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Development and Validation of Reverse Phase High-Performance Liquid Chromatography Method for Quantitative Estimation of Abemaciclib in Tablet Dosage Form

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ABSTRACT

A simple, accurate, precise, and reproducible Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the determination of Abemaciclib in tablet dosage form was developed. The chromatographic separation was achieved on the Thermo Synchronis C18 column (250 mm × 4.6 mm id, 5 µ) and a mobile phase comprising of 0.1% Trifluoroacetic acid in water and Acetonitrile in the ratio of (70:30 v/v) was used. The flow rate was kept at 0.7 ml/min and detection was carried out at 320 nm. The retention time was found to be about 4.0 min. The method was linear over the range of 5-100 µg/ml with a regression coefficient of 0.9999. The method was validated as per ICH guidelines. The developed method is better with respect to robustness, reproducibility, and superior system suitability parameter.

INTRODUCTION

The chemical 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 and its Molecular formula is $C_{27}H_{32}F_2N_8$. Abemaciclib is an Anti-Cancer used for Advanced or metastatic breast cancer [2]. Fig. 2.

Literature survey reveals that the HPLC method is not available for Abemaciclib in the tablet dosage form. Hence, attempts were made to develop a simple, rapid, precise, and accurate reverse phase chromatographic method to estimate Abemaciclib in the tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents:

An analytically pure Abemaciclib working standard was procured from CDTL, Mumbai with defined potency [99.99 % as is basis]. RAMIVEN 100 mg Abemaciclib market formulation was used for analysis. HPLC Grade Acetonitrile from Merck, Trifluoroacetic acid AR Grade from Merck, and Water- milli-Q Grade were used.

Instrumentation:

Perkin Elmer UV/ VIS Spectrophotometer Lambda 25 connected to a computer loaded with Perkin Elmer UV Win Lab software was used for all the spectrophotometric measurements. Thermofischer system HPLC using software chromoleon 7.2.1 with LC instrument control was used for chromatography. The column used was the Thermo Synchronis C18 column (250 mm x 4.6 mm x 5 µ).

Selection of solvent (diluent):

Based on the Molecular structure and chemical nature of Abemaciclib, the mobile phase (0.1% TFA in water and Acetonitrile in the ratio of (70:30) was selected as a diluent for preparation of standard and sample solutions.

Selection of wavelength: Standard Stock solution:

About 10.0 mg of Abemaciclib was transferred to the 100 ml volumetric flask and the volume was made up to the mark with diluent and aliquot portions of standard stock solutions of Abemaciclib were diluted appropriately with diluent to obtain the concentration of 10 µg/ml

of the drug. The solution was scanned in the range of 200-400 nm. The absorbance maximum of Abemaciclib was found at 319 nm as shown in Fig. 3.

Preparation of standard drug solution:

Accurately weighed about 10 mg of Abemaciclib working standard was transferred in a 100 ml volumetric flask and dissolved by sonication in enough diluent then volume made with diluent (100 µg/ ml). Then 5 ml from the above stock solution was diluted up to 20 ml with the same diluent (25 µg/ ml).

Preparation of sample solution:

Twenty tablets of RAMIVEN (100 mg) were weighed and the average weight was calculated. The tablet contents were crushed to a fine powder. An accurately weighed quantity of tablet powder equivalent to 100 mg of Abemaciclib was dissolved in diluent and sonicated for 15 min. Final dilution was made up to 200ml with diluent (500 µg/ ml). Then 5 ml from the above stock solution was diluted up to 100 ml with the same diluent (25µg/ ml).

Method optimization:

The chemical structure of the Abemaciclib shows that the drug is basic hence base deactivated column Thermo synchronis column C18 column was selected for the retention of Abemaciclib. Initial trials were started with 0.1% TFA in water and Acetonitrile of different proportions until to get, good peak shape with better SST parameters. The mobile phase consisting of a mixture of 0.1% TFA in water, Acetonitrile in the ratio of (70:30 v/v) was found to be suitable for the retention of Abemaciclib, the Flow rate was kept at 0.7 ml/min and UV detection wavelength of 320 nm and column oven temperature maintained at 40˚C. Chromatographs are shown in Fig. 4 and 5.

