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Stability Demonstrating Validated High Pressure Liquid Chromatographic Method for the Determination of Trilaciclib in Bulk and Pharmaceutical Formulation

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Authors' contributions

This work was carried out in collaboration between both authors. Author KR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SR managed the analyses of the study managed the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: New validated method for the estimation of Trilaciclib using HPLC and study of its degradation
Place and Duration of Study: Department of Chemistry, RVR & JC College of Engineering, Chowdavaram, Guntur, Andhra Pradesh, between February 2021 and August 2021.
Methodology: Using an inertsil ODS column (150 mm x 4.6 mm, 3.5 µ), acetonitrile, and 0.1 percent ortho phosphoric acid (OPA) (50:50 v/v) as a mobile phase, the proposed method successfully achieved effective chromatographic separation with a flow rate of 1 mL/min and a wave length of 220 nm. Trilaciclib had a retention time of 4.358 minutes. The isocratic chromatography was performed at room temperature and took approximately six minutes to complete.
Results: Analysis was achieved within 6 min over an honest linearity within the concentration range from 3-45 µg/ml of Trilaciclib. Using a mathematical process, the suitability parameters of the

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system were investigated, and the results were found to be in acceptable limits. In a linear analysis, stages with regression coefficients of 0.999 were used. LOD and LOQ values were 0.038 µg/ml and 0.124 g/ml for trilaciclib. The drug was recovered at a rate of 98-102 percent, which means that the recovery is within reasonable limits.

Conclusion: The validation results were satisfactory, and the approach was found to be suitable for bulk and formulation analysis. The recommended procedure was found to be warranted according to ICH guidelines.

Keywords: Trilaciclib; development; validation; RP-HPLC; stability studies.

1. INTRODUCTION

Trilaciclib (brand name-Cosela) is a drug [1] used to inhibits chemotherapy [2,3] induced bone marrow suppression [4]. Common side effects include fatigue [5,6], low levels of calcium, potassium, and phosphate, as well as elevated levels of the enzyme aspartate aminotransferase [7]. Additional possible side effects include headache [8], infection in the lungs (pneumonia) [9,10], and peripheral neuropathy. Cyclin-dependent kinase 4/6 (CK4/6) is an enzyme involved in chemotherapy-induced bone marrow [11,12] cell damage, and trilaciclib may help to protect the bone marrow cells from damage by inhibiting CK4/6 [13]. Even though chemotherapy drugs are designed to specifically target cancer cells, they can also harm noncancerous cells and tissues. Additionally, the bone marrow is vulnerable to chemotherapy-induced damage. Red blood cells [14,15], white blood cells [16], and platelets [17] are made in the bone marrow. They are responsible for transporting oxygen, fighting infection, and clotting wounds. The bone marrow, when damaged, makes fewer of these cells, which results in fatigue, increased susceptibility to infection, and bleeding. The normal bone marrow cells are protected from the damaging effects of chemotherapy by trilaciclib. Two randomised, double-blind, placebo-controlled studies were performed to test the effectiveness of trilaciclib for patients with extensive-stage small cell lung cancer [18,19]. Prior to chemotherapy, 245 participants were randomly assigned to receive either a placebo or an infusion of trilaciclib in their veins. After the studies comparing the two groups for the number of people with severe neutropenia [20] (a low count of white blood cells called neutrophils) and the length of time of severe neutropenia in the first cycle of chemotherapy, the participants were further categorised according to the presence of this condition. Trilaciclib was found to have a lower risk of having severe neutropenia compared to those who received a placebo in all three studies. Average trilaciclib treatment durations among participants who had severe neutropenia were, on average, shorter than those of participants who received a placebo. Chemical structure of Trilaciclib Fig. 1.

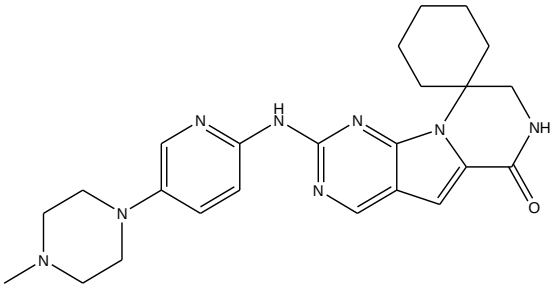

Fig. 1. Chemical structure of Trilaciclib

To date, there have been no HPLC methods for Trilaciclib estimation. Thus, the goal of the study is to predict Trilaciclib, which is a pharmaceutical component, using RP-HPLC.

