ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ESTIMATION OF TRILACICLIB DRUG IN BULK AND INJECTABLES USING RP-UPLC

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Abstract:

A simple, Accurate, precise method was developed for the estimation of the Trilaciclib in bulk and pharmaceutical dosage form. Chromatogram was run through X-bridge (100x 2.1mmx 3.5µm). Mobile phase containing 0.1% OPA: Acetonitrile taken in the ratio 72.2(%v/v) and 27.78 was pumped through column at a flow rate of 0.29 ml/min. Temperature was maintained at 27.5°C. Optimized wavelength selected was ACQUITY TUV ChA 219.0 nm. Retention time of Trilaciclib was found to be 1.757 min. %RSD of the Trilaciclib was found to be 0.2. %Recovery was obtained as 99.85% for Trilaciclib. Regression equation of Trilaciclib is y = 9698x + 2551.2 and correlation coefficient was found to be 0.999. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: Trilaciclib, Method development, ICH guidelines, validation, RP-UPLC
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1. INTRODUCTION

Most medications in multicomponent dosage forms may be analysed by the UPLC system thanks to its many benefits, including speed, specificity, consistency, accuracy, precision, and simplicity of automation. The UPLC method saves repeating extraction and isolation procedures. There are several modes of differentiation in UPLC. These are Size Exclusion Chromatography, Chromatography of Reversed Phase Ion Phase, Chromatography of Affinity, Normal Phase Mode, and Inverted Phase Mode.[1].

The effectiveness and safety of a medicine are significantly influenced by the quality of the drug. For customers to have access to safe and effective medicinal formulations, quality assurance and control of pharmaceutical and chemical formulations are crucial. Hence When determining whether a chemical is suitable for use in patients, analysis of both the pure drug material and its pharmaceutical dose forms is crucial. The calibre of the processes used to generate the data are what determines the calibre of the analytical data . To ensure that pharmaceuticals and their formulations are legally certified by regulatory bodies, it is crucial to establish tough and reliable analytical procedures. [2-3].

Based on the review of literature, the present work selected based on the significance of the cytotoxic injectable usage in the Pharmaceutical Market, the RP-UPLC was selected due to its sensitivity, selectivity, separation efficiency, speed, robustness, and compatibility of the compound analysis.

Trilaciclib is indicated to reduce the incidence of chemotherapy induced myelosuppression in patients prior to receiving platinum and etoposide-containing or topotecan-containing chemotherapy regimens for extensive-stage small cell lung cancer.it is chemically 12'-{[5-(4-methylpiperazin-1-yl)pyridin-2-yl]amino}-2',5',11',13'-tetraazaspiro[cyclohexane-1,3'-tricyclo[7.4.0.0^{2,7}]tridecane]-1'(9'),7',10',12'-tetraen-6'-one.[4].

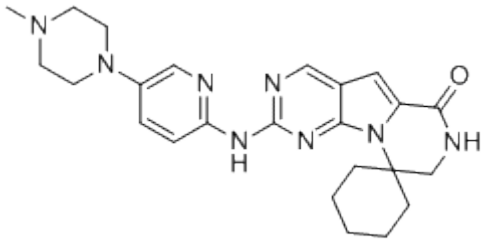


Figure1: Chemical strcuture of Trilaciclib

Experimental Work:

2. MATERIALS AND METHOD

Chemicals and reagents

Pure Trilaciclib was procured from Spectrum pharma lab (Hyderabad). Hydrochloric acid AR grade (HCL) and sodium hydroxide AR grade (NaOH) were obtained from Rankem, India. Hydrogen Peroxide (H_2O_2) was purchased from Qauligens. Acetic acid AR grade was purchased from Fisher scientific, India and S.D. Fine chem Ltd. Respectively. Potassium dihydrogen orthophosphate and orthophosphoric acid were obtained from S.D. Fine chem Ltd and Merck India Pvt Ltd. Respectively. UPLC grade Acetonitrile (ACN) and methanol (MeOH) were purchased from Fischer scientific.

UPLC grade water used throughout analysis was obtained from the Merck milli-Q water purification unit.[5].

