Toxicology International, *30(3) : 371-382; 2023. DOI: 10.18311/ti/2023/v30i3/33573*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Development and Application of a Validated HPLC Method for the Determination of Alpelisib and its Process-Related Impurities in Pure Drug and Pharmaceutical Formulations

Jammu Harish[1], Bodasingi Sai Kumar[1], Uppu Naga Babu[2], Gattupalli Subhakara Rao[3] and Venkateswara Rao Anna[1]*

[1]*Department of Engineering Chemistry College of Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur - 522302, Andhra Pradesh, India; avrchemistry@gmail.com*

[2]*Department of Engineering Chemistry, S.R.K.R. Engineering College, Chinna Amiram, Bhimavaram - 534204, Andhra Pradesh, India*

[3]*Department of Engineering English, Koneru Lakshmaiah Education Foundation, Green Fields, Vaddeswaram, Guntur - 522302, Andhra Pradesh, India*

Abstract

Impurity analysis plays a significant role in the manufacture of a safe pharmaceutical product that ensures the safety of consumers. Keeping this in consideration, the present study was intended to develop a simple and sensitive HPLC method for the resolution and quantification of 4 process-related impurities namely impurities 1, 2, 3 and 4 in alpelisib pure drug and formulations. The method consumes greener solvents as a mobile phase that resolves the analytes on ProntoSIL ODS-C18 (250×4.6 mm; 5 µ id) column at room temperature as stationary phase, ethanol and 0.1% aqueous acetic acid in 65:35 (V/V) at pH 4.5 as mobile phase at 0.8 mL/min flow rate, UV detection at 246 nm. The method can detect the analytes at retention times of 7.05, 4.52, 6.09, 2.88 and 8.14 min respectively for alpelisib, impurity 1, 2, 3 and 4. The analysis was completed with a run time of 15 min that consume less solvent and the same analysis time. The linearity of the proposed method was perceived in the range of 12.5 to 100 µg/mL for alpelisib and 0.0125 to 0.10 µg/mL for impurities. The method can effectively resolve the unknown stress degradation products generated during the stress exposure of alpelisib along with its known impurities in the study. The outcomes of other validation studies were likewise satisfactory and proven adequate for regular analysis of alpelisib and its process-related impurities in bulk drug and pharmaceutical tablet doses.

Keywords: Alpelisib, Formulation Analysis, HPLC Analysis, Method Development, Process Related Impurities

**Author for correspondence*

Article Received on: 22.04.2023 Revised on: 20.06.2023 Accepted on: 30.06.2023

1. Introduction

The analytical methods that don't contain harmful, toxic chemicals and reagents with simple pre-treatment, little waste generation, little energy consumption, as well as quantification steps, were considered as green analytical methods[1]. The green analytical method development has gained significant attention in recent times due to its positive impact on the environment. In this context, during the development of any analytical method for the quantification of an analyte, two core features must be considered[2]. The first future includes the metrological outcomes such as sensitivity, selectivity, precision, and accuracy whereas the second one includes method greenness[3]. The guidelines related to the establishment of the green analytical method provide the best idea about greener chemicals that are to be considered during the development of green analytical techniques. The uses of green analytical techniques were still inadequate for regular quality control of pharmaceuticals and the High-Performance Liquid Chromatographic methods (HPLC) have the advantage of being non-hazardous to the environment, operator safety, and much more[4,5]. This study aimed to develop a novel chromatographic method that utilizes a less harmful ethanol solvent that was considered an ecologically sound alternative to acetonitrile and methanol.

Alpelisib belongs to the kinase inhibitors class of antineoplastic drugs prescribed to treat certain types of breast cancer[6]. It was administered with fulvestrant for treating PIK3CA-mutated, advanced, or metastatic breast cancer for both postmenopausal women and men with hormone receptor-positive and human epidermal growth factor receptor 2 negative[7]. Alpelisib works by inhibiting the Phosphatidylinositol-3-kinase-α enzyme responsible for cell proliferation and reducing cancer. It was available in the form of tablets and administered orally[8]. The possible side effects of alpelisib include high blood sugar, low blood cells, kidney problems, diarrhoea, pancreatitis, rash, vomiting, liver problems and hair loss. It has a Molecular Mass (MM) of 441.47 g/mol with a Molecular Formula (MF) of $C_{19}H_{22}F_3N_5O_2S$ and its chemical structure was presented in Figure 1.

The literature survey was conducted for various available analytical methods for the quantification of alpelisib in various samples. One UPLC-MS/MS method reported alpelisib characterization in rat plasma and reported the drug-drug interaction[9]. One HPLC method

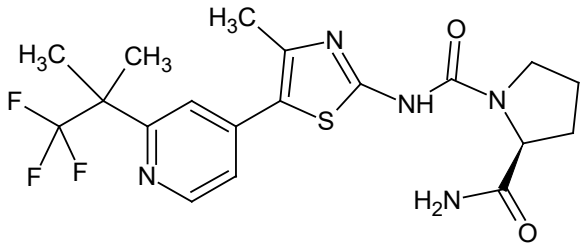


Figure 1. Chemical structure of alpelisib.

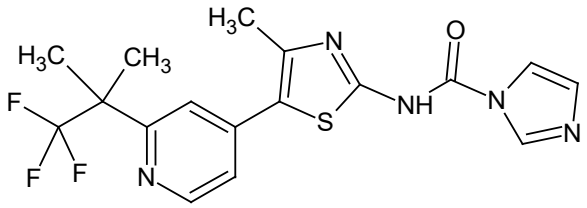
was reported for quantification as well as metabolism and pharmacokinetic study of alpelisib rat plasma[10]. LCMS/MS methods reported for quantification as well as the pharmacokinetic profile of alpelisib in rabbit[11], human and mouse plasma samples[12]. The literature survey suggested that an analytical method has been published for quantifying potential impurities in alpelisib. The presence of pharmaceutical impurities in any pharmaceutical product may cause unwanted side effects and some are hazardous in nature. Hence there is a need to develop a simple and sensitive analytical method for the quantification of impurities. As the literature doesn't have a method to analyze the pharmaceutical impurities, the present study aimed to develop a simple and sensitive HPLC method for the quantification of alpelisib impurities. The impurities selected in the study were presented in Figure 2.

