obtain 2500µg/mL.

Working Standard solution:

5ml of the primary standard stock solution of 2500µg/mL was taken in 50 ml volumetric flask and thereafter made up to 50 ml with mobile phase to get a concentration of 250µg/ml.

Preparation of Sample solution:

20 Tablets of Daclatasvir (Declahep ® 60 mg, Hetero Health Care, Tablets,) were and then powdered. A sample of the blended tablet powder, equivalent to 250 mg of the active ingredient, was mixed with 70 ml of mobile phase in 100 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by addition of mobile phase up 100 ml to obtain a stock solution of 2500µg/mL. The resultant solution was further diluted by taking 5 ml of the stock solution with 50 ml of mobile phase to get the concentration of 250µg/mL

RESULTS AND DISCUSSION:

Validation for the method was carried out as per ICH Q2(R1) guidelines. The validation parameters such as system suitability, linearity, recovery studies, robustness, detection limit , quantitation limit were studied.[7,8]

System Suitability:

The system suitability tests were carried out on freshly prepared standard stock solution of Daclatasvir. The system was suitable for use, the tailing factors for Daclatasvir were 1.23 and USP theoretical plates were found to be significantly high around 16305.

ISSN- 2394-5125 VOL 06, ISSUE 05, 2019

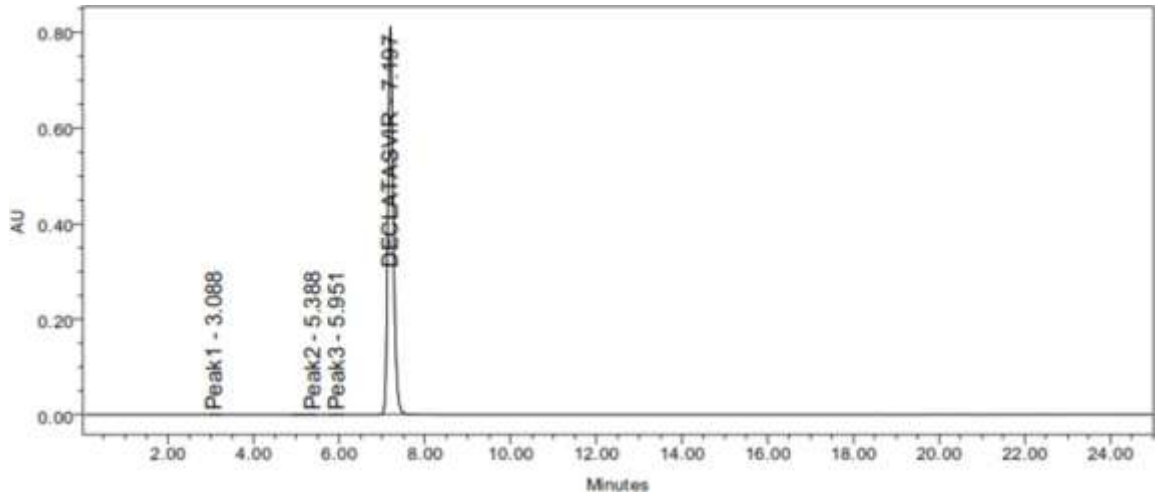

Fig 2: Typical System suitability Chromatogram of Daclatasvir Working standard solution.