Apparatus and Equipment

UPLC studies were carried out on WATERS UPLC 2965 SYSTEM with a Photo diode array detector (PDA) set at 219 nm for uv detection. columns, viz; Agilent C18 (150×4.6 mm, 5 µm), Discovery C18(150×4.6mm,5 µm), Zodiac (150×4.6mm,5 µm) ,BDS (150×4.6mm,5 µm), Phenomenex(150×4.6mm,5 µm) and X-Bridge (100 × 2.1 mm, 3.5 µm)column were utilized in the study. pH meter (Eutech instruments pH tutor, pH meter, India) was used to check the pH of all solutions. sonicator (ePEI ultrasonic generator), Analytical balance (Mettler Toledo), vortex meter (IKA Vortex), Hot air oven (Yorco scientific).[6]

Methodology:

Diluent: The Water and Acetonitrile in the ratio 50:50 was used as diluent in this method.

Preparation of buffer

0.01N Potassium dihydrogen ortho phosphate

Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Orthophosphoric acid solution[7].

0.1% Ortho phosphoric acid buffer:

1ML of Ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

Preparation of drug solution

Preparation of Standard stock solutions: Accurately weighed 60mg of Trilaciclib and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (1200µg/ml of Trilaciclib)

Preparation of Standard working solution (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (120µg/ml of Trilaciclib).

Preparation of Sample stock solutions: Five vials of single dose vial contain 10 ml (300 mg) Trilaciclib were collected and then mixed thoroughly. A sample of the blended sterile liquid of 1vial taken (equivalent to 300 mg) transferred into a 100 mL volumetric flask, 25mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered and The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined and Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-µm nylon filter. (3000µg/ml of Trilaciclib) 3000µg/ml of Trilaciclib.[8]

Preparation of Sample working solution: From the filtered solution 0.4 ml was pippeted out into a 10 ml volumetric flask and made upto 10ml with diluent. (120µg/ml of Trilaciclib)

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Trilaciclib (120ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Methodology for Validation Parameters:

Specificity: The concentration of the 120-ppm from the standard solution used for the specificity to Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these

drugs in this method. So, this method was said to be specific

Precision:

Preparation of Standard stock solutions: Accurately weighed 60mg of Trilaciclib and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (1200µg/ml of Trilaciclib)

Preparation of Standard working solution (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (120µg/ml of Trilaciclib).

Preparation of Sample working solution: From the filtered solution 0.4 ml of the sample stock solution was pippeted out into a 10 ml volumetric flask and made up to 10ml with diluent. (120µg/ml of Trilaciclib).

Linearity:

Preparation of Standard stock solutions: Accurately weighed 60mg of Trilaciclib and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (1200µg/ml of Trilaciclib)

25% Standard solution: 0.25ml each from two standard stock solutions was pipette out and made up to 10ml. (30µg/ml of Trilaciclib)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (60µg/ml of Trilaciclib)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (90µg/ml of Trilaciclib,)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (120µg/ml of Trilaciclib)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (150µg/ml of Trilaciclib)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml. (180µg/ml of Trilaciclib)

Accuracy:

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102

LOD sample Preparation: 0.25ml Standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.25ml Trilaciclib, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.9ml Trilaciclib of, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were

no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.1ml/min), Flow plus (0.3ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

Degradation studies:[10-14]

Oxidation:

To 1 ml of stock solution of Trilaciclib, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For UPLC study, the resultant solution was diluted to obtain 120µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution Trilaciclib, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c.The resultant solution was diluted to obtain 120µg/ml solution and 10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Trilaciclib, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 120µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 1h to study dry heat degradation .For UPLC study, the resultant solution was diluted to 120µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 200µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1hrs or 200 Watt hours/m^2 in photo stability chamber. For UPLC study, the resultant solution was diluted to obtain 120µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxingthedruginwaterfor1hrs at a temperature of 60º. For UPLC study, the resultant solution was diluted to 120µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

3. RESULTS AND DISCUSSION

UV Spectrum for Trilaciclib drug:

Trilaciclib sample is soluble in the Diluent (Water and Acetonitrile in the ratio 50:50) UV Spectrum solution was run at 200-400 nm.