The schematic representation for the synthesis of alpelisib was presented in Figure 3[13]. During the process of synthesis of alpelisib, there is a possibility of the formation of a greater number of process-related impurities that originated during the process of manufacture of the pure drug. Based on the availability, impurity 1 (compound 6 in synthesis scheme), impurity 2 (compound 4 in synthesis scheme), impurity 3 (compound 3 in synthesis scheme) and impurity 4 (compound 2 in synthesis scheme) were selected for the study.

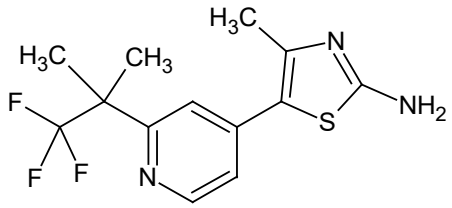
2. Materials and Methods

2.1 Chemicals and Reagents

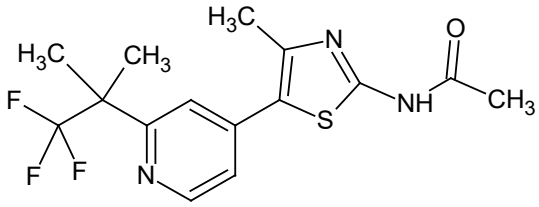
The pure analytical standard compound alpelisib with purity of 98.28%, its impurities 1, 2, 3 and 4 along with its marketed formulation with brand Piqray® (50 mg) were procured from Novartis Pharmaceuticals India Limited, Hyderabad. HPLC grade methanol, acetonitrile, Milli-Q® water were purchased from Merck Chemicals, Mumbai. The analytical reagent grades chemicals such as ethanol,



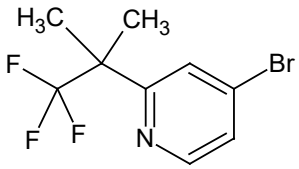
Impurity 1 [*N-(4-Methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)-1H-imidazole-1-carboxamide*]
MF: $C_{17}H_{16}F_3N_5OS$; MM: 395.4 g/mol



Impurity 2 [*4-Methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-amine*]
MF: $C_{13}H_{14}F_3N_3S$; MM: 301.3g/mol



Impurity 3 [*N-(4-Methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)acetamide*]
MF: $C_{15}H_{16}F_3N_3OS$; MM: 343.4g/mol



Impurity 4 [*4-Bromo-2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridine*]
MF: $C_9H_9BrF_3N$; MM: 268.1g/mol

Figure 2. Molecular structure of impurities in the study.

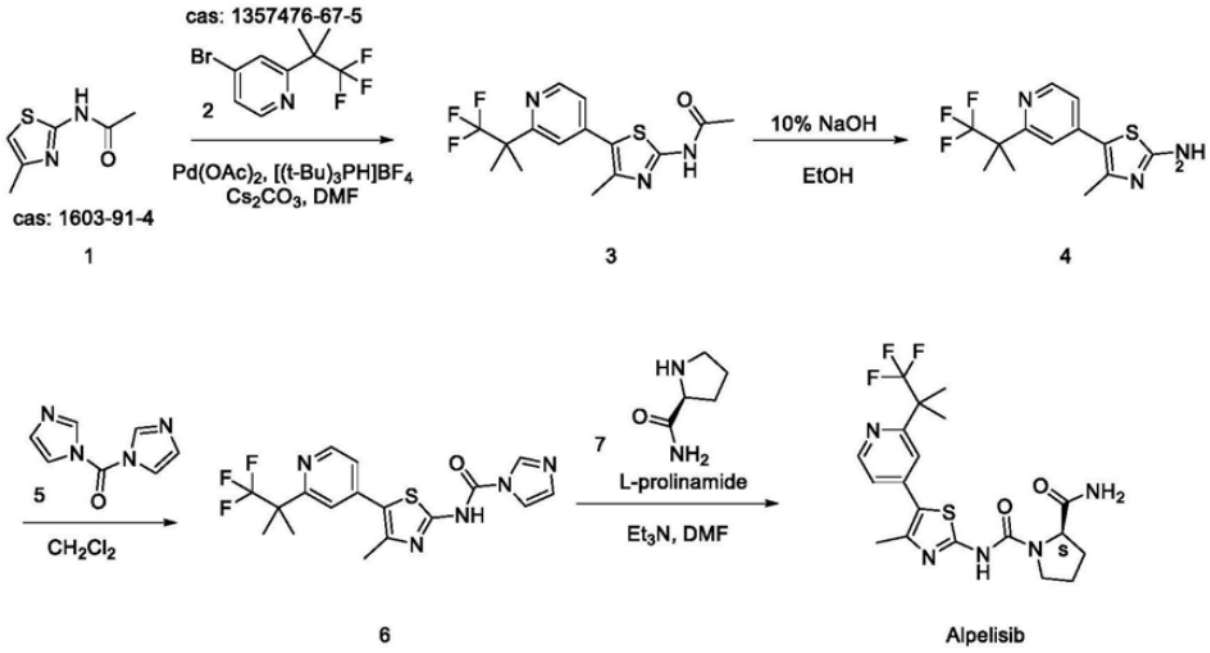


Figure 3. Schematic representation for the synthesis of alpelisib.

acetic acid, sodium acetate, Hydrochloric Acid (HCl), Sodium Hydroxide (NaOH), and hydrogen peroxide were purchased from Fisher Scientific, Mumbai.

