Gaddey and Sundararajan
Future Journal of Pharmaceutical Sciences (2024) 10:57
https://doi.org/10.1186/s43094-024-00629-x

Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

Bioanalytical method development and validation for quantification of amivantamab in rat plasma by LC-MS/MS

Pridhvi Krishna Gaddey[1] and Raja Sundararajan[1*]

Abstract

Background Amivantamab is a monoclonal bispecific anti-EGFR-MET antibody used to treat non-small cell lung cancer. There were no published methods using a liquid chromatographic—tandem mass spectrometric approach to develop and validate a feasible, novel, and thoroughly validated method for quantifying amivantamab in rat plasma.

Results The liquid–liquid extraction method was used to extract the analyte from rat plasma. The analyte was separated using acetonitrile–ammonium formate buffer (40:60) as a mobile phase on waters, alliance e-2695 model high-pressure liquid chromatographic system having Agilent eclipse C_{18}, 150 mm × 4.6 mm, 3.5 µm column. The overall runtime was 6 min at a 1.0 ml/min flow rate. The method showed significant sensitivity and acceptable linearity over the 5.00–100.00 ng/ml concentration range. Accuracy was proved by mean percent recovery ranging from 98.03 to 99.99%. The intraday precision coefficient of variation (%) ranged between 0.31 and 5.43. Also, the findings such as C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and half-life values of amivantamab showed that the technique was helpful for pharmacokinetic studies.

Conclusions All the validated parameters were found to be within the acceptable range. The validated method was found to be simple, accurate, precise, and reproducible and hence can be used for the routine analysis of amivantamab, such as in-process quality control by liquid chromatographic—tandem mass spectrometry.

Keywords Amivantamab, LC-MS/MS, Rat plasma, Validation

Background

One of the most frequent malignancies linked to occupational exposures is lung cancer. Mesothelioma and lung cancer incidence have been related to the usage of asbestos in manufacturing and industries [1]. The two main subtypes of the disease are small cell lung carcinoma and non-small cell lung carcinoma (NSCLC), which account for 15% and 85% of all instances of lung cancer, respectively. Three other subtypes of NSCLC include squamous-cell carcinoma, adenocarcinoma, and large-cell carcinoma [2]. The prevailing type of lung cancer, advanced non-small cell lung cancer (NSCLC), has a terrible prognosis and no recognized treatment. Due to the few therapy choices available, survival times are frequently brief [3]. There are risk factors for NSCLC that can be prevented and some that cannot be avoided. Tobacco inhalation is the most prevalent preventable risk factor for NSCLC [4]. Alcohol consumption, environmental exposure to second-hand smoke, asbestos, radon, arsenic, chromium, nickel, exposure to ionizing radiation, and polycyclic aromatic hydrocarbons are other causes of lung cancer [5]. One of the treatments for non-small cell lung cancer is the epidermal growth

*Correspondence:
Raja Sundararajan
sraja61@gmail.com
[1] Department of Pharmaceutical Analysis, GITAM School of Pharmacy, GITAM (Deemed to Be University), Visakhapatnam, Andhra Pradesh 530 045, India

factor receptor (EGFR) gene. The tyrosine kinase cell-surface receptor EGFR can open up pathways that are involved in cell growth and proliferation when it is active [6]. EGFR mutations in malignancies lead to unregulated cell division because of persistent activation. 10–15% of people with lung cancer adenocarcinoma with European and Asian ancestry, those who never smoked, and female patients had EGFR gene mutations [7]. The first targeted treatment for non-small cell lung cancer patients, amivantamab, is a monoclonal bispecific anti-EGFR-MET antibody [8].

The quantitative assessment of drugs and their metabolites in efficient and robust techniques is important for the fruitful evaluation of biopharmaceutical, preclinical, and clinical research. The protocol referred to in the demonstration of a technique for quantitative assessment of analytes in biological matrices such as plasma, urine, blood, and urine is called bioanalytical method validation [9]. These techniques are dependable and repeatable. A review of the literature was conducted. Bioanalytical methods for the determination of amivantamab in biological samples have not yet been reported. There were no analytical methods reported for determination of amivantamab in pure form and in different matrices. The goal of this work was to create a sensitive and specific analytical approach for the quantitation of amivantamab in rat plasma. The developed technique was validated by ICH M10 regulations [10]. The results of this study provide a robust and reliable analytical strategy for the rapid identification of amivantamab and provide a helpful basis for further research.

Methods

Solvents and chemicals

The amivantamab sample (99.99% purity) was provided as a gift sample from Shree Icon Labs, Vijayawada, Andhra Pradesh, India. All other chemicals, including HPLC grade acetonitrile and methanol, were purchased from Merck Chemical Division in Mumbai. The Milli-Q water purification system's HPLC-grade water was used throughout the study. All chemicals and reagents were used as received without further purification. Trastuzumab (99.98% purity) was used as an internal standard (IS). It was obtained from Glenmark Pharmaceuticals Pvt Ltd, Mumbai, India.

Instrumentation

The liquid chromatography system comprised of Waters, alliance e-2695 model HPLC armed with column oven, autosampler, and degasser was employed for analysis. The SCIEX QTRAP 5500 mass spectrometer was connected to the HPLC system. REMI centrifuge was used for centrifugation. Cyclo Mixer was used for mixing.

Chromatographic conditions

The autosampler was maintained at ambient temperature. An Agilent eclipse C_{18}, 150 mm × 4.6 mm, 3.5 µm column was used for elution. Isocratic elution was employed with a mobile phase comprising acetonitrile and 0.1 M ammonium formate in the proportion of 40:60. With an injection volume of 10 µl, the chromatographic flow rate was set at 1.0 ml/min.

Mass spectrometer conditions

The mass spectrometric system comprised a SCIEX QTRAP 5500 mass spectrometer armed with an electrospray ionization interface ionization mode. The collision gas used was nitrogen. The compound-specific parameters working are displayed in Table 1. The ions were detected using multiple-reaction monitoring mode (MRM). Sciex Analyst software was used to process the data.

Selection of internal standard (IS)

Trastuzumab was selected as an internal standard to reduce the inaccuracy at the processing level or the ongoing analysis level caused by the instrument for quantifying both analytes by technique. Trastuzumab was chosen as the IS due to its near molecular weight with the analyte.

Preparation of solutions

Preparation of amivantamab stock and working solutions

Five milligrams of amivantamab working standard was weighed and transferred into a 100-ml volumetric flask and then diluted to volume with diluent. Further, 1 ml from the above solution was transferred to a 10-ml volumetric flask and made up with diluent. 0.4 ml of the above solution was taken into a 10-ml volumetric flask and made up to the mark with diluent.

