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Novel HPTLC Method Development and Validation for Estimation of Abemaciclib in Tablet Dosage Form

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Abstract:

A novel, selective, precise, and high performance thin layer chromatographic technique has been developed and validated for estimating Abemaciclib in tablet dosage form. TLC aluminium plates precoated with silica gel 60 F-254 were used as the stationary phase employed TLC Aluminium plates precoated with silica gel 60 F- 254 as the stationary phase. Linear ascending development with Toluene: acetone: methanol: ammonia in the ratio 8:2:2:0.1 (v/v) as the mobile phase. It was concluded at room temperature (25± 2^0C) phase was performed at room temperature (25 ± 2°C) in a twin trough glass chamber saturated with the mobile phase. Abemaciclib has compact bands (R_F 0.42 ± 0.02). Scanning was done in absorbance mode at 298 nm. Linear regression analysis of calibration plots revealed a strong linear association between peak area and peak height (R^2=0.9995 and 0.997) in the concentration range of 50-400 ng/band. The method was tested for specificity, precision, accuracy, robustness, detection and quantification limits. The detection and quantification limits were 9.75 and 29.70 ng/band, respectively. The approach achieved recovery rate of 100.28%. This approach can be used as a quality control method to check the purity of Abemaciclib in its tablet dosage form.

Keywords: Abemaciclib, HPTLC, Method development, Validation.

Introduction:

The molecular name of Abemaciclib is N-[5-[(4-ethylpiperazin-1-yl) methyl]. pyridine-2-yl]-5- fluoro-4-(7-fluoro-2-methyl-3-propan-2-benzimidazole-5-yl pyrimidine-2-amine has the empirical formula $C_{27}H_{32}F_2N_8$. The molecular weight is 506.06 g/ml and melting point is 175-181°C. It is a light yellow crystalline powder with a pKa of 10.27 and

soluble in organic solvents like acetonitrile, methanol, and slightly soluble in water. [1]

Abemaciclib is an oral cancer therapy that targets HR+ and HER2- progressed or metastatic breast cancer. This medicine is an anti-tumor agent as well as a dual inhibitor of cyclin dependent kinase 4 (CDK4) and 6 (CDK6), which are involved in the cell cycle and, when deregulated, promote cancer cell proliferation. [2-3] On September 28, 2017, the FDA approved Abemaciclib therapy, marketed as Verzenio, for the treatment of HR-positive and HER2-negative advanced or metastatic breast cancer. [4] It is administered alone to patients who have undergone endocrine therapy and chemotherapy following cancer metastases, or in conjunction with Fulvestrant. Abemaciclib, administered daily without interruption as a solo drug or in combination with antiestrogen, reduced tumor size. Abemaciclib improved progression-free survival and objective response rates in patients with HR-positive, HER2-negative breast cancer after oral therapy. [5-6]

According to the literature review, the monograph for Abemaciclib is not officially included in any pharmacopoeia, and no HPTLC methods for estimating Abemaciclib in bulk or medicinal formulations have been reported. As a result, there was a need to create a new, simple, speedy, precise, and accurate HPTLC method for estimating Abemaciclib in tablet dosage form. The proposed technique was optimized and validated using the ICH Q2 R1 recommendations.

