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Bioanalysis of alpelisib using liquid chromatography–tandem mass spectrometry and application to pharmacokinetic study

Seop Lee[1,2], Min-Soo Kim[3], Jong-Woo Jeong[1], Jung-woo Chae[2], Tae-Sung Koo[4], Han-Joo Maeng[5], Suk-Jae Chung[3], Kyeong-Ryoon Lee[1,6*] and Yoon-Jee Chae[7*]

Abstract

Alpelisib is the first alpha-specific phosphatidylinositol-3-kinase (PI3K) inhibitor indicated for the treatment of hormone receptor-positive, human epidermal growth factor receptor 2-negative, PI3K catalytic subunit alpha-mutated, advanced, or metastatic breast cancer. Substantial attempts have been made to extend its clinical use to other types of cancer. Analytical methods proven to accurately quantify alpelisib would improve the reliability of the preclinical and clinical data of alpelisib. Therefore, we developed and validated a quantification method based on liquid chromatography–tandem mass spectrometry for alpelisib in mouse and human plasma samples. Alpelisib and an internal standard (IS; enzalutamide) were separated from endogenous substances using an XTerra MS C18 column with a linear gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Multiple reaction monitoring transitions for alpelisib and the IS were *m/z* 442.1 > 328.0 and *m/z* 465.0 > 209.1, respectively. The calibration curve for alpelisib was confirmed to be linear in the range of 1–2000 ng/mL in both mouse and human plasma. The intra- and inter-day accuracy and precision met the acceptance criteria, and no significant matrix effects were observed. Alpelisib was stable under various storage and handling conditions, and the carryover effect was overcome using the injection loop flushing method. We successfully used this assay to study the in vitro metabolic profiles and in vivo pharmacokinetics of alpelisib in mice. Here, to the best of our knowledge, we report for the first time a valid quantitative method for alpelisib in mouse and human plasma, which could aid in providing valuable pharmacokinetic information on alpelisib to increase its clinical availability.

Keywords: Alpelisib, Liquid chromatography–tandem mass spectrometry, Method validation, Pharmacokinetics

Introduction

Phosphatidylinositol-3-kinase (PI3K) is a plasma membrane-associated lipid kinase composed of three subunits: the p85 regulatory, p55 regulatory, and p110 catalytic subunits (Yu et al. 1998). The catalytic subunit p110 can also be divided into four isotypes: alpha, beta, gamma, and delta forms encoded by the PI3K catalytic subunit (PI3KC) A, PIK3CB, PIK3CG, and PIK3CD, respectively (Reif et al. 2004). PI3K forms part of the PI3K/protein kinase B (PKB, also known as AKT)/mammalian target of rapamycin (mTOR) signaling pathway, which is critical for cellular functions such as cell growth, motility, metabolism, survival, and angiogenesis (Davis et al. 2015). Additionally, activation of the PI3K/AKT/mTOR pathway has been reported to be associated with tumor development and resistance to anticancer agents (Liu et al. 2009). Thus, enormous efforts have been dedicated to evaluating the potential therapeutic effects of PI3K inhibitors, both alone and in combination, against

*Correspondence: kyeongrlee@kribb.re.kr; yjchae@woosuk.ac.kr

[1] Laboratory Animal Resource Center, Korea Research Institute of Bioscience and Biotechnology, Cheongju 28116, Republic of Korea
[7] College of Pharmacy and Research Institute of Pharmaceutical Sciences, Woosuk University, Wanju 55338, Republic of Korea
Full list of author information is available at the end of the article

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various cancer types (Wu et al. 2009; Heavey et al. 2014). The first approved PI3K inhibitor for the treatment of certain blood cancers was idelalisib (Zydelig®, developed by Gilead Sciences), which was approved in 2014 (US FDA 2014). Since then, several anticancer drugs have been approved by the Food and Drug Administration (FDA) of the USA, including copanlisib (Aliqopa®, developed by Bayer) and duvelisib (Copiktra®, developed by Verastem), which are pan-PI3K inhibitors, in 2017 and 2018, respectively (Brown 2019).

In 2019, the FDA approved the first alpha-specific PI3K inhibitor, alpelisib (Piqray®, developed by Novartis), for the treatment of hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated, advanced, or metastatic breast cancer in combination with fulvestrant (Markham 2019; Narayan et al. 2021). Alpelisib inhibits the alpha isoform of PI3K with much higher potency than other isoforms (IC_{50} values for alpha, beta, delta, and gamma forms: 4.6, 1156, 250, and 290 nM, respectively) (Juric et al. 2018). Patients with HR-positive/HER2-negative/PIK3CA-mutated, advanced, or metastatic breast cancers exhibited a statistically significant improvement in progression-free survival (PFS) when co-treated with fulvestrant and alpelisib as compared to that with fulvestrant alone (median PFS: 11.1 months vs 3.7 months) (André et al. 2019). Considering that the PI3K pathway is the most frequently activated oncogenic pathway in breast cancers and unselective blockage of the PI3K pathway by pan-PI3K inhibitors leads to unexpected adverse effects (Mukohara 2015), the alpha-specific PI3K inhibitor alpelisib can be considered as a promising anticancer agent for treating breast cancer.

As PIK3CA mutations are also frequently observed in various cancers other than breast cancer (Samuels and Waldman 2010), substantial attempts have been made to extend its clinical use to other cancer types. Oropharyngeal cancer (NCT03601507), meningiomas (NCT03631953), and gastric cancer (NCT04526470) are representative examples of the target diseases of ongoing or planned clinical trials with alpelisib, and the target cancer types are expected to be extended further based on recent reports (Mollon et al. 2018; Tayyar et al. 2021; Zaryouh et al. 2021). The extension of its use to new cancer targets starts with various preclinical studies; in particular, in vivo tumor xenograft mouse studies to show robust anticancer efficacy against the target cancer type are essential. To conduct safe clinical trials, it is imperative to understand the in vivo pharmacokinetics of the drug to select the appropriate dose. Thus, the use of valid analytical methods to determine drug concentrations is inevitable in preclinical and clinical studies. To the best of our knowledge, valid analytical methods for the quantification of alpelisib in biological matrices have only been reported for rat plasma (Seo et al. 2021; Wang et al. 2021). However, large plasma sample volumes (100 μL) were used in these studies; therefore, the methods are not feasible for pharmacokinetic studies in mice, the most commonly used species for xenograft studies, considering that a small volume of blood is available from mice. Although Wang et al. (2021) applied a simplified pretreatment method for rat plasma compared to that reported by Seo et al. (2021), improvement of the peak shape for alpelisib in a chromatogram is required owing to the shoulder peak to produce reproducible results. Several clinical studies on alpelisib have been reported (Mayer et al. 2017; Juric et al. 2018); however, to the best of our knowledge, there are no valid analytical methods for the quantification of alpelisib in human plasma.

Therefore, we developed a rapid and sensitive analytical method for quantifying alpelisib in mouse and human plasma using liquid chromatography–tandem mass spectrometry (LC–MS/MS). We validated the use of the developed method in line with FDA and European Medicines Agency (EMA) guidelines. Using a valid analytical method, we successfully quantified alpelisib in in vitro and in vivo pharmacokinetic studies in mice.

