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Analytical Method Development and Validation of RP-HPLC method for the Estimation of Abemaciclib Content in Tablet Dosage Form

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Abstract

A simple, precise, accurate and reproducible RP-HPLC technique has been developed and validated for the quantitative measurement of Abemaciclib in Tablet dosage form. The drug was analyzed isocratically and separated using an Inertsil ODS C18 column (250mm X 4.6mm, 5 µm). The mobile phase consisted of 25 mM di-potassium hydrogen orthophosphate and 0.1% triethylamine in water pH adjusted to 2.5 with orthophosphoric acid: Acetonitrile in a 60:40% v/v ratio, and the temperature of the column was kept at 40ºC and a flow rate of 0.7 ml/min was used. With the same mobile phase as the diluent, 10 µl of the solution was injected into the system and the peak area was measured at 298 nm. The developed RP-HPLC technique was validated in accordance with the ICH Q2 (R1) guidelines. The retention period of Abemaciclib was to be approximately 5.60 minutes. The drug demonstrated linearity within the concentration range of 5-100 µg/ml, with a correlation coefficient of 0.9999. The mean percent recovery of Abemaciclib was determined to be 99.96%. The limit of detection (LOD) and limit of quantification (LOQ) of Abemaciclib were determined to be 1.14µg/ml and 3.47µg/ml. The validation exercise revealed that the developed HPLC technique was accurate, specific, precise, rapid, reliable, and reproducible; hence, it can be used for quality control analysis of Abemaciclib in unitdosage form.

Keywords: Abemaciclib, RP-HPLC, Method Development, Validation

Introduction

The IUPAC 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. Itsempirical formula is $C_{27}H_{32}F_2N_8$ and the molecular weight is 506.06 g/mL. Its melting point is 175-181ºC. It is a light yellow crystalline powder with a pKa of 10.27 that dissolves in organic solvents such as acetonitrile and methanol but just slightly soluble in water [1-2].

Abemaciclib is an orally administered anti-cancer drug that targets HR^+ and HER^{2-} progressed or metastatic breast cancers. 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. On September 28, 2017, the FDA approved abemaciclib therapy, marketed as Verzenio, for the treatment of HR-positive and HER-negative advanced or metastatic breast cancer [3-4]. It is used alone in patients who have undergone hormone therapy and chemotherapy following cancer metastases, or in conjunction with Fulvestrant. Abemaciclib administered daily as a single drug or in combination with anti-estrogen, reduces tumor size. It improves progression free survival and objective response rates in patients with HR-positive, HER2-negative breast cancer after oral therapy [5].

A review of literature revealed that the monograph for abemaciclib is not official in any pharmacopoeia, and only a few chromatographic methods has been established for estimating abemaciclib in bulk and pharmaceutical formulation [6-11]. As a result, there was a need to develop new, simple rapid, precise, and accurate reverse phase chromatographic method for estimating abemaciclib in tablet dosage form. The proposed method has been validated using the ICH Q2 R1 guidelines.

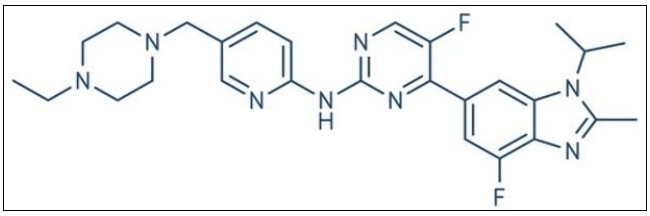

Fig 1: Chemical Structure of Abemaciclib

Materials and Methods

Chemicals and reagents:

CDTL, Mumbai provided an analytically pure Abemaciclib working standard with a stated potency of 99.90% (as is basis). The RAMIVEN 50 mg Abemaciclib commercial formulation was used for analysis. Acetonitrile and di-potassium hydrogen orthophosphate (HPLC grade) from Finar Ltd. were utilized.The Milli-Q® water purification unit (Millipore, Miliford, MA, USA) produced ultra-purified HPLC grade distilled water. A 0.45 µm high flow nylon membrane filter was acquired from Millipore (USA).

