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Development and validation of LC–MS/MS method for alpelisib quantification in human plasma: Application to pharmacokinetics in healthy rabbits

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ABSTRACT

A new sensitive and specific liquid chromatography-tandem mass spectrometry methodology was needed for the estimation of alpelisib in plasma samples. Drug components were extracted by liquid–liquid extraction technique utilizing ethyl acetate. Liquid chromatographic system was processed with Zorbax C18 (50 × 4.6 mm, 5 µ) reverse phase analytical column with isocratic mobile solvent system consisting of acetonitrile and 0.1% formic acid in the ratio of 90:10 (%V/V). Analytes were detected on mass system equipped with triple quadrupole system and electrospray ionization, functioning in multiple reaction monitoring, with the transitions of m/z 442.15 → 70.06, m/z 409.14 → 391.13 for alpelisib, dapagliflozin, respectively, in the positive ionization mode. Method has given more than 90.0% recovery values and accuracy values were present in between −4.32% and 4.37% of relative error. All the relative standard deviation findings were less than 4.21%. Alpelisib pharmacokinetic parameters were determined from the curve obtained by taking time on *X*-axis and concentration of plasma on *Y*-axis. Alpelisib has mean *T*max of 2.0 ± 0.21 and AUC0$_{\rightarrow\alpha}$, AUC0t and mean *C*max, for test formulation is 3,624.21 ± 315, 3,075.20 ± 316, and 269 ± 12.17, respectively.

INTRODUCTION

Alpelisib chemically designated as (2*S*) -1- *N*- [4-Methyl -5- [2- (1,1,1- trifluoro-2-methylpropan-2-yl) pyridin-4-yl] -1,3-thiazol-2-yl] pyrrolidine-1, 2-dicarboxamide. Its chemical formula and molecular mass are C19H22F3N5O2S and 441.47 g/mol, respectively (Fig. 1). Alpelisib inhibits the enzyme, phosphatidylinositol 3-kinase (PI3K) and shows effective antitumor action (Konstantinopoulos *et al*., 2019; Yang *et al*., 2017). It acts by the inhibition of class-I PI3K p110α specifically, a lipid kinase which shows an important role in different biological processes, like survival, proliferation, metabolism, and differentiation (James *et al*., 2015; Rodon *et al*., 2018).

This drug is approved by the United States Food and Drug Administration (USFDA) in the month of May 2019, as the first PI3K inhibitor designated for human epidermal growth factor receptor 2-negative, treatment of hormone receptor-positive, advanced or metastatic breast cancer, PIK3CA-mutated in combination with fulvestrant for postmenopausal women and male patients. To start alpelisib treatment, it is essential that the existence of PIK3CA transmutation in tissue or liquid biopsy sample collection should be established through diagnostic tests approved by Food & Drug Administration (FDA). The drug is marketing under the Piqray trade name and is existing as oral tablets. Studies assessing the therapeutic efficiency of drug in other cancer types, such as colorectal cancer and ovarian cancer, are under continuing research (De Buck *et al*., 2014; James *et al*., 2017).

The literature on alpelisib reveals that no single method was reported on liquid chromatography-tandem mass spectrometry (LC–MS/MS) for the estimation of alpelisib in plasma (Seo *et al*., 2021). Current research work was directed to develop a

