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ENHANCED SELECTIVITY IN ALPELISIB QUANTIFICATION IN HUMAN PLASMA: A VALIDATED LC-MS/MS METHOD WITH DEUTERATED INTERNAL STANDARD

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ABSTRACT

Background: To enhance efficacy and manage dose-limiting toxicities such as hyperglycemia, therapeutic drug monitoring of alpelisib (ALB), a phosphatidylinositol 3-kinase alpha (PI3Kα) inhibitor used in advanced breast cancer, is essential. This study aimed to develop and validate a selective, sensitive, and high-throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of ALB in human plasma to support pharmacokinetic studies and therapeutic drug monitoring. **Methodology:** Alpelisib-D3 was used as a deuterated internal standard (IS). Analytes were extracted from 50 µL of plasma by liquid-liquid extraction using n-hexane and methyl tert-butyl ether (20:80, v/v). Chromatographic separation was performed on an X-Terra RP8 column using an isocratic mobile phase of 5 mM ammonium acetate buffer (pH 5.00) and acetonitrile (10:90, v/v) at 0.4 mL/min. Detection was carried out in positive electrospray ionization mode using multiple reaction monitoring of m/z 442.15→141.07 for ALB and m/z 445.36→144.05 for the IS. Validation included linearity, precision, accuracy, recovery, matrix effect, carryover, dilution integrity, and stability. **Results and discussion:** The method was linear over 50.0–10,000 ng/mL. Intra- and inter-day precision (CV%) was <4.2%, and accuracy ranged from 94.5% to 106.2%. Mean extraction recovery exceeded 90%, with no significant matrix effects. Carryover, stability, and dilution integrity also met acceptance criteria. **Conclusion:** A reliable, sensitive, and selective LC-MS/MS method was successfully developed and validated. The method is suitable for high-throughput pharmacokinetic studies and therapeutic drug monitoring of ALB in human plasma.

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INTRODUCTION

Breast cancer remains the leading cause of cancer-related mortality among women worldwide. [1–3]. The crucial biological pathway involved in the progression of this disease is the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, which has been discovered to be hyperactivated in breast cancers that are hormone receptor-positive and Human Epidermal growth factor Receptor 2 (HER2)-negative [4–7]. This hyperactivation is mainly attributable to mutations in the PIK3CA gene. ALB is broken down through multiple pathways: Hydrolysis, Oxidation by cytochrome P450 3A4 (CYP3A4), and glucuronidation by UDP-glucuronosyltransferase 1A9 (UGT1A) [8–10]. The pharmacokinetic changes and high drug-drug interactions emphasize the need for precise bioanalytical techniques that provide accurate mass in patient plasma, which is vital for informing dosing decisions in both medical and research scenarios.

Few analytical techniques, notably HPLC, have been reported for the measurement of ALB, with UPLC and LC-MS also reported [11-17]. These methodologies in LC-MS/MS bioanalysis often require considerable plasma volumes, such as 500 µL, which limits applicability in studies with restricted sample availability, such as intensive pharmacokinetic investigations requiring repeated sampling in adult patients. In addition, these methods frequently encounter variability in peak shape and lack a stable isotope-labeled internal standard. It is crucial for minimizing variability in the extraction and ionization processes inherent to the bioanalytical technique. A rapid, sensitive, and selective LC-MS/MS method was developed for measuring ALB in human plasma using 50 µL of sample. The internal standard was deuterated ALB (alpelisib-D3). The method was validated according to FDA and EMA requirements. [18], [19].

MATERIALS AND METHODS

The compound alpelisib and alpelisib-D3 were sourced from Clearsynth Labs Ltd. in Mumbai, India. All other chemicals and solvents were purchased from S.D. Fine Chemicals, Mumbai. The control human plasma sample was procured from Doctors Pathological Labs (Hyderabad, India).

Instrumentation and Chromatographic conditions

The LC-MS/MS system is composed of an Agilent 1200 series High-Performance Liquid Chromatography (HPLC) system manufactured by Agilent Technologies linked to an API 4000 triple quadrupole mass spectrometer. Data acquisition and analysis are facilitated through Analyst® version 1.4.1 software provided by SCIEX; an X-terra® RP8 column (4.6 mm × 50 mm with a particle size of 5 µm) is employed and maintained at 40°C. The mobile phase consists of a 5 mM ammonium acetate buffer, with the pH fine-tuned to 5.00 ± 0.05 using glacial acetic acid and acetonitrile, in a 10:90 volume/volume ratio. The system operates under isocratic conditions at a flow rate of 0.4 mL/min, and the entire analytical run is completed in 3.5 minutes. Although both analytes elute at approximately 2.1 minutes, an additional 1.4-minute post-peak interval was maintained to ensure complete elution of late-eluting endogenous components, minimize carryover, and re-equilibrate the column before the subsequent injection, utilizing an injection volume of 5 µL.

Specific Mass Spectrometric parameters

Ionization was carried out using electron spray ionization (ESI) in the positive ion mode, with specific source parameters optimized for sensitivity and accuracy. The parameters included a source temperature of 450°C and an ion spray voltage of 5500 V. Nitrogen was used for both curtain and collision gas, with pressures set at 20 psi & 5 psi, respectively. Nebulizer and heater gas pressures were maintained at 35 psi each. For quantification, MRM was utilized. The ion transitions of Precursor to product noted were m/z 442.15 -141.07 for ALB and m/z 445.36 - 144.05 for the IS, as shown in Figures 1 and 2. The specific optimized parameters were a de-clustering potential (DP) of 85 V, an entrance potential (EP) of 10 V, a collision energy (CE) of 55 V, and a collision cell exit potential (CXP) of 22 V for ALB and 10 V for the internal standard. These parameters were important for ensuring the accuracy and reliability of the mass spectrometry results.

