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A VALIDATED STABILITY INDICATING METHOD DEVELOPMENT & VALIDATION FOR ESTIMATION OF TRILACICLIB IN PHARMACEUTICAL DOSAGE FORM BY RP-UPLC.

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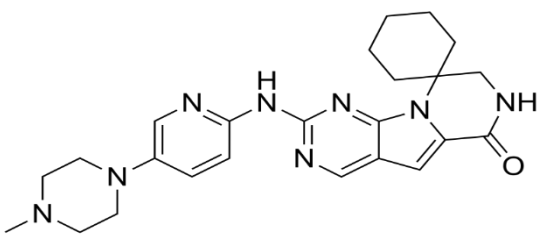
Abstract: An easy, sensitive, specific, and precise RP-UPLC method for the pharmaceutical dose estimation of Trilaciclib in injection form was developed. The chromatogram was run through an ACQUITY UPLC HSS T3 Column 1.8 µm, 2.1 mm X 150 mm. The mobile phase containing Ammonium Acetate: Methanol in the ratio 70:30 was pumped through the column at a 0.2 ml/min flow rate. The temperature was maintained at 46.0°C, and the optimized wavelength selected was 214.0 nm. The retention time of Trilaciclib was found to be 1.192 min. The %RSD of Trilaciclib was found to be 1.3, and the %RSD of repeatability precision of Trilaciclib was found to be 0.5. A recovery of 100.76% for Trilaciclib and an assay of 100.07% for Trilaciclib were obtained. The LOD and LOQ values obtained from the regression equation of Trilaciclib were 0.79 and 2.39, respectively. The regression equation of Trilaciclib is y = 58735x + 78569. Retention times were decreased, and the run time was lessened, making the developed method simple and economical, which can be adopted for regular quality control tests in industries.

Key Words: Trilaciclib, RP-UPLC,

INTRODUCTION:

Trilaciclib, also known as G1T28, is a promising CDK4 and CDK6 inhibitor specifically designed to address chemotherapy-induced myelosuppression in patients undergoing platinum and etoposide-containing or topotecan-containing regimens for extensive-stage small cell lung cancer. This novel drug received FDA approval on February 12, 2021, due to its ability to selectively inhibit CDK4 and CDK6, which are key regulators of cell cycle progression. Its chemical structure is characterized by its CAS Number (1374743-00-6), Molecular Weight (Average: 446.559; Monoisotopic: 446.254257618), Chemical Formula (C24H30N8O), and IUPAC Name (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). Pharmacokinetically, Trilaciclib demonstrates dose-dependent increases in maximum plasma concentration (Cmax) and area under the curve (AUC), indicating predictable absorption and distribution properties. It undergoes extensive metabolism and is primarily eliminated through feces (79.1%) and urine (14%) with a half-life of approximately 14 hours, supporting practical dosing intervals in clinical practice. In addition to its primary indication, Trilaciclib's mechanism of action suggests potential synergies with immune checkpoint inhibitors by enhancing T-cell activation and antigen presentation pathways. This dual action not only mitigates myelosuppression but also augments the antitumor immune response, promising enhanced therapeutic outcomes in cancer treatment.



STRUCTURE OF TRILACICLIB

Although generally well-tolerated, common adverse effects of Trilaciclib may include diarrhea, headache, and mild gastrointestinal disturbances. Overall, Trilaciclib represents a significant advancement in cancer supportive care, offering new avenues to improve treatment outcomes and quality of life for patients with extensive-stage small cell lung cancer.

Furthermore, there have been limited previous attempts to identify and quantify pure Trilaciclib. In light of this, a study aims to develop a more precise, accurate, and sensitive method for Trilaciclib compared to previous research. The study will also involve validating the method and conducting stability studies using RP-UPLC. This research is crucial for advancing the understanding and application of Trilaciclib in clinical settings.

MATERIAL AND METHOD:

Material:

The materials used for developing the RP-HPLC method for Trilaciclib include pure Trilaciclib drug (API), Trilaciclib Injection (**Cosela**), distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen ortho phosphate buffer, and ortho-phosphoric acid. These chemicals and solvents were sourced from Rankem.

