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ORIGINAL ARTICLE

Development and validation of the quantitative determination of avapritinib in rat plasma by a bioanalytical method of UPLC-MS/MS

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KEYWORDS

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Abstract Avapritinib, an orally consumed, highly selective inhibitor of platelet-derived growth factor receptor alpha (PDGFRA), is approved in the USA for PDGFRA exon 18 (including D842V) mutant gastrointestinal stromal tumour (GIST). The research conducted investigates an advanced reliable and fast UPLC-MS/MS method that could verify and determine avapritinib concentration in plasma of rats. An addition of acetonitrile was incorporated along with the plasma sample in order to precipitate protein with the analyte separated from the matrix by a gradient elution procedure on a Waters Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm). The active stage in mobile phase consists of a mixture of 0.1% formic acid in water and acetonitrile with a 0.40 mL/min flow rate. UPLC-MS/MS detection was done employing a mode of multiple reaction monitoring (MRM), and the ion transitions of avapritinib and imatinib (internal standard, IS) was *m*/*z* 499.10 → 482.09, and *m*/*z* 494.30 → 394.20, respectively. This method has good linearity within 2–4000 ng/mL of avapritinib calibration range and a lower limit of quantification (LLOQ) of 2 ng/mL verified. Avapritinib precisions in both intra-day and inter-day were below 15% with the determined accuracy of −12.9% to 12.0%. The recoveries, stabilities, and matrix effect of avapritinib and IS were credible. 30 mg/kg avapritinib was administered as a single dose orally

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to rats. The determination of avapritinib level in pharmacokinetic studies was accomplished by efficiently applying a newly optimized UPLC-MS/MS assay.

1. Introduction

Avapritinib (also known as BLU-285, Fig. 1A) is an antitumor drug with high safety, selectivity, and tolerability (Heinrich et al., 2020), and is approved in the USA for PDGFRA exon 18 (including D842V) mutant GIST and is undergoing regulatory assessment in the USA as a 4th-line treatment for GIST (Dhillon, 2020; Joseph et al., 2020). The original purpose was to formulate a drug was for it to be capable to aim for KIT and PDGFRA active conformation by type 1 inhibition component, which has been well known as systemic mastocytosis driver mutation (Evans et al., 2017; Heinrich et al., 2020; Klug, Kent, & Heinrich, 2018). Avapritinib has the ability to disrupt KIT signaling in vitro. This is accomplished by KIT phosphorylation inhibition disrupting the activation of AKT and STAT3 downstream proteins present in leukemia cell lines as well as human mast cells (Gebreyohannes et al., 2019; Wu et al., 2019). Avapritinib also has the ability to inhibit the development of tumors depending on applied doses in vivo in systemic mastocytosis mice models (Gebreyohannes et al., 2019). In addition, it can also lead to PDGFRA p.D842V inhibition which is the source of 1/5 primary gastric GIST (gastrointestinal stromal tumor) mutations that were resistant to imatinib (Evans et al., 2017; Gebreyohannes et al., 2019). Oral avapritinib is rapidly absorbed, with a median time to C_{max} of 2.1–4.1 h after single doses of 30–400 (0.1–1.33 times the approved recommended dose) (Heinrich et al., 2020). After once daily administration, steady state of avapritinib was reached by day 15 and the mean accumulation ratio was 3.1 to 4.6. It was largely metabolized by CYP3A4 and to a lesser extent by CYP2C9 in in vitro studies (Alzofon & Jimeno, 2020).

Given that cancer patients often receive multiple drug treatments, it remains to be explored whether the combination of avapritinib and these drugs would cause drug-drug interaction (DDI). Therefore, it is necessary to establish a quantitative analysis method of avapritinib to study its pharmacokinetics and DDI in clinical applications.

At present, there is no relevant literature reported to characterize the pharmacokinetic profile of avapritinib using LC-MS/MS methods. In other words, there is no analytical assay for the quantification of avapritinib in biological media. However, a reliable and sensitive bioanalytical assay is disparately needed for quantitation of avapritinib in routine pharmacokinetic, DDI and therapeutic drug monitoring studies. Therefore, in the research conducted, the aim is to establish and verify the credibility and rapidness of ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method in order to measure avapritinib concentration in rat plasma. In addition, the use of novel developed UPLC-MS/MS method in studying avapritinib in rats was already established in pharmacokinetics.

