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Original Article

BIO-ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AVELUMAB, AXITINIB AND ITS APPLICATION TO PHARMACOKINETIC STUDIES IN RABBIT PLASMA BY USING LCMS/MS

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ABSTRACT

Objective: An easy, quick, precise, active and reproducible LC-MS/MS technique was developed for the bioanalytical method of Avelumab and Axitinib using Cytarabine as an internal standard.

Methods: This article summarizes the recent progress on bioanalytical LC-MS/MS methods using waters x-bridge phenyl column (150x4.6 mm, 3.5µ) column and organic mobile phase of 0.1% Tri fluoro acetic acid and Acetonitrile in 50:50 ratio.

Results: The calibration curve was linear in the range of 2-40 ng/ml for avelumab and 0.5-10 ng/ml axitnib. Accuracy, precision, recovery, matrix effect and stability results were found to be within the suitable limits. Simple and efficient method was developed and utilized in pharmacokinetic studies to see the investigated analyte in body fluids.

Conclusion: The application denotes all the parameters of system suitability, specificity, linearity and accuracy are in good agreement with USFDA guidelines and applied effectively for the investigation of pharmacokinetic studies in rabbit.

Keywords: Avelumab, Axitinib, LC-MS/MS, USFDA guidelines, Rabbit plasma

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INTRODUCTION

Avelumab is a fully human monoclonal antibody medication for the treatment of merkel cell carcinoma, urothelial carcinoma, and renal cell carcinoma [1]. In adults and children at least 12 y of age, treatment of a particular form of skin cancer, metastatic merkel cell carcinoma (MCC) [2]. Up to 12% of patients with MCC have incorrectly prognosed distant metastatic disease (mMcc). And progression to mmolcc is fruequentin up to 21 percent in patients with local or regional disease [3]. Although no prospective clinical chemotherapy [4] have been performed and no regime has been officially approved for mmolCC treatment, combinations of platinum/etoposide have been commonly used and reasonably high objective response rates (ORRs) have been achieved, response time, however, is limited and no significant survival benefit has been reported. Highlighting the need for alternative treatments. Recently, clinical trials with immune checkpoint inhibitors targeting the programmed death-ligand 1(PD-L1)/programmed death 1(PD-1) interaction have shown clinical activity and durable responses in patients with advanced MCC [5]. Avelumab is given by an infusion into the vein through a special filter over 60 min every two weeks. Avelumab gives side effects to a few patients after discontinuation, such as immune-related side effects and other common side effects, such as feeling tired, muscle pain, muscles, joints, tendons, ligaments, nerves, and increased liver enzymes [6-10].

Axitinib is a small molecule Tyrosine kinase inhibitor [11] developed by Pfizer under the trade name Inlyta which take orally. It has been shown to significantly inhibit the growth of breast cancer in animal models [12]. And has shown partial response in clinical trials with Renal cell carcinoma (RCC) and several other tumour types [13]. It was approved for RCC by the U. S. Food and drug Administration after showing a modest increase in Progression-free survival. There have been reports of fatal adverse effects. Most common effects are Diarrhea, High blood pressure [14], Fatigue, Loss of appetite, Anemia [15].

In drug discovery and production, bioanalysis is an integral component. Bioanalysis is related to the analysis of analytes in biological samples (drugs, metabolites, biomarkers) and requires several phases from sample collection to analysis of samples and reporting of results. The first phase is the selection of samples from clinical or preclinical trials, then sending the samples for analysis to the laboratory. Sample clean-up is the second step and it is a very critical step in bioanalysis. A robust and stable sample preparation system should be implemented in order to reach accurate results. The task of sample preparation is to remove interference from the matrix of the sample and improve the efficiency of the analytical method. Preparation of samples is often labour intensive and time-consuming. The last step is the examination and detection of samples. The method of choice in bioanalytical laboratories is liquid chromatography-tandem mass spectrometry (LC-MS/MS) for separation and detection. This is attributed to the high selectivity and high sensitivity of the LC-MS/MS technique. In addition, the information about the analyte chemical structure and chemical properties is important to be known before the start of bioanalytical work. This review provides an overview of bioanalytical method development and validation. The main principles of method validation will be discussed. Commonly used sample preparation techniques will be presented. In addition, the role of LC-MS/MS in modern bioanalysis will be discussed. In the present review, we have our focus on the bioanalysis of small molecules. Till date, no method is available for bioanalysis of eliglustat in any type of biological matrix. This is the first time to report a bioanalytical method for these drugs.

MATERIALS AND METHODS

Chemicals and reagents

Acetonitrile and Tri fluoro acetic acid-water (HPLC grade) were purchased from Merck (India) Ltd, Wroli, Mumbai, India. All APIs of Avelumab and Axitinib as reference standards were procured from spectrum pharma research solutions pvt ltd, Hyderabad.