Instrumentation:

All spectroscopic measurements were performed on a Perkin Elmer UV/VIS Spectrophotometer Lambda 45 connected to Perkin Elmer UV Win Lab software. Thermo Scientific Ultimate 3000 HPLC system equipped with Chromeleon 7.4.2 was used for the analysis.The column utilized was an Inertsil ODS C18 column (250mm x 4.6mm, 5µ).

Selection of solvent:

Considering the chemical nature of Abemaciclib, the diluent for standard and sample preparations was 25 mM di-potassium hydrogen orthophosphate and 0.1% triethylamine in water pH adjusted to 2.5 with orthophosphoric acid: Acetonitrile in the ratio of 60:40% v/v.

Selection of wavelength:

Abemaciclib standard 10 mg was weighed accurately and transferred to a 100 ml volumetric flask. The volume was made up with diluent (100 µg/ml) and subsequently diluted to reach a concentration of 10 µg/ml. The solution was then scanned from 400.0 to 200.0 nm. Abemaciclib exhibited an absorption maximaat 298 nm.

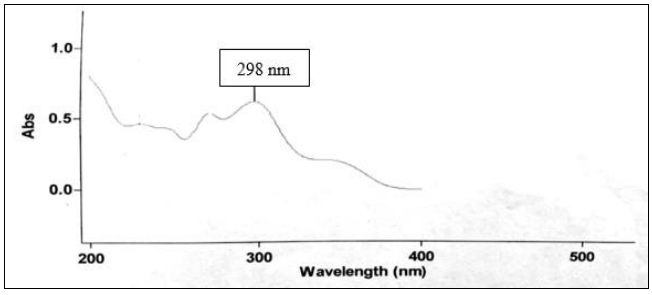

Fig 2: UV spectrum of Abemaciclib

Preparation of buffer solution:

A solution of 25 mM di-potassium hydrogen orthophosphate and 0.1% triethylamine in water was prepared and the pH was adjusted to 2.5 with orthophosphoric acid. It was transferred to a 1000 ml mobile phase bottle and the volume was made up with water. The solution was sonicated for a few minutes using an ultra sonicator and vacuum filtered using a 0.45 µm high flow nylon membrane filter.

Preparation of standard solution:

Mobile phase was used as a diluent to obtain the 50µg/ml concentration of the Abemaciclib standard solution.

Analysis of abemaciclib tablet:

The average weight was calculated using twenty tablets of Ramiven (50 mg). Sample equivalent to 50 mg of Abemaciclib was precisely weighed and transferred into a 100 ml volumetric flask, and dissolved in 25-30 ml of diluent. After 20 minutes of sonication the volume was adjusted with diluent. The sample was further diluted to obtain a concentration of 50µg/ml.

To estimate the drug content in the tablets, 10µl of both the standard and sample solutions were injected in triplicate.Percent assay, mean, SD and %RSD were calculated.

Method optimization:

Since Abemaciclib has basic molecular structure (pK_a=10.27 and pK_b=7.94), base deactivated column Inertsil ODS C_{18} was chosen to retain Abemaciclib. The Kromasil C18 column was used for the initial trials, which included a mobile phase of 50 mM ammonium acetate in water pH 5.5 adjusted with triethylamine: Acetonitrile (50:50% v/v). The injection volume was 10µl, and the flow rate was 1ml/min. However, there was unsatisfactory peak shape seeneven after the mobile phase component ratio was changed. The Luna phenomenex C18 column was subjected to additional trials with a mobile phase of25 mM di-potassium hydrogen orthophosphate and 0.1% triethylamine in water pH adjusted to 3.3 with orthophosphoric acid: Acetonitrile (70:30% v/v). However, the peak shape failed to match the system suitability standards. Using Inertsil ODS C18 column, additional trials were conducted with a mobile phase of 25 mM di-potassium hydrogen orthophosphate, and 0.1% triethylamine in water pH adjusted to 2.5 with orthophosphoric acid: Acetonitrile (60:40% v/v). A satisfactory peak shape and appropriate system suitability parameters were observed from the chromatogram under these conditions.

