(RESEARCH ARTICLE)

Development and validation of stability indicating HPTLC method for estimation of Daclatasvir Dihydrochloride in pharmaceutical dosage form

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Abstract

A high-performance thin-layer chromatographic method was developed and validated for estimation of Daclatasvir Dihydrochloride in pharmaceutical dosage form. The proposed method was applied successfully to the pharmaceutical analysis of the recently approved dosage form of Daclatasvir Dihydrochloride which is available in market as a brand name of 'NALDAC 60' tablets. The drugs were satisfactorily show peak with RF 0.38 for Daclatasvir Dihydrochloride. Method was validated according to the ICH guidelines. The calibration plot was linear between 50-300 ng per band for Daclatasvir Dihydrochloride. The LOD and LOQ for Daclatasvir Dihydrochloride were found to be 0.171 μg per band and 0.521 μg per band, respectively. Accuracy and precision of the proposed method was evaluated by recovery studies (% recovery for Daclatasvir Dihydrochloride was 99.88%) and intra-day and inter-day precision studies (standard deviation value for precision studies was found to be 0.453).

In stability testing, Daclatasvir Dihydrochloride was found susceptible to acid hydrolysis and alkaline degradation. Because the method could effectively separate the drugs from their degradation products, it can be used as a stability indicating method. The proposed validated stability indicating assay for the sensitive determination of the mentioned drugs is suitable for Quality control laboratories as a simple fast economic method. Degradation product of Daclatasvir Dihydrochloride in alkaline condition was carried out.

Keywords: Daclatasvir Dihydrochloride; High performance thin layer chromatography; Stability indicating assay; 'NALDAC 60' tablets

1. Introduction

Daclatasvir Dihydrochloride highly potent and selective direct-acting antiviral (DAA) that targets the hepatitis C virus… (IUPAC name: 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)(Fig.1).[3] It interferes with viral replication by inhibiting nonstructural protein 5A (NS5A), leading to suppression of RNA synthesis, vision assembly and secretion. Daclatasvir is active against HCV genotypes 1a, 1b, 2 and 3. It is also expected to be efficacious in patients with genotype 4 infection (based on extrapolations from studies with Daclatasvir in combination with peg interferon and ribavirin). Daclatasvir has not been studied in patients with HCV genotypes 5 and 6. In combination studies using cell-based HCV replicon system, daclatasvir demonstrated synergistic effects with interferon alfa and anti-HCV agents with different pharmacologic targets, including nonstructural protein 3 (NS3) protease inhibitors, nonstructural protein 5B (NS5B) nucleoside and non-nucleoside inhibitors.

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Literature review of Daclatasvir Dihydrochloride should contain all the following methods either in biological fluids or in pharmaceutical dosage form, also either alone or in combination with other drugs like, Sofosbuvir or in combination with metformin [1-7]. Literature survey revealed HPLC [4] and UV Spectrophotometry [2] method for estimation of Daclatasvir Dihydrochloride in pharmaceutical dosage form. An oral dose formulation containing Daclatasvir Dihydrochloride (60 mg) is available in the market with a brand name 'NALDAC 60'Quantitative Estimation of daclatasvir Dihydrochloride in drug substances has been studied by UPLC [5]

However, there is no stability indicating analytical method reported for estimation of this drug in pharmaceutical formulation. So it was thought worthwhile to develop a new stability indicating HPTLC method for Daclatasvir Dihydrochloride. Which may be simple, economical and more specific than the previously reported method for drugs in combination? The present work describes a simple, precise, rapid, selective, and economic high-performance thin-layer chromatographic procedure for determination of Daclatasvir Dihydrochloride formulation. The proposed method was optimized and validated as per ICH guidelines [7-9]
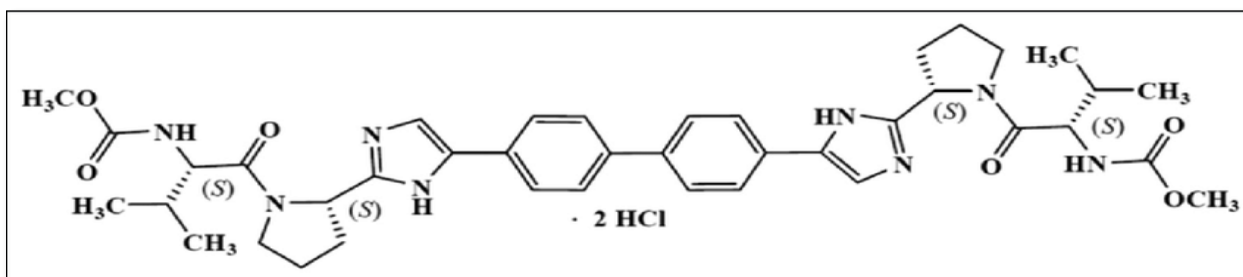
Figure 1 Structure of Daclatasvir Dihydrochloride