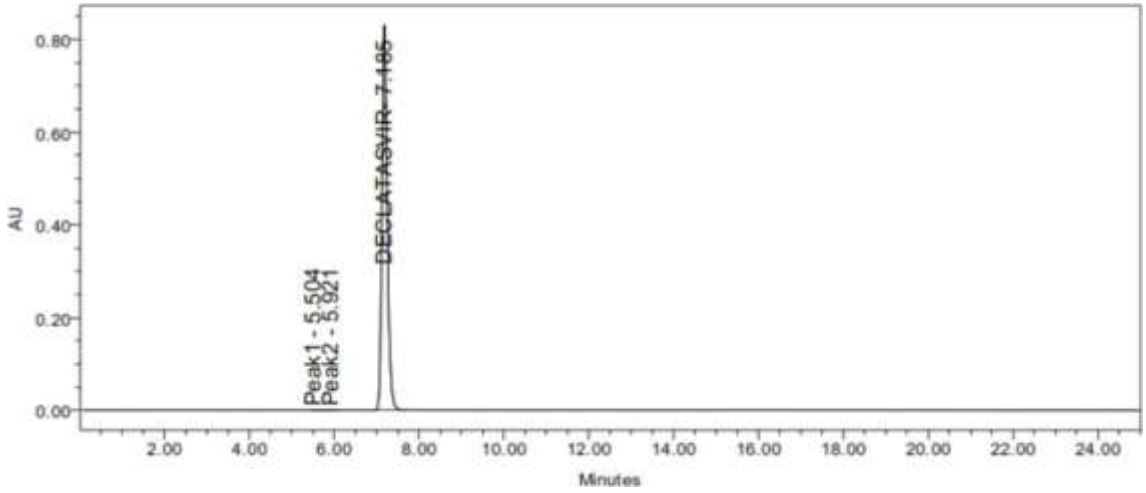

Fig 3: Typical Chromatogram of Daclatasvir Working standard solution

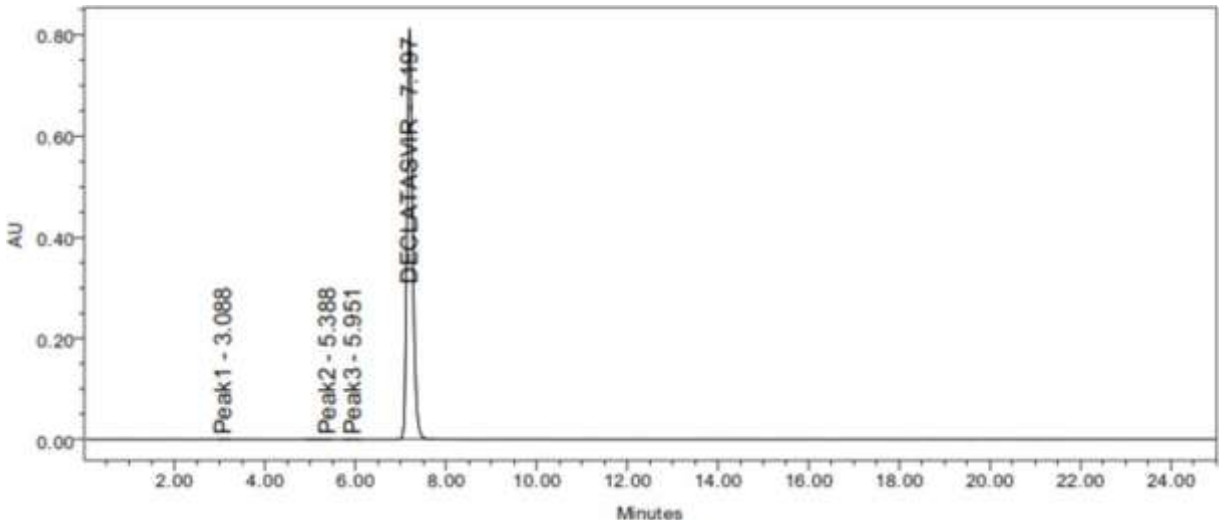

Fig 4: Typical Chromatogram of Daclatasvir Working sample (Declahep®-60 mg tablets) solution.

ISSN- 2394-5125 VOL 06, ISSUE 05, 2019

Linearity:

Aliquots of standard Daclatasvir stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Daclatasvir are in the range of 100-300 µg/ml. Each of these drug solutions (10 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 284 nm and a Calibration graph was obtained by plotting peak area versus concentration of Daclatasvir (Figure 5) The linearity Chromatograms presented in Figure 6.