Equipment

An HPLC system (waters alliance e2695 model) connected with mass spectrometer QTRAP 5500 triple quadrupole instrument (sciex) was used. By the Empower 2.0 software operation was performed [16-18].

Pharmacokinetic study

Selection of animals

In vivo pharmacokinetic studies, 6 healthy white New Zealand rabbits (2.0-2.5 kg) were obtained from Biological E Limited,

Hyderabad, India. The protocol of the animal study was approved by institute of animal ethics committee (Reg. No: 1074/PO/ Re/S/05/CPCSEA).

Chromatographic conditions

Chromatographic separation, using x-bridge phenyl (150 x 4.6 mm, 3.5 micron) columns, was administered in isocratic mode at room temperature. As a mobile phase, a mix of 0.1 percent trifluoroacetic acid and acetonitrile at 50:50 v/v with a flow of 1.0 ml/min was used. 10 µl was the injection rate and the run time was 8 min.

Preparation of standard and internal control samples

Preparation of standard stock solution

Take 20 mg of the Avelumab and 5 mg of Axitinib working standards is taken into a 100 ml volumetric flask and 70 ml of diluents and sonicate for 10 min to dissolve the contents completely and makeup to the mark with diluent. Further dilution by taking 0.1 ml into 100 ml volumetric flask. From the above solution 4 ml of the solution is taken into the 10 ml volumetric flask and makeup to the mark with the diluent.

Preparation of internal standard

Take 50 mg internal standard of Cytarabine into a 100 ml volumetric flask and makeup to the mark with diluent and sonicate for ten minutes to dissolve the contents completely. From this solution, take 0.2 ml of the solution into 50 ml volumetric flask. From the above solution 1 ml is taken into the 10 ml volumetric flask and makeup to the mark with the diluent.

Preparation of standard solution

For standard preparation, 200 µl of plasma was taken and 300 µl of ACN into a 2 ml centrifuge tube and 500 µl of standard stock solutions and 500 µl of IS and 500 µl of diluents were added and vortexed for 10 min. These samples were further subjected for centrifuge at 5000rpm for 30 min. Collect the solution and filter through 0.45µ nylon syringe filter and the clear solution was transferred into vial and injected into a system.

Bioanalytical Method validation

The method was validated [19-27] in selective, sensitive, linearity, accuracy and precise, matrix condition, recovery study, re-injection reproducibility and stability.

Selectivity

By analyzing the six different rabbit's plasma samples and to check interference at the retention time, selectivity was conducted.

Matrix effect

By comparing the height area ratio from the six various drug free plasma samples for avelumab and axitinib to get matrix effect. Experiments were performed at MQC levels in triplicate with six different plasma lots with a suitable precision of ≤ 15 %.

Precision and accuracy

It was determined by replicate analysis of internal control samples at a lower limit of quantification (LLOQ), low-quality control (LQC), medium quality control (MQC), top quality control (HQC) levels. The half of CV should be less than 15 % and accuracy should be within 15% except LLOQ where 20%.

Recovery

The analysis of six samples reproduce at each internal control concentration is by extracting the avelumab and axitinib. By comparing the height areas of extracted standards to the height areas of unextracted standards, recovery is evaluated.

Carryover

Carryover [28, 29] deals with the analyte retained by the chromatographic system during the matrix with an analyte concentration ULOQC and above the diluting this sample with blank matrix.

Dilution integrity

By spiking the matrix with an analyte concentration above the ULOQC and diluting this sample with a blank matrix, the dilution integrity should be explained.

Stability

By comparing the act of stock solution stability [30] under the stability sample with the sample from the fresh stock sample preparation. Sample Stability studies in plasma were performed at the LQC and HQC concentration levels using six replicates at each level. Analyte was considered stable if the change is smaller amount than 15 % as per US FDA guidelines. The perfectness of spiked rabbit plasma stored at room temperature was evaluated for twenty-four hrs. The stability of spiked rabbit plasma stored at RT in autosampler was evaluated for 24 h. The autosampler stability (LQC, MQC and HQC) was evaluated by comparing the extract plasma samples that were injected immediately with the samples that were re-injected after storing with wet extract stability at room temperature after 12 h and 18 h at 2-8 °C. The reinjection reproducibility was evaluated by comparing the extracted plasma samples that were injected immediately with the samples that were re-injected after storing in the dry extract stability at room temperature after 12 h and 18h at-20 °±3 °C. The freeze-thaw stability was conducted by comparing the steadiness samples that had been frozen at-31 °C and thawed 3 times with freshly spiked internal control samples. The short-term stability was conducted 7 d at 7 °C. For long-term stability evaluation, the concentrations obtained after 24 h were compared with the initial concentration.

