RESEARCH ARTICLE

Development and Validation of a Novel Stability-Indicating Reverse Phase High-Performance Liquid Chromatography Method for the Quantification of Capivasertib in Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT

The quantification of capivasertib has been achieved using a Waters Symmetry column with UV detection at 260 nm through a novel stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method that has been established and verified. The analysis can be completed in just 5 minutes. The separation and collection of capivasertib occurred at a retention time of 3.3475 minutes. The concentration range of capivasertib was shown to be linear, ranging from 50 to 300 µg/mL. The regression equations for capivasertib were determined as y = 13485.85x + 1723.04. The detection limit for capivasertib was determined to be 0.6 µg/mL, while the quantification limits were established at 2.00 and 0.5000 µg/mL, respectively.

Keywords: Capivasertib, Acetonitrile, Ammonium acetate, ICH, HPLC, Wavelength.

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INTRODUCTION

As a result of its sensitivity to estrogen but insensitivity to HER2, estrogen receptor-positive and HER2-negative breast cancer is the most common metastatic subtype of the disease. More than 400 thousand people die every year from this subtype. Once resistance develops to endocrine-based therapy, the first line of treatment, chemotherapy becomes the final resort. However, it is often fruitless. As a result, developing drugs that are specific to an individual's genetic makeup has been an area of intense study.[1] The development of capivasertib, a pan-AKT kinase inhibitor, suggests that focusing on the PIK3/AKT pathway may one day be an approach to treating breast cancer.[2,3] In November 2023, capivasertib received approval for use in medicine inside the United States.[4] The application for capivasertib fast track designation was approved by Food and Drug Administration (FDA).[5] Figure 1 shows that capivasertib with empirical formula is $C_{21}H_{25}ClN_6O_2$ with molecular weight of 428.915. According to the literature survey, only one bioanalytical method was developed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).[6-8] The International Council of Harmonization (ICH) recommendations acceptability range was followed while validating the chromatographic parameters.[9]

MATERIALS AND METHODS

Reagents and Chemicals

For this work, a Waters Alliance HPLC system with a 2695 pump with Empower 2 software, an auto-injector, a UV detector, a Shimadzu UV-visible spectrophotometer, and a Phoenix 4.5 L digital ultrasonic cleaner was utilized. The API sample for capivasertib was procured from the Pharma Life Research facility located in Hyderabad, India. Additionally, the chemicals used were of AR-grade, as reported by Rankem Chemicals, India.

Standard Solution Procedure

Precisely measure 20 mg of the capivasertib standard into a 10 mL, dry, clean volumetric flask. After that, include the diluent and use sonication to ensure complete dissolution of the entire mixture. Finally, ensure that this is done correctly by using the same solvent (Order of stocks). The 10 mL volumetric flask should contain 1-mL of the stock solution mentioned earlier. This solution needs to be diluted with diluents until the desired final volume is reached capivasertib, 200 ppm.

Sample Solution Preparation

Accurately weigh 44 mg of capivasertib and transfer it into a 10 mL dry volumetric flask. To dissolve it, add the diluent

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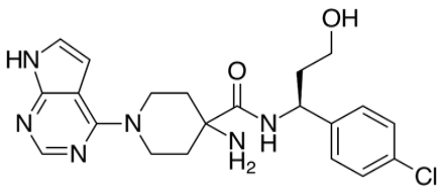

Figure 1: Structure of capivasertib

and sonicate it for up to half an hour. After centrifuging it for half an hour to completely dissolve it, use the same fluid to fill the flask to the brim. Next, it is cleaned using a 0.45-micron syringe filter (Stock solution). Next, fill a 10 mL volumetric flask with 1-mL of the aforementioned stock solutions using a pipette. Completely fill the flask with the diluent (200 parts per milliliter of Capivasertib).