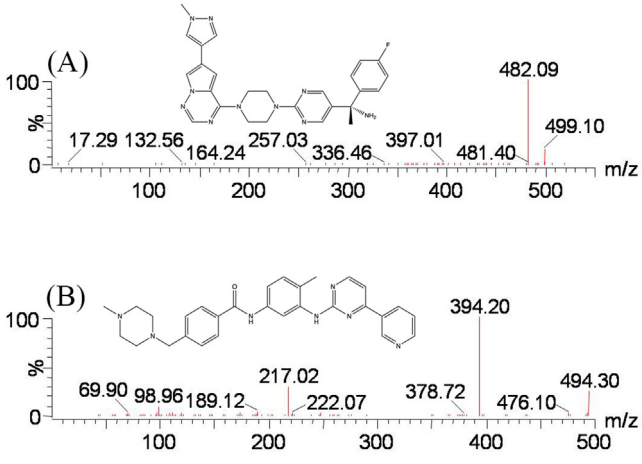

Fig. 1 Mass spectra of avapritinib (A) and imatinib (IS, B) in this study.

2. Experimental

2.1. Chemicals materials

The purity > 98% of avapritinib and imatinib (used as internal standard, IS, Fig. 1B) were used and were purchased from Shanghai Chuangsai Technology Co., Ltd. (Shanghai, China), including formic acid (analytical grade). Chromatographic grade methanol and acetonitrile were used in this study and were purchased from Merck (Darmstadt, Germany). Milli-Q purification system (Millipore, Bedford, USA) was used to generate ultrapure water and used to prepare the mobile phase and all other solutions.

2.2. Animal experiments

The Laboratory Animal Center of Wenzhou Medical Universtiy (Zhejiang, China) have supplied six rats weighed 200 ± 20 g. They were allowed to obtain food and water at their own free will in a feeding room which has a controlled environment for over a week. All experimental operations of the rats were examined and approved by the Institutional Ethics Committee of Wenzhou Medical Universtiy (Zhejiang, China) that strictly followed the rules and regulations in regards to the Care and Use of Laboratory Animals.

After 12 h of fasting, all rats were given orally 30 mg/kg of avapritinib, which was prepared in a 0.5% sodium carboxymethylcellulose (CMC-Na) solution. Blood samples (0.3 mL) were obtained at 0, 0.333, 0.667, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 h after administration and stored within a 1.5 mL polyethylene tube containing heparin. Subsequently, all plasma samples were immediately put under centrifugation for 8 min at 4,000 × g in 25 °C conditions. Then the plasma was collected into clean test tubes before frozen at −80 °C for storage prior to later tests. In this study, levels of avapritinib

in rat plasma concentration were assessed via a bioanalysis method founded on UPLC-MS/MS technology The Drug and Statistics (DAS) 2.0 software (Mathematics and Pharmacology Professional Committee, Shanghai, China) was applied for inspection and calculation of exact pharmacokinetic boundaries of avapritinib through non-compartmental analysis.

2.3. Instrumentations and analytical conditions

Waters ACQUITY UPLC I-Class system (Milford, MA, USA), Waters Xevo TQ-S triple quadrupole tandem mass spectrometer paired with electrospray ionization (ESI) source (Milford, MA, USA) was utilized in order to establish UPLC-MS/MS system for the conducted research. Masslynx 4.1 software along with Quanlynx programme (Milford, MA, USA) was used for data collection and processing for the entire experiment.

Chromatographic separation of avapritinib and IS were done using ACQUITY UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm). And the mobile phase in this study was composed of solvent A (acetonitrile) and solvent B (0.1% formic acid solution). Linear gradient elution of 0.40 mL/min flow rate was done as follows: 0–0.5 min (A, 10–90%), 0.5–1.0 min (A, 90–90%), and 1.0–1.1 min (A, 90–10%). Then, 10% A was used for equilibration from 1.1 to 2.0 min. Each individual volume of the injection was 2.0 μL, and the time for each analysis costs 2.0 min. All samples in the autosampler were kept at 10 °C, while the column was at 40 °C.

