BIOANALYTICAL METHOD FOR ESTIMATION OF RIBOCICLIB IN HUMAN PLASMA USING ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROSCOPY

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ABSTRACT

To quantify Ribociclib in plasma samples employing Gefitinib as an internal standard (IS), a straightforward, efficient, and selective LC-MS/MS approach was designed. Using an Agilent, Zorbax, XDB C18 (2.1 x 50 mm ID, 5 µm) column, the process of separating was carried out on an isocratic mobile phase entailing acetonitrile (ACN) and 0.1% formic acid in water (70:30) at 0.120 mL/min. In the context of MRM positive mode, ribociclib and Gefitinib were discovered to have parent ions at m/z 435.60 and 447.10, correspondingly, while the daughter masses were determined to be 322.10 and 127.90, correspondingly. The medication and IS were extracted via the protein precipitation (PP) technique. The correlation value (r2) was 0.99934, validating the approach throughout linear concentration levels of 1.0-1000.0 ng/mL. The accuracy spanned between 96.00 - 102.08 and the precision spanned between 0.008 - 8.5%, according to this strategy. Benchtop, postoperative, and long-term stability investigations all revealed that ribociclib was stable.

Keywords: Ribociclib, Gefitinib, Internal standard, Flow rate

INTRODUCTION

The method known as bioanalysis is utilized to find the quantity of medication and its metabolites present in biological matrix including plasma, saliva, serum, CSF, urine, etc. [1]. Bioavailability and bioequivalence investigations need the use of bioanalytical techniques [2]. The use of bioanalytical techniques and validation helps to establish that a validated analysis strategy may be linked to the biochemical pathway [1, 3]. An evaluation of the method's suitability and repeatability for the envisaged applications takes the form of a laboratory test [4]. It is used to evaluate clinical pharmacokinetics, novel drug discovery, quantifiable drug and

metabolite assessment, bioavailability and bioequivalence investigations, research methodologies, and therapeutic drug monitoring (TDM). Technology-leading advancements and alterations are always being made to bioanalytical methods [5].

A cancer-fighting medication called ribociclib (RCB) is administered in conjunction with hormone therapy for managing those having metastatic breast cancer. Ribociclib is an extremely selective orally given CDK4 and CDK6 inhibitor that transiently interacts with cyclin D. Bipartite complex formed by these components phosphorylates essential transcriptional factors and antioncogene, promoting cell growth continuation. Individuals having late-stage breast cancer who are ER-positive/HER2-negative had longer progression-free survival (PFS) while using ribociclib plus letrozole (LET) [6-7]. The USFDA authorized ribociclib in 2017[8–10]. Ribociclib is also known by its chemical name, 7-cyclopentyl-N, N-dimethyl-2-[(5-piperazin-1-ylpyridin-2-yl) amino] pyrrolo[2,3-d] pyrimidine-6-carboxamide (Fig 1). The chemical formula is C23H30N8O. The weight of the molecules is 434.5 g/mol. [11]

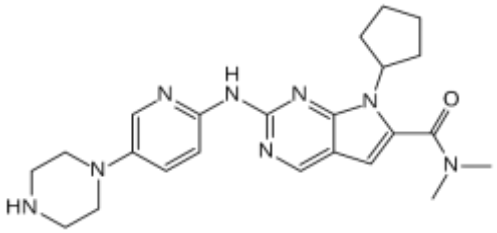

Figure No. 1: Structure of RCB

The USFDA authorized ribociclib in March 2017 [12]. In August 2017, it received EMA (European Medicines Agency) approval. [13] In February 2021, NICE authorized its usage in the NHS. [14] For treating late-stage or metastatic HR-positive, HER2-negative breast cancer, it may be administered in conjunction with an aromatase inhibitor (particularly Letrozole). [15]

Ribociclib substantially increased PFS, or the period during which the tumor did not worsen, in the clinical study related to the drug's licensure. At the time of 2017 analysis, PFS among individuals getting ribociclib with Letrozole was 25 months, as against a median PFS of 16 months for those taking a placebo with Letrozole. [16-17] September 2020 completion date has been set for the investigation. [18-19]

A review of the literature found no approach for estimating the concentration of ribociclib in human plasma employing UPLC and liquid chromatography. According to ICH criteria, the suggested approach was optimized and verified. [20-21].