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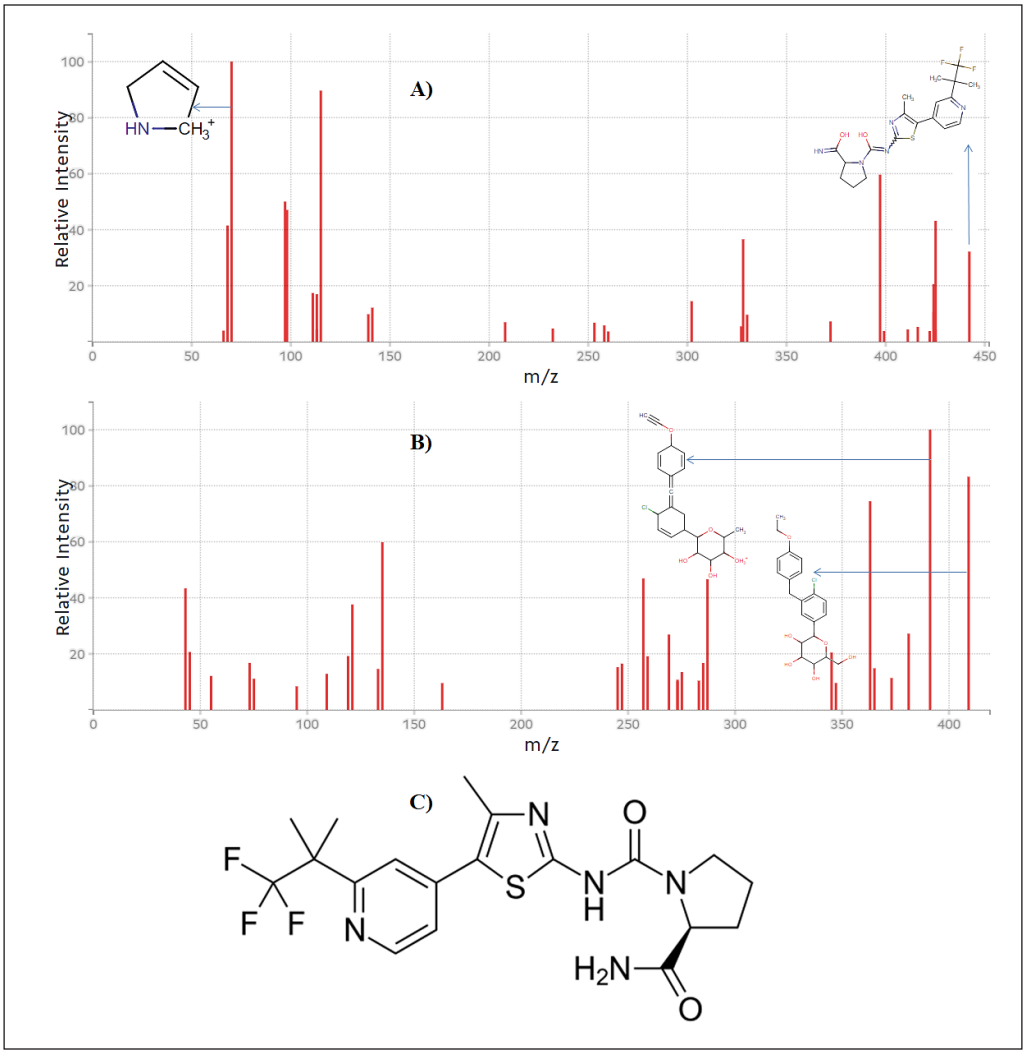

Figure 1. Mass spectrum of A) Alpelisib, B) Dapagliflozin, and C) Chemical structure of Alpelisib.

new sensitive and specific technique for the estimation of alpelisib in human plasma. Few analytical methods on LC-MS/MS were reviewed for method development and validation.

MATERIALS AND METHODS

Reagents and chemicals

The standards of alpelisib (purity: 99.51%) and dapagliflozin (purity: 99.82%) utilized as internal standard (IS) were obtained from the Dr. Reddy's Lab, Hyderabad, India. High Performance Liquid Chromatography (HPLC) grade methanol and acetonitrile were procured from AB enterprises, Mumbai, India. Deionized water is processed by a Milli-Q water system. Human plasma samples were procured from Vivekananda blood blank, Hyderabad, India.

Equipment and LC–MS/MS parameters

The LC–MS/MS instrument comprised of an Agilent1200 liquid chromatographic system with a SL-binary pump and 6460-Agilent triple-quadrupole mass system with electrospray ionization. Mass-Hunter software B0104 version was utilized for the data acquisition and for the controlling the LC-MS/MS system. The analyte elution was executed at 35.0°C through Zorbax C18 (50 × 4.6 mm, 5 µ) reverse phase analytical column. The mobile phase consisted of acetonitrile and 0.1% formic acid in the ratio of 90:10 (%V/V) with 0.90 ml/minute flow rate. The auto-sampler temperature was maintained at 10.0°C, and infusion volume was 10.0 µl. Mass system was ran in positive ionization mode. MS/MS study was organized under scan mode of multiple reaction monitoring (MRM). The MS/MS constraints were adjusted as: 5.0 kV capillary voltage; 400.0°C source temperature; 12.0 l/minute N2 drying gas flow and 40.0 psi nebulizer gas pressure. The optimized voltage for the fragmentation of both alpelisib and IS was 135.0 V. Data acquisition was processed in MRM mode utilizing transitions of m/z 442.15 → 70.06 for alpelisib with 12.0 eV collision energy and m/z 409.14 → 391.13 for IS with 15.0 eV collision energy.

