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Research Article

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Estimation of Ribociclib in Human Plasma Samples by LC-ESI-MS/MS

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ABSTRACT

A validated Ribociclib bioanalytical method was developed by HPLC-ESI-MS/MS using stationary phase Zorbax SB-C18, 4.6 × 75 mm, 3.5 µm, 80 Å column and mobile phase was used 5mM ammonium formate: acetonitirle (10:90 v/v). The Ribociclib and Ribociclib-D6 were identified using ESI mode at m/z 435.5 /112.1 and Ribociclib-D6 (IS) at 441.7/112.1. The correlation coefficient is ≥ 0.998 with linearity range 50.00-10000.00 pg/mL.

Keywords: HPLC-ESI-MS/MS; Ribociclib; Human plasma

INTRODUCTION

Ribociclib is cyclin-dependent kinase (CDK) 4 and 6 inhibitor. The molecular formula is C23H30N8O·C4H6O4 and with molecular weight 552.64 g/mol. The IUPAC name is Butanedioic acid—7-cyclopentyl-N,N-dimethyl-2-{(5-(piperazin-1-yl) pyridin-2-yl)amino}-7H-pyrrolo(2,3-d)pyrimidine-6-carboxamide [1-18]. Based on the literature survey as per best of our knowledge very few methods were reported on pharmacokinetics of Ribociclib [1-5] and none of the method was reported on estimation of Ribociclib in biological samples by HPLC-MS/MS. The main objective of this work is to develop and validate Ribociclib in biological samples (plasma) by HPLC-ESI-MS/MS using deuterated internal standard.

MATERIALS AND METHODS

Chemicals and Reagents

Reference standard of Ribociclib (RC) (purity, 99.5%) and Ribociclib-D6 is gift sample of Symed labs (Figure 1). All solvents were HPLC grade (Purchased from JT baker) like extraction solvent (Tertiary butyl methyl ether-TBME) and methanol and acetonitirle. Buffer salts were used AR-grade (purchased from Merck, Mumbai) like KH_2PO_4 and NH_4HCO_2.

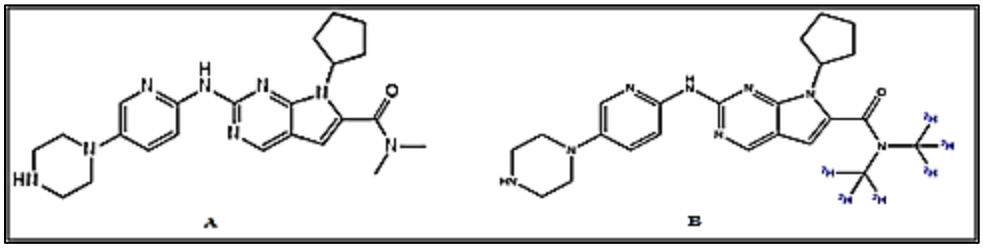

Figure 1: Chemical structures of A) Ribociclib (RC) B) Ribociclib-D6 (RCD6)

Instrumentation

The ABI-SCIEX, Toronto, Canada, HPLC-MS/MS and Analyst 1.4.1 software was used to detect and estimate mass ions *m*/*z* 435.5/112.1 and *m*/z 441.7/112.10 Ribociclib and Ribociclib-D6 in biological matrix samples (Figures 2 and 3).