Experimental study

Solutions and reagents

The APIs for ribociclib and Gefitinib were made available by Supriya Life science, Mumbai. Merck, Mumbai, provided the acetonitrile (ACN) (99.99 % pure), and 5 mM Ammonium Formate acetate buffer containing 0.1% formic acid, in addition to water (Milli Q).

Instrumentation:

A LC-MS/MS procedure was carried out using a liquid chromatographic device designed using a Waters Acquity UPLC system connected to a Waters Quattro Premier XE mass spectrometer featuring electrospray ionization (ESI) employed for detection and Mass Lynx 4.1 SCN 805 software which enabled processing and data collection. As stationary phase, Agilent, Zorbax, XDB C18 2.1 50 mm, 5 µm, was employed.

Preparation of standard and internal samples

Preparation of stock solution

10 mg of pure Ribociclib powder were precisely weighed and added to a volumetric flask. A little over 70 ml of the dilutant was introduced, dissolved using a sonicator for 15 minutes, and afterwards diluted to the required level. The aforementioned solution was subsequently added to a milliliter to a flask and diluted to 100ml.

Preparation of standard stock solution

1 ml of stock solution containing was placed in a flask and diluted to 10 ml.

Preparation of internal standard stock solution

10 milligrams of pure Gefitinib powder were precisely weighed before being added to a volumetric flask. A little over 70 ml of the dilutant was introduced, dissolved using a sonicator for 15 minutes, and afterwards adjusted to the required level. 1 ml of aforementioned solution was subsequently moved to to a flask and diluted to 100ml. Following that, one milliliter of the aforementioned solution was transferred and diluted to the required amount in a 10-milliliter volumetric flask.

Preparation of standard solution

For preparing standard solution, 200 µl of plasma plus 300 µl of ACN were placed in a 2 ml centrifuge tube. Next, 500 µl each of standard stock solution, IS, and dilutants were mixed, and the mixture was refluxed for 10 min. Subsequent centrifugation of these materials was done for half an hour at 5000 rpm. The transparent solution was placed into a vial and loaded into the column after being filtered through a 0.45-µ nylon syringe filter, and deposited.

Bioanalytical method validation

The technique was verified for selectivity, matrix effect, accuracy, and precision, recovery studies, and repeatability and stability of re-injections.

Selectivity

It was performed by assessing the plasma samples from 6 distinct individuals with the aim of checking for interference from unrecognized substances at retention durations of ribociclib and IS,

Matrix effect

It was assessed by contrasting the peak zone fraction in the extracted samples of 6 distinct blank plasma samples with unextracted samples. Six different batches of plasma were employed to conduct the trails, which were run in replicates and had an average accuracy (% CV) of 15%.

Precision and accuracy

These parameters were assessed at a LLOQ, LQC, MQC, and HQC levels by repeated investigation of QC samples (n=6). With the exception of LLOQ, where it ought to be under 20%, CV must be below 15%.

Recovery

It is accomplished through the extraction of the Ribociclib after evaluating the six samples that were reproduced at every internal control dose. Recovery was assessed using a comparison of the height areas of extracted versus unextracted standards.

Carry over

It was carried out by the analyte held by the chromatographic column during the matrix having an analyte level ≥ ULOQC, and its dilution using blank matrix.

Dilution integrity

It can be determined by adding analyte to the matrix beyond the ULOQC and mixing this sample with a blank matrix.

Stability

By contrasting the analyte's area response in stability samples with the specimen made from newly made stock solution, it was possible to determine the stock solution's stability. Plasma was used for the sample stability analyses, which were carried out using 6 repetitions for every level of LQC and HQC concentrations. In accordance with US FDA recommendations, an analysis was deemed stable if the difference was below 15%. After being kept at ambient temperature for 24 hrs, stability of spiked plasma samples was assessed. Samples maintained for 7 days at 7 °C

were used to perform the short-term stability. The concentrations acquired 24 hours later were contrasted to the starting concentration for the purpose of evaluating long-term stability.