VALIDATION OF METHOD: -

Validation of the developed method was done as per the ICH Q2 (R1) 22 guidelines with respect to various parameters such as linearity, accuracy, specificity, precision, and robustness. A standard solution of Abemaciclib was used for the comparison of results.

Linearity:

Appropriate aliquots from standard Abemaciclib stock solutions were prepared to obtain concentrations of 5- 100 µg/ml. The linear calibration plot was constructed by analyzing the

concentrations over the selected range versus the peak area of the sample. The response for the drug was linear in the concentration range between 5- 100 µg/ml. The linearity was observed in the expected concentration range, demonstrating its suitability for analysis Table 1 and Fig. 1.

Accuracy (Standard Addition method):

Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. A known amount of Abemaciclib (110, 120, and 130%) of the standard solution was added to the pre-analyzed solution of the formulation. This solution was analyzed as previously described. The assay was repeated over 3 injections of each concentration to obtain data. The resultant % RSD for this study was found to be < 2.0 % with a corresponding percentage recovery value as shown in Table 2.

Precision:

The precision of the System was ascertained by five replicate analyses of 25 µg/ml Standard solution of Abemaciclib. The precision of the method was ascertained by five replicate analyses of homogeneous samples of tablet powder at a concentration 25 µg/ml. The precision was studied by injecting freshly prepared working standard solution of the Abemaciclib table (3, 4, and 5).

Specificity:

Specificity of the method was established to ascertain how accurately and specifically the analyte of interest to be estimated in presence of excipients. The results of the specificity done by injecting Blank (Mobile phase), showed there was no interference and co-elution of any other peaks with the retention of Abemaciclib. The peak purity of Abemaciclib in Tablet dosage forms was found within the limit which proved that there was no interference of the blank peaks and excipients peaks at the retention time of Abemaciclib Fig. 6.

Robustness:

The Robustness of the method was established by the deliberate change in detection wavelength by ±2 nm, change in the temperature by ± 2°C, and flow rate by ±0.2 ml in the estimation of the tablet. The reproducible results were obtained which proves that method is robust as shown in Table no 6.

System suitability test:

As the system suitability test was an integral part of chromatographic methods development and was used to verify that the system is adequate for the analysis to be performed, the system suitability parameters for Abemaciclib were evaluated.

The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values, with the acceptance criteria of the ICH guidelines, such as RSD, tailing factor, theoretical plates, and retention time as shown in Table 7.

Assay:

For the quantitative estimation of Abemaciclib in tablet dosage form, six sample preparations of Abemaciclib were prepared and injected into the HPLC. The Mean, Standard deviation, and % RSD of Assay percentage of Abemaciclib sample solution were calculated. Results are shown in Table 8.

RESULTS AND DISCUSSION:

Novel and simple RP HPLC methods have been developed for the determination of Abemaciclib in Tablet dosage forms. The chromatographic conditions were optimized by taking into consideration the chemical structures of Abemaciclib, choice of the column with respect to chemistry of packing material, dimension of the column, composition, pH, flow rate of mobile phase, the wavelength of detection, and injection volume.

The optimized chromatographic condition was found satisfactory to yield a well retained, sharp, and symmetrical peak at 4.0 min. The number of Average theoretical plates was above 13,000 and the tailing factor was 1.504 for Abemaciclib, which indicates the efficient performance of the column.

The results of linearity studies over the concentration range 5-100 µg/mL showed the linear detector response with a correlation coefficient of 0.999 and the regression equation of y=10022x – 1372.

Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. Mean Recovery was observed to be 100.04% representing the accuracy of the method and non-interference of excipients.