Pharmacokinetic study

Before experimentation, all animals are starved overnight and had water ad-libitum. Topical anesthetic procedure was used. Pharmacokinetic evaluation was performed for avelumab and axitinib formulations. The samples were administered to each rabbit under fasting conditions. After oral administration of avelumab and axetinib, blood samples were collected from rabbit marginal ear vein using a 25-guage, 5/8 inch needle by clipping the marginal ear vein with a paper clip shown in fig. 1 with the volume of 0.5 ml to 1.0 ml at 0.5,2,4,8,12,16,20,24,28,32 and 36 h. The blood was collected in Eppendorf containing 10% EDTA solution. Blood was centrifuged at 5000 rpm for 30 min at 2-8 °C temperature. The clear supernatant plasma were collected and stored at-30 °C till its analysis. The plasma samples were treated for liquid-liquid phase extraction and analyzed for drug content with a developed analytical method. After the study, the animals were returned to the animal house for rehabilitation.

The pharmacokinetic parameters for avelumab and axetinib oral administration were determined from plasma concentration data. Pharmacokinetic parameters like AUC, Cmax, Tmax the time at which Cmax occurred, Kel, t½, Ka and MRT were calculated using the data. Data was measured by the trapezoidal rule method from time zero to infinity of the concentration-time curve. Cmax and Tmax were obtained from the graph. All values are expressed in mean±SD.

Fig. 1: **Sampling of rabbit**

RESULTS AND DISCUSSION

The maximum response on air pressure chemical ionization mode selected in this method is by having the electrospray ionization. The

mobile phase flow of 10 µl/min avelumab and axitinib are highly responsive in the positive ion mode to offer sensitivity and signal stability with the continuous flow to the electrospray ion.

Specificity

The specificity of the method to research Avelumab and Axitinib simultaneously is proved. The chromatograms of blank and standard as shown in fig. 2, 3. The chromatograms of blank rabbit plasma and standard having no interference peaks were observed.

Matrix effect

Percent RSD for within the signal, ion suppression/enhancement was observed as 1.0 percent for Avelumab and Axitinib in LC-MS/MS, suggesting that under these circumstances, the matrix effect [31] on analyte ionization is within an acceptable range of ionization. In matrix effect, LQC and HQC of Avelumab were 99.6 and 99.9 and axitinib were 99.4, 99.8%. %CV of both drugs at LQC level were 1.31, 1.28 and HQC level is 0.28, 1.61 respectively. It indicates that the matrix effect on the ionization of the analyte is within the suitable limit.

Linearity

The peak area ratio of calibration standards was proportional to the concentration. The concentration range of Avelumab is 2-40 ng/ml and Axitinib is 0.5-10 ng/ml. Linearity results of Avelumab and Axitinib were shown in following table 1 and their calibration plots were shown in fig. 4 [32]. The calibration curves were appeared linear and the coefficient of correlation was found to be 0.999 for Avelumab and Axitinib.

Precision and accuracy

By pooling all individual assay results of different internal control samples, the accuracy and precision were calculated. It was obvious, based on the data provided, that the strategy was precise and effective. The precision results of avelumab and axitinib were shown in table 2, 3. avelumab accuracy results in quality control samples 98.8-99.9 and axitinib accuracy results in quality control samples 99.4-99.8. Half of Avelumab and Axitinib CV is<5% of total internal control samples.