Methods

Chemicals and reagents

Alpelisib and enzalutamide, which was used as an internal standard (IS), were purchased from MedChemExpress (Monmouth Junction, NJ, USA). High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). Formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water, used as a component of the mobile phase, was obtained using a Milli-Q water system (Merck Millipore, Burlington, MA, USA). Heparinized blank mouse and human plasma was obtained from Innovative Research, Inc. (Novi, MI, USA), and heparinized capillaries were purchased from Kimble Chase Life Science and Research Products, LLC (Rockwood, TN, USA). All other reagents were of analytical grade and were used without further purification.

Instruments

An LC–MS/MS system comprising an Agilent 1260 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) and an API 3200 mass spectrometer (AB SCIEX, Framingham, MA, USA) equipped with a turbo electrospray interface was used in this study. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode with positive electrospray ionization (ESI+) for the quantification of alpelisib and the IS. The optimized collision energy voltages were 33 and

35 V for alpelisib and the IS, respectively, and the source temperature was set at 550 °C. The most abundant product ions were at *m/z* 328.0 from the precursor ion at *m/z* 442.1 for alpelisib, and at *m/z* 209.1 from the precursor ion at *m/z* 465.0 for the IS. Chromatographic resolution of alpelisib and the IS was performed using a reversed-phase HPLC column (XTerra MS C18, 50 mm × 2.1 mm, 5 μm; Waters Corporation, Milford, MA, USA) with a linear gradient of 0.1% formic acid in water and 0.1% formic acid acetonitrile (90%:10% → 5%:95%, v/v) at a flow rate of 0.4 mL/min for 5 min. The autosampler temperature was set at 10 °C. Instrument control and data analysis were performed using Analyst software version 1.5.2 (Applied Biosystems, Foster City, CA, USA).

Sample preparation

A 20 μL aliquot of mouse or human plasma sample was added to 80 μL of acetonitrile containing 300 ng/mL of the IS to induce the precipitation of plasma proteins. After vortexing for 10 min, the mixture was centrifuged at 13,500×*g* for 10 min at 4 °C. The supernatant was transferred to a 96-well sample plate, and 10 μL was injected into the LC–MS/MS system.

Preparation of standard and quality control samples

Standard stock solutions (1000 μg/mL) of alpelisib and the IS were prepared in dimethyl sulfoxide (DMSO). Standard working solutions of alpelisib were prepared by serial dilution of the standard stock solution with methanol. The IS solution was prepared in acetonitrile at a final concentration of 300 ng/mL. Calibration standard samples for alpelisib were prepared by spiking 2 μL of standard working solution into 18 μL of blank mouse or human plasma to produce final concentrations of 1, 2, 10, 50, 200, 400, 800, and 2000 ng/mL. In addition, mouse and human plasma quality control (QC) alpelisib samples of 3 ng/mL (low QC; LQC), 150 ng/mL (middle QC; MQC), and 1600 ng/mL (high QC; HQC) were prepared using the same process as that for the standard samples.

Method validation

The analytical method of alpelisib was evaluated in terms of selectivity, sensitivity, linearity, and intra-/inter-day accuracy and precision in accordance with the FDA and EMA guidelines (EMA CHMP 2012; US FDA 2018). The selectivity, defined as the extent to which an analyte in the plasma can be determined without interference from the matrix, was evaluated using six blank mouse or human plasma samples obtained from different individual sources. Interference from endogenous compounds present in mouse or human plasma was investigated by comparing the chromatograms obtained from blank plasma, plasma spiked with only alpelisib, plasma spiked only with the IS (zero blank), and plasma spiked with both alpelisib and the IS. Sensitivity, which is the lowest analyte concentration in the matrix that can be measured with acceptable accuracy and precision, was evaluated by the lowest limit of quantification (LLOQ) showing adequate accuracy and precision and ≥ 5 times the analyte response of the zero blank. The linearity of the assay was determined using standard samples containing 1–2000 ng/mL alpelisib. Standard samples were processed as described above, and linear regression analysis was performed using a constructed calibration curve. The accuracy and precision of the analytical method were determined at four different concentrations (LLOQ, LQC, MQC, and HQC), which were prepared independently from the calibration samples, by repeating the experiments for three days ($n = 6$/day). The intra- and inter-day accuracy was determined by the deviation of the calculated concentrations from the nominal concentrations and presented as relative error [RE (%) = (calculated concentration-nominal concentration)/nominal concentration × 100]. The acceptance criteria of accuracy for inter- and intra-assay were defined within ± 15% of nominal concentrations, except for LLOQ, where the calculated concentration should be within ± 20% of nominal concentrations. The intra- and inter-day precision was determined using the relative standard deviation [RSD (%) = standard deviation of the calculated concentration/mean calculated concentration × 100] at each concentration level. Precision was confirmed by the acceptance criteria of RSD ≤ 15%, except for LLOQ (RSD ≤ 20% for LLOQ).

Recovery, matrix effect, and process efficiency

The recovery, matrix effect, and process efficiency were evaluated in triplicate at three different QC levels, following a previously reported method (Chae et al. 2012; Lee et al. 2012). The recovery was calculated by dividing the mean peak area of an analyte added before precipitation (set 3) by the mean peak area ratio of an analyte spiked in the post-precipitation matrix (set 2). The mean peak area of an analyte spiked in the post-precipitation matrix (set 2) was compared with that of an analyte spiked in the mobile phase (set 1) to calculate the matrix effect. The ratio of the mean peak area of an analyte added before precipitation (set 3) to that of an analyte in the mobile phase (set 1) was defined as the process efficiency. The recovery, matrix effect, and process efficiency were calculated for the IS (300 ng/mL) in the same manner.

Stability

The stability of alpelisib in mouse and human plasma was assessed using three QC samples in triplicate under various storage and handling conditions: bench-top,

long-term, freeze–thaw, and autosampler storage. For the assessment of bench-top stability, QC samples containing alpelisib were maintained at room temperature for 6 h prior to sample preparation. The freeze–thaw stability assessment was performed by repeating three cycles of freezing at − 20 °C for at least 12 h followed by thawing at room temperature. To evaluate long-term stability, the samples were stored at − 20 °C for 1 month and then processed as described above. To determine the autosampler stability, QC samples were processed by protein precipitation and allowed to stand at 10 °C for 10 h. Stability was determined by comparing the measured concentrations of samples stored under the conditions mentioned above with nominal concentrations. The stability of stock solutions of alpelisib and the IS was also assessed after storage at − 20 °C for 6 months.

Dilution effect

The dilution effect was evaluated for samples with concentrations above the upper limit of quantification (ULOQ). Plasma samples containing tenfold or fivefold concentrations of HQC were prepared from mouse or human plasma, respectively, and then diluted using blank plasma. The mixture was processed according to the method described above, and the analyte concentration was determined. Accuracy was determined by comparing the calculated concentrations to nominal concentrations and was considered acceptable when the deviation was within ± 15%. The acceptance criterion for precision was defined as RSD ≤ 15%.

In vitro and in vivo pharmacokinetic study of alpelisib

Metabolic stability of alpelisib was determined using mouse and human liver microsomes. Alpelisib dissolved in DMSO was diluted to a final concentration of 1 μM in a reaction mixture consisting of 160 μL potassium phosphate buffer, 1 mM β-nicotinamide adenine dinucleotide phosphate, and 0.5 mg/mL human or mouse liver microsomes. This mixture was incubated at 37 °C, and the reaction was terminated at 0, 10, 30, and 60 min by adding ice-cold acetonitrile containing the IS. After centrifugation of the samples, the supernatant was stored at − 20 °C until LC–MS/MS analysis.

