Int J Biomed Investig (2025) Vol 8 (2)

International Journal of Biomedical Investigation

journal homepage: https://ijbi.edwiserinternational.com

Original Article

Method Development, Validation for Simultaneous Estimation of Ribociclib and Letrazole in Human Plasma by Using LC-MS/MS

Deepthi Kuturu*[1], Anandarajagopal Kal Usalingam[2], Ashok Kumar Uppuluru[3], Abdullah Khan[4], Mohammad Yosuf Malik Damani[5], Roohi Jamal[6], Rakesh SA[5], Mohammed Jaffer Sadik[7], Tazneem B[8]

[1]*Bojjam Narasimhulu Pharmacy for Woman, Vinay Nagar, IS Sadan Cross road, Saidabad, Hyderabad-500059, Telangana, India.*
[2]*School of Pharmacy, KPJ University, 71800, Nilai, Negeri Sembilan, Malaysia*
[3]*Max Institute of Pharmaceutical Sciences, Khammam, Telangana, India.*
[4]*Quest International University, 30250 Ipoh, Perak, Malaysia.*
[5]*GM Institute of Pharmaceutical Sciences and Research, Davangere, Karanataka-577006, India.*
[6]*K B H S S trust's institute of Pharmacy, Bhaygon road, Malegaon, Nashik, Maharashtra, India, 423105.*
[7]*Sri Indu Institute of Pharmacy, Sheriguda, Ibrahimpatanam, R.R Dist, Hyderabad, Telangana, India.*
[8]*Deccan School of Pharmacy, Osmania University, Hyderabad - 500001, Telangana State, India.*

ARTICLE INFO

Received 03 November 2025
Revised 08 December 2025
Available Online 13 December 2025

Keywords:
Letrazole
Ribociclib
LC-MS/MS
Vitex negundo
In-silico studies
Method Validation
Bioanalytical method
Chemotherapeutic agents

ABSTRACT

Ribociclib (RIBO) and Letrozole (LETRO) are chemotherapeutic agents commonly used in combination therapy. In this study, a novel and robust bioanalytical LC–MS/MS method was developed for the simultaneous quantification of both drugs in human plasma. The LC–MS/MS system was operated in positive ionization mode using the following source parameters: collision gas 20, cone gas 20, desolvation gas 700, and source temperature 150°C. Chromatographic separation was achieved using Mobile Phase A, consisting of a mixture of 0.1% formic acid (pH adjusted to 3.5 with triethylamine) and methanol (80:20, v/v), and Mobile Phase B, consisting of 5% acetonitrile. The gradient program was initiated at 95% A and 5% B at 0.8 min, shifted to 5% A and 95% B at 2.2 min, and subsequently returned to 95% A and 5% B at 2.4 min, which was maintained until 3.5 min. An additional gradient step was incorporated to effectively eliminate carry-over. The developed method was fully validated within the relevant linearity ranges that encompassed the anticipated plasma concentrations of both analytes, with correlation coefficients ranging from 0.9999 to 0.9939. Interday precision (%CV) for RIBO ranged from 3.53% to 5.44%, while LETRO showed values between 6.12% and 13.12%. Intraday precision ranged from 3.58% to 8.77% for RIBO and 2.49% to 14.14% for LETRO. These results comply with the EMA and ICH guidelines for bioanalytical method validation, confirming the reliability and reproducibility of the method.

Introduction

Over the past few decades, mass spectrometry (MS) analysis in conjunction with liquid chromatography (LC) strategies has had a significant effect on the creation of new medications. Drugs are being analyzed using LC-MS instruments at different phases during research and development. It is an excellent technique

for identifying unknown substances; it requires pure samples for optimal results. Mass spectrometers also produce three-dimensional data, including signal strength and mass spectral information, which provides valuable insights into the molecular weight, structure, identity, quantity, and purity of a sample [1, 2]. According to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), specifically ICH Q2 (R1), method validation is a structured process aimed at generating documented evidence. As per FDA (Food and Drug Administration) guidelines, in production and process control, validation is an essential step that guarantees pharmaceutical goods retain the goal identity, durability, value, and impurity. Ribociclib (RIBO): It is a class of drug that helps slow down cancer progression by inhibiting two proteins that are called cyclin-dependent kinase 4 and 6 (CDK4/6). This drug is called a selective cyclin-dependent kinase inhibitor. The molecular formula of the drug is $C_{23}H_{30}N_8O$, and its molecular weight is 434.5 g/mol. The IUPAC name of the compound is 7-cyclopentyl-N, N-dimethyl-2-[(5-piperazin-1-ylpyridin-2-yl) amino]. pyrrolo[2,3-d] pyrimidine-6-carboxamide. Figure 1 [3]. Letrozole (LETRO): This drug's molecular formula is C17H11N5, and its molecular weight is 285.30 g/mol. This drug's mechanism of action is inhibiting tumor growth and regulation of aromatase in breast cancer. The IUPAC name is 4-[(4-cyanophenyl) -(1,2,4-triazol-1-yl) methyl] benzonitrile. 7. Chemical/molecular formula and its molecular formula is C17H11N5. [Figure 1] [4].