RESULTS AND DISCUSSION

Our key goal is to create a straightforward, repeatable procedure that produces excellent peak shapes with minimal baseline noise and rapid recovery. For this, it was required to modify the chromatographic settings and MS parameters in addition to developing a reliable extraction technique that yields predictable and reproducible analyte yield from plasma. By directly injecting solutions of ribociclib into the MS's ESI source, the MS optimization is carried out. A number of crucial variables, including temperature, ionizing mode, voltage, nebulizer gas, heating gas, collision cell exits potential (CXP), Declustering potential (DP), focusing potential (FP), entry potential (EP), collision energy (CE), were improved to achieve greater ionizing to generate protonated ionic Ribociclib and IS molecules. In the context of MRM positive mode, ribociclib and gefitinib were discovered to have parent ions at m/z 435.60 and 447.10, correspondingly, while the daughter masses were determined to be 322.10 and 127.90, correspondingly. (Fig 2–4). To get the greatest resolution and to boost the signal of Ribociclib and Gefitinib, chromatographic parameters, particularly the content and makeup of the mobile phase, were improved via a series of experiments.

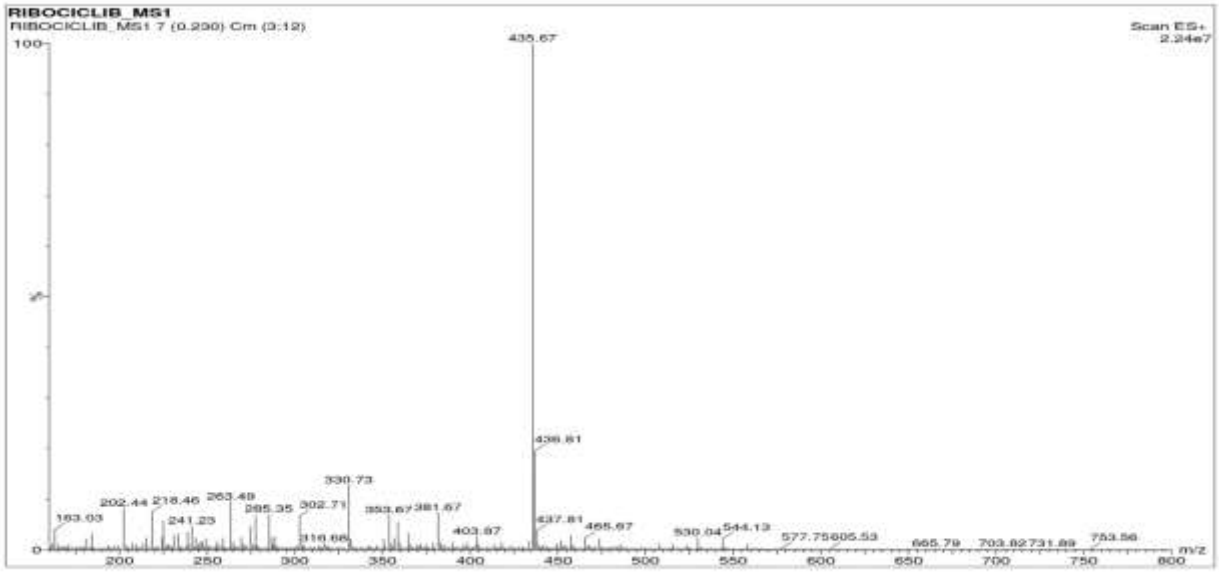

Fig. 2. Mass spectra of Ribociclib (Parent ion: 435.67)

