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BIOANALYTICAL VALIDATION METHOD FOR CAPMATINIB AND SPARTALIZUMAB IN RABBIT PLASMA BY USING HIGHLY EFFECTIVE MASS SPECTROPHOTOMETRIC METHOD

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ABSTRACT
LC-MS was developed and verified as a quick, sensitive, and easy approach for the simultaneous measurement of Capmatinib and Spartalizumab. C18 column separation was carried out (150 x 4.6 mm, 3.5) using isocratic elution with a buffer made of 1 mL of formic acid in 1 lit of water and a mixture of two components, such as buffer and acetonitrile, in a 50:50 ratio, with a flow rate of 1 mL/min and room temperature as the mobile phase. In less than eight minutes, the analysis was completed. For Capmatinib and Spartalizumab, within the concentration range of 1.0 ng/mL to 20 ng/mL, the calibration curve was linear. (r2 = 0.999 and 0.99, respectively). All of the system appropriateness, specificity, linearity, and accuracy characteristics are successfully utilized for the analysis of rabbit pharmacokinetic studies and are in good accordance with USFDA recommendations.
Keywords: LC-MS, Capmatinib, Spartalizumab, Validation, Rabbit Plasma.

INTRODUCTION
For those who have non-small cell lung cancer that has spread (NSCLC)[1,2], Capmatinib, offered under the trade name Tabrecta, is an FDA-certified medicine in order to treat cancers[3] with a mutation in the MET gene, which codes for membrane receptor HGFR.[4] Peripheral edoema[5], nausea[6,7], tiredness[8,9], vomiting, dyspnea[10], and reduced appetite are the most prevalent side effects. Malignant cancer cells[11,12] occur in the lung tissues of people with lung cancer that is not tiny cell (NSCLC). The main factor causing non-small cell lung cancer is up to 90% of all cases of the disease, making it the most prevalent. NSCLC is cancer that develops when normal cells undergo an aberrant growth phase. Cancer cells may easily travel from the lungs[13] to other organs and body parts in this stage of the disease. There is a sequence of events that must occur before cancer may spread, and MET exon 14 skipping is one of them.[14,15] Mutations that skip exon 14 in MET are detected in 3-4 percent of lung cancer patients.[16,17] Spartalizumab is a checkpoint inhibitor and a monoclonal antibody[18,19] that is being studied for the treatment of melanoma. Novartis is working on this medication. Phase III studies[20] of spartalizumab began in 2018 and are ongoing. It is very uncommon for pyrexia to be a typical symptom of people with non-small cell lung cancer (NSCLC)[21], tiredness, nausea, vomiting, diarrhoea[22], dry skin[23], reduced appetite, hemorrhage[24], rash, and chills. Bio-analysis is an essential part of drug discovery and manufacture. To do bio-analysis, one must first collect biological samples (drugs, metabolites, biomarkers) and then analyze and report the findings of the analysis. The first step is to pick samples from clinical or preclinical studies and submit them to a laboratory for examination. In bio-analysis, the second stage is sample cleaning, and it's a crucial part of the process. For reliable findings, a consistent and robust sample preparation procedure must be created. The goal of sample preparation is to eliminate sample matrix interference and enhance the analytical method's efficiency. Sample preparation may be time-consuming and labor-demanding. The last stage is to examine and identify the samples. For separation and detection in bio-analytical labs, LC-MS is the technique of choice. In part, this may be ascribed to the LC-MS method's excellent selectivity and sensitivity. Additionally, prior to beginning bioanalytical work, it is critical to have knowledge about the analyte's chemical structure and characteristics. The development and validation of bioanalytical methods are discussed in this paper.

Method validation will be explored in detail. We'll go through some of the most common methods for getting samples ready for testing. In addition, LC-MS/MS will be examined in relation to its function in contemporary bioanalysis. The bio-analysis of tiny compounds is the topic of this review. Capmatinib and Spartalizumab have yet to be bio-analyzed in any form of the biological matrix. Bioanalytical methods for these medicines have never been described before.

EXPERIMENTAL

Chemicals
Merck Worli, Mumbai, India, supplied acetonitrile, formic acid, and water (HPLC grade) for the experiment. Dr. Reddy's laboratory, Hyderabad, supplied APIs of Capmatinib and Spartalizumab as reference standards.