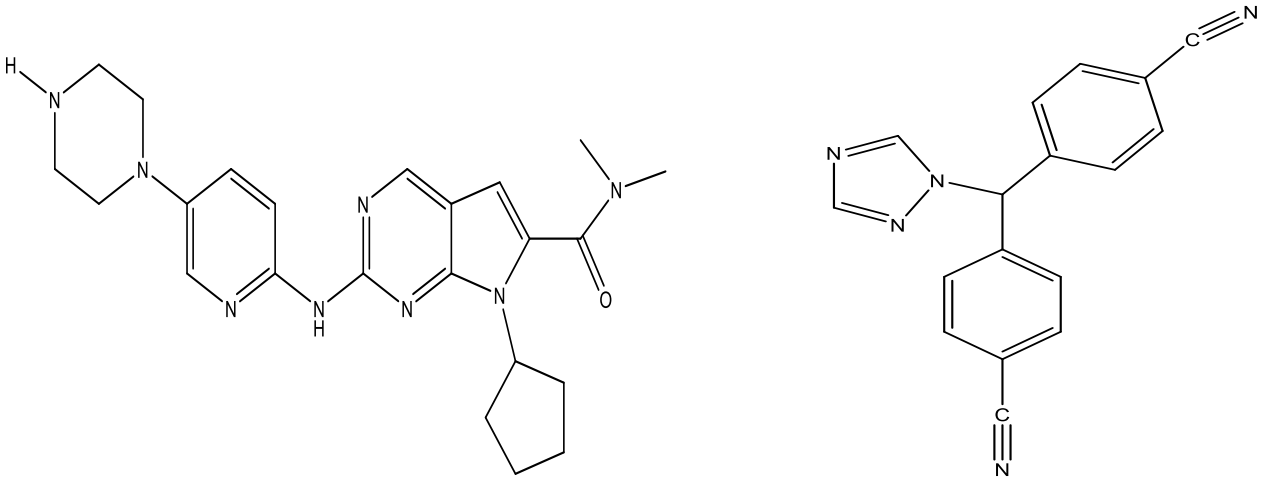

Figure 1: Chemical structure of Ribociclib and Letrozole.

The most frequent type of cancer among women globally is breast cancer. Based on the presence or lack of molecular markers for human epidermal growth factor 2 (ERBB2; formerly HER2), progesterone, or estrogen receptors. Breast cancer is classified into three major subtypes: triple-negative (tumors lacking all three standard molecular markers; 15%), ERBB2 positive (15–20%), and hormone receptor positive/ERBB2 negative (70% of patients) [5].

Quantification of RIBO and LETRO has been performed by HPLC-UV in human plasma; the developed method was validated according to ICH guidelines. This bioanalytical method supports the bioavailability bioequivalence studies.

In a run time of 5 minutes, both drugs are identified in a mobile phase of 10 mM phosphate buffer–acetonitrile (60:40, v/v) adjusted to pH 3.0 in a column of Orochem orosil C18 (4.6 mm × 250 mm, 5 µ). The flow rate was 0.9 ml/min and λmax 260 nm [6]. By the LC-MS/MS method developed and validated, this method has been employed to examine the plasma pharmacokinetics and CNS penetration of RIBO in patients with malignant brain cancer. The optimized chromatographic conditions were linearity range 0.5–1000 nM, flow rate 0.8 mL/min, mobile phase acetonitrile and ammonium formate (10 mM, pH 3.0, 75:25), triple quadrupole mass spectrometer, and hypersil Gold C18 (50 mm x 3.0 mm, 5 µm) [7].

