RESEARCH ARTICLE

Novel Quantitative Estimation of Rucaparib and Bevacizumab in Rat Plasma Using LC-MS/MS & Study of Pharmacokinetics

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ABSTRACT

Rucaparib and Bevacizumab's bioanalytical evaluation was performed using an easy-to-use, effective, and repeatable LC-MS/MS technique, with D6-Rucaparib and D6-Bevacizumab serving as internal standards. This work provides a summary of current developments in bioanalytical LC-MS/MS techniques using an organic mobile phase consisting of 50:50 formic acid and acetonitrile IN 0.1% as well as a Waters Symmetry C18 column. The results for stability, matrix effect, accuracy, precision, and recovery were all within allowable bounds. To test the targeted analytes in body fluids using pharmacokinetic research, a simple and efficient approach was created. This application included all accuracy, system appropriateness, linearity, and specificity characteristics that were used successfully for pharmacokinetic investigations in rats and that complied well with USFDA guidelines.

Keywords: Rucaparib, Rat plasma, Bevacizumab, USFDA guidelines LC-MS/MS.

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INTRODUCTION

Rucaparib, marketed as the brand Rebraca, functions as a PARP inhibitor[1,2] and is utilized as an anti-cancer agent. It is administered orally in tablet form. Common adverse effects may include fatigue,[3] nausea, elevated levels of creatinine[4,5] (indicating potential kidney issues), elevated liver enzymes (indicative of potential liver damage), vomiting, anemia, decreased appetite, dysgeusia[6,7] (alterations in taste perception), diarrhea, thrombocytopenia[8] (reduced platelet levels), and abdominal pain.

Rucaparib is used in the treatment of adults with epithelial ovarian,[9,10] fallopian tube,[11] or peritoneal cancer[12] and in chemotherapy.[13]

Bevacizumab, commercially known as Avastin among other brands, is a medication employed in the treatment of various cancers and a specific eye condition. When used to combat cancer, it is administered through slow intravenous injection and is indicated for colon cancer,[14,15] lung cancer,[16] glioblastoma,[17,18] and renal-cell carcinoma.[19] In many instances, it serves as a first-line therapy for these conditions. For age-related macular degeneration, it is administered via intravitreal injection directly into the eye.

Specifically, bevacizumab inhibits angiogenesis[20] and functions as anti-VEGF therapy by inhibiting VEGF-A (vascular endothelial growth factor A)[21] and thereby preventing new blood vessels formation.[22]

METHODS AND MATERIALS

Reagents and Chemicals

Acetonitrile, formic acid, HPLC grade water procured from the Merck, Worli, India. All the reference standards of Rucaparib and Bevacizumab APIs were procured from the Glenmark Pharmaceuticals Pvt Ltd. in Hyderabad, India.

Equipment

The analysis was conducted using a Waters Alliance e2695 model HPLC system coupled with triple quadruple mass spectrometer (QTRAP 5500; Sciex-software). Tandem mass spectrometry has been acknowledged as a primary methodology for the precise analysis of endogenous steroid hormones in biological samples.

Study of Pharmacokinetics

Selection of animals

Six healthy rats (250–300 g) were utilized for in vivo pharmacokinetic investigations, procured from Hyderabad, India and approval obtained from the animal ethics committee.

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Chromatographic conditions

Separation by chromatography was carried out at the room temperature using symmetric C18 columns. The mobile phase was conducted and comprised a 50:50 v/v combination of formic acid and acetonitrile. The runtime was 6 minutes, and volume was 10 µL.

Preparation of Standard Stock Solutions

Preparation of rucaparib parent stock solution

A quantity of 20 mg of rucaparib working standard was dissolved in 70 mL of diluent and diluted to 100 mL. The flask was sonicated for 10 minutes for complete dissolution, followed by dilution to a volume with diluent. A portion of 0.1 mL of this diluted to 10 mL.

Preparation of bevacizumab parent stock solution

About 5 mg of bevacizumab working standard was dissolved in 70 mL of diluent and sonicated for 10 minutes, followed by addition of diluent to reach the desired concentration. Subsequently, 0.5 mL of this diluted to 100 mL.