Protocol for quality control (QC) and standard solutions

1.0 mg/ml alpelisib and IS individual stock solutions were processed in 80% acetonitrile (diluent) in water separately. The alpelisib stock solution was then subjected for serial dilution to acquire the working standards. The IS 500 ng/ml working solution was processed by diluting IS stock solution with diluent. Calibration curve standards of alpelisib (145, 410, 1,050, 1,950, 2,900, 3,900, 4,900, and 5,800 ng/ml) were processed by spiking the suitable working standards to blank plasma. QC samples at low, medium, and high concentrations (406, 2,900, and 4,350 ng/ml) were executed distinctly in the same way.

Protocol for sample solution

50.0 µl of sample plasma was employed in 10.0 ml plastic tube and into that 125.0 µl of IS working standard was added to all the sample solutions except blank. Mixture was subjected for extraction with 4.0 ml of diethyl ether by vortexing for 5.0 minutes and shaking for 25.0 minutes. Then, samples are subjected for centrifugation at 5.0°C for 15 minutes at 4,500 rpm. Organic layer is relocated to fresh glass tubing and vaporized to dryness with the help of stream of N2. The resulting residue was re-formed with 100.0 µl of movable solvent and a 10.0µl sample was infused in to LC–MS/MS instrument for the examination.

Pharmacokinetic study

Six male rabbits of about 2.50 to 3.0 kg were chosen for the pharmacokinetic study of alpelisib. Before 12 hours of a drug direction into the rabbits, food was evaded. Water has provided for the rabbits under the study throughout the work and 37.5 mg/kg drug dose has given to healthy rabbits and taken 0.60 ml of blood sample solutions from marginal ear vein of rabbits prior to dosing (zero-time) and at different time intervals between 0 and 24 hours. The resultant solution was exposed to 4,500 rpm in the centrifuge for 15.0 minutes and plasma samples were separated and relocated into labelled polypropylene tubings at −20.0°C (Patel *et al*., 2011; Ravi *et al*., 2021). The study was approved by Institutional animal ethical committee with No: 1447/PO/Re/8/11/CPCSEA/18/A.

Method validation

The developed analytical process was subjected for the validation as per the guidelines of FDA (EMA, 2005; Shankar and Bhikshapathi, 2021) to meet the acceptance limit.

RESULTS AND DISCUSSION

Method optimization

Current research work was aimed to develop a highly sensitive and selective LC–MS/MS method for the estimation of alpelisib in human plasma. Initially, a mobile phase consisting of methanol, acetonitrile and formic acid, in varying combinations were tried, but less resolution and low response was observed. Finally chromatographic separation was performed on a Zorbax C18 (50 × 4.6 mm id, 5 µm) analytical column with a simple isocratic mobile phase composed of 0.1% formic acid and acetonitrile, (10:90, *v/v*). The LC system was operated at a flow rate of 0.90 ml/minute with total single run time of 6.5 minutes. The column and auto-sampler temperatures were maintained at 40.0°C and 10.0°C, respectively.

Mass system optimization

During the optimization of product and parent ions in the mass instrument, neat alpelisib solution was injected under the positive ionization mode. Precursor ion was observed at m/z 442.15 and fragments of m/z 397.13, 115.08 and 70.06 were noticed upon parent ion fragmentation. Fragment of alpelisib ion having m/z 70.06 was identified with the utmost intensity. Alpelisib isotopes were not available commercially, so we have searched for variable possible IS, and elected dapagliflozin as IS. MRM transitions of m/z 442.15 → 70.06 for alpelisib, and m/z 409.14 → 391.13 for IS were monitored after optimization of the conditions.

Specificity and selectivity

Interference peaks were not identified in the alpelisib and dapagliflozin samples of plasma. The representative chromatograms of spiked plasma at 145 ng/ml and blank of alpelisib (lower limit of quantification) and IS were presented in Figure 2. The retaining timings of alpelisib and IS were 2.50 and 1.18 minutes, respectively (Patel *et al*., 2017).