Simultaneous quantification of CDK4/6, M2, M20, and letrozole by LC-MS/MS in human plasma has been reported. This method is simple, accurate, and robust. The mobile phase was methanol and pyrrolidine-pyrrolidinium formate (0.005:0.005 mol/L) buffer (pH 11.3). The column used was the X Bridge BEH C18 column (2.5 mm, 3.0X75 mm XP) [8]. A bioanalytical method has been reported by LC-MS/MS for the

*Corresponding author: Deepthi Kuturu, Bojjam Narasimhulu Pharmacy for Woman, Vinay Nagar, IS Sadan Cross road, Saidabad, Hyderabad-500059, Telangana, India.

https://doi.org/10.31531/2581-4745.1000174

simultaneous quantification of multiple drugs: abemaciclib, palbociclib, RIBO, anastrozole, LETRO, and fulvestrant in human plasma. This is novel and reliable for sample analysis of breast cancer patients. A calibration curve was prepared over the range of 25 ppb to 150 ppb. In the analysis the retention of RIBO and LETRO was 0.96 min and 1.23 min, respectively. Mobile phase was A: Acetonitrile; B: 2 mM Ammonium acetate with 0.2% formic acid in a ratio of 75:25. The column used was X Bridge C18 (150x4.6 mm, 3.5 µm, Waters) with a PDA detector [9]. By the hyphenated technique HPLC-PDA, the quantification of LETRO and Palbociclib in rat plasma has been reported; that method is simple, accurate, and novel. Also, different chromatographic conditions were reported that are helpful to design the new method [10,11]. An LC-MS/MS method has been developed for the clinical analysis of cancer patients' samples for therapeutic drug monitoring of palbociclib, RIBO, and LETRO [12]. A novel and simple method has been developed for the quantification of the CDK4/6 inhibitors by LC-MS/MS that has been validated according to ICH guidelines [13]. Also, the quantification of the CDK4/6 inhibitor palbociclib by HPLC-UV [14] and the estimation of RIBO, a CDK4/6 inhibitor, by LC-MS/MS [15] have been reported. From the existing literature, a new, simple, and accurate method has been developed for the quantification of RIBO and LETRO in human plasma by LCMS/MS.

Materials and Methods

Chemical and reagent: RIBO and LETRO are purchased from Sigma and Aldrich, and ACN (HPLC and LC-MS grade) and MeOH (HPLC grade) were purchased from J. T. Baker (Phillipsburg, NJ, USA). Water was purified using a Merck Mili-Q IQ 7000 water purification system (Darmstadt, Germany). Human plasma from a blood bank.

LC-MS/MS instrumentation and chromatographic conditions: LC-MS/MS is used to separate mixture components based on changes in affinity between the stationary and mobile phases, as well as to eliminate unwanted contaminants. The online DGU-20A3 soluble degasser, segment boiler CTO-20A, LC-20AD drives-2, detectors, and column make up the LC setup. For retention, a C18 column (5 µm, 50x4.6 mm) (Phenomenex Zorbax SB) is employed. Electron spray ionization was employed to address the problem of hard ionization in the LC-MS/MS equipment. It should have the sample in solution and be able to employ inline separation as well as liquid chromatography. Multiple charged ions are produced by ESI. The LC-MS/MS system works in the positive ion mode with the following settings: collision gas: 20, cone gas: 20, desolvation gas: 700, and source temperature: 1500°C. Mobile phase A (80:20 ratio of 0.1% formic acid, pH adjusted with triethylamine up to 3.5, and methanol) and B is 5% acetonitrile. In the primary phase it is 95% A and 5% B at 0.8 min and altered to 5% A and 95% B at 2.2 min. Then gradually it is slowly transformed to 95% A and 5% B at 2.4 min and then remains the same at 3.5 min.

Optimization of MS/MS detection conditions and parameters: The optimal multi-reaction monitoring (MRM) parameters were found using infusion of each analyte at 500 ng/ml concentration. For each analyte, MRM transitions and optimum compound-dependent parameters were identified. Optimization of MS conditions is presented in table 1.

Table 1: Positive ion mode (for RIBO and LETRO).

Parameter	Value
Ionization type and polarity	ESI, +ve ion mode
Ion source	Turbo spray
Scan type	MRM
Ion spray voltage	5500V
Q1Resolution	Unit (1)
Q3Resolution	Unit (0.75)
Temperature	550^{0}C
Gas 1	50
Gas 2	50
CUR gas	20
CAD gas	4
Ihe	ON

Preparation of stock solution: To make a 1 mg/ml solution, dissolve 100 mg of RIBO and LETRO in a 1:1 mixture of acetonitrile and water. Store the solution at 80°C. The normal stock solution is held at -2 to 20°C in the mobile phase.

Internal standard selection: Imatinib was employed as the internal standard (IS) for the simultaneous analysis of Ribociclib (RIBO) and Letrozole (LETRO). During sample preparation, Imatinib remained stable under the extraction conditions, exhibiting an acidic pH profile, whereas the extracted samples of RIBO and LETRO showed a neutral pH after processing. Following protein precipitation and extraction, the resulting supernatant was uniformly diluted with water to achieve consistent matrix conditions for all analytes and the IS. This dilution step ensured improved ionization efficiency, minimized matrix effects, and facilitated accurate and reproducible quantification of RIBO and LETRO using Imatinib as the internal standard.