Preparation of internal standard stock solution (200 ng/ml)

Five milligrams of trastuzumab working standard was weighed and transferred into a 100-ml volumetric flask

Table 1 Compound-specific parameters

Parameter	Value
Declustering potential (DP)	40 V
Collision energy (CE)	14 V
Entrance potential (EP)	10 V
Collision cell exit potential (CXP)	7 V
Source temperature	550 °C
Drying gas temperature	120–250 °C
Drying gas flow stream	5 ml/min

Table 2 Preparation of standards

Stock solution (ng/ml)	Volume taken (µl)	Made up to volume (µl)	Final concentration (ng/ml)	Identification
200	50	2000	5.0	Standard 1
200	125	2000	12.5	Standard 2
200	250	2000	25.0	Standard 3
200	375	2000	37.5	Standard 4
200	500	2000	50.0	Standard 5
200	625	2000	62.5	Standard 6
200	750	2000	75.0	Standard 7
200	1000	2000	100.0	Standard 8

Table 3 Preparation of QC samples

Stock solution (ng/ml)	Volume (µl)	Made up to volume (µl)	Final concentration (ng/ml)	Identification
200	750	2000	75.0	HQC
200	500	2000	50.0	MQC
200	250	2000	25.0	LQC
200	50	2000	5.0	LLQC

and, then, diluted to volume with diluent. Further, 1 ml was pipetted into a 10-ml volumetric flask and made up with diluent. 0.4 ml of the above solution was taken into a 10-ml volumetric flask and made up to the mark with diluent. This IS added to the sample processing (50 µl) resulted in a final concentration of 50 ng/ml corresponding with the MQC concentration of amivantamab.

Preparation of plasma samples

Transferred 500 µl of amivantamab stock solution into a 2-ml Eppendorf tube. To this, 200 µl of plasma, 500 µl of internal standard, 300 µl of acetonitrile, and 500 µl of diluent were added (50 ng/ml). Calibration standards were prepared by spiking blank rat plasma with appropriate amounts of amivantamab and trastuzumab. Calibration standards for final concentration were 5.00, 12.50, 25.00, 37.50, 50.00, 62.50, 75.00, 100.00 ng/ml for amivantamab. The preparation of calibration standards and QC samples are displayed in Tables 2 and 3.

Diluent

Mobile phase of acetonitrile and ammonium formate in the ratio 40:60 was used as diluent.

Sample preparation

The 2000 µl samples prepared above were vortexed with the vortex cyclo mixture. The solution was centrifuged at 4000 RPM for 15 to 20 min. Then, the supernatant fluid was collected in an HPLC vial. Double blank samples (i.e., without analyte and IS) were prepared by mixing 1000 µl of acetonitrile with 200 µl of rat plasma samples.

Bioanalytical method validation

Method validation was done according to ICH M10 guidelines.

System suitability

Six replicates of high-quality control (QC) standard solution were injected into the chromatographic apparatus to analyze the system suitability parameters.

Linearity

The calibration curve was generated by analyzing eight concentrations of amivantamab in plasma. Samples were measured by comparing the peak area of amivantamab to that of trastuzumab. The plot of peak area ratios vs plasma concentrations was made.

Specificity

The specificity of the developed approach was evaluated in the current study by analyzing the chromatograms of blank plasma and spiked plasma samples (amivantamab, trastuzumab).

Sensitivity

The method's sensitivity was assessed by analyzing 6 replicates of rat plasma comprising a lower limit of quantification (LLOQ) sample (5.0 ng/ml) of amivantamab.

Accuracy and precision

Four QC samples—LLOQ, low-quality control (LQC), medium-quality control (MQC), and high-quality control (HQC)—each with six replicates were used to assess the proposed bioanalytical method's precision and accuracy. The precision and accuracy of the proposed method were represented as mean accuracy (%) and coefficient of variance (CV) (%), respectively.

LOD and LOQ

The signal-to-noise ratio was used to calculate the bioanalytical method's limit of detection (LOD) and limit of quantification (LOQ).

Autosampler carryover

Autosampler carryover was assessed by injecting a blank sample, followed by an HQC sample and then an LLOQ sample followed by a blank sample.

Dilution integrity

The dilution integrity of plasma samples was evaluated by evaluating amivantamab samples above ULQC. Those

samples were taken and diluted with a blank matrix to MQC and ULQC.

Matrix effect

The matrix effect of plasma on the response of amivantamab was assessed by blank plasma samples that were extracted from six different lots and reconstituted to form working standards of LQC and HQC. These samples in triplicate were quantified against the calibration curve.

Recovery of analyte

Six replicates of the amivantamab QC low-, medium-, and high samples were created by spiking the relevant concentrations of the drug and an internal standard into either unextracted or supernatant-recovered blank rat plasma (extracted). Recovery was calculated by comparing its response in multiple samples to neat standard solution responses.

Recovery of internal standard

Trastuzumab (50.0 ng/ml) samples in blank plasma were prepared and examined in six repetitions.

Ruggedness

Low, medium, and high QCs of amivantamab in plasma samples were reinjected into the system. % CV and accuracy were assessed to determine ruggedness.

Reinjection reproducibility

Low, medium, and high QCs of amivantamab in plasma samples were reinjected into the system. % CV and accuracy were assessed to determine reinjection reproducibility.

Stability studies

Benchtop stability

Amivantamab's stability in rat plasma was assessed by exposing six replicates of three different concentrations (LQC, MQC, and HQC) for 8 h on a benchtop and injecting them into the system.

Short-term and long-term stability

Short-term and long-term stability was assessed for amivantamab. Three different analyte concentrations were spiked into six duplicates of rat plasma for QC. LQC, MQC, and HQC samples were prepared and stored at 5 ± 3 °C for 7 days, and short-term stability was assessed. LQC, MQC, and HQC samples were prepared and stored at − 20 ± 3 °C. These samples were injected from day 1 to 28 days for every seven days (as day 1,7,14 21, and 28), and long-term stability was assessed.

Freeze–thaw stability

The stability of amivantamab was evaluated after freeze–thaw cycles, respectively. Each LQC, MQC, and HQC had six duplicates that were held at − 20° C, totally thawed at ambient temperature, and then immediately refrozen at − 20° C. After this cycle was done twice, the samples were removed for injection into the LC-MS.

Autosampler stability

LQC, MQC, and HQC samples of amivantamab in plasma were injected at one-hour up to 24-h intervals. Mean accuracy (%) and CV (%) were calculated.

Dry extract and wet extract stability

Wet extract stability was evaluated by assessing the six sets of LQC, MQC, and HQC after 12 h and 18 h that were stored at 2–8 °C. The dry extract stability test used six sets of LQC, MQC, and HQC after 12 h and 18 h that were stored at 22 °C.

Assay

Assay was done to define the applicability of the bioanalytical method to the marketed formulation. MQC (50.0 ng/ml) sample was prepared from the marketed formulation (Rybrevant) and injected into the LC-MS system.

Method applicability to rats

Six healthy white female albino rats (body weight in between 250 and 350 g) were taken from Flair Labs, Gujarat, India. Before the experiment was directed, rats were adapted to laboratory environments for seven days. Diet was restricted for 12 h before the experiment, although water was freely available. A single dose of amivantamab (0.83 mg/ml) was administered to six rats. Samples were collected at different intervals, such as 10, 20, 30, 40, 50, and 60 min. K2 EDTA vacutainer tubes were used to collect blood at each interval. A predose sample was also taken to check for any potential plasma interferences. The plasma was obtained by centrifuging the collected samples kept at 10 °C. The liquid–liquid extraction method was used to isolate amivantamab in rat plasma. The animal study protocol was approved by the Institute of the Animal Ethics Committee (Reg.No: 1250/PO/RcBi/S/09/CPCSEA). Phoenix Win Nonlin (Version 5.2) software was used to analyze the data.