The pharmacokinetic properties of alpelisib were investigated in vivo using mice. Animal experiments were performed in accordance with the Guidelines for Animal Experiments of the Korea Research Institute of Bioscience and Biotechnology (approval number: KRIBB-AEC-20309). Male ICR mice (8 weeks old) were obtained from Orient Bio (Seongnam, Gyeonggi, Republic of Korea). They were maintained under a controlled environment (12 h light/dark cycle; temperature, 22 ± 2 °C; relative humidity, 50 ± 5%) with free access to water and food for at least 1 week. Mice were fasted for at least 8 h with free access to water before the experiment. A dosing solution of alpelisib for intravenous (IV) administration was prepared in a solvent composed of saline, PEG400, ethanol, and DMSO (44:50:5:1) at a concentration of 2 mg/mL. A dosing solution of alpelisib for oral administration was prepared in a solvent of 20% 2-hydroxypropyl-beta-cyclodextrin in water, saline, and ethanol (50:45:5) at a concentration of 5 mg/mL. Thereafter, alpelisib was injected into the tail vein (10 mg/kg) or administered orally (50 mg/kg) to mice. Following IV administration of alpelisib, blood was collected from the retro-orbital sinus using heparinized capillaries at 0 (pre-dose), 5, 15, and 30 min, and 1, 2, 4, 6, 8, and 24 h post-dose. In the group assigned to oral administration, blood was obtained at 0 (pre-dose), 15, and 30 min, and 1, 2, 4, 6, 8, and 24 h post-dose. The blood was centrifuged immediately after collection at 13,500 × g for 10 min at 4 °C, and the plasma fractions were stored at − 20 °C until LC–MS/MS analysis.

A standard non-compartmental analysis was performed to calculate the pharmacokinetic parameters for alpelisib using WinNonlin® Professional 8.1 software (Pharsight Corporation, Mountain View, CA, USA). The area under the plasma concentration–time curve from time zero to the last quantifiable point (AUC_{last}) was calculated using the linear trapezoidal method, and the area under the plasma concentration–time curve from time zero to infinity (AUC_{inf}) was obtained using the linear trapezoidal method with standard extrapolation. The terminal half-life ($T_{1/2}$) was obtained by dividing 0.693 by λ, where λ represents the slope of the terminal phase in the log-linear phase of the concentration–time profile. The total clearance (CL) was calculated using dose/AUC_{inf}, and the steady-state volume of distribution (V_{ss}) was calculated using moment analysis. The maximum plasma concentration (C_{max}) and T_{max}, which represents the time to reach C_{max}, were directly obtained from the plasma concentration–time profiles from individual mice. Oral bioavailability (F) was calculated by dividing the dose-normalized AUC_{inf} after oral administration by that after IV administration.

Results and discussion

LC–MS/MS conditions

The LC–MS/MS conditions were optimized to produce a symmetric peak shape and reproducible results without significant interference. The ESI + mode was applied because of the lower noise and higher signal intensity for alpelisib compared to that for the negative ion mode. Enzalutamide was selected as the IS owing to its structural similarity to alpelisib. The most abundant precursor ions $[M+H]^+$ for alpelisib and the IS were observed at

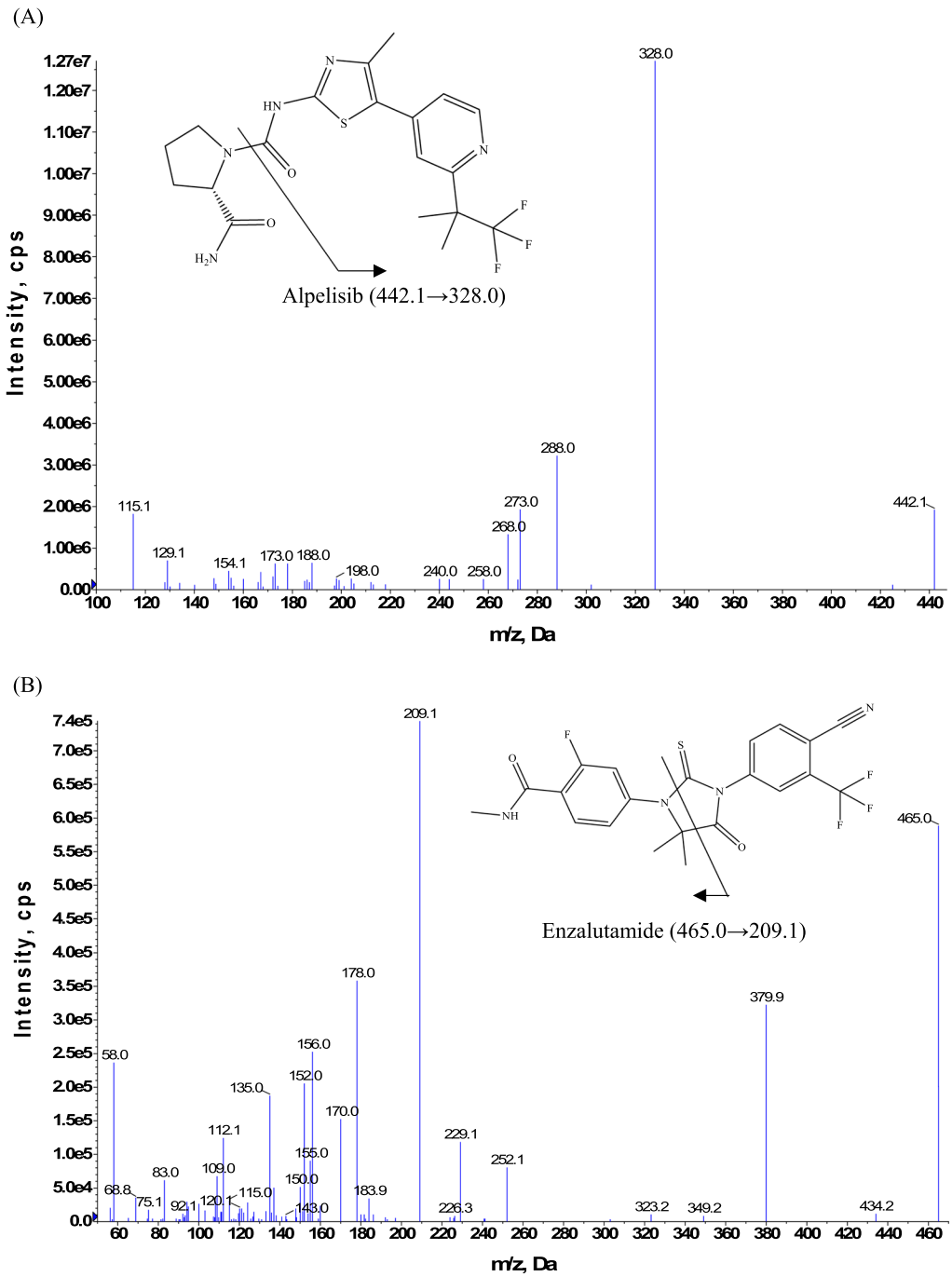

Fig. 1 Structures and mass fragmentation of **a** alpelisib and **b** the internal standard (enzalutamide)