Instruments:
- Electronics Balance-Denver
- pH meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS ACQUITY UPLC SYSTEM equipped with Binary pumps, TUV detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software

Method :

Diluent: Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 60:40.
Preparation of buffer:
0.01N Ammonium Aceatate Buffer: Accurately weighed 0.77gm of Ammonium Aceatate 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 PH adjusted to 5.4 with dil. Orthophosphoric acid solution.
Preparation of Standard stock solutions: Accurately weighed 30mg of Trilaciclib transferred 50ml and volumetric flasks, 3/4 Th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (600µg/ml of Trilaciclib).
Preparation of Standard working solutions (100% solution): 1ml of Trilaciclib was transferred from each stock solution into a 10ml volumetric flask and diluted with diluent to achieve a concentration of 60µg/ml of Trilaciclib.
Preparation of Sample stock solutions: one Injection vial were taken was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, Then, the flask was filled to volume with diluent and filtered using HPLC filters to obtain a solution containing 600 µg/ml of Trilaciclib.
Preparation of Sample working solutions (100% solution): 0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (60µg/ml of Trilaciclib)

Validation :

System suitability parameters:
The system suitability parameters were determined by preparing standard solution of Trilaciclib (60ppm) and the solution 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%.
Specificity: To ensure method specificity, interference was evaluated in both blank and placebo samples. No interfering peaks were detected at the retention times corresponding to Trilaciclib in this optimized method, confirming its specificity.
Preparation of Sample stock solutions: one Injection vial were taken was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, Then, the flask was filled to volume with diluent and filtered using HPLC filters to obtain a solution containing 600 µg/ml of Trilaciclib.
Preparation of Sample working solutions (100% solution): 0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (60µg/ml of Trilaciclib)
Precision
The precision were determined by preparing sample solution of Trilaciclib (60ppm) and the solution were injected six times, The % RSD for the area of six standard injections results should not be more than 2%.
Linearity:
Preparation of Standard stock solutions: Accurately weighed 30mg of Trilaciclib transferred 50ml and volumetric flasks, 3/4 Th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (600µg/ml of Trilaciclib).
25% Standard solution: 0.25ml each from standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Trilaciclib)
50% Standard solution: 0.5ml each from standard stock solutions was pipetted out and made up to 10ml. (30µg/ml of Trilaciclib)
75% Standard solution: 0.75ml each from standard stock solutions was pipetted out and made up to 10ml. (45µg/ml of Trilaciclib)
100% Standard solution: 1.0ml each from standard stock solutions was pipetted out and made up to 10ml. (60µg/ml of Trilaciclib)
125% Standard solution: 1.25ml each from standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Trilaciclib)
150% Standard solution: 1.5ml each from standard stock solutions was pipettede out and made up to 10ml. (90µg/ml of Trilaciclib)

Accuracy:

Preparation of Sample stock solutions: The contents of one injection vial were transferred into a 100 ml volumetric flask. 50 ml of diluent was added, and the mixture was sonicated for 25 minutes. The flask was then filled up to volume with diluent and filtered using HPLC filters to obtain a solution containing 600 µg/ml of Trilaciclib.

Preparation of Standard working solutions (100% solution): 1ml of Trilaciclib from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (60µg/ml of Trilaciclib).

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

Robustness: Small intentional variations in method parameters such as flow rate, mobile phase ratio, and temperature were introduced, but no significant changes in results were observed, all of which fell within the acceptable ranges as per ICH guidelines. Robustness conditions including variations such as Flow rate minus (0.1 ml/min), Flow rate plus (0.3 ml/min), mobile phase composition minus, mobile phase composition plus, temperature minus (41.0°C), and temperature plus (51.0°C) were maintained. Samples were injected in triplicate, and system suitability parameters remained largely unaffected, with %RSD within acceptable limits.

LOD Sample Preparation: 0.25 ml from each of two standard stock solutions was pipetted into separate 10 ml volumetric flasks and diluted with diluent. From these solutions, 0.25 ml each of Trilaciclib solutions was transferred to 10 ml volumetric flasks and made up to volume with the same diluent.