Chromatographic Conditions

Different ratios for the mobile phase, which is made up of acetonitrile and ammonium acetate, were examined in order to obtain a sharp peak and ensure that capivasertib was well resolved. The first solution tested was acetonitrile: ammonium formate pH 2.5 adjusted with OPA (40:60% v/v); this resulted in a longer peak retention duration of 9.054 minutes. Acetonitrile was then changed, and the following modifications were made: ammonium acetate pH 3.0 corrected with OPA (60:40% v/v); Rt at 3.986 minutes with several unknown peaks generated. The selected ultimate mobile phase, consisting of a mixture of acetonitrile and ammonium acetate with a pH of 3.0 that was adjusted with OPA buffer in a ratio of 60:40 (volume/volume), resulted in a clearly defined and sharp peak. Capivasertib exhibited a prominent peak with a retention time of 3.347 (Figure 2).

Analytical Method Validation

System suitability

System suitability studies were conducted on a recently created standard solution of capivasertib to analyze optimal parameters, including theoretical plates, resolution, and tailing factor.

Accuracy

The traditional additional method was used to perform the accuracy method. The mean recovery of capivasertib was measured using drug concentrations at different levels (50, 100 and 150%).

Precision

On the same day, capivasertib was taken for intraday precision. On three separate days, different operators performed interday precision at a similar concentration. Six times the regular solution was injected and the area of each injection was calculated in high-performance liquid chromatography (HPLC).

Linearity

The linearity was achieved by diluting the stock solution of capivasertib with the mobile phase to a concentration range of

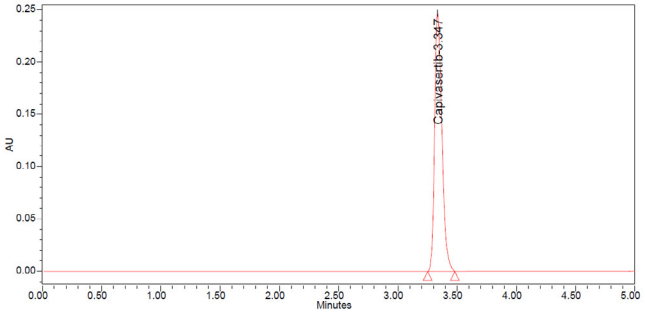

Figure 2: Capivasertib optimized chromatogram with a retention time of 3.347 minutes

50 to 300 µg/mL. An assessment of linearity was conducted using the least square approach and linear regression analysis.

Limit of detection and Limit of quantitaion

The limit of detection (LoD) was determined using the ocular assessment method, which involves identifying the minimum concentration at which the analyte can be detected. The limit of quantification (LoQ) is the lowest concentration of analytes that can be properly and precisely detected and quantified.

Forced degradation studies

The ICH guidelines recommend stress testing as a method to assess the inherent stability of drug substances. The solution of the standard underwent various degradation methods in these investigations, including "acid degradation, alkali degradation, peroxide degradation, thermal degradation, photolytic degradation, and hydrolysis". The peak areas of the samples under stress were determined and compared to the peak areas of the standard.

RESULTS AND DISCUSSION

Many mobile phases with varying compositions and flow rates were tested in order to establish a specific, exact, and accurate stability indicating reverse-phase high-performance liquid chromatography (RP-HPLC) technique for capivasertib quantification utilizing stressed samples. Chromatographic conditions were refined and developed following several compositions and permutations. The mobile phase that produced clear and defined peaks was Acetonitrile: Ammonium acetate of pH-3.0 adjusted with OPA (60:40 v/v). The injection volume was 20 µL, and the flow rate was set at -mL/min. There was a clear peak of capivasertib observed at a retention time of 3.3475 minutes.

System Suitability Test

Validating analytical techniques and confirming resolution among numerous peaks of interest requires the system suitability test. Given the conditions outlined in Table 1, a tailing factor of less than two and a theoretical plate of greater than 2000 indicate that the technique was effective.

Specificity

The retention period for capivasertib was 3.347 minutes. The retention times of the medications, including those of the blank and placebo, did not show any interfering peaks when using this

Table 1: System suitability parameters for capivasertib

Parameter	*Capivasertib*
Retention time	3.347
Plate count	17854
Tailing factor	0.96
%RSD	0.21

method. This procedure is known for its specificity. The sample and blank chromatograms are displayed in Figures 3 and 4.

LoD and LoQ

The limit of detection values for capivasertib were determined to be 0.6 µg/mL. The limit of quantification values for capivasertib were found to be 2.0 µg/mL, respectively.