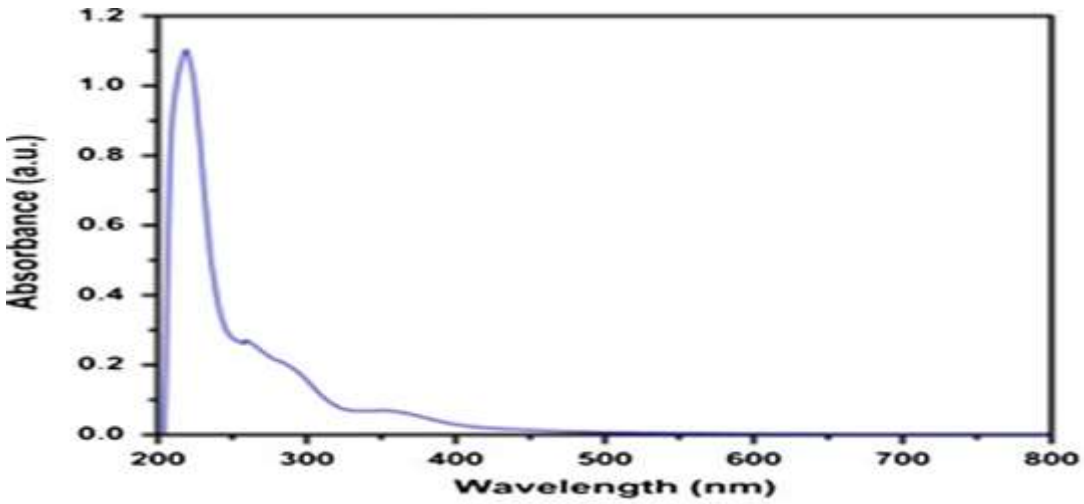


Figure2: UV Spectrum of Trilaciclib at 219 nm

Optimized Chromatography Conditions:
Column: X-bridge C18 Column (100x 2.1mmx 3.5µm).
Mobile Phase: 0.1% OPA: Acetonitrile (72.2: 27.78)
Flow rate: 0.29 ml/min
Column Temperature: 27.5 ^{o}C
Injection volume: 2 µl
PDA detector wave length: 219 nm

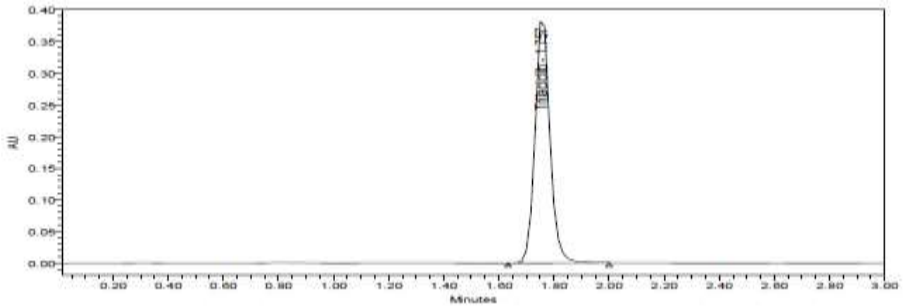


Figure 3: Chromatogram of final optimized method

System suitability for optimized method development

S.No	Drug	Retention time	No of theoretical plates	Tailing Factor
1		1.757	5985	1.18

2	Trilaciclib	1.752	5988	1.17
3		1.748	5965	1.15
4		1.755	5966	1.17
5		1.75	5984	1.17
6		1.75	5992	1.18

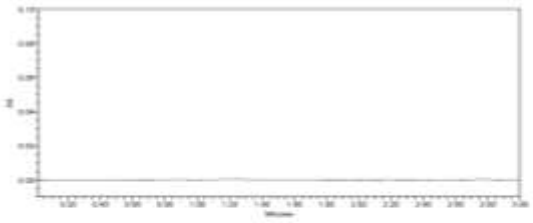
Table 1: System Suitability

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

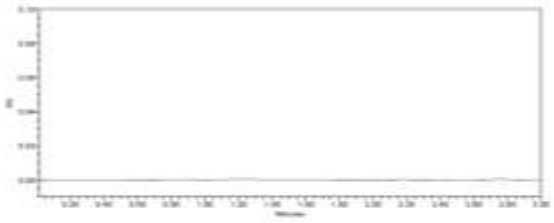
Method validation:

Specificity:

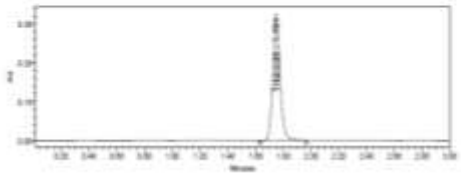
Retention time of Trilaciclib was 1.741min. We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.



