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Research Article

METHOD DEVELOPMENT AND VALIDATION OF TRILACICLIB BY RP- HPLC METHOD

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ABSTRACT
Employing the RP-high- approach, an uncomplicated, clear, and reliable way of figuring out Trilaciclib was discovered. the circumstances applied are outlined below: Eluent used is 0.1% TFA and MeOh (55:45), Solid Adsorbent used is SunFire C18 Column, 5 µm, 4.6 mm X 150 cm], an ideal technique was used to finalize the parameters. The ambient tem of the S.p was set to 25 °C, as well as the threshold spectrum was 212 nm. a consistency investigates encompassing 7.5µg to 45µg took place and the R2 value was found to be 0.999. 2.042 records proved to be an Elutant rate. % variance was found to be 0.5%. The Repeatability exactitude RSD was calculated to be 0.6% .99.95% repossession was accomplished. % 99.47% assay was gathered. The equation for regression yielded sensitivity values of 0.03, 0.09. The equation for R2 is y = 210008x + 42435. The strategy that originated cost-effective, so it's appropriate for conventional inspections in industries. Both the rate of retention and the amount of time running were diminished.
Key Words: Trilaciclib, Method development, Validation, RP-HPLC.

INTRODUCTION[1-10]

Trilaciclib is a first-in-class cyclin-dependent kinase 4/6 (CDK4/6) inhibitor developed to protect hematopoietic stem and progenitor cells (HSPCs) from chemotherapy-induced myelosuppression. Myelosuppression, a common side effect of chemotherapy, can lead to severe complications such as neutropenia, anemia, and thrombocytopenia, limiting the dose and duration of chemotherapy and adversely affecting patient outcomes. Trilaciclib is unique among CDK4/6 inhibitors because, rather than targeting cancer cells directly, it is designed to shield healthy bone marrow cells during chemotherapy by transiently inhibiting the cell cycle in these cells.

Trilaciclib was granted FDA approval in February 2021 for use in patients with extensive-stage small cell lung cancer (ES-SCLC) undergoing chemotherapy with a platinum/etoposide regimen or topotecan. This approval was based on its ability to reduce the incidence and severity of chemotherapy-induced myelosuppression, thus improving patient tolerance to chemotherapy and reducing the need for supportive care interventions such as granulocyte colony-stimulating factors (G-CSFs), red blood cell transfusions, and erythropoiesis-stimulating agents (ESAs).

Trilaciclib works by inhibiting CDK4 and CDK6, enzymes that play a critical role in regulating the transition from the G1 phase to the S phase of the cell cycle. CDK4/6 inhibitors block this progression in HSPCs, temporarily arresting them in the G1 phase, making them less susceptible to the cytotoxic effects of chemotherapy. This targeted cell-cycle arrest spares the bone marrow from damage while allowing chemotherapy to effectively target rapidly dividing cancer cells, particularly in cancers such as SCLC, where proliferation is not as dependent on CDK4/6 activity.

The efficacy of trilaciclib in protecting against myelosuppression was demonstrated in three randomized, double-blind, placebo-controlled Phase II clinical trials in patients with ES-SCLC. Across these studies,

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trilaciclib significantly reduced the occurrence of severe neutropenia, the need for G-CSF support, and the number of chemotherapy dose reductions compared to patients receiving chemotherapy alone.

For example, in one of the trials, trilaciclib reduced the occurrence of grade 4 neutropenia (the most severe form) by nearly 50%, from 60.2% in the placebo group to 31.5% in the trilaciclib group. Additionally, patients receiving trilaciclib required fewer interventions to manage myelosuppression, leading to a better overall quality of life during treatment.

Trilaciclib has a generally favorable safety profile, with adverse effects mostly related to its pharmacological action of transiently inhibiting the bone marrow cell cycle. The most common side effects include fatigue, hypocalcemia, and headaches, which are typically mild to moderate in severity. Importantly, trilaciclib does not appear to interfere with the efficacy of chemotherapy or other concomitant cancer treatments, making it an attractive option for managing chemotherapy-induced myelosuppression.