Sensitivity and linearity

Linearity graphs were executed for each lot, over concentration between 145 and 5,800 ng/ml for alpelisib (Table 1) in plasma sample. The average regression line equation gained for alpelisib was $0.0018 + 0.0056\,x = y$ ($n = 6$) for alpelisib, where x is the plasma concentrations and y is meant for fractions of analytes to dapagliflozin. The Lower Limit of Quantification (LLOQ) standard of alpelisib was set to 145 ng/ml with less than 3.54% accuracy and precision values and the S/N findings were more than 10.0 (Shah *et al*., 2017).

Matrix effects

Table 4 represents findings of matrix effect processed at LQC and HQC levels (Titier *et al*., 2007). The peak response ratios of drug/IS, which were liquefied with plasma blank extractions to those liquefied with movable phase ranges between 93.87% and 102.85% for alpelisib at LQC level and 94.21% to 102.89% at HQC level. These findings recommended that matrix effect of alpelisib were nil under current LC–MS/MS circumstances.

Recovery, precision, and accuracy

Table 2 and Figure 3 were represents findings of intraday and inter-day accuracy and precision. Intraday precision values were ranged between 1.75% and 3.19% relative standard deviation (RSD) for drug component, while the accuracy values were present in between −0.71% and 3.25% of relative error. Likewise, for inter-day experimentations, accuracy values were present in between 0.67% and 3.45% of relative error, while the precision differ in between 1.67% and 3.17% (RSD) for alpelisib. The findings were evidenced that the technique was accurate and

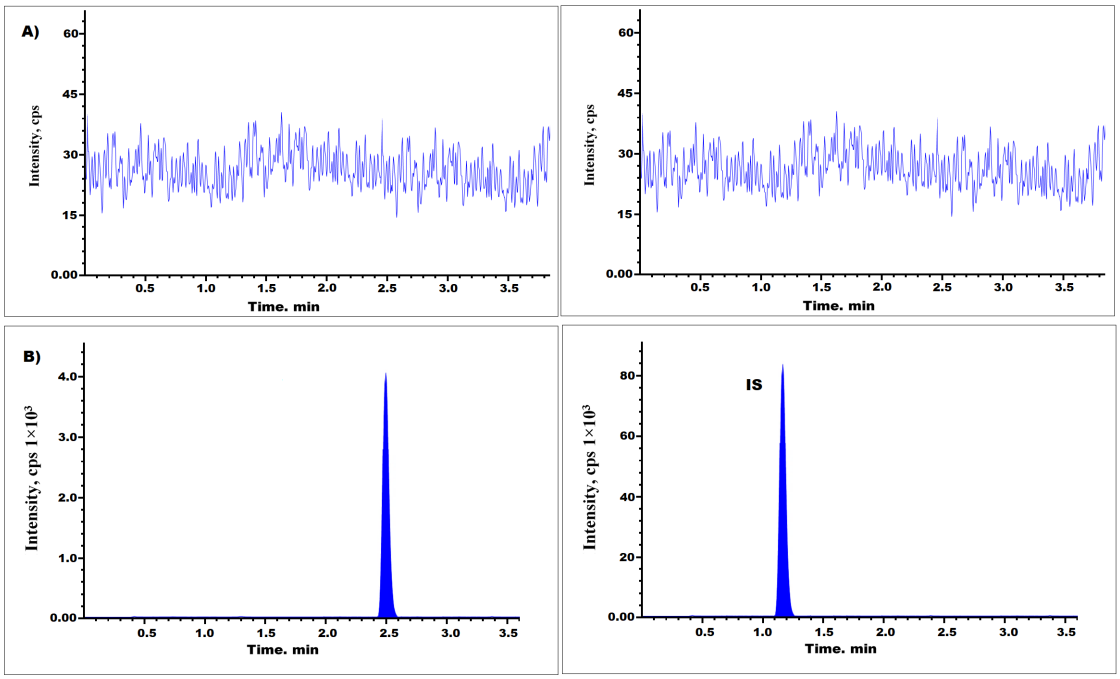

Figure 2. Representative chromatograms of drug A) Blank and B) LLOQ samples.

Table 1. Calibration standards for alpelisib.

