RESEARCH ARTICLE

Stability-Indicating RP-HPLC Method Development and Validation for the Estimation of Inavolisib in Bulk and Pharmaceutical Dosage Form

Selvaraja Elumalai*, Meenakshi Sharma

Department of Chemistry,School of Basic and Applied Sciences, Raffles University, Neemrana, Alwar, Rajasthan-301705, India

Received: 29th May, 2025; Revised: 26th Jun, 2025; Accepted: 18th Jul, 2025; Available Online: 25th Sep, 2025

ABSTRACT

A robust and stability-indicating RP-HPLC method was developed and validated for the estimation of Inavolisib in bulk and its pharmaceutical formulations. Separation was achieved on a Symmetry Shield RP-18 column (150 × 4.6 mm, 3.5 µm) using an isocratic mobile phase comprising acetonitrile and 0.1% triethylamine (pH adjusted to 2.5 with orthophosphoric acid) in a 1:1 ratio. The method utilized a 1.0 mL/min flow rate with detection at 245 nm. Validation, as per ICH Q2(R1) guidelines, demonstrated excellent specificity, linearity (R^2 = 0.99967), precision (%RSD < 2%), and accuracy (recovery 99.8–99.9%). The method also proved to be robust and sensitive, with LOD and LOQ values of 0.54 µg/mL and 1.80 µg/mL, respectively. The assay of the marketed product Itovebi confirmed 100.1% of the labeled content. Forced degradation studies under various stress conditions confirmed the method's capability to separate the drug from its degradation products, supporting its application as a reliable and stability-indicating analytical method for routine quality control of Inavolisib.

Keywords: Inavolisib, RP-HPLC, Method validation, Stability-indicating, Forced degradation

How to cite this article: Selvaraja Elumalai, Meenakshi Sharma. Stability-Indicating RP-HPLC Method Development and Validation for the Estimation of Inavolisib in Bulk and Pharmaceutical Dosage Form. International Journal of Drug Delivery Technology. 2025;15(3):1114-18. doi: 10.25258/ijddt.15.3.29

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Inavolisib is a selective oral PI3Kα inhibitor designed to treat PIK3CA-mutant, HR-positive, HER2-negative breast cancer[1], Inavolisib is a synthetic small molecule with a molecular formula of $C_{27}H_{28}F_2N_6O_3$ and an approximate molecular weight of 522.55 g/mol. It demonstrates favorable lipophilicity, enhancing its ability to cross cell membranes and supporting its oral bioavailability[2]. The structure includes fluorinated aromatic moieties and nitrogen-rich heterocyclic groups, which contribute to its high selectivity and potency toward the PI3Kα isoform[3]. It shows moderate solubility in water and maintains chemical stability in both acidic and basic environments, making it well-suited for solid oral formulations and targeted intracellular action[4,5]. Compared to earlier pan-PI3K inhibitors, Inavolisib offers improved tolerability and reduced toxicity due to its high specificity[6,7]. Its continued evaluation in clinical trials holds promise for personalized cancer therapy, especially in PIK3CA-mutant breast cancers[8]. The present study is the first to address this unmet need, aligning with ICH guidelines for method validation and forced degradation studies.

MATERIALS AND METHODOLOGY

Reagents and Chemicals

HPLC-grade acetonitrile (Merck), Milli-Q water (in-house), and AR-grade triethylamine and orthophosphoric acid (Merck) were used for mobile phase preparation. The Inavolisib reference standard was purchased from MedChemExpress (MCE), USA, and was used without further purification.

All chemicals conformed to analytical quality standards.

Instruments

A Waters Alliance HPLC system operating in isocratic mode was employed for chromatographic separation. Complementary instruments used were a Eutech pH700 pH meter, a precision Sartorius BSA224S-CW balance, a UV-1700 spectrophotometer from Shimadzu, and a UCA 701 ultrasonicator from Unichrome.

Standard and Sample Solutions

A 90-ppm standard solution of Inavolisib was prepared by dissolving 9 mg of the standard in diluent, sonicating, and diluting to 10 mL, followed by a 1:10 dilution. The sample solution was made by dissolving 6.9 mg of Inavolisib in diluent, sonicating for 30 minutes, diluting to volume, and filtering through a 0.45 µm membrane to achieve the same concentration[9].