Utilizing an Inertsil ODS C18 (250 mm × 4.6 mm × 5 µ) column, the chromatographic conditions were set by injecting a standard solution of Abemaciclib (concentration 50µg/mL) with a mobile phase made up of 25 mM di-potassium hydrogen orthophosphate and 0.1% Triethylamine in water pH adjusted to 2.5 with orthophosphoric acid and acetonitrile in the ratio of (60:40% v/v) at 40ºC temperature. 10 µL injection volumes were chosen, and the flow rate was maintained at 0.7 ml/min with detection at 298 nm using a UV visible detector. The drug eluted at 5.60 min.

Validation of Method:

The developed chromatographic method was validated as per ICH guidelines for specificity, system suitability, linearity, precision, accuracy, recovery, LOD, LOQ, and robustness [12-14].

System suitability studies:

Studies on system suitability were conducted to evaluate the system's functionality. Six replicates of Abemaciclib standard solution (50µg/ml) and one injection of blankpreparation were injected into the HPLC. Chromatograms were recorded, the mean, SD, %RSD of

area and retention time were calculated. The tailing factor and theoretical plates were also calculated.

Table 1: Result of system suitability studies

System suitability				
S. No	**Peak area**	**Retention time**	**Theoretical plates**	**Tailing factor**
1	2179861	5.608	5174	1.36
2	2186505	5.606		
3	2192151	5.603		
4	2184665	5.608		
5	2189106	5.603		
6	2187675	5.607		
Average	2186661	5.606		
SD	3816.51	0.002		
%RSD	0.71	0.04		
Limit	NMT 2.0%	NMT 2.0%		

Specificity:

To determine specificity, a blank, standard drug solution (50µg/ml), and sample solution (50µg/ml) were injected into the HPLC and chromatograms were run. It establishes that the peaks observed in the standard and sample solutions at working concentrations are solely due to the drug, as the blank shows no peak at the retention time of Abemaciclib. As a result, it can be to conclude that the developed method is specific.

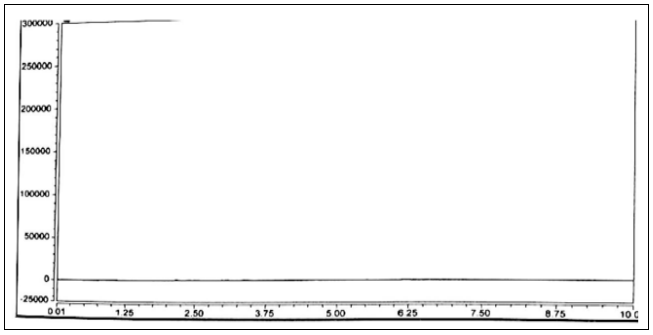

Fig 3: Chromatogram of blank solution

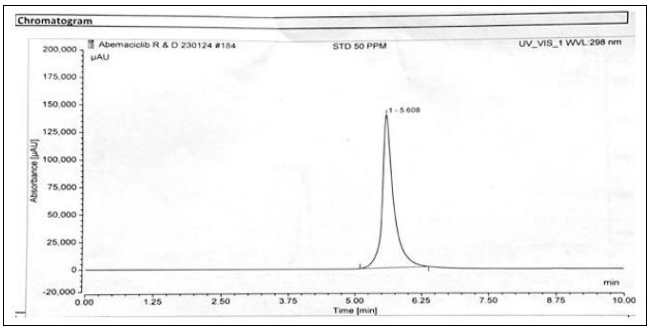

Fig 4: Chromatogram of Abemaciclib standard solution (50µg/ml)

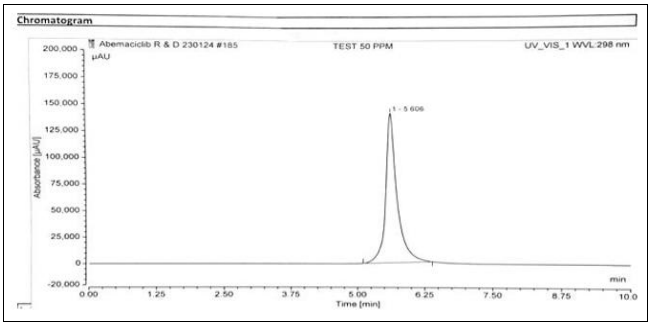

Fig 5: Chromatogram of Abemaciclib sample solution (50µg/ml)

Linearity:

Abemaciclib was studied for linearity in the concentration range of 5-100 µg/ml, with a correlation coefficient of 0.9999. It was discovered that y= 44229x-24932 is the regression equation. Plotting the linearity graph involved taking the drug's concentration on the X-axis and the corresponding peak area on the Y-axis, as illustrated in Fig 6.