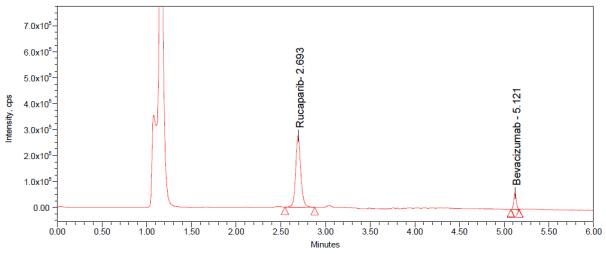

Figure. 1: Blank sample

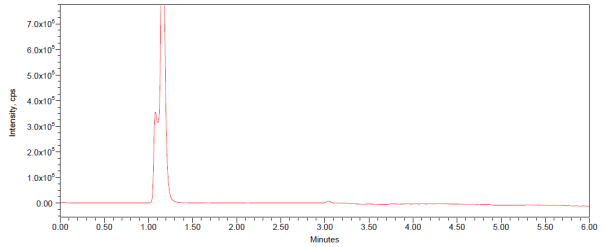

Figure. 2: Standard sample

Table 1: Linearity result

Linearity	*Rucaparib*		*Bevacizumab*	
	Concentration (ng/mL)	*Ratio of area response*	*Concentration (ng/mL)*	*Ratio of area response*
1	20.00	0.367	2.50	0.074
2	50.00	0.687	6.25	0.164
3	100.00	1.527	12.50	0.322
4	150.00	2.108	18.75	0.526
5	200.00	2.834	25.00	0.684
6	250.00	3.571	31.25	0.897
7	300.00	4.187	37.50	1.045
8	400.00	5.642	50.00	1.465
Slope:		0.0048		0.0366
Intercept:		0.02952		0.00580
CC:		0.99930		0.99924

Table 2: Accuracy, precision: Rucaparib

Quality Control Name	*LLQC*	*LQC*	*MQC*	*HQC*
Concentration (ng/ml)	20 ng/ml	100 ng/ml	200 ng/ml	300 ng/ml
Quality Conrol sample -1	20.152	100.132	200.185	300.143
QC sample -2	20.317	100.648	200.116	300.125
QC sample -3	20.543	100.322	200.124	300.231
QC sample -4	20.452	100.485	200.204	300.317
QC sample -5	20.143	100.819	200.557	300.433
QC sample -6	20.984	100.563	200.629	300.547
Mean	20.432	100.495	200.303	300.299
SD	0.314	0.243	0.229	0.167
%CV	1.54	0.24	0.11	0.06
Accuracy	97.89	99.51	99.9	99.91

Mean ± SD (n=6)

Table 3: Accuracy, precision: Bevacizumab

Quality contol name	*LLQC*	*LQC*	*MQC*	*HQC*
Concentration (ng/mL)	2.5	12.5	25	37.5
Quality Ccontrol sample -1	2.527	12.542	25.157	37.542
QC sample -2	2.515	12.517	25.214	37.517
QC sample -3	2.535	12.543	25.263	37.655
QC sample -4	2.562	12.522	25.154	37.732
QC sample -5	2.538	12.569	25.115	37.541
QC sample -6	2.552	12.514	25.346	37.567
Mean	2.538	12.535	25.208	37.592
Stddev	0.017	0.021	0.085	0.084
%CV	0.67	0.17	0.34	0.22
Accuracy%	98.50	99.72	99.17	99.76

Mean ± SD (n = 6)

Preparation of rucaparib and bevacizumab stock solution

A 4 mL each of the parent stock solutions of bevacizumab and rucaparib were transferred to a 10 mL flask and diluted to appropriate concentration. A stock solution was prepared in a similar manner.