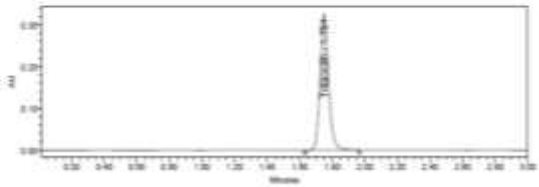
blank Chromatogram



Placebo Chromatogram



Standard Chromatogram



Sample Chromatogram

Precision:

System Precision: Six working sample solutions of 120 ppm are injected and the % Amount found was calculated and %RSD was found to be 0.2 and chromatogram was shown in fig

S.No	Peak Area
1	1177228
2	1179905
3	1175063
4	1179878
5	1178953
6	1180829

AVG	1178643
STDEV	2135.9
%RSD	0.2

Table 2 Repeatability data

Method precision: Six working sample solutions of 120 ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.3.

S.No	**Peak Area**
1	1179428
2	1180125
3	1175315
4	1177938
5	1177822
6	1171157
AVG	1176964
STDEV	3292.1
%RSD	0.3

Table 3 Method precision data

Intermediate precision: Six working sample solutions of 120ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.2 .

S.No	**Peak Area**
1	1171327
2	1168574
3	1175389
4	1172725
5	1170593
6	1172414
AVG	1171837
STDEV	2288.8
%RSD	0.2

Table4 Intermediate precision data

LINEARITY:

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 30ppm to 180ppm of Trilaciclib. Plot a graph to concentration versus peak area. Slope obtained was y = 9698x + 2551.2 and Correlation Co-efficient was found to be 0.999.

S.No	**Concentration (ppm)**	**Area**
1	0	0
2	30	284705

3	60	588295
4	90	888380
5	120	1164991
6	150	1464681
7	180	1736566

Table 5: Linearity data for Trilaciclib

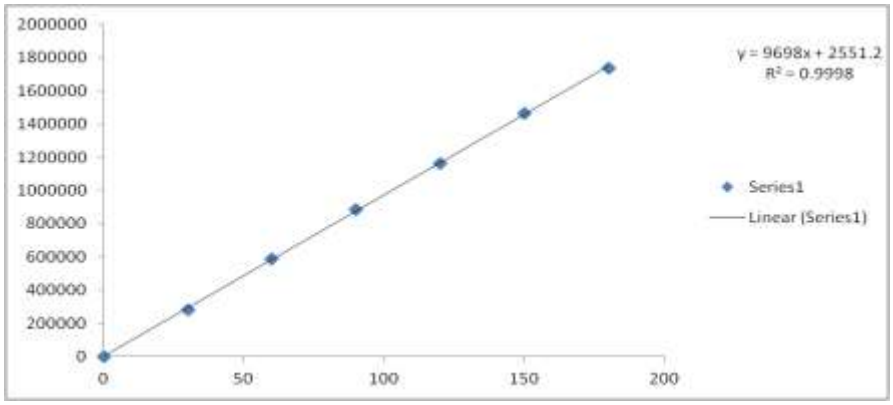


Figure6: Linearity curve for Trilaciclib

Accuracy: Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 99.85%.

% Level	**Amount Spiked (µg/mL)**	**Amount recovered(µg/mL)**	**% Recovery**	Mean %Recovery
50%	60	59.69	99.48	99.85%
	60	59.87	99.79	
	60	59.74	99.57	
100%	120	119.81	99.84	
	120	120.53	100.44	
	120	119.44	99.54	
150%	180	180.06	100.04	
	180	179.40	99.66	
	180	180.61	100.34	

Table 6 Accuracy data for Trilaciclib

Robustness: Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions is calculated.