Table 2: Linearity data of Abemaciclib

Concentration (ppm)	Area (AU)
5	205895
12.5	517852
25	1076992
37.5	1620534
50	2192151
62.5	2742932
75	3318121
100	4380063

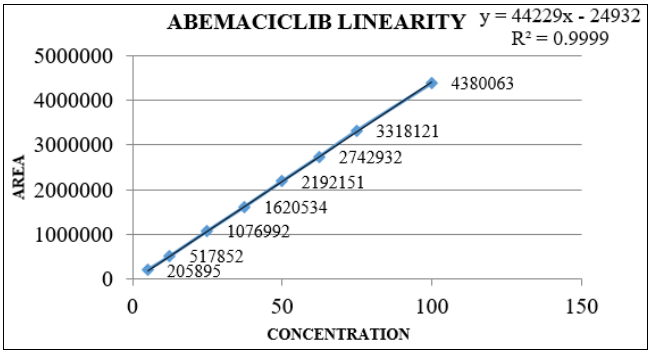

Fig 6: Linearity curve of Abemaciclib

Precision:

System precision: Six replicate injections of the standard solution (50µg/ml) were used to measure this parameter. Six replicate injections peak areas were measured, and the results included the mean, SD, and %RSD.

Method precision: Six replicates of the Abemaciclib sample solution (50µg/ml) were injected to the HPLC apparatus. Six replicate injections peak areas%assay, mean, SD, and %RSD were calculated and reported.

Intermediate precision: Here, six replicates of the standard solution (50µg/ml) and three replicates of the sample solution (50µg/ml) were injected into the HPLC system for the intermediate precision test, which was conducted on two separate days. It'spercent assay, mean, % SD, and % RSD were calculated. Table 5 provides a summary of the findings.

Table 3: System precision data of standard

System Precision	
Standard	**Area**
1	2115763
2	2106915
3	2118637
4	2119499
5	2122670
6	2116697
Mean	2116697
SD	4896.45
%RSD	0.23
Limit: NMT 2%	

Table 4: Method precision data of sample

Method Precision	
Standard	**%Assay**
1	99.13
2	99.64
3	99.58
4	98.87
5	99.36
6	99.10
Mean	99.28
SD	0.273
%RSD	0.27
Limit: NMT 2%	

Table 5: Abemaciclib Intermediate precision data

Intermediate precision		
	Day 1	**Day 2**
S. No	**%Assay**	**%Assay**
1	99.13	99.93
2	99.64	99.14
3	99.58	99.78
Average	99.45	99.62
Standard deviation	0.28	0.42
%RSD	0.278	0.418
Limit: NMT 2%		

Recovery and Accuracy (standard addition method)

Accuracy was assessed at three distinct levels of 110%, 120% and 130%, using addition method. Three assessments were made at each level. Calculations and reports of %Recovery and %RSD were made. Table 6 describes the results of accuracy and recovery.

Table 6: Accuracy data of Abemaciclib

% level	Standard Spiked (ml)	Amount recovered (mg/Tab)	% Amount recovered	% Recovery	% Mean recovery
100	0	49.65	99.30	99.43	99.96
110	0.5	54.94	109.88	100.06	
120	1.0	60.19	120.38	100.32	
130	1.5	65.09	130.18	100.54	

LOD and LOQ

The following formula was used to determine the limit of detection (LOD) and Limit of Quantification (LOQ) of abemaciclib based on the calibration curve method:

$$LOD = 3.3x\sigma/s,\ LOQ = 10 \times \sigma/s$$

Where σ, which is derived from the calibration curve, stands for the standard deviation of the response of the regression line and determined from the calibration curve. After calculations, the appropriate concentration solutions were made and injected.