Method Validation: The approach was verified in compliance with the EMA and ICH bioanalytical technique validation requirements [16-19].

Precision: Intraday precision was assessed for three different levels, i.e., lower, middle, and higher levels. Inter-day precision was evaluated for three different days where the mean values obtained were recorded. Intermediate precision/ruggedness—assess the method's intermediate precision, also referred to as ruggedness; precision tests were conducted on multiple days. The acceptable range of % RSD should be less than 2%.

Accuracy: The developed method's accuracy has been performed by recovery studies at concentration level LOQ level, 50%, 100%, and 150%. Pre-analyzed solutions of the indicated levels were spiked with a known quantity of RIBO and LETRO, separately. The LC-MS equipment was used to inject every spiked level three times, and the percentage

Linearity: A relationship between concentration and test result that is directly proportional to each other. Plotted the curve between peak area and concentration over the strength of the LOQ level (3 ppm) to about 150% of the limit (10 ppm) for both drugs of five or six dilutions by injecting each level of concentration two times and finding out the regression coefficient value. The value of the correlation coefficient should not be less than 0.99.

Stability studies: To conduct stability studies, multiple drug samples were kept at room temperature for a full day. The initial analyte concentrations of freshly generated samples were correlated with the drug concentrations following each storage period. If the sample's concentration is within the permitted ranges for precision and accuracy, the samples are deemed stable.

Result

Preparation of Calibration Curve: By using the same plasma and dilute solution, 5 sets (same) of quantitation standards were made, each on a separate day, to assess the linearity of response. This is referred to as inter- and intra-day data, and it will demonstrate the test is reliable and repeatable on dissimilar days. Figure 2.

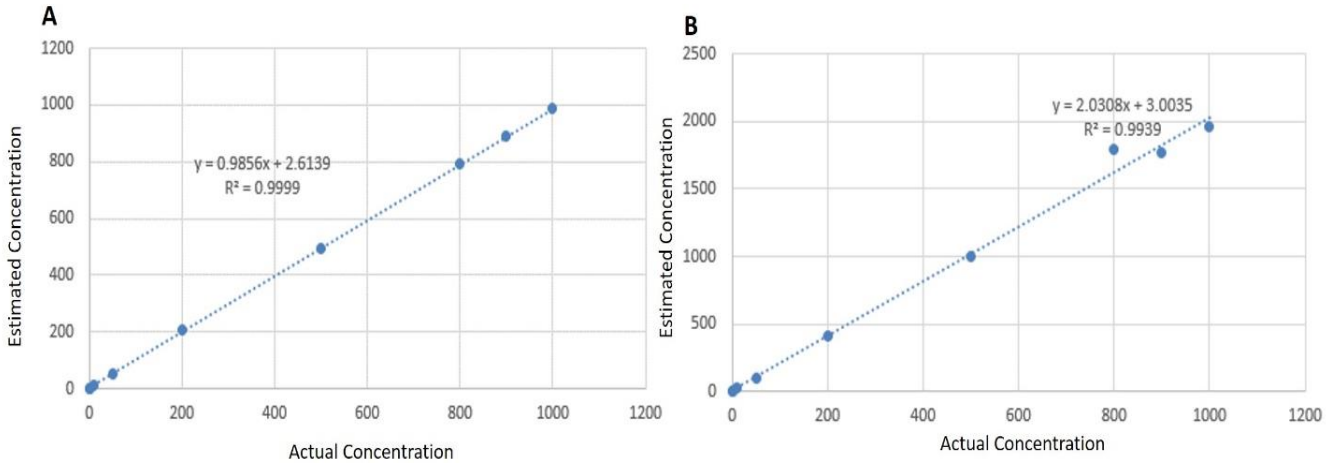

Figure 2: Calibration curve of RIBO and LETRO.

Carry over: The sample devoid of an internal standard is introduced right after the batch's highest standard to guarantee that, in terms of peak area, carryover is less than 20% of the LLOQ.

Method selectivity (matrix selectivity): When six independent batches of unspiked K_2EDTA human plasma were analyzed under the optimized LC-MS/MS conditions, the MRM transitions monitored for each analyte demonstrated excellent selectivity and specificity. No endogenous peaks or interfering signals were observed at the respective retention times of the target analytes or their internal standards. This confirms that the method can reliably distinguish the analytes of interest from endogenous plasma components, ensuring accurate quantification even in complex biological matrices.

Table 2: Summary of MRM transition conditions.