Results

Method development

MS/MS analysis

The mass spectra of amivantamab and trastuzumab were obtained by preparing each analyte in diluent and injecting it into the liquid chromatography-tandem

mass spectrometer with positive ionization mode. Scan displayed that precursor ions of amivantamab and an m/z value of 145.66 were chosen. During MRM optimization, the product ion 112.10 showed the best response and was selected as the daughter fragment. The collision energy was optimized as 14 V using the edit ramp function. The scan presented that precursor ions of trastuzumab have an m/z value of 148.57. During MRM optimization, the product ion 126.01 showed the best response and was chosen as the daughter fragment. The mass transitions are shown in Figs. 1 and 2.

Liquid chromatography

Several elution conditions were tested for the chromatographic separation. An isocratic flow profile was devised to get the finest peak separation with a minimal overall run time for all the analytes. The liquid chromatographic settings were improved to prevent the matrix effect, provide better peak shapes for all analytes, and increase sensitivity. Agilent eclipse C_{18}, 150 mm × 4.6 mm, 3.5 µm column, was chosen as the stationary phase for the analyzed compounds. Acetonitrile and 0.1 M ammonium formate buffer (40:60) were used for the mobile phase. The optimized method chromatogram is presented in Fig. 3. The finest outcomes were found with a flow rate of 1.0 ml/min.

Optimization of the sample preparation

Based on the pKa value of the compound and the ease of sample extraction, the liquid–liquid extraction method was chosen for sample preparation. Acetonitrile was selected as the extractant because of its low ionization suppression and excellent extraction efficiency compared to other organic solvents frequently employed in LC-MS/MS analysis. High sensitivity, linear calibration range, and low matrix effect were attained for amivantamab.

Bioanalytical method validation

System suitability

System suitability was examining a set of reference standards to determine an instrument's performance, which was conducted before the analytical run. The CV (%) for amivantamab and trastuzumab area ratio was 0.13. The CV (%) of retention time of amivantamab and trastuzumab was found to be 0.63 and 0.23, respectively. The system suitability parameters like tailing factor, plate count, and resolution were within the limit. System suitability parameters are displayed in Table 4.

Linearity

The peak area obtained from the analysis was used to calculate the area response ratio. A calibration curve was plotted by taking concentration on the X-axis and area response ratio on the Y-axis. The slope, intercept, and correlation coefficient were obtained from the

