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Research Article

ESTIMATION OF TRILACICLIB BY USING RP-HPLC METHOD

A. Kanaka Durga Valli[1], B. Madhu Kumar[2], Dr. K. Atchuta Kumar[3]

[1]Assoc.Professor, Srinivasa Rao College of Pharmacy, P.M Palem, Vishakapatnam.

[2]M. Pharmacy, Department of Pharmaceutical Analysis and Quality Assurance, Srinivasa Rao College of Pharmacy, P.M Palem,Affiliated to Andhra University, Vishakapatnam - 530041, Andhra Pradesh, India.

[3]Professor, Srinivasa Rao College of Pharmacy, P.M Palem, Vishakapatnam.

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ABSTRACT

A simple, precise, and accurate RP-HPLC method was devised to estimate Trilaciclib. Stationary phase Agilent C18 (150mm*4.6mm3.6m), mobile phase 0.01N KH2PO4: Methanol in the ratio 55:45, flow rate 0.9ml/min, detection wave length 253nm, column temperature 30oC, and diluent mobile phase. Optimized conditions were set. System appropriateness characteristics were examined by injecting the standard six times and scoring considerably below acceptability. R2 was 0.999 for linearity study between 25% and 150%. Precision was 0.9 for repeatability and 0.7 for intermediate. The LOD and LOQ are 0.14µg/ml and 0.41µg/ml, respectively. The aforesaid approach assayed commercial formulation and found 100.31%. All Trilaciclib degradation investigations showed purity thresholds greater than purity angle and within acceptable limits. Full length approach was not conducted; if done, it may be utilized for ordinary Trilaciclib analysis.

Keywords: HPLC, Trilaciclib, Method development. ICH Guidelines.

INTRODUCTION

Chemotherapy often causes myelosuppression, or bone marrow suppression, which reduces blood cell synthesis.[1] Trilaciclib (G1T28), a CDK4 and CDK6 inhibitor, reduces chemotherapy-induced myelosuppression in extensive stage small cell lung cancer patients before topotecan- or platinum- and etoposide-containing treatment.Since the mid-1990s,[5] CDK4 and CDK6 inhibitors have been studied for cancer and chemotherapy.[3] Literature first described trilaciclib in 2016.[2] Trilaciclib received FDA clearance on 12 February 2021.[4] Trilaciclib reduces chemotherapy-induced bone marrow suppression in people with extended stage small lung cancer (ES-SCLC) following platinum/etoposide or topotecan treatment.

The FDA approved trilaciclib after three randomized placebo-controlled phase 2 trials showed that it decreased severe neutropenia and DSN in chemotherapy cycle 1 in ES-SCLC patients [6,7] NCCN recommendations propose Trilaciclib to reduce chemotherapy-induced myelosuppression in ES-SCLC.[8] CDK4/6 inhibitors with immunotherapy may treat cancer, according to many studies. To overcome T cell cytotoxic function reduction-induced immunity treatment resistance, Deng et al. tested tiny inhibitors to boost immunological checkpoints. PD-1 overexpressing human T cells treated with CDK4/6 inhibitors palbociclib and trilaciclib secrete more IL-2,

Address for Correspondence: A. Kanaka Durga Valli, Assoc.Professor, Srinivasa Rao College of Pharmacy, P.M Palem, Vishakapatnam **E-Mail:** bmadhukumar111@gmail.com

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a sign of T cell activity. In genetically engineered animal models of non-small cell lung cancer, CDK4/6 inhibitors boost CD4+ and CD8+ T cell tumor invasion.Another research found that CDK4/6 inhibitors reduce immunosuppressive pathways to boost immunotherapy.[9] In preclinical experiments, trilaciclib caused temporary and reversible G1 cell cycle arrest of murine and human HSPCs and protected them against chemotherapy-induced fatigue [13]. Trilaciclib, an HSPC protector, is given a few hours before chemotherapy. The trilaciclib clinical trials have a 10–50% G-CSF rescue rate for neutropenia.[10–12,14.]Trilaciclib had dose-proportional pharmacokinetics in healthy volunteers[13] and was well tolerated throughout the clinical development program, with minimal discomfort in the bones and fewer grade 3 or higher AEs than placebo-treated categories, mostly attributable to less hematologic toxicities [10–12,14]. The most prevalent trilaciclib AEs were tiredness (9.6%), nausea (7.7%), anaemia (5.8%), and infusion-related events (5.8%).[10] Trilaciclib has shown myelopreservation against other hematopoietic lineages in addition to neutrophil benefits [10–12]. RBC transfusion on/after week five, and erythropoiesis-stimulating agents. Generic Name Trilaciclib Drug Bank Commercially, G1 Therapeutics, Inc., company produces and markets Trilaciclib.