While trilaciclib is currently approved for use in ES-SCLC, research is ongoing to assess its protective effects in other tumor types, such as triple-negative breast cancer (TNBC) and colorectal cancer (CRC), where myelosuppression is a common side effect of chemotherapy. Additionally, studies are investigating the potential of trilaciclib to enhance immune responses when used in combination with immune checkpoint inhibitors. Early data suggest that by protecting immune cells from chemotherapy-induced damage, trilaciclib may improve anti-tumor immunity and boost the efficacy of immunotherapy in certain cancers.

Trilaciclib represents a novel approach to managing chemotherapy-induced myelosuppression by targeting and protecting healthy bone marrow cells without compromising the effectiveness of chemotherapy. Its approval for ES-SCLC marks an important step forward in supportive cancer care, and ongoing studies will determine its broader potential across different cancer types and therapeutic regimens. By reducing the hematologic toxicity of chemotherapy, trilaciclib has the potential to improve patient outcomes and enhance the tolerability of cancer treatments.

ANALYTICAL BACKGROUND[11]

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 known as 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.

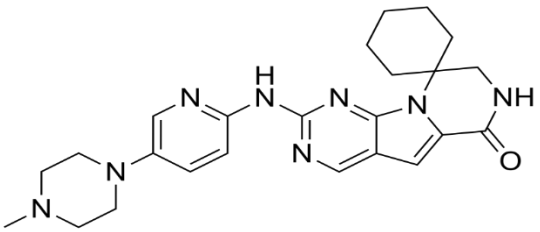

Figure 1 structure of Trilaciclib

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Trilaciclib, In the review of literature, more economical methods were observed[13-17], hence a simple, cost-effective stability-indicating simultaneous estimation of Trilaciclib by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

MATERIALS:

Trilaciclib pure drug (API), Trilaciclib formulation (Cosela), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

INSTRUMENTATION

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Table 1: Chromatographic Conditions:

Mobile phase	Methanol : 0.1% TFA (55:45 v/v)
Flow rate	0.9 ml/min
Column	Sunfire C18 (4.6 x 150mm, 5µm)
wave length	212 nm
Column temperature	30°C
Injection volume	10µL
Run time	10.0 min
Buffer	0.1% TFA

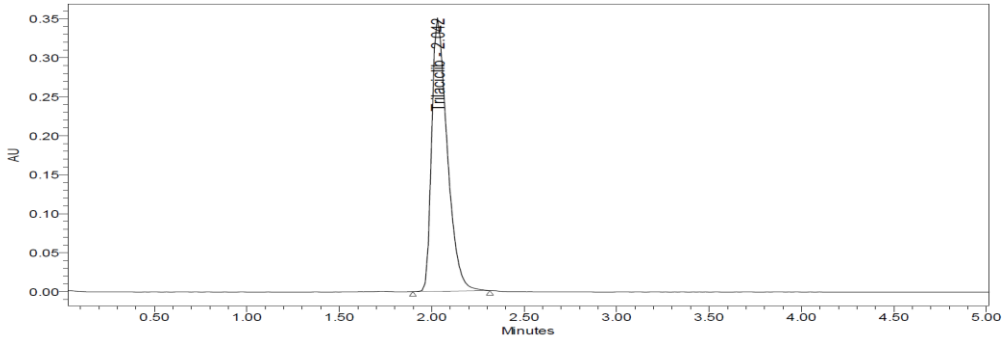

Figure 2: Optimized Chromatogram

Methods:

Preparation of Standard stock solutions: Accurately weighed 15mg 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 (300µg/ml of Trilaciclib).

Preparation of Standard working solutions (100% solution): 1ml of Trilaciclib from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (30µg/ml of Trilaciclib).