m/z 442.1 and 465.0, respectively. The *m/z* of 328.0 was selected as the product ion of alpelisib because it showed greater sensitivity than that shown by other fragmentations. Thus, the final selected MRM transition for alpelisib was 442.1 > 328.0, and that for the IS was 465.0 > 209.1 (Fig. 1), which resulted in the highest signals and stable product ions. Various compositions of the mobile phase (e.g., 0.1% formic acid in water/0.1% formic acid in acetonitrile, 10 mM ammonium acetate in water/10 mM ammonium acetate in acetonitrile, and 5 mM ammonium acetate in 0.1% formic acid in water/methanol/acetonitrile) were investigated to determine the optimal

peak shape and sensitivity, along with acceptable selectivity. The final chosen conditions for the mobile phase comprised a linear gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (90%:10% → 5%:95%, v/v). In addition to the mobile phase, the column type is a critical component that produces high sensitivity and selectivity. Reverse phase columns including an XTerra MS C18 column (2.1 mm × 50 mm, 5 μm, 125 Å; Waters Corporation), ZORBAX Eclipse XDB-C8 column (2.1 × 50 mm, 3.5 μm, 80 Å; Agilent Technologies), and ZORBAX Eclipse XDB-Phenyl column (2.1 × 50 mm, 5 μm, 80 Å; Agilent Technologies) were tested in this study considering the physicochemical properties of alpelisib. The ZORBAX Eclipse XDB-C8 column showed the highest sensitivity; however, carryover issues could not be resolved even with various injection loop flushing methods [0.2% formic acid in 50% acetonitrile, 0.1% formic acid in 70% methyl alcohol, isopropyl alcohol: acetonitrile: methyl alcohol: water mixed at a ratio of 1:1:1:1 (v/v/v/v), 0.1% formic acid in 100% methyl alcohol, and 100% methyl alcohol]. In contrast, the XTerra MS C18 column produced an appropriate sensitivity without a carryover effect when the injection loop was flushed with 100% methanol; therefore, this column was finally selected for the analysis of alpelisib in plasma. To the best of our knowledge, this is the first study to report a method for overcoming the carryover effects in chromatographic analysis. In addition, improved symmetricity and reduced tailing effect were observed with this method compared to those in a previously reported study (Wang et al. 2021).

Sample preparation

We attempted to develop a simple and rapid sample preparation method compared to a previously reported method (Seo et al. 2021), while providing sufficient sensitivity and negligible interference from the matrix. Various ratios of plasma and acetonitrile for sample preparation were investigated, and the final selected ratio was 1:4 (20 μL of plasma and 80 μL of acetonitrile), which allowed high sensitivity and selectivity and provided an efficient process for sample preparation.

Selectivity and sensitivity

Figure 2 shows chromatograms for blank plasma, plasma spiked only with the IS (zero blank), plasma samples at a concentration of 1 ng/mL (LLOQ) of alpelisib, and mouse plasma samples obtained 1 h after oral administration of alpelisib at a dose of 50 mg/kg. The retention times for alpelisib and the IS were 2.8 and 2.9 min, respectively, and no significant interference was identified at the retention time of alpelisib or the IS in blank plasma obtained from six individual mice or humans. The LLOQ, which was confirmed to meet the requirement (≥ 5 times the response at the retention time of the analyte in the zero blank) in accordance with the guidance, was determined to be 1 ng/mL with adequate accuracy and precision from six replicates in three runs. Therefore, we confirmed that this analytical method is sufficiently selective and sensitive for the detection of alpelisib in mouse and human plasma.

Calibration curve

The calibration curve for alpelisib was confirmed to be linear in the range of 1–2000 ng/mL in mouse and human plasma. The typical regression equation for the curve was $y = 0.0224x + 0.00154$, where x is the concentration ratio of alpelisib/IS and y is the peak area ratio of alpelisib/IS. The coefficient of determination (r) was higher than 0.990 in each validation run, confirming the acceptable linearity of this analysis method. In addition, more than 75% of the nonzero standard samples met the acceptance criteria in each validation run, satisfying the acceptance criteria for the calibration curve defined in the guidelines.

Accuracy and precision

Accuracy and precision were determined using LLOQ, LQC, MQC, and HQC samples (1, 3, 150 and 1600 ng/mL, respectively). The RE was within ±5%, and the RSD was less than 10% in both matrices (Table 1). These results indicate that the analytical method developed in this study is accurate and precise enough to be applied for the quantification of alpelisib in mouse and human plasma.

Recovery, matrix effect, and process efficiency

The recovery, matrix effect, and process efficiency of this method were determined at three QC levels for alpelisib (3, 150, and 1600 ng/mL) and one concentration for the IS (300 ng/mL) (Table 2). The recoveries for alpelisib were 90.8–100% and 91.1–101% in mouse and human plasma, respectively, and those for the IS were 102% and 98.0% in mouse and human plasma, respectively, indicating that the present sample processing method provides adequate recoveries for both the analyte and IS. Mean peak areas of alpelisib spiked post-precipitation were 96.8–99.9% and 96.8–101% of the mean peak areas in a neat solution in mouse and human plasma, respectively, and those for the IS were 99.8% in both matrices, suggesting that no significant matrix effect exists for the analysis of alpelisib and the IS using this method. Overall, the process