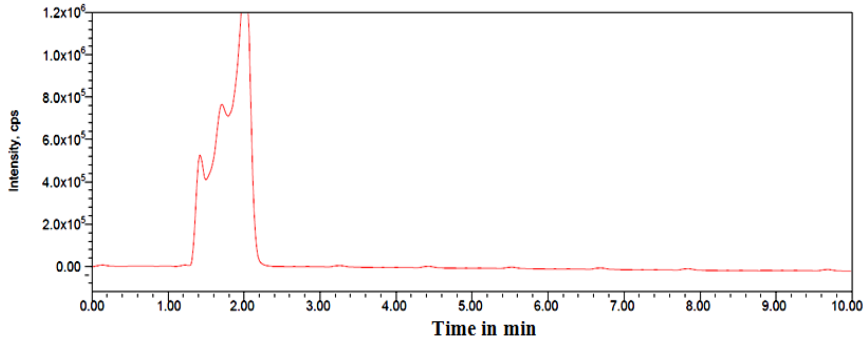


Fig. 2: Chromatogram of blank

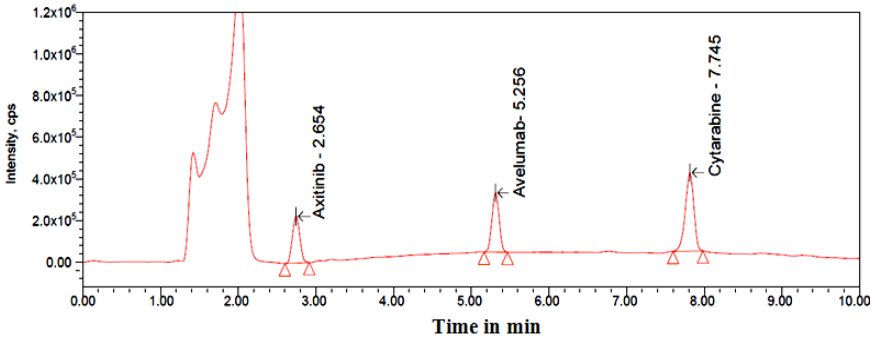
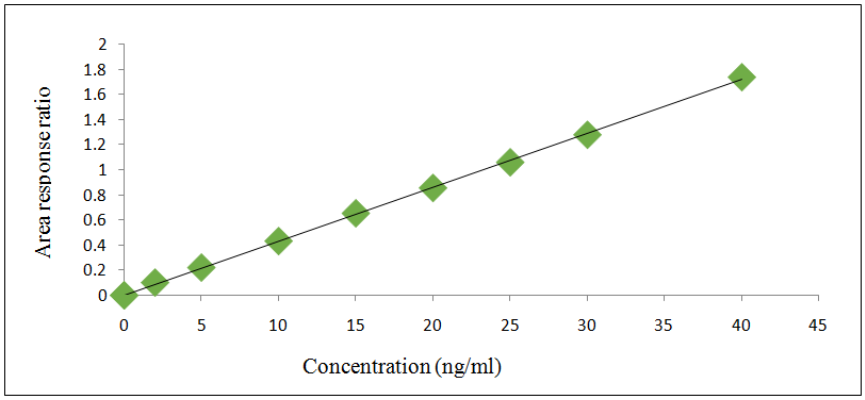


Fig. 3: Chromatogram of standard

Table 1: Results of linearity

Linearity	Avelumab		Axitinib	
	Conc. (ng/ml)	Area response ratio	Conc. (ng/ml)	Area response ratio
1	2	0.102	0.5	0.054
2	5	0.221	1.25	0.119
3	10	0.432	2.5	0.240
4	15	0.653	3.75	0.362
5	20	0.855	5	0.513
6	25	1.059	6.25	0.613
7	30	1.278	7.5	0.733
8	40	1.737	10	0.996
Slope		0.0420	Slope	0.0970
Intercept		0.01672	Intercept	0.00395
CC		0.99958	CC	0.99915



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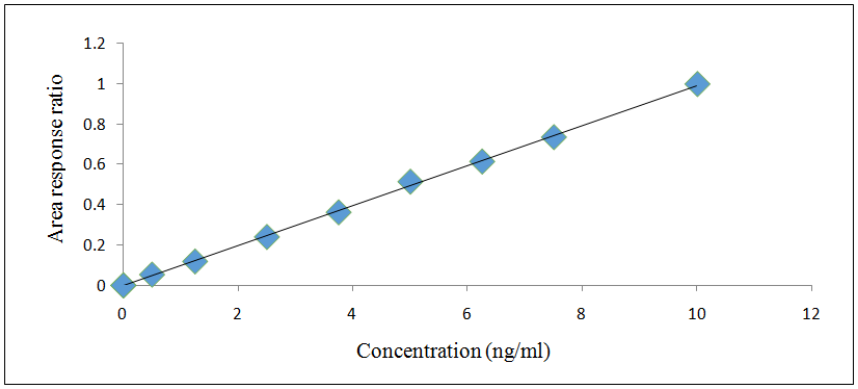

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Fig. 4: Calibration plots of (A) Avelumab and (B) Axitinib

Table 2: Precision and accuracy of avelumab

QC name	LLQC	LQC	MQC	HQC
Conc.(ng/ml)	2 ng/ml	10 ng/ml	20 ng/ml	30 ng/ml
QC sample-1	2.182	10.194	20.165	30.138
QC sample-2	2.314	10.652	20.125	30.125
QC sample-3	2.568	10.353	20.145	30.242
QC sample-4	2.478	10.485	20.256	30.356
QC sample-5	2.121	10.865	20.569	30.458
QC sample-6	2.957	10.586	20.685	30.567
Mean	2.4133	10.506	20.313	30.308
SD	0.302	0.262	0.251	0.185
%CV	1.124	0.965	0.975	0.856
Accuracy	99	99.624	99.182	100

Mean+SD (n=6)