Parameter	%RSD
Flow Minus	0.3
Flow Plus	0.3
Mobile phase Minus	0.4
Mobile phase Plus	0.5
Temperature minus	0.3
Temperature plus	0.4

Table 7 Robustness Data Trilaciclib

ASSAY OF MARKETED FORMULATION

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula.

Preparation of Sample stock solutions: The Marketed formulation COSELA brand injections used for the assay of the drug, and it contain Trilaciclib 300MG. Taken Five vials of single dose vial contain 10 ml (300 mg) Trilaciclib were collected and then mixed thoroughly. A sample of the blended sterile liquid of one vial taken (equivalent to 300 mg) transferred into a 100 mL volumetric flask, 25mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered and the resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined and Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-µm nylon filter. (3000µg/ml of Trilaciclib)

Preparation of Sample working solution: From the filtered solution 0.4 ml was pippeted out into a 10 ml volumetric flask and made upto 10ml with diluent. (120µg/ml of Trilaciclib)

Formula for calculation of % of drug content in any particular dosage form by UPLC method.

Assay content by UPLC:

Assay (%w/w) (on as is basis) = S_A / Std_A x T_D/ S_D x Potency of Drug / 100 x Avg wt of drug / LB x 100.

Where,

S_A - Area of the sample,

Std_A - Area of the Standard,

T_D- Test Dilution

D_D- Sample Dilution

Lb –Label claim of drug,

Wt - Weight of the Sample,

P - Potency or Assay of Drug .

Sample No	Standard Area	Sample Area	%Assay
1	1177228	1179428	99.67

2	1179905	1180125	99.73
3.	1175063	1175315	99.32
4.	1179878	1177938	99.54
5.	1178953	1177822	99.53
6.	1180829	1171157	98.97
AVG	1178643	1176964	99.46
STDEV	2135.9	3292.1	0.28
%RSD	0.2	0.3	0.3

Table 8 Assay of Standard and marketed formulation for Trilaciclib

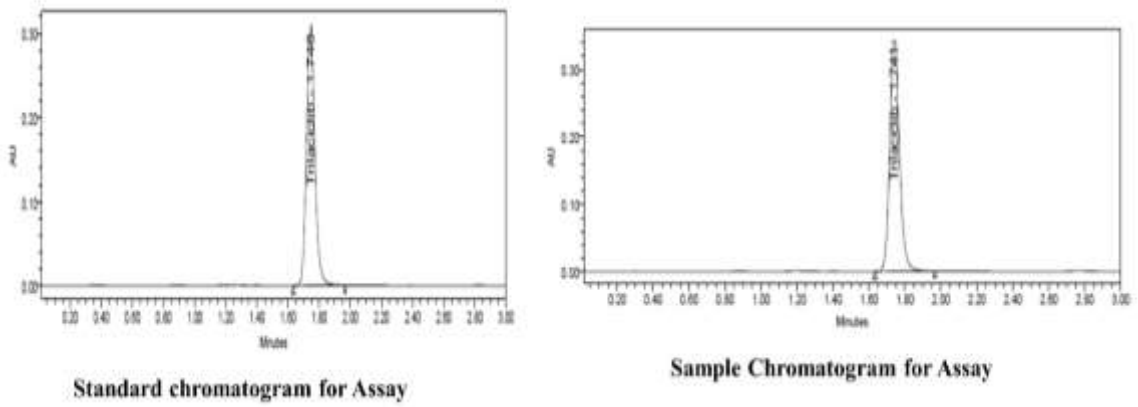


Figure 7 Assay chromatogram of the standard and sample of Trilaciclib

Stability studies:

Acid hydrolysis:

The drug substance was exposed to 2N HCl, kept for reflux in Radley apparatus at 70 0C temperature for 8 hrs, it was showing there is 7.76% of Trilaciclib degradation in acid hydrolysis. The blank solutions also subjected to stress study in the same fashion as the drug solution. The exposed stress sample and blank solutions were analyzed by UPLC system.