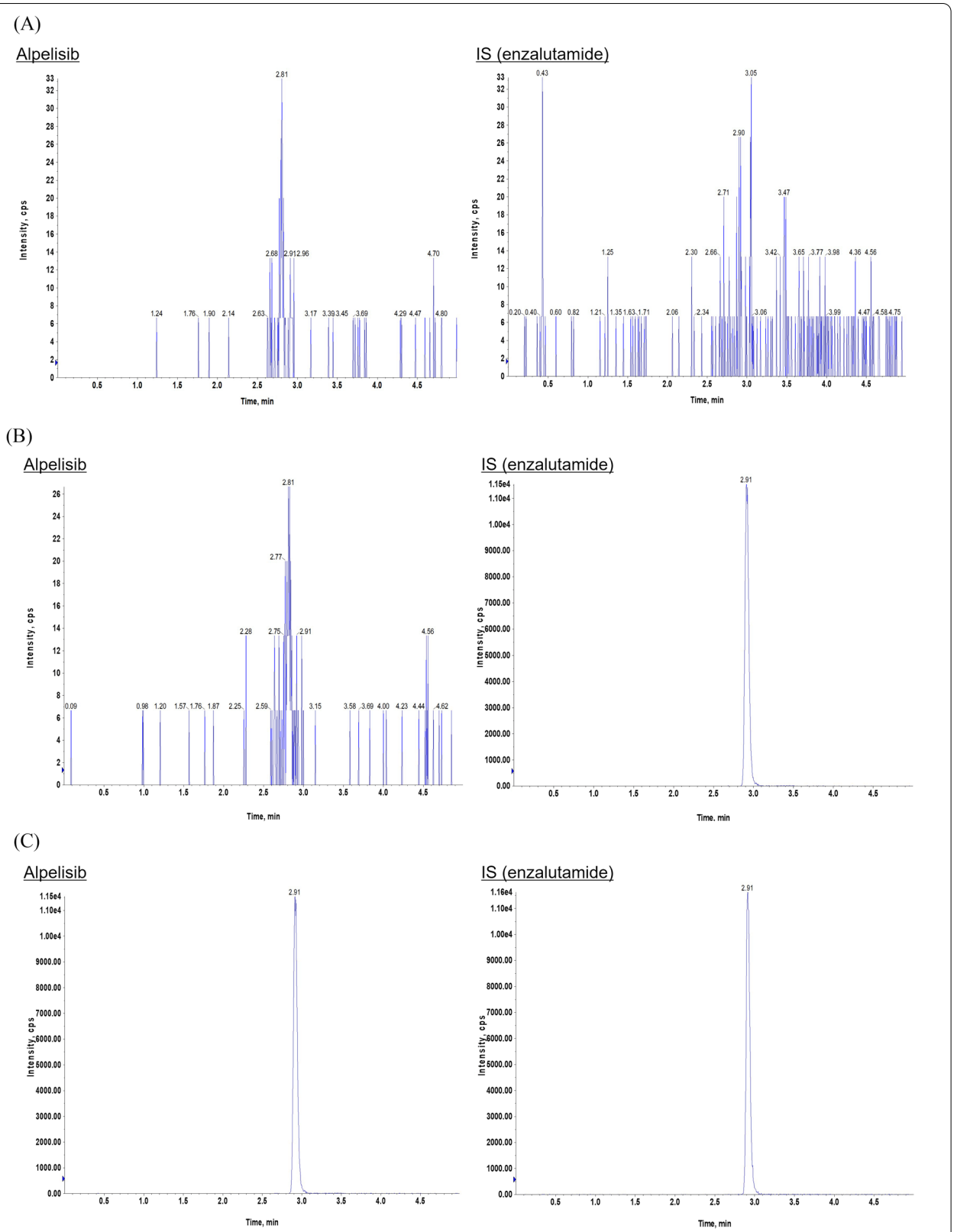

Fig. 2 Typical multiple reaction monitoring chromatograms of alpelisib and the internal standard (IS; enzalutamide). **a** Blank mouse plasma; **b** blank mouse plasma spiked with the IS (300 ng/mL); **c** lowest limit of quantification (LLOQ; 1 ng/mL) sample in mouse plasma; **d** mouse plasma sample collected at 1 h after oral administration of alpelisib at a dose of 50 mg/kg in mice; **e** blank human plasma; **f** blank human plasma spiked with the IS (300 ng/mL); and **g** LLOQ (1 ng/mL) sample in human plasma

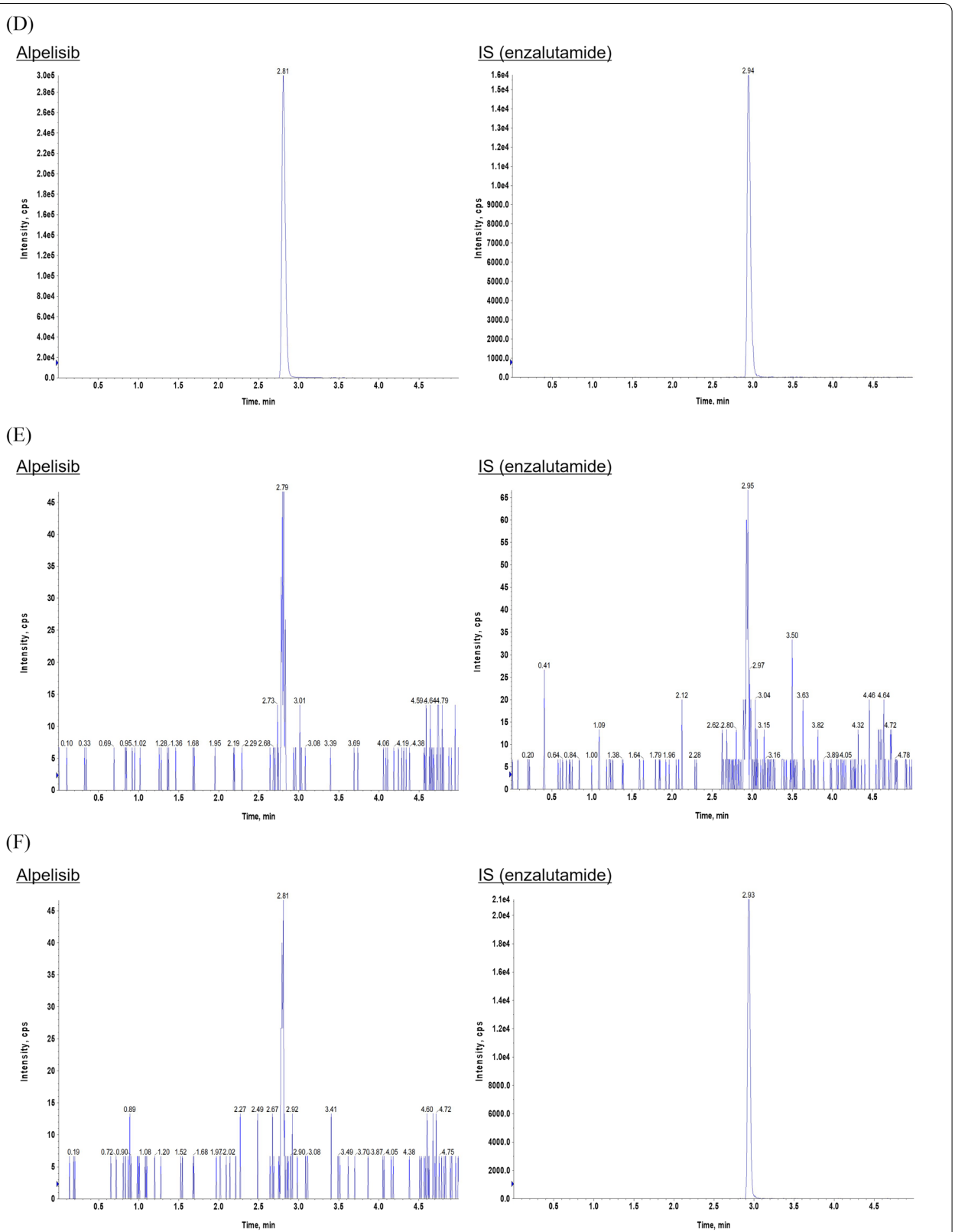

Fig. 2 continued

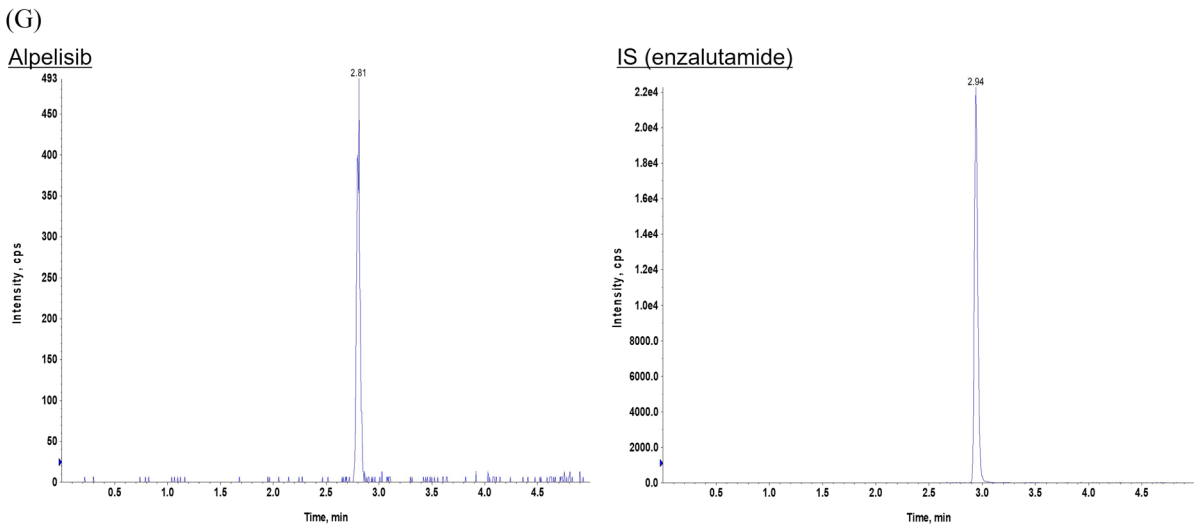

Fig. 2 continued

Table 1 Intra-/inter-day accuracy and precision of alpelisib in mouse and human plasma