Preparation of Sample stock solutions: one Injection vial was taken was transferred into a 100 ml volumetric flask, 70ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (3000 µg/ml of Trilaciclib).

Preparation of Sample working solutions (100% solution): 0.1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (30µg/ml of Trilaciclib)

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Trilaciclib (30 ppm) 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 (Selectivity): 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. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 2

Table: 2 System suitability parameters for Trilaciclib

S no	Trilaciclib		
Inj	**RT(min)**	**USP Plate Count**	**Tailing**
1	2.032	8586	1.53
2	2.034	8658	1.54
3	2.034	8696	1.53
4	2.035	8648	1.56
5	2.035	8685	1.52
6	2.036	8654	1.54

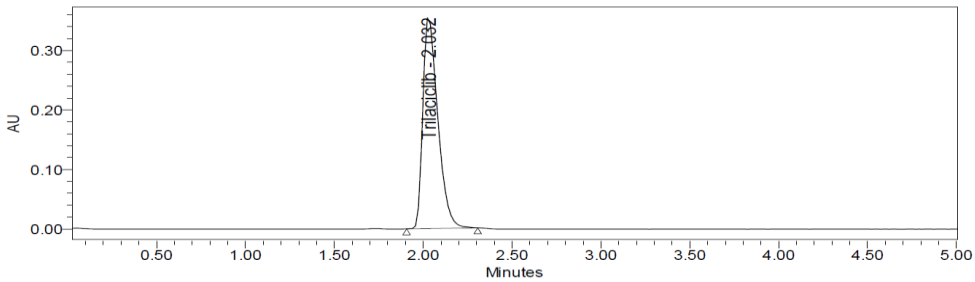

Figure 3: System Suitability Chromatogram of Trilaciclib

Table 3: Specificity Data

Peak name	Rt	Area	USP plate count	Tailing
Trilaciclib	2.042	6394636	8647	1.54

Specificity:

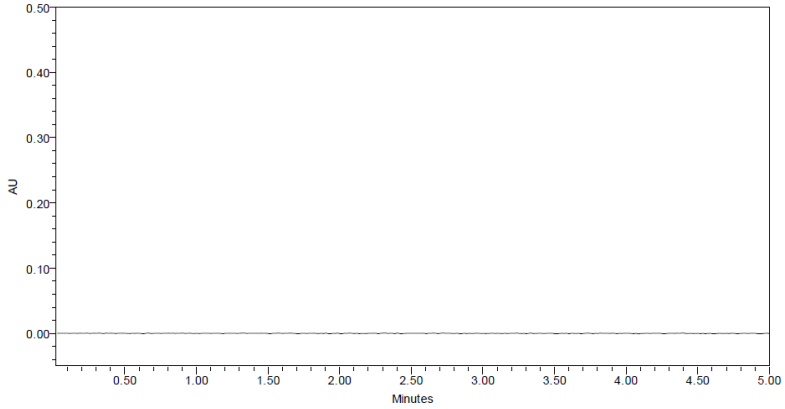

Figure 4 Chromatogram of blank.

The forced degradation conditions are mentioned in Table 4 and the results are mentioned in Table 5

Table 4: Forced degradation conditions for Trilaciclib

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxidation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

From the results, degradation peaks were observed when the samples were exposed to acid. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 5: Degradation profile results

Degradation Condition	% Drug Un Degraded	% Drug Degraded
Acid	92.66	6.81
Base	93.78	5.69
Oxidation	92.66	6.81
Thermal	98.45	1.02
Photolytic	98.16	1.31
Hydrolytic	99.10	0.37