Preparation of Standard and Quality Control Samples

Methanol was employed to generate primary stock solutions of ALB and an internal standard, each at a concentration of 1.0 mg/mL. Calibration standards and quality control (QC) stock solutions were formulated through serial dilution in a methanol-water mixture (50:50, v/v). Calibration standards (CS) at varying concentrations were created by adding specific volumes of working solutions to blank human plasma, resulting in concentrations of 50.0, 100.0, 200.0, 400.0, 800.0, 1000.0, 2000.0, 4000.0, 6000.0, and 10,000.0 ng/mL. QC samples were established at four distinct concentration levels: the lower limit

of quantification (LLOQ) at 50.0 ng/mL, low QC (LQC) at 150.0 ng/mL, medium QC (MQC) at 5000.0 ng/mL, and high QC (HQC) at 9000.0 ng/mL. An additional working IS solution was developed at 500 ng/mL in a 50% methanol solution. All stock and working solutions were maintained at 2–8 °C, while plasma standards and quality controls were stored at -80 °C.

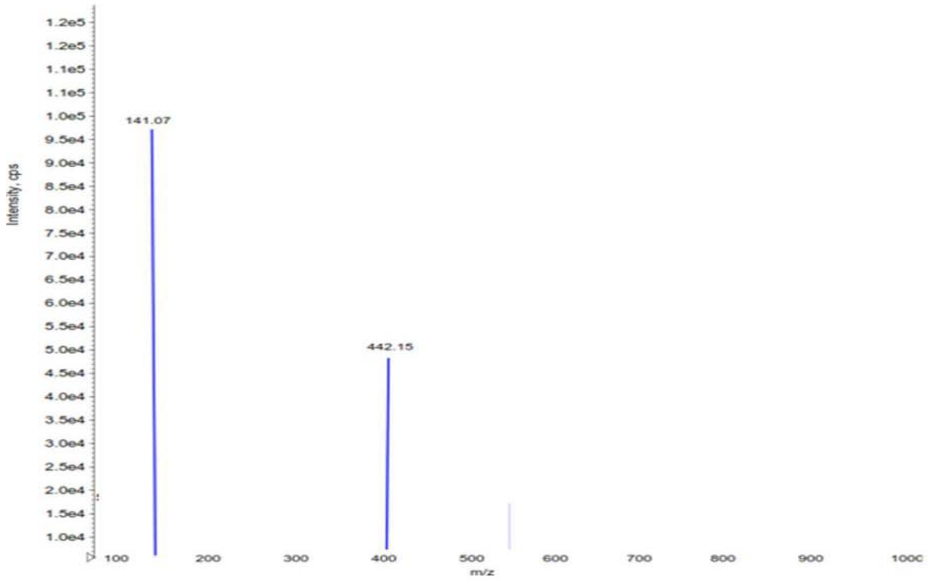

Figure 1: Mass spectra (Q1 and Q3) of Alpelisib

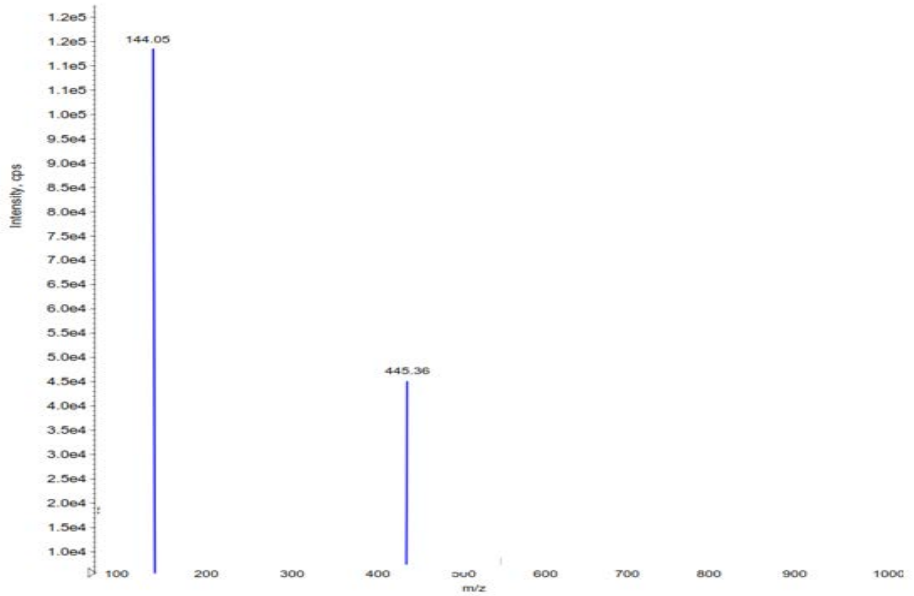

Figure 2: Mass spectra (Q1 and Q3) of Alpelisib-d3

Preparation of Sample

A 50 µL plasma sample, designated as either a calibrator, quality control, or research sample, was placed into a polypropylene tube. To this, 50 µL of an internal standard working solution at a concentration of 500 ng/mL was added alongside 2.0 mL of an extraction solvent composed of n-hexane and MTBE in a ratio of 20:80 (v/v). The resulting mixture was vortexed vigorously for 2 minutes. The sample was then centrifuged at 4000 × g for 10 minutes at 20 °C to facilitate phase separation. The top organic layer, which contained the extracted analytes, was carefully transferred to a sterile tube and subsequently evaporated to dryness