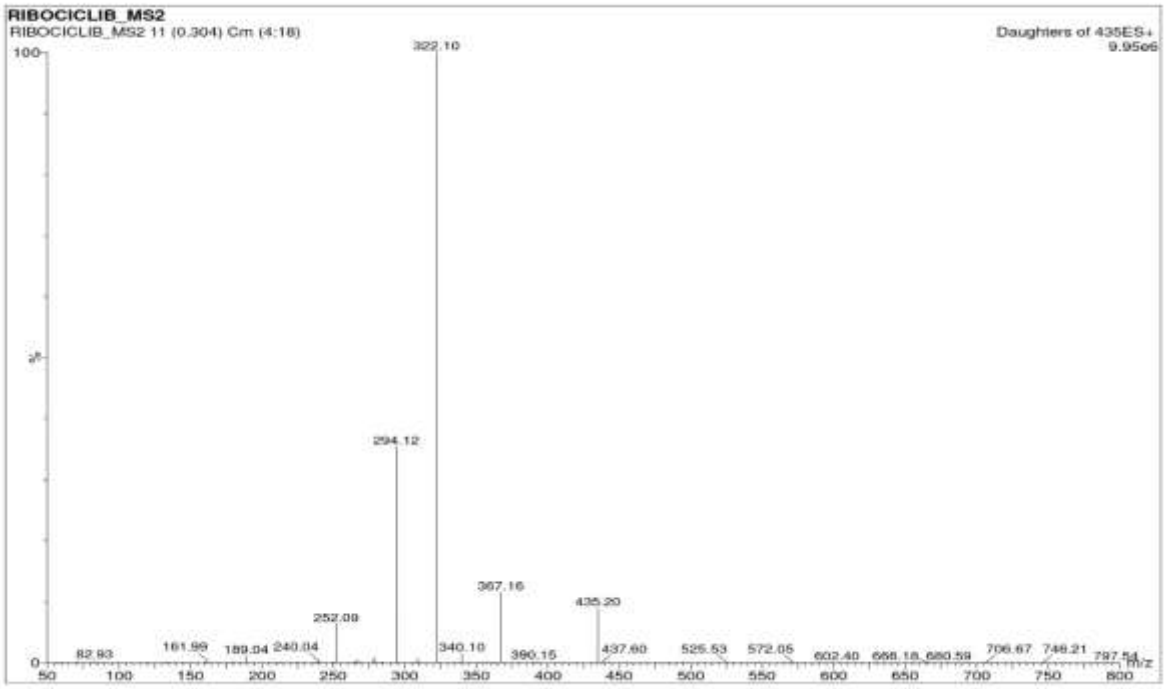

Fig. 3. Mass spectra of Ribociclib (Product ion: 322.10)