	Nominal concentration (ng/mL)			
	LLOQ	LQC	MQC	HQC
	1	3	150	1600
(A) Mouse plasma				
Intra-day ($n=6$)				
Measured concentration (ng/mL; mean ± SD)	1.00 ± 0.03	3.05 ± 0.27	149 ± 6	1530 ± 70
Accuracy (RE, %)	0.000	1.61	− 0.667	− 4.17
Precision (RSD, %)	3.23	8.85	4.29	4.42
Inter-day ($n=18$)				
Measured concentration (ng/mL; mean ± SD)	0.998 ± 0.030	2.97 ± 0.19	145 ± 9	1570 ± 60
Accuracy (RE, %)	− 0.189	− 1.11	− 3.26	− 1.98
Precision (RSD, %)	3.02	6.37	6.15	3.80
(B) Human plasma				
Intra-day ($n=6$)				
Measured concentration (ng/mL; mean ± SD)	0.998 ± 0.010	3.08 ± 0.09	154 ± 1	1550 ± 10
Accuracy (RE, %)	− 1.13	2.56	2.33	− 3.13
Precision (RSD, %)	0.961	2.97	0.798	0.816
Inter-day ($n=18$)				
Measured concentration (ng/mL; mean ± SD)	0.977 ± 0.019	3.03 ± 0.12	153 ± 6	1610 ± 70
Accuracy (RE, %)	− 2.29	0.963	2.30	0.451
Precision (RSD, %)	1.98	3.83	3.76	4.09

RE, Relative error, calculated as (calculated concentration − theoretical concentration)/theoretical concentration × 100%; RSD, relative standard deviation, calculated as standard deviation of concentration/mean concentration × 100%; and SD, standard deviation

efficiency was within 85–115% for alpelisib and the IS in both matrices.

Stability

The stability of alpelisib was evaluated at three different QC levels (LQC, MQC, and HQC; 3, 150, and 1600 ng/mL, respectively) in both mouse and human plasma under various storage and handling conditions, which were relevant to the present LC–MS/MS analytical method (Table 3). When QC samples were maintained at room temperature for 6 h, deviations in the measured concentrations were less than 5% compared to those of

Table 2 Recovery, matrix effect, and process efficiency of alpelisib and the internal standard in mouse and human plasma (mean ± SD, $n = 3$)

	Nominal concentration (ng/mL)		
	LQC	**MQC**	**HQC**
	3	**150**	**1600**
(A) Alpelisib			
Mouse plasma			
Recovery (%)[a]	90.8 ± 1.3	91.0 ± 0.3	100 ± 0
Matrix effect (%)[b]	99.9 ± 1.5	96.8 ± 0.7	99.8 ± 0.8
Process efficiency (%)[c]	92.2 ± 1.4	88.4 ± 0.7	98.9 ± 0.6
Human plasma			
Recovery (%)	93.1 ± 0.7	91.1 ± 0.3	101 ± 1
Matrix effect (%)	101 ± 1	96.8 ± 0.3	97.3 ± 0.4
Process efficiency (%)	94.7 ± 1.0	88.0 ± 0.7	98.5 ± 1.6
	Nominal concentration (ng/mL)		
	300		
(B) Internal standard (enzalutamide)			
Mouse plasma			
Recovery (%)	102 ± 2		
Matrix effect (%)	99.8 ± 1.3		
Process efficiency (%)	100 ± 1		
Human plasma			
Recovery (%)	98.0 ± 2.2		
Matrix effect (%)	99.8 ± 1.5		
Process efficiency (%)	96.6 ± 1.9		

[a] Matrix effect (%) = mean peak area of an analyte added post-precipitation (set 2)/mean peak area of an analyte in neat analyte solution (set 1) × 100

[b] Recovery (%) = mean analyte peak area of an analyte added before precipitation (set 3)/mean peak area of an analyte added post-precipitation (set 2) × 100

[c] Process efficiency (%) = mean analyte peak area of an analyte added before precipitation (set 3)/mean peak area of an analyte in neat analyte solution (set 1) × 100

Table 3 Stability of alpelisib in mouse and human plasma (mean ± SD, $n = 3$)

Storage condition	**Nominal concentration (ng/mL)**		
	LQC	**MQC**	**HQC**
	3	**150**	**1600**
(A) Stability in mouse plasma			
Bench top (room temperature for 6 h)	104 ± 4	102 ± 0	99.1 ± 1.1
Long term (− 20 °C for 1 month)	96.0 ± 1.6	101 ± 2	100 ± 2
Freeze–thaw (3 cycles)	104 ± 1	110 ± 1	99.5 ± 4.1
Autosampler (10 °C for 10 h)	99.9 ± 3.3	91.0 ± 4.9	93.7 ± 7.5
(B) Stability in human plasma			
Bench top (room temperature for 6 h)	102 ± 1	103 ± 1	102 ± 1
Long term (− 20 °C for 1 month)	104 ± 1	100 ± 3	100 ± 1
Freeze–thaw (3 cycles)	105 ± 1	104 ± 1	97.9 ± 1.0
Autosampler (10 °C for 10 h)	108 ± 1	106 ± 1	102 ± 1

nominal concentrations. Long-term storage of mouse and human QC samples at − 20 °C for 1 month and three freeze–thaw cycles was determined to be adequate in terms of the stability of alpelisib, as the measured concentrations were close to the theoretical values. The stability in post-preparative samples (storage at 10 °C for 10 h, which is identical to the conditions in the autosampler) ranged from 91.0–99.9% and 102–108% in mouse and human plasma, respectively, with RSD less than 8%. In addition, the stock solutions of alpelisib and the IS were stable with storage at − 20 °C for 6 months (97.9–106% of the nominal concentrations). Collectively, these results implied that alpelisib was stable under all conditions tested in this study.