Linearity

The calibration curve data was used to assess the linearity of the technique, and for capivasertib, the linear value was found to be between 50 and 300 µg/mL. The findings showed a significant correlation between drug concentration levels within the concentration range and detector response. A linear response for capivasertib is seen in Figure 5. The capivasertib results are listed in Table 2.

Precision

The precision investigation revealed that the total %RSD of system method precision was below 2, indicating effective achievement of precision within the specified limit. Table 3 provides the outcomes of system precision and technique precision.

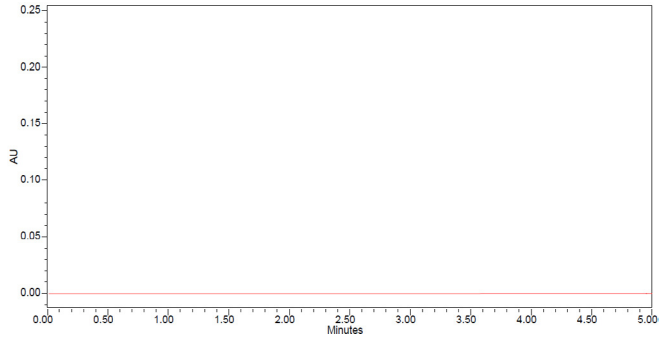

Figure 3: Chromatogram of blank

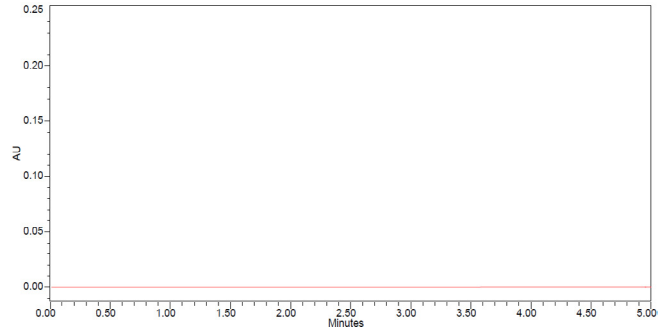

Figure 4: Chromatogram of placebo

Table 2: Results of linearity for capivasertib

S. No	*Capivasertib*	
	Concentration (µg/mL)	*Peak area*
1	50.00	681818
2	100.00	1388822
3	150.00	1943374
4	200.00	2745546
5	250.00	3350828
6	300.00	4061815
Regression equation	y =13485.85x + 1723.04	
Slope	13485.85	
Intercept	1723.04	
R^2	0.99957	

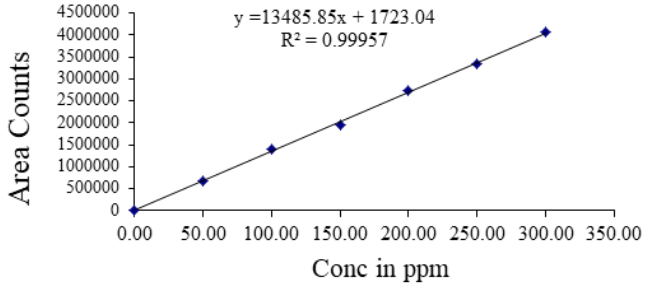

Figure 5: Calibration curve for capivasertib at 260 nm

Accuracy

Then recovery was estimated at 50, 100 & 150% of the selected concentrations. Capivasertib recovery values were determined to be 99.8 to 100.02%, respectively, as stated in Table 4.

Assay of tablets

The market currently offers only one manufacturer brand name, TRUQAP (AstraZeneca). According to Table 5, the percentage assay for capivasertib was determined to be 99.6%.

Robustness

The analytical procedure's robustness was established and developed through adjustments to the flow rate, pH, wavelength, and mobile phase composition. The data on the robustness of both drugs has been included in Table 6.