Bioanalytical Method Validation

The development of a bioanalytical method involves designing, establishing operating conditions, and identifying limitations to ensure its suitability for the intended purpose and optimization for validation.[23,24] The technique was assessed[19-21] in accordance with USFDA guidelines.[25]

Serum Concentration-Time Curves

The serum concentration-time profiles of CIP were constructed using measured serum concentrations at various time intervals. Pharmacokinetic parameters such as were determined from these curves utilizing PK-software.[26]

RESULTS AND DISCUSSION

By using electrospray ionization, the best response has been obtained. Rucaparib and Bevacizumab showed enhanced positive ion mode responsiveness at a mobile phase flow rate of 10 µL/min, which guaranteed signal stability and sensitivity.

Table 4: The Stability assessment result: Rucaparib

Stability experiment spiked plasma		*Spiked plasma Concentration (n = 6 ng/mL)*	*Concentration measured (n = 6 ng/mL)*	*%CV*
Stability of Bench top	LQC	100	100.352	1.21
	MQC	200	200.748	0.54
	HQC	300	300.685	0.74
Stability of Auto sampler	LQC	100	100.427	0.53
	MQC	200	200.184	0.27
	HQC	300	300.065	0.45
Stability of Long term (Day28)	LQC	100	100.215	0.41
	MQC	200	200.325	0.79
	HQC	300	300.547	0.82
Stability of Wet extract	LQC	100	100.554	0.51
	MQC	200	200.254	0.74
	HQC	300	300.315	0.36
Stability of Dry extract	LQC	100	100.285	1.53
	MQC	200	200.463	0.87
	HQC	300	300.587	0.96
Freeze thaw stability	LQC	100	100.158	0.24
	MQC	200	200.325	0.15
	HQC	300	300.457	0.78
Short term stability	LQC	100	100.526	1.02
	MQC	200	200.214	1.52
	HQC	300	300.321	1.11

Mean ± SD (n = 6)

Table 5: The stability assessment results: bevacizumab

Stability experiment spiked plasma		*Spiked plasma Concentration (n = 6,ng/mL)*	*Concentration measured (n = 6,ng/mL)*	*%CV*
Stability of Bench top	LQC	12.5	12.248	1.25
	MQC	25	25.136	0.65
	HQC	37.5	37.247	0.14
Stability of Auto sampler	LQC	12.5	12.548	0.51
	MQC	25	25.163	0.63
	HQC	37.5	37.247	0.87
Stability of Long term (Day 28)	LQC	12.5	12.524	0.92
	MQC	25	25.531	0.53
	HQC	37.5	37.214	0.87
Wet extract stability	LQC	12.5	12.325	0.42
	MQC	25	25.487	0.53
	HQC	37.5	37.596	0.27
Dry extract stability	LQC	12.5	12.263	1.45
	MQC	25	25.147	1.37
	HQC	37.5	37.285	0.78
Freeze thaw stability	LQC	12.5	12.432	0.64
	MQC	25	25.125	1.32
	HQC	37.5	37.056	1.24
Short term stability	LQC	12.5	12.284	0.44
	MQC	25	25.495	0.63
	HQC	37.5	37.325	1.47

Mean ± SD (n = 6)

Specificity

The specificity of the approach is established for the simultaneous detection of bevacizumab and rucaparib. Figures 1 and 2 showed the samples of standard and blank chromatograms that were analyzed. The absence of interference peaks in the chromatograms of the standard solutions and blank rat plasma was noteworthy and confirmed the specificity of the procedure.

Matrix Effect

The goal of the matrix effect assessment was to determine how various plasma lots affected the back-calculated values of the QC normal concentrations.[27,28] For bevacizumab and rucaparib in LC-MS/MS, the percent relative standard deviation (%RSD) for the signal within the matrix indicated ion suppression/enhancement of 1.0%. This implies that the influence of matrix stays within an acceptable range. Rucaparib's showed a matrix impact of 99.7 and 99.2%, respectively, while bevacizumab's samples showed a matrix effect of 99.5 and 99.3%. The impact of matrix on analyte was shown by the %CV for both medications at the LQC level, which was 1.24 and 1.15%, and at the HQC level, which was 0.56 and 1.03%, respectively.