2.2 Instrumentation

The HPLC analysis was performed on Agilent 1100 (USA) instrument that coupled with a quaternary pump (G1311 A) for solvent delivery. The analytes were injected through a temperature-adjustable autosampler (G 1329A) having an injection capacity of 0.1–1500 µL. The column eluents were detected using programmable Ultraviolet (UV) detector (G 1314 A) and the chromatographic integrations were carried out using Agilent chem-station software.

2.3 Preparation of Solutions

2.3.1 Alpelisib and Impurity Solutions

The standard alpelisib and its impurities at 1 mg/mL (1000 µg/mL) were prepared separately by accurately weighing 25 mg of analyte in 25 mL of a volumetric flask containing 15 mL of ethanol. The analytes were dissolved in the solvent using an ultrasonic bath sonicator. Then the analytes were filtered through a 0.2 µ membrane filter and the final volume was made up to the mark using the same solvent to obtain a 1000 µg/mL concentration of alpelisib and its impurities separately. During the analysis, the selected volume of the required concentration of individual analytes was mixed separately.

2.3.2 Formulation Solution

The tablets of alpelisib (Piqray® - 50 mg) were powdered using a clean, sterile mortar and pestle to obtain a uniform, fine powder. The tablet powder equivalent to 25 mg of alpelisib was weighed and taken in a 25 mL volumetric flask containing 15 mL of methanol. The flask was kept in an ultrasonic bath sonicator for 5 min to dissolve alpelisib completely in a solvent. The un-dissolved tablet particles were removed by filtration using a 0.2 µ membrane filter. Then the concentration of the solute was brought to a precision level in the calibration curve and the solution was used for the study.

2.4 Method Development

The method development was initiated by identifying a suitable wavelength for the detection of alpelisib and its impurities using a UV detector. The UV-visible spectrophotometer was used for the identification of a suitable wavelength for the detection of alpelisib and its impurities. The standard solution at 10 µg/mL concentration of alpelisib and its impurities was scanned individually at a scan range of 400-200 nm. The overlay UV absorption spectra of alpelisib and its impurities confirm the iso-absorption wavelength that was suitable for the detection of alpelisib and its impurities.

The suitable stationary phase for the separation of alpelisib and its impurities in the study was selected based on the resolution and chromatographic responses of analytes in each column studied. Various C18 columns such as prontoSIL ODS, zodiac, zorbax XDB and phenomenex luna with 250 mm and 100 mm column lengths were studied. The green solvent ethanol was selected as an organic modifier and various strengths of aqueous acetic acid and sodium acetate were selected as pH modifiers. Various compositions of these solvents were pumped at a flow range of 0.5 to 1.5 mL/min was studied for the best resolution of alpelisib and its impurities studied. The condition that gives the best resolution of alpelisib and its impurities with acceptable system suitability was considered suitable for the study.

2.5 Method Validation

The method developed for the analysis of alpelisib and its impurities were validated as per the available literature[14-17] and ICH guidelines[18].

2.6 System Suitability

The 100% concentration level solution was analysed six times in the proposed method for establishing the system suitability of the developed method. The chromatographic response of the resultant chromatograms in each study was summarized for evaluating the system suitability of the developed method. The chromatographic parameters such as Relative Retention Time (RRT), asymmetric factor (tail factor), plate count (number of theoretical plates) and resolution factor were used to evaluate method system suitability.

2.6.1 Sensitivity

The method sensitivity of the proposed method for the detection of impurities was evaluated by assessing the Detection Limit (LOD) and Quantification Limit (LOQ). The signal (s) to noise (n) ratio method was

adopted for evaluating the sensitivity levels of impurities in the developed method. The minimal concentration of impurities of alpelisib was analysed in the developed method and the chromatographic response (signal) along with baseline (noise) response was summarized. The signal-to-noise ratio of 3 and 10 were considered as LOD and LOQ respectively.

2.6.2 Linearity and Range

The calibration concentrations were prepared such that the alpelisib solution contains 0.1% of impurity. Various levels of alpelisib standard solution spiked with 0.1% of studied impurities were analysed in the developed method. The chromatographic response of each analyte was tabulated, and a calibration curve was plotted individually by considering obtained peak area response on the y-axis and its prepared concentration on the x-axis. The best-fitted calibration range for each analyte was considered a suitable range of analysis in the developed method.

2.6.3 Precision

The 100% concentration level in the linearity level spiked with 0.1% of each impurity was used for evaluating the repeatability and reproducibility of the developed method. The solution was prepared and injected six times on the same day (intraday), six times in three days (interday) and six times by three different analysts on the same day (ruggedness). The peak area response of each analyte in each study was tabulated and the % RSD was calculated for each analyte in each study. The % RSD of less than 2 was acceptable in each study as per the guidelines.

2.6.4 Robustness

The influence of minor variations in the developed method conditions for the separation and quantification of alpelisib and its impurities was evaluated in robustness. The ±5 mL variation in the composition of the mobile phase, ±5 nm variation in the wavelength of the detector and ±0.1 factor variation in mobile phase pH was made intentionally and the 100% concentration of alpelisib containing 0.1% of each impurity was injected in each changed method condition. The chromatographic response and the system suitability of the obtained chromatograms in each condition were summarized. The % change in the peak area response of each analyte was calculated by comparing its corresponding regression equation and a % change of less than 2 was considered as acceptable.

2.6.5 Recovery

In the recovery/accuracy study, 50%, 100% and 150% spiked levels of known standard concentration (100%) in the calibration range were performed and the % recovery was calculated in each spiked level by comparing with calibration results. The % recovery of 98-102 was considered acceptable.