LOQ Sample Preparation: 0.25 ml from each of two standard stock solutions was pipetted into separate 10 ml volumetric flasks and diluted with diluent. From these solutions, 0.9 ml each of Trilaciclib solutions was transferred to 10 ml volumetric flasks and made up to volume with the same diluent.

Stability studies:

Oxidation:

To 1 ml of stock solution of Trilaciclib, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solution was kept for 30 min at 60^0c. For UPLC study, there resultant solution was diluted to obtain 60µg/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 60µg/ml solution and 10µlsolution were injected in to the system and the chromate grams 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 60µg/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 subjected to dry heat degradation by placing it in an oven at 105°C for 6 hours. After exposure, the resultant solution was diluted to a concentration of 60 µg/ml and 10 µl of this solution were injected into the UPLC system. Chromatograms were recorded to evaluate the stability of the sample under these conditions.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 600µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For UPLC study, the resultant solution was diluted to obtain 60µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

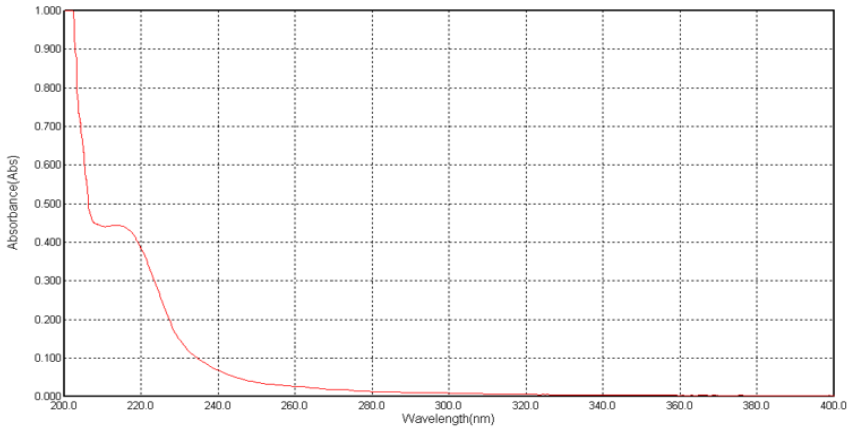
Neutral Degradation Studies:

The drug was subjected to stability testing under neutral conditions by refluxing it in water for 6 hours at a temperature of 60°C. After this exposure, the resulting solution was diluted to a concentration of 60 µg/ml. Subsequently, 10 µl of this solution were injected into the UPLC system to analyze and assess the sample's stability using chromatographic techniques.

RESULT AND DISCUSSION :

UV Spectrum For Trilaciclib:

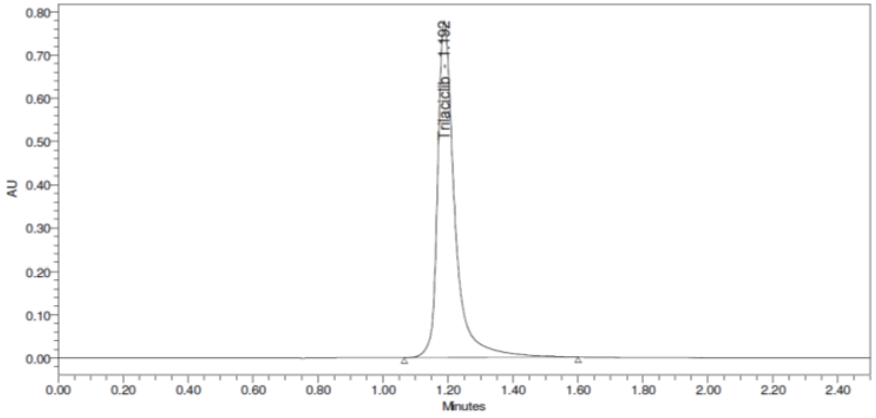
Trilaciclib sample is soluble in the Diluent (Methenol and Water in the ratio 60:40) UV Spectrum solution was run at 200-400 nm. After scanning from 400 to 200nm in UV-VIS spectrophotometer, Trilaciclib showed absorption maxima at 214nm.