Compound	Mode of ionization	Q_1mass (m/z)	Q_3 mass (m/z)	CE	Cone voltage
Ribociclib	ESI +ve	435.5	112.1	4	92
Letrozole	ESI -ve	284.1	242.0	22	32
Imatinib	ESI +ve	494	394	37	96

LC-MS/MS spectra: A stock solution of RIBO and LETRO in a 1:1 mixture of acetonitrile and water was used for the LC-MS/MS estimation. The initial processes, like spectra, mobile phase, stationary phase, and sample preparation, were selected to carry out analysis by LC–MS/MS in human plasma. As shown in Figure 3 and Figure 4, the mass spectra for RIBO and LETRO with their specific retention times are given, respectively. The molecular ion peak for RIBO and LETRO was observed at 149 g/mol and 242 g/mol. The area of the curve corresponds to the concentration of RIBO and LETRO. The LC-MS/MS system works in the positive ion mode.

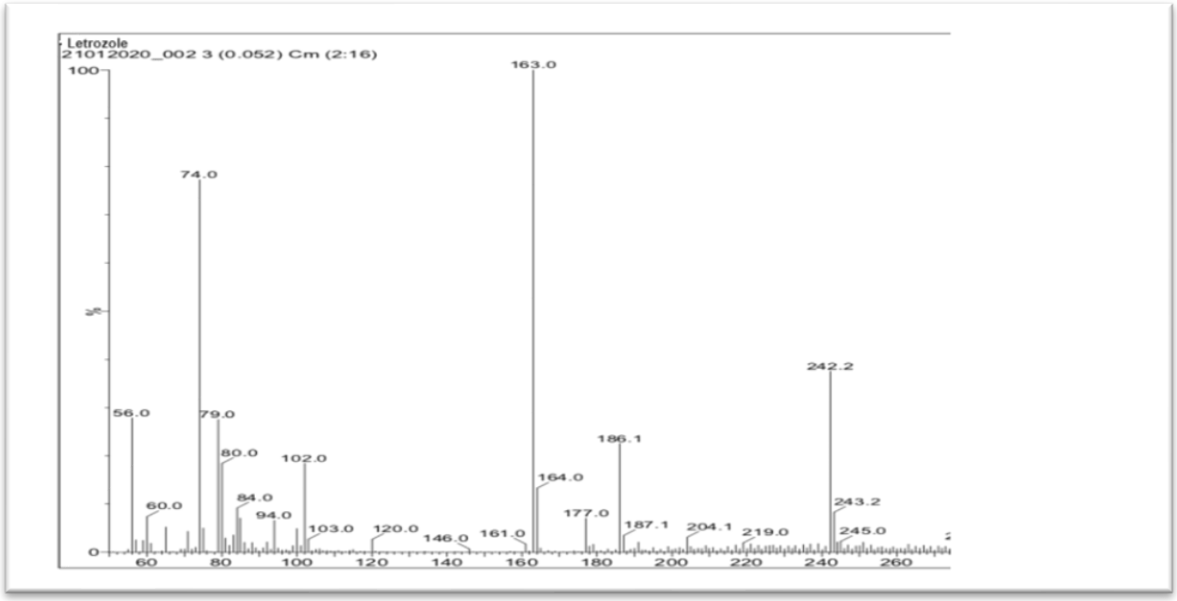

Figure 3: Product ion mass spectra of LETRO in positive ionization mode.