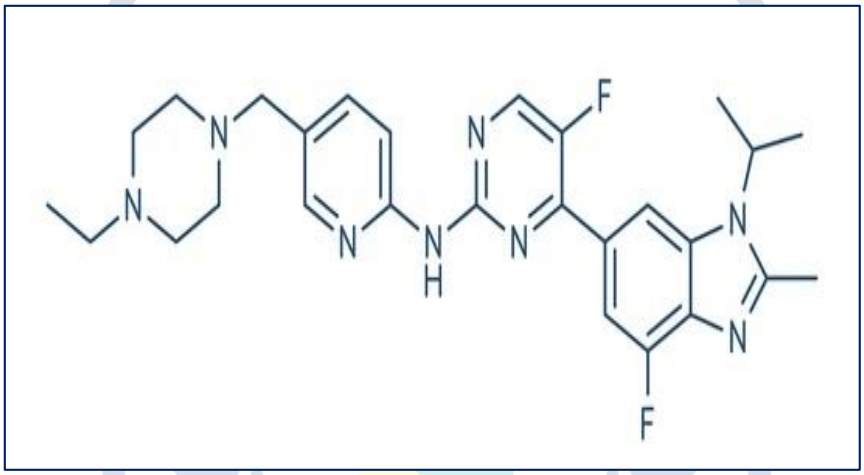

Figure no. 1: Abemaciclib chemical structure

MATERIALS AND METHOD

Chemicals and reagents:

An empirically pure Abemaciclib working standard with defined potency 99.90% (as is basis) and formulation (brand name: Ramiven was procured from CDTL (Mumbai, India) whereas Methanol from Merck Life Science, Toluene from Finar chemicals, Acetone and Ammonia solution from Sisco research laboratories. The silica gel 60 F-254 plates were purchased from Sigma-Aldrich, Germany.

Instrumentation:

The HPTLC system included a chamber with a dual wavelength UV light, a CAMAG Hamilton Bonaduz syringe (100 µl), a CAMAG Linomat 5 sample applicator (CAMAG, Switzerland), and a CAMAG TLC scanner 4 with CATS software for vision spot recognition. The medication has been separated analytically using pre-coated HPTLC plates (silica gel 60 F-254, 250 µm thicknesses).

Selection of solvent:

For standard and sample preparations, methanol was selected as a diluent due to its chemical composition of Abemaciclib.

Selection of Wavelength:

After carefully weighing 10 mg of Abemaciclib standard was added to a 100 ml volumetric flask. The volume was adjusted using diluent (100 µg/ml) and then diluted to a concentration of 10 µg/ml. After that, a scan was performed using the previously described solution from 400.0 to 200.0 nm. The highest absorbance of Abemaciclib was measured at 298 nm.

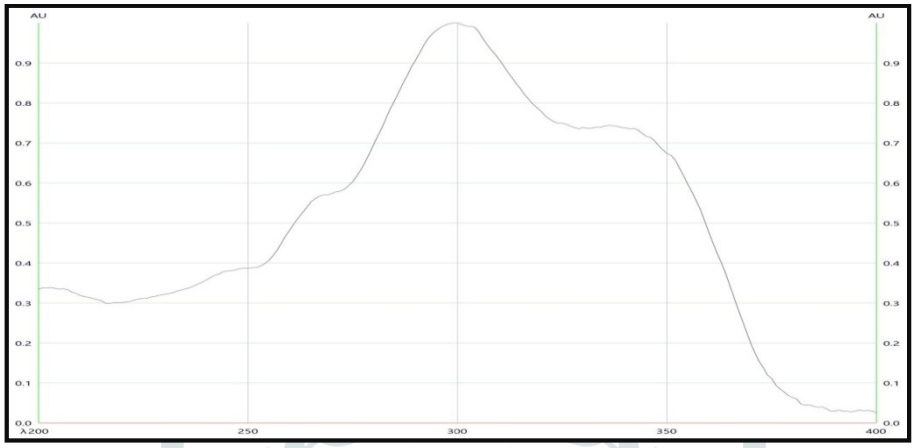

Figure no. 2: Abemaciclib UV spectrum at 298 nm.

Preparation of Solutions:

Preparation of standard drug solution:

Methanol was used as a diluent to obtain the 250ng/band concentration of Abemaciclib standard drug solution.

Preparation of sample solution:

Using 20 Ramiven tablets (50 mg each), the average weight was determined. A precise weight of 50 mg of Abemaciclib was obtained, and the sample was then put into a 100 ml volumetric flask and dissolved in 25 ml of diluent. The volume was changed with diluent after 20 minutes of sonication. Additional dilution was carried out to achieve a concentration of 250ng/band.

Three injections of 5µl each of the standard and sample solutions were made in order to perform the assay on the tablets mentioned above. The following were calculated and reported: %Assay, mean, SD, and %RSD.