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

The reagents were purchased from Merck (India) Ltd., Mumbai, India: Acetonitrile, Ortho phosphoric acid (OPA) (purity-99.9 percent) and water (HPLC grade). Glenmark Pharmaceutical Private Ltd., Andheri (E), Mumbai, India provided an API (purity-99.9%) for Trilaciclib as a reference standard.

2.2 Equipment

Using an e-2695 chromatographic system and a PDA 2996 detector, we utilised a quaternary pump and a PDA. Empower software version 2.0 was used to analyse the chromatographic data.

2.3 Chromatographic Conditions

To conduct chromatography using isocratic conditions, an inertsil ODS (150 mm x 4.6 mm, 3.5 µ) column was utilised at temperature using a Chromatographic conditions separation was

administered in isocratic mode at temperature employing an inertsil ODS (150 mm x 4.6 mm, 3.5 µ) column. Ortho phosphoric acid (0.1%) and acetonitrile (50:50 v/v) with a flow rate of 1 mL/min were used as a mobile phase in this experiment. Injection volume was 10 µl, and the eluent was found at 220 nm, as the maximum concentration of Trilaciclib was found at this wavelength. So, it was decided to use the wave length of 220 nm.

2.4 Preparation of standard solution

30 mg of Trilaciclib working standard was added to 100 ml of the flask and the solution was diluted to the required volume with the diluent. Dilute 5 ml of the prepared solution with diluents to a final volume of 50 ml.

2.5 Preparation of Sample Solution

Mix 30 mg of Trilaciclib with 100 ml of diluents and sonicate to dissolve it. Then, add the remaining 70 ml of diluents to the mark. Use more diluents to dilute the sample solution, mixing thoroughly.

3. RESULTS AND DISCUSSION

Following optimization of the chromatography conditions for specificity, count, tailing factor, and retention time, the best isocratic condition for eluting Trilaciclib with Inertsil ODS Column was a mobile phase consisting of 0.1% OPA and Acetonitrile in the ratio of 50:50. Back ground noise or peaks indicating the tailing effect can be seen in the resulting chromatogram if a higher percentage of mobile phase was used. Trilaciclib was eluted after four minutes and thirty-eight seconds based on the previously mentioned parameters. Table 1 depicts the chromatographic parameters applied for the method.

3.1 Specificity

There was no blanketing of Trilaciclib until the molecules had been retained for the set period of time. The chromatogram in Fig. 2 shows an empty chromatogram [21].

Table 1. Optimized chromatographic conditions

Parameter	Proposed method
Stationary Phase	Inertsil ODS (150 x 4.6 mm, 3.5 µ)
Mobile Phase	0.1% OPA : Acetonitrile (50:50)
Injection Volume	10 µl
Flow Rate	1.0 mL/min
Column Temperature	Ambient
Wave Length	220 nm
Run Time	6.0 min
Retention time of Drospirenone	4.358 min