Instrumentation
Waters Alliance's e2695 HPLC equipment was utilized in conjunction with Sciex's QTRAP 5500 triple quadrupole mass spectrometer. The procedure was carried out by use of the SCIEX software. Ion pair mass monitoring in MRM mode was used their mass: m/z 547.26 → 128.39, m/z 145782.56→38425.16 for Capmatinib and Spartalizumab, m/z 419.12→250.37, m/z 145788.34→169538.48 for D_6-Capmatinib and D_6-Spartalizumab (Internal standards of Capmatinib and Spartalizumab). After optimization, the following are the mass spectrometry working parameters: Drying gas temperature ranges from 120 to 250°C, and the collision gas is Nitrogen. The flow rate of 5mL/min, 55psi, delustering potential 40V, 45 volts, 15 volts, capillary voltage 5500 volts, and dwell period of 1 sec.

Preparation of Buffer
Filter through a 0.45 filter paper after dissolving 1Lt of HPLC-grade water and 1ml of formic acid.

Preparation of Mobile phase
Use acetonitrile as the mobile phase and add a buffer in a 50:50 ratio. Mix completely, sonicate for 5 minutes, and then filter through a 0.45 filter paper.

Diluent
The diluent was the mobile phase.

Chromatographical Circumstances
The separation was done at room temperature in isocratic mode using column C18 (150 x 4.6 mm, 3.5). As a mobile phase, 0.1 percent formic acid and acetonitrile were combined at a 50:50 ratio with a 1.0 mL/min flow. The experiment lasted for 8 minutes, and the injection volume was 10 L.

Preparation of Capmatinib and Spartalizumab Stock Solution
Put 5 mg Capmatinib and 5 mg Spartalizumab putting standards into a volumetric flask with 100 ml, accurately balance them out, and add approx. 30 minutes of sonication with 70 ml of diluent to dissolve them, and then add enough diluent to make up the difference. Take 1 ml aforementioned solution, pour it into a volumetric flask of 100 ml, and add diluents to the desired strength. Take 0.8 ml aforementioned solution, transfer it to 10 ml into a volumetric flask, and diluted it to the proper strength. The stock solution is the name of this formulation.

Preparation of Capmatinib and Spartalizumab Standard Procedure
200 liters of plasma sample, 300 liters of acetonitrile, 500 liters of internal standard, 500 liters of diluents, and 500 liters of standard stock were used to prepare the standard, which was then mixed in the vortex cyclo mixture after all the proteins were precipitated. 30 minutes of centrifuging at 500 rpm. Inject the supernatant solution into the chromatogram after collecting it in an HPLC vial.

Preparation of D_6-Capmatinib and D_6-Spartalizumab (IS) Stock Solution
5 mg D6-Capmatinib and 5 mg D6-Spartalizumab working standards should be put into a 100 ml volumetric

flask after being precisely weighed. Add roughly 70 ml of diluent, then sonicate the mixture for 30 minutes to dissolve it, and then add diluent to the mark to the required concentration. The aforesaid solution is diluted to an appropriate concentration in a volumetric flask (100 ml) by adding diluents to 1 ml. Transfer

0.8 ml of the aforementioned diluted solution to a volumetric flask of 10 ml desired concentration using diluents to complete the experiment. This is referred to be an "IS stock".

RESULTS AND DISCUSSION

Different ratios of buffers with acetonitrile as the mobile phase were tested for isocratic and gradient mode in order to get the optimal chromatographic conditions. At each experiment, the mobile phase's composition was tweaked to optimize resolution and retention time. Isocratic mode with 0.1% formic acid and ACN at 50:50 v/v ratios was chosen as the mobile phase since it provides the best response for each of the chosen medicines. We employed C_{18}, C_8, and CN-propyl as stationary phases in the optimization procedure. A PDA detector attached to a C_{18} column of 150mmx4.6mm, 3.5µ provides us with nice peak shapes for Capmatinib and Spartalizumab. 1 mL/min of the mobile phase was pushed through the system. The retention periods of Capmatinib and Spartalizumab were 2.24 and 5.098 minutes, respectively, after applying the indicated conditions. As a result of six duplicate injections, we get a percent CV of 0.31% Capmatinib and 0.29% Spartalizumab, and we draw the conclusion that the recommended method is very specific based on the percent CV data. In accordance with USFDA requirements, a validation study of the proposed procedure is now being conducted.