CS-ID	Concentration (ng/ml)	Mean[a] (ng/ml)	%RSD	%RE
CS-1	145	143.23	3.14	1.22
CS-2	410	400.45	2.95	2.33
CS-3	1,050	1,011.38	3.45	3.68
CS-4	1,950	1,986.44	4.01	−1.87
CS-5	2,900	2,964.56	2.56	3
CS-6	3,900	4,038.35	3.08	−3.55
CS-7	4,900	4,742.23	2.47	3.22
CS-8	5,800	5,973.392	1.94	−2.99

a 6 replicates.

Table 2. Intraday and inter-day accuracy and precision of alpelisib.

Spiked conc. (ng/ml)	Intraday[a]			Interday[b]		
	Measured conc. (mean ± SD; ng/ml)	Precision (RSD %)	Accuracy (RE %)	Measured conc. (mean ± SD; ng/ml)	Precision (RSD %)	Accuracy (RE %)
145	146.03 ± 3.49	2.38	−0.71	144.03 ± 2.98	2.06	0.67
406	395.97 ± 12.77	3.22	2.47	391.97 ± 11.21	2.85	3.45
2,900	2,845.64 ± 49.77	1.75	1.87	2,832.64 ± 47.26	1.67	2.32
4,350	4,128.45 ± 134.65	3.19	3.25	4,215.45 ± 133.52	3.17	3.09

a 6 replicates.
b 6 × 3 replicates.

precise (Patel *et al*., 2011). The alpelisib average recovery values were varied in between 94.85% and 102.35% at three levels of QC standards (Table 3).

Stability analysis

Alpelisib stability studies were processed by exposing the QC standards to variable storage environments. The subjected

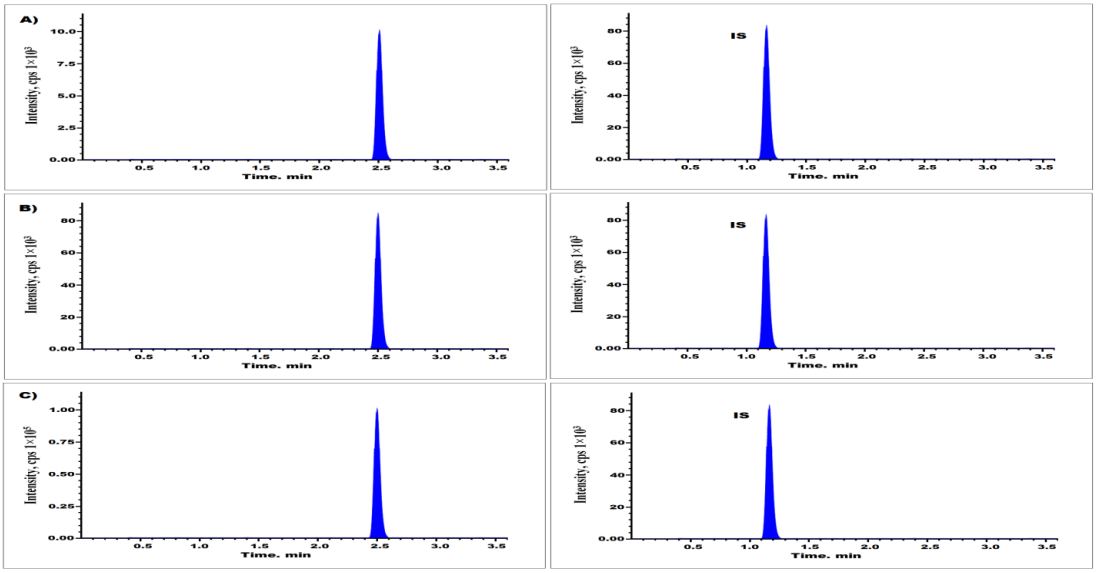

Figure 3. QC standards of alpelisib at A) LQC, B) MQC, and C) HQC levels.

Table 3. Extraction recovery rates of analytes.

Concentration level	A	B	% Recovery	% Mean recovery	%RSD
LQC	11,636	11,070	95.14	97.45	3.56
MQC	83,120	78,839	94.85		
HQC	124,680	127,610	102.35		
IS	85,846	84,489	98.42		

A, average recovery values of unextracted sample; B, average recovery of extraction sample.

Table 4. Matrix effect for alpelisib at HQC and LQC level.