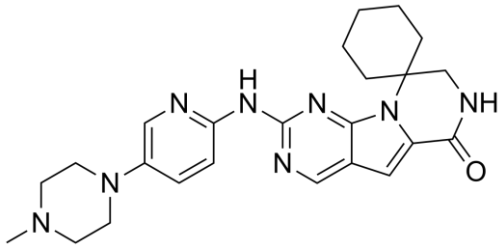

Figure 1: Structure of Trilaciclib

A study of the literature says that there are different ways to measure these medicines at the same time, as well as ways to measure them separately or in combination with other medicines. Using RP-HPLC and UV-Spectrophotometry A review of the literature shows that there isn't a standard way to measure Trilaciclib RP-HPLC simultaneously while showing stability in pharmacy dosage form. The main goal of this work is to come up with an RP-HPLC method that is quick, easy, and accurate for figuring out the amount and type of Trilaciclib medicines. As suggested by the ICH, a tried-and-true method was also used to guess how much Trilaciclib was present.[15-23]

MATERIALS AND REAGENTS

Spectrum Pharma Research Solutions in Hyderabad sent us pure Trilaciclib drugs. Trilaciclib (Cosela), a mixture drug, was bought at a nearby pharmacy. All of the materials and buffers used in this method came from Rankem in India. These included acetonitrile, phosphate buffer, methanol, potassium dihydrogen ortho phosphate buffer, ortho-phosphoric acid, distilled water, and phosphate buffer.

Instrumentation and Chromatographic Conditions

For the development and validation method, an automated sample injector was employed with a WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector. For the separation, a Discovery 150 (C18 250 mm x 4.6 mm, 5µm) column was employed. Acetonitrile is employed as mobile phase B, while 0.1% ortho phosphoric acid is used as mobile phase A. (35:65 Ratio). The analysis was done in isocratic mode with an injection volume of 10 mL and a flow rate of 1 mL/min. The duration was six minutes. The measurements were made at 254 nm.

PREPARATION OF SOLUTIONS

Diluent: Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50

Preparation of buffer:

0.01N KH2PO4 Buffer: Accurately weighed 1.83gm of Disodium 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 PH adjusted to 4.8 with dil.

Preparation of Standard stock solutions: Accurately weighed 10 mg of Trilaciclib, transferred to 50ml volumetric flasks and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (600µg/ml of Trilaciclib)

Preparation of Standard working solutions (100% solution): 1ml 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 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 (3000 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).

METHOD VALIDATION

To prove that the technique is suggested for routine analysis, the HPLC method's validation was done for the simultaneous estimation Trilaciclib drug material in accordance with the ICH criteria.

Specificity: 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.

Linearity: stock solutions of Trilaciclib is taken in to 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Accuracy: That is sometimes term of trueness. The Accuracy should be established across the specified range of the analytical procedure.