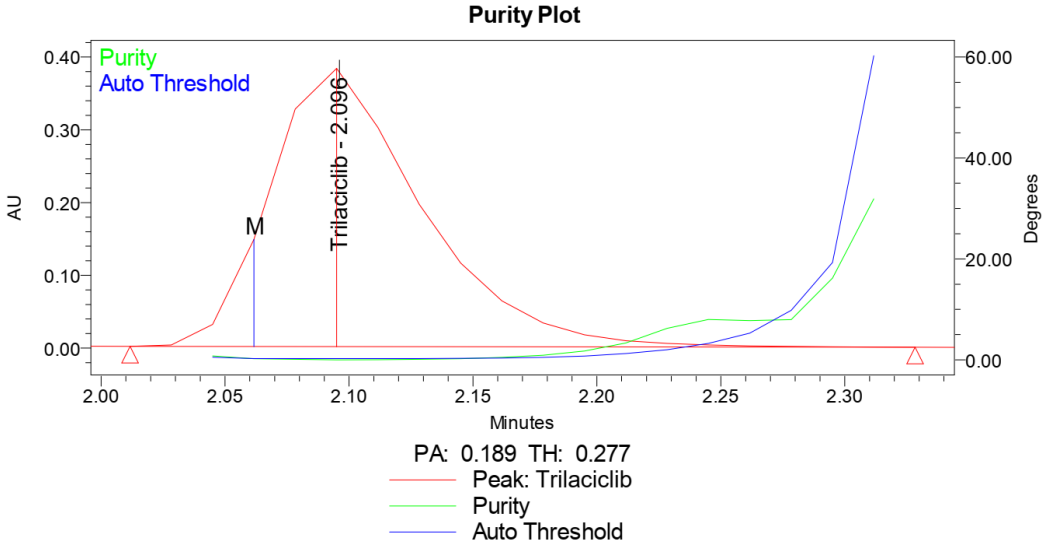

Figure 5: Purity Plot of Acid

Limit of detection (LOD) The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated.

Limit of quantitation (LOQ): The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy of the method.

The LOD values obtained for Trilaciclib are listed in Table 6.

Table 6: Summary of limit of detection

Sample	Conc (µg/ml)
LOD	0.03
LOQ	0.09

Linearity: The linearity of the method was demonstrated for Trilaciclib by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 7). The correlation coefficient for Trilaciclib was 0.999. This indicates good linearity

Linearity:

Calibration data is given in table 7 and regression data in table 8 and calibration curve in figure 6

Table 7: Calibration data of Trilaciclib

Trilaciclib	
Conc (µg/mL)	**Peak area**
0	0
7.5	1561057
15	3206136
22.5	4796183
30	6375216
37.5	7994692
45	9397512

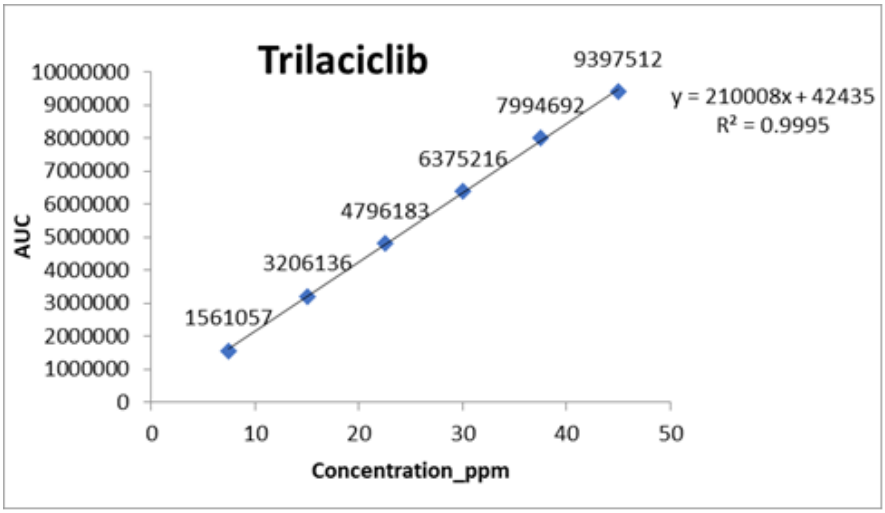

Figure 6: Calibration curve of Trilaciclib

Table 8: regression data

Parameter	Trilaciclib
Conc range (µg/mL)	7.5-45µg/ml
Regression Equation	y = 210008x + 42435
Co-relation	0.999