2. Material and methods

2.1. Reference substances, reagents and chemicals

Pure drugs, Daclatasvir Dihydrochloride are procured from Tajo mahal Vision Chemicals Mumbai. Tablet formulation NALDAC 60 (60 mg of Daclatasvir Dihydrochloride,), were purchased from local market. All chemicals and reagents used were oar grade. In HPTLC method Toluene and methanol used was of AR grade (Merck specialties Pvt. Ltd.).'

2.2. Instrumentation

The HPTLC system used for analysis was of CAMAG, with Camag Linomat V sample applicator, Camag syringe of capacity of 100 µl (Muttenz, Switzerland), with UV detector, plate scanner used was of Camag TLC scanner III, software WinCAT's software version 1.4.3.6336 was used, TLC Plates Used was of size 10x10Aluminium plates precoated with silica gel 60 F254 plates (E.Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India).

2.3. Chromatographic conditions

2.3.1. Selection of mobile phase

From the standard stock solutions 10 µl of Daclatasvir Dihydrochloride, had been applied on Thin Layer Chromatographic plates in band form (band size: 6 mm) and these plates were put for run under different solvent systems. The attempts were made to achieve the preferred Rf value range (0.1-0.8) with a compact band, various trials were performed by using different solvent systems which contain non-polar solvents as well as comparatively polar solvents like toluene: methanol; toluene: methanol: ammonia; methanol: ethyl acetate: Glacial acetic acid by varying its concentration levels and was tried to determine the suitable conditions for the efficient separation of Daclatasvir Dihydrochloride. From the different mobile phase combinations tested, Toluene: Methanol (2:8v/v) yields compact bands which showed symmetrical peak on chromatogram and expected Rf value. The Rf values with their standard deviation were 0.38± 0.05 for DDH was found.

2.3.2. Sample preparation

Accurately weighed quantity of 10.0 mg Daclatasvir Dihydrochloride was transferred to 10.0 ml volumetric flask, dissolved and diluted up to the mark with mobile phase. From above solution, 1.0 ml of solution was diluted to10.0 ml

with methanol. (Concentration 100 µg/ml for Daclatasvir Dihydrochloride). The solution was filtered through 0.2 µ membrane filter.

2.4. Analysis of marketed formulation

Preparation of standard solution A: Accurately weighed quantity of 10.0 mg Daclatasvir Dihydrochloride was transferred to 10.0 ml volumetric flask, dissolved and diluted up to the mark with mobile phase. From above solution, 1.0 ml of solution was diluted to10.0 ml with methanol. (Concentration 100 µg/ml for Daclatasvir Dihydrochloride). The solution was filtered through 0.2 µ membrane filter.

Preparation of sample solution: Accurately weigh 10.0 mg of Daclatasvir Dihydrochloride and then transferred to 10.0 ml volumetric flask; 5 ml methanol was added and ultrasonicated for 15 min., volume was then made upto the mark with methanol. The solution was filtered by using Whatmann filter paper No. 42. From this solution, 1.0 ml of solution was then diluted to 10.0 ml with methanol. The resulting solution was used as sample solution. On the TLC plate two bands of standard stock solution A and four bands of sample solution of band width, 5.0 µl each, were applied and the plate was developed and scanned under the optimum chromatographic condition. After scanning the peak obtained for standard and sample bands were integrated. Amount of drug present in sample was calculated by comparing the mean peak area of sample band with that of the standard band.

2.5. Method validation

2.5.1. Accuracy

An accurately weighed quantity of a sample equivalent to 10 mg Daclatasvir Dihydrochloride was transferred individually in nine different 10.0 ml volumetric flasks, added 8.0 /10.0 and /12.1 mg of DDH. Accuracy was determined as per ICH guidelines and expressed as % recovery.

2.5.2. Precision

To establish reproducibility and repeatability of the method precision studies were performed. The sample solution was prepared and then analyzed similarly as described under analysis of the marketed formulation. Intra-day precision and Interday precision studies were performed for three consecutive days.