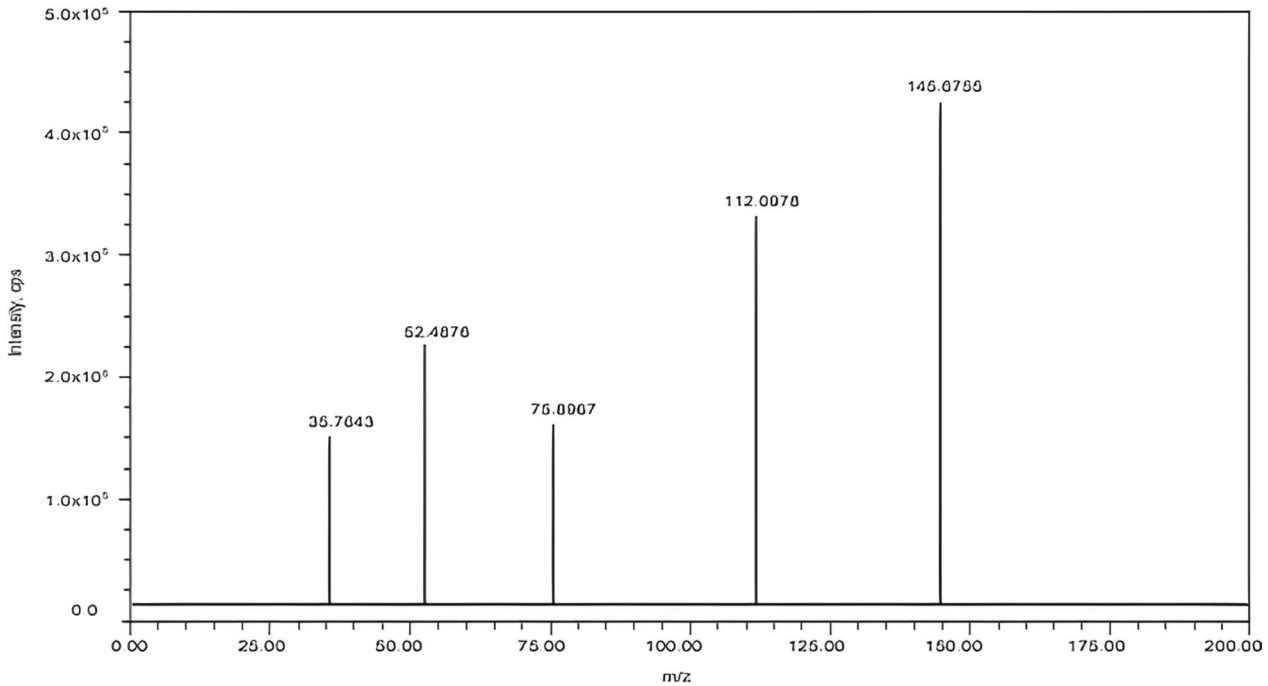

Fig. 1 Product ion scan of amivantamab using positive polarity

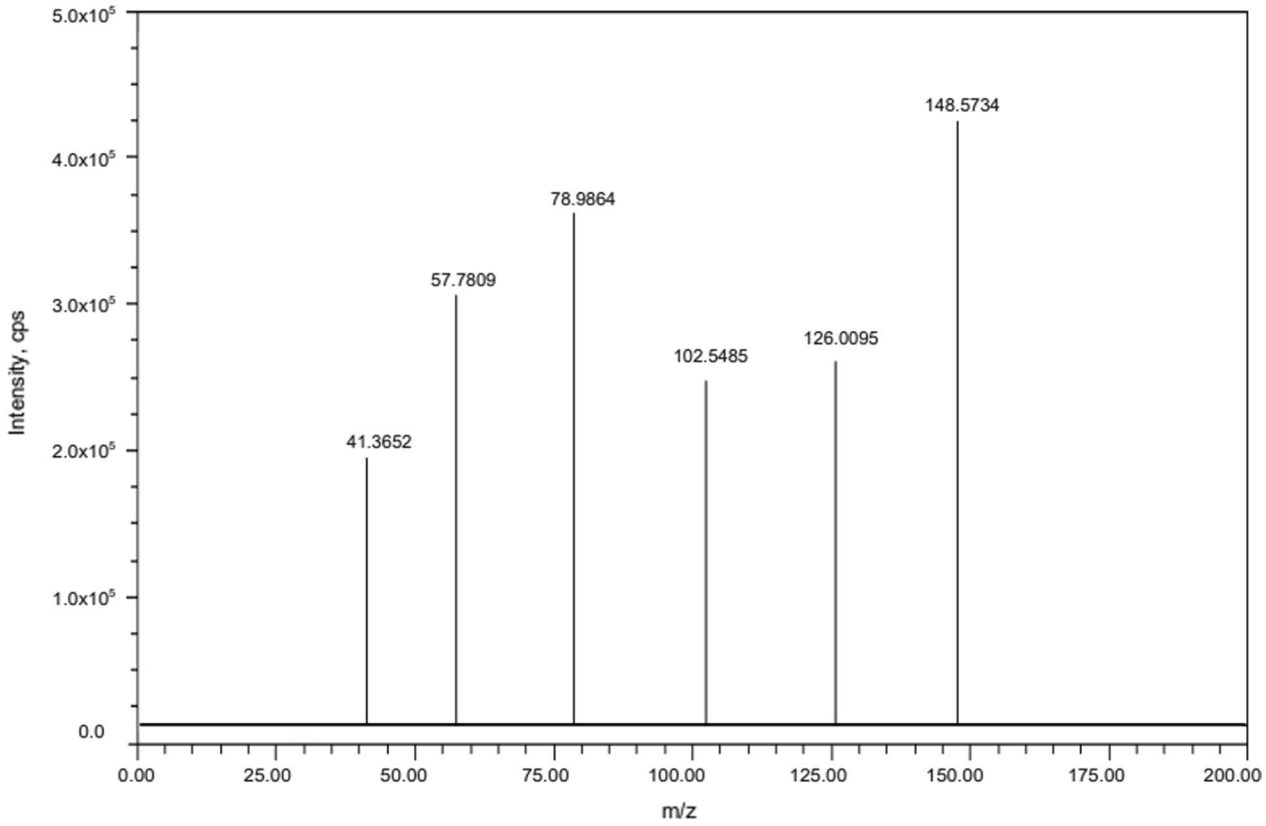

Fig. 2 Product ion scan of trastuzumab using positive polarity

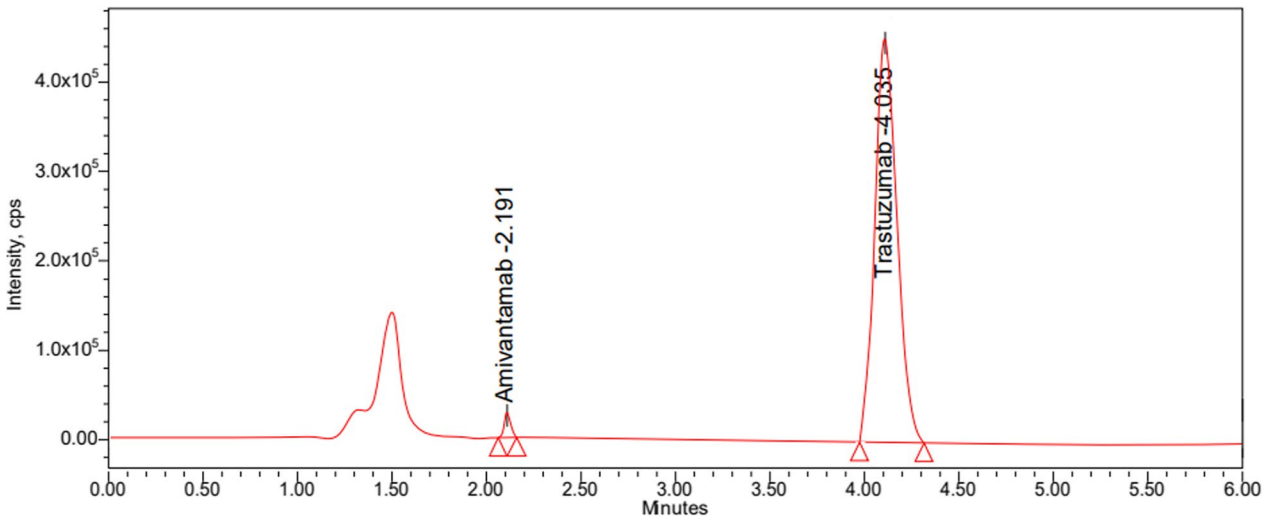

Fig. 3 LLOQ chromatogram of amivantamab

plot. The best linearity of the calibration curve for amivantamab was secured over the concentration ranges of 5.0 – 100.0 ng/ml. The correlation coefficient was 0.99949. The linearity range of solutions and respective area response ratios are tabulated in Table 5. A representative calibration curve for amivantamab is presented in Fig. 4.

Table 4 System suitability results of amivantamab

	Name	Retention time	Area	USP tailing	Theoretical plate	USP resolution
1	Amivantamab	2.128	3265124	1.07	6594	
2	Trastuzumab	4.136	4485769	1.01	7318	8.19

Table 5 Linearity results of amivantamab

Concentration (ng/ml)	Area	Area response ratio
0	0	0
5	0.345×10^5	0.082
12.5	0.852×10^5	0.202
25.0	1.754×10^5	0.416
37.5	2.601×10^5	0.616
50.0	3.457×10^5	0.819
62.5	4.305×10^5	1.020
75.0	5.211×10^5	1.233
100.0	6.712×10^5	1.589
Slope	0.01610	
Intercept	0.00584	
R^2 value	0.99949	

Specificity

Specificity results exhibited that the process developed was highly selective for amivantamab. No discernible endogenous chemicals interfered at the retention times for amivantamab and trastuzumab were seen in six different types of blank plasma. The ability to clearly distinguish the analyte in biological fluids, which comprised various components, including the matrix, was known as specificity. The specificity results are tabulated in Table 6.

Sensitivity (LLOQ)

The current approach achieved an LLOQ of 5.00 ng/ml for amivantamab in rat plasma. The CV (%) and mean accuracy (%) were 6.30% and 93.10%, respectively. Table 7 displays the sensitivity test results.

Accuracy and precision

Accuracy was shown as % mean recovery and precision as % CV. The degree to which the experimental value and the actual value were similar depends on the accuracy of the analytical approach. LLQC, LQC, MQC, and HQC solutions were prepared in replicates and injected into the system. The intraday accuracy and precision of amivantamab were 94.2–99.9% and 0.9–5.3, respectively. Results were summarized in Table 8.

Autosampler carryover

The chromatograms of standard blank samples were observed, and no significant carryover of amivantamab was detected. Similarly, carryover of trastuzumab was also not found.

LOD and LOQ

The LOD (1.67 ng/ml) solution was prepared so that the S/N ratio ranged around 3:1. Respective chromatograms of LOD and LOQ (5.0 ng/ml) are shown in Figs. 5 and 6. The method was found to be sensitive and specific.