Table 6: Assessment of pharmacokinetics: Rucaparib and bevacizumab

Pharmacokinetic parameters	*Rucaparib*	*Bevacizumab*
AUC_{0-t}	983 ng-hr/mL	890 ng-hr/mL
C_{max}	185.3 ng/mL	93.9 ng/mL
$AUC_{0-\infty}$	9832 ng-hr/mL	890 ng-hr/mL
t_{max}	1.5 hours	3 hours
$T_{1/2}$	16 hours	20 hour

Linearity

The relationship between the peak area and the ratio of calibration standards and their concentrations exhibited proportionality. Rucaparib covered a concentration limit of 20 to 400 ng/mL, while bevacizumab ranged from 2.5 to 50 ng/mL. Detailed linearity outcomes for rucaparib and bevacizumab can be found in Table 1, with their respective plots illustrated in Figure 3. These curves displayed linearity, characterized by correlation coefficients of 0.999 for both rucaparib and bevacizumab.

Precision and Accuracy

Accuracy and precision were assessed by compiling individual assay results obtained from a range of internal control sample. Precision in the analytical method reflects the degree of agreement (or dispersion) between multiple measurements obtained under specified conditions from homogeneous samples. Precision results for rucaparib and bevacizumab are given in Tables 2 and 3. Accuracy of rucaparib in quality control samples, ranged from 97.89 to 99.91%, while for bevacizumab, it ranged from 98.5 to 99.76%. The variation coefficient (CV) for both rucaparib and bevacizumab was less than 5%.

Recovery

The recovery results for rucaparib and bevacizumab at different levels (LQC, MQC, and HQC) indicated excellent extraction efficiency in the bioanalytical method, independent of concentration. The recoveries for rucaparib ranged from 99.24 to 100.35%, and for bevacizumab, they ranged from 99.08 to 100.11%. The %CV ranged from 0.54 to 0.97% for rucaparib and from 0.23 to 1.05% for bevacizumab, demonstrating good extraction efficiency.

Assessment of Ruggedness

To evaluate the robustness of both the chromatographic method and the extraction procedure, quality control samples and calibration standards were analyzed using a different column of the same type, operated by a separate analyst. The percentage recovery and %CV of rucaparib and bevacizumab obtained in acceptable limits across various concentration levels, confirming the robustness of the method. Percent recoveries ranged from 99.34 to 100.48% for rucaparib and from 99.15 to 99.65% for bevacizumab. The %CV values ranged from 0.18 to 0.72% for rucaparib and from 0.34 to 1.68% for bevacizumab, indicating the method's ruggedness.

Autosampler Carryover

Carryover testing was conducted and showed that peak area responses of rucaparib and bevacizumab were not detected in blank samples after injections of low and ultra-low quality control samples at the retention times of rucaparib and bevacizumab, indicating the absence of autosampler carryover in this method.

Stability Assessment

For stability analysis, solutions of rucaparib and bevacizumab were prepared using appropriate diluents. Comparisons have been done between freshly prepared stock solutions and those prepared 24 hours prior. Stability assessments were conducted for benchtop and autosampler plasma conditions, demonstrating consistent results over a 24-hour period at room the temperature and 20°C in autosampler. Additionally, further testing confirmed the stability of rucaparib and bevacizumab when stored at -30°C for up to 24 hours. Detailed stability findings for both compounds are outlined in Tables 4 and 5.

In-vivo Pharmacokinetic Assessment

Figure 4 showed concentration-time patterns of bevacizumab and rucaparib in rats. A curve may be seen in experimental compositions. Following oral dosing, rutapib and bevacizumab were detectable in the bloodstream for 16 and 20 hours, respectively, suggesting an effective release from formulation. Table 6 provides further information on pharmacokinetic parameters that were calculated.

CONCLUSION

A novel HPLC-ESI-LCMS/MS technique was innovated and verified with high sensitivity for detecting rucaparib and bevacizumab in rat plasma. This approach demonstrates robustness, speed, and reproducibility suitable for bioanalytical purposes. Validation of this method was conducted following USFDA guidelines. It offers a straightforward and effective means to assess the specified analytes in bodily fluids, particularly useful for pharmacokinetic investigations.

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