2.6.6 Force Degradation Studies

The method applicability for the separation and analysis of stress degradation compounds generated during the stress exposure of alpelisib was confirmed by performing forced degradation studies. In this, the standard alpelisib at a quantity of 50 mg was separately mixed with 50 mL of 0.1 N HCl, 0.1 N NaOH and 3% hydrogen peroxide solution for acid, base, and peroxide degradation study respectively. The stressed samples were incubated for 24 hours to induce degradation in alpelisib drug. Then the solution was neutralized, diluted to 100% concentration level, and then analysed in the developed method. The standard alpelisib was taken in a petri dish and exposed to 60°C for 24 h in an air oven for thermal degradation and exposed to UV light at 254 nm for 24 h for a photolytic degradation study. Then the stressed sample was diluted to a 100% concentration level and the dilute solution was analysed in the developed method. The chromatograms observed for each stress sample analysis were observed for evaluation of method efficiency for the separation and analysis of stress degradation compounds. The % degradation of alpelisib was calculated by comparing the peak area response of the stressed sample with the un-stressed sample of the same concentration level.

2.6.7 Sample Analysis

The formulation solution of alpelisib with brand Piqray® - 50 mg was analysed in the developed method. The developed method also analysed the formulation solution spiked with the known concentration of the impurities. The % assay was calculated by comparing the formulation results with that of the calibration curve results.

3. Results and Discussions

The literature survey for the available analytical methods proved that there is no method reported for the resolution and quantification of process-related impurities of alpelisib in synthetic drugs as well as in pharmaceutical formulations. Given the above, this study was intended to develop a simple and sensitive HPLC method for the quantification of 4 process-related impurities namely impurities 1, 2, 3 and 4 along with standard alpelisib in formulations. The systematic method development strategies, results achieved in each trailed condition, and method rejection reasons were summarized in Table 1.

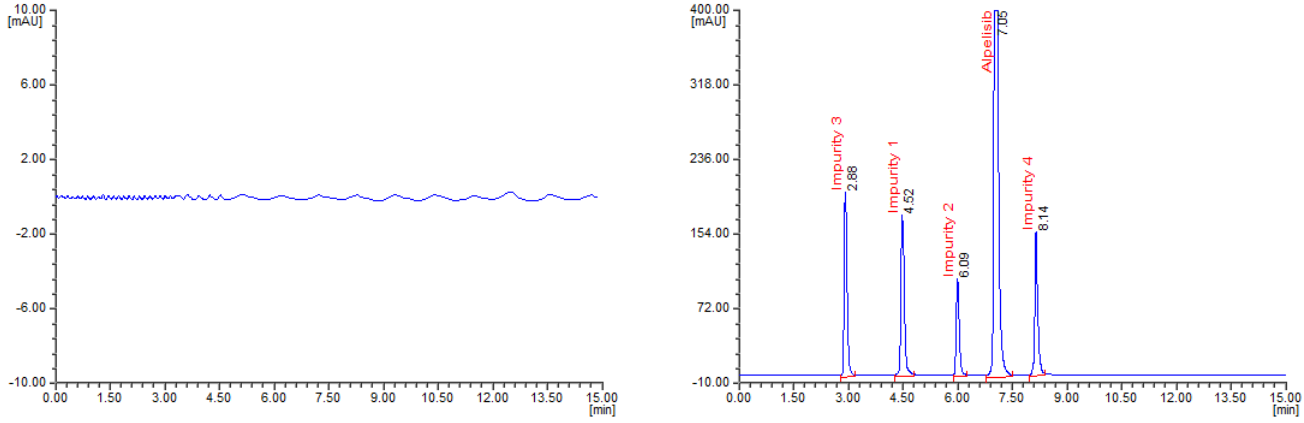
The method optimization was concluded by achieving the best resolution of analytes with acceptable system suitability. The mobile phase composition of ethanol and 0.1% aqueous acetic acid in 65:35 (V/V) at pH 4.5 as mobile phase at 0.8 mL/min flow rate, ProntoSIL ODS-C18 (250×4.6 mm; 5 µ id) column at room temperature as stationary phase and 246 nm as detector wavelength. In this proposed method conditions, the chromatogram of blank (Figure 4a) doesn't show any chromatographic detection throughout the run time whereas 100% solution of alpelisib spiked with 0.1% impurities were noticed to be well resolved and retained peaks corresponding to analytes in the study Figure 4b. This result proved that the method was specific for the detection of alpelisib and its impurities in the study.

Before validation of the developed method, repeatability and system suitability of the developed method was determined at recovery levels of alpelisib and its impurities. From the results obtained, the system

Table 1. Method development conditions tried during the optimization process

S. No.	Mobile Phase composition	Result	Conclusion
1	MP: 0.1% aqueous sodium acetate andethanol 50:50 (V/V); SP: Zodiac c18 (250×4.6 mm; 3.5 µ id) column; WL: 246 nm; FR: 0.8 mL/min	No clear identification of analytes was noticed and the baseline was observed to be disturbed throughout the run time	Method Rejected
2	MP: 0.1% aqueous sodium acetate andethanol in 75:25(V/V); SP: Zorbax XDB (250×4.6 mm; 3.5 µ id) column; WL: 246 nm; FR: 0.8 mL/min	Proper separation of analytes was not identified and split/merged peaks were identified for the analytes. Baseline was disturbed	Method Rejected
3	MP: 0.1% aqueous sodium acetate andethanol in 25:75 (V/V); SP: phenomenex luna C18 (250×4.6 mm; 5 µ id); WL: 246 nm; FR: 0.8 mL/min	Peaks were eluted very closely with very poor resolutions. Peak symmetry and area response was noticed to be very poor with nominal disturbances in the baseline.	Method Rejected
4	MP: 0.1% aqueous acetic acid and ethanol in 50:50 (V/V); SP: ProntoSIL ODS-C18 (250×4.6 mm; 5 µ id); WL: 246 nm; FR: 0.8 mL/min	Peaks corresponding to analytes were identified but the resolution was noticed to be very poor	Method Rejected
5	MP: 0.1% aqueous acetic acid and ethanol in 80:20 (V/V); SP: ProntoSIL ODS-C18 (250×4.6 mm; 5 µ id); WL: 246 nm; FR: 0.8 mL/min	Peaks for alpelisib, impurities 2 and 4 were eluted closely with poor system suitability. Peaks of impurity 1 and 3 were well resolved and retained. The baseline throughout the run time was noticed to be clear without any additional detection.	Method Rejected
6	MP: 0.1% aqueous acetic acid and ethanol in 10:90(V/V); SP: ProntoSIL ODS-C18 (250×4.6 mm; 5 µ id); WL: 246 nm; FR: 0.8 mL/min	Peaks correspond to alpelisib and its impurities were detected. The peak area response was poor with poor peak symmetry.	Method Rejected
7	MP: ethanol and 0.1% aqueous acetic acid in 65:35 (V/V) at pH 4.5; SP: ProntoSIL ODS-C18 (250×4.6 mm; 5 µ id); WL: 246 nm; FR: 0.8 mL/min	Acceptable separation of analytes with acceptable system suitability was noticed	Method Accepted