Specificity

It has been shown that the approach used to study Capmatinib and Spartalizumab at the same time is very specific. Figures 1, 2, and 3 show the chromatograms of blank, standard, and internal standard, and Fig. 4 to 8 show the mass spectra of Capmatinib, D_6-Capmatinib, Spartalizumab, D_6-Sparatalizumab. It was possible to have a look at the chromatograms of rabbit plasma and a standard that didn't have any interference peaks.
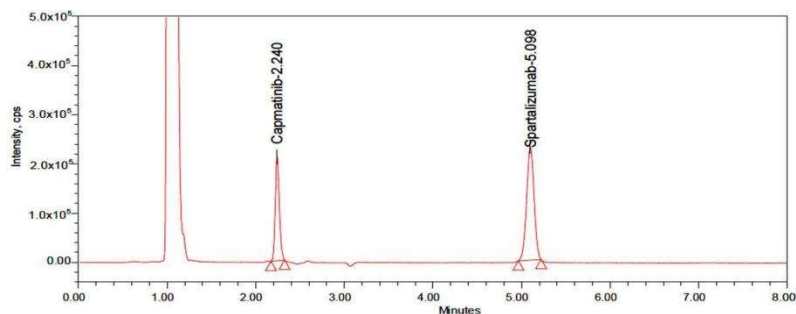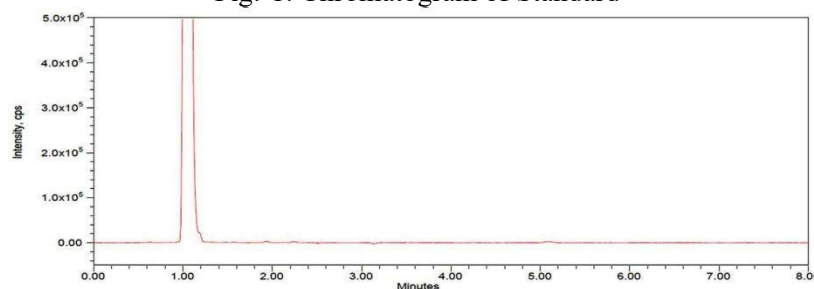

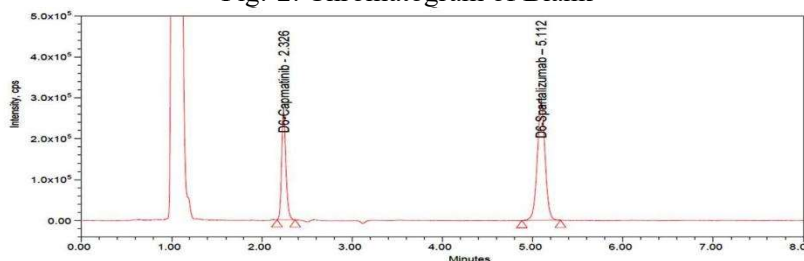Fig.-1: Chromatogram of Standard

Fig.-2: Chromatogram of Blank

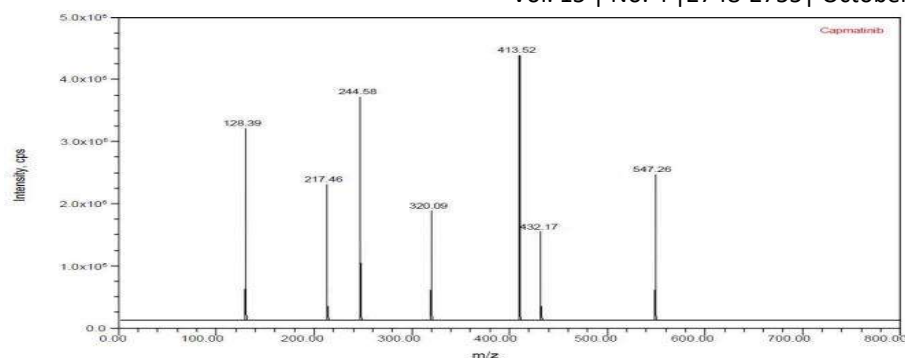Fig.-3: Chromatogram of Blank Plasma Spiked with Internal Standard