Method Development

A series of trials were conducted to optimize the chromatographic conditions for the analysis of Inavolisib. In Trials 1–3, using the Luna Phenyl Hexyl column with varying mobile phase ratios of acetonitrile and 0.1% OPA or TEA (pH 2.5), issues such as poor retention time, unsatisfactory peak shape, and inadequate baseline were observed. Switching to a Symmetry Shield RP-18 column in Trials 4 and 5 improved performances; however, unknown peaks and excessive peak response were noted.

Method Validation

**Author for Correspondence:selvarrraj@gmail.com*

Table 1: Evaluation of System Suitability for Inavolisib

S. No.	Parameter	Inavolisib
1	Retention time	2.875
2	Plate count	10123
3	Tailing factor	0.91
4	%RSD	0.25

Table 2: Precision Evaluation of Inavolisib by HPLC

S. No.	Conc. Inavolisib (µg/ml)	System Area of Inavolisib	Method Area for Inavolisib	Intermediate Area for Inavolisib
1.	90	3137713	3142627	3132402
2.	90	3130428	3131058	3157828
3.	90	3125609	3124689	3144673
4.	90	3141520	3155640	3151910
5.	90	3129754	3148213	3110817
6.	90	3145622	3150779	3122406
Mean	3135108		3142168	3136673
S.D	7736.180		12011.198	18077.730
%RSD	0.25		0.38	0.58

Table 3: HPLC Linearity Data for Inavolisib

Inavolisib	Conc.(µg/ml)	Peak area
	22.50	783451
	45.00	1572870
	67.50	2350643
	90.00	3140256
	112.50	3805727
	135.00	4543101
Regression equation	y = 33716.26x+37873.89	
Slope	33716.26	
Intercept	37873.89	
R^2	0.99967	

The RP-HPLC method established for Inavolisib underwent validation following the protocols defined by ICH Q2(R1) standards[9-11].

System Suitability

Prior to initiating the method validation process, system suitability testing was carried out to confirm the reliability and consistency of the chromatographic system. Six successive injections of standard Inavolisib were used to assess retention time, plate count, tailing factor, and % RSD, ensuring system performance and consistency.

Specificity

The specificity of the proposed HPLC method was established by analyzing blank, placebo, and standard drug solutions independently. These injections ensured there was no interference from excipients or matrix components at the retention time of Inavolisib (2.875 min). The absence of overlapping or co-eluting peaks confirmed the method's ability to selectively quantify the analyte in the presence of other constituents.

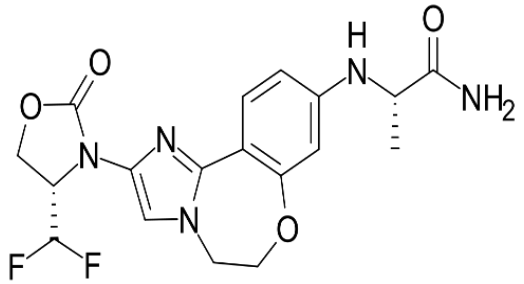

Figure 1: Chemical Structure of Inavolisib

Table 4: Accuracy results of Inavolisib

Conc.	Area	% Recovery	Mean % Recovery
80%	5620143	99.6	99.9
	5634780	99.9	
	5656592	100.2	
100%	6248746	99.7	99.9
	6264731	99.9	
	6280994	100.2	
120%	6871421	99.6	99.8
	6885966	99.8	
	6890123	99.9	

Table 5: Robustness results of Inavolisib

Parameters	Condition	Peak area	Tailing	%RSD
Flow rate Change (mL/min)	Less flow (0.9ml)	2976746	1.04	0.56
	Actual flow (1.0ml)	3137713	0.91	0.25
	More flow (1.1ml)	3265984	0.90	0.50
Organic Phase change	Less Org (45:55)	2824561	0.99	0.25
	Actual (50:50)	3130428	0.95	0.25
	More Org (55:45)	3452879	0.88	0.46

Table 6: Stress Degradation Study Outcomes for Inavolisib

Degra. Conditions	Area	% Assay	% Degra.
Control	3132456	100	0
Acid	2704517	86.3	13.7
Alkali	3091245	98.7	1.3
Peroxide	2645122	84.4	15.6
Reduction	2780461	88.7	11.3
Thermal	3090418	98.6	1.4
Photolytic	3025213	96.6	3.4
Hydrolysis	3009175	96.0	4.0

Precision

System precision was assessed by injecting the same standard solution six times and determining the %RSD of peak areas to confirm consistency. Method precision involved preparing six separate sample solutions from the same batch and analyzing them under identical conditions. Intermediate precision was tested on a different day using a different analyst and HPLC system, confirming reproducibility across varying conditions[12].