Method optimization:

Because of the chemical composition and polarity of Abemaciclib (pKa=10.27 and pKb=7.94), TLC Silica gel 60 F254 aluminum plate were utilized as the stationary phase. Initial trials were made on TLC plates and then same conditions with respect to that of HPTLC were performed. The conditions for chromatography were set by applying a standard solution of Abemaciclib (concentration is 250ng/band). Various mobile phase proportions were tested utilizing experimental methods, with methanol serving as a diluent. Initially, attempts with the mobile phase n-hexane: ethyl acetate: methanol: glacial acetic acid (80:15:5:0.1 v/v/v/v) resulted in improper separation of bands. The mobile phase consisting n-hexane: dichloromethane: methanol: ammonia (80:15:5:0.1 v/v/v/v) was tried, but peak shape was not symmetrical and finally the mobile phase consisting toluene: acetone: methanol: ammonia in the ratio (8:2:2:0.1 v/v/v/v), which yielded a satisfactory peak shape with suitable R_F.

The mobile phase for method development and validation of Abemaciclib was composed of toluene: acetone: methanol: ammonia (8:2:2:0.1 v/v/v/v). A 5 µl of the Abemaciclib (250ng/band) standard solution were applied using the Camag Linomat 5 sample applicator to a TLC plate, with 15 minutes saturation duration. A Camag HPTLC scanner 4 equipped with a 298 nm deuterium lamp was used to carry out the detection. The result showed an RF value of 0.42 ± 0.02.

VALIDATION OF METHOD:

The developed RP-HPLC technique was validated for system applicability, specificity, linearity, precision, accuracy, LOD, LOQ, and robustness in accordance with the ICH recommendations [7-9].

System suitability studies:

Table no. 1 provided evidence of the applicability of the HPTLC method by showing that the percentages RSD of both the retention factor and peak area were found to be within the limits.

Table no. 1: Abemaciclib system suitability data

System Suitability test		
Sr. No.	Area (AU)	R_F
1	0.00661	0.422
2	0.00667	0.423
3	0.00663	0.425
4	0.00673	0.421
5	0.00669	0.422
MEAN	0.006666	0.4226
SD	4.775E-05	0.001517
%RSD	0.7163118	0.358868
LIMIT	NMT 2.0%	NMT 2.0%

Specificity:

The specificity of the procedure was demonstrated by analyzing the Abemaciclib sample and reference solutions. The band for Abemaciclib can be found by comparing its R_F value with the band spectrum of the standard. It is verified that Abemaciclib works. To ascertain Abemaciclib peak purity, the spectra were examined at three different band regions: peak apex (M), peak start (S), and peak end (E).