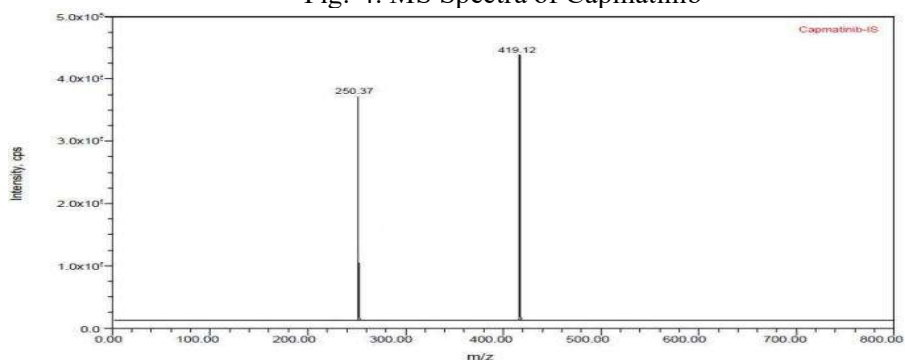2750

Fig.-4: MS Spectra of Capmatinib
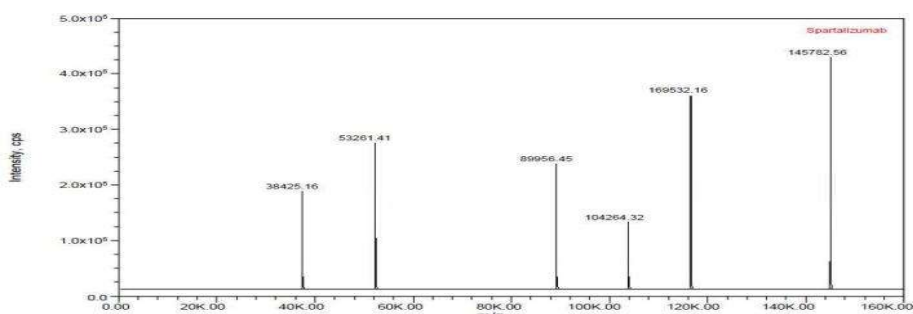

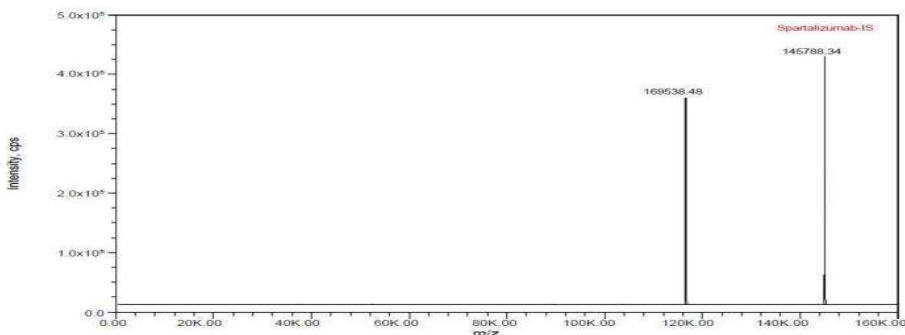Fig.-5: MS Spectra of Capmatinib IS

Fig.-6: MS Spectra of Spartalizumab

Fig.-7: MS Spectra of Spartalizumab IS

Matrix Impact

Capmatinib and Spartalizumab were shown to have a 1.0% ion suppression/enhancement CV at the MQC level. As a result, the analyte's ionization was not adversely affected by the matrix effect. In matrix effect, the results of LQC and HQC of Capmatinib were 99.8 and 99.1 and Spartalizumab was 99.3, 99.9%. %CV of both drugs at LQC level was 0.77, 0.69 and HQC level is 0.39, 0.36 respectively. It shows that the matrix influence on the analyte's ionization is within the acceptable range.

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Linearity

The calibration curve made it clear that peak area ratios were inversely proportional to substance concentrations. Capmatinib has a concentration range of 1.0-20ng/ml, whereas Spartalizumab has a concentration range of 1-20ng/ml. The correlation coefficient was 0.999 for Capmatinib and Spartalizumab in their calibration curves. Table-1 shows the linearity findings of Capmatinib and Spartalizumab. Figures 8 and 9 show the calibration plots of Capmatinib and Spartalizumab.
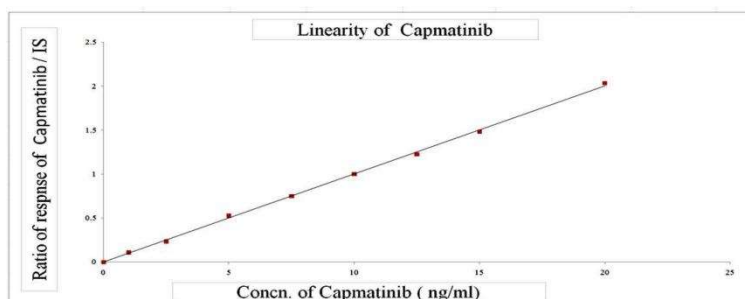