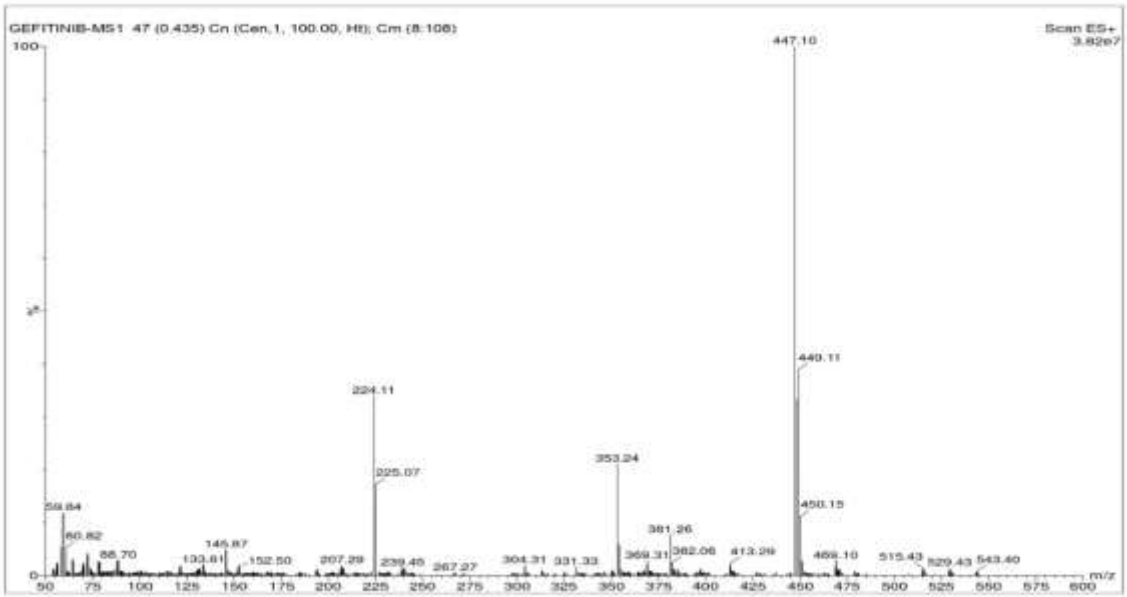

Fig. 4. Mass spectra of Gefitinib (Parent ion: 447.10)

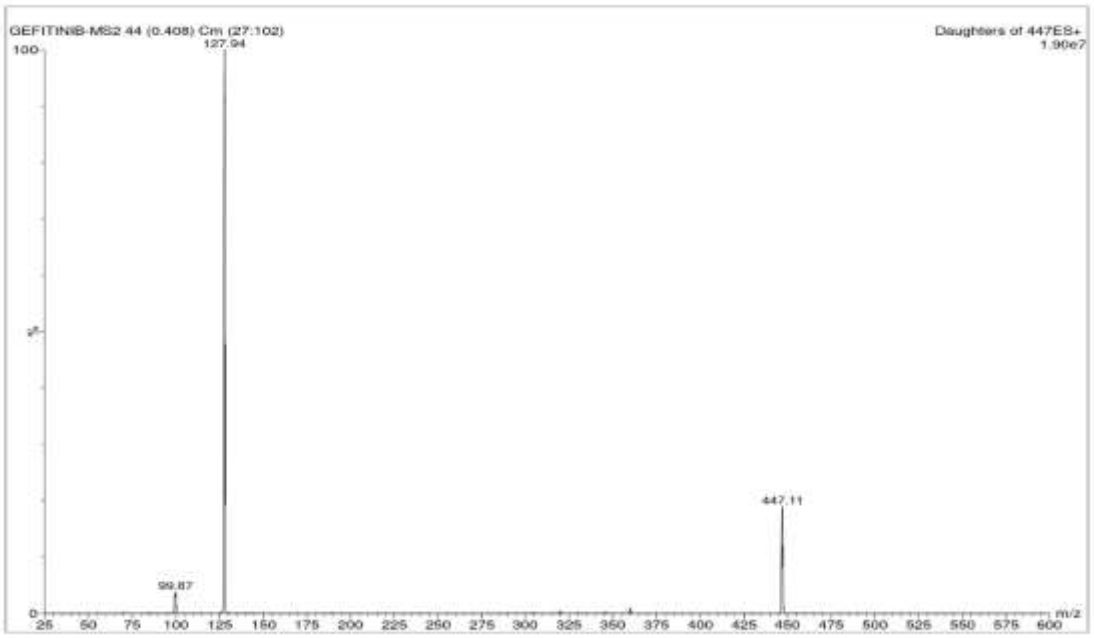

Fig. 5. Mass spectra of Gefitinib (Product ion: 127.94)

We investigated isocratic and gradient modes using various buffers with ACN as the solvent system in varying proportions to obtain the optimal chromatographic settings. For every trial, the mobile phase's makeup was changed to improve resolution and provide respectable retention times. Lastly, the mobile phase entailing ACN and 0.1% formic acid in water at 0.120 ml/min was chosen since it maximizes the responsiveness of the chosen medicines. Using Agilent, Zorbax, XDB C18 (2.1 x 50 mm ID, 5 μm) column, it was possible to obtain Ribociclib peak shapes from several experiments. Positive ion mode atmospheric pressure ESI-MS was used for detection. The RTs of Ribociclib and IS are 2.63 minutes and 2.23 minutes, correspondingly, after implementing the aforementioned parameters. The technique in use has been verified in accordance with USFDA regulations. Figure 6 depicts the typical chromatogram.