using a moderate nitrogen stream at 40 °C. The resulting desiccated residue was then reconstituted in 500 µL of a reconstitution solution consisting of 5 mM ammonium acetate buffer and acetonitrile (10:90, v/v), matching the mobile phase composition to minimize solvent mismatch, prevent peak broadening, and avoid injection solvent effects during high-throughput analysis. After vortexing this for 30 seconds, a 5 µL aliquot was finally injected into the LC-MS/MS apparatus for analysis.

Method Validation

The method was validated according to FDA and EMA guidelines [18,19] for the parameters described below.

Selectivity and Specificity

Selectivity was assessed by evaluating blank plasma samples from six different individual donors. Each blank sample was meticulously processed to evaluate any interference at both the internal standard (IS) and ALB retention times. For a sample to be considered acceptable, the response at the ALB retention time must remain below 20% of the mean lower limit of quantitation (LLOQ) response, while the IS response should be under 5%. The document includes chromatograms illustrating the LLOQ response in blank plasma samples (Figure 3 to Figure 4), and further chromatographs depict the quality control (QC) levels across additional figures (Figure 5 through Figure 7).

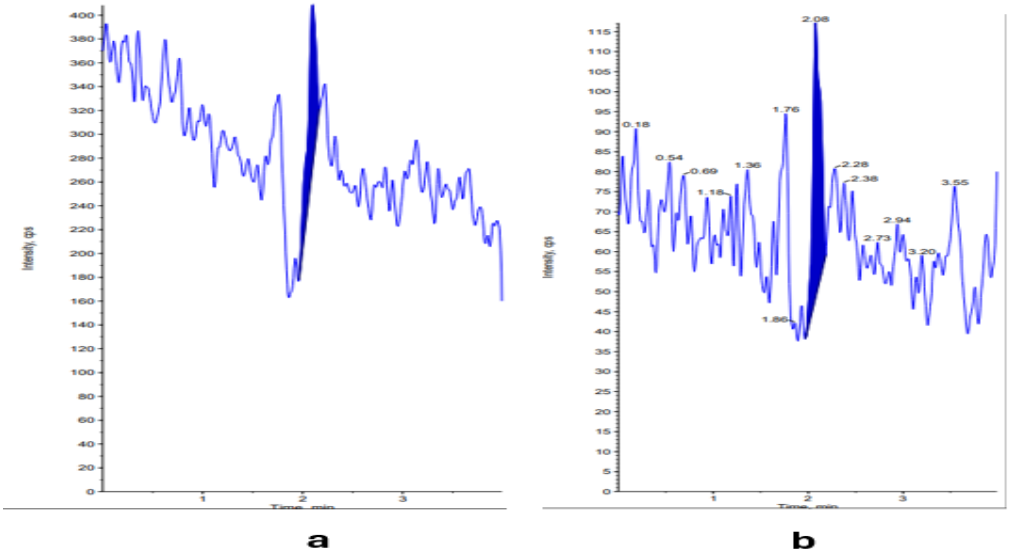

Figure 3: Blank plasma chromatogram of interference-free (a)ALB and (b) ALB –IS

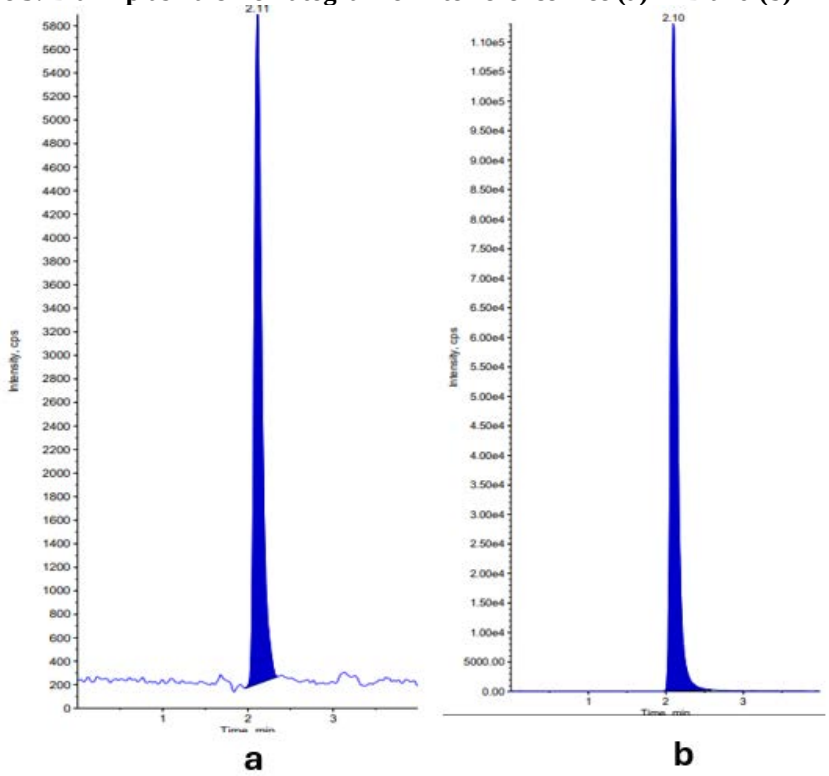

Figure 4: Chromatogram of LLOQ sample (a) ALB and (b) ALB –IS

Carryover

Carryover was assessed by administering a processed blank plasma sample immediately after the injection of a standard at the upper limit of quantification (ULOQ, 10,000 ng/mL). The response during the retention times of ALB and IS was required to be ≤20% of the LLOQ response for ALB and ≤5% for the IS.

