RESEARCH PAPER

Stability indicating Method Development and Validation of Inavolisib and its impurities by RP-HPLC Method

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ABSTRACT

Aim: The present study describes about the development and validation of novel RP-HPLC analytical method for the analysis of Inavolisib and its impurities and to carry out stability studies by forced degradation to estimate the stability of drug and its impurities. The proposed method was novel for the estimation of anti cancer drug. Materials and Methods: Based on the solubility studies carried out at 25oC using variety of solvents and buffers, Acetonitrile and Ammonium formate pH-2.5/formic acid (40:60) were found to be suitable mixture of solvents for mobile phase system. The analysis of drug is accomplished by using Waters X-Terra RP-18 column (150mm x 4.6mm ID, 3.5µm) at 259 nm. Results and Discussions: The method is then validated according to the ICH guidelines. The analytical results revealed that the method developed is within the limits for all the validated parameters. The forced degradation studies revealed that 3.25% and 3.46% of Inavolisib degradation was observed in presence of peroxide and alkali conditions. Summary and Conclusion: From the results of the study, the sample recoveries were found within the limits. Hence the proposed method can be conveniently and easily adopted to the determination of Inavolisib and its impurities...

Keywords: Inavolisib, RP-HPLC, Method Development, Validation, Forced Degradation....

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INTRODUCTION

Breast cancer is one of the most common cancers that affect women. It happens when cancerous cells in your breasts multiply and become tumors. About 80% of breast cancer cases are invasive (a tumor may spread from your breast to other areas of your body). Breast cancer typically affects women age 50 and older, but it can also affect women who are younger than 50.[1,2] Inavolisib is a PIK3CA inhibitor[3] and is used in combination with other antineoplastic agents to treat selected adults with locally advanced or metastatic breast cancer. It is indicated in combination with palbociclib and fulvestrant for the treatment of adults with endocrine-resistant, PIK3CA-mutated, hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, locally advanced or metastatic breast cancer.

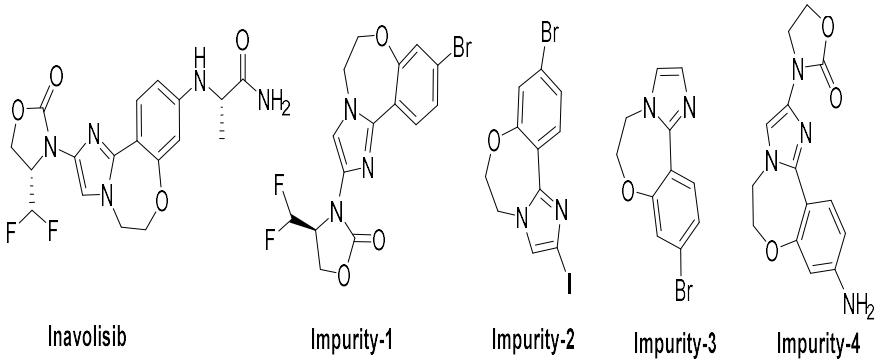

Figure 1: Structures of Inavolisib and its impurities.

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The present study is aimed to develop a stability indicating RP-HPLC method for simultaneous estimation and validation of Inavolisib with its impurities. The results were shown to be within the acceptable limits for all the validated parameters and the drug was found to be stable under various forced degradation conditions.

Materials and Methods:
Inavolisib and its impurities were procured from Genentech, Inc. Hyderabad. Acetonitrile (HPLC grade), Ammonium formate (AR grade), Formic acid (HPLC grade) were purchased from Spectrochem. HPLC of Alliance (Waters e 2695-Empower software 2.0 version) equipped with PDA detector, Shimadzu/AY220 Analytical balance, ultrasonicate water bath from PCI Analytics-Mumbai and pH meter of Digisun electronics were used in the study.

Chromatographic Conditions:
The identification of Inavolisib and its impurities were achieved by using Waters X-Terra RP-18 (150mm x 4.6mm ID, 3.5µm) column with isocratic elution using Acetonitrile and Ammonium formate pH-2.5/formic acid (40:60) as solvent. 1.0 mL/min is the flow rate set with a run time of 10 min. The wavelength for detection is set at 259 nm with the temperature of the column is set at 25°C. 10 µL is the injection volume used for the determination.

Preparation of standard stock solution:
Accurately weigh and transfer 10mg of Inavolisib working standard into a 10 ml volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of impurity stock solution:
Weigh accurately 5mg each of Inavolisib impurity-1, Inavolisib impurity-2, Inavolisib impurity-3 and impurity-4 and transferred into 10 mL volumetric flask and add 7 mL of diluent and sonicate up to 30 mins and make volume up to the mark with the same diluent. Further dilute 1mL of the above solution into a 10 mL volumetric flask and make up to the mark with diluent.