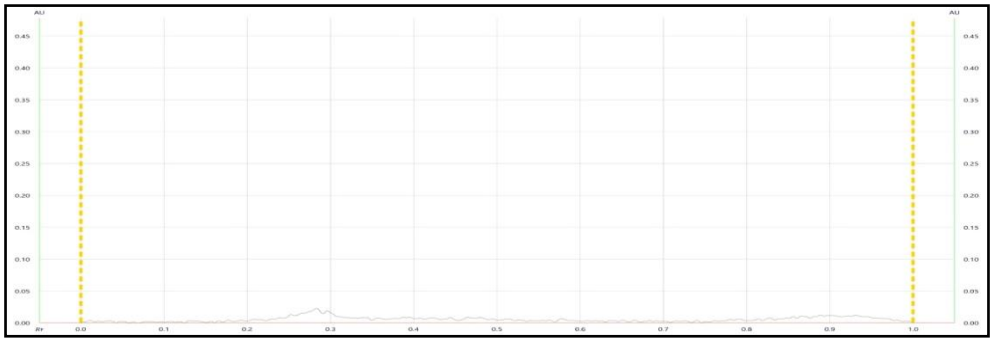

Figure no. 3: HPTLC Densitogram of blank solution

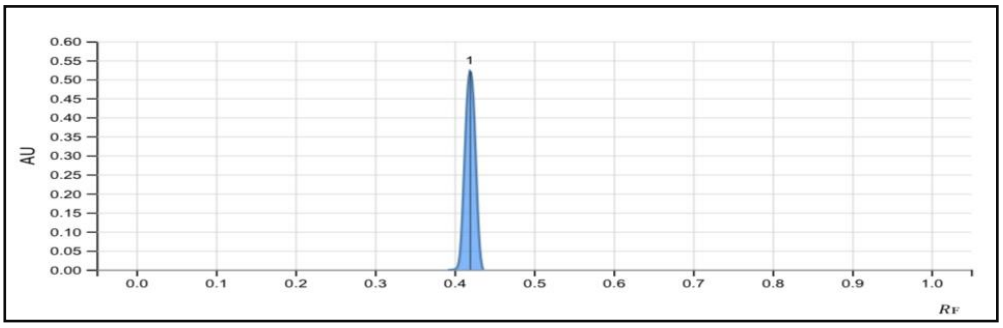

Figure no. 4: HPTLC Densitogram of standard solution of Abemaciclib (250ng/band)

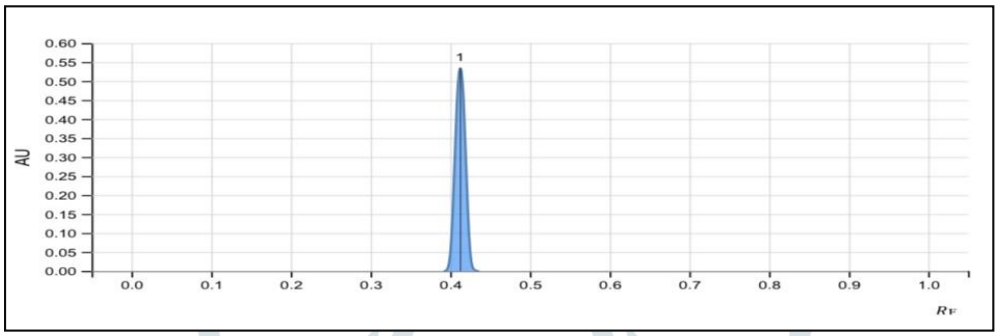

Figure no. 5: HPTLC Densitogram of sample solution of Abemaciclib (250ng/band)

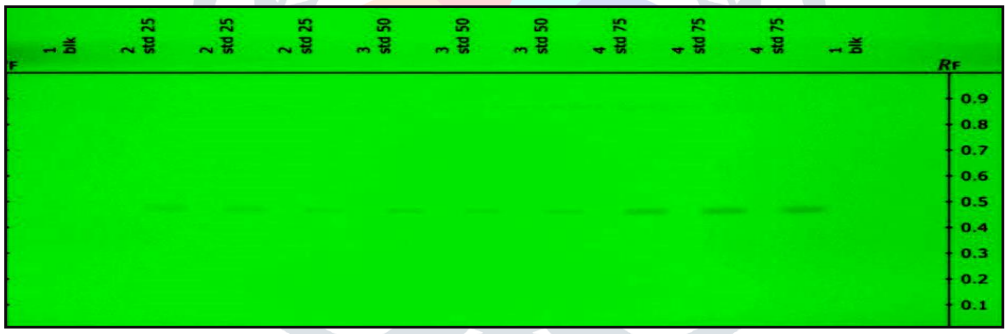

Figure no. 6: Visualization of HPTLC plate at 298 nm

Linearity:

The linearity of Abemaciclib was tested by applying eight different concentrations (50-400ng/band) of Abemaciclib reference standard solution in triplicate. The areas versus concentrations plots were used to demonstrate Abemaciclib linearity. It was found that the correlation coefficient (R^2) was 0.9995, which is less than one; hence the approach was classified as linear. Table No. 2 and Figures 7, 8 illustrate the results.