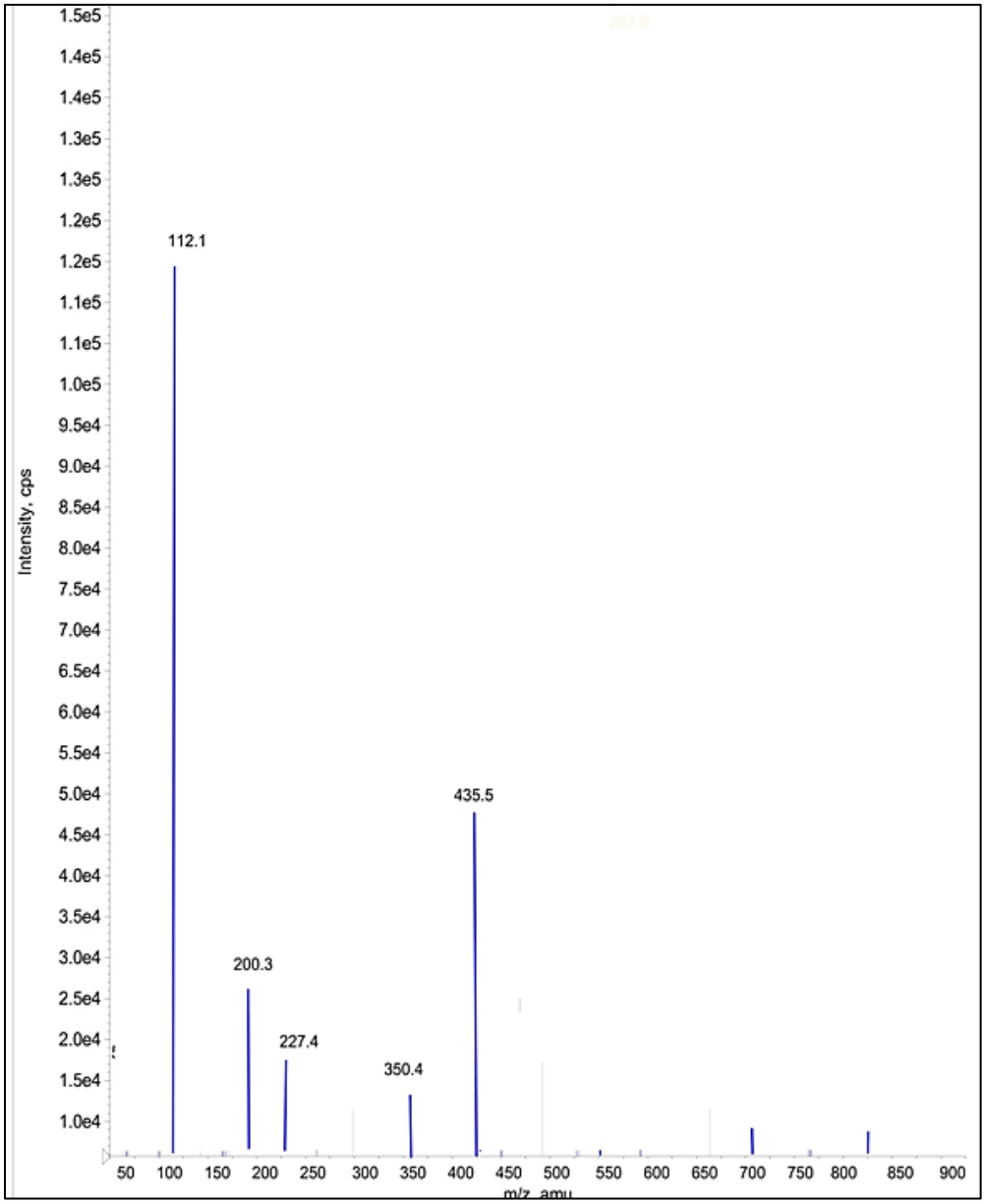

Figure 2: Mass spectrum of Ribociclib (RC) Q1 and Q3 ions

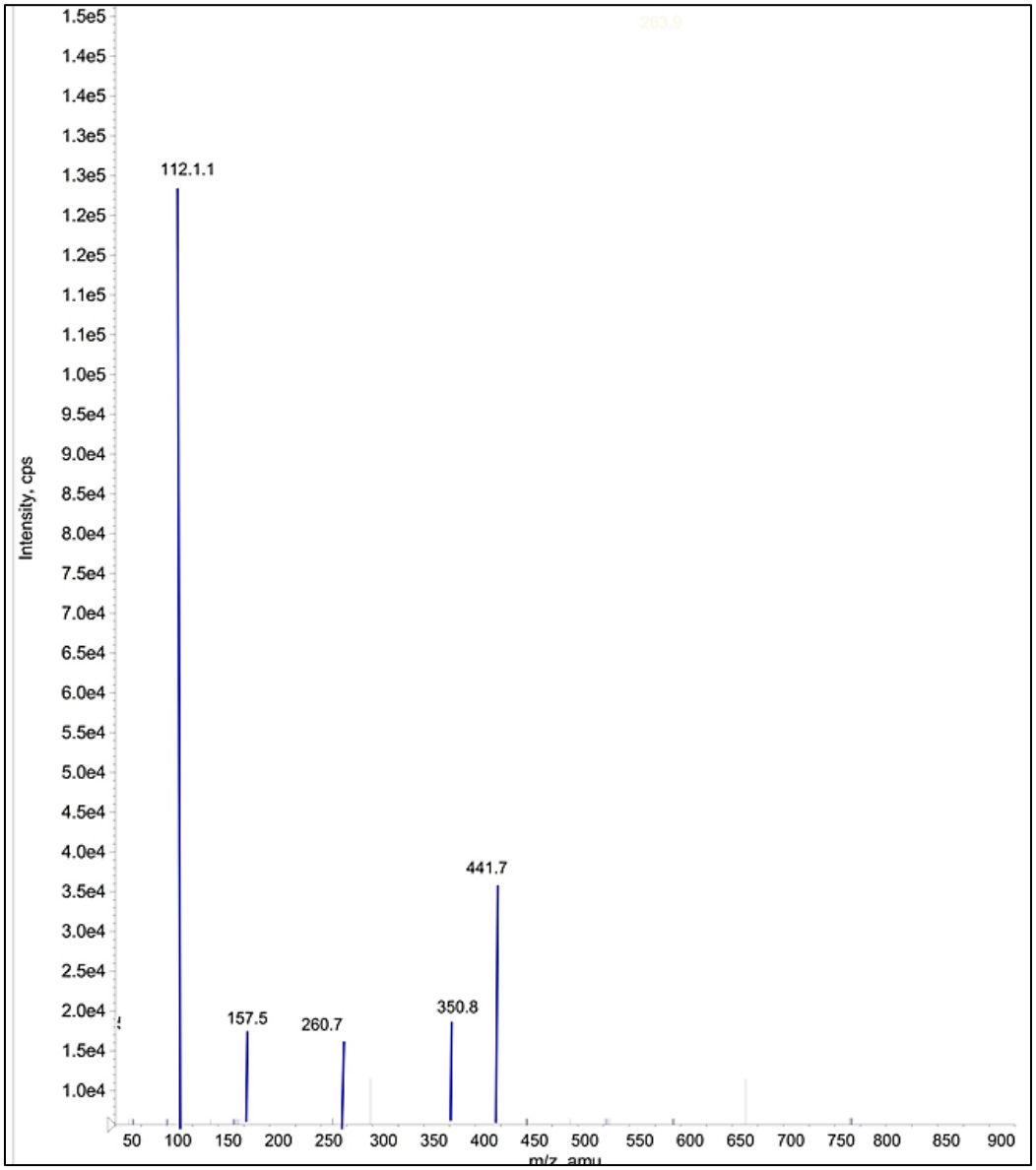

Figure 3: Mass spectrum of RibociclibD6 (RCD6) Q1 and Q3 ions

Chromatographic Conditions

For chromatographic resolution 5 mM ammonium formate: acetonitirle (10:90 v/v) was used as mobile phase and analytical column Zorbax SB-C18, 4.6 × 75 mm, 3.5 µm 80 Å. The total analysis time was 13 min and flow rate was set to 0.6 mL/min. The temperature was set to 40°C for the column oven. The retention times for Ribociclib and Ribociclib-D6 was found to be 9.2 and 8.2.

Preparation of Calibration Curve Standards and Quality Control Samples

Calibration curve (50.0, 100.0, 500.0, 1000.0, 2000.0, 4000.0, 6000.0, 8000.0 and 10000.0 pg/mL) and quality control standards (50.0, 150.0, 3000.0 and 8000.0 pg/mL-LLOQ, LQC, MQC and HQC) were prepared in biological matrix.