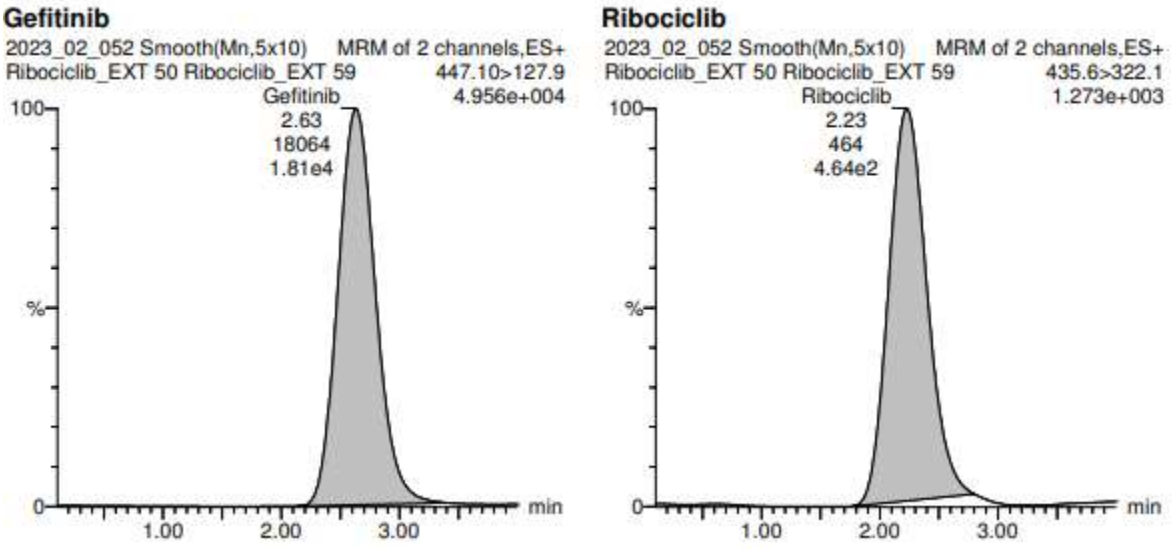

Fig. 6: Chromatogram of standard

Specificity

It is established that the approach used to study ribociclib is specific. The chromatograms for a blank are displayed in Figure 7. It was noticed that neither the standard nor the human plasma chromatograms had any interfering peaks.