Preparation of Standard stock solutions: Accurately weighed 10 mg of Trilaciclib and transferred to 50ml volumetric flasks and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (200µg/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 deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guidelines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/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 affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml of Trilaciclib, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml of Trilaciclib, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Trilaciclib (60ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Degradation studies:

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 600c. For HPLC study, the resultant solution was diluted to obtain (60ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of s tock s solution Trilaciclib 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 1c. The resultant solution was diluted to obtain (60ppm) solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.7

Alkali Degradation Studies: To 1 ml of stock solution Trilaciclib 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain (60ppm) solution and 10 µl 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 1050c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (60ppm) solution and10µl 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 (600ppm) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200-Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (60ppm) solutions and 10 µl 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 refluxing the drug in water for 6hrs at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to (60ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample

RESULTS AND DISCUSSIONS:

Table 1. System suitability table

S.No	Trilaciclib		
Injection	RT(min)	USP Plate Count	Tailing
1	2.441	3107	1.20
2	2.444	2867	1.20
3	2.448	2771	1.23
4	2.448	3036	1.21
5	2.452	3402	1.21
6	2.452	3126	1.23

Table 2. Specificity data

Sample name	Retention time(Mins)	Area
Trilaciclib	2.067	373382

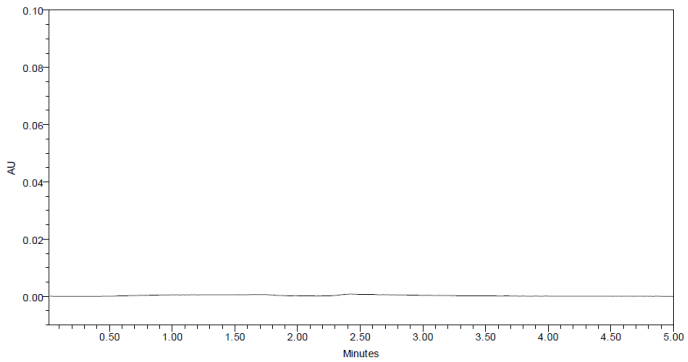

Figure 2. Blank Chromatogram

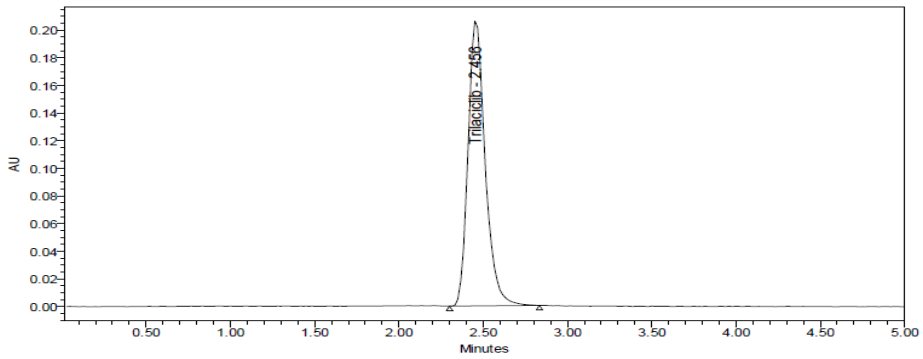

Figure 3. Specificity Chromatograms of Trilaciclib

Linearity

Table 2: Linearity table for Trilaciclib:

Trilaciclib		
Linearity level (%)	**Conc (µg/mL)**	**Peak area**
0	0	0
25	15	1066416
50	30	1925671
75	45	3028643
100	60	3970103
125	75	4975021
150	90	6013506

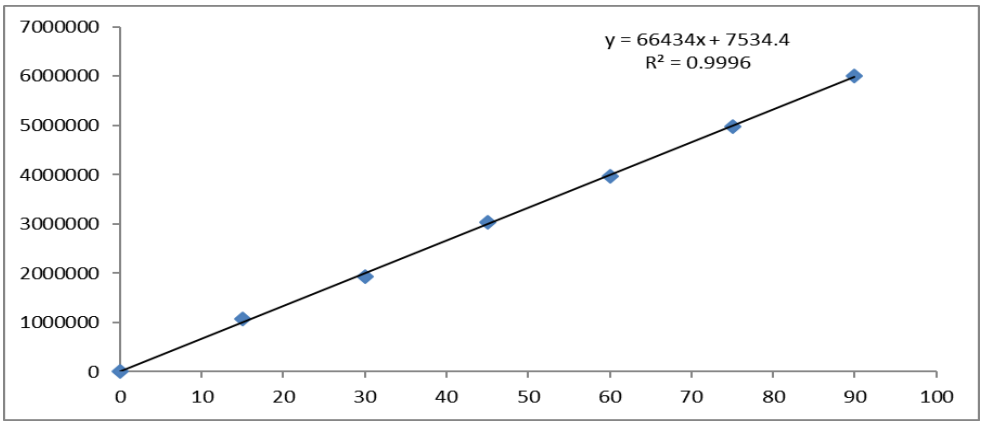

Figure 4. Trilaciclib calibration Curve

Accuracy:

Table 3. Accuracy table of Trilaciclib

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	30	60	101.08	**100.45%**
	30	60	101.06	
	30	60	100.03	
100%	60	60	101.86	
	60	60	101.74	
	60	60	100.72	
150%	90	60	98.73	
	90	60	99.91	
	90	60	98.94	

System Precision: With regard to the working strength of Trilaciclib, six duplicate injections of the standard

solution at 100% of the prescribed limit were analysed to determine the system accuracy. In Table 5, the results of the peak area are compiled.

Table 4. System precision

S. No	Area of Trilacilib
1.	3900986
2.	3940420
3.	3895207
4.	3855454
5.	3922067
6.	3923727
Mean	**3906310**
S.D	**29843.3**
%RSD	**0.8**

The % RSD for the peak areas of Trilaciclib obtained from six replicate injections of standard solution was within the limit of (<2%).

Method precision: Analyzing a sample of Trilaciclib allowed researchers to gauge the method's accuracy (Six individual sample preparations). Table 6 provides a summary of the data.

Table 5. Method precision

S.no	Trilaciclib
1	3950268
2	3908540
3	3899032
4	3895431
5	3984869
6	3918533
Avg	**3926112**
Std dev	**34861.9**
%RSD	**0.9**

Results shows, the % RSD of Repeatability study was within the range for **Trilaciclib** is (<2%)

Table 6. Robustness

S.No.	Condition	%RSD of Trilaciclib.
1	Flow rate (-) 0.9ml/min	0.6
2	Flow rate (+) 1.1ml/min	0.5
3	Mobile phase (-) 60B:40A	1.1
4	Mobile phase (+) 70B:30A	1.1
5	Temperature (-) 25°C	1.2
6	Temperature (+) 35°C	0.5

Table 7. Forced degradation for Trilaciclib

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

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Table 8. Degradation results of Trilaciclib

Type of degradation	Trilaciclib	
	% Recovered	% Degraded
Acid	93.29	6.71
Base	97.11	2.89
Peroxide	94.59	5.41
Thermal	97.25	2.75
Uv	98.61	1.39
Water	99.88	0.12