UV SPECTRA FOR TRILACICLIB (214nm)

Optimized Chromatographic conditions:

Mobile phase : Methanol: 0.01N AmmoniumAceatate (30:70 v/v)
Flow rate : 0.2 ml/min
Column : ACQUITY UPLC HSS Column, 1.8 µm, 2.1 mm X 150 mm
Detector wave length : 214nm
Column temperature : 46.0°C
Injection volume : 5.0µL
Run time : 2.5 min
Results :Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.



Optimized Chromatogram

Observation: Trilaciclib were eluted at 1.192 min with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability:

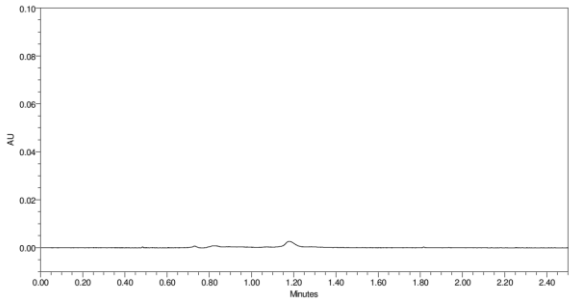
System suitability parameters for Trilaciclib

S no	Trilaciclib		
Inj	RT (min)	USP Plate Count	Tailing
1	1.181	3234	1.61
2	1.182	3276	1.60
3	1.184	3256	1.61
4	1.184	3256	1.60
5	1.186	3254	1.61
6	1.187	3265	1.60

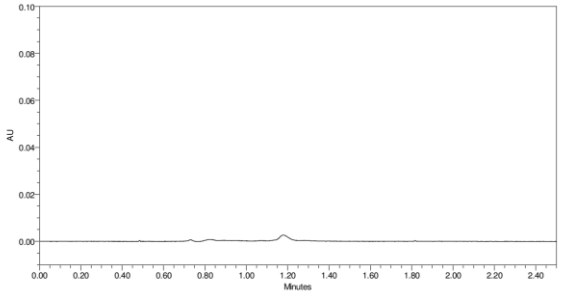
Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

VALIDATION:

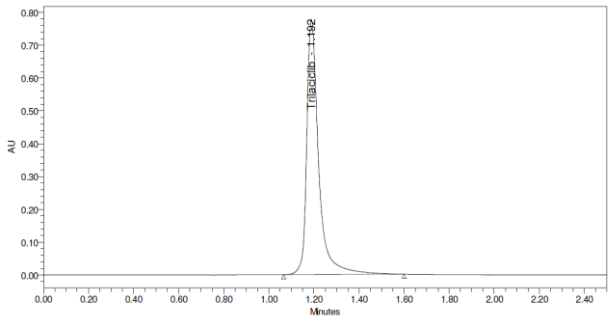
Specificity:



Chromatogram of blank



Chromatogram of Placebo



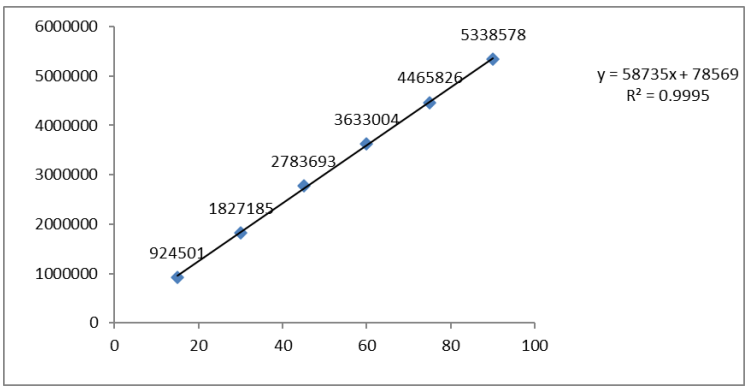
Optimized Chromatogram

Discussion: Retention time of Trilaciclib was 1.192 min. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity:

Linearity table for Trilaciclib.