MP = Mobile Phase composition; SP = Stationary Phase; WL = Detector wavelength; FR = Flow Rate of mobile phase



a) Blank

b) Standard chromatogram of alpelisib spiked with 0.1 % of all impurities

Figure 4. System suitability chromatogram in the developed method.

Table 2. System suitability results

S. No.	Studied parameter	Results observed for				
		Alpelisib	Impurity 1	Impurity 2	Impurity 3	Impurity 4
1	Concentration in µg/mL	37.5	0.0375	0.0375	0.0375	0.0375
2	Retention time (min)	7.05	4.52	6.09	2.88	8.14
3	Peak Area	625656.7	42498.6	23625.8	52815.3	34849.1
4	Theoretical plates	7950	5471	6573	3107	9628
5	Tail factor	0.93	0.98	1.03	1.04	0.95
6	Resolution	4.85	7.63	5.12	--	4.02

suitability parameters like RRT, tail factor, number of theoretical plates and resolution were evaluated, and results confirm that all the parameters are within the acceptable limit (Table 2) hence the method was found to be acceptable.

The method sensitivity for the detection of impurities was assessed in terms of LOD and LOQ that were performed by adopting the s/n approach. The detection limit was observed to be 0.012 µg/mL for impurity 2, 0.011 µg/mL for impurity 1, 4 and 0.009 µg/mL for impurity 3 whereas the LOQ was observed to be 0.0375 µg/mL for impurity 2, 0.0325 µg/mL for impurity 1, 4 and 0.0297 µg/mL for impurity 3. The higher quantification limit concentration of impurities i.e., 0.0375 µg/mL was considered as the lowest concentration for constructing the calibration curve for impurities. The standard concentration was prepared such that the solution contains 0.1% of each impurity in standard alpelisib.

The accurate fit calibration curve was obtained in the concentration level of 125-100 µg/mL for alpelisib and 0.0125-0.10 µg/mL for impurities in the study. The regression equation was found to be y = 16137x + 21343 (R^2 = 0.9995), y = 1E+06x - 775.07 (R^2 = 0.9993), y = 704249x - 2056.7 (R^2 = 0.9997), y = 1E+06x + 1880.8 (R^2 = 0.9995) and y = 979175x - 2147.8 (R^2 = 0.9994) for alpelisib, impurities 1, 2, 3 and 4 respectively. The calibration curve was found to be linear with a very high correlation coefficient for both impurities and standard alpelisib. Linearity results were given in Table 3.

The repeatability and reproducibility of the developed method were performed at 100 µg/mL of concentration of alpelisib spiked with 0.1% of each impurity in the study. The % RSD of the peak area response was calculated and was observed to be 0.11, 0.48, 0.81, 0.42 and 0.36 in intraday precision, 0.29, 0.53, 0.99, 0.37 and 0.41 in interday precision and 0.44, 0.55, 0.73, 0.28 and

Table 3. Linearity results

S. No.	Alpelisib		Con# of impurity	Peak area (mV) observed for impurity			
	Con#	Peak Area (mV)		1	2	3	4
1	12.5	216025.5	0.0125	12516.8	7057.5	19795.6	10223.4
2	25	416858.3	0.025	28595.1	15995.5	33894.1	22656.2
3	37.5	625656.7	0.0375	42498.6	23625.8	52815.3	34849.1
4	50	848591.2	0.05	54841.2	32948.5	68946.1	46894.7
5	62.5	1040130.9	0.0625	68495.4	41875.6	86155.9	58147.3
6	75	1224979.2	0.075	83484.2	50491.3	103317.3	70018.1
7	87.5	1434913.6	0.0875	97682.5	59965.6	118710.2	84191.7
8	100	1625148.9	0.10	112636.4	68498.5	136959.7	96465.8

#Con = Concentration in µg/mL

Table 4. Robustness results

S. No.	Condition changed	% Change in peak area response				
		Alpelisib	Impurity 1	Impurity 2	Impurity 3	Impurity 4
1	MP 1	0.64	0.96	0.34	0.24	0.89
2	MP 2	0.92	0.33	0.95	0.62	0.77
3	pH 1	0.56	0.12	0.31	0.80	0.77
4	pH 2	0.96	0.30	0.83	0.23	0.36
5	WL 1	0.48	0.95	0.64	0.81	0.85
6	WL 2	0.98	0.13	0.62	0.41	0.76

0.63 in ruggedness for alpelisib, impurities 1, 2, 3 and 4 respectively.