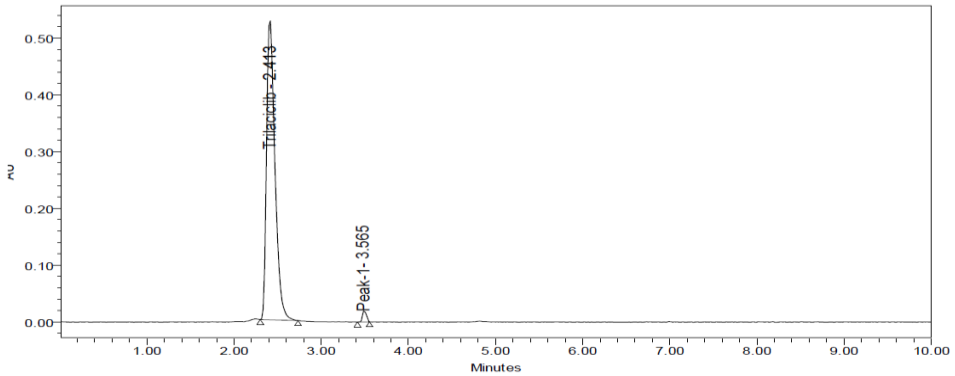

Figure 5. Acid chromatogram of Trilaciclib

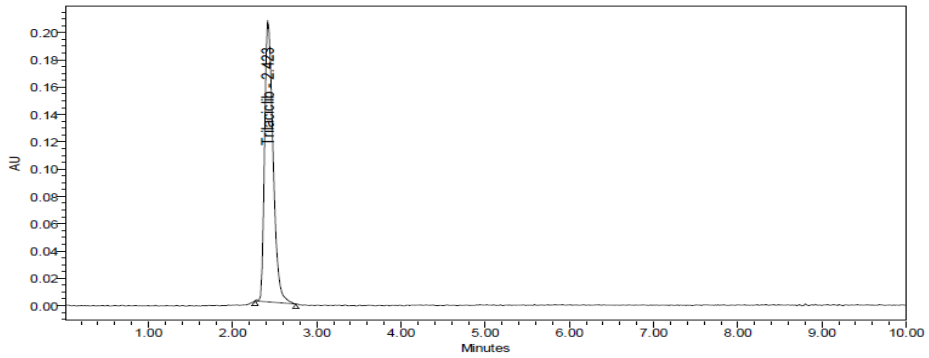

Figure 6. Base chromatogram of Trilaciclib

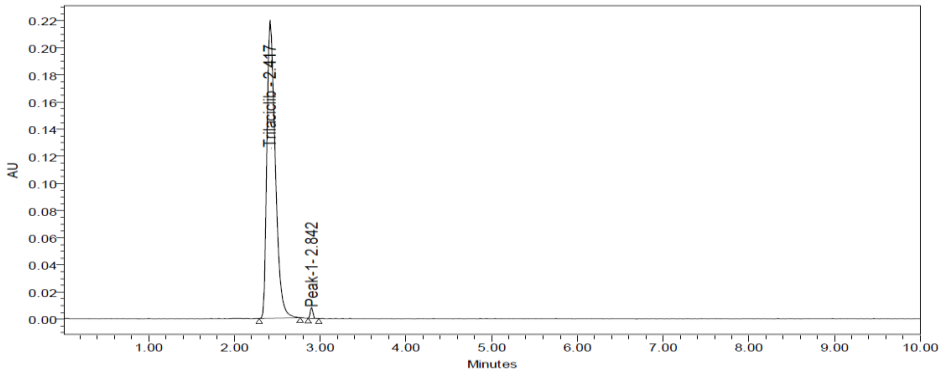

Figure 7. Peroxide chromatogram of Trilaciclib

According to the results, samples were degraded when they were subjected to an acid, base, and oxidation interaction. Hydrolysis reaction, heat reaction, and light reaction all showed no deterioration. According to the stress research, none of the degradants co-eluted with the maxima of the active medication.

Assay: (Cosela) bearing label claim, Trilaciclib 300mg, assay was carried out by injecting sample into HPLC System.

Table 9: Assay data of Trilaciclib

S.No.	Standard Area	Sample area	% Assay
1	3900986	100.92	100.92
2	3940420	99.86	99.86
3	3895207	99.61	99.61
4	3855454	99.52	99.52
5	3922067	101.81	101.81
6	3923727	100.11	100.11
Avg	**3906310**	**100.31**	**100.31**
Stdev	**29843.2**	**3033.4**	**0.39**
% RSD	**0.8**	**0.4**	**0.4**

Table 10: Assay outcome for Trilaciclib

Drug Name	Label claim dose	%Assay	Brand Name
Trilaciclib	300mg	100.31	**Cosela**

CONCLUSION

The proposed HPLC method was validated as per ICH guidelines and applied for the determination of Trilaciclib in tablet dosage form. Chromatographic conditions used are stationary phase Agilent C18 (150mm*4.6mm2.8m), Mobile phase 0.01N KH2PO4: Methanol in the ratio of 55:45 and flow rate was maintained at 1.0ml/min, detection wave length was 253nm, column temperature was set to 30 oC and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150 % levels, R2 value was found to be as 0. 999.Precision was found to be 0.9 for repeatability and 0.7 for intermediate precision. LOD and LOQ are 0.14µg/ml and 0.41µg/ml respectively. By using above method assay of marketed formulation was carried out 100.31% was present. Degradation studies of Trilaciclib were done, in all condition's purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Trilaciclib.

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