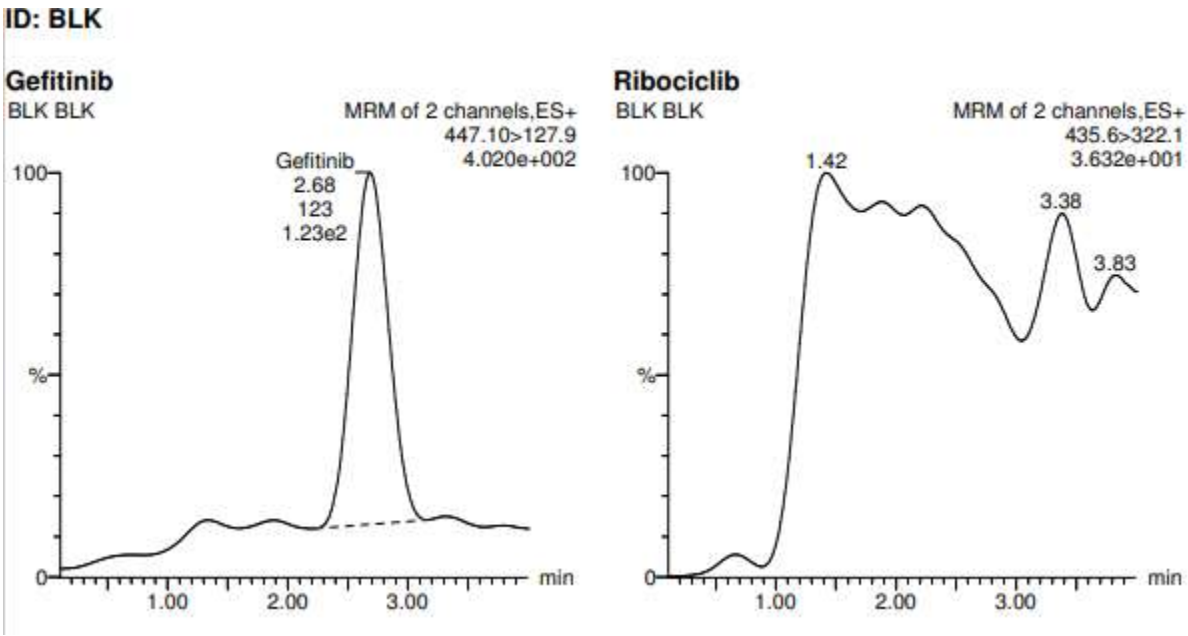

Fig. 6: chromatograms of blank

Matrix effect

Ribociclib had matrix effects at LQC and HQC levels of 99.76 and 103.89%, respectively. Although LQC and HQC values of 0.68 and 0.18, respectively, were discovered for both medications'% CV. The findings show that the internal interferences and matrix effect on analyte ionization were both within established boundaries.

Linearity

The area was under considerable attention when it came to adjustment rules for height ratios. This technique has a linearity range of 1.0-1000 ng/ml (fig. 4). With Ribociclib at various QC levels, the standard curves were visible across the linearity range, and the R2 was determined to be higher than 0.99953. Table 1 displays the linearity and correlation data for ribociclib.

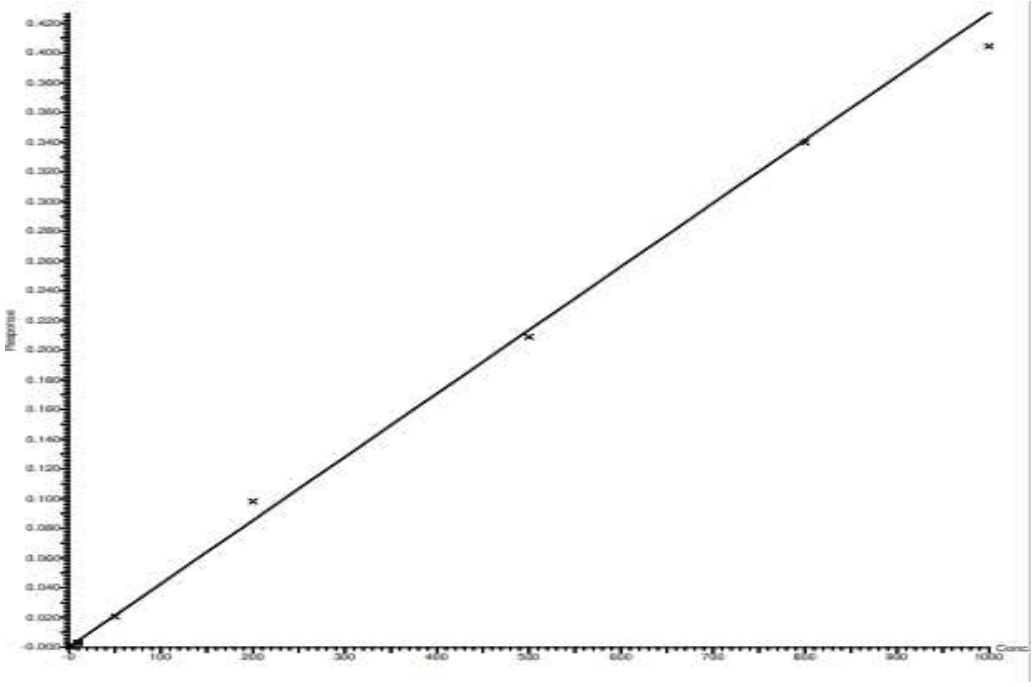

Fig. 7: Calibration plots of Ribociclib

Table 1: Findings of linearity

Linearity	Conc.(ng/ml)	Area response ratio
1	1.0	0.000
2	2.0	0.001
3	10.0	0.003
4	50.00	0.021
5	200.00	0.098
6	500.00	0.209
7	800.00	0.340

8	1000.00	0.405
Intercept		**0.000426968**
CC		**0.993494**

Precision and accuracy

The accuracy and precision were determined by adding together each of the separate test findings from the various QC samples. The statistics presented made it understandable that the approach was precise and efficient. Table 2 displays the Ribociclib precision outcomes. QC samples for ribociclib provide accuracy values between 96.0 and 102.0.