Table 3: Precision results of capivasertib (200 µg/mL)

	Concentration capivasertib (200 µg/mL)		
Parameters	*System precision*	*Repeatability*	*Intermediate precision*
Mean	2748189	2744069	2740100
S.D	5764.790	12140.70	9817.55
%RSD	0.21	0.44	0.36

Table 4: Results of capivasertib accuracy determined using the HPLC method

%Concentration	*Average area*	*Average %Recovery*	*Mean %Recovery*
	1371822	99.8	
50	1385478	100.8	100.2
	1373937	100.0	
	2751471	100.1	
100	2747241	100.0	99.9
	2738687	99.7	
	4098617	99.4	
150	4077314	98.9	99.8
	4161436	100.9	

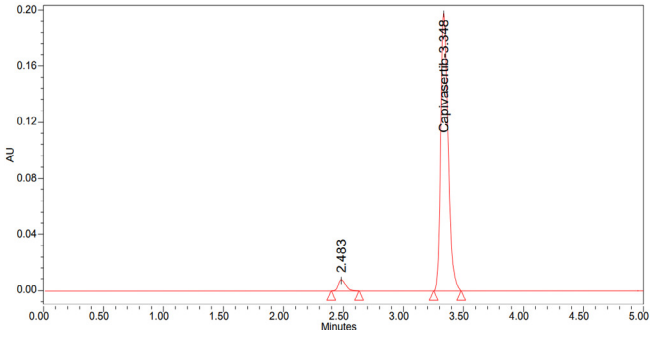

Figure 6: Acid degradation

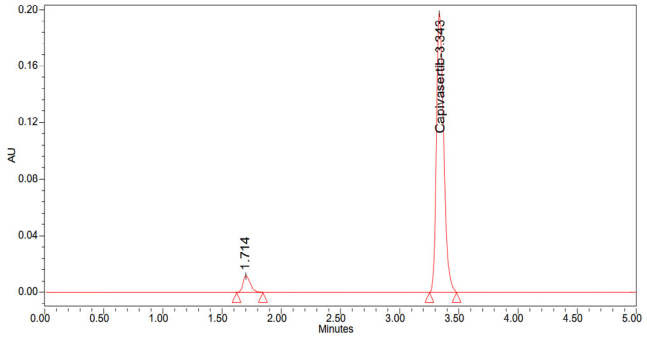

Figure 7: Alkali degradation

Forced degradation studies

The suggested HPLC approach was employed to periodically monitor the behavior of deterioration. Furthermore, the drug exhibited increased degradation when exposed to acidic, alkaline, peroxide, and thermal conditions. The elution of peaks of degradation occurred under various conditions, including acidic, thermal, peroxide, and basic. Table 7 displays the findings of the stability investigation. Figures 6–12 show the chromatogram peaks of degradation research.