Table 3: Precision and accuracy of axitinib

Qc name	LLQC	LQC	MQC	HQC
Conc.(ng/ml)	0.5	2.5	5	7.5
QC sample-1	0.512	2.528	5.1	7.526
QC sample-2	0.534	2.678	5.248	7.589
QC sample-3	0.538	2.798	5.384	7.682
QC sample-4	0.548	2.854	5.468	7.763
QC sample-5	0.553	2.93	5.528	7.542
QC sample-6	0.567	2.961	5.687	7.524
Mean	0.542	2.791	5.402	7.604
Stddev	0.0171	0.149	0.190	0.089
%CV	1.895	0.985	0.885	0.985
Accuracy %	99.145	98.354	99.568	100.128

Mean+SD (n=6)

Recovery

The recoveries for Avelumab and Axitinib at LQC, MQC and HQC levels the results demonstrated that the bioanalytical method had good extraction efficiency. This also showed that the recovery wasn't hooked into concentration. The recoveries for Avelumab (98.81%-100.62%) and Axitinib (99.41%-100.18%) at LQC, MQC and HQC levels and % CV ranged from 0.21-0.72 for Avelumab and 0.84-1.83 for Axitinib. The results demonstrated that the bioanalytical method had good extraction efficiency.

Ruggedness

The percent recoveries and percent CV of Avelumab and Axitinib determined with two different analysts and on two different columns were within acceptable criteria in HQC, LQC, MQC and LLQC samples. The results proved method is ruggedness. The percent recoveries ranged from 99.61–100.73% for Avelumab and 99.24%-99.91% for Axitinib. The %CV values ranged from 0.09-0.31for Avelumab and 0.61–1.11 for Axitinib. The results proved method is ruggedness.

Autosampler carryover

Peak area response of Avelumab and Axitinib, wasn't observed within the blank rabbit plasma samples after successive injections of LLQC and ULQC at the retention times of Avelumab and Axitinib. In autosampler carryover this method doesn't exhibit autosampler carryover.

Stability

Avelumab and Axitinib solutions were prepared with diluents for solution stability analysis and placed in a refrigerator at 2-8 °C. Fresh stock solutions were associated with stock solutions that were prepared 24 h earlier. The plasma stability of the benchtop and autosampler was stable for twenty-four hours, and 24 h at 20 °C in the autosampler. It became apparent from future stability that Avelumab and Axitinib were stable at a storage temperature of-30 °C for up to 24 h. The overall stability results of avelumab and axitiniib have been stated in the below table 4, 5.

Table 4: Stability results of avelumab

Stability experiment spiked plasma		Spiked plasma conc.(n=6, ng/ml)	Conc. Measured (n=6, ng/ml)	%CV
Bench top stability	LQC	10	10.135	1.246
	MQC	20	20.257	0.858
	HQC	30	30.458	0.968
Auto sampler stability	LQC	10	10.897	0.952
	MQC	20	20.589	0.856
	HQC	30	30.124	0.977
Long term(Day28) stability	LQC	10	10.368	0.985
	MQC	20	20.354	0.856
	HQC	30	30.126	0.746
Wet extract stability	LQC	10	10.328	0.789
	MQC	20	20.856	0.852
	HQC	30	30.175	0.845
Dry extract stability	LQC	10	10.689	0.963
	MQC	20	20.657	0.784
	HQC	30	30.821	0.894
Freeze thaw stability	LQC	10	10.628	0.854
	MQC	20	20.145	0.874
	HQC	30	30.286	0.745
Short term stability	LQC	10	10.369	0.841
	MQC	20	20.486	1.456
	HQC	30	30.289	1.025

mean±SD (n=6)

Table 5: Stability results of axitinib

Stability experiment spiked plasma		Spiked plasma conc.(n=6,ng/ml)	Conc. Measured (n=6,ng/ml)	%CV
Bench top stability	LQC	2.5	2.534	1.042
	MQC	5	5.12	0.986
	HQC	7.5	7.548	0.974
Auto sampler stability	LQC	2.5	2.525	0.981
	MQC	5	5.321	0.874
	HQC	7.5	7.584	0.954
Long term	LQC	2.5	2.587	0.845
(Day 28)stability	MQC	5	5.874	0.768
	HQC	7.5	7.582	0.734
Wet extract stability	LQC	2.5	2.574	0.861
	MQC	5	5.369	0.827
	HQC	7.5	7.514	0.965
Dry extract stability	LQC	2.5	2.542	1.142
	MQC	5	5.841	1.254
	HQC	7.5	7.586	0.964
Freeze thaw stability	LQC	2.5	2.564	0.985
	MQC	5	5.684	1.246
	HQC	7.5	7.521	1.103
Short term stability	LQC	2.5	2.574	0.824
	MQC	5	5.231	0.987
	HQC	7.5	7.541	1.485

mean±SD (n=6)