Preparation of spiked standard solution:
Transfer 1 mL of standard stock solution and 1 mL of impurity Stock solution in 10 mL volumetric flask and add diluent upto the mark. Filter through 0.45µ filter paper.(100ppm of Inavolisib and 5ppm of impurity-1, impurity-2, impurity-3 and impurity-4)

Preparation of sample stock solution:
Accurately weigh and transfer 70 mg of Inavolisib sample into a 10 ml volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of sample solution:
Pipette 1ml of the above sample stock solution and 1ml of imp stock solution into a 10 ml volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter through 0.45µ filter paper. (100ppm of Inavolisib and 5ppm of impurity-1, impurity-2, impurity-3 and impurity-4)

Preparation of Mobile Phase:
40 volumes of acetonitrile, 60 volumes of ammonium formate (pH adjusted to 2.5) are combined to create the combination. For the purpose of degassing the mobile phase, the mobile phase has been sonicated for 10 min in order to remove gases.

Method Validation:
Linearity:
Inavolisib (10.0 mg) and impurities 1 to 4 (5.0 mg each) is weighed accurately in volumetric flask of 10 mL. The concentrations of 25, 50, 75, 100, 150 ppm and 1.25, 2.50, 3.75, 5.0, 6.25, 7.25 ppm were prepared for Inavolisib and impurities 1 to 4 respectively for linearity studies.
Accuracy:
"Standard addition method" was used to determine the accuracy of both the developed methods. Standard addition at three addition levels i.e. 80% Low Concentrations (LC), 100% Intermediate Concentrations (IC) and 120% High Concentrations (HC) were carried out as per ICH quality guidelines. Percent recoveries were calculated as per following formula:
% Recovery = [Conc.(spiked)-Conc.(unspiked)x100] / Conc. (added)

Method precision
Inavolisib and impurities 1-4 sample preparations were made in compliance with the test technique and administered 6 times to the column. The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 100ppm of Inavolisib, 5ppm of Impurities 1-4. The % RSD for the absorbance of six replicate injections results should not be more than 2%.

System Suitability:
Tailing factor for the peak due to impurity of Inavolisib and its impurities (1-4) in Standard solution should not be more than 2.0. Theoretical plates for the impurity of Inavolisib and its impurities peak in Standard solution should not be less than 2000.

Robustness:
As part of the Robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

Flow rate Variation:
Standard solution 100ppm of Inavolisib, 5ppm of Imp-1, imp-2, imp-3 and imp-4 were prepared and analysed using the varied flow rates along with method flow rate.

Organic Phase ratio Variation:
Standard solution 100ppm of Inavolisib, 5ppm of Imp-1, imp-2, imp-3 and imp-4 were prepared and analysed using the varied in mobile phase ratio.

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Limit of detection (LOD) and limit of quantification (LOQ):

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines.

LOD = 3.3 X σ /S

LOQ = 10 X σ /S

Where, σ=Standard deviation of response, S = Slope of calibration curve.

Forced degradation Studies:

A procedure known as forced degradation involves subjecting a medicinal product to varied stresses, which causes a variety of degradation products to be produced. These studies are sometimes named to be stress test and stress degradation studies. These methods are mostly employed for determining a molecule's stability under time-sensitive conditions. In the present study, the selected compounds were subjected to stress conditions like Thermal degradation, Photolytic degradation, Acid degradation, Base degradation and Peroxide degradation

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Results:

In the present work, a novel RP-HPLC method is developed for the simultaneous estimation of Inavolisib and impurities 1-4. The method employed uses fewer organic solvents and maintain a minimal mobile phase without the addition of buffer salts or pH correction. Inavolisib retention time was determined to be 8.049 min, while impurities 1-4 was 2.605, 3.733, 5.358 and 6.619 min respectively (Figure XX). The created approach was validated using ICH Q2 (R1) guidelines to see if it was accurate for analyzing Inavolisib and its impurities.

Method Development:

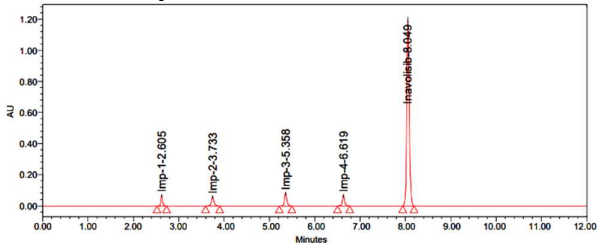

Figure 2: Chromatogram of Inavolisib and its impurities.