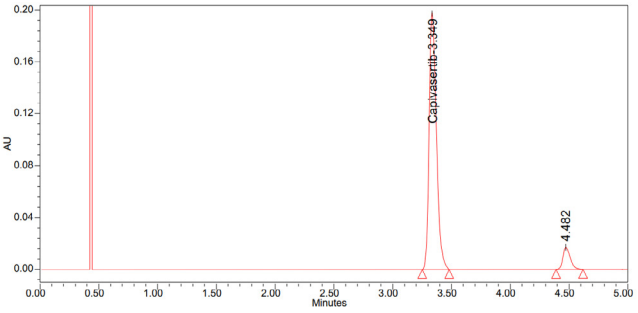

Figure 8: Peroxide degradation

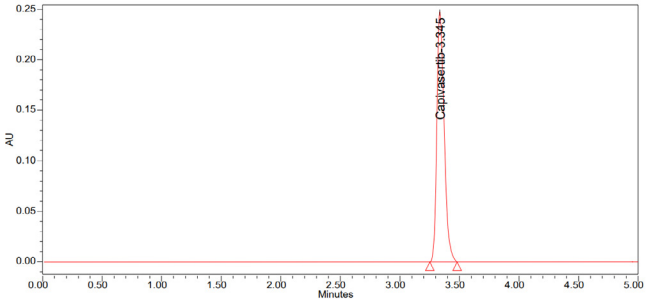

Figure 9: Reduction degradation

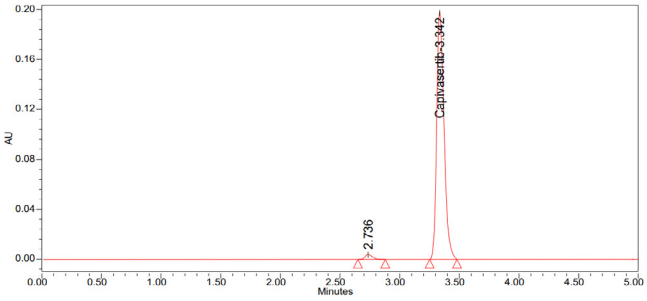

Figure 10: Thermal degradation

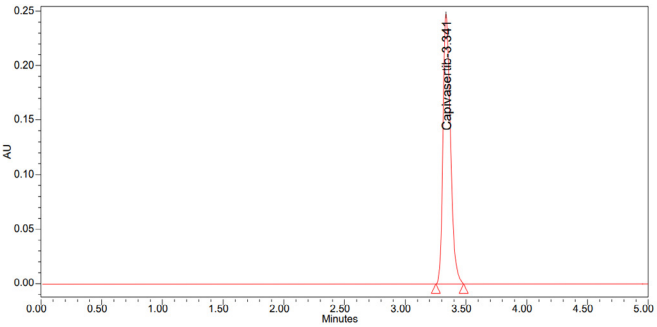

Figure 11: Photolytic degradation

Table 5: Assay results of capivasertib

Drug	*Average sample area*	*Std. wt. (mg)*	*Sample wt. (mg)*	*Label amount (mg)*	*Amount found (µg/mL)*	*%Assay*
Capivasertib	2737955	20	44	200	19.93	99.6

Table 6: Robustness results of capivasertib by HPLC

Parameter	*Condition*	*Tailing*	*%RSD*
Flow rate change (mL/min)	Less flow (0.9 mL)	0.95	0.65
	More flow (1.1 mL)		
Organic phase change	Less Org (54:46)	0.89	0.40
	More Org (66:44)	0.88	0.15
		0.97	0.31

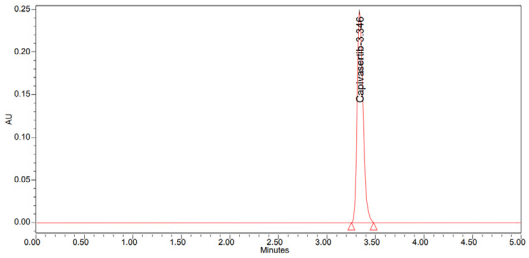

Figure 12: Hydrolysis degradation

Table 7: Forced degradation results for capivasertib

Degradation condition	*%Assay*	*%Degradation*	*Purity angle*	*Purity threshold*
Acid	87.2	11.7	0.451	7.656
Alkali	86.8	12.1	0.454	7.629
Peroxide	83.3	15.6	0.463	7.616
Reduction	98.4	0.5	0.447	7.608
Thermal	88.4	10.5	0.413	7.633
Photolytic	97.7	1.2	0.429	7.667
Hydrolysis	95.2	3.7	0.452	7.682

In this work, the HPLC method was used to efficiently analyze and achieve a successful separation of capivasertib. To achieve this separation, a mixture of acetonitrile and ammonium acetate with a pH of 3.0, which had been adjusted with OPA at a ratio of 60:40 v/v, was utilized. Under these circumstances, capivasertib showed a strong peak with a retention time of 3.347. It was discovered that they exhibited linearity within the respective range of 50.00 to 300 µg/mL capivasertib. There is now just one manufacturer brand name TRUQAP (AstraZeneca) on the market, and for capivasertib, its percentage assay was found to be 99.6%. The recommended HPLC method was applied in order to routinely track the deterioration's behavior. Furthermore, the drug was more easily broken down in alkaline, thermal, peroxide, and acidic environments. Degradation peaks were eluted in heat, peroxide, acidic, and basic environments.

CONCLUSION

The experimental modalities were effectively validated in accordance with ICH guidelines and routine analysis procedures. Recovery studies and preliminary analysis of a standard sample were utilized to validate the proposed method. A validated RP-HPLC method that indicates stability has been devised to quantify capivasertib in both pharmaceutical dosage form and bulk. The proposed technique is characterized by its simplicity, speed, accuracy, and precision. The statistical assessment of the suggested methodology unveiled its favorable linearity and validation across various parameters, leading us to the deduction that it might be implemented for the expeditious and dependable quantification of capivasertib in pharmaceutical formulations.

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