Xevo TQ-S triple quadrupole tandem mass spectrometer which was equipped with ACQUITY UPLC system set in positive ion mode was executed. Recognition was done under multiple reaction monitoring (MRM) mode at m/z 499.10 → 482.09 for avapritinib and m/z 494.30 → 394.20 for IS, respectively. The collision energy and cone voltage were respectively 30 eV and 30 V of avapritinib, and that of IS was 25 eV and 10 V respectively. The general parameters after optimizing MS were: 1000 °C desolvation temperature, 2.0 kV capillary voltage, 0.15 mL/min collision gas, 200 L/h cone gas and 1000 L/h desolvation gas.

2.4. Standard solutions, calibration curves and quality control (QC) samples

Avapritinib and IS stock solutions was respectively formed via liquifying each compound in methanol to form a 1.0 mg/mL separate concentration. A dilution was done to the stock solution using methanol in order to gain a working solution, calibration curve and QC samples. Final concentrations of avapritinib in blank plasma were prepared in the range of 2–4000 ng/mL, and the concentration of IS present in the methanol was 200 ng/mL. Additionally, the three final concentrations of QC samples were 5 (LQC), 160 (MQC) and 3200 (HQC) ng/mL, along with the lower limit of quantification (LLOQ) of 2 ng/mL. Both stock and working solutions were formulated and aliquoted in 1.5 mL polypropylene tubes, and stored at −80 °C in advance.

2.5. Sample preparation

A 1.5 mL EP centrifuge tube was obtained before the addition of 100 μL plasma sample, 20 μL IS solution and 300 μL acetonitrile. This mixture was then put into the vortex for a time frame of 1.0 min before centrifugation for 10 min at 13,000 × g at room temperature condition. A new autosampler vial was then used to obtain 100 μL supernatant before the injection of 2.0 μL aliquot supernatant to the chromatographic system for quantitative examination.

2.6. Method validation

The entire method validation procedures for this analytical method, including the calibration curve, selectivity, LLOQ, stability, precision, accuracy, recovery and matrix effects were accomplished under acquiescence with bioanalytical method justification in FDA ethics (Shi et al., 2020; Tang et al., 2020; Xu et al., 2019).

The selectivity of the assay was investigated by checking the absence of interferences from the blank (neither analyte nor IS from six different rats), standard solutions and real rat plasma in correspondence to the retention times of avapritinib and IS.

Peak area ratio plotting avapritinib to IS in contrast with their nominal concentrations was done in order to gain calibration curves. This was accomplished via the use of a weighted ($1/x^2$) least square regression mode. LLOQ was used for evaluation of the sensitivity of the relating method, of which the signal-to-noise ratio (S/N) was above 10, the acceptable accuracy (RE) inside ± 20% and accuracy below 20% of the nominal value.

QC samples (n = 6) with levels of low, medium and high dilutions were detected after undergone 3 continuous cycles in order to complete the evaluation of intra- and inter-day precision and accuracy. Recovery from present approach of sample preparation was studied by comparing the peak area ratio of the extraction before and after. Matrix effect (ME) was also analyzed using the comparison of the responses of the analyte in the plasma matrix after extraction (A) with neat solutions (B) in 6 replicates, and ME = A/B × 100%.

LQC, MQC and HQC samples (5, 160 and 3200 ng/mL) were detected under different storage conditions in order to assess the stability of the spiked plasma analyte. An ambient temperature for 3 h and at −80 °C over a time period of 5 weeks was respectively established in order to assess short-term and long-term stability. In addition, the stability of the spiked sample was evaluated at autosampler storage for 12 h at 10 °C conditions, and 3 complete freeze–thaw stability tests (-80 °C to room temperature) were also being carried out.