Linearity

A linear relationship was exhibited in the calibration curve between the area of peak and concentration ranging from 25-150 µg/mL. Chromatograms of six preparations have been obtained as shown in Figures 3, 4, 5, 6, 7 and 8. Linear function gave the best correlation (0.999 ± 0.002)

Accuracy:

A Study of Accuracy for Inavolisib and its impurities in triplicate (80%, 100% and 120%) as per test method with equivalent amount of drug containing Inavolisib and its impurities into each volumetric flask for each spike level to get the concentration of Inavolisib and its impurities equivalent to 80%, 100% and 120% of the labeled amount as per the test method. The average % recovery was calculated. (Table 1).The mean % recovery of the Inavolisib and its impurities at each level should be not less than 98% and not more than 102%.

Method precision:

The average % assay of six injections in triplicate found to be 99.72% for Inavolisib and 100.15, 99.53, 100.11, 100.38% for impurities respectively (Table 2). The % RSD is found to be less than 2.0%.

System suitability:

The percentage RSD for peak area is found to be 0.48 for Inavolisib and 0.13, 0.19, 0.23, 0.28 for impurities respectively (Table 3). The other factors such as theoretical plates and tailing factor is found to be within the limits.

Robustness:

The percentage RSD for two different parameters such as flow rate (±10%) and organic composition (±10%) is found to be less than 2.0% as shown in Table 4.

Forced degradation studies:

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive, thermal degradation, photolytic and hydrolysis degradation. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

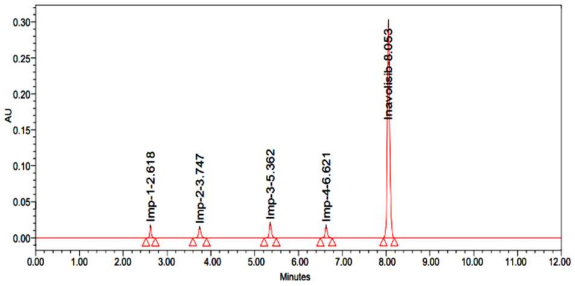

Figure 3: Chromatogram of Inavolisib and Impurities (Preparation-1).

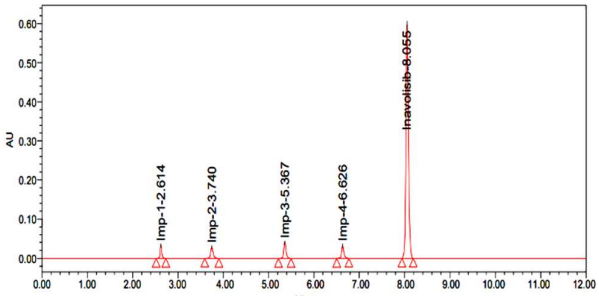

Figure 4: Chromatogram of Inavolisib and Impurities (Preparation-2).

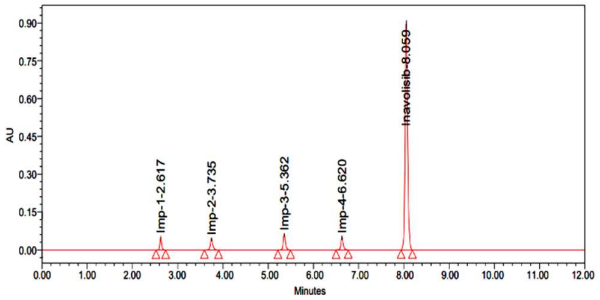

Figure 5: Chromatogram of Inavolisib and Impurities (Preparation-3).

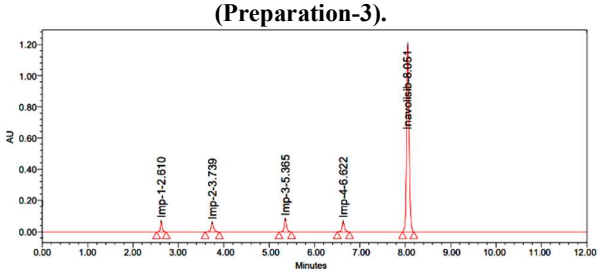

Figure 6: Chromatogram of Inavolisib and Impurities (Preparation-4).