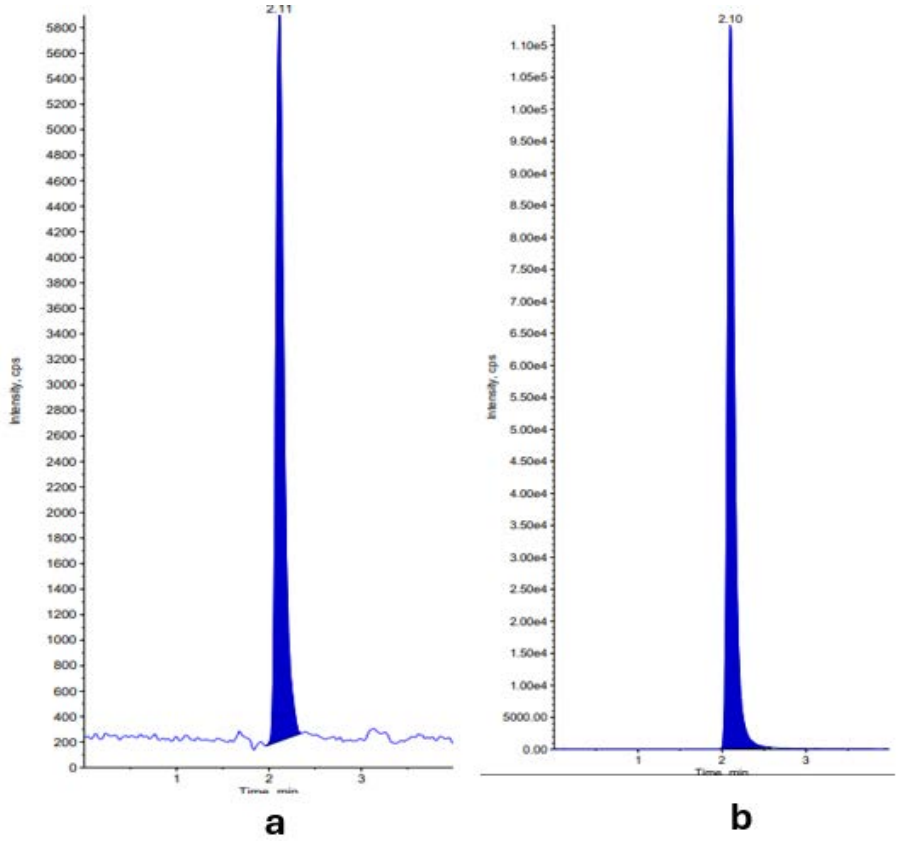

Figure 5: Chromatogram of LQC sample (ALB and ALB -IS)