Linearity

A linear calibration range from 22.5 to 135 µg/mL was established for Inavolisib by analyzing standard solutions at six different concentrations. Triplicate readings were used to ensure accuracy, and a regression line was plotted. The correlation coefficient of above 0.999 confirmed that the method demonstrated excellent linearity throughout the evaluated range.

Accuracy

To verify the method's accuracy, known quantities of Inavolisib were added to previously analyzed sample

solutions at three concentration levels: 80%, 100%, and 120% of the target concentration. Each level was tested in triplicate using the established chromatographic conditions, assessing the method's capability to precisely quantify the drug in the presence of excipients[13].

Robustness and Sensitivity

Robustness was assessed by intentionally varying the flow rate (±0.1 mL/min) and organic content of the mobile phase (±5%). The method consistently maintained acceptable retention time, peak shape, and system suitability, demonstrating its reliability under slight operational changes. LOD and LOQ were calculated per ICH guidelines using response standard deviation and calibration curve slope, confirming the method's sensitivity for detecting low levels of Inavolisib[13,14].

Forced Degradation Studies

Forced degradation studies of Inavolisib were conducted in accordance with ICH guidelines to evaluate the stability-indicating power of the developed HPLC method. The drug was subjected to stress conditions such as acidic, alkaline, oxidative, thermal, hydrolytic, and photolytic environments. After appropriate exposure durations, the samples were neutralized when required and analysed[15-17]. This helped in assessing the method's capability to separate Inavolisib from its potential.

RESULTS AND DISCUSSIONS

Method Optimization

The optimized chromatographic method was developed using a Waters Alliance e-2695 HPLC system (Fig 2). Separation was achieved on a Symmetry Shield RP-18 column (150 × 4.6 mm, 3.5 µm particle size) under isocratic conditions. The mobile phase consisted of a 50:50 mixture of acetonitrile and 0.1% triethylamine (TEA) adjusted to pH 2.5 with ortho-phosphoric acid. The flow rate was maintained at 1 mL/min, with a detection wavelength of 245 nm, an injection volume of 10 µL, and ambient column temperature (25°C).

System Suitability

The system suitability for Inavolisib was established by evaluating key chromatographic parameters. The retention time was observed at 2.875 min, with a plate count of 10,123 indicating good column efficiency. The tailing factor (0.91) and %RSD (0.25) were well within ICH-specified limits, confirming method suitability (Table 1).

Specificity

The blank chromatogram showed no peaks, and the placebo exhibited no signals overlapping with the drug's retention time at 2.875 minutes (as depicted in Fig. 3 and Fig. 4), indicating the absence of interference from the sample matrix. These findings confirmed that the method is highly specific, enabling reliable and accurate quantification of Inavolisib without any contribution from endogenous substances or formulation excipients.

Precision

The precision studies including system, method, and intermediate precision showed %RSD values of 0.25, 0.38, and 0.58 respectively, all well within the acceptable limit of ≤2.0, confirming the reproducibility and consistency of the method (Table 2).