2.5.3. Robustness

The Robustness study for the proposed method was evaluated. As making change of composition, in the mobile phase volume, time from spotting to development (15 min, 40 min and 1.2 hrs) and time from development to scanning (15 min, 40 min and 1.2 hrs), the effects of these parameter on Rf value and peak area of drugs were examined. The composition of mobile phase was changed slightly (± 0.1 ml for component).

2.5.4. The limit of detection and limit of quantitation

The Limit of detection (LOD) and Limit of Quantitation (LOQ) were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ of the developed method.

2.5.5. Forced degradation studies

The forced degradation studies, was carried by keeping a sample to expose to the following stress conditions: acidic (0.1 M HCl), alkaline (0.1 M NaOH), and oxidation (3 % H2O2). For degradation. Contents of the flasks were refluxed in a water bath at 80°C for 2hr. For heat degradation and photo degradation a sample was kept at 60°C and in UV light (254 nm) for 24 hr, respectively. After the respective time intervals all the flasks were removed and allowed to cool. The samples were then analyzed in similar manner as described under analysis of DDH in formulation % degradation of drug was estimated.

3. Results and discussion

3.1. Optimization of procedures

Different solvent combinations like methanol, toluene- butanol, ethyl acetate and ammonia in varying polarity and proportion were tried during selection of mobile phase. Among the different combinations tested, Toluene: Methanol (8:2, v/v) was selected as mobile phase. The bands developed were dense and compact with acceptable Rf values for

DDH (Rf=0.38). Peaks were symmetrical in nature and no tailing was observed when plates were scanned at 315 nm. Typical densitogram of Daclatasvir Dihydrochloride was shown in Figure.2
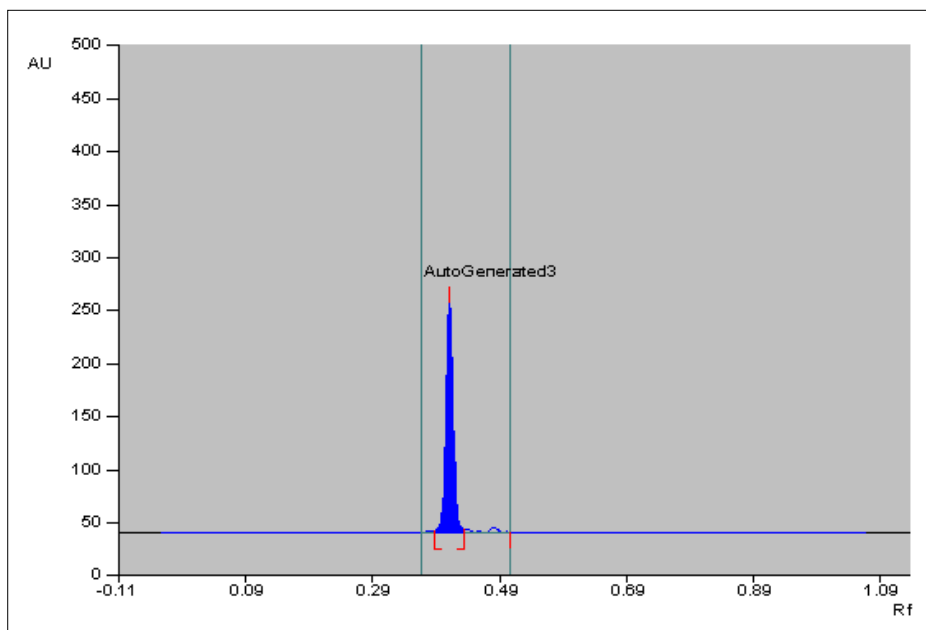
Figure 2 Densitogram of Daclatasvir Dihydrochloride