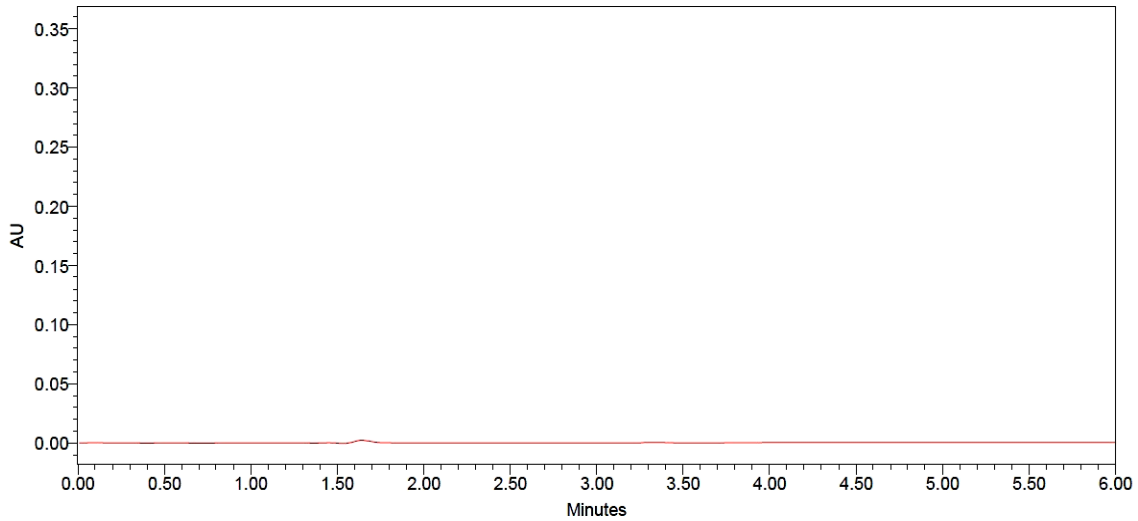

Fig. 2. Chromatogram of blank

3.2 System Suitability

Stabilization was performed for 60 minutes to encourage a constant bottom line. The system suitability was checked by dispensing six Trilaciclib-branded injections, which each contained 30 µg/ml of Trilaciclib, and assessing the results. A theoretical plate count of 7451 was derived for Trilaciclib, while the tailing factor was 1.05. These values were deemed acceptable. To gather all the data, the chromatography software will be utilised (Empower 2.0). Fig. 3 shows Satandard chromatogram and Table 2 gives System precision results [22].

3.3 Linearity

A standard solution containing 30 micrograms per millilitre of Trilaciclib l was prepared to determine the linearity of the tactic (100 percent of the targeted level of the assay concentration). For this problem, it was necessary to perform sequential dilutions of the given solutions at concentrations ranging from 10 percent, 25 percent, 50 percent, 100 percent, 125 percent, 150 percent of the target concentrations. Because they were pumped, the peaks are used to map calibration curves on to the data points. It was found that the correlation coefficient between these analytes was 0.999. The results of the linearity tests and the Fig. 4, which displays the calibration plot of Trilaciclib, are shown in Table 3. The values of slope, intercept and correlation coefficient were acquired from the linearity calculation sheet.

3.4 Limit of Detection and Quantification

The concentration level at which the analyte are reliably detected and quantified is the limit of detection and quantification. Trilaciclib had a LOD concentration of 0.038 µg/ml and a S/N value of 7. The concentration of trilaciclib in the LOQ was 0.124 µg/ml, and the S/N value was 25. S/N is the ratio of signal to noise.

3.5 Precision

Six samples of an identical batch were prepared, and then the method precision of the process was examined. After injecting these six samples, the maximum responses from these six separate samples were used to calculate mean and percentage RSD values [23]. This method was found to be precise, with an RSD of 2%, and the RSD percentage of the specimen or share assay values was nearly 100%. Table 4 gives the method precision results. Sampling chromatogram (Fig. 5).

Table 2. Results of system suitability

Parameter	Trilaciclib
Theoretical plate count	7451
Tailing factor	1.05
Resolution	-
Retention time	4.358 min

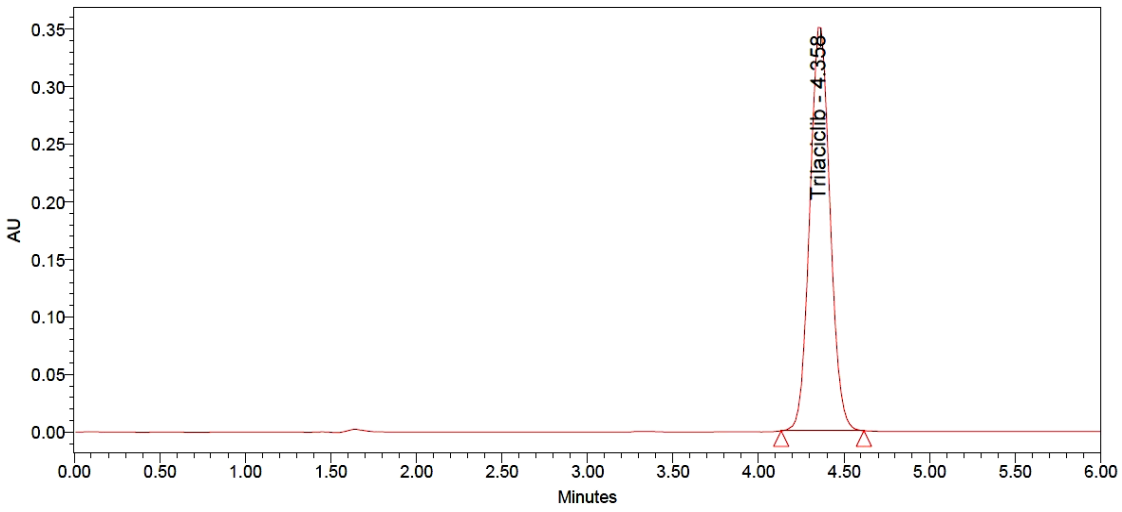

Fig. 3. Chromatogram of standard

Table 3. Results of linearity

S. No	Trilaciclib	
	Concentration (µg/mL)	Area
1	3.00	273054
2	7.50	743514
3	15.00	1406209
4	30.00	2834505
5	37.50	3578517
6	45.00	4255617
CC	0.99994	
Slope	94760.55	
Intercept	2065.71	