Sample Preparation

To each Ribociclib spiked plasma samples like calibration and quality control standard samples (100 µL) fixed concentration 30 ng/ml of Ribociclib-D6 (50 µL) internal standard was added and extracted with 2.5 ml of TBME along with buffer solution (100 µL of 10 mM KH_2PO4). After completion of extraction process samples were

centrifuged and supernatant was evaporated at 40°C. Finally, dried residues were dissolved in mobile phase and injected to HPLC-MS/MS.

Bioanalytical Method Validation

The method was validated according to US food and drug administration bioanalytical method validation guidelines includes system suitability, selectivity and specificity, LOQ (limit of quantification or sensitivity), injector carryover, linearity, precision and accuracy (P&A; Intraday and Inter day), recovery, matrix effect, dilution integrity, re-injection reproducibility, ruggedness (analyst and column), sample stability studies include auto sampler, freeze-thaw, bench top, long term in plasma and short term stock solution stabilities were carried out during method validation [19] .

Selectivity and Specificity

Six different lots of blank biological matrices (plasma) were extracted and analysed by HPLC-MS/MS to qualify the level of matrix interference for each lot of sample.

Precision and Accuracy

Six replicates of quality control samples like LQC (low quality control), MQC (medium quality control) and HQC (high quality control) were in biological matrix (plasma) and quantified the level precision.

Matrix Effect

Three equal concentrations of MQC samples were prepared in different lots of biological matrices (plasma) to measure matrix interferences.

Recovery

The recovery was performed by using drug spiked quality control plasma samples like LQC and HQC and the percent recovery was calculated.

Stability Studies

Spiked quality control plasma samples (LQC and HQC) were prepared in six replicates and different stability parameters were evaluated, like, bench top stability (48 h) and autosampler stability (20°C for 55.5 h), reinjection reproducibility (20°C for 27 h) The stability of spiked human plasma samples stored at room temperature (bench top stability) and freeze-thaw stability (at -30°C) and long term stability (71 days) was evaluated for 48 h.

RESULTS AND DISCUSSION

Different mass parameters were optimized like DP, EP, FP, CE and CXP to quantify the drug concentration in plasma samples. After series trials, chromatographic conditions were achieved with 5 mM ammonium formate: acetonitirle (10:90 v/v) as the mobile phase, at a flow-rate of 0.6 mL/minutes. Whereas, high extraction efficiency was achieved with TBME extraction solvent (Figure 4). From the results it was observed that chromatograms were free from matrix interferences and linearity was achieved with concentration range of 50.0-10000.0 pg/mL (Tables 1-3). Intraday day precision (1.2 to 4.5 and 91.7 to 105.5%) and inter day precision (3.5 to 7.4 and 103.9 to 110.6%) values shows developed method was precise and accurate matrix effect results (% CV 1.27) indicating samples were free from interferences and %recovery 99.6 ± 3.53, 88.2 ± 2.7 and 97.60 ± 4.7% for LQC, MQC and HQC values shows method was highly efficient to extract drug from biological samples. Limit of quantification and limit of detection was found to be 31.95 pg/ml and 10.5 pg/ml. Stability of drug and internal standard in biological samples was evaluated with Freeze - thaw, Auto sampler, Room temperature, Long term studies and found to be stable at various environmental conditions.

Table 1: Calibration curve details of Ribociclib

Spiked plasma concentration (pg/mL)	Concentration measured(mean) (pg/mL) (n = 5)	Precision RSD. (n = 5)	Accuracy%
50	51.0 ± 1.3	2.5	102
100	96.6 ± 4.7	4.9	96.6
500	498.4 ± 24.7	5	99.68
1000	1000.0 ± 17.1	1.7	100
2000	2013.0 ± 74.6	3.7	100.65
4000	4008.4 ± 206.6	5.2	100.21
6000	5956.5 ± 190.7	3.2	99.28
8000	7952.2 ± 165.6	2.1	99.4
10000	10317.1 ± 487.6	4.7	103.17