3.2. Linearity

The peak areas were found to have good linear relationship with the concentration. The linearity of detector response was determined in the concentration range of 500-300 μg /band for the drug. The coefficient of correlation for DDH on calibration curves was found to be 0.992. The calibration curve graph for DDH at 315 nm was shown in Figure.3

$$y = 964.19x + 830.15$$
$$R^2 = 0.9867$$
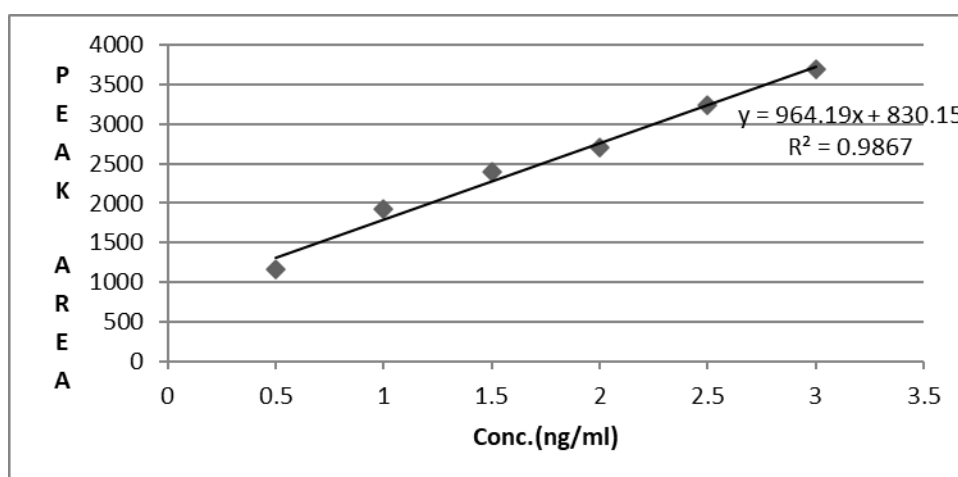
Figure 3 Calibration graph of Daclatasvir Dihydrochloride

3.3. Analysis of marketed formulation

Analysis of marketed tablet formulation containing Daclatasvir Dihydrochloride (60 mg) was performed and results were expressed as percentage amount of the label claim. The amount of DDH was estimated by comparing the peak area of sample with that of the standard bands. The DDH content was found to be close to 100 % and the results were summarized in Table 1. There was no interference of the Excipients was found during analysis of drug formulation. The low value of SD indicated the suitability of this method for routine analysis

Table 1 Results of analysis of marketed formulation

Injection Estimation	%
1	100.7
2	100.1
3	100.1
4	99.99
5	101.1
6	101.4
Mean	100.55
S.D	0.609
R.S.D	0.606

3.4. Recovery studies

To carry out the accuracy of proposed method, recovery studies were carried out by standard addition method and the results are expressed as percent recovery. The mean percentage recovery for each compound was calculated at each concentration level and reported with its standard deviation. The percentage recovery at three levels (80,100 and 120 %) for the drug was found to be satisfactory. Accuracy of developed method was shown in Table. 2.

Table 2 Results of recovery studies

Level of Recovery	% Recovery
80	99.98
	99.70
	97.85
100	99.99
	98.27
	102.75
120	99.83
	100.26
	100.66
Mean of recovery	99.60
S.D	0.50
R.S.D	0.78

3.5. Precision

Precision study was performed by carrying out six independent sample preparations of a single lot of formulation. Standard deviation and percentage relative standard deviation (% RSD) was found to be less than 2 % for intraday and inter day precision. Intraday and interday determination indicating satisfactory repeatability and reproducibility of the developed Method. Results for precision study were shown in Table.3

Table 3 Result of precision study

| Result of Interday Precision			
Conc(ug/ml)	% Label claim	S.D	% RSD
100	100.06	0.533	0.5
Result of Intraday Precision			
Conc(ug/ml)	% Label claim	S.D	%RSD
100	100.72	0.614	0.6

3.6 Robustness

The effect of change in optimized chromatographic conditions like change in mobile phase composition (± o.1 ml), chamber saturation time, (± 20 %), time from application to development (0, 15, 40, 1.2 hr), time from development to scanning (0, 10, 30, 1 hr). effect of this parameters on the Rf value of drugs was studied. The method was found to be robust because it was unaffected by small changes in method parameters with % RSD for Rf values under varied method parameters less than 2 %. The developed method is considered to be robust.

3.6. Forced degradation studies

The Forced degradation of DDH was tried under different stress conditions such as acid hydrolysis, alkaline hydrolysis, oxidation, thermal and exposure photo degradation. DDH was found to degrade in acid, alkaline stress conditions. The percent assay of active substance and the Rf values of degradation products are given in Table 4. Densitogram of acid, alkaline, oxidation, Neutral, thermal; and photo degradation treated samples were shown in Figure 5 to 10 respectively.