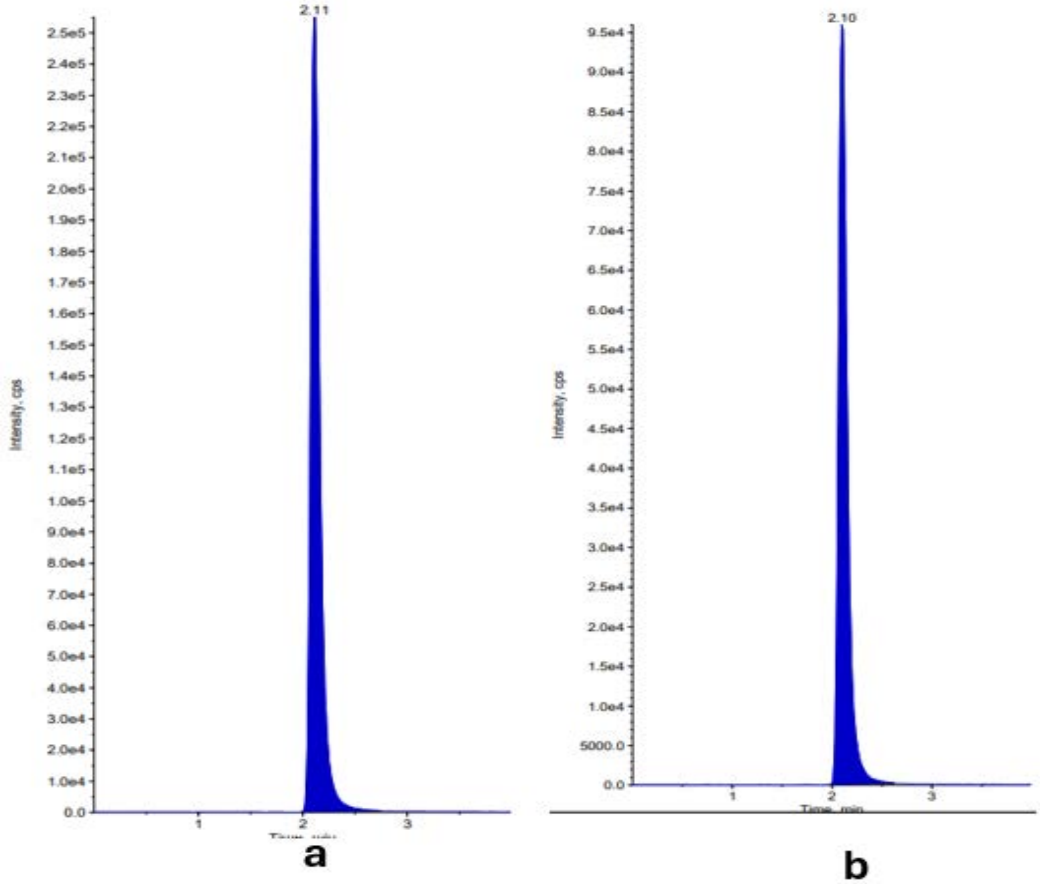

Figure 6: Chromatogram of MQC sample (ALB and ALB -IS)

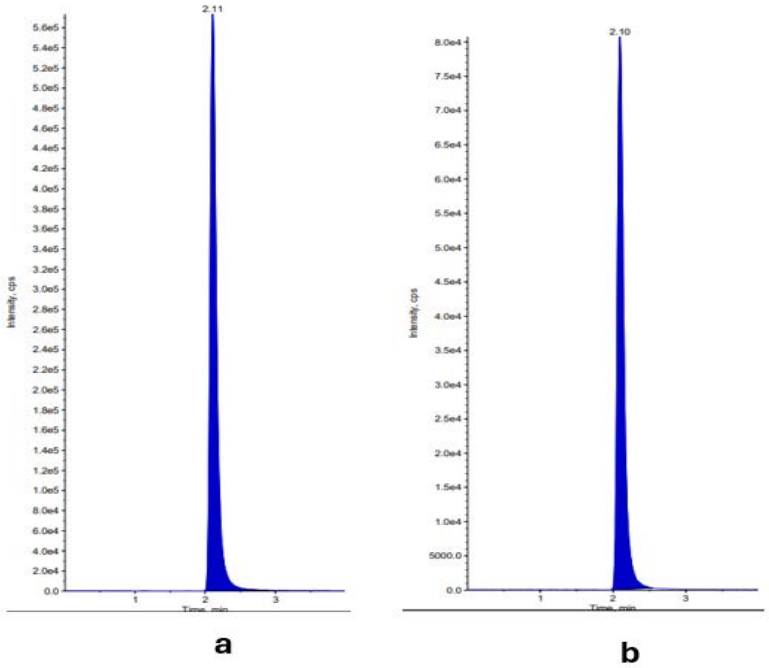

Figure. 7: Chromatogram of HQC sample (ALB and ALB -IS)

Linearity, LLOQ, and ULOQ

Linearity was tested by evaluating calibration curves in duplicate across three separate runs. Graphing the peak area ratio of ALB to IS against the nominal concentration of ALB using a weighted ($1/x^2$) least-squares quadratic regression model, as shown in Figure 8, produced the calibration curve. The LLOQ was created as the smallest concentration on the calibration curve that could be determined with an accuracy and precision (CV%) within ±20%. The ULOQ was the highest concentration that remained within the linear range and fulfilled the accuracy and precision criteria of ±15%. To assess intra-day accuracy and precision, six samples of LLOQ, LQC, MQC, and HQC were analyzed in a single run. We evaluated inter-day accuracy and precision by measuring these QC levels three times over three consecutive days. The accuracy of the measurements was represented by the percentage deviation of the mean observed concentration from the expected value. Precision was demonstrated using the coefficient of variation (CV%).

Extraction Recovery and Matrix Effect

The study involved a detailed comparison of analyte regions in plasma samples spiked before extraction against those spiked after extraction, with specific focus on low quality control (LQC), medium quality control (MQC), and high-quality control (HQC) levels. Each quality control level comprised six samples, ensuring a robust dataset. The recovery of the internal standard (IS) was assessed at its standard operational levels. Additionally, the matrix effect (ME) was quantified through the IS-normalized matrix factor (MF) using a post-column infusion method. Various blank plasma types were tested and spiked with ALB at both low- and high-quality control levels in conjunction with the internal standard after extraction. The analysis focused on the peak area differences between the tested samples and standard solutions introduced to a replacement solution. Importantly, the coefficient of variation (CV%) remained within 15%, ensuring consistency and reliability of the measurements.