Table no. 2: Results of Abemaciclib linearity

Sr. No.	Concentration (ng/band)	Area
1	50	0.00369
2	100	0.00438
3	150	0.00495
4	200	0.00562
5	250	0.00626
6	300	0.00684

7	350	0.00744
8	400	0.00803

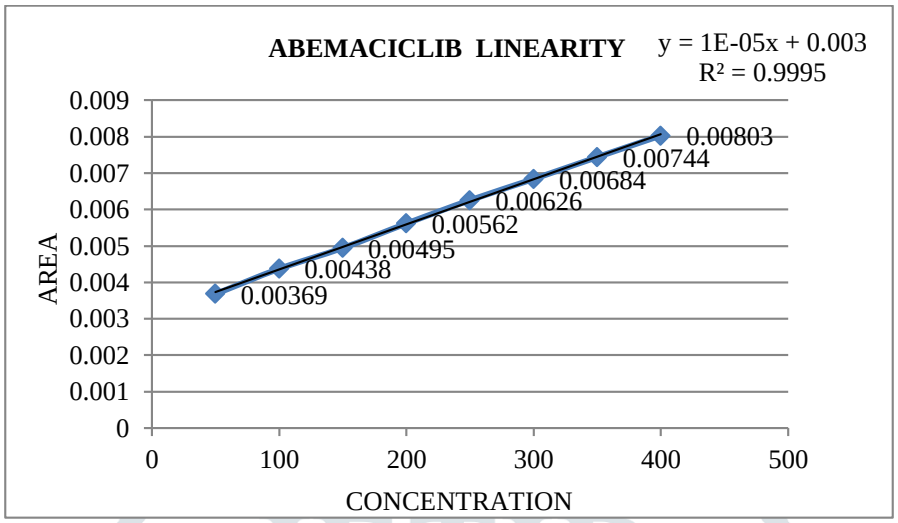

Figure No.7: Calibration curve of Abemaciclib

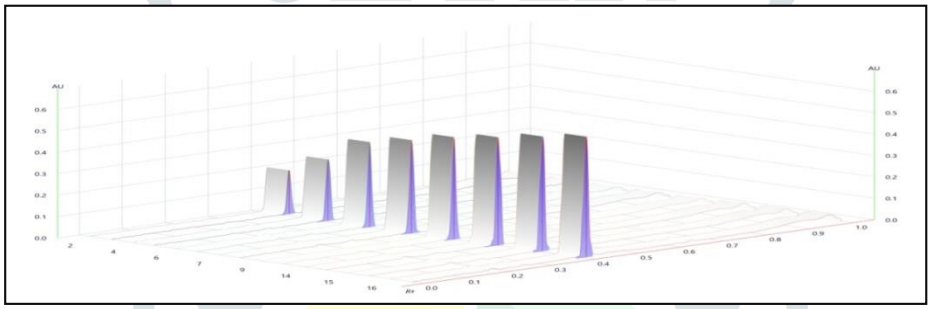

Figure No. 8: Three dimensional Densitogram of Abemaciclib linearity

Precision:

An analytical technique's precision is defined as the degree of agreement between measurements made from many samples of the same homogenous sample under predetermined conditions. The method's precision was evaluated by inter-day, intraday, and repeatability examinations. The %RSD was determined by analyzing Abemaciclib at 250ng/band. Each band on the HPTLC plate was 5 µl. Abemaciclib was analyzed at three concentration levels (low, medium, and high) to assess intra-day precision (%RSD).