Replicate estimations (n=6) of Abemaciclib by the proposed method have yielded 100.76% indicating substantially high precision of the method.

The intermediate precision study was ascertained based on intra-day and inter-day data obtained by analyzing Abemaciclib by the proposed method and it is found to be very much reproducible. This indicates the precision and Repeatability of the method. The method was sufficiently robust for normally expected variations in chromatographic conditions as per ICH guidelines such as wavelength, Temperature, and Flow rate.

CONCLUSION:

The developed HPLC method is simple, specific, accurate, and precise for Abemaciclib in the tablet dosage form. It was successfully validated in terms of linearity, accuracy, precision, specificity, and recovery in accordance with ICH Q2 (R1) guidelines. Thus, the described method is suitable for routine analysis and quality control of Abemaciclib in the tablet dosage form.

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TABLE 1: Abemaciclib Linearity study in the range of 5– 100 µg/ml

Linearity level	Concentration (µg/ml)	Area	r^2
1	5	511505	0.9999
2	10	101818	
3	25	2516187.67	
4	50	475218.33	
5	75	7479977.33	
6	100	10067811.3	

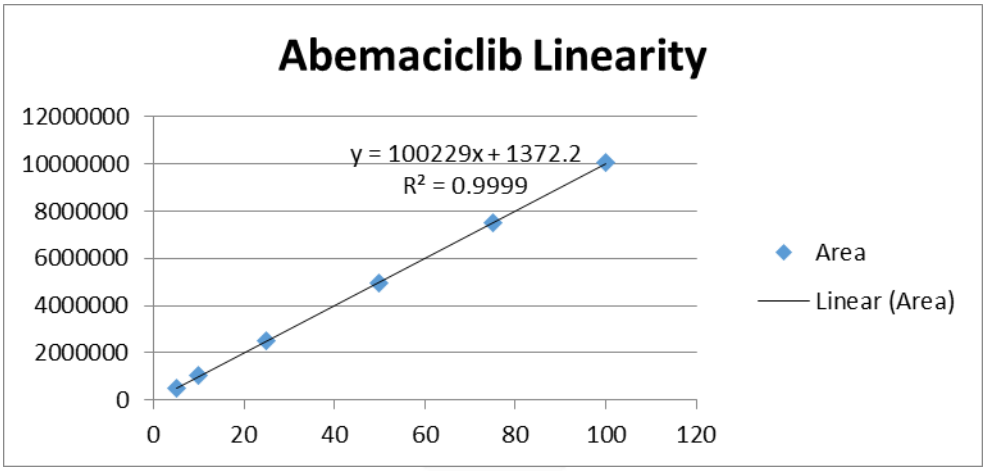

Fig. 1: Abemaciclib Linearity study in the range of 5– 100 µg/ml

TABLE 2: ACCURACY STUDY BY STANDARD ADDITION METHOD

% Level added	Sample spiked (µg/ml)	Amount Recovered (mg)	% Recovery	Mean % Recovery
110	5	109.48	99.55	99.6
110	5	109.53	99.6	
110	5	109.43	99.5	
120	10	120.01	100.0	100.01
120	10	120.15	100.1	
120	10	119.91	99.93	
130	15	130.75	100.6	100.5
130	15	130.62	100.5	
130	15	130.57	100.4	
			Mean	100.04
			±SD	0.30
	Limit	NMT 2%	%RSD	0.30313

TABLE 3: STUDY OF SYSTEM PRECISION DATA BY AREA

Injection No.	Area at 320nm	Limit
1	2363430	NMT 2.0 %
2	2373596	
3	2377182	
4	2373550	
5	2374712	
Mean	2372494	
± S.D.	5276.895489	
% RSD.	0.22	

TABLE 4: STUDY OF METHOD PRECISION DATA BY AREA

Injection No	Area at 320nm	Limit
1	2377508	NMT 2.0 %
2	2302076	
3	2399842	
4	2338460	
5	2377385	
6	2365693	
Mean	2360161	
SD	3477.07	
% RSD	1.47	