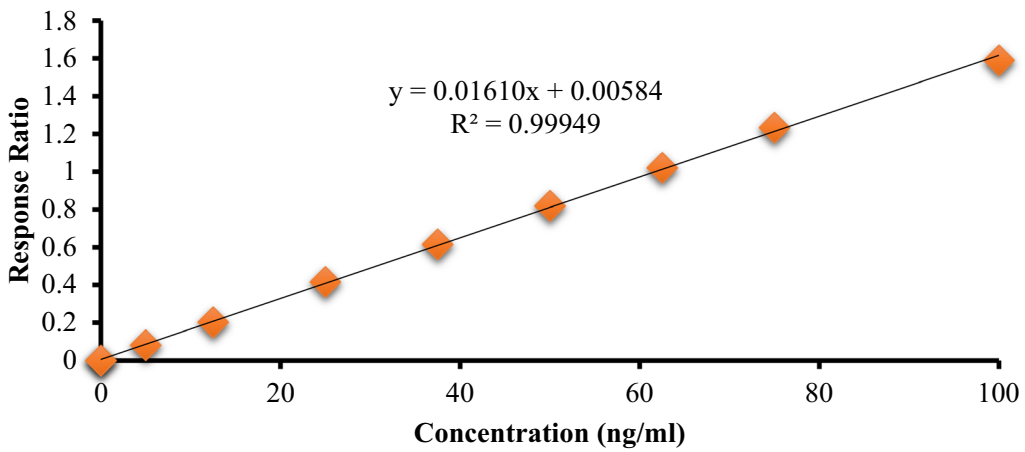

Fig. 4 Calibration curve of amivantamab

Table 6 Specificity results of amivantamab

S. no	Sample ID	Intensity (cps)		% Interference		Pass/fail
		Amivantamab	Trastuzumab	Amivantamab	Trastuzumab	
1	Std Blank 1	0	0	0	0	Pass
2	LLOQ 1 (5 ng/ml)	0.369×10^5	4.237×10^5	0	0	Pass
3	Std Blank 2	0	0	0	0	Pass
4	LLOQ 2 (5 ng/ml)	0.361×10^5	4.221×10^5	0	0	Pass
5	Std Blank 3	0	0	0	0	Pass
6	LLOQ 3 (5 ng/ml)	0.365×10^5	4.234×10^5	0	0	Pass
7	Std Blank 4	0	0	0	0	Pass
8	LLOQ 4 (5 ng/ml)	0.367×10^5	4.225×10^5	0	0	Pass
9	Std Blank 5	0	0	0	0	Pass
10	LLOQ 5 (5 ng/ml)	0.363×10^5	4.211×10^5	0	0	Pass
11	Std Blank 6	0	0	0	0	Pass
12	LLOQ 6 (5 ng/ml)	0.364×10^5	4.240×10^5	0	0	Pass

Table 7 Sensitivity results of amivantamab

Replicate number	LLOQ (5.0 ng/ml)
1	4.3 ng/ml
2	4.9 ng/ml
3	4.4 ng/ml
4	4.9 ng/ml
5	4.9 ng/ml
6	4.5 ng/ml
Mean	4.7 ng/ml
SD	0.3
% CV	6.3
% Mean accuracy	93.1%

Dilution integrity

Dilution integrity was the evaluation of the sample dilution technique to ensure that it does not affect the precision and accuracy of the measured concentration of the analyte, as needed. The CV (%) and mean accuracy (%) for MQC and ULQC were found to be 0.5, 0.5, and 100.1 and 98.5, respectively. The results are displayed in Table 9.

Matrix effect

A matrix effect describes the changes observed in detecting or quantifying an analyte when other substances are present in the sample. The CV (%) for LQC

Table 8 Precision and accuracy results of amivantamab

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	MQC (50.0 ng/ml)	LQC (25.0 ng/ml)	LLQC (5.0 ng/ml)
1	74.8	49.9	24.5	4.6
2	74.8	49.9	25.1	4.6
3	75.4	48.7	25.1	4.6
4	75.4	49.3	25.1	5.2
5	73.6	49.9	25.1	4.6
6	74.2	49.9	25.1	4.6
Mean	74.7	49.6	24.9	4.7
SD	0.7	0.5	0.3	0.3
% CV	0.9	1.0	1.0	5.3
% Mean accuracy	99.6%	99.3%	99.9%	94.2%