S. No.	LQC			HQC		
	Peak response in absence of matrix	Peak response in existence of matrix	Matrix factor	Peak response in lack of matrix	Peak area in presence of matrix	Matrix factor
1	388.14	399.20	102.85	4,368.22	4,115.3	94.21
2	383.34	373.91	97.54	4,318.38	4,112.83	95.24
3	399.56	392.25	98.17	4,302.61	4,426.96	102.89
4	434.82	451.52	103.84	4,047.47	3,852.79	95.19
5	385.82	364.83	94.56	4,013.63	3,837.43	95.61
6	401.57	376.95	93.87	4,449.12	4,637.76	104.24
Mean			98.47			97.89
± SD			4.13			4.44
% RSD			4.19			4.53

Table 5. Stability findings of alpelisib (n = 3).

Storage condition	LQC 406 ng/ml		MQC 2,900 ng/ml		HQC 4,350 ng/ml	
	Accuracy (Mean%)	Precision (RSD%)	Accuracy (Mean%)	Precision (RSD%)	Accuracy (Mean%)	Precision (RSD%)
Room temp., 8 hours	92.34	3.86	101.85	2.64	94.32	3.86
30 day at −20°C	96.73	4.71	98.37	3.86	99.24	4.72
Three freeze-thaw cycles	103.65	2.39	92.65	4.12	94.61	3.81
Extract, 24.0 hours at 4.0°C	93.16	1.87	95.28	2.66	94.55	2.91

RSD: relative standard deviation.

Table 6. Measured plasma concentration values in healthy rabbits.

Time (hours)	Plasma concentration (ng/ml)							
	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Avg	SD
0	0	0	0	0	0	0	0	0
0.15	43	44	39	41	42	40	41.5	1.7075
0.25	92	90	89	93	92	90	91	1.414
0.5	129	128	121	124	128	123	125.5	2.986
0.75	189	175	185	180	179	178	181	4.654
1	217	209	211	215	206	214	212	3.741
1.5	226	221	219	226	221	229	223.6667	3.543
2	261	253	275	278	279	241	264.5	4.139
2.5	185	188	179	185	186	184	184.5	2.7537
3	163	169	160	158	160	169	163.1667	4.3702
4	139	130	132	136	138	132	134.5	3.3541
5	123	115	119	120	118	116	118.5	2.629
6	106	100	101	106	105	104	103.6667	2.3570
8	101	99	98	102	97	99	99.33333	1.699
12	79	75	76	74	72	77	75.5	2.217
16	60	62	59	63	64	60	61.33333	1.795
20	42	44	39	46	48	45	44	2.886
24	0	0	0	0	0	0	0	0

Table 7. pk parameter values of test animals.

Parameters	Animal-1	Animal -2	Animal -3	Animal -4	Animal -5	Animal -6	Average	SD
*C*max	261	253	275	278	279	241	264.5	14.14
log *C*max	2.4166	2.403	2.43	2.44	2.445	2.382	2.42	0.02
*T*max	2.08	2.02	2.04	2.07	2.05	2.03	2.05	0.02
log *T*max	0.318	0.305	0.309	0.315	0.311	0.307	0.31	0.0044
*t*1/2	7.24	7.16	7.21	7.15	7.25	7.08	7.18	0.058
log *t*1/2	0.8597	0.8543	0.853	0.854	0.860	0.85	0.86	0.003
Ke	0.059	0.063	0.081	0.075	0.074	0.068	0.07	0.008
log Ke	−1.22914	−1.2003	−1.0915	−1.1249	−1.13073	−1.16911	−1.16	0.05
AUC0→last	3,610.19	3,331.84	3,810.87	3,501.02	3,885.02	3,606.9	3,624.306	184.39
log AUC0→last	3.55753	3.5226	3.581	3.5441	3.5893	3.5571	3.55866	0.022

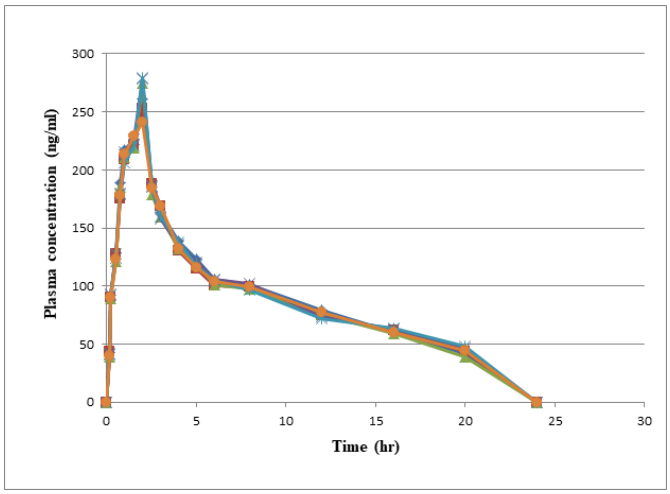

Figure 4. Profile for test rabbits' plasma concentrations and time.