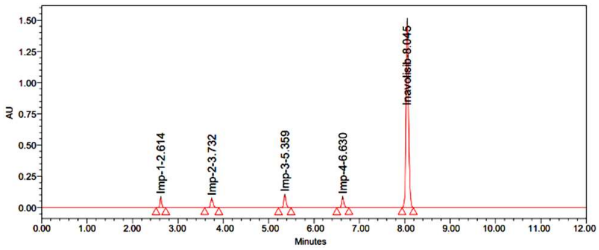

Figure 7: Chromatogram of Inavolisib and Impurities (Preparation-5).

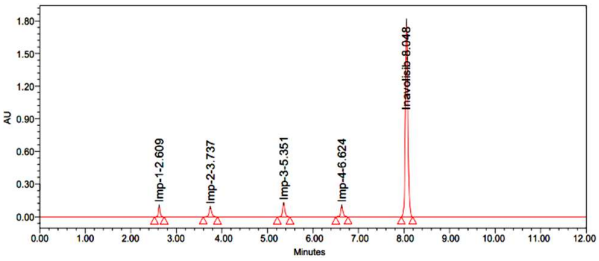

Figure 8: Chromatogram of Inavolisib and Impurities (Preparation-6).

Accuracy:

Drug name	80% Recovery			100% Recovery			120% Recovery		
	Trail 1	Trail 2	Trail 3	Trail 1	Trail 2	Trail 3	Trail 1	Trail 2	Trail 3
Impurity -1	99.5	99.3	98.8	99.8	99.6	99.4	98.8	99.2	99.3
Impurity -2	98.8	98.5	99.3	99.8	99.4	99.2	100.5	100.2	99.2
Impurity -3	98.8	99.3	99.8	100.2	99.8	99.4	98.5	98.3	98.2
Impurity -4	100.3	98.5	99.3	100.8	100.6	100.2	100.8	100.3	100.5
Inavolisib	98.8	98.9	99.1	100.1	99.4	98.5	98.5	100.3	99.6

Table 1: Recovery results of Inavolisib and its impurities.

Method Precision:

Drug name	% RSD	% Recovery
Impurity-1	0.24	100.15
Impurity-2	0.36	99.53
Impurity-3	0.17	100.11
Impurity-4	0.41	100.37
Inavolisib	0.96	99.72

Table 2: Method Precision results of Inavolisib and its impurities.

System Suitability:

Parameter	Imp-1	Imp-2	Imp-3	Imp-4	Inavolisib
Retention Time	2.612	3.745	5.364	6.621	8.053
Theoretical Plates	3748	5102	8013	7299	11324
Tailing Factor	1.01	1.05	0.98	1.13	0.87
USP Resolution	-	5.27	8.12	6.88	7.45
%RSD	0.13	0.19	0.23	0.28	0.48

Table 3: System Suitability results of Inavolisib and its impurities.

Robustness:

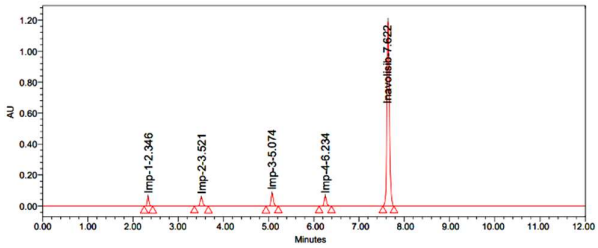

Figure 9: Chromatogram of Inavolisib and Impurities (Flow +10%)

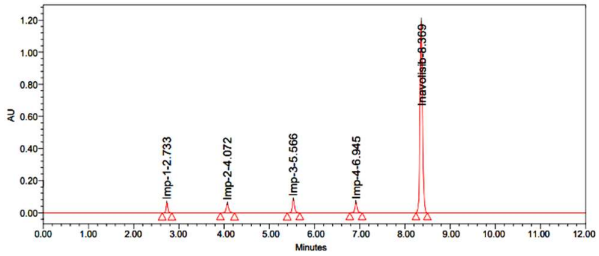

Figure 10: Chromatogram of Inavolisib and Impurities (Flow -10%)

Forced Degradation Studies:

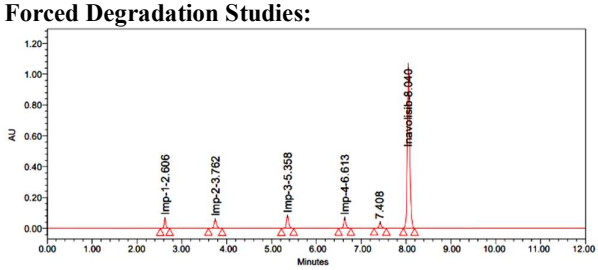

Figure 11: Chromatogram for Acid degradation.