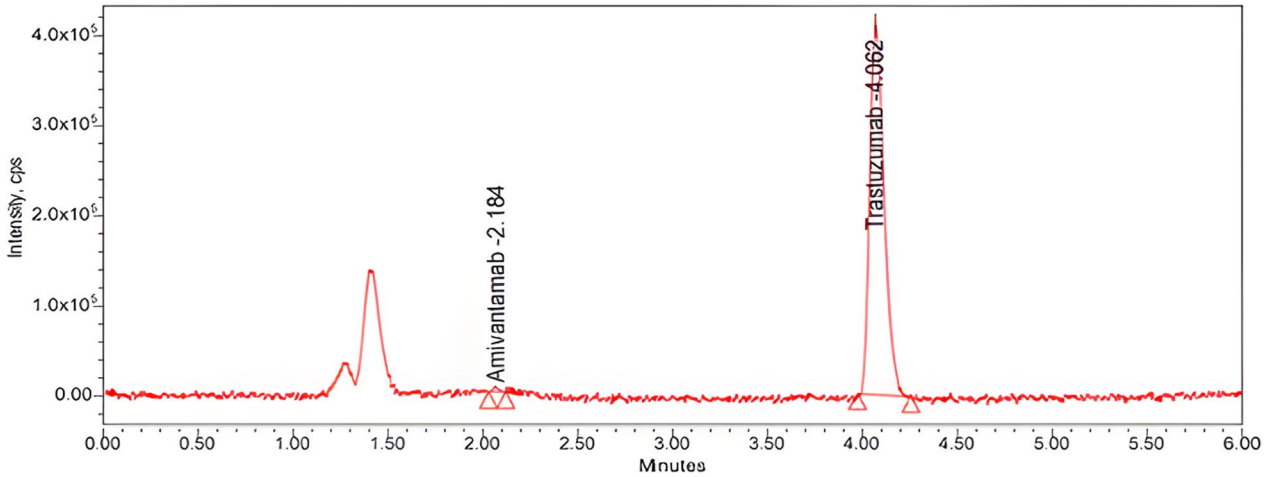

Fig. 5 Chromatogram of LOD

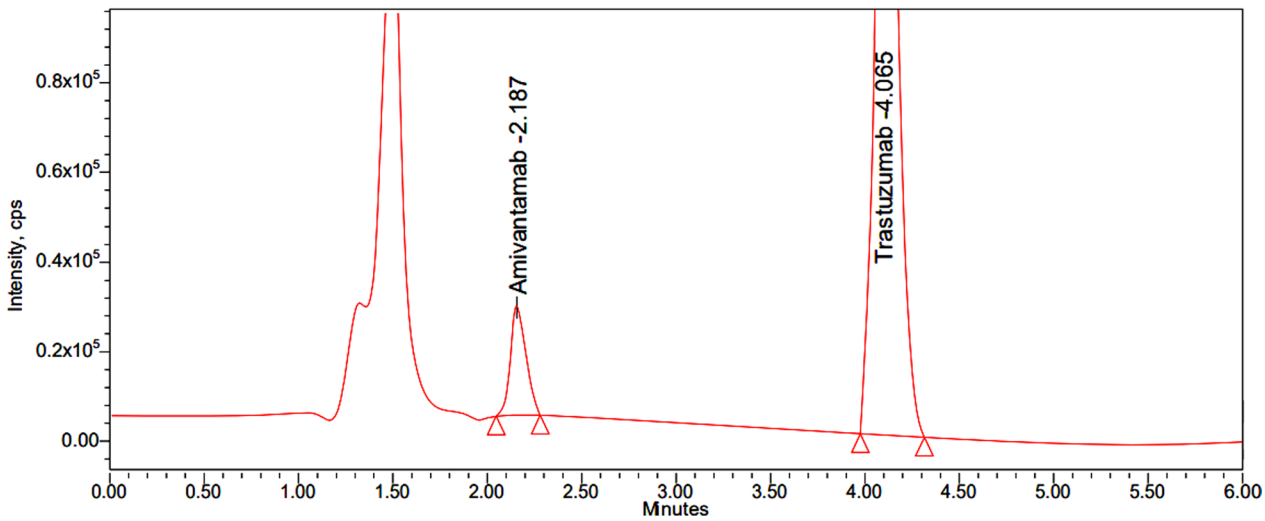

Fig. 6 Chromatogram of LOQ

and HQC was observed to be 0.73 and 1.20, respectively. The mean accuracy was 99.71% for low-quality control samples and 99.88% for high-quality control samples. Data was included in the Additional file 1.

Recovery of analyte

The effectiveness of separating analytes from samples was described by recovery. The CV (%) for extracted and unextracted samples at the HQC level was found to be 0.99 and 0.98, respectively. The mean recovery (%) for extracted and unextracted samples at the HQC level was found to be 99.79 and 99.94%, respectively. Data was incorporated in the Additional file 1.

Recovery of internal standard

The CV (%) of unextracted and extracted methods was found to be 0.17 and 0.19, respectively. The CV (%) of trastuzumab's recovery was around 15.00%. Data was integrated in the Additional file 1.

Ruggedness

Ruggedness is a measure of the susceptibility of a method to small changes that might occur

Table 9 Results for dilution integrity

Replicate number	MQC (50.0 ng/ml)	ULQC (100.0 ng/ml)
	Concentration	
1	49.9	97.8
2	49.9	98.4
3	49.9	99.0
4	49.9	99.0
5	50.6	98.4
6	49.9	98.4
Mean	50.1	98.5
SD	0.3	0.5
% CV	0.5	0.5
% Mean accuracy	100.1%	98.5%

during routine analysis, like small changes in pH values, mobile phase composition, temperature, analysis, etc. The mean accuracy (%) for LQC, MQC, and HQC was 99.13–99.88%. Data was included in the Additional file 1.

Reinjection reproducibility

Six duplicates of low, medium, and high QCs of amivantamab were reinjected into plasma samples to see if samples could be reinjected in the event of instrument failure or other issues. The % CV for HQC, MQC, and LQC was found to be 0.7, 0.3, and 0.9%, respectively. Data was added in the Additional file 1.

Stability studies

Bench-top stability

Bench-top stability is the stability of an analyte in a matrix under sample handling conditions during sample processing. The CV (%) of HQC, LQC, and MQC was found to be 0.9, 1.3, and 0.5, respectively. The mean accuracy (%) of HQC, LQC, and MQC was found to be 99.7, 98.8, and 98.4%, respectively. The results of bench top stability are shown in Table 10.

Short-term stability and long-term stability

Long-term stability assesses the degradation of an analyte in the matrix relative to the starting material after periods of frozen storage. The results showed that amivantamab QC low, medium, and high samples were stable in short-term and long-term stability. Short-term stability results are displayed in Table 11, and long-term stability results are summarized in Tables 12, 13, 14, 15, 16.

Freeze–thaw stability

Freeze–thaw stability refers to the stability of the analyte in the matrix upon freezing and thawing. The CV (%) of HQC, LQC, and MQC was found to be 1.2, 1.9, and 0.8, respectively. The mean accuracy (%) of HQC, LQC, and MQC was found to be 99.75, 99.94, and 99.94%, respectively. The results are tabulated in Table 17.

Autosampler stability

Autosampler stability is the stability of the analyte in the processed sample under the conditions in the autosampler. The CV (%) of HQC, LQC, and MQC was found to be 1.09, 0.46, and 1.24, respectively. The mean accuracy (%) of HQC, LQC, and MQC was found to be 99.7, 98.3, and 99.9%, respectively. The outcomes are summarized in Table 18.

Dry extract stability and wet extract stability

Extract stability assesses the degradation of the processed sample relative to the starting material. The results of wet

Table 10 Bench top stability results of amivantamab

Replicate no	HQC (75.0 ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
	Concentration (ng/ml)		
1	75.4	24.5	48.7
2	74.2	24.5	49.3
3	75.4	25.1	49.3
4	75.4	24.5	49.3
5	74.2	25.1	49.3
6	74.2	24.5	49.3
Mean	74.8	24.7	49.2
SD	0.7	0.3	0.3
% CV	0.9	1.3	0.5
% Mean accuracy	99.7%	98.8%	98.4%

Table 11 Short-term stability results of amivantamab

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	74.8	23.2	49.3
2	74.8	23.2	49.3
3	74.2	22.6	48.7
4	74.2	23.2	49.3
5	74.8	22.6	48.7
6	75.4	23.2	48.7
Mean	74.7	23.0	49.0
SD	0.5	0.3	0.3
% CV	0.6	1.4	0.7
% Mean accuracy	99.6%	92.1%	98.0%

Table 12 Long-term stability results of amivantamab—Day 1

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	75.4	25.1	49.3
2	73.6	24.5	49.9
3	74.2	24.5	49.3
4	76.0	24.5	49.3
5	76.0	23.8	49.9
6	74.2	25.1	49.9
Mean	74.9	24.6	49.6
SD	1.1	0.5	0.3
% CV	1.4	1.9	0.7
% Mean accuracy	99.9%	98.3%	99.3%

Table 13 Long-term stability results of amivantamab—Day 7

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	75.4	23.2	48.7
2	75.4	23.2	48.7
3	75.4	22.6	48.1
4	74.8	22.6	48.7
5	74.8	23.2	48.1
6	74.8	22.3	48.7
Mean	75.1	22.9	48.5
SD	0.3	0.3	0.3
% CV	0.5	1.5	0.7
% Mean accuracy	100.1%	91.7%	96.9%

Table 14 Long-term stability results of amivantamab—Day 14

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	73.6	22.6	48.7
2	72.9	22.6	48.1
3	73.6	21.9	48.1
4	74.2	22.6	48.7
5	73.6	22.6	48.1
6	74.8	23.2	48.7
Mean	73.8	22.6	48.4
SD	0.6	0.4	0.3
% CV	0.9	1.7	0.7
% Mean accuracy	98.3%	90.5%	96.8%

Table 15 Long-term stability results of amivantamab—Day 21

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	73.6	21.9	46.8
2	73.6	21.9	47.5
3	73.6	22.6	47.5
4	74.2	22.6	47.5
5	72.9	22.6	48.1
6	73.6	21.9	46.8
Mean	73.6	22.3	47.4
SD	0.4	0.3	0.5
% CV	0.5	1.5	0.9
% Mean accuracy	97.6%	89.2%	94.7%

Table 16 Long-term stability results of amivantamab—Day 28

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	72.3	21.4	45.6
2	72.3	21.4	46.2
3	72.9	21.4	46.2
4	72.9	21.4	46.2
5	72.9	21.9	46.2
6	72.9	20.8	45.6
Mean	72.7	21.4	46.0
SD	0.3	0.4	0.3
% CV	0.4	1.8	0.7
% Mean accuracy	96.9%	85.5%	92.0%

extract stability are tabulated in Tables 19, 20. Similarly, the results of dry extract stability were summarized in Tables 21, 22.