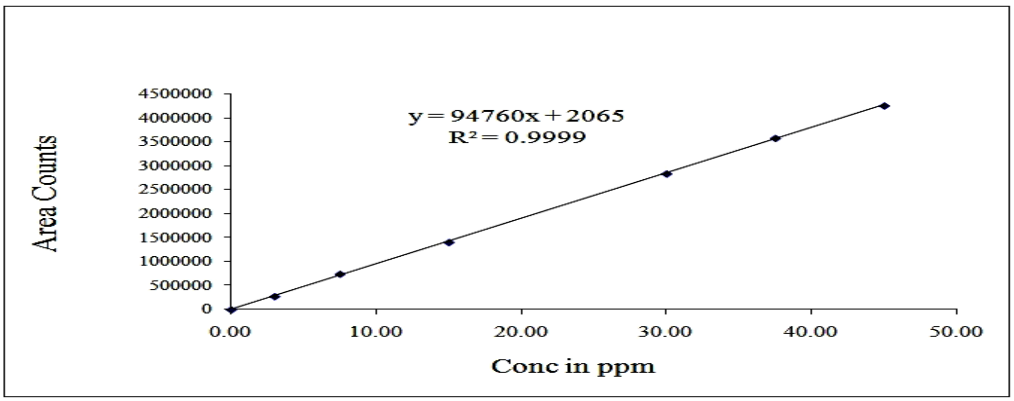

Fig. 4. Calibration plot of Trilaciclib

Table 4. Results of method precision

S. No.	Area of Trilaciclib
1	2850839
2	2842476
3	2874127
4	2857333
5	2866629
6	2857396
Mean	2858133
Std. dev	11202.83
% RSD	0.392

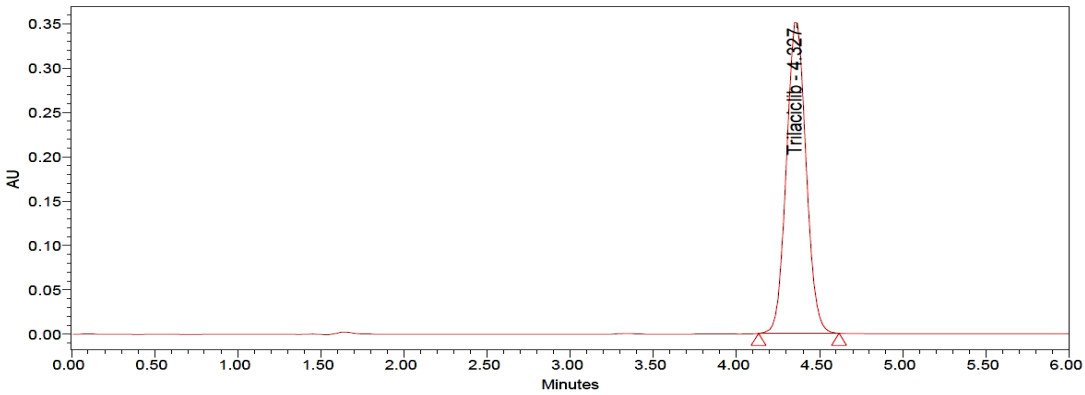

Fig. 5. Chromatogram of sample

3.6 Accuracy

Effectiveness was established through recovery studies that were conducted in 3 separate concentrations (50 percent, 100 percent and 150 percent). 15, 30, and 45 µg/ml concentrations of API were made. According to the specified test method, the solution was injected into three solutions of increasing concentration, which allowed for the assay to be performed. In between 99.5 and 100.6 percent of Trilaciclib, the recovery values were observed. The recovery values for the share price were found to be two percent. Table 5 presents the accuracy results [24].

3.7 Ruggedness

The HPLC method, observer, and column were investigated to see if the chromatographic patterns changed significantly when a different tactic was used. It is proof of the quality of the long-standing process that the RSD percentage was less than 2 percent. Ruggedness results Table 6.