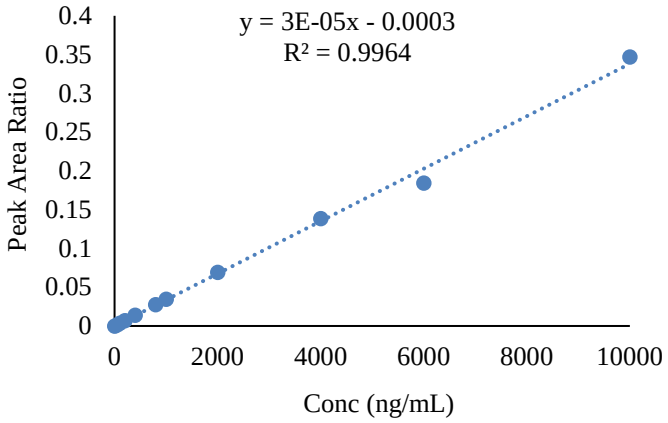

Figure 8: Calibration curve data of ALB

Dilution Integrity

The feasibility of measuring plasma samples above the upper limit of quantitation (ULOQ) was evaluated by preparing a sample at a concentration of 20,000 ng/mL, which is twice the ULOQ threshold. The study involved diluting this sample two times and four times with blank plasma, with a total of six

replicates for each dilution factor. The requirement for the processed diluted samples is that they must achieve a precision level within ±15%

Stability

The stability of ALB in human plasma and samples was assessed under diverse conditions:

Bench-top stability (BT), Freeze-thaw stability (FT), and **Long-term stability (LT)**

LQC and HQC samples were kept at room temperature (~25 °C) for 24 hours for BT. LQC and HQC samples were subjected to three complete freeze (-80 °C) and thaw (room temperature) cycles for FT & LQC & HQC samples were stored at -80 °C for 50 days for LT.

Processed sample (autosampler) stability: Extracted LQC and HQC samples were kept in the autosampler at 10 °C for 60 hours.

Stock solution stability: Stock solutions were stored at 2–8 °C for 30 days. Stability was confirmed if the mean concentration of the stability samples was within ±15% of the nominal concentration.

Robustness

Robustness is a procedural approach; planned modifications were introduced to key variables, specifically adjusting the pH of the mobile phase buffer by ±0.1 units from a baseline of 5.00. The mobile phase was composed of approximately 2% absolute organic content, chiefly consisting of acetonitrile, which accounted for about 88% to 92% of the solution. The sample retrieval process took nearly two minutes, one minute longer than the usual timeframe. Six samples were tested under different conditions: three were Low Quality Control (LQC), and three were High Quality Control (HQC). The method was considered robust if accuracy stayed within ±15% and precision, measured by the coefficient of variation (CV%), was 15% or less in all tests.

RESULTS AND DISCUSSION

Method Development and Optimization

The objective was to design a high-throughput, sensitive, and fast LC-MS/MS technology. Alpelisib-D3, a deuterated internal standard, was employed to monitor the analyte accurately during sample preparation and ionization, thereby reducing variability. Positive electrospray ionization (ESI) mode generated a robust signal for both alpelisib (ALB) and the internal standard (IS). Due to its high intensity and specificity, the MRM transition m/z 442.15→144.05, which indicates a significant fragment loss, was chosen for ALB (Figure 1). We adjusted the chromatographic parameters to produce a sharp, symmetrical peak in a short time. We used a low-pH ammonium acetate buffer to help protonate the analyte and ensure compatibility with positive ESI. Compared to C18 columns, the X-terra RP8 column offers an optimal balance between retention and analytical speed. The retention times for ALB and the internal standard (IS) using the final isocratic method were approximately 2.10 and 2.11 minutes, respectively.

Liquid-liquid extraction (LLE) with n-hexane: MTBE (20:80) was employed instead of protein precipitation because it produced purer extracts, significantly reduced matrix effects, and provided improved and more consistent recovery for both ALB and the internal standard from the 50 µL plasma sample.

METHOD VALIDATION RESULTS

Selectivity, Specificity, and Carryover

Chromatograms obtained from six independent plasma sources demonstrated no significant interference peaks at the retention times of ALB or the internal standard. This result indicates high selectivity, with blank reactions remaining below 5% for ALB and below 2% for the internal standard at the lower limit of quantification (LLOQ). Furthermore, analysis of a blank sample following the upper limit of quantification (ULOQ) standard showed a carryover reaction of less than 1% of the LLOQ for ALB, which is substantially below acceptable thresholds and does not affect subsequent sample analysis.

Linearity, LLOQ, and ULOQ

The calibration curve demonstrated linearity over the range of 50.0 to 10,000 ng/mL. An example of a regression equation is $y = 0.00125x + 0.000812$ ($r^2 = 0.9987$), where y denotes the peak area ratio and x signifies the concentration. The lower limit of quantification (LLOQ) was established at 50.0 ng/mL, with an accuracy of 102.4% and a precision (coefficient of variation) of 4.8% (n = 6).

Accuracy and Precision

To assess intra-day accuracy and precision, six samples of LLOQ, LQC, MQC, and HQC were analyzed in a single run. We evaluated inter-day accuracy and precision by measuring these QC levels three times over three consecutive days. The accuracy

of the measurements was represented by the percentage deviation of the mean observed concentration from the expected value. Precision was demonstrated using the coefficient of variation (CV%) as shown in Table 1.

Table 1: Intra-day and inter-day accuracy and precision for the quantification of Alpelisib in human plasma.

Nominal Concentration (ng/mL)	Intra-day (n=6) Mean Concentration (ng/mL)	Inter-day (n=6 per day, 3 days) Accuracy (%)	Precision (CV%)	Mean Concentration (ng/mL)	Accuracy (%)	Precision (CV%)
50.0 (LLOQ)	51.2	102.4	4.8	49.8	99.6	5.2
150.0 (LQC)	146.7	97.8	3.1	152.1	101.4	3.5
5000.0 (MQC)	5103.4	102.1	2.7	4945.2	98.9	2.9
9000.0 (HQC)	8844.0	98.3	2.3	9126.0	101.4	2.1

Recovery and Matrix Effect

The average extraction recovery of ALB across different quality control (QC) levels was quite consistent. It recorded 94.5% for low-quality control (LQC), 96.5% for medium-quality control (MQC), and 99.3% for high-quality control (HQC). The internal standard (IS) was retrieved with a high rate of 95.9%. An analysis of six plasma batches showed that the IS-normalized Matrix factor had a coefficient of variation (CV%) of 6.7% at LQC and 5.2% at HQC. This suggests minimal ion suppression or enhancement and confirms that the IS effectively compensates for minor variations in the matrix.