Trilaciclib	
Conc (µg/mL)	**Peak area**
0	0
15	924501
30	1827185
45	2783693
60	3633004
75	4465826
90	5338578



Calibration curve of Trilaciclib

Discussion: Six linear concentrations of Trilaciclib (15-90µg/ml) were injected in a Duplicate manner. Average areas were mentioned above and linearity equations obtained for Trilaciclib was y = 58735x + 78569. Correlation coefficient obtained was 0.999 for the drug.

System Precision:

System precision table of Trilaciclib

S. No	Area of Trilaciclib
1.	2413367
2.	2472139
3.	2488910
4.	2415511
5.	2460335
6.	2467573
Mean	2452973
S.D	31299.2
%RSD	1.3

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated % RSD obtained as 1.3% for Trilaciclib. As the limit of Precision was less than "2" the system precision was passed in this method.

Repeatability:

Repeatability table of Trilaciclib

S. No	Area of Trilaciclib
1.	2464351
2.	2440587
3.	2450668
4.	2469069
5.	2476286
6.	2456332
Mean	2459549
S.D	12981.5
%RSD	0.5

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated and obtained as 0.5% for Trilaciclib. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day_ Day Precision):

Inter day precision: Inter day precision was performed with 24 hrs time lag.

Intermediate precision table of Trilaciclib

S. No	Area of Trilaciclib
1.	2486807
2.	2411176
3.	2448682

4.	2430573
5.	2435702
6.	2449418
Mean	2443726
S.D	25337.0
%RSD	1.0

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated and obtained as 1.0% for Trilaciclib. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy:

Accuracy table of Trilaciclib

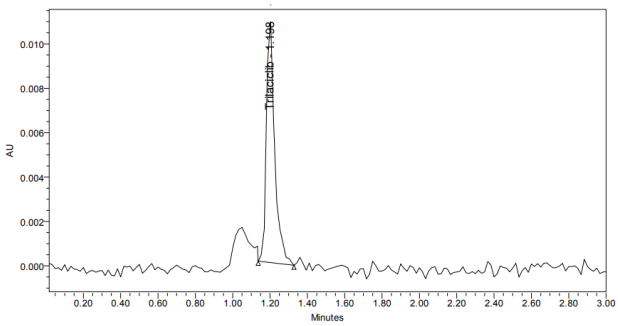
% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	30	30.26	100.88	100.76%
	30	30.21	100.71	
	30	29.98	99.94	
100%	60	61.01	101.68	
	60	60.09	100.14	
	60	59.99	99.98	
150%	90	91.31	101.46	
	90	90.44	100.49	
	90	91.38	101.53	

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 100.76% for Trilaciclib.

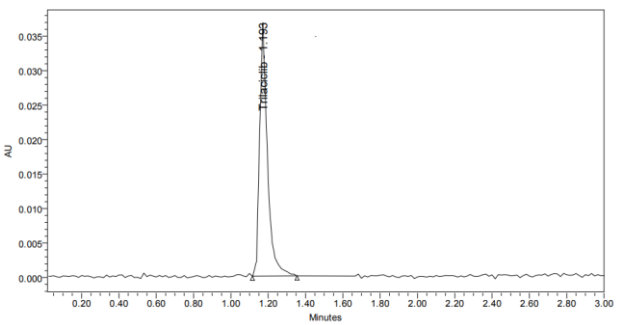
Sensitivity:

Sensitivity table of Trilaciclib

Molecule	LOD	LOQ
Trilaciclib	0.79	2.39



LOD Chromatogram



LOQ Chromatogram

	Peak Name	RT	Area	s/n	USP Plate Count	USP Tailing
1	Trilaciclib	1.198	774	3.9	6014.1	1.3

	Peak Name	RT	Area	s/n	USP Plate Count	USP Tailing
1	Trilaciclib	1.193	1936	29.3	6567.5	1.6

Observation for LOD: S/N (signal & noise) is found to be 3.9 for LOD of Trilaciclib which according to acceptance criteria is S/N > 2 or 3. Therefore Passed

Observation for LOQ: S/N (signal & noise) is found to be 3.9 for LOQ of Trilaciclib which according to acceptance criteria is S/N > 10 . Therefore Passed.

LOD: Limit of detection for Trilaciclib was calculated & was found to be 0.79 which in consideration of the Acceptance Criteria should be less more than 2 or 3. Thus Obtained limit of detection is passed in this test.