Base hydrolysis:

The drug substance was exposed to 2N NaOH, kept for reflux in Radley apparatus at 70 ^{0}C temperature for 8 hrs, it was showing 4.63% of Trilaciclib degradation in base hydrolysis with two degradation products. The blank solutions also subjected to stress study in the same fashion as the drug solution. The exposed stress sample and blank solutions were analyzed by UPLC system.

Neutral hydrolysis

The drug substance was exposed to water, kept for reflux in Radley apparatus at 70 ^{0}C temperature for 24 hrs; it was showing there was 0.88% degradation in neutral hydrolysis. The blank solutions also subjected to stress study in the same fashion as the drug solution. The exposed stress sample and blank solutions were analyzed by UPLC system.

Oxidative degradation

The drug was exposed to 20% H_2O_2, at room temperature for 24 hours. Samples were withdrawn at different time intervals and injected into the UPLC system and chromatogram was recorded 4.46% of Trilaciclib degradation in 20% H2O2 solution at the end of 24 hrs .The blank solutions also subjected to stress study in

the same fashion as the drug solution. The exposed stress sample and blank solutions were analyzed by UPLC system.

Thermal degradation

The drug sample was exposed to 70°C for 1 day in a hot air oven and samples were withdrawn at different time intervals from 1day. Samples were injected into the UPLC system and chromatogram was recorded. 2.72% of Trilaciclib degradation was found at the end of 3 days of exposure. Hence the drug can be regarded as thermostable at 70°C.

Photo degradation

The drug sample was exposed to direct sunlight for 24 hours. Samples were injected into the UPLC system and chromatogram was recorded. 1.78% of Trilaciclib degradation was found at the end of 24hrs of exposure. Hence the drug can be considered as photostable.