Table 4 Forced Degradation Studies

| Stress condition	Assay of active substances	Rf values of Degraded Products
Acid(0.1M HCL)	78.03	0.32,0.36,0.56
Base(0.1 M NaOH)	80.04	0.32,0.63
Oxide(3 % H2O2)	82.92	0.39,0.47,0.49
Neutral(Distilled Water)	99.80	-
Thermal	73.80	0.40,0.41
UV Degradation	83.23	0.89,0.90,0.91
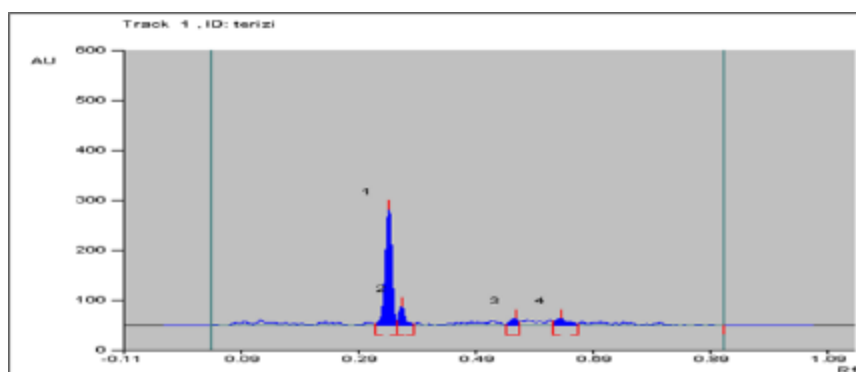
Figure 5 Densitogram of 0.1 N HCL treated sample
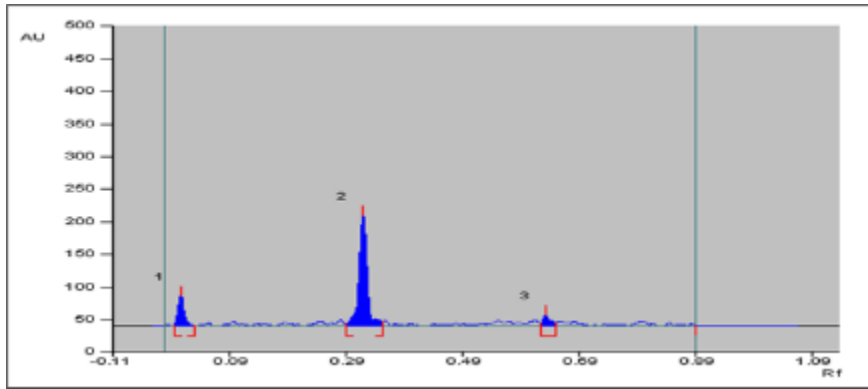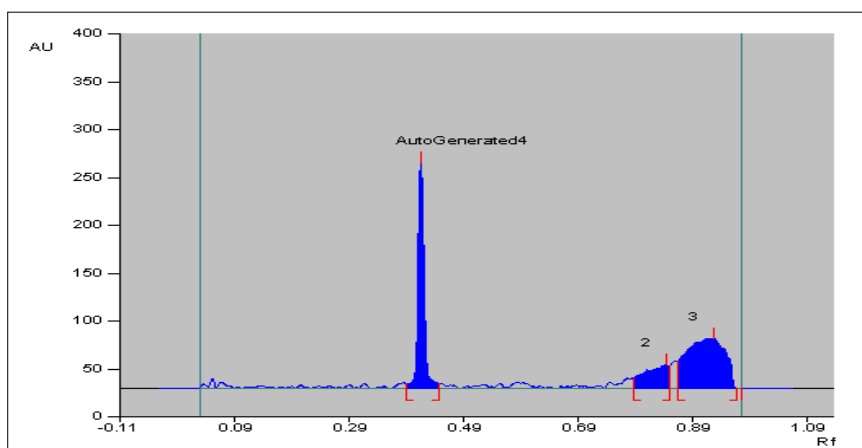
Figure 6 Densitogram of 0.1 N NaOH treated sample
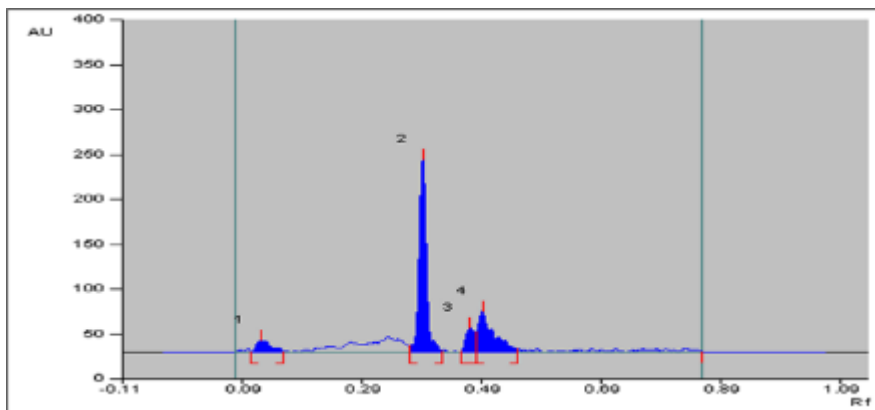
Figure 7 Densitogram of oxide (3% H2O2) treated sample
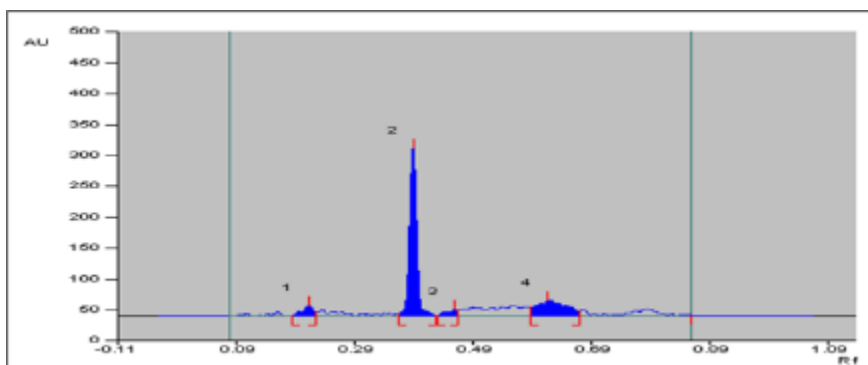
Figure 8 Densitogram of sample exposed to neutral hydrolysis
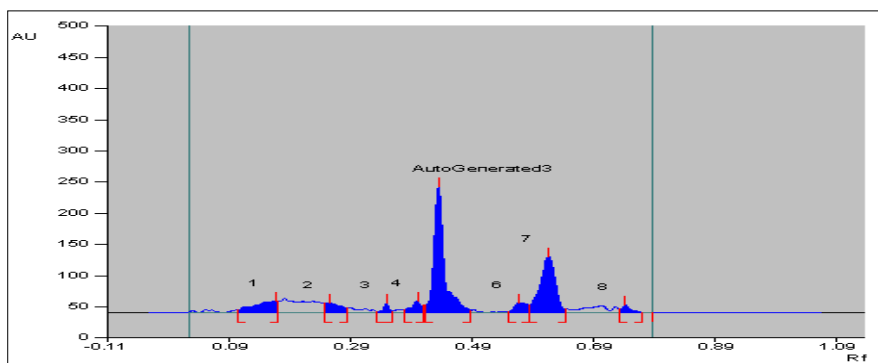
Figure 9 Densitogram of sample exposed to heat