Assay

The developed method's applicability for quantifying amivantamab in the marketed formulation was assessed.

Table 17 Freeze–thaw stability results of amivantamab

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	74.8	24.5	50.6
2	75.4	24.5	49.9
3	74.2	24.5	49.9
4	76.0	25.1	49.9
5	73.6	25.1	49.9
6	74.8	23.9	49.9
Mean	74.8	24.6	49.9
SD	0.9	0.5	0.4
% CV	1.2	1.9	0.8
% Mean accuracy	99.7%	98.3%	99.9%

Table 18 Autosampler stability results of amivantamab

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	75.4	25.1	48.7
2	75.4	25.1	48.7
3	75.4	24.5	49.3
4	75.4	25.1	49.3
5	75.4	25.1	48.7
6	75.4	25.1	48.7
7	76.0	25.1	48.7
8	74.8	25.1	49.3
9	75.4	25.1	49.3
10	76.0	24.5	49.3
11	76.0	24.5	49.3
12	76.0	24.5	49.3
13	74.8	24.5	49.3
14	73.6	25.1	49.9
15	74.2	24.5	49.9
16	74.2	25.1	49.9
17	74.2	25.1	49.9
18	74.8	25.7	49.9
19	74.2	25.7	49.9
20	74.8	25.1	49.9
21	74.2	25.1	49.9
22	74.2	25.1	49.9
23	74.8	25.1	50.6
24	74.8	25.1	50.6
Mean	74.9	24.9	49.5
SD	0.7	0.4	0.6
% CV	0.9	1.4	1.1
% Mean accuracy	99.9%	99.9%	99.1%

Table 19 Wet extract stability results of amivantamab at 12 h

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	75.4	25.1	49.9
2	73.6	25.1	49.3
3	76.0	25.1	49.3
4	76.7	24.5	49.3
5	74.8	23.9	50.6
6	76.7	24.5	49.9
Mean	75.5	24.7	49.7
SD	1.2	0.5	0.5
% CV	1.6	2.1	1.0
% Mean accuracy	100.7%	98.8%	99.5%

Table 20 Wet extract stability results of amivantamab at 18 h

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	76.0	25.1	49.9
2	76.7	25.7	50.6
3	76.7	24.5	49.9
4	76.0	24.5	50.6
5	74.8	25.1	50.6
6	76.0	24.5	50.6
Mean	76.0	24.9	50.4
SD	0.7	0.5	0.3
% CV	0.9	2.0	0.6
% Mean accuracy	101.4%	99.6%	100.7%

Table 21 Dry extract stability results of amivantamab at 12 h

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	75.4	25.7	49.9
2	75.4	25.1	49.3
3	76.0	25.1	49.3
4	74.2	24.5	49.9
5	76.7	24.5	49.9
6	74.8	23.8	49.9
Mean	75.4	24.8	49.7
SD	0.9	0.7	0.3
% CV	1.2	2.6	0.6
% Mean accuracy	100.6%	99.2%	99.5%

The assay (%) of amivantamab was found to be 99.96%. The results of the assay are shown in Table 23.

Method application to rat plasma samples

The developed and validated procedure was applied to study in rats. The concentrations of amivantamab in rat

Table 22 Dry extract stability results of amivantamab at 18 h

Replicate no	HQC (75.0 ng/ml) Concentration (ng/ml)	LQC (25.0 ng/ml)	MQC (50.0 ng/ml)
1	76.0	25.1	50.6
2	75.4	25.1	49.9
3	74.8	25.1	49.9
4	76.7	24.5	49.9
5	73.6	24.5	49.3
6	73.6	24.5	49.9
Mean	74.9	24.8	49.9
SD	1.3	0.3	0.4
% CV	1.7	1.4	0.8
% Mean accuracy	99.9%	99.2%	99.9%

Table 23 Results of assay

Injection	Area counts
1	3.439×10^5
2	3.474×10^5
Mean	3.457×10^5
% Assay	99.96

Table 24 Concentration of amivantamab in rat samples

Time intervals (minutes)	Amivantamab (ng/ml)	SD	% CV
10	46.216	0.731	1.581
20	28.151	0.640	2.275
30	12.117	0.793	6.548
40	6.517	0.587	9.014
50	2.155	0.414	19.209
60	0	0	0

Table 25 Pharmacokinetic parameters of amivantamab

Pharmacokinetic parameters	Amivantamab
$AUC_{0\text{-}t}$	15.16 ng-h/ml
C_{max}	46.22 ng/ml
$AUC_{0\text{-}\infty}$	15.57 ng-h/ml
T_{max}	10.00 min
$T_{1/2}$	7.80 min

plasma samples are tabulated in Table 24. The pharmacokinetic parameters of amivantamab were calculated using Phoenix Win Nonlin (Version 5.2) software. The results of pharmacokinetic parameters are tabulated in Table 25. The recovery plot of amivantamab in rat plasma is shown in Fig. 7.

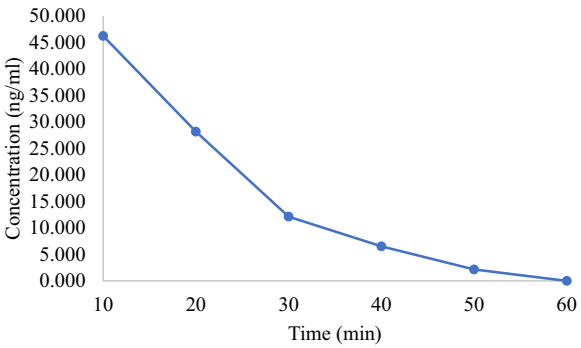

Fig. 7 Recovery plot for amivantamab in rat plasma

Discussion

LC-MS/MS is a sensitive method for the quantification of monoclonal antibodies. Several elution conditions were tested for the chromatographic separation. In trial 1, a mobile phase composition of acetonitrile and triethylamine buffer in the ratio 60:40 was used. Peak splitting was observed, so further trial was carried out. In trial 2, a mobile phase ratio of acetonitrile and ammonium formate buffer (60:40) was used. The plate count was not within the limit. Hence, further trial was carried out. A mobile phase ratio of acetonitrile and ammonium formate buffer (50:50) was used in trial 3. Peak heights were not within the limit, so further trial was conducted. In trial 4, a mobile phase ratio of acetonitrile and ammonium formate buffer (40:60) was used. System suitability parameters were within the limit, so this method was validated. The developed method quantified amivantamab in a biological matrix. The system was deemed suitable for usage if the area