Fig.-8: Calibration plot of Capmatinib
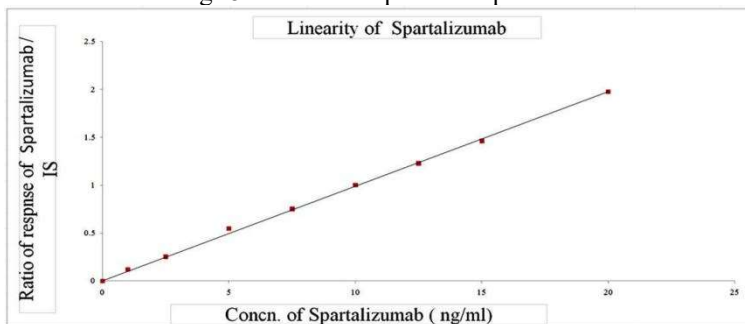
Fig.-9: Calibration plot of Spartalizumab

Table-1: Linearity results of Capmatinib and Spartalizumab

Linearity	Capmatinib		Spartalizumab	
	Conc. ng/ml	Peak response	Conc. ng/ml	Peak response
1	1.00	0.223	1.00	0.263
2	2.50	0.482	2.50	0.548
3	5.00	1.076	5.00	1.182
4	7.50	1.524	7.50	1.623
5	10.00	2.036	10.00	2.152
6	12.50	2.514	12.50	2.635
7	15.00	3.027	15.00	3.159
8	20.00	4.152	20.00	4.276
Slope	0.0991		0.0979	
Intercept	0.00766		0.02232	
CC	0.99911		0.99927	

Table-2: Precision and Accuracy of Capmatinib

QC Name	LLQC	LQC	MQC	HQC
Conc.(ng/ml)	1 ng/ml	5 ng/ml	10 ng/ml	15 ng/ml
QC sample -1	0.224×10^5	1.016×10^5	2.024×10^5	3.032×10^5
QC sample -2	0.216×10^5	1.017×10^5	2.027×10^5	3.035×10^5
QC sample -3	0.222×10^5	1.015×10^5	2.021×10^5	3.031×10^5
QC sample -4	0.222×10^5	1.019×10^5	2.022×10^5	3.037×10^5
QC sample -5	0.221×10^5	1.021×10^5	2.029×10^5	3.033×10^5
QC sample -6	0.229×10^5	1.013×10^5	2.025×10^5	3.035×10^5
Mean	0.222×10^5	1.017×10^5	2.025×10^5	3.034×10^5
SD	0.00423	0.00286	0.00301	0.00223

%CV	1.90	0.28	0.15	0.07
Accuracy (%)	99.2%	99.32%	99.8%	98.7%

Table-3: Precision and Accuracy of Spartalizumab

QC Name	LLQC	LQC	MQC	HQC
Conc.(ng/ml)	1 ng/ml	5 ng/ml	10 ng/ml	15 ng/ml
QC sample -1	0.262×10^5	1.128×10^5	2.132×10^5	3.152×10^5
QC sample -2	0.266×10^5	1.126×10^5	2.133×10^5	3.154×10^5
QC sample -3	0.267×10^5	1.122×10^5	2.136×10^5	3.157×10^5
QC sample -4	0.275×10^5	1.125×10^5	2.137×10^5	3.151×10^5
QC sample -5	0.268×10^5	1.123×10^5	2.131×10^5	3.155×10^5
QC sample -6	0.264×10^5	1.127×10^5	2.138×10^5	3.158×10^5
Mean	0.267×10^5	1.125×10^5	2.135×10^5	3.155×10^5
SD	0.00447	0.00232	0.00288	0.00274
%CV	1.67	0.21	0.13	0.09
Accuracy	99.8%	99.4%	99.1%	99.2%

Table-4: Results of Recovery (%)

Analyte	% Recovery				
	LQC	MQC	HQC	Mean	% RSD
Capmatinib	98.72	99.40	99.65	99.26	0.69
Spartalizumab	98.61	99.94	99.65	99.40	0.81

Precision and Accuracy
By combining the test findings from many QC specimens, they were able to regulate the accuracy and exactness. Quality control samples' accuracy findings for Capmatinib were determined to be 98.7-99.8% and for Spartalizumab, they were 99.1-99.8%. For all quality control samples at different concentrations, and %CV of capmatinib and spartalizumab was less than 5%. All precision and accuracy scores were within the permitted range of quantification. Details of the precision and accuracy results.