***In vivo* pharmacokinetic evaluation**

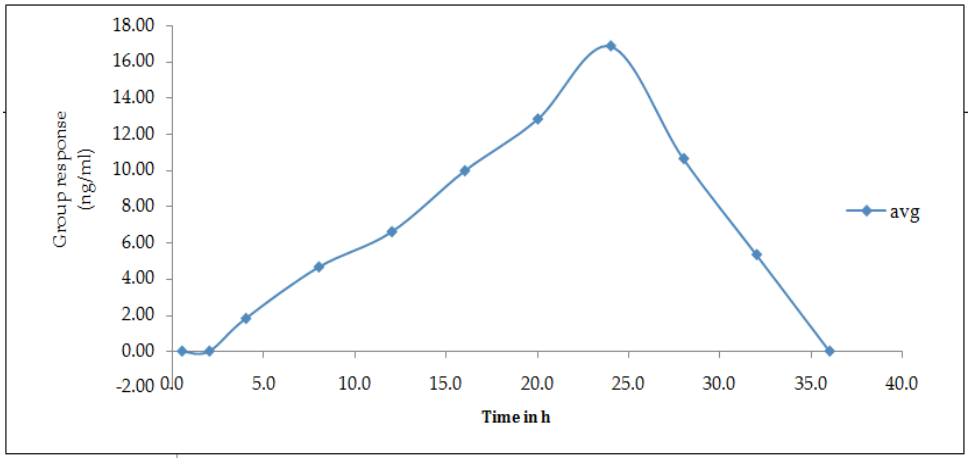
The plasma concentration-time profiles of avelumab and axitinib in rabbit are shown in fig. 5. The graph indicated a bell-shaped curve in both cases of the experimental formulation. Avelumab and axitinib could be traced to be present in the blood for 24 h and 4 h after oral administration, which indicates the effectiveness of drug release from the formulation.

The pharmacokinetic parameters Cmax, Tmax, T1/2, Kel, Ka, AUC0-t, AUC0-∞, AUMC 0-24, AUMCt-∞, MRT0-24, MRT0-∞ were calculated and the data is shown in table 6. The Cmax for avelumab and axitiniib were found to be 16.9 ng/ml and 4.9 ng/ml, respectively. The Tmax for avelumab and axitiniib were found to be 24h and 4h, respectively. The t½ values were 32h and 24h respectively for avelumab and axitiniib. The Kel for avelumab and axitiniib 0.41 and 0.005 h-1. The Ka values of avelumab and axitiniib were found to be 0.13 and 1.41 h-1, respectively. The AUC0-t for avelumab and axitiniib were found to be 273 and 63 ng-h/ml, respectively. The values of AUMC0-∞ and AUMC0-t for ng-hr/ml were found to be 352.18, 141.11 µg h ml-1 and 273.06, 63.18 µg h ml-1. The MRT0-24 and MRT0-∞ for ng-hr/ml were found to be 24.17, 6.31 and 36.0, 36.0 respectively, shown in table 6.

Table 6: Pharmacokinetic parameters of avelumab and axitinib

Pharmacokinetic parameters	Avelumab	Axitinib
AUC_{0-t}	273 ng-h/ml	63 ng-h/ml
C_{max}	16.9 ng/ml	4.9 ng/ml
$AUC_{0-\infty}$	352 ng-h/ml	141 ng-h/ml
t_{max}	24 h	4h
$T_{1/2}$	32 h	24h
k_{el}	0.41 h-1	0.005 h-1
Ka	0.13 h-1	1.41 h-1
MRT0-24	24.17 ng-h/ml	6.31 ng-h/ml
MRT0-∞	36.0 ng-h/ml	36.0 ng-h/ml

$AUC_{0-\infty}$: Area under the curve extrapolated to infinity, AUC_{0-t}: Area under the curve up to the last sampling time, C_{max}: The maximum plasma concentration, T_{max}: The time to reach peak concentration, $T_{1/2}$: Time the drug concentration, Kel: Elimination rate constant, Ka: Absorption rate constant, MRT: Mean residence time.