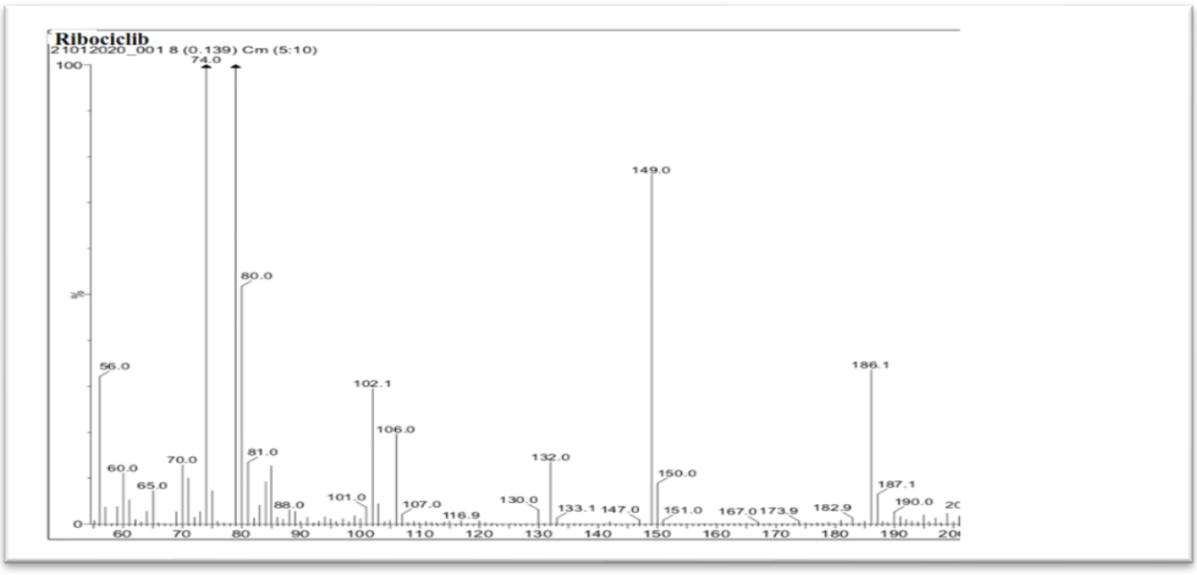

Figure 4: Product ion mass spectra of RIBO in positive ionization mode.

Linearity: Back-calculated accuracy ranges from 100±15% of the nominal concentration. All calibration curves' correlation coefficient values were not more than 0.99. Table 3.

Table 3: Back-calculated standard curve data in human plasma.

Concentration (ng/mL)								
RIBO					LETRO			
Std conc.	Mean (N3)	SD	% CV	% Accuracy	Mean (N3)	SD	% CV	% Accuracy
1	1	0.01	1	100	0.95	0.072342	3.807462	95
2	1.94	0.137477	7.086457	97	2.05	0.240208	5.931068	102.5
10	9.99	0.215716	2.159318	99.9	11.37	0.255408	1.318576	113.7
50.01	52.2	1.630164	3.122919	104.4	49.66	8.164321	8.446432	99.30
200.03	208.38	8.600333	4.127235	104.19	212.71	11.53786	2.795634	106.355
500.06	496.38	23.34151	4.702347	99.276	494.54	26.67813	2.682459	98.89
800.1	792.35	22.40601	2.827792	99.04375	792.05	211.1126	11.78051	98.99
900	888.24	59.6434	6.714785	98.69333	910.21	64.73354	3.656828	101.11
1000	986.5	45.98623	4.661554	98.65	998.7	40.40989	2.052618	99.87

Accuracy & precision: The results demonstrate good accuracies, especially when considering the multistep sample preparation technique, with the majority of samples falling between 90 and 110% of the nominal concentration. The intra- and inter-day coefficients of variation for each analyte are likewise considerably below the nominal requirement of less than 15%. The concentration of both the drugs RIBO and LETRO was estimated in the human plasma. The drugs were evaluated for 3 different batches with 3 different concentrations i.e. LQC, MQC and HQC. The % CV, SD and mean values obtained for RIBO and LETRO are shown in table 4.

Table 4: Accuracy - calculated concentrations of drugs in human plasma (n=6).

RIBO									
	Batch-1			Batch-2			Batch-3		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
MEAN	3.43	541.68	836.27	2.98	495.1	807.2	3.33	497.93	858.46
SD	0.33	21.46	26.38	0.3	17.12	9.65	0.24	23.31	54.79
%CV	9.62	3.96	3.16	10.11	3.46	1.19	7.14	4.68	6.38
LETRO									
MEAN	5.77	1133.04	1822.73	5.36	883.25	1621.55	5.96	1094.17	1776.28
SD	0.40	37.76	25.67	0.20	18.77	45.81	0.43	404.50	57.72
% CV	7.00	3.33	1.40	3.86	2.12	2.82	7.36	6.96	3.24

The precision was assessed for both the drugs by conducting inter-day and intra-day evaluations. Three different concentration levels were evaluated for both the drugs' low-level quality control (LQC), middle-level quality control (MQC), and high-level quality control (HQC) solutions. Each experiment's precision was evaluated using % RSD values in table 5.

Recovery: The average recovery for RIBO and LETRO was found to be 98.3% and 94.3% (n=6), respectively. The total of all losses that occur prior to, during, and following sample extraction through the point at which the samples are examined by the LC-MS/MS system is known as total recovery. An acceptable total loss during bioanalysis is indicated if the recovery rate is more than 85–90%. Thus, before moving on to method validation, no additional condition optimization is required (Table 6).

Table 5: Intra- and inter-run precision and accuracy for drugs in human plasma.