TABLE 5: INTERMEDIATE PRECISION

Sr no	Analyst A	Analyst B	% RSD
1	99.13	99.19	
2	99.06	99.16	NMT
3	99.00	99.24	2.0 %
MEAN	99.06	99.20	
SD	0.06	0.04	
%RSD	0.063	0.039	

TABLE 6: ROBUSTNESS DATA

Parameter	Change in Parameter (±)	% Estimation	Mean	S.D.	% R.S.D.
Wavelength (± 2 nm)	318	98.5	98.4	0.11	0.11
	320	98.3			
	322	98.5			
Temperature (± 2° C)	38	98.48	98.4	0.10	0.11
	40	98.31			
	42	98.50			
Flow rate (±0.2ml/min)	0.5	98.33	98.3	0.04	0.04
	0.7	98.31			
	0.9	98.39			
Change in Column		98.99 98.81 98.0	98.96	0.14	0.138

TABLE 7: SUMMARY OF SYSTEM SUITABILITY STUDY

Sr. No.	Area	Retention Time	Theoretical plate	Tailing Factor
1	2363430	3.980	13731	1.47
2	2373596	3.981	13137	1.50
3	2377182	3.978	13171	1.52
4	2373550	3.975	13282	1.51
5	2372712	3.971	13276	1.52
Mean	2372494	3.977	13319.4	1.504
SD	5276.895489	0.004062	238.7411	0.020736
% RSD	0.22	0.10	1.79	1.38
Limit	NMT 2.0 %	NMT 1.0 %	NMT2.0%	NMT2.0%

TABLE 8: QUANTITATIVE STUDY OF ABEMACILIB IN TABLET DOSAGE FORM

Sr. No.	Weight of standard (mg)	Sample Weight (equivalent to 100mg)	*Area of the standard at 320 nm	Area of the sample at 320nm	% Assay
1	10.16	293.59	2372494	2377508	101.61
2		293.77		2302076	98.32
3		295.30		2399842	101.97
4		293.49		2338460	99.97
5		293.76		2377385	101.54
6		293.45		2365693	101.15
Mean					100.76
± SD					1.38
% RSD					1.37

*Average of 6 determinations; SD is standard deviation; %RSD is relative standard deviation