In the robustness study, the mobile phase composition of ethanol and 0.1% aqueous acetic acid at 60:40 in MP 1, and 70:30 in MP 2 was studied. The pH of the mobile phase was varied to 4.4 (pH 1) and 4.6 (pH 2) whereas the detector wavelength was altered as 241 nm (WL 1) and 251 nm (WL2). The % change in all the changed conditions in the robustness study was found to be with the acceptable limit of less than 2 (Table 4) for alpelisib and its impurities studied. This confirms that the method is found to be robust as there is no considerable change in the separation and detection of alpelisib and impurities when small change in the developed method conditions.

The accuracy of the method was determined in spiked recovery studies by spiking 50%, 100% and 150% concentrations of target 100 µg/mL for alpelisib spiked with 0.1 µg/mL for impurity 1, 2, 3 and 4. The % recovery in was calculated standard and all impurities in each analysis and the % RSD was calculated for each spiked level. The acceptable % recovery of 98-102 was observed for alpelisib and impurities studied and the % RSD in each spiked level was found to be less than 2 (Table 5) which is the acceptable limit. Hence the method was found to be accurate.

The different stress-exposed and unstressed standard solution of alpelisib was analysed in the optimised conditions. In acid degradation, the % degradation was calculated to be 8.51%. The chromatogram in this condition shows three degradation products identified at 1.9 min, 3.4 min and 6.1 min. In this, the impurity 2 and 3 were also detected (Figure 5). In base degradation, the % degradation of 7.35% was noticed with three additional degradation products retained at 3.8 min, 5.5 min and 11.9 min (Figure 6). In this condition, impurities 1, 3 and 4 in the study were also retained. Figure 7 represent the oxidative degradation chromatogram of alpelisib that resolves two degradation products along with impurity 2 and 3 in the study. The % degradation was calculated to be 5.35%. A very high % degradation of 9.93% was

Table 5. Recovery results

S. No.	Compound	Recovery Level	Concentration in µg/mL	Amount found Mean ± SD*	% recovered Mean ± SD*	% RSD of Recovery
1	Alpelisib	50%	25	24.948±0.026	99.79±0.104	0.10
2		100%	50	49.772±0.101	99.54±0.202	0.20
3		150%	75	74.613±0.270	99.48±0.360	0.36
4	Impurity 1	50%	0.025	0.0247±0.0001	98.95±0.522	0.53
5		100%	0.05	0.0493±0.0002	98.53±0.475	0.48
6		150%	0.075	0.0745±0.0001	99.34±0.086	0.09
7	Impurity 2	50%	0.025	0.0246±0.0001	98.37±0.224	0.23
8		100%	0.05	0.0496±0.0004	99.22±0.705	0.71
9		150%	0.075	0.0740±0.0004	98.62±0.558	0.57
10	Impurity 3	50%	0.025	0.0248±0.0001	99.02±0.395	0.40
11		100%	0.05	0.0494±0.0004	98.88±0.828	0.84
12		150%	0.075	0.0739±0.0003	98.53±0.428	0.43
13	Impurity 4	50%	0.025	0.0248±0.0001	99.12±0.551	0.56
14		100%	0.05	0.0495±0.0004	98.90±0.829	0.84
15		150%	0.075	0.0752±0.0004	100.29±0.552	0.55

*Values given in the table are the average±standard deviation for three replicate experiments

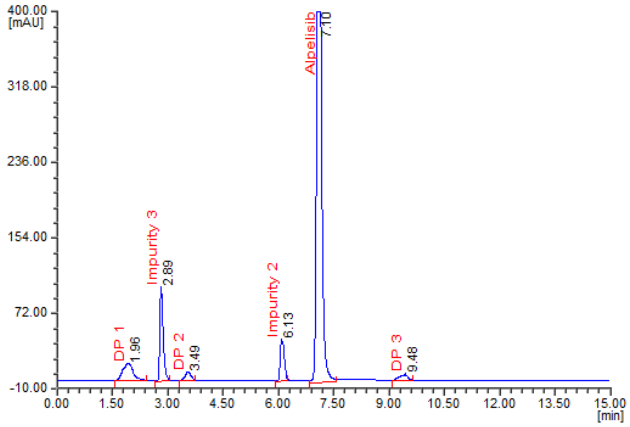


Figure 5. Chromatogram identified in acid degradation study.

calculated in the photolytic degradation study. The chromatogram obtained in this condition (Figure 8) shows well-resolved 4 degradation products at a retention time of 1.47 min, 3.79 min, 10.01 min and 13.47 min. The impurity 1, 2 and 3 in the study were also detected in this stress study and these impurities were well resolved from degradation products. In the thermal degradation study, the % degradation was calculated to be 5.91% and three additional degradation products were well resolved from impurities 2, and 4 in the study along with standard alpelisib. In all the degradation conditions, there is no change in the retention time of the alpelisib along with its known impurities in the study. Hence the method can separate and quantify the process-related impurities in alpelisib.

The developed HPLC method was applied for the quantification of process-related impurities of alpelisib in pharmaceutical formulation. The formulation sample was directly analysed for the evaluation of impurities present in it and the formulation sample spiked with impurities was analysed for evaluating the effectiveness of the method for the resolution and quantification of impurities in the formulation. The chromatogram obtained for impurities spiked formulation solution (Figure 10a) shows clear identification of peaks corresponding to impurities in the study. Whereas the chromatogram observed for the unspiked formulation solution (Figure 10b) shows peaks corresponding to impurities 1, 3 and 4. 2 was not identified in the chromatogram proving that the quantity of impurity

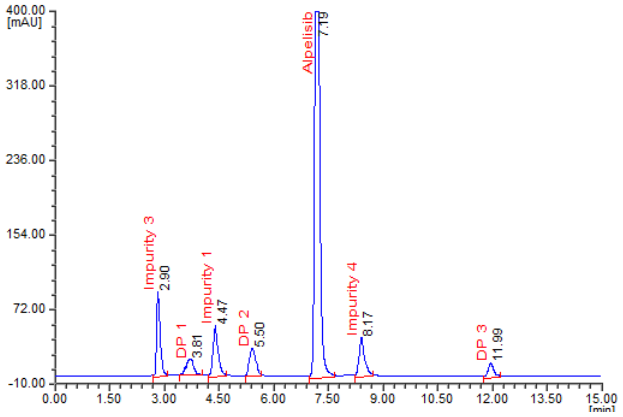


Figure 6. Chromatogram identified in base degradation study.