		RIBO			LETRO		
		Concentration (ng/mL)					
		LQC (4.2)	MQC (500)	HQC (900)	LQC (4.2)	MQC (500)	HQC (900)
Intra Batch-1 (N=6)	mean	3.43	541.68	836.27	5.77	1133.04	1822.73
	SD	0.33	21.46	26.38	0.40	37.76	25.67
	% CV	9.6	3.96	3.16	7.00	3.33	1.40
	%Accuracy	114.33	108.33	104.53	192.33	226.60	227.84
Intra Batch-2 (N=6)	mean	3.15	494.87	809.82	5.36	883.25	1621.55
	SD	0.3	17.12	9.65	0.20	18.77	45.81
	% CV	9.57	3.46	1.19	3.86	2.12	2.82
	%Accuracy	99.33	99.02	100.90	178.83	176.65	202.69
Intra Batch-3 (N=6)	mean	3.33	497.93	858.46	5.96	1094.17	1776.28
	SD	0.24	23.31	54.79	0.43	404.50	57.72
	% CV	7.14	4.68	6.38	7.36	36.96	3.24
	%Accuracy	111.16	99.58	107.30	198.66	218.83	222.03
Inter- Batch (N=18)	mean	3.31	509.28	838.15	5.80	1037.35	1749.3
	SD	0.15	27.72	29.62	0.37	136.09	107.08
	% CV	4.39	5.44	3.53	6.39	13.12	6.12
	%Accuracy	104.54	96.45	95.24	95.81	102.91	104.12

Table 6: Drug Recovery Data for RIBO and LETRO.

Analyte	Concentration			Avg. Recovery	SD	% CV
	LQC	MQC	HQC			
Ribociclib	98.6	97.7	98.4	98.3	0.47	0.48
Letrozole	92.3	94.9	95.9	94.3	1.82	1.93

Stability: Stability studies were conducted to ensure the reliability of the analytical method under various storage and handling conditions. No instability was observed for either analyte during benchtop stability assessment, within the autosampler during the entire analytical run, or during repeated freezing and thawing cycles. The freeze–thaw stability evaluation, performed over three complete cycles, confirmed that both drugs retained their integrity, with no significant degradation or variability detected. Furthermore, long-term stability studies in human plasma demonstrated that the analytes remained stable when stored under the prescribed conditions for the duration of the study.

In addition to stability, key validation parameters—including linearity, specificity, accuracy, precision, extraction recovery, and overall method robustness—were thoroughly assessed in accordance with regulatory guidelines. The method showed excellent linearity across the tested concentration ranges, high specificity without interference from endogenous plasma components, and consistent recovery of both analytes from the biological matrix. A consolidated summary of all validation outcomes, covering linearity, specificity, accuracy, mean recovery, and the various stability assessments (benchtop, freeze–thaw, and autosampler), is presented in Table 7.

Table 7: Stability Data for Ribociclib and Letrozole.

	RIBO			LETRO		
	LQC	MQC	HQC	LQC	MQC	HQC
Bench Top Stability (%)	5.39	8.97	11.21	5.39	8.97	11.21
Freeze and Thaw Stability (%)	13.28	11.81	5.99	13.28	11.81	5.99
Auto sampler stability (%)	12.26	4.36	2.99	12.26	4.36	2.96

Conclusion

The development and validation of a method for the simultaneous measurement of LETRO and RIBO in human plasma using liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the main focus of this work. RIBO and LETRO are commonly used together in the treatment of hormone receptor-positive, HER2-negative breast cancer. Accurate and reliable quantification of these drugs in plasma is essential for pharmacokinetic studies, therapeutic drug monitoring, and ensuring effective and safe clinical outcomes.

The used method was accurate, reliable, and simple. The isocratic mode, which was used, followed by a short runtime of 3.0 minutes, makes the procedure suitable and economical for the analysis of drugs at the same time. The validation was carried out as per the USFDA guidelines. Henceforth the conclusion depicts the suitability of the method for routine analysis of both the drugs and marketed preparation. The study successfully develops and validates a robust, sensitive, and specific LC-MS/MS method for quantification of RIBO and LETRO in human plasma. The method demonstrates excellent linearity, accuracy, precision, recovery, and stability, adhering to established regulatory criteria. This method can be used in clinical and pharmacokinetic studies, facilitating therapeutic drug monitoring and ensuring optimized treatment regimens for patients undergoing combined RIBO and LETRO therapy. In conclusion, the validated LC-MS/MS method provides a reliable analytical tool for the detection of both the drugs RIBO and LETRO simultaneously in human plasma, contributing to improved patient care in oncology settings.

Conflict of Interest

The authors declare no conflict of interest.

Funding

The authors did not receive any fundings from any private or government sources.

Ethical Approvals

This study does not involve experiments on animals or human subjects.