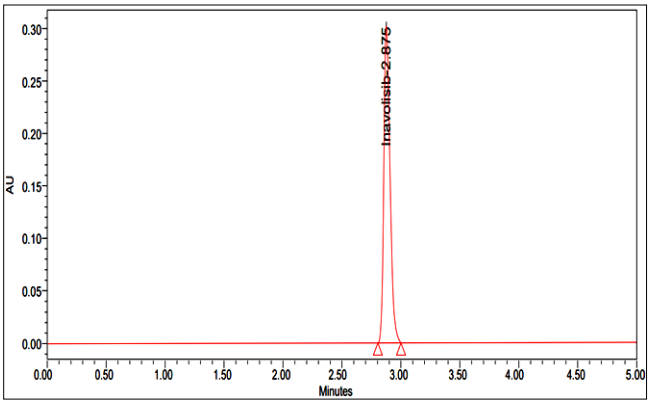

Figure 2: Optimized chromatogram of Inavolisib

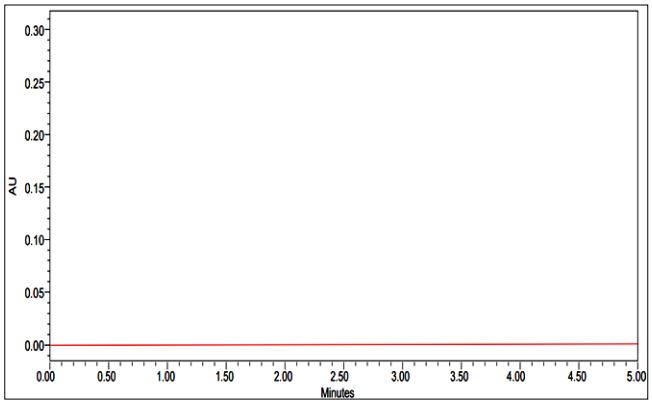

Figure 3: Blank Chromatogram

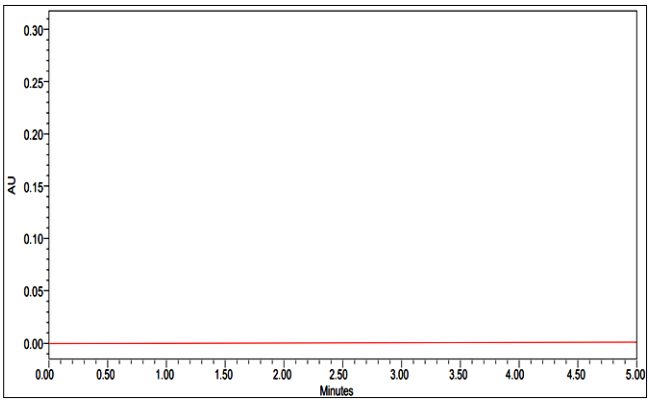

Figure 4: Placebo Sample Chromatogram

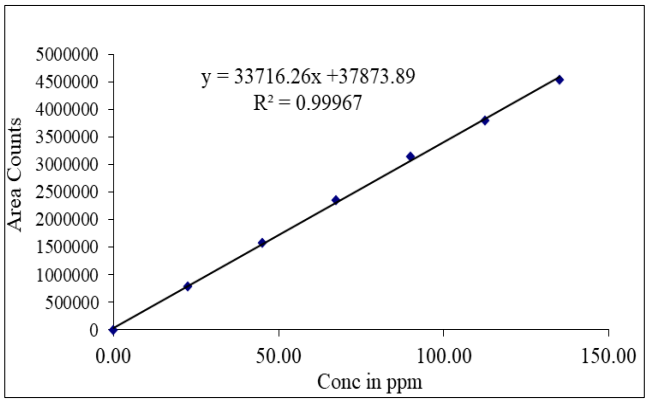

Figure 5: Calibration curve for Inavolisib at 245 nm

Linearity

The developed method demonstrated outstanding linearity for Inavolisib across a wide concentration range of 22.5 to 135 µg/mL. The R^2 was found to be 0.99967, reflecting a highly linear response between concentration and peak area. This strong linearity confirms the method's suitability for accurate and reliable quantification of Inavolisib in both lower and higher concentration levels, ensuring consistent analytical performance as illustrated in Table 3 and Figure 5.

Accuracy

Accuracy studies for Inavolisib showed mean recovery values ranging from 99.8% to 99.9% at 80%, 100%, and 120% concentration levels, demonstrating the method's reliability and reproducibility (Table 4).

Assay, LOD and LOQ

The assay of Inavolisib in the marketed formulation (Itovebi) demonstrated a percent assay of 100.1%, indicating excellent accuracy and label claim compliance. Inavolisib showed an LOD of 0.54 µg/mL and an LOQ of 1.80 µg/mL.

Robustness

The robustness study of Inavolisib confirmed that minor deliberate changes in flow rate and organic phase composition did not significantly affect the method performance. All parameters remained within acceptable limits, as shown in Table 5.

Forced Degradation Studies

The forced degradation studies demonstrated that Inavolisib was most susceptible to oxidative and acidic conditions, showing 15.6% and 13.7% degradation respectively, while other conditions like thermal and alkali resulted in minimal degradation (Table 6).

CONCLUSION

The developed RP-HPLC method provides a reliable and efficient approach for routine analysis of Inavolisib in bulk and dosage forms, meeting all ICH validation criteria such as system suitability, linearity, precision, accuracy, and robustness. The forced degradation studies establish its applicability as a stability-indicating assay. The assay results confirm its accuracy for quantifying Inavolisib in marketed formulations like Itovebi. Moreover, the method's simplicity, short runtime (5 minutes), and ease of execution make it highly suitable for routine quality control in pharmaceutical laboratories. The inclusion of degradation profiling further enhances its utility in regulatory submissions and stability testing protocols.