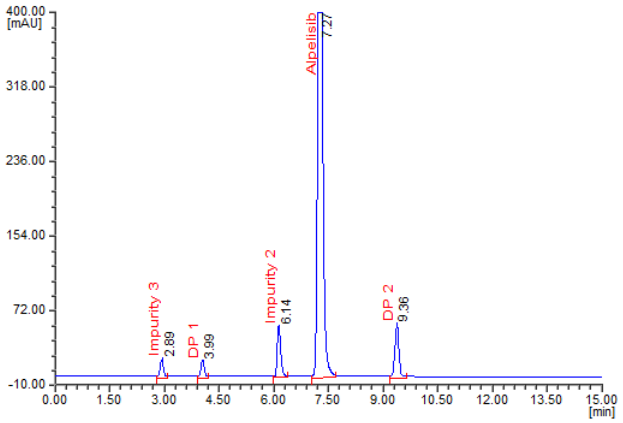


Figure 7. Chromatogram identified in oxidative photolytic degradation study.

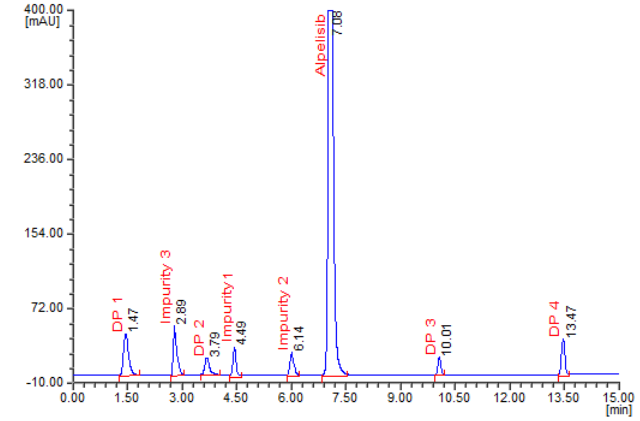


Figure 8. Chromatogram identified in photolytic degradation study.

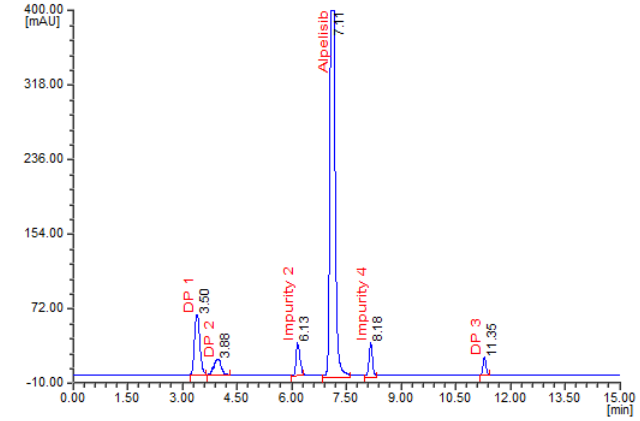
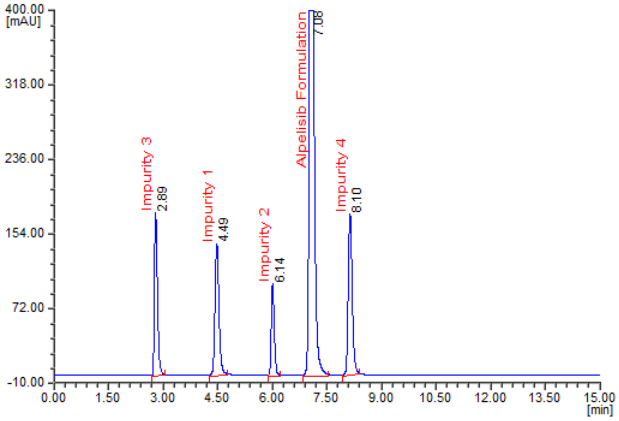
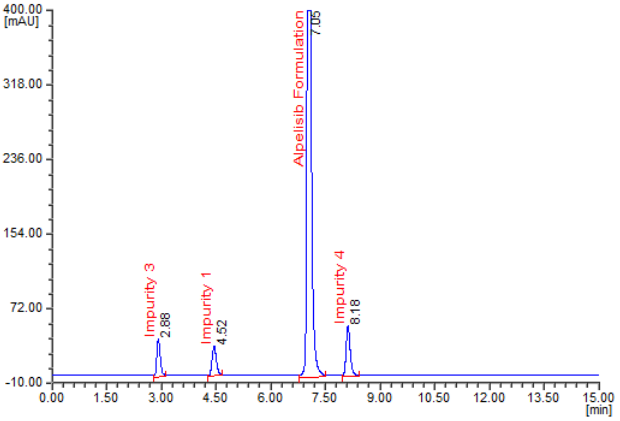


Figure 9. Chromatogram identified in thermal degradation study.



(a) Spiked sample chromatogram.



(b) Un- spiked sample chromatogram.

Figure 10. Formulation chromatogram of alpelisib in the developed method.

in the sample was less than the detection limit of impurity B. The peak area response of impurities 1, 3 and 4 was substituted in its corresponding regression equation and the % assay of impurities was calculated. The % assay of impurities was estimated to be 0.03%, 0.02% and 0.04% respectively for impurities 1, 3 and 4. This confirms that the quantity of impurities in the sample was noticed to be under the acceptable level and the proposed method can be successfully applicable for the quantification of process-related impurities in alpelisib.