3. Results and discussion

3.1. Method development and optimization

During this study, both ESI negative and positive were explored to discover the most sensitive ionization mode for the analyte. The research proved positive ion mode is able to exhibit higher mass response of avapritinib and IS when compared with negative ion mode. The positive molecular ions $[M + H]^+$ of avapritinib and IS was respectively m/z 499.10 and m/z 494.30 (as indicated in Fig. 1), with the richest product ions being m/z 482.09 and 394.20, respectively. Therefore, the precursor-to-daughter of quantifier transitions were m/z 499.10 → 482.09 for avapritinib, and m/z 494.30 → 394.20 for IS, respectively.

Different combinations of organic phase (such as methanol and acetonitrile) and aqueous phase (water, 0.1% acetic acid, 0.1% formic acid and 1 mM ammonium acetate buffer) were assessed. Given that adding formic acid into mobile phase might improve the ionization efficiency and sensitivity of avapritinib and IS, the final suitable mobile stage consisted of acetonitrile and water (containing 0.1% formic acid). Furthermore, different LC columns were investigated and the comparisons of chromatographic separation were carried out. Finally, BEH C18 (2.1 mm × 50 mm, 1.7 μm) column was chosen due to high separation efficiency, lower background noise and good peak symmetry.

3.2. Method validation

3.2.1. Selectivity

Fig. 2 indicates that avapritinib retention time was at 0.71 min while IS equaled 0.68 min. Comparison of the representative MRM chromatograms of 6 individual sourced blank rat plasma, blank plasma added with additional avapritinib and IS standard solutions, and the actual plasma sample from rats demonstrated that no endogenous interfering substances were found, which suggested that the analytical method had good selectivity for the determination of avapritinib and IS in the plasma of rats.

3.2.2. Calibration curve and LLOQ

The calibration curve of avapritinib in the range of 2 to 4000 ng/mL exhibited outstanding linearity. $Y = 0.332162 \times X \pm 0.132643$ ($r^2 = 0.9992$) was the regression equation gained with Y as the peak ratio of avapritinib to IS and X as the matching concentration. At 2 ng/mL (LLOQ), the precision was lower than 15%, and the accuracy was more than −12.9%, which indicated that the method had good sensitivity (as shown in Table 1).

3.2.3. Precision and accuracy

The precision and accuracy of established UPLC-MS/MS analysis were calculated through detecting QC samples in three days (n = 6) at four different concentration levels of HQC, MQC, LQC and LLOQ. As listed in Table 1, under the four determined QC concentration levels, intra and inter-day precision and accuracy of avapritinib were lower than 14.8% and ± 12.9%, respectively. This data showed an indication of described UPLC-MS/MS method providing suitable accuracy and precision for determining avapritinib in rat plasma.

3.2.4. Recovery and matrix effect

Mean recovery of avapritinib at 3 different concentration levels (LQC, MQC and HQC) are presented in Table 2, showing data within the range of 85.2–88.9% and the matrix effect values for avapritinib were 93.5–101.9%, which indicated that no obvious matrix effect was observed in rat plasma under the optimized UPLC-MS/MS conditions.

3.2.5. Stability

The stability of avapritinib in rat plasma from diverse preserved and processing environments was investigated for different stability experiments. As listed in Table 3, it suggested that the plasma avapritinib samples were stable when placed at 10 °C conditions in the autosampler for no less than 12 h, at ambient temperature for 3 h, 5 weeks at −80 °C, and 3 complete freeze (-80 °C)/thaw cycle tests.