Dilution Integrity: The supra-ULOQ sample (20,000 ng/mL) was diluted two and four times. This yielded 98.5% and 101.2% accuracy with 3.2% and 3.8% precision, respectively. These results confirm that, following appropriate dilution with blank plasma, samples exceeding the upper limit of quantification can be quantified with accuracy.

Table 2: Stability of Alpelisib in human plasma under various conditions (n=3).

Stability Condition	LQC (150 ng/mL) Accuracy (%)	LQC (150 ng/mL) Precision (CV%)	HQC (9000 ng/mL) Accuracy (%)	HQC (9000 ng/mL) Precision (CV%)
Bench-top (24 h)	98.7	2.9	99.1	1.8
Three freeze-thaw cycles	97.2	3.4	101.5	2.1
Long-term (-80°C, 50 days)	99.5	2.7	98.8	2.3
Autosampler (10°C, 60 h)	101.2	3.1	97.9	2.5
Stock Solution (2-8°C, 30 d)	99.8	1.5	100.3	1.7

Stability Assessment

ALB demonstrates stability across all testing situations, as detailed in Table 2. The stability quality controls indicated that all measured concentrations remained within ±10% of their nominal levels.

Robustness

The method was stable when tested under various conditions. Its accuracy ranged from 96.8% to 103.7% for both low-quality control (LQC) and high-quality control (HQC) samples under various conditions. These included pH levels of 4.9 and 5.1, acetonitrile concentrations of 88% and 92%, and extraction times of 1 and 3 minutes. The method's precision did not exceed 4.5%.

Comparison with Existing Methods: A comparison of the proposed methodology with previously reported bioanalytical techniques for ALB is shown in Table 3.

The present methodology offers significant benefits. It notably reduces the plasma volume requirement to 50 µL compared to the usual ≥100 µL. It also uses a deuterated internal standard to improve precision, has a short run time of 3.5 minutes, and offers thorough validation that covers robustness and dilution integrity, which are not always reported.

Comparison with Existing Methods

Table 3 presents a comparison between the proposed methodology and previously reported bioanalytical techniques for ALB. Significantly lower plasma volume needs (50 µL as opposed to the usual ≥100 µL), the application of a deuterated internal standard for enhanced precision, a short run time of 3.5 minutes, and thorough validation covering robustness and dilution integrity aspects that are not consistently reported during previous tests are merely a few advantages of the suggested method.

Table 3: Comparison of the present LC-MS/MS method with reported methods for alpelisib quantification.

Ref.	Method	Sample Volume	Internal Standard	Linear Range (ng/mL)	LLOQ (ng/mL)	Run Time (min)	Key Advantages of Present Work
[13]	HPLC-FLD	100 µL	None	50-5000	50	15	MS detection, deuterated IS, smaller volume, faster
[14]	UPLC-MS/MS	50 µL	Structural analog	1-1000	1	5	Wider range, full validation per guidelines
[15]	LC-MS/MS	500 µL	Deuterated	2-2000	2	6	Much smaller plasma volume required (50 µL)
This work	LC-MS/MS	50 µL	Alpelisib-D3	50-10,000	50	3.5	Rapid, robust, validated for full PK scope, low volume

CONCLUSION

A selective, sensitive, and rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for detecting alpelisib in human plasma. The method uses a deuterated internal standard, a simple liquid-liquid extraction, only 50 µL of plasma, and fast isocratic chromatography. All validation parameters, including selectivity, linearity, accuracy, precision, recovery, matrix effects, stability, and robustness, met regulatory standards. The technique also effectively addresses carryover and dilution integrity issues.

Overall, this reliable and high-throughput assay is intended to enable therapeutic drug monitoring and pharmacokinetic investigations of alpelisib, applicable in both clinical and research contexts. Enhanced selectivity was achieved through the use of a stable isotope-labeled internal standard (Alpelisib-D3), which compensated for extraction variability and matrix-related ion suppression.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Venkateswararao Agraharapu contributed to conceptualization, study design, experimental investigation, LC-MS/MS method development and validation, data collection, data analysis, and preparation of the original manuscript draft. Suryadevara Vidyadhara contributed to the supervision of the research work, guidance, data interpretation, and critical review of the manuscript. Venkatarao Vutla contributed to project supervision, validation of the analytical approach, manuscript editing, and final review of the manuscript.

DECLARATION OF USE OF AI TOOLS

The authors used ChatGPT (OpenAI) to assist with language editing, improving readability, and enhancing the clarity of the manuscript. All scientific content, data analysis, interpretations, and conclusions were carefully reviewed and verified by the authors. The authors take full responsibility for the originality, accuracy, authenticity, and integrity of the work.