Table 2: Precision and accuracy (analysis with spiked plasma samples at three different concentrations)

Spiked plasma concentration (pg/mL)	Within-run (n=6)			Between-run (n=6)		
	Concentration measured (pg/mL) (mean ± S.D.)	Precision RSD	Accuracy%	Concentration measured (pg/mL) (mean ± S.D.)	Precision RSD	Accuracy%
50	51.4 ± 2.3	4.5	102.7	55.5 ± 4.1	7.4	110.6
150	154.9 ± 3.4	2.2	105.5	157.9 ± 5.7	3.6	105.2
3000	3103.8 ± 102.0	3.3	103.2	3133.0 ± 108.2	3.5	104.3
8000	7297.1 ± 89.9	1.2	91.7	7278.7 ± 275.5	3.8	103.9

Table 3: Stability of Ribociclib in human plasma samples

Stability experiments	Storage condition	Spiked plasma concentration (pg/ml)	Concentration measured (n=6) Mean ± SD	RSD (n=6) (%)	Accuracy (%)
Bench top in plasma	RT 48 hr	150	148.3 ± 8.1	5.5	98.9
		8000	6728.3 ± 206.3	3.1	81.5
Processed (extracted sample)	Autosampler 55.5 hr	150	162.3 ± 2.4	1.5	108.2
		8000	7536.7 ± 294.5	3.9	90.4
Freeze/Thaw stability	-30°C Cycle-3	150	156.5 ± 4.0	2.5	104.3
		8000	7381.7 ± 173.4	2.3	90.4
Long-term stability in human plasma	- 30°C 71 days	50	160.3 ± 13.2	8.2	106.9
		8000	7450.0 ± 229.1	3.1	90.5

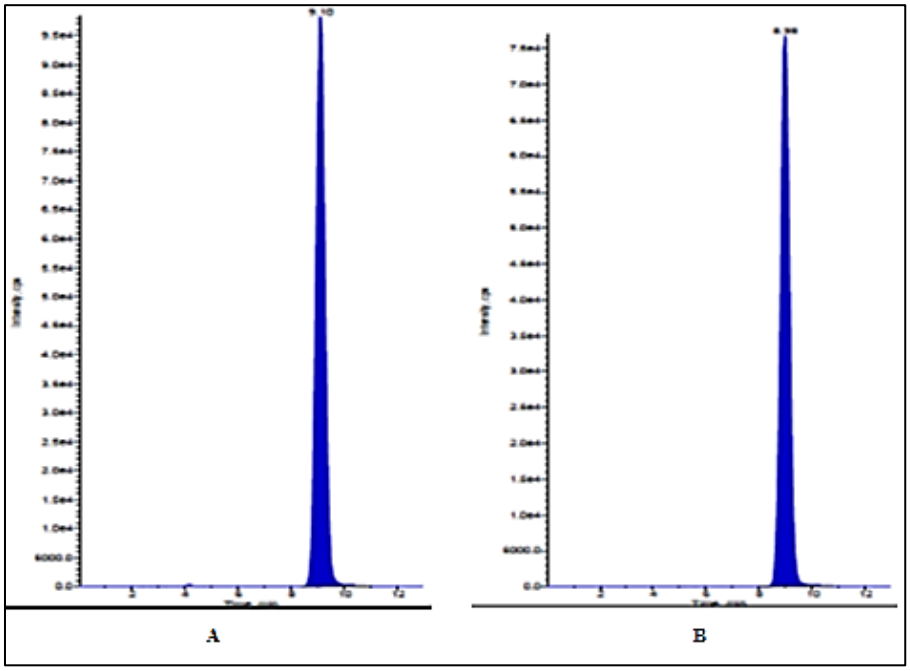

Figure 4: Chromatograms of A) Ribociclib (RC) B) Ribociclib-D6 (RCD6)

CONCLUSION

From the results it was concluded that developed method was Selective, linear, precise and accurate, sensitive, reproducible and stable in biological matrices. Hence, the optimized can suitable for routine quality control analysis for estimation of Ribociclib in biological samples by HPLC-MS/MS.

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