Table no. 3: Repeatability data of Abemaciclib (n=6)

Sr. No.	Peak area of Abemaciclib standard
1	0.00649
2	0.00654
3	0.00657
4	0.00651
5	0.00648
6	0.00659
Mean	0.00653
SD	4.427E-05
% RSD	0.678

Table no. 4: Intermediate precision data of Abemaciclib

Concentration (ng/band)	Intraday precision		Interday precision	
	Mean area ± SD (n=3)	% RSD	Mean area ± SD (n=3)	% RSD
125	0.00459 ± 0.0000404	0.665	0.00491 ± 0.0000351	0.714
250	0.00683 ± 0.0000493	0.554	0.00701 ± 0.0000306	0.435
375	0.00805 ± 0.0000721	0.826	0.00817 ± 0.0000361	0.441

Accuracy:

The degree of agreement between the produced value and the recognized true value, or reference value, is referred to as the accuracy of an analytical procedure. Table no. 5 illustrates that there were three degrees of percentage recovery and percentage mean recovery (110%, 120%, and 130%), with a percentage mean recovery of 100.28%. The technique was correct, as evidenced by the mean recovery, which was found to be within the range of 99-101%, beyond the limit.

Table no. 5: Abemaciclib accuracy results

%level	Standard spiked (ml)	Amount recovered (mg/Tab)	%Amount recovered	%Recovery	%Mean recovery
100	0	50.16	100.32	100.32	100.28
110	0.5	55.12	110.24	100.21	
120	1	60.35	120.06	100.58	
130	1.5	65.03	130.07	100.04	

LOD and LOQ:

The smallest quantity of analyte in a sample that can be detected but not accurately measured is referred to as the analytical method's detection limit. The lowest concentration of analyte in a sample that can be precisely and accurately measured is known as the quantitation limit of an analytical procedure. Using the calculations below, the LOD and LOQ are obtained based on the slope of the calibration curves and the regression line.
LOD= 3.3 x σ/s, LOQ = 10 x σ/s

Table No. 6: Sensitivity studies of Abemaciclib

Concentration range	50-400 ng /band
Regression equation	y = 6E-05x + 0.003
Correlation coefficient	R^2 = 0.999
Slope	6.18929E-05
Intercept	0.003116
Standard response of Regression line	3.89E-05
Limit of Detection (LOD)	9.75 ng/band
Limit of Quantification (LOQ)	29.70 ng/band

Robustness:

Robustness was assessed in triplicate at 125 and 375ng/band by varying the volume, saturation time, and composition of the mobile phase. RSD (%) of peak areas were calculated to assess the impact of these changes on the results. Toluene, Methanol, Acetone, and Ammonia mobile phases in the ratio 8:2:2:0.1 (v/v/v/v) were used for chromatography. The

distance travelled by solvent front and duration of saturation were 80 ± 5 mm (75, 80, and 85 ml) and 15 ± 5 minutes (10, 15 and 20 minutes), respectively.

Table no. 7: Abemaciclib robustness data

Change in Mobile Phase Ratio (8:2:2:0.1% v/v/v/v ± 0.2% in toluene content)					
Ratio	R_F Value	Area	Average Area	SD	%RSD
7.8:2:2 0.1	0.423	0.00672			
8:2:2:0.1	0.421	0.00675	0.00676	0.0000513	0.75873
8.2:2:2:0.1	0.422	0.00682			
Change in chamber saturation time (15 min ± 5 min)					
Ratio	R_F Value	Area	Average Area	SD	%RSD
10	0.425	0.00671			
15	0.422	0.00675	0.00669	0.0000611	0.91240
20	0.421	0.00663			
Change in distance travelled by solvent front (80 mm ± 5 mm)					
Ratio	R_F Value	Area	Average Area	SD	%RSD
75	0.425	0.00679			
80	0.421	0.00681	0.00679	0.0000436	0.64195
85	0.432	0.00682			

Assay:

The developed method was successfully used to analyze an Abemaciclib tablet formulation. The tablet formulation contained 100.15% of Abemaciclib. The results are shown in Table no. 8.