REFERENCES

André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. *N. Engl. J. Med.*, **380(20)**, 1929–1940 (2019) https://doi.org/10.1056/NEJMoa1813904

Tan A, Fanaras JC. Use of high-pH (basic/alkaline) mobile phases for LC-MS or LC-MS/MS bioanalysis. *Biomed. Chromatogr.*, **33(1)**, e4409 (2019) https://doi.org/10.1002/bmc.4409

Peleg Hasson S, Rubinek T, Ryvo L, Wolf I. Endocrine resistance in breast cancer: Focus on the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling pathway. *Breast Care*, **8(4)**, 248–255 (2013) https://doi.org/10.1159/000354757

Markham A. Alpelisib: First global approval. *Drugs*, **79(11)**, 1249–1253 (2019) https://doi.org/10.1007/s40265-019-01161-6

Rugo HS, André F, Yamashita T, Cerda H, Toledano I, Stemmer SM, et al. Time course and management of key adverse events during the randomized phase III SOLAR-1 study of PI3K inhibitor alpelisib plus fulvestrant in patients with HR-positive advanced breast cancer. *Ann. Oncol.*, **31(8)**, 1001–1010 (2020) https://doi.org/10.1016/j.annonc.2020.05.001

Armaghani AJ, Han HS. Alpelisib in the treatment of breast cancer: A short review on the emerging clinical data. *Breast Cancer Targets Ther.*, **12**, 251–258 (2020) https://doi.org/10.2147/BCTT.S219436

Miricescu D, Totan A, Stanescu-Spinu II, Badoiu SC, Stefani C, Greabu M. PI3K/AKT/mTOR signaling pathway in breast cancer: From molecular landscape to clinical aspects. *Int. J. Mol. Sci.*, **22(1)**, 173 (2021) https://doi.org/10.3390/ijms22010173

Srivastava S, Upadhye V J, Prasad M E, Singh P. Applications of bioactive compounds of traditional Chinese medicine in breast cancer management. *Journal of Applied Pharmaceutical Research*, **13(4)**, 1-15 (2025) https://doi.org/10.69857/joapr.v13i4.935

Tankova T, Mrinakova B, Frolova M. Management strategies for hyperglycemia associated with the α-selective PI3K inhibitor alpelisib for the treatment of breast cancer. *Cancers*, **14(7)**, 1598 (2022) https://doi.org/10.3390/cancers14071598

Costa RLB, Han HS, Gradishar WJ. Targeting the PI3K/AKT/mTOR pathway in triple-negative breast cancer: A review. *Breast Cancer Res. Treat.*, **169(3)**, 397–406 (2018) https://doi.org/10.1007/s10549-018-4697-y

James A, Hazell K, Trandafir L, et al. Absorption, distribution, metabolism, and excretion of BYL719 (alpelisib) in healthy male volunteers. *Cancer Chemother. Pharmacol.*, **76(4)**, 751–760 (2015) https://doi.org/10.1007/s00280-015-2835-9

De Buck SS, Jakab A, Boehm M, et al. Population pharmacokinetics and pharmacodynamics of BYL719, a phosphoinositide 3-kinase antagonist, in adult patients with advanced solid malignancies. *Br. J. Clin. Pharmacol.*, **78(3)**, 543–555 (2014) https://doi.org/10.1111/bcp.12354

Seo SW, Kim JM, Han DG, Geum D, Yun H, Yoon IS. A sensitive HPLC-FLD method for the quantification of alpelisib in rat plasma: Drug metabolism and pharmacokinetic evaluation in vitro and in vivo. *J. Chromatogr. B*, **1163**, 122508 (2021) https://doi.org/10.1016/j.jchromb.2020.122508

Wang Q, Lan X, Zhao Z. Characterization of alpelisib in rat plasma by a newly developed UPLC-MS/MS method: Application to a drug-drug interaction study. *Front. Pharmacol.*, **12**, 725384 (2021) https://doi.org/10.3389/fphar.2021.725384

Lee S, Kim MS, Jeong JW. Bioanalysis of alpelisib using liquid chromatography–tandem mass spectrometry and application to pharmacokinetic study. *J. Anal. Sci. Technol.*, **13**, 31 (2022) https://doi.org/10.1186/s40543-022-00340-7

James AD, Marvalin C, Luneau A, et al. Comparison of ^19F NMR and ^14C measurements for the assessment of ADME of BYL719 (alpelisib) in humans. *Drug Metab. Dispos.*, **45(8)**, 900–907 (2017) https://doi.org/10.1124/dmd.117.075051

Cai HL, Deng Y, Fang PF, Cao S, Hou ZY, Wu YQ, et al. A sensitive LC-MS/MS method for analysis of pericyazine in presence of 7-hydroxypericyazine and pericyazine sulphoxide in human plasma and its application to a comparative bioequivalence study in Chinese healthy volunteers. *J. Pharm. Biomed. Anal.*, **135**, 67–74 (2017) https://doi.org/10.1016/j.jpba.2016.12.007

Tiwari G, Tiwari R. Bioanalytical method validation: An updated review. *Pharm. Methods*, **1(1)**, 25–38 (2010) https://doi.org/10.4103/2229-4708.72226

Kaza M, Karaźniewicz-Łada M, Kosicka K, Siemiątkowska A, Rudzki PJ. Bioanalytical method validation: New FDA guidance vs. EMA guideline. Better or worse? *J. Pharm. Biomed. Anal.*, **165**, 381–385 (2019) https://doi.org/10.1016/j.jpba.2018.12.030