Figure 10 Densitogram of sample exposed to heat

3.8 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ values for DDH were 0.171and 0.521 µg/band, respectively. The low LOD and LOQ values for Daclatasvir Dihydrochloride indicate the reasonably good sensitivity of the method to estimate the drug in marketed formulation .

4. Conclusion

The developed method describes a simple, sensitive and selective stability indicating HPTLC method for estimation of Daclatasvir Dihydrochloride in pharmaceutical Tablet dosage form. Based on the results obtained and statistical analysis, it is concluded that the method is suitable for estimation of these drug in marketed tablet formulation without any interference of the excipients present in formulation. Moreover, the method was validated as stability indicating assay for estimation of Daclatasvir Dihydrochloride in presence of its degradation products. Therefore, the method can be employed as a stability indicating one. As HPTLC method have several advantages like simultaneous processing of sample and standard, multiple sample handling, no prior treatment for solvents like filtration and degassing, low mobile phase consumption per sample etc., the developed HPTLC method represents a good alternative to the existing HPLC methods. Hence, the method can be applied for routine quality control of pharmaceutical formulation containing Daclatasvir Dihydrochloride. Identification of degradation product might be a future study of this work. The degradation pathway of drug can help in future to identify the impurities and for impurity profiling of Daclatasvir Dihydrochloride.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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