Dilution effect

The reported concentration of alpelisib after oral administration of 3 mg/kg in mice is 686 ng/mL (EMA CHMP 2020). Assuming a dose-proportional increase in systemic exposure to alpelisib, the expected mouse plasma concentrations after oral or IV administration of the

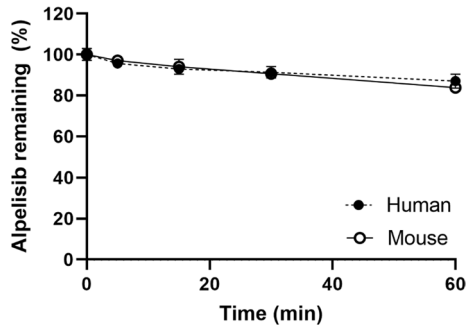

Fig. 3 Residual levels of alpelisib (%) after incubation with β-nicotinamide adenine dinucleotide phosphate in human (closed circles) and mouse liver microsomes (open circles). Each data point represents the mean ± SD ($n = 3$ for each group)

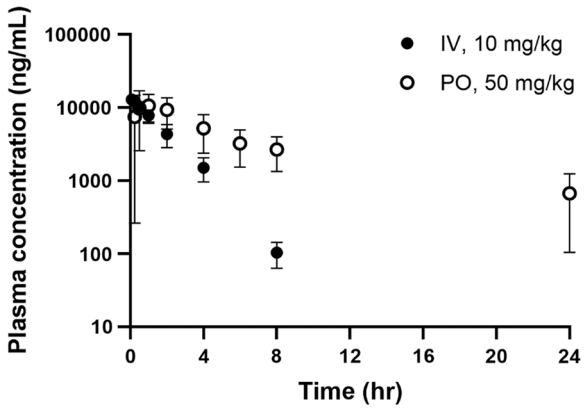

Fig. 4 Plasma concentration–time curve (semilogarithmic scale) of alpelisib after intravenous (IV, 10 mg/kg; closed circles) or oral administration (PO, 50 mg/kg; open circles) in mice. Each data point represents the mean ± SD ($n = 4$ for each group)

planned dose (50 or 10 mg/kg for oral or IV administration, respectively) were above the ULOQ of the present method, highlighting the necessity of evaluating dilution integrity in mouse plasma. The RE and RSD for tenfold dilution in mouse plasma were 0.217 and 0.474%, respectively. In addition, considering that the reported C_{max} of alpelisib after oral administration of 300 mg (a currently approved dose) in humans is 2380 ng/mL (Bertho et al. 2021), the integrity of the fivefold dilution was tested in human plasma. The RE and RSD for fivefold dilution in human plasma were 0.833 and 2.34%, respectively. These results indicated that the dilution method was accurate and precise for samples with concentrations of up to 20,000 and 10,000 ng/mL in mice and humans, respectively.

Pharmacokinetic study of alpelisib in mice

Cytochrome P450 (CYP)-mediated metabolism is the most prevalent metabolic process in drugs. Thus, we determined the metabolic stability of alpelisib using mouse and human microsomes that exhibit CYP activity to investigate the mechanism of alpelisib elimination. The residual levels of alpelisib after incubation in the mouse and human microsomes are shown in Fig. 3. The remaining amount of alpelisib was 83.8% and 87.2% after incubation in mouse and human microsome reaction mixtures for 60 min, respectively, indicating that phase I metabolism mediated by CYPs is not likely a major route for the elimination of alpelisib. Other metabolic processes may be involved in the degradation of alpelisib, but further investigation of the metabolic pathway of alpelisib is required.

To investigate the in vivo pharmacokinetic properties of alpelisib, it was administered intravenously or orally at a dose of 10 or 50 mg/kg, respectively, and plasma samples collected at designated times were used to determine the alpelisib concentration. In each run, to quantify the concentrations of alpelisib in mouse plasma, it was confirmed that ≥ 67% of the QC samples and ≥ 50% of the QCs per level were within ± 15% of the nominal concentrations in accordance with the FDA and EMA guidelines. Samples with concentrations above the ULOQ were diluted tenfold to be within the linear range of the calibration curve. The plasma concentration–time profiles following IV and oral administration of alpelisib are shown in Fig. 4, and the pharmacokinetic parameters are summarized in Table 4. After IV administration of 10 mg/kg, the plasma concentration of alpelisib decreased almost monoexponentially with an estimated $T_{1/2}$ of 1.09 ± 0.07 h. AUC_{inf} and V_{ss} were 25,700 ± 5,700 ng·h/mL and 648 ± 87 mL/kg, respectively. The calculated CL

Table 4 Pharmacokinetic parameters of alpelisib after intravenous (10 mg/kg) or oral administration (50 mg/kg) in mice (mean ± SD, $n = 4$)

Parameter	Intravenous	Oral
T_{max} (h)	–	0.875 ± 0.250
C_{max} (ng/mL)	–	11,400 ± 6,000
$T_{1/2}$ (h)	1.09 ± 0.07	7.86 ± 4.93
AUC_{last} (ng·h/mL)	25,600 ± 5,600	73,500 ± 24,300
AUC_{inf} (ng·h/mL)	25,700 ± 5,700	84,000 ± 16,400
CL (mL/h/kg)	402 ± 85	–
V_{ss} (mL/kg)	648 ± 87	–
F (%)[a]	–	65.4

[a] Calculated by $(AUC_{PO}/Dose_{PO})/(AUC_{IV}/Dose_{IV}) \times 100$

AUC, Area under the plasma concentration–time curve; CL, total clearance; C_{max}, maximum plasma concentration; F, bioavailability; SD, standard deviation; $T_{1/2}$, terminal half-life; T_{max}, time to reach C_{max}; and V_{ss}, steady-state volume of distribution

value following IV administration was 402 ± 85 mL/h/kg, which was similar to the reported value for alpelisib (EMA CHMP 2020), showing a relatively slow rate compared to the hepatic blood flow rate in mice (5400 mL/h/kg) (Davies and Morris 1993). Following oral administration, the plasma concentrations increased rapidly with a T_{max} of 0.875 ± 0.250 h and C_{max} of 11,400 ± 6,000 ng/mL, which was very close to the expected value with the assumption of dose proportionality of systemic exposure (11,433 ng/mL expected using results with 3 mg/kg administration assuming a dose-proportional increase in systemic exposure). The $T_{1/2}$ was estimated to be 7.86 ± 4.93 h after oral administration of alpelisib, showing differences from that after IV administration (1.09 h). When we assume a dose-independent increase in systemic exposure to alpelisib after oral administration, as mentioned above, mechanisms other than saturation in the elimination process may explain the different $T_{1/2}$ values depending on the dosing route. Thus, further investigation is warranted to elucidate the observed differences in $T_{1/2}$. The percentage of the AUC extrapolated from the last quantifiable drug concentration to infinity was less than 20% for IV and oral administration (0.626% and 12.6%, respectively), indicating that the sampling schedule of the study was well determined and the LLOQ of the present analytical method was sufficiently low for application in this pharmacokinetic study. With the assumption that absolute bioavailability with IV administration is 1, the oral bioavailability (F) of alpelisib was calculated as 65.4% in mice.

Conclusion

An LC–MS/MS method was developed and validated to quantify alpelisib in mouse and human plasma samples. Selectivity, sensitivity, linearity, intra-/inter-day accuracy, and precision were determined to be adequate according to the FDA and EMA guidelines. Alpelisib was stable under various handling and storage conditions, and no significant matrix effect or carryover was observed. We successfully used this assay to study the *in vitro* metabolic profiles and *in vivo* pharmacokinetics of alpelisib in mice. Here, to the best of our knowledge, we report for the first time a valid quantitative method for alpelisib in mouse and human plasma, which could aid in providing valuable pharmacokinetic information on alpelisib to increase its clinical availability.