LOQ: Limit of Quantification for Trilaciclib was calculated & was found to be 2.39 which in consideration of the Acceptance Criteria should be less more than 10. Thus obtained the limit of Quantification is passed in this test.

Robustness:

Robustness data for Trilaciclib

S.no	Robustness Condition	%RSD of Trilaciclib
1	Flow rate (-) 0.1ml/min	0.8
2	Flow rate (+) 0.3ml/min	0.5
3	Mobile phase (-) 65B:35A	0.8
4	Mobile phase (+) 70B:30A	0.5
5	Temperature (-) 41°C	1.5
6	Temperature (+) 50°C	0.9

Discussion: Robustness conditions like Flow minus (0.1ml/min), Flow plus (0.3ml/min), mobile phase minus (65:35A), mobile phase plus (70B:30A), temperature minus (41°C) and temperature plus(50°C) was maintained and samples were injected in Triplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

ASSAY OF MARKETED DRUG: bearing the label claim Trilaciclib 300mg.Assay was performed with the above formulation. Average % Assay for Trilaciclib obtained was 100.07%.

Preparation of Sample stock solutions: one Injection vial were taken was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (600 µg/ml of Trilaciclib).

Preparation of Sample working solutions (100% solution): 0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (60µg/ml of Trilaciclib)

The Assay was determined by preparing sample solution of Trilaciclib (60ppm) and the solution were injected six times, The % RSD for the area of six standard injections results through assay formula calculated it should not be more than 2%.

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{50} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{0.2} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of Trilaciclib in test solution
AS	Mean peak area of Trilaciclib in standard solution
WS	Weight of Trilaciclib working standard taken in mg
P	Assay of Trilaciclib working standard in % on dried basis
L.C	Label Claim
FV	Filled volume(1ml of a vail)

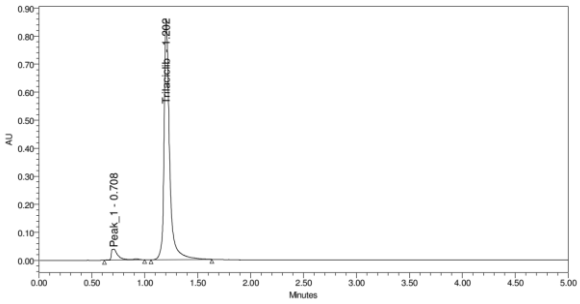
Assay Data of Trilaciclib

S.no	Standard Area	Sample area	% Assay
1	2413367	2464351	100.26
2	2472139	2440587	99.30
3	2488910	2450668	99.71
4	2415511	2469069	100.45
5	2460335	2476286	100.75
6	2467573	2456332	99.94
Avg	2452973	2459549	100.07
Std dev	31299.2	12981.5	0.5282
%RSD	1.3	0.5	0.5

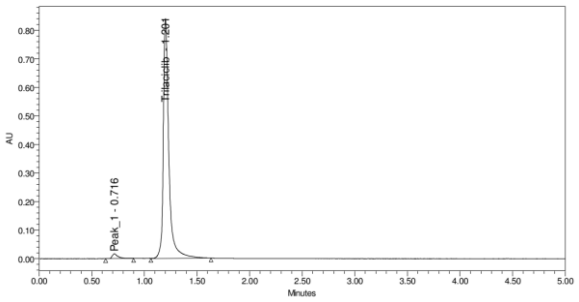
STABILITY STUDIES :

Degradation Data of Trilaciclib

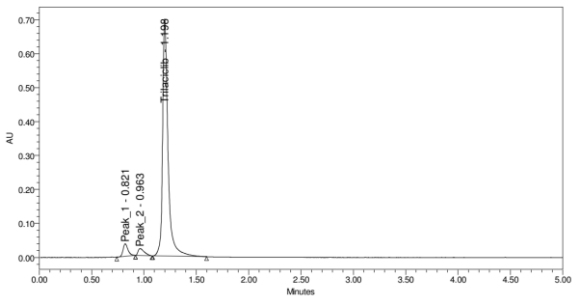
S.NO	Degradation Condition	% Drug UnDegraded	% Drug Degraded
1	Acid	93.31	6.69
2	Alkali	94.76	5.24
3	Oxidation	92.14	7.86
4	Thermal	97.51	2.49
5	UV	97.91	2.09
6	Water	99.22	0.78