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Trilaciclib at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analysed. The percentage recovery results obtained for each impurity was listed in Table 9

Table 9 Accuracy table of Trilaciclib

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% recovery
50%	15	15.02	100.15
	15	14.91	99.43
	15	14.92	99.48
100%	30	15.02	100.15
	30	14.91	99.43
	30	14.92	99.48
150%	45	44.78	99.52
	45	45.36	100.81
	45	45.26	100.57
Mean % recovery			**99.95**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Trilaciclib. Results of peak area are summarized in Table 10

Table 10 System precision table of Trilaciclib

S. No	Area of Trilaciclib
1.	6364747
2.	6345479
3.	6394636
4.	6384633
5.	6355474
6.	6304858
Mean	**6358305**
S.D	**31886.4**
%RSD	**0.5**

Method Precision: The precision of the method was determined by analyzing a sample of Trilaciclib). Data obtained is summarized in Table 11

Table 11 Repeatability table of Trilaciclib

S. No	Area of Trilaciclib
1.	1647208
2.	1646907
3.	1644783
4.	1651256
5.	1645671
6.	1649307
Mean	**1647522**
S.D	**2388.9**
%RSD	**0.1**

Intermediate precision: It is differently from the repeatability, the precision obtained within a single laboratory over a longer period (generally at least several months) and considers more changes than repeatability. Data obtained is summarized in Table 12

Table 12 Intermediate precision table of Trilaciclib

S. No	Area of Trilaciclib
1.	6384654
2.	6357464
3.	6394636
4.	6357464
5.	6384646
6.	6358475
Mean	**6372890**
S.D	**16930.4**
%RSD	**0.3**

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase, and system suitability also complied as per methodology. The robustness results are summarized in Table 13.

Table 13 Robustness data for Trilaciclib

Condition	%RSD of Trilaciclib
Flow rate (-) 0.9ml/min	0.5
Flow rate (+) 1.1ml/min	0.5
Mobile phase (-) 55B:45A	0.5
Mobile phase (+) 65B:35A	o,5
Temperature (-) 27°C	0.4
Temperature (+) 33°C	0.3

Assay data: -

Cosela Tablet bearing the label claims Trilaciclib 300 mg. Assay was performed with the above formulation. Average % Assay for Trilaciclib obtained was 99.47%. Assay data shown in table no 14.

Figure 7: Trilaciclib Marketed Drug

Formula to calculate assay:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{10}{1} \times \frac{P}{5} \times \frac{FV}{100} \times \frac{}{L.C} \times 100$$

AT	Avergage peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of sa,ple working standard taken in mg
P	Assay of sample working standard in % in dried basis
L.C	Label claim
FV	filled volume (1ml of a vail)

Table 14: Assay Data of Trilaciclib

S.no	Standard Area	Sample area	% Assay
1	6364747	6324477	99.09
2	6345479	6324847	99.96
3	6394636	6395443	98.68
4	6384633	6314747	100.16
5	6355474	6394363	99.26
6	6304858	6326477	99.68
Avg	**6358305**	**6346726**	**99.47**
Stdev	**31886.4**	**37547.3**	**0.559**
%RSD	**0.5**	**0.6**	**0.6**

CONCLUSION

The Trilaciclib HPLC study findings reveal that this technique can accurately gauge the concentration and purity of the drug. Because it can be repeatedly utilised with crisp peak resolutions and uniform retention periods, this approach is excellent for both pharmacokinetic research and frequent quality control. Making sure Trilaciclib performs as expected and is safe for medical usage as well as confirming its chemical composition depend on HPLC.

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