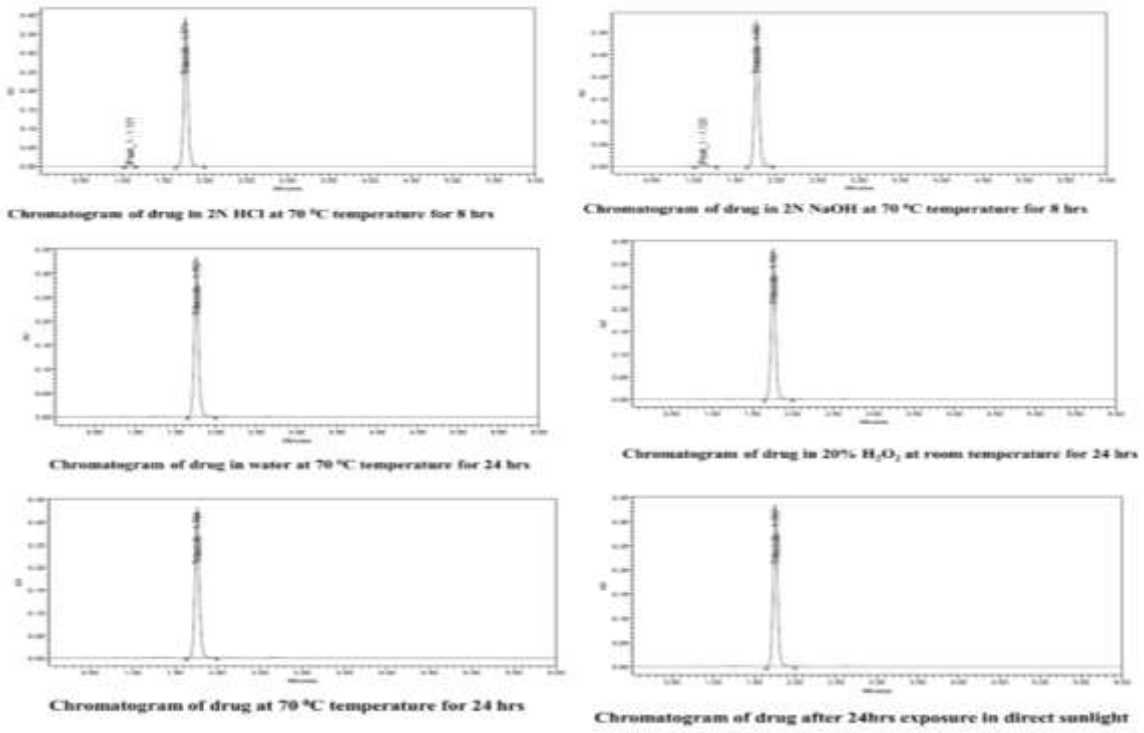


Figure 8 Stability studies of the Trilaciclib

Sr.No.	Condition of degradation study	% of drug degraded	Retention time of degradant
1.	2N HCl, 8 hrs	7.76	1.101
2.	2N NaOH, 8hrs	4.63	1.120
3.	Oxidative degradation, 24 hrs	4.46	1.185
4.	Thermal degradation, 1 days	2.72	-
5.	Photo degradation, 24 hrs	1.78	-

6.	Neutral hydrolysis, 24 hrs	0.88	-

Table 9:Summary of degradation studies of Trilaciclib

4. DISCUSSION

The RP-UPLC method for the analysis of Trilaciclib in bulk drug and pharmaceutical dosage forms. In order to affect proper elution of the component peaks, mixtures of Acetonitrile with Orthophosphoric Acid in different combinations were tested as mobile phase on a non-polar Acquity UPLC X-Bridge C18 (100 x 2.1 mm, 3.5 µm) column. A mixture of Ortho Phosphoric Acid (pH 4.2) and Acetonitrile (72.2:27.78 v/v) was proved to be the most suitable of the combination since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention time for Trilaciclib was at 1.757 ± 10% min respectively injected at a flow rate of 0.29 mL/min. The detection wave length was fixed at 219.0 nm as the peak areas were consistent and reproducible over a run time of 3.00 minutes. The injection volume was optimized at 2.0 µL. The method obeyed linearity in the range of 30-180 µg/mL for Trilaciclib respectively as observed from the linearity curves. The regression equations for Trilaciclib were found to be y = 9698x + 2551.2 (R2 = 0.999) respectively. The specificity of the method was established by studying the Trilaciclib peaks in the presence of excipients. The commonly present excipients did not pose any interference at the retention time of the drug as they were not identified. The repeatability and intermediate precision were studied by analyzing the sample solutions of Trilaciclib. The low coefficients of variation obtained in the intraday (0.3 %) and inter day precision (0.2 %) study are indicative of the precision of the method. High recovery values for Trilaciclib (99.85%) obtained from the analysis of combined dosage forms by the proposed method indicates the accuracy of the method. The deliberate changes in the method (flow rate and temperature) have not much affected the peak tailing, theoretical plates, resolution and percent assay. This indicates that the present method is robust (% RSD < 2). The limit of detection and limit of quantitation for Trilaciclib were found to be 0.08 µg/mL and 0.23 µg/mL respectively. The low values of LOD and LOQ as obtained by the proposed method indicate the sensitivity of the method. The developed UPLC method was applied for the analysis of Trilaciclib in marketed formulations. The marketed dosage form was found to contain an average of 99.46 ± 10 % w/v of Trilaciclib as stated on the label claim. The absence of additional peaks indicates non-interference of common excipients used in the Sample preparations. The proposed UPLC method was also applied for the forced degradation studies on Trilaciclib under a variety of conditions like acid and base hydrolysis, oxidation, and heat and photo stability. The drugs were found to be stable except in acidic, Basic, Peroxide stress conditions. The drug peaks in these degradations were found to be homogenous and no other peaks merged which could be confirmed from the peak purity index (1.0000) and similarity index values (> 0.99987). No major degradants were found in Neutral stress, photo stability and Thermal degradation studies. As the developed method could effectively separate Trilaciclib from the degradants, it can be employed as a stability indicating assay.

5. CONCLUSION

The developed UPLC method is specific, sensitive, precise, accurate and stability indicating. The method is linear over a

wide range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for quantification of Trilaciclib in bulk drugs and pharmaceutical dosage forms without any interference from degradants or excipients. This method can also be used for the regular quality control analysis of Trilaciclib, Results which were obtained from the validation of developed analytical method were within limit as per ICH guidelines.

Conflict of interest:

The authors declare no conflicts of interest.

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