Recovery
Capmatinib and Spartalizumab were evaluated for recovery at low, medium, and high concentration focal levels are 98.72%, 99.4%, 99.65% and 98.61%, 99.94%, 99.65% at 10,20, 30 ng/mL and 10, 20, 30 ng/mL concentrations in rabbit plasma.

Ruggedness
In HQC, LQC, MQC, and LLQC samples, Capmatinib and Spartalizumab % recoveries and %CV were within acceptable limits when assessed by two separate analysts and on two distinct columns. The % recoveries ranged from 98.42 – 99.91% for Capmatinib and 98.25% -98.58% for Spartalizumab. Results demonstrated that the approach is robust.

Auto Sampler Carryover
After repeated LLQC and ULQC injections at the retention times of Capmatinib and Spartalizumab, there was no peak area response in the blank rabbit plasma samples.

Stability
Capmatinib and Spartalizumab solutions were produced with diluents and kept chilled at 2–8°C for research on solution stability. There was a correlation between new stock solutions and older stock solutions created over the previous 24 hours. When kept at 2-8°C, the stock solutions remained stable for up to 24 hours. Capmatinib and Spartalizumab for 24 hours at ambient temperature and 24 hours were stable in an auto-sampler at 20 °C in plasma stored at room temperature. The stability of plasma spiked with Capmatinib and Spartalizumab was unaffected by freezing and thawing at LQC and HQC values, as shown by this study. Capmatinib and Spartalizumab showed long-term stability for up to 24 hours at a storage temperature of -

30°C. Tables-5 and 6 summarize the stability data for Capmatinib and Spartalizumab.

Table-5: Stability Results of Capmatinib

Stability experiment spiked plasma		Spiked plasma Mean conc. (n=6, ng/ml)	% Recovery	%CV
Bench top stability	LQC	5.075	98.6	0.38
	HQC	15.423	98.2	0.09
Auto sampler stability	LQC	5.064	98.5	1.80
	MQC	10.425	98.2	0.62
	HQC	15.367	98.3	0.60
Long term stability (Day 28)	LQC	5.214	99.1	0.82
	HQC	15.425	98.7	0.39
Freeze thaw stability	LQC	5.119	98.6	0.28
	HQC	15.324	98.2	0.08
Wet extract stability	LQC	5.024	98.6	0.24
	HQC	15.326	98.2	0.10
Dry extract stability	LQC	5.105	99.6	0.88
	HQC	15.174	98.9	0.42
Short term stability	LQC	5.243	99.5	0.57
	HQC	15.163	99.6	0.96

Table-6: Stability Results of Spartalizumab

Stability experiment spiked plasma		Spiked plasma Mean conc. (n=6, ng/ml)	% Recovery	%CV
Bench top stability	LQC	5.214	98.5	0.31
	HQC	15.321	98.8	0.08
Auto sampler stability	LQC	5.016	98.4	1.22
	MQC	10.223	98.6	0.62
	HQC	15.452	98.8	0.60
Long term stability	LQC	5.119	99.2	0.74
	HQC	15.421	98.8	0.63
Freeze thaw stability	LQC	5.347	98.5	0.17
	HQC	15.229	98.8	0.11
Wet extract stability	LQC	5.130	98.5	0.17
	HQC	15.242	99.8	0.08
Dry extract stability	LQC	5.046	99.2	0.45
	HQC	15.174	99.7	0.16
Short term stability	LQC	5.069	98.3	0.83
	HQC	15.223	99.5	0.71

CONCLUSION

Using Capmatinib-D_6 and Spartalizumab-D_6 as internal standards, we set out to establish a simple, cost-effective, robust, and sensitive technique for LCMS-based Capmatinib and Spartalizumab determination. When compared to other articles, the work has a shorter run time. Capmatinib and Spartalizumab had a retention time of 2.241 and 5.098 minutes respectively, in a total chromatographic run duration of 8.0 minutes. Over a dynamic linear range of 1-20ng/mL each of Capmatinib and Spartalizumab, the approach has been verified and the correlation value is r^2 0.999. According to US Food and Drug Administration (FDA) rules.

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