Table 2: Precision and accuracy of RCB

QC name	LLQC	LQC	MQC	HQC
Conc.(ng/ml)	**1.100**	**2.200**	**480.00**	**780.00**
1	0.967	2.185	490.58	779.54
2	0.959	2.206	487.24	769.24
3	1.088	2.198	481.54	789.99
4	1.203	2.187	485.22	785.72
5	1.123	2.210	495.54	782.22
6	0.985	2.007	499.25	788.54
Mean	**1.054**	**2.165**	**489.895**	**782.541**
SD	**0.090**	**0.071**	**6.035**	**6.923**
% CV	**8.5**	**3.27**	**1.23**	**0.008**
Accuracy	**96.00**	**98.37**	**102.08**	**100.15**

Recovery

The findings showed that the bio-analytical approach had high extraction yield based on the recovery for ribociclib at three concentration (LQC, MQC, and HQC) levels. Furthermore, this signified that recovery was unrelated to drug quantity. The recovery values varied between 98.83% through 103.85% at LQC, MQC, HQC levels, and% CV spanned between 0.004 and 2.28 for ribociclib.

Ruggedness

In samples from the HQC, LQC, MQC, and LLQC, the % recovery and % CV of ribociclib as measured by two analyzers and using two dissimilar columns were within allowable limits. Results shown that approach is robust. With ribociclib, the % recovery varied between 95.23 - 104.19%, while the % CV values varied from 0.015 to 3.98.

Auto sampler carryover

Following sequential loadings of LLQC and ULQC at the RTs of RCB and LET, peak area responses of these drugs were not seen in the blank rabbit plasma samples. This approach fails to display auto sampler carryover in terms of sampling.

Stability

A stock solution was made and maintained at ambient temperature for 18 days to study the benchtop stability of the drug. The stock solution was then placed in a freezer for 28 days to study long-term stability before being injected into the column. The short-term stability of the medication was shown to be strong when kept at 5°C for 7 days. Comparing newly prepared stock solutions with those prepared earlier than 24 hours will reveal which solution is more stable. This led us to notice that the percentage change for ribociclib was, correspondingly, 1.18% and 0.88%, indicating that solutions remain stable for as long as 24 hours. Ribociclib was stable in plasma under various circumstances at room temperature. It was determined that the amounts of LQC, MQC, and HQC did not affect the stability of plasma samples that had been spiked with ribociclib while they continued to freeze and thaw. Ribociclib remained stable at a potential temperature of -30 °C for approximately 24 h, as was evident by long-term stability. Table 3 displayed the Ribociclib findings for overall stability.

Table 3: Stability findings of RCB

Stability experiment spiked plasm	Quality control	**Mean conc. (n=6,ng/ml)**	**Std dev (n=6,ng/ml)**	**%CV**
Bench top stability	LQC	2.225	0.026	1.16
	HQC	795.21	7.521	0.94
Stock solution stability	LQC	2.193	0.026	1.18
	HQC	785.32	6.923	0.88
Long term stability	LQC	2.232	0.022	0.98
	HQC	788.81	7.626	0.96

CONCLUSION:

For the purpose of quantifying ribociclib in plasma samples with the Protein Precipitation technique, an LC-MS/MS approach was designed. The approach is quick, quick, selected, specialized, accurate, precise, and economical. By employing the LC-MS/MS technology, this technique may be utilized for routinely analyzing the presence of Ribociclib in plasma samples.

CONFLICTS OF INTEREST: Nil.

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