3.8 Robustness

According to RSD's tests, the robustness of the tactic brought in only 2% of RSD. The slightly varied parameters such as flow (±0.2 mL/min) and organic content in the mobile phase (±10 percent) were eliminated in favour of the optimised methods. Robustness results Table 7.

3.9 Stability

The ordinary and sample solutions were studied from initial to 24 hours, stored at RT, by examining the stability techniques. Injections were given at different time intervals, and the percentage of the assay made at the time of the first injection was about 2 percent less than that made 24 hours later. In storage conditions, there is no effect for Trilaciclib. Stability results Table 8 [25].

Table 5. Results of accuracy

Accuracy	Amount of Trilaciclib	% Recovery
50*****	15	99.9
100*	30	100.6
150*	45	99.4

** Results are mean recovery of three sample preparations*

Table 6. Results of intermediate precision

S.No.	Area of Trilaciclib	% RSD
1	2842841	0.71
2	2860823	
3	2899252	
4	2846210	
5	2866629	
6	2870798	

Table 7. Results of robustness

Parameter	% RSD of Trilaciclib
Flow (0.8 mL/min)	0.11
Flow (1.2 mL/min)	0.38
Organic phase (45:55)	1.56
Organic phase (55:45)	0.75

Table 8. Stability results of Trilaciclib

Time intervals	Trilaciclib (% assay)	% Deviation
Initial	100.2	0.00
6 Hrs	99.3	-0.90
12 Hrs	99.1	-1.10
18 Hrs	98.5	-1.70
24 Hrs	97.3	-2.89

Table 9. Results of forced degradation

Stress Parameter	% Degradation of Trilaciclib
Acid degradation (1N HCl)	13.7
Alkali degradation (1N NaOH)	14.2
Peroxide degradation (30% Peroxide)	15.7
Reduction degradation (30% sodium bi sulphate)	13.0
Thermal (sample, 70°C, 6 Hrs)	12.4
Hydrolysis (1 ml HPLC water)	11.7
Photolytic degradation (UV-Vis light)	12.9

3.9 Forced Degradation

As far as release and stability studies are concerned, this proposed technique is an improvement on previous techniques, as it enables the use of both of these approaches. The following process steps are all part of the forced degradation study required by the ICH guidelines: acid, base, oxidation, reduction, photo and thermal degradation. In conclusion, it appears that the drugs under consideration were stable even though degraded peaks were observed, as they are dependent on the type of chromatography used. Results of forced degradation (Table 9).

3.9.1 Acid degradation

1 ml of the sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, to which 1 ml of 1N HCl was added and left to stand for 15 minutes. After 15 min add 1 ml of 1N NaOH and make up to the diluent mark. Filter the solution using syringe filter and injected into HPLC system.

3.9.2 Alkali degradation

1 ml of the sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, 1 ml of 1N NaOH was added, and the mixture was left to stand for 15 minutes. After 15 minutes, add 1 mL of 1N HCl to bring the solution up to the required concentration. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

3.9.3 Peroxide degradation

1 ml of sample stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 30% hydrogen peroxide solution and make up to the mark with diluents. Filter the solution using syringe filter and injected into HPLC system.

3.9.4 Reduction degradation

Using a volumetric flask with a capacity of 10 ml, transfer 1 ml of sample stock solution and add 1 ml of 30% hydrogen peroxide solution, then dilute to the required concentration with diluents. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

3.9.5 Thermal degradation

During the 6 hour baking period, the sample solution was kept at 105°C. The resulting solution was injected into a high-performance liquid chromatography system.

3.9.6 Hydrolysis degradation

1 ml of sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, to which 1 ml of HPLC water was added, and the volume was brought up to the required level with diluents. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

3.9.7 Photolytic degradation

100mg of sample was exposed to sunlight for 6 hrs and the exposed sample was analysed. Prepare the sample solution by using this sample and inject into HPLC system.

4. CONCLUSION

This methodology details Trilaciclib's quantification as it applies to bulk and pharmaceutical dosage form in accordance with ICH recommendations. The evolved technique was accurately, precisely, linearly, and reliably shown to be correct. Additionally, less expensive reagents were used, thus reducing the cost of the product. To ensure adequate resolution, the proposed HPLC conditions have been instituted. The testing results show that the precision and reproducibility data are sufficient. Routine drug testing using the developed chromatographic technique became widespread.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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