environments comprise short term stability at long-term stability after storage at −20.0°C for 30 days, room temperature for 8.0 hours, the prepared extract sample stability after 24.0 hours at 4.0°C and three complete freeze thaw cycle (freeze temperature at −20.0°C for 12.0 hours) (Titier *et al*., 2007). The findings of stability studies at QC levels at processed and plasma sample were represented in Table 5. The evaluated accuracy findings for alpelisib were present in between 92.34%–103.65% of the nominal concentrations which were within the satisfactory limits. As an end result, alpelisib was considered to be more stable under dissimilar storage environments.

Pharmacokinetics

The Pharmacokinetic parameters of alpelisib were estimated from the plot drawn by taking time on *X*-axis and plasma concentration on *Y*-axis by utilizing Pk-solver software. In the present work trapezoidal rule has been elected for the computing

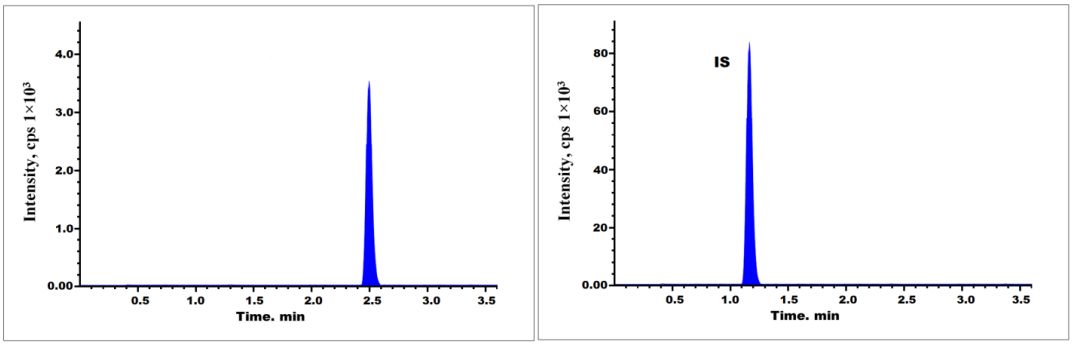

Figure 5. Chromatogram of rabbit plasma sample.

of an area beneath the plot from 0.0 to 24.0 hours(AUC0–24). Alpelisib has an average *T*max of 2.0 ± 0.21 and AUC0$_{\rightarrow\alpha}$, AUC0$_{\rightarrow}$t and mean *C*max, for test formulation is 3,624.21 ± 315, 3,075.20 ± 316, and 269 ± 12.17, respectively. The resultant finding were represented in Tables 6 and 7, Figures 4 and 5.

CONCLUSION

A specific, reliable, and validated LC-MS/MS technique was developed for the quantification of alpelisib drug component in human plasma. The subjected validation parameters in obedience to FDA procedures were fully satisfied. Developed technique was specific and sensitive with 145 ng/ml as LLOQ and the total run time of 3.5 minutes. The intra-day and inter-day accuracy results existed in between −0.71% and 3.45% of relative error and the RSD findings related to precision were below 3.25%. Alpelisib was effectively stable beneath variable analytical environments. liquid liquid extraction (LLE) process was finalized for alpelisib extraction from plasma with average percentage recoveries of 97.45% by utilizing the dapagliflozin as an IS. Alpelisib has an average *T*max of 2.0 ± 0.21 and average *C*max, AUC0t and AUC0$_{\rightarrow\alpha}$ for drug was 269 ± 12.17, 3,075.20 ± 316, and 3,624.21 ± 315, respectively. The specificity, accuracy, and higher percentage recovery from plasma of the current technique considers it suitable for pharmacokinetic and bioequivalence studies.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

FUNDING

There is no funding to report.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

ETHICAL APPROVALS

The study was approved by Institutional animal ethical committee with No: 1447/PO/Re/8/11/CPCSEA/18/A.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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