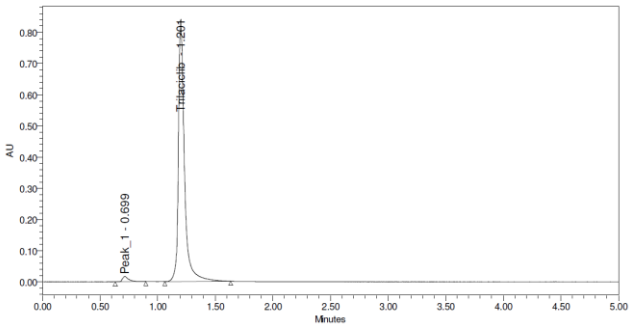
Acid chromatogram of Trilaciclib



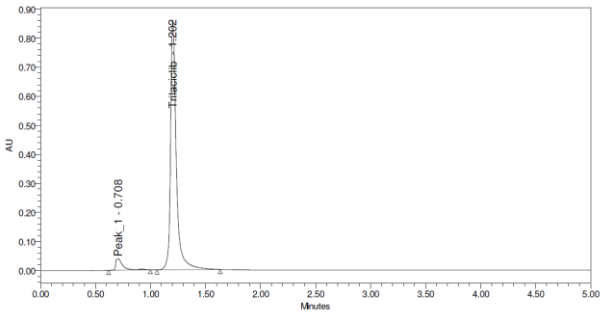
Base chromatogram of Trilaciclib



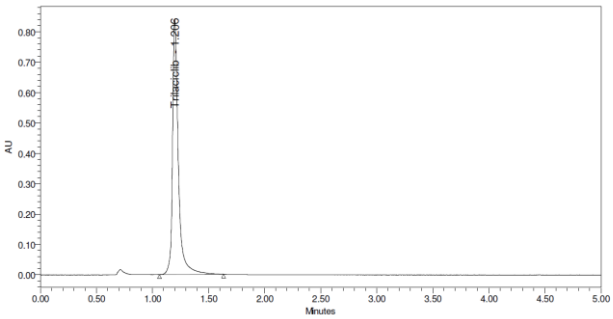
Peroxide chromatogram of Trilaciclib



Thermal chromatogram of Trilaciclib



UV chromatogram of Trilaciclib



Water chromatogram of Trilaciclib

Discussion: Regarding the pH adjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time.

Summary Table with Acceptance criteria

Parameters		Trilaciclib	LIMIT
Linearity Range(µg/ml)		15-90µg/ml	R< 1
Regression coefficient		0.999	
Slope(m)		58735	
Intercept(c)		78569	
Regression equation (Y=mx+c)		y = 58735x + 78569	
Assay (% mean assay)		100.07%	90-110%
Specificity		Specific	No interference of any peak
System precision %RSD		1.3	NMT 2.0%
Method precision %RSD		0.5	NMT 2.0%
Accuracy% recovery		100.76%	98-102%
LOD		0.79	NMT 3
LOQ		2.39	NMT 10
Robustness	**FM**	0.8	%RSD NMT 2.0
	FP	0.5	
	MM	0.8	
	MP	0.5	
	TM	1.5	
	TP	0.9	

CONCLUSION:

An Easy, sensitive, specific, and precise RP-UPLC method for the pharmaceutical dose estimation of Trilaciclib in Injection form. Retention time of Trilaciclib was found to be 0.941 min. %RSD of the Trilaciclib were and found to be 1.3. %RSD of Repeatabily precision of Trilaciclib was found to be 0.5. %Recovery was obtained as 100.76% for Trilaciclib. %Assay was obtained as 100.07% for Trilaciclib. LOD, LOQ values obtained from regression equation of Trilaciclib were 0.79, 2.39. Regression equation of Trilaciclib is y = 58735x + 78569. 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.

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