The findings reported in the study were compared with the literature available for the analysis of alpelisib. In the literature methods only reported for quantification of alpelisib in biological samples and evaluation of its pharmacokinetic profile. This reported method is not able to resolve and quantify process-related impurities of alpelisib and is also not suitable for the resolution of stress degradation compounds of alpelisib. Hence the present study is significantly helpful for the quantification of process-related impurities and of alpelisib in formulations.

4. Conclusion

A novel stability-indicating RP-HPLC method was developed for the separation, qualitative and quantitative determination of 4 process-related impurities of alpelisibin bulk and dosage forms. The method was adequately suitable for the resolution and identification of various stress degradation compounds. The method possesses an extensive and sensitive calibration range with high recoveries and less analysis time. The method can efficiently resolve the impurities in the formulation and can be suitable for the quantification of process-related impurities in bulk drugs and formulations.

5. References

1. Armenta S, Garrigues S, Guardia DM. Green analytical chemistry. TRAC-Trend Anal Chem. 2008; 27(6): 497-511. https://doi.org/10.1016/j.trac.2008.05.003
2. Koel M, Kaljurand M. Application of the principles of green chemistry in analytical chemistry. Pure Appl Chem. 2006; 78(11):1993-2002. https://doi.org/10.1351/pac200678111993
3. Tobiszewski M, Mechlinska A, Namiesnik J. Green analytical chemistry – theory and practice. Chem Soc Rev. 2010; 39(8):2869-78. https://doi.org/10.1039/b926439f PMid:20502819
4. Gałuszka A, Migaszewski Z, Namiesnik J. The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices. TRAC-trend Anal Chem. 2013; 50:78-84. https://doi.org/10.1016/j.trac.2013.04.010
5. Płotka J, Tobiszewski M, Sulej AM, Kupska M, Gorecki T, Namiesnik J. Green chromatography. J Chromatogr A. 2013; 1307:1-20. https://doi.org/10.1016/j.chroma.2013.07.099 PMid:23932374
6. André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, *et al*. PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med. 2019; 380(20):1929–40. https://doi.org/10.1056/NEJMoa1813904 PMid:31091374
7. André F, Ciruelos EM, Juric D, Loibl S, Campone M, Mayer IA, Rubovszky G, *et al*. Alpelisib plus fulvestrant for PIK3CA-mutated, hormone receptor-positive, human epidermal growth factor receptor-2–negative advanced breast cancer: Final overall survival results from SOLAR-1. Ann Oncol. 2021; 32(2):208-17. https://doi.org/10.1016/j.annonc.2020.11.011 PMid:33246021
8. Ye Y, Huang Z, Zhang M, Li J, Zhang Y, Lou C. Synergistic therapeutic potential of alpelisib in cancers (excluding breast cancer): Preclinical and clinical evidence. Biomed Pharmacother. 2023; 159:114183. https://doi.org/10.1016/j.biopha.2022.114183 PMid:36641927
9. Wang Q, Lan X, Zhao Z, Su X, Zhang Y, Zhou XY and Xu RA. Characterization of Alpelisib in rat plasma by a newly developed UPLC-MS/MS method: Application to a drug-drug interaction study. Front Pharmacol. 2021; 25(12):743411. https://doi.org/10.3389/fphar.2021.743411 PMid:34899297 PMCid:PMC8656162
10. Seo SW, Kim JM, Han DG, Geum D, Yun H, Yoon IS. A sensitive HPLC-FLD method for the quantification of alpelisib, a novel phosphatidylinositol 3-kinase inhibitor, in rat plasma: Drug metabolism and pharmacokinetic evaluation *in vitro* and *in vivo*. J Chromatogr B Analyt Technol Biomed Life Sci. 2021; 1163:122508. https://doi.org/10.1016/j.jchromb.2020.122508 PMid:33387858
11. Majumder T, Gubbiyappa SK. Development and validation of LC–MS/MS method for alpelisib quantification in human plasma: Application to pharmacokinetics in healthy rabbits. J Appl Pharm Sci. 2023; 13(03):089-96. https://doi.org/10.7324/JAPS.2023.75269
12. Lee S, Kim MS, Jeong JW, *et al*. Bioanalysis of alpelisib using liquid chromatography–tandem mass spectrometry and application to pharmacokinetic study. J Anal Sci Technol. 2022; 31:1603. https://doi.org/10.7324/JAPS.2023.75269

13. Yong X, Yanping Y, Jing Y, Zhaoze F, Wei D, Long C. Novel synthesis method of PI3K inhibitor Alpelisib. 2020; Patent No. CN111057051A, China.
14. Bikshal BK, Useni RM, Venkateswara RA, Maheshwara RL. Intended high-performance liquid chromatography procedure for the quantification of norfloxacin and its potential impurities in active pharmaceutical ingredient and tablet dosage forms. Thai J Pharm Sci. 2018; 42(1):27-36.
15. Mallu UR, Anna VR, Kasimala BB. Rapid stability indicating HPLC method for the analysis of leflunomide and its related impurities in bulk drug and formulations. Turk J Pharm Sci. 2019; 16:457-65. https://doi.org/10.4274/tjps.galenos.2018.34635 PMid:32454750 PMCid:PMC7227885
16. Babu BK, Rao RA, Reddy UM. Stability-indicating reversed-phase HPLC method for the separation and estimation of related impurities of cilnidipine in pharmaceutical formulations. Indian Drugs. 2018; 55(12):41-9. https://doi.org/10.53879/id.55.12.11185
17. Girija SK, Babu BK, Rao VA. A new high-performance liquid chromatography method for the separation and simultaneous quantification of eptifibatide and its impurities in pharmaceutical injection formulation. Int J App Pharm. 2021; 13(2):165-72. https://doi.org/10.22159/ijap.2021v13i2.39895
18. ICH validation of analytical procedures: Text and methodology Q2(R1); 1994. p. 1-13.