Abbreviations

AUC_{inf}: Area under the plasma concentration–time curve from time zero to infinity; AUC_{last}: Area under the plasma concentration–time curve from time zero to the last quantifiable point; CL: Total clearance; C_{max}: Maximum plasma concentration; CYP: Cytochrome P450; DMSO: Dimethyl sulfoxide; EMA: European medicines agency; ESI: Electrospray ionization; F: Bioavailability; FDA: Food and Drug Administration; HER2: Human epidermal growth factor receptor 2; HPLC: High-performance liquid chromatography; HQC: High-quality control; HR: Hormone receptor; IS: Internal standard; IV: Intravenous; LC–MS/MS: Liquid chromatography–tandem mass spectrometry; LLOQ: Lowest limit of quantification; LQC: Low-quality control; MQC: Middle-quality control; MRM: Multiple reaction monitoring; mTOR: Mammalian target of rapamycin; PFS: Progression-free survival; PI3K: Phosphatidylinositol-3-kinase; QC: Quality control; RE: Relative error; RSD: Relative standard deviation; $T_{1/2}$: Terminal half-life; T_{max}: The time to reach C_{max}; V_{ss}: Steady-state volume of distribution.

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Author contributions

SL, M-SK, J-WJ, JC, Y-JC, and K-RL designed the research, and SL and J-WJ performed experiments. SL, M-SK, J-WJ, JC, H-JM, and T-SK analyzed and interpreted the data. SL, M-SK, J-WJ, JC, H-JM, T-SK, K-RL, and Y-JC were involved in writing the manuscript, and all authors reviewed it. All authors have read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Laboratory Animal Resource Center, Korea Research Institute of Bioscience and Biotechnology, Cheongju 28116, Republic of Korea. [2]College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea. [3]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 08826, Republic of Korea. [4]Graduate School of New Drug Discovery and Development, Chungnam National University, Daejeon 34134, Republic of Korea. [5]College of Pharmacy, Gachon University, Incheon 21936, Republic of Korea. [6]Department of Bioscience, University of Science and Technology, Daejeon 34113, Republic of Korea. [7]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Woosuk University, Wanju 55338, Republic of Korea.

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References

André F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med. 2019;380:1929–40. https://doi.org/10.1056/nejmoa1813904.

Bertho M, Patsouris A, Augereau P, et al. A pharmacokinetic evaluation of alpelisib for the treatment of HR+, HER2-negative, PIK3CA-mutated advanced or metastatic breast cancer. Expert Opin Drug Metab Toxicol. 2021;17:139–52. https://doi.org/10.1080/17425255.2021.1844662.

Brown JR. PI3K inhibitors: present and future. Cancer J. 2019;25:394. https://doi.org/10.1097/PPO.0000000000000414.

Chae YJ, Koo TS, Lee KR. A sensitive and selective LC–MS method for the determination of lurasidone in rat plasma, bile, and urine. Chromatographia. 2012;75:1117–28. https://doi.org/10.1007/s10337-012-2294-5.

Davies B, Morris T. Physiological parameters in laboratory animals and humans. Pharm Res. 1993;10:1093–5.

Davis WJ, Lehmann PZ, Li W. Nuclear PI3K signaling in cell growth and tumorigenesis. Front Cell Dev Biol. 2015;3:24. https://doi.org/10.3389/FCELL.2015.00024.
Heavey S, O'Byrne KJ, Gately K. Strategies for co-targeting the PI3K/AKT/mTOR pathway in NSCLC. Cancer Treat Rev. 2014;40:445–56. https://doi.org/10.1016/J.CTRV.2013.08.006.
Juric D, Rodon J, Tabernero J, et al. Phosphatidylinositol 3-kinase a—selective inhibition with alpelisib (BYL719) in PIK3CA-altered solid tumors: results from the first-in-human study. J Clin Oncol. 2018;36:1291–9. https://doi.org/10.1200/JCO.2017.72.7107.
Lee JH, Chae YJ, Lee KR, et al. Development of a LC-MS method for quantification of FK-3000 and its application to in vivo pharmacokinetic study in drug development. J Pharm Biomed Anal. 2012;70:587–91. https://doi.org/10.1016/j.jpba.2012.05.030.
Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov. 2009;8:627–44.
Markham A. (2019) Alpelisib: first global approval. Drugs. 2019;7911(79):1249–53. https://doi.org/10.1007/S40265-019-01161-6.
Mayer IA, Abramson VG, Formisano L, et al. A phase Ib study of alpelisib (BYL719), a PI3Kα-specific inhibitor, with letrozole in ER+/HER2− metastatic breast cancer. Clin Cancer Res. 2017;23:26–34. https://doi.org/10.1158/1078-0432.CCR-16-0134.
Mollon L, Aguilar A, Anderson E, et al. Abstract 1207: a systematic literature review of the prevalence of PIK3CA mutations and mutation hotspots in HR+/HER2− metastatic breast cancer. Cancer Res. 2018;78:1207–1207.
Mukohara T. PI3K mutations in breast cancer: prognostic and therapeutic implications. Breast Cancer (Lond). 2015;7:111. https://doi.org/10.2147/BCTT.S60696.
Narayan P, Prowell TM, Gao JJ, et al. FDA approval summary: alpelisib plus fulvestrant for patients with HR-positive, HER2-negative, PIK3CA-mutated, advanced or metastatic breast cancer. Clin Cancer Res. 2021;27:1842–9. https://doi.org/10.1158/1078-0432.CCR-20-3652.
Reif K, Okkenhaug K, Sasaki T, et al. Cutting edge: differential roles for phosphoinositide 3-kinases, p110γ and p110δ, in lymphocyte chemotaxis and homing. J Immunol. 2004;173:2236–40. https://doi.org/10.4049/JIMMUNOL.173.4.2236.
Samuels Y, Waldman T. Oncogenic mutations of PIK3CA in human cancers. Curr Top Microbiol Immunol. 2010;347:21. https://doi.org/10.1007/82_2010_68.
Seo SW, Kim JM, Han DG, et al. 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 Anal Technol Biomed Life Sci. 2021;1163:122508. https://doi.org/10.1016/j.jchromb.2020.122508.
Tayyar Y, Idris A, Vidimce J, et al. Alpelisib and radiotherapy treatment enhances Alisertib-mediated cervical cancer tumor killing. Am J Cancer Res. 2021;11:3240.
Wang Q, Lan X, Zhao Z, et al. Characterization of Alpelisib in rat plasma by a newly developed UPLC-MS/MS method: application to a drug-drug interaction study. Front Pharmacol. 2021. https://doi.org/10.3389/fphar.2021.743411.
Wu P, Liu T, Hu Y. PI3K inhibitors for cancer therapy: What has been achieved so far? Curr Med Chem. 2009;16:916–30. https://doi.org/10.2174/092986709787581905.
Yu J, Zhang Y, McIlroy J, et al. Regulation of the p85/p110 phosphatidylinositol 3′-kinase: stabilization and inhibition of the p110α catalytic subunit by the p85 regulatory subunit. Mol Cell Biol. 1998;18:1379–87.
Zaryouh H, De PI, Baysal H, et al. Recent insights in the PI3K/Akt pathway as a promising therapeutic target in combination with EGFR-targeting agents to treat head and neck squamous cell carcinoma. Med Res Rev. 2021. https://doi.org/10.1002/MED.21806.
EMA CHMP (2012) Guideline on bioanalytical method validation. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Accessed 2 Feb 2022
EMA CHMP (2020) EPAR public assessment report (piqray). https://www.ema.europa.eu/en/documents/assessment-report/piqray-epar-public-assessment-report_en.pdf. Accessed 29 May 2021
US FDA (2014) Prescribing information of Zydelig. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/206545lbl.pdf. Accessed 20 Jul 2021
US FDA (2018) Guidance for Industry: bioanalytical method validation. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm and/or http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm. Accessed 2 Feb 2022

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