Table no. 8: Analysis of Abemaciclib marketed formulation

Sr. No.	Label claim (mg/Tab)	Weight of standard (mg)	Weight of sample (mg)	Average area of standard at 298nm	Area of sample at 298nm	%Assay
1	50 mg	10.39mg	143.83	0.00620	0.00592	100.35
2			143.78		0.00591	100.26
3			143.89		0.00592	100.42
4			144.46		0.00590	100.07
5			143.79		0.00597	99.50
6			144.38		0.00589	100.30
Mean						100.15
SD						3.40E-01
%RSD						0.339

RESULT AND DISCUSSION:

By examining the Abemaciclib standard and sample solutions, the method's specificity was demonstrated. The band for Abemaciclib was verified by comparing the RF value and the standard's band spectrum. The spectra were compared at three distinct band regions—peak apex (M), peak start (S), and peak end (E) in order to ascertain the peak purity of Abemaciclib. With a correlation coefficient of 0.9995, the technique demonstrated good linearity over the concentration range of 50-400 ng/band. The average % assay result for the sample solution containing Abemaciclib was determined to be within the acceptable range. As a result, the accuracy of the procedure was determined. The mean percent recovery of the Abemaciclib sample solution was found to be 100.28%, which was within the allowed range, indicating that the procedure was accurate.

Table no. 9: combined result of all parameters

Parameters	Abemaciclib
Linearity:	
Linearity range	50-400 ng/band
Correlation coefficient	0.9995
Precision:	
%RSD	0.67
Intraday precision	0.66-0.826
Interday precision	0.435-714
Sensitivity:	
LOD	9.75 ng/band
LOQ	29.70 ng/band
Assay:	
Mean recovery	100.15%
%RSD	0.33
Robustness	
Change in Mobile phase ration	0.75
Change in Saturation time	0.91
Change in distance travelled by solvent	0.64
Accuracy (%level)	% Mean recovery
110	100.21
120	100.58
130	100.04

CONCLUSION:

For the purpose of estimating Abemaciclib in the tablet dosage form, a novel, straightforward, appropriate, precise, and accurate HPTLC approach was created. In accordance with ICH Q2 (R1) requirements, the suggested HPTLC method was successfully verified, and it was discovered to be easy to use, precise, and accurate for quantifying Abemaciclib in tablet dosage form without interfering with excipients. Every validation parameter was discovered to be within allowable bounds. Higher sensitivity and improved resolution between the medicines and excipients are provided by this approach. There has never been a published HPTLC method for the analysis of Abemaciclib in tablet form. Thus, it makes sense to use this strategy. Because of the method's high capacity (18 bands per plate), ease of sample preparation, and flexibility in running both qualitative and quantitative experiments at the same time, using it can be very advantageous. As a result, the technique may be applied consistently to the study of Abemaciclib in dosage form as pharmaceutical tablets.

REFERNCES:

1. Bantley, Driver JE. 2008. Text-book of Pharmaceutical Chemistry, Oxford University Press, New Delhi, India, 9-103.
2. Corona SP, Generali D. 2018. Abemaciclib: a CDK4/6 inhibitor for treatment of HR+/HER2-advanced breast cancer. Drug Des Dev Ther, 12:321-30
3. Finn RS, Aleshin A, Slamon DJ. 2016. Targeting the cyclin-dependent kinase (CDK) 4/6 in estrogen receptor-positive breast cancers. Breast Cancer Res Treat, 18:17.
4. Kim ES. 2017. Abemaciclib: first global approval. Drugs, 77:2063-2070.
5. Johnston S, Shaughnessy J, Martin M, Huober J, Toi M, Sohn J, Andre VA, Martin HR, Hardebeck MC, Goetz MP. 2021. Abemaciclib as initial therapy for advance breast cancer: MONARCH 3 updated results in prognostic subgroups. NPJ Breast cancer, 7(1):80.
6. Sethi PD. 2013. Quantitative Analysis of Pharmaceutical formulations by HPTLC.CBS publishers & distributors, 3: 950-951.
7. ICH guideline for Validation of analytical procedures: text and methodology. Q2 (R1). 2005; 1(20):05.
8. Ravisankar P, Navya CN, Pravallika D, Sri DN. 2015. A review on step-by-step analytical method validation. IOSR J Pharm, 5(10):7-19.
9. Bhardwaj SK, Dwivedia K, Agarwala DD. 2015. A review: HPLC method development and validation. Int J Analyt Bioanalyt Methods, 5(4):76-81.