References

1. Pitt JJ. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. Clin Biochem Rev. 2009;30(1):19-34.
2. Thomas SN, French D, Jannetto PJ, Rappold BA, Clarke WA. Liquid chromatography–tandem mass spectrometry for clinical diagnostics. Nature Reviews Methods Primers. 2022 Dec 8;2(1):96.
3. Tripathy D, Bardia A, Sellers WR. Ribociclib (LEE011): Mechanism of Action and Clinical Impact of This Selective Cyclin-Dependent Kinase 4/6 Inhibitor in Various Solid Tumors. Clin Cancer Res. 2017;23(13):3251-3262.
4. Caciolla J, Bisi A, Belluti F, Rampa A, Gobbi S. Reconsidering Aromatase for Breast Cancer Treatment: New Roles for an Old Target. Molecules. 2020;25(22):5351.
5. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, Vignat J, Gralow JR, Cardoso F, Siesling S, Soerjomataram I. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast. 2022; 66:15-23.
6. Shelke RU, Rishipathak DD. Development and validation of a new bioanalytical method for quantification of CDK4/6 inhibitor in Spiked Human Plasma by HPLC-UV. Current Issues in Pharmacy and Medical Sciences. 2023;36(2):81-6.
7. Bao X, Wu J, Sanai N, Li J. Determination of total and unbound Ribociclib in human plasma and brain tumor tissues using liquid chromatography coupled with tandem mass spectrometry. Journal of pharmaceutical and biomedical analysis. 2019; 166:197-204.
8. Posocco B, Zanchetta M, Orleni M, Gagno S, Montico M, Peruzzi E, Roncato R, Gerratana L, Corsetti S, Puglisi F, Toffoli G. Therapeutic Monitoring of Palbociclib, Ribociclib, Abemaciclib, M2, M20, and Letrozole in Human Plasma: A Novel LC-MS/MS Method. Therapeutic Drug Monitoring. 2022:10-97.
9. Turković L, Bočkor L, Ekpenyong O, Silovski T, Lovrić M, Crnković S, Nigović B, Sertić M. Development and validation of a novel LC-MS/MS method for the simultaneous determination of abemaciclib, palbociclib, ribociclib, anastrozole, letrozole, and fulvestrant in plasma samples: a prerequisite for personalized breast cancer treatment. Pharmaceuticals. 2022;15(5):614.
10. Al-Shehri M, Hefnawy M, Abuelizz H, Alzamil A. Evaluation of the pharmacokinetics of the simultaneous quantification of letrozole and palbociclib in rat plasma by a developed and validated HPLC–PDA. Acta Chromatographica. 2020;32(3):170-8.
11. Dange Y, Bhinge S, Salunkhe V. Optimization and validation of RP-HPLC method for simultaneous estimation of palbociclib and letrozole. Toxicology Mechanisms and Methods. 2018;28(3):187-94.
12. Poetto AS, Posocco B, Gagno S, Orleni M, Zanchetta M, Iacuzzi V, Canil G, Buzzo M, Montico M, Guardascione M, Basile D. A new

dried blood spot LC-MS/MS method for therapeutic drug monitoring of palbociclib, ribociclib, and letrozole in patients with cancer. Journal of Chromatography B. 2021; 1185:122985.

13. Martínez-Chávez A, Rosing H, Hillebrand M, Tibben M, Schinkel AH, Beijnen JH. Development and validation of a bioanalytical method for the quantification of the CDK4/6 inhibitors abemaciclib, palbociclib, and ribociclib in human and mouse matrices using liquid chromatography-tandem mass spectrometry. Analytical and bioanalytical chemistry. 2019; 411:5331-45.
14. Nalanda RB, Rao AS, Sankar DG. Determination of palbociclib in human plasma using high performance liquid chromatography-ultraviolet detection. International journal of pharmaceutical sciences and research. 2018 1;9(9):3883-8.
15. Kala A, Patel YT, Davis A, Stewart CF. Development and validation of LC–MS/MS methods for the measurement of ribociclib, a CDK4/6 inhibitor, in mouse plasma and Ringer's solution and its application to a cerebral microdialysis study. Journal of Chromatography B. 2017 Jul 1; 1057:110-7.
16. European Medicines Agency, Guideline on Bioanalytical Method Validation. 2015. [(accessed on 18 February 2022)]. Available online: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf
17. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, ICH Harmonised Guideline Bioanalytical Method Validation M10. 2019. [(accessed on 18 February 2022)]. Available online: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-ich-guideline-m10-bioanalytical-method-validation-step-2b_en.pdf
18. Mittal A, Imam SS, Parmar S, Gilani S, Taleuzzaman M, Design of Experiment based Optimized RP-HPLC Method for Simultaneous Estimation of Amlodipine and Valsartan in Bulk and Tablet Formulations. Austin J Anal Pharm Chem, 2015, 2 (6),1057.
19. Mittal A, Parmar S, Gilani SJ, Imam SS, Taleuzzaman M, Optimization and Validation for Simultaneous Estimation of Citicoline and Piracetam in bulk and tablet formulations using RP-HPLC method: Analytical quality by design approach, Asian Journal of Research in Chemistry, (2017), 10 (2),198-205.