The plot of peak area of each sample was found to be linear in the range of 100-300µg/ml with correlation coefficient of 0.999. Linear regression least square fit data obtained from the measurements are given in Table 1 and Table 2 .The respective linear regression equation being

Y= 28817.742X-14741.2.The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table I.**

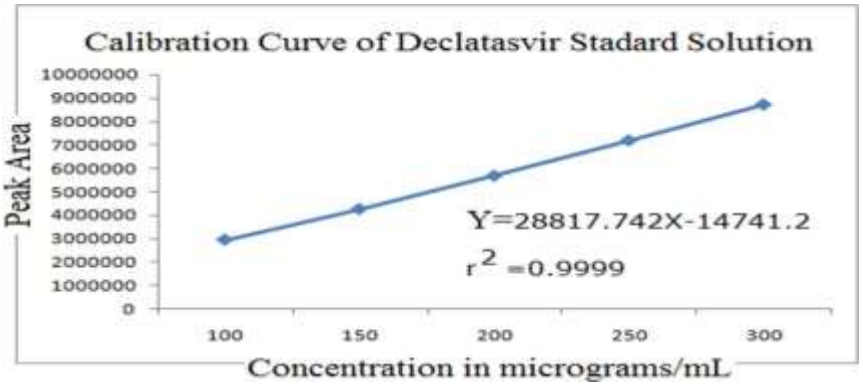

Fig 5 : Calibration curve of daclastavir

Table 1: Standard calibration values of daclastavir

Concentration of drug (µg/mL)	Peak Area
100	2951349
150	4256089
200	5667685
250	7170168
300	8698745

Table 2: Optical & Regression Characteristics of HPLC method

Parameter	Results of HPLC Method
Detection wavelength (nm)	284
Linearity range (µg/mL)	100-300
Regression Equation (y=mx + c)	Y=28817.742X-14741.2
Slope (m)	28817.742
Intercept (c)	-147412
Correlation coefficient	0.9999
Relative Standard deviation*	1.1
% error in bulk samples	0.234

ISSN- 2394-5125 VOL 06, ISSUE 05, 2019

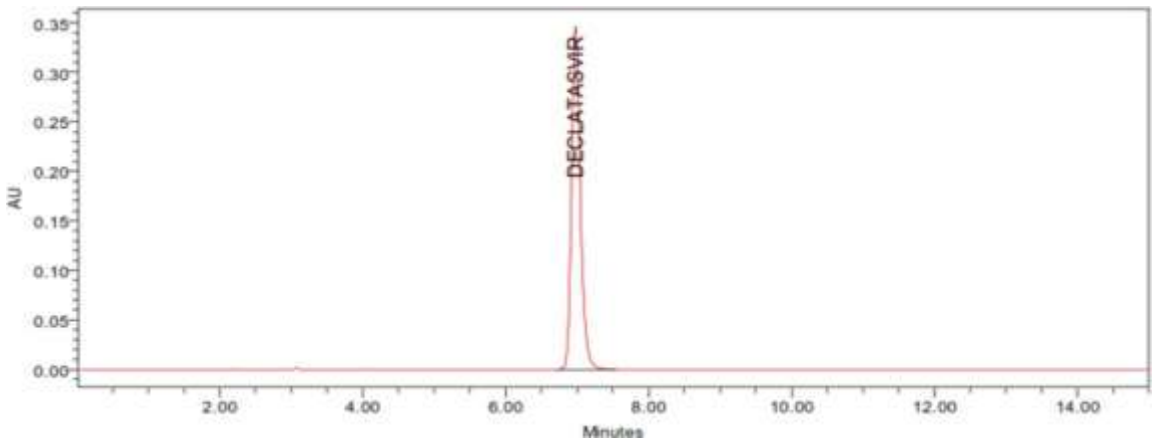

Fig 6: Linearity Chromatograms of Daclatasvir in standard dilution:

Assay and recovery studies:

Recovery studies were conducted by analyzing pharmaceutical formulation in the first instance for the active ingredient in the concentration of 80% of the working standard (contains 200 µg/mL of Daclatasvir); 100% of the working standard solution (contains 250 µg/mL of Daclatasvir) and 120% of the working standard solution (contains 300 µg/mL of Daclatasvir) by the proposed method. Each concentration was injected 3 times and the peak area was recorded. Known amounts of pure drug [10% of the working standard solution contains 25 µg/mL of Daclatasvir for 80% of the working standard, for 100% of the working standard, for 120% of the working standard] was then added to each 3 previously analyzed formulation and the total amount of the drug was once again determined by the proposed method (each concentration was again injected 3 times) after keeping the active ingredient concentration within the linearity limits (Table 3).

Robustness:

A method is robust if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at two different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the mobile phase flow rate was varied by ±10%) and different column had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. When the chromatographic conditions were deliberately altered, system suitability results remained within acceptance limits and selectivity for individual substance was not affected. The results of the study prove the robust nature of the method (Table 4).

Limit of Detection [LOD] and Limit of Quantification [LOQ]:

The detection limit of the method was investigated by injecting standard solutions Daclatasvir into the HPLC column. By using the signal-to-noise method the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated. A signal-to-noise ratio (S/N) of 3 is generally accepted for estimating LOD and signal-to-noise ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise. Chromatograms illustrating the LOD are shown in figure 2.10. The limit of detection (LOD) and limit of quantification (LOQ) for Daclatasvir were found to be 0.05µg/ml and 0.15 µg/ml respectively (Table 5).

ISSN- 2394-5125 VOL 06, ISSUE 05, 2019

Table 3: Recovery Peak areas of Daclatasvir by Accuracy studies

S.No	Recovery at 80% dilution level Peak areas		Recovery at 100% dilution level Peak areas		Recovery at 120% dilution level Peak areas	
	Standard	Spiked	Standard	Spiked	Standard	Spiked
1	6078549	6883692	7137688	8171910	8693037	9402205
2	6094909	6936077	7254913	8123507	8737102	9487382
3	6117299	6939025	7199150	8025701	8581219	9334452
Avg	6096919.0	6919598.0	7197250.3	8107039.3	8670452.7	9408013.0
SD	19453.0	31130.4	58635.6	74482.6	80358.1	76630.3
%RSD	0.3	0.4	0.8	0.9	0.9	0.8
% Recovery	102.0%		119.10%		93.50%	

Table 4: Robustness study of Daclatasvir Standard solution at 100 % level (250 µg/mL)

Parameter	Peak areas of Daclatasvir in Flow increase study	Peak areas of Daclatasvir in Flow decrease study	Peak areas of Daclatasvir in Variable column Study
Injection-1	6666481	8007918	7281195
Injection-2	6630941	7931643	7281896
Injection-3	6667925	7947609	7252862
Mean	6655115.7	7962390.0	7271984.3
% RSD	20948.3	40228.4	16564.1
Std. Dev	0.3	0.5	0.2

Table 5: Performance & Detection Characteristics of HPLC method:

Parameter	Results of the proposed HPLC method	
	Daclatasvir Standard solution	Daclatasvir Sample (Declahep®-60 mg tablets) Solution
Retention time (min)	7.185	7.197
Theoretical plates (n)	16633.23	16304.73
Plates per meter (N)	66532.8	65218.92
HETP	$1.5030x10^{-5}$	$1.5333 x10^{-5}$
Peak asymmetry (T)	1.23	1.23
Linearity range (µg/mL)	100-300	
Limit of Detection (µg/mL)	0.05	
Limit of Quantification (µg/mL)	0.15	

CONCLUSION:

There are no reports on the HPLC determination of Daclatasvir in pharmaceutical formulations in the literature prior to commencement of this work. The author has developed a sensitive, accurate and precise HPLC for the estimation of Daclatasvir in bulk drug and in tablet dosage form. From the typical chromatogram of Daclatasvir as shown in fig 3.1.2, it was it found that the retention time was 7.185 min. The contents of the mobile phase were Buffer: Acetonitrile 15: 85 (v/v). solvent-A (Buffer) is 3.48 gms of di Potassium hydrogen ortho-phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute orthophosphoric acid and solvent-B is acetonitrile in a isocratic mode of separation was used to resolute the Daclatasvirat a flow rate of 1.0 ml/min and eluents were monitored at 284 nm, was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r^2=0.9999) was observed between the concentration range of 100-300 µg/mL. The assay of Daclatasvir in bulk was found to be 99.85%. From the recovery studies it was found that about 191.10 % on average of Daclatasvir was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the Tablets. This

ISSN- 2394-5125 VOL 06, ISSUE 05, 2019

demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and sterile powder for injection dosage form of Daclatasvir within a short analysis time.

It can be seen from the results presented that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations.

REFERENCES:

1. American Association for the Study of Liver Diseases; Infectious Diseases Society of America. HCV guidance. http://hcvguidelines.org. Accessed June 12, 2017.
2. Daclatasvir label" (PDF). FDA. April 2016.
3."Daklinza film-coated tablets - Summary of Product Characteristics (SPC) - (eMC)". Electronic Medicines Compendium. September 2016.
4. Lee C: Daclatasvir: potential role in hepatitis C. Drug Des Devel Ther. 2013 Oct 16;7:1223-33. doi: 10.2147/DDDT.S40310. eCollection 2013. [PubMed:24204123]
5. www.drugbank.com /Daclatasvir.
6. www.wikipedia.com/ Daclatasvir.
7. Kapil Kalra. Method development and validation of Analytical Procedures. Dev Bhoomi Institute of Pharmacy and Research Dehradun, Uttarakhand, India
8. Szepesi G.. J. Chromatogra, 1989, 464,265 -278
9.Ashok chakravarthy v1*, Sailaja bbv1, Praveen kumar a, Method development and validation of ultraviolet-visible spectroscopic method for the estimation of hepatitis-c drugs - daclatasvir and sofosbuvir in active pharmaceutical ingredient form, Vol 9, Suppl. 3, 2016.
10.Jeevana juothi, Padmaja, UV Spectrophotometric method for estimation of new drug, daclatasvir dihydrochloride, October 2016
11. Vikas Kekan, Sachin Gholve*, Omprakash Bhusnure Development, Validation and Stability Study of UV Spectrophotometric Method for Determination of Daclatasvirin Bulk and Pharmaceutical Dosage Forms, Vol.10 No.5, pp 281-287, 2017
12. Manel Aouria, , Darius Moradpour , Matthias Cavassini , Thomas Mercier , Thierry Buclin , Chantal Csajka , Amalio Telenti , Andri Rauch , Laurent A. Decosterd A Multiplex LC-MS/MS Assay for the Simultaneous Therapeutic Drug Monitoring 2 of Ribavirin, Boceprevir and Telaprevi,2013
13. HaoJianga, HamzaKandoussia , JianingZenga , JianWanga ,Multiplexed LC-MS/MS method for the simultaneous quantitation of three novel hepatitis C antivirals, daclatasvir, asunaprevir, and beclabuvir in human plasma, JOPBA,vol 107,2015.
14. K. Sumathi, K. Thamizhvanan and S. Vijayraj, Development and validation of stability indicating RP-HPLC method for the estimation of Daclatasvir in bulk and formulation, Der Pharmacia Lettre, 2016, 8 (15):107-113
15. Baker MM1, El-Kafrawy DS2, Mahrous MS2, Belal TS3.Validated stability-indicating HPLC-DAD method for determination of the recently approved hepatitis C antiviral agent daclatasvir. Epub 2017 Feb 8