REFERENCES

1. Juric D, Janku F, Rodon J, Burris HA, Mayer IA, Schellens JHM, et al. Alpelisib plus fulvestrant in PIK3CA-altered and PIK3CA-wild-type estrogen receptor-positive advanced breast cancer. *JAMA Oncol*. 2018;4(3):e173598.
2. Hanker AB, Kaklamani V, Arteaga CL. Challenges for the clinical development of PI3K inhibitors: Strategies to improve their impact in solid tumors. *Cancer Discov*. 2019;9(4):482–491.
3. Fritsch C, Huang A, Chatenay-Rivauday C, Schnell C, Reddy A, Liu M, et al. Characterization of the novel and specific PI3Kα inhibitor NVP-BYL719 and development of the patient stratification strategy for clinical trials. *Mol Cancer Ther*. 2014;13(5):1117–1129.
4. Baselga J, Im SA, Iwata H, Cortés J, De Laurentiis M, Jiang Z, et al. Buparlisib plus fulvestrant versus placebo plus fulvestrant in postmenopausal, hormone receptor-positive, HER2-negative, advanced breast cancer (BELLE-2): A phase 3, randomized, double-blind, placebo-controlled study. *Lancet Oncol*. 2017;18(7):904–916.
5. Janku F, Yap TA, Meric-Bernstam F. Targeting the PI3K pathway in cancer: Are we making headway? *Nat Rev Clin Oncol*. 2018;15(5):273–291.
6. Juric D, Smith D, Garrett M, Deegan B, Li B, Patel P, et al. First-in-human phase I dose-escalation study of the α-specific PI3K inhibitor GDC-0077 in patients with advanced solid tumors. *J Clin Oncol*. 2020;38(15_suppl):TPS1110.
7. André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-mutated, hormone receptor–positive advanced breast cancer. *N Engl J Med*. 2019;380(20):1929–1940.
8. Vasan N, Toska E, Scaltriti M. Overview of the relevance of PI3K pathway in HR-positive breast cancer. *Ann Oncol*. 2019;30(Suppl_10):x3–x11.
9. Chandramore KR, Sonawane SS, Ahire RS, Reddy H, Ahire SB, Jadhav PB, et al. Development and Validation of Stability Indicating LC Method for Selexipag : In-Silico Toxicity Study and Characterization of its Degradation Products. Chem Methodol. 2025;9:427–47.
10. Konatham TK. A Systematic Review on Method Development and Validation of Few Antiviral Drugs by Using RP-HPLC. Ijppr. Human. 2021;21(3):651-61.
11. Konatham TK, Anuradha M, Narmada A. a stability indicating method development and validation of Telmisartan and Nifedipine in pure form using RP-HPLC. International Journal of Pharmaceutical, Biological and Chemical Sciences. 2020;9(3):36-44.
12. Yelampalli SR, Gandla K, Reddy KTK, Ibrahim AE, El Deeb S. Determination of Sodium, Potassium, and Magnesium as Sulfate Salts in Oral Preparations Using Ion Chromatography and Conductivity Detection. Separations. 2023; 10(2):99.
13. Nagda D, Captain A. Validated Green Densitometric Method for Estimation of Naringin Biomarker in Hydroalcoholic Extract of Leaves of Ceiba Pentandra (L .) Gaertn. Asian J Green Chem. 2025;9:427–42.
14. Reddy KTK, Haque MA. Development and Validation of Aducanumab by Bioanalytical Method Using Liquid Chromatography-Tandem Mass Spectroscopy. Adv J Chem Sect A. 2025;8(3):456–68.
15. Mohammadzadeh Jahani P, Zaimbashi R, Beitollahi H, Tajik S. An Electrochemical Sensor Based on SnSe nanosheets and ionic liquids for the Selective Detection of N-acetylcysteine. Chem Methodol. 2025;9(3):158–71.

16. Preeti N. Yadav, Chhalotiya Usmangani K, Patel Kesha M, Tandel Jinal N. Quantification of A β Adrenergic Receptor Drug Mirabegron by Stability Indicating LC Method andUv–visible Spectroscopic Method in Bulk and Pharmaceutical Dosage Form. Chem Methodol [Internet]. 2020;4(53):340–58. Available from: http://chemmethod.com
17. Elumalai S, Sharma M, Dantinapalli VLS, Palanisamy M. Novel Stability Indicating UPLC Method Development and Validation for Simultaneous Quantification of Perindopril Arginine and Bisoprolol Fumarate in Pure and Pharmaceutical Dosage Form. Adv J Chem Sect A. 2025;8(9):1488–507.