Table 7: Abemaciclib LOD and LOQ data

Parameters	Abemaciclib
Linearity range (µg/ml)	5-100
Regressionequation (y= mx + c)	44229x – 24932
Slope (m)	44229
Intercept (C)	-24932
Correlation coefficient (R^2)	0.9999
LOD = 3.3 σ/S (µg/ml)	1.14
LOQ= 10σ /S (µg/ml)	3.47

Robustness

The degree of robustness was assessed by minor variations in method parameters, such as the ratio of mobile phase (±2% v/v), flow rate (± 0.02ml/min), column temperature (± 2ºC), and wavelength (± 2 nm). Under various chromatographic conditions, six replicate injections of the standard solution (50µg/ml) andsix replicate injections of the sample preparations (50µg/ml) were made and injected. Its mean, assay percentage, SD, %RSD wasdetermined and the results are summarized in Table 8.

Table 8: Results of Abemaciclib Robustness

Parameters	Change in parameter	Abemaciclib Estimation %	Mean	SD	%RSD	Limit of %RSD
Mobile phase ratio (±2% v/v)	58:42	100.96	100.21	0.31	0.31	NMT 2%
	60:40	99.61				
	62:38	99.53				
Flow rate (±0.2 ml/min)	0.5	100.06	99.80	0.48	0.48	
	0.7	99.59				
	0.9	99.77				
Temperature (±2ºC)	38	99.22	99.28	0.37	0.37	
	40	99.68				
	42	98.95				
Wavelength (±2 nm)	296	99.02	99.09	0.59	0.37	
	298	99.72				
	300	98.55				

Assay:

Six abemaciclib sample preparations were made and injected into the HPLC. The assay % of the Abemaciclib sample solution was calculated, along with the mean, standard deviation, and percent RSD.The amount of Abemaciclib in the Tablet formulation was 99.28%. The outcomes are shown in Table 9.

Table 9: Result of Abemaciclib Assay

S. 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	10.27 mg	143.83	2116697	1982502	99.13
2			143.78		1992288	99.64
3			143.89		1992297	99.58
4			144.46		1992295	98.87
5			143.79		1986588	99.36
6			144.38		1988679	99.10
Mean						99.28
SD						0.273
%RSD						0.273

Result and Discussion

Abemaciclib content of a marketed tablet dosage form has been determined using a simple, precise, and accurate RP-HPLC method. The optimized chromatographic conditions were found to be adequate for producing a well retained, sharp, and symmetrical peak at 5.60 min. The method was confirmed to be linear in the concentration range of 5-100 µg/ml, with a correlation coefficient of 0.9999.Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate.Percent Recovery was observed to be 99.96 % representing the accuracy of the method and non-interference of excipients. The method was sufficiently

robust for normally expected variations in chromatographic conditions.

In the terms of specificity, no interference from the blank (diluent) was detected at the abemaciclib peak retention time period. The abemaciclib sample solution's mean percent recovery and accuracy was determined to be 99.96%, falling between the range 98 to 102%. It was discovered that the LOD and LOQ values were 1.14µg/ml and 3.47µg/ml, respectively. Since the developed method can detect and quantify the analyte at very small concentrations, the lower values of LOD and LOQ showed that it is accurate, precise, and sensitive.

The robustness of method was demonstrated by the reproducible results produced and the %RSD of several robustness parameters being less than 2.0. The new approach is suitable for routine analysis of abemaciclib in its tablet dosage form, as confirmed by high recovery values and low SD and %RSD.

Conclusion

The suggested RP-HPLC method was simple, selective, sensitive, rapid, and precise for qualitative and quantitative determination of abemaciclib in tablet dosage form. Regarding superior system suitability characteristics like theoretical plates, tailing factor, the developed method performs better than previously published studies. All of the validated acceptance criteria were met, and the method was validated accordance with ICH guidelines. As a result the method presented here is appropriate for routine quality analysis and quality controls of pharmaceutical formulation, specifically tablet unit dosage form.

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