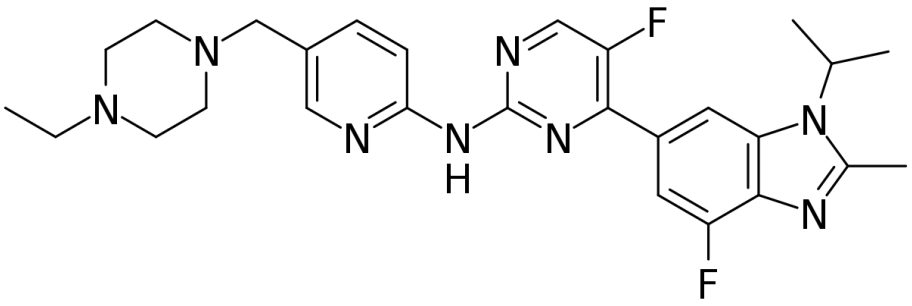

Fig. 2: Structure of Abemaciclib

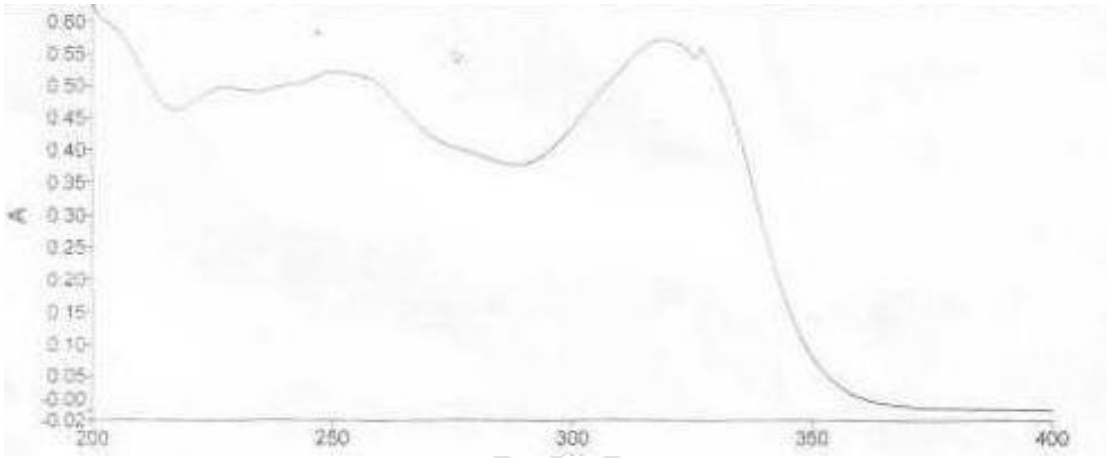

Fig. 3: Abemaciclib UV spectrum scanned in the range of 200 – 400 nm Wavelength about 320nm