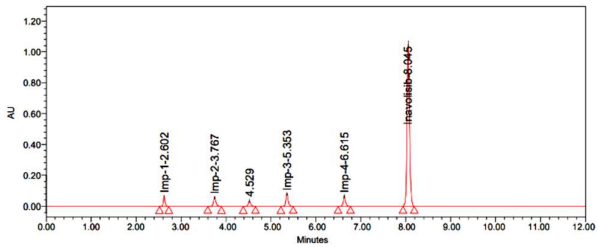

Figure 12: Chromatogram for Alkali degradation.

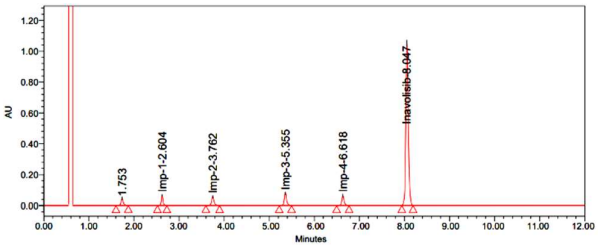

Figure 13: Chromatogram for Peroxide degradation.

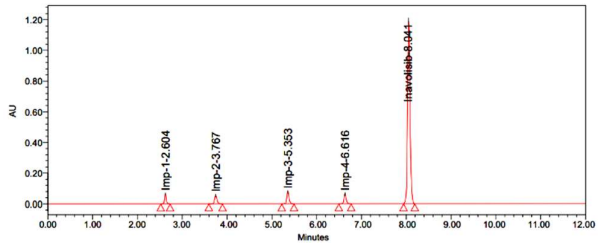

Figure 14: Chromatogram for Hydrolysis degradation.

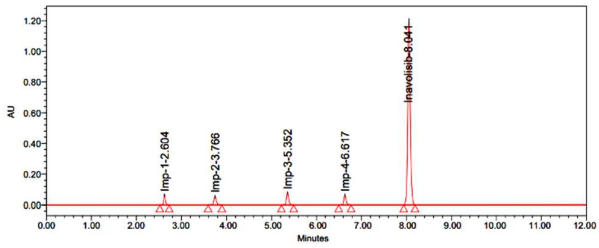

Figure 15: Chromatogram for Reduction degradation.

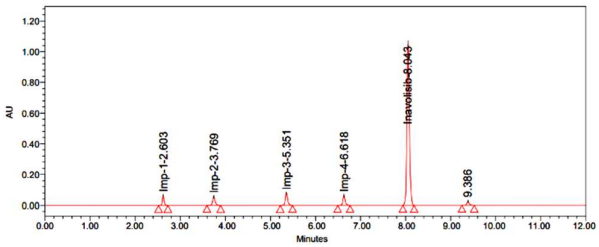

Figure 15: Chromatogram for Photolytic degradation.

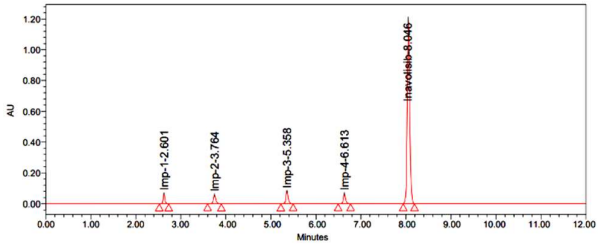

Figure 15: Chromatogram for Thermal degradation

Discussion:

The correlation coefficient for the linearity produced between concentration versus area is 0.99952. It was discovered that the correlation coefficient is within acceptable bounds. Inavolisib have mean percentage recovery rate of 99.72%. From the method precision studies, it was observed that all the parameters like %RSD of retention time and peak area were within the limits. The recovery results are also indicating that the test method has an acceptable level of accuracy. The results were found to be within the limits. The procedure was tough, as evidenced by the ruggedness results, which reveal that the %RSD between two analysis measurements is not larger than 2.0%. The chosen compounds were subjected to stability indicating studies under a variety of stress conditions, including thermal, peroxide, acid, alkali, peroxide reduction

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and photolytic degradation. The main analyte peak purity was also passed, demonstrating the electiveness of this method as a stability indicating method.

Summary and Conclusion:

As per the findings of the study for the chosen combination, sample recoveries in all formulations were in line with the claims made on each drug's label, and the suggested method was found to be accurate, sensitive, quick, and affordable for the detection of Inavolisib and impurities. Satisfactory findings were obtained after validating the suggested approach in accordance with guidelines of ICH and comparing the acquired values with the standard values.

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