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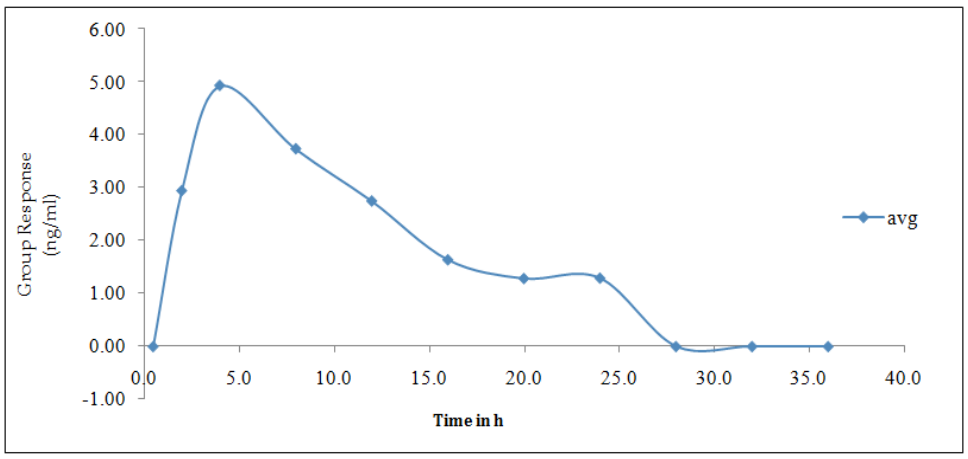

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Fig. 5: Recovery plot (A) Avelumab and (B) Axitinib

CONCLUSION

For the primary time higher sensitive HPLC-ESI-LCMS/MS method was developed and validated for the determination of Avelumab and Axitinib in rabbit plasma. Here the described method is rugged, fast, reproducible bioanalytical method. This method was validated according to USFDA guidelines. Simple and efficient method was developed and may be utilized in pharmacokinetic studies and to see the investigated analyte in body fluids.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Author declares that there have been no conflicts of interest.