ratio's CV (%) was less than five and the retention time's CV (%) was less than 2. It thus passed the system suitability test. The calibration curve was deemed agreeable when % accuracy for all calibration curve standards ranged from 85.00 to 115.00%. The correlation coefficient (R^2) was 0.99 or better. The method was found to be linear. The response of any interfering peaks at the analyte retention time was to be ≤ 20.00% of amivantamab at LLOQ and ≤ 5.00% of that in LLOQ in the case of trastuzumab. The method was found to be specific and selective. Sensitivity acceptance criteria were to be as 4 out of 6 samples, or at least 67.00%, fell within the 80.00–120.00% range. The recommended range for mean accuracy (%) was 80.00–120.00%. The CV's (%) accuracy was to be 20.00%. The outcomes fell within the permitted range. The method was found to be sensitive. The standards for data acceptance included accuracy (%) within 85.00–115.00% of the actual values and precision within 15.00% relative standard deviation (RSD). These findings demonstrated that the accuracy and precision were reproducible and dependable for quantifying amivantamab in rat plasma. If the analyte concentration detected in the double blank sample was less than 20.00% for amivantamab, carryover was deemed significant. Hence, there was no carryover effect. CV (%) and mean accuracy in dilution integrity were within the limits for amivantamab. The minimum acceptance standard required that two out of three samples at every level fell under the 85.00 to 115.00% range. The matrix lot was to be within the agreeable criteria in at least 80.00% (5 out of 6 cases). The results were within the tolerable range. Hence, the matrix effect was found to be negligible. For each QC level, the CV (%) of recovery was to be under 15.00%. For all QC levels, the mean recovery CV (%) was to be under 20.00% overall. All of the results fell within desirable limits. The overall mean recovery (%) and CV (%) were less than 20.00% for all QC levels. The range of the mean accuracy for low-, medium-, and high-quality control samples was between 85.00 and 115.00%. The results were found within tolerable limits. The results were within the tolerable range. This specifies that the extraction technique used was effective. The limitations were all met in reinjection reproducibility. The method was found to be reproducible. The CV (%) of low- and high-quality control samples was ≤ 15.00%. The CV (%) and mean accuracy were within the standard limits. Any condition, time period, or analyte concentration examined had less than 15.00% of CVs. All the stability results were within the tolerable range. The range for the LQC and HQC samples' mean concentration accuracy was between 85.00 and 115.00%. LQC and HQC samples were to have a CV (%) of less than 15.00%. The results showed that amivantamab was stable in rat plasma. CV (%) and mean accuracy (%) were within the limits. Samples were deemed stable if the CV (%) for the low-, medium-, and high-quality control samples was less than 15.00%. It showed that the stability of the autosampler was determined to be within limits. Moreover, the mean accuracy and CV (%) were within limits. The CV (%) and mean accuracy (%) for amivantamab passed the wet and dry extract stability. As a result, the approach was accurate in various conditions. Through the study of three QC samples of amivantamab the application of various storage conditions, stability of the drug was evaluated. The findings were consistent throughout the studies conducted. These stability results indicate that amivantamab was stable during benchtop, freeze–thaw, autosampler, short term, long term, wet extract, and dry extract stability studies. Also, amivantamab was stable during the storage and handling of samples in rat plasma matrix. The study confirmed that the bioanalytical method was accurate and can be used to study pharmaceutical dosage forms. The validated technique was sensitive enough to quantify analyte in plasma samples in experimental rats accurately. The pharmacokinetic findings illustrate less absorption and metabolism effects on amivantamab in rats. These findings will be helpful in further pharmacokinetic assessments.

Conclusions

This was the first fully validated stability, indicating that the LC-MS/MS technique was developed to measure amivantamab in pharmaceutical preparations and rat plasma. A precise, easy, and repeatable method for measuring amivantamab in rat plasma was developed and validated by ICH M10 guidelines. The validation parameters' findings inferred that the current analysis technique could be used to carry out bioavailability studies with high sensitivity, precision, and accuracy. Also, from the recovery studies, it was found that there was less interaction from the matrix to monoclonal antibody and less absorption or distribution in the rats. It was strongly advised to evaluate the quality of medications during routine analyses or stability studies. Rat plasma samples can be analyzed in clinical investigations using this fully validated approach.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-024-00629-x.

Additional file 1. Supporting data of few validation parameters.

Acknowledgements
The authors are thankful to the management of GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India, for providing necessary facilities and M.V.V.S Murthi fellowship grants to carry out the research work.

Author contributions
PKG conceived and designed the analysis, collected samples, collected data, performed the analysis, and wrote the manuscript. SR conceived and designed the analysis. All authors have read and approved the manuscript.

Funding
Not applicable.

Availability of data and material
Data will be made available on request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The authors declare no conflict of interest.

Competing interest
The authors declare that there are no conflicts of interest.

Received: 15 December 2023 Accepted: 29 March 2024
Published online: 05 April 2024

References

1. Xia Y, Han H, Gu R, Tao R, Lu K, Sun Z, Shen S, Wang A, Lu Y (2023) The efficacy and hemorheological indexes of ginseng and its active components for patients with non-small cell lung cancer: a systematic review and meta-analysis. J Oncol 2023:3144086
2. Sher T, Dy GK, Adjei AA (2008) Small cell lung cancer. Mayo Clin Proc 83(3):355–367
3. Chen R, Manochakian R, James L, Azzouqa AG, Shi H, Zhang Y, Zhao Y, Zhou K, Lou Y (2020) Emerging therapeutic agents for advanced non-small cell lung cancer. J Hematol Oncol 13:58
4. Wu AH, Henderson BE, Pike MC, Yu MC (1985) Smoking and other risk factors for lung cancer in women. J Natl Cancer Inst 74:747–751
5. Alberg AJ, Samet JM (2003) Epidemiology of lung cancer. Chest 123:21–49
6. Wang J, Chi Y, Chen H, Jia B, Zhai X, Ma M, Li J, Zhuo M (2022) Analysis of the efficacy and safety of amivantamab in non-small cell lung cancer patients with EGFR/MET gene abnormalities: a single center's experience. Chin J Cancer 25(7):493–500
7. Russell MC, Garelli AM, Reeves DJ (2023) Targeting EGFR Exon 20 insertion mutation in non-small cell lung cancer: amivantamab and mobocertinib. Ann Pharmacother 57(2):198–206
8. Vyse S, Huang PH (2022) Amivantamab for the treatment of EGFR exon 20 insertion mutant non-small cell lung cancer. Expert Rev Anticancer Ther 22(1):3–16
9. Eswarudu MM, Rao AL, Vijay K (2022) Development and validation of a LC-MS/MS method for simultaneous quantification of Ivabradine and metoprolol in rat plasma. J Pharmacol Toxicol Methods 116:107186
10. Booth B, Vazvaei F, Fluhler E, Myler H, Woolf E (2019) AAPS Workshop Report on ICH M10. AAPS 22:10

Publisher's Note