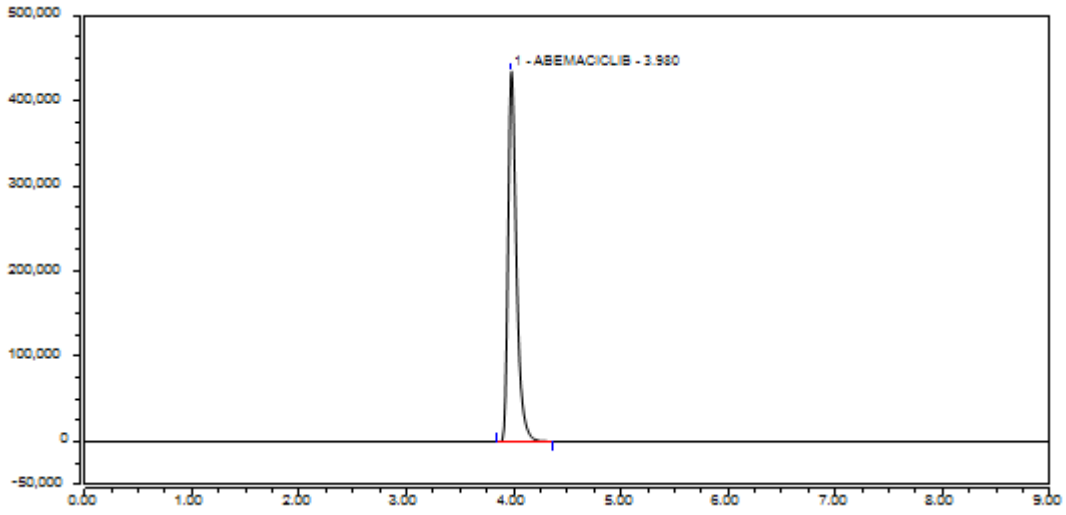

Fig. 4: Chromatogram of Abemaciclib standard solution of 25µg/ml

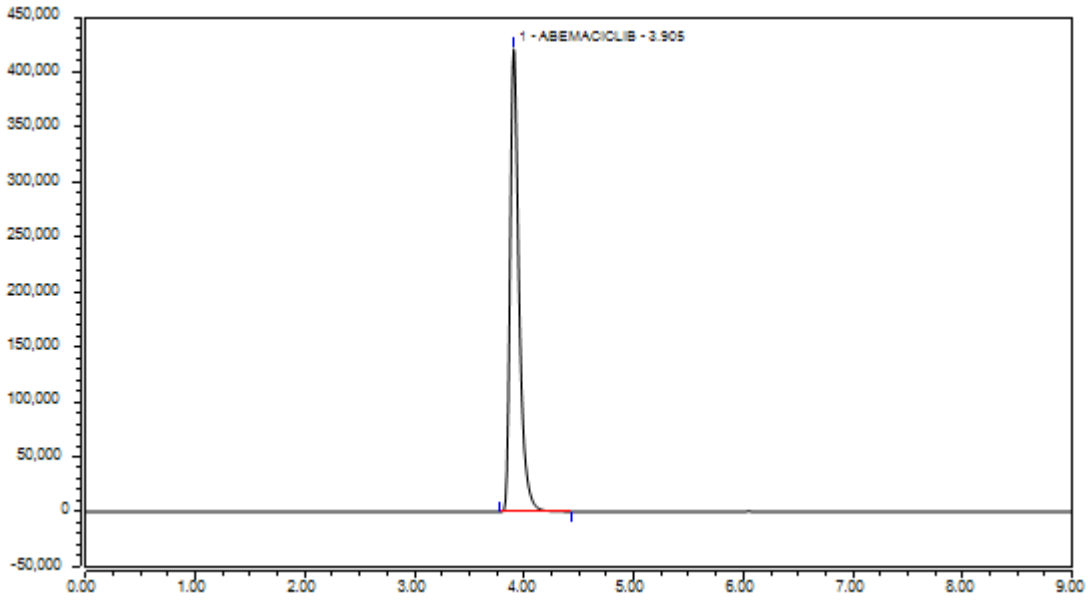

Fig. 5 Chromatogram of Abemaciclib sample solution 25 µg/ml

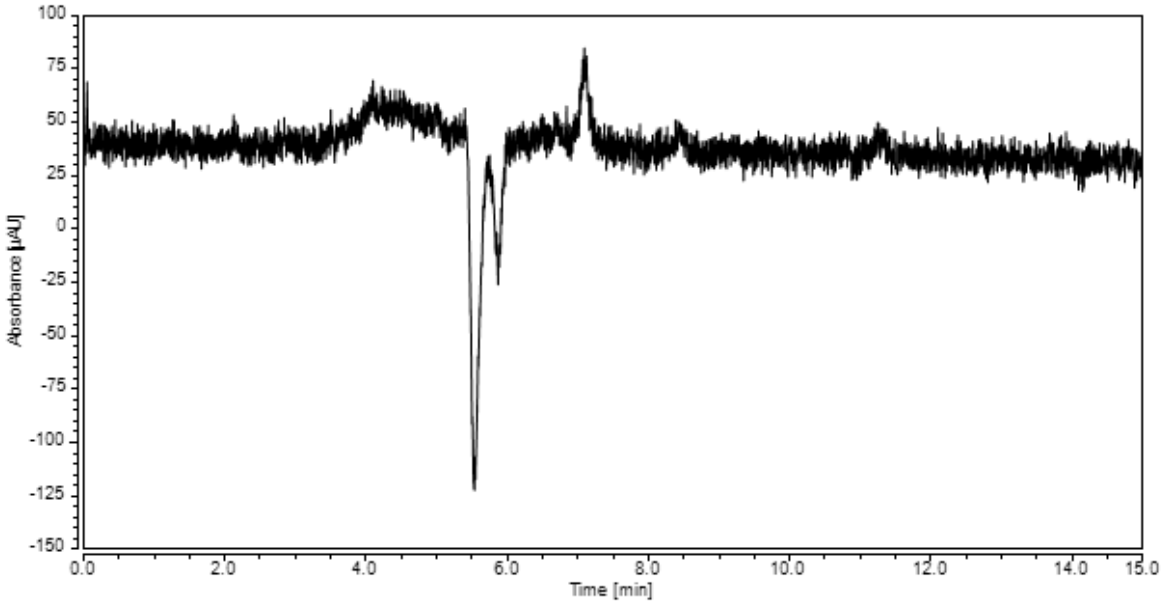

Fig. 6: Chromatogram of blank solution