REFERENCES

1. Quinn DI, Lara PN. renal-cell cancer-targeting an immune checkpoint or multiple kinases. N Engl J Med 2015;373:1872–4.
2. Pulitzer, Melissa. Merkel cell carcinoma. Surgical Pathol Clin 2017;10:399–408.
3. C Lebbe, JC Becker, JJ Grob, J Malvehy, V Del Marmol, H Pehamberger, *et al.* Diagnosis and treatment of merkel cell carcinoma. European consensus-based interdisciplinary guideline. Eur J Cancer 2015;5:2396-403.
4. Beumer JH, Chu E, Salamone SJ. Body-surface area-based chemotherapy dosing: appropriate in the 21st century? J Clin Oncol 2012;30:3896–7.
5. D Schadendorf, C Lebbe, A ZurHausen, MF Avril, S Hariharan, M Bharmal, *et al.* Merkel cell carcinoma: epidemiology, prognosis, therapy and unmet medical needs. Eur J Cancer 2017;71:53-69.
6. PJ Allen, WB Bowne, DP Jaques, MF Brennan, K Busam, DG Coit. Merkel cell carcinoma: prognosis and treatment of patients from a single instituition. J Clin Oncol 2005;23:2300-9.
7. Johnston DE. Special considerations in interpreting liver function tests. Am Fam Physician 1999;59:2223–30.
8. P Nghiem, HL Kaufman, M Bharmal, L Mahnke, H Phatak, JC Becker. Systematic literature review of efficacy, safety and tolerability outcomes of chemotherapy regimens in patients with metastatic Merkel cell carcinoma. Future Oncol 2017;13:1263-79.
9. PT Nghiem, S Bhatia, EJ Lipson, RR Kudchadkar, NJ Miller, L Annamalai, et al. PD-1 blockade with pembrolizumab in advanced Merkel cell carcinoma. N Engl J Med 2016;374:2542-52.
10. Kwo, Paul Y, Cohen, Stanley M, Lim, Joseph K. acg clinical guideline: evaluation of abnormal liver chemistries. Am J Gastroenterol 2017;112:18–35.
11. Rivera Torres J, Jose ES. Src tyrosine kinase inhibitors: new perspectives on their immune, antiviral, and senotherapeutic potential. Front Pharmacol 2019;10:1011.
12. LJ Wilmes, MG Pallavicini, LM Fleming, J Gibbs, D Wang, KL Li, et al. AG-013736, a novel inhibitor of VEGF receptor tyrosine kinases, inhibits breast cancer growth and decreases vascular permeability as detected by dynamic contrast-enhanced magnetic resonance imaging. Magn Reson Imaging 2007;25:319-27.
13. B Rini, O Rixe, R Bukowski, MD Michaelson, G Wilding, G Hudes, et al. AG-013736, a multi-target tyrosine kinase receptor inhibitor, demonstrates anti-tumor activity in a phase 2 study of cytokine-refractory, metastatic renal cell cancer (RCC). J Clin Oncol 2005;23:4509.
14. Poulter NR, Prabhakaran D, Caulfield M. Hypertension. Lancet 2015;386:801–12.
15. Guagnozzi D, Lucendo AJ. Anemia in inflammatory bowel disease: a neglected issue with relevant effects. World J Gastroenterol (Review) 2014;20:3542–51.
16. Potturi Ramadevi, Kantipudi Rambabu. Bioanalytical method development and validation for ezetimibe and pitavastain and its applications to pharmacokinetic studies in rabbit plasma by using LCMS/MS. Int J Res Pharm Sci 2020;11:7854-62.
17. Asha Eluru, Surendra Babu K. Bioanalytical method development and validation for aplidine in rat plasma and their pharmacokinetic studies by LCMS. WJPPS 2019;8:1201-9.
18. D Ramchandran, Anita Kethipalli, Mannam Krishnamurthy. Bio analytical method development and validation of daunorubicin and cytrarabine in rat plasma by LC-MS/MS and its application in pharmacokinetic studies. J Pharm Sci Res 2020;12:381-6.
19. Mukta D Naykode, Durgacharan A Bhagwat, Swapnil D Jadhav, Harinath N. Analytical and bioanalytical method for quantification of pure azilsartan, not its salt by RP-HPLC. Res J Pharm Tech 2017;10:708-14.
20. Mayanka Singh, Manoj Charde, Rajesh Shukla, Rita MC. Determination of calcipotriene in calcipotriene cream 0.05% w/w by RP-HPLC method development and validation. Res J Pharm Tech 2011;4:1219-23.
21. Malathi S, Arunadevi N. Development and validation of stability-indicating simultaneous estimation of metformin and alogliptin in tablets by high-performance thin-layer chromatography. Int J Pharm Pharm Sci 2020;12:68-73.
22. Senthil Rajan D, Muruganathan G, Shivkumar K, Ganesh T. Development and validation of hplc method for simultaneous quantification of vasicine, glycyrrhizin and piperine in polyherbal cough syrup. Int J Curr Pharm Res 2020;12:15-9.
23. Palani Shanmugasundaram, Kamarapu SK. RP-HPLC method for the simultaneous estimation and validation of amlodipine besylate and atenolol in bulk and tablet dosage form in biorelevant dissolution medium (Fassif). Res J Pharm Tech 2017;10:3379-85.
24. Gomathy S, Narenderan ST, Meyyanathan SN, Gowramma B. Development and validation of hplc method for the simultaneous estimation of apigenin and luteolin in commercial formulation. J Crit Rev 2020;7:4785-90.
25. Ashutosh Kumar S, Manidipa Debnath, Seshagiri Rao JVLN, Gowri Sankar D. Development and validation of a sensitive RP-HPLC method for simultaneous estimation of rosuvastatin and fenofibrate in tablet dosage form by using PDA detector in Gradient mode. Res J Pharm Tech 2016;9:549-54.
26. Malak Y, Al-Bathish AA, Gazy MK El-Jamal. Rp-hplc and chemometric methods for the determination of two anti-diabetic mixtures; metformin hydrochloride-canagliflozin and metformin hydrochloride-gliclazide in their pharmaceutical formulation. Int J Pharm Pharm Sci 2020;12:83-94.
27. Gadhvi MP, Bhandari A, Suhagia BN, Desai UH. Development and validation of RP-HPLC method for simultaneous estimation of atazanavir and ritonavir in their combined tablet dosage form. Res J Pharm Tech 2013;6:200-3.
28. Koya Prabhakara Rao, Namburi LA Amara Babu, Kalyani Koganti, Babji Palakeeti, Koduri SV Srinivas. Related substances method development and validation of an LC-MS/MS method for the quantification of selexipag and its related impurities in rat plasma and its application to pharmacokinetic studies. SN Appl Sci 2021;3:321.
29. Hasanah YIF, Harahap Y, Suryadi H. Development and validation method of cyclophosphamide and 4-hydroxy cyclophosphamide with 4-hydroxy cyclophosphamide-D_4 as internal standard in dried blood spots using UPLC-MS/MS. Int J Appl Pharm 2021;13:148-52.
30. Naveen VMK, Veeraswami B, Srinivasa Rao G. High response bio analytical validation approach of nadolol and bendroflumethiazide by LC-MS/MS on rat plasma. Int J Res Pharma Sci 2020;11:2272-9.
31. Gowri Kusuma Kumari, Rambabu Kantipudi. Bioanalytical method development and validation for avapritinib in rat plasma by LC-MS/MS. J Pharm Sci Res 2021;13:134-7.
32. Hemanth Kumar AK, Sudha V, Vijayakumar A, Padmapriyadarsini C. Simultaneous method for the estimation of bidaquiline and delamanid in human plasma using high-performance liquid chromatography. Int J Pharm Pharm Sci 2021;13:36-40.
33. Siva Madhu Chaitanya, Srinath Nissankararao, Satya Lakshmi Gandham. A sort of validated bioanalytical method developed for the estimation of etoposide and cisplatin in rat plasma by using two different advanced liquid chromatographic techniques like HPLC and UPLC and its application in bioequivalence studies. Int J Res Pharma Sci 2021;12:708-17.