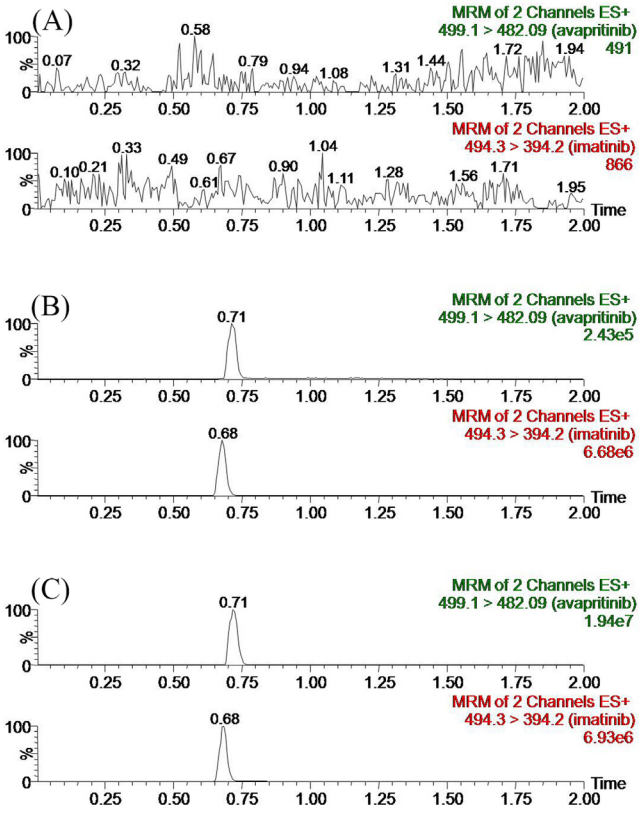

Fig. 2 Representative chromatograms of avapritinib and IS in rat plasma: (A) blank plasma; (B) blank plasma spiked with standard solutions; (C) sample obtained from rat at 1.0 h after oral administration of 30 mg/kg avapritinib.

3.3. Animal study

The recognized UPLC-MS/MS method was effectively utilized for measuring plasma concentration of avapritinib in rats post a single dose of 30 mg/kg orally administration. Fig. 3 showed the mean avapritinib concentrations in rat plasma versus time curve. After analysis of the non-compartment model, the main pharmacokinetic parameters were summarized in Table 4.

Post a single dose of 30 mg/kg avapritinib orally administration, fast absorption was observed reaching maximum plasma concentration (C_{max}) at 3161.31 ± 710.32 ng/mL. Moreover, the peak time (T_{max}) in rat plasma was about 5.5 0 ± 1.76 h. Besides, its elimination half-life ($t_{1/2}$) from rats was 8.43 ± 1.68 h. Considering our study was carried out in rats with only 6 animals, more researches are needed to determine the pharmacokinetic characteristics of of avapritinib accurately.

4. Conclusions

In short, the fully optimized UPLC-MS/MS method was reliable and rapid which was used for the first time for the detection of avapritinib concentration in rat plasma. Significant

Table 1 The precision and accuracy of avapritinib in rat plasma.

Analyte	Concentration (ng/mL)	Intra-day		Inter-day	
		RSD%	RE%	RSD%	RE%
Avapritinib	2	10.9	−12.9	14.8	−8.0
	5	8.8	6.5	13.9	12.0
	160	6.4	5.1	10.6	4.2
	3200	4.5	−5.0	6.3	−2.2

Table 2 Recovery and matrix effect of avapritinib in rat plasma (n = 6).

Analyte	Concentration added (ng/mL)	Recovery (%)		Matrix effect (%)	
		Mean ± SD	RSD (%)	Mean ± SD	RSD (%)
Avapritinib	5	85.2 ± 8.7	10.2	101.9 ± 8.7	8.5
	160	88.6 ± 5.8	6.5	95.1 ± 5.9	6.2
	3200	88.9 ± 2.7	3.0	93.5 ± 4.5	4.8

Table 3 Stability results of avapritinib in plasma under different conditions (n = 5).

Analyte	Added (ng/mL)	Room temperature, 3 h		Autosampler 10 °C, 12 h		Three freeze–thaw		−80 °C, 5 weeks	
		RSD (%)	RE (%)	RSD (%)	RE (%)	RSD(%)	RE(%)	RSD(%)	RE(%)
Avapritinib	5	9.6	12.7	14.8	8.0	12.3	8.4	12.5	7.9
	160	9.5	10.0	6.8	3.9	9.2	−2.0	8.6	12.0
	3200	5.1	2.0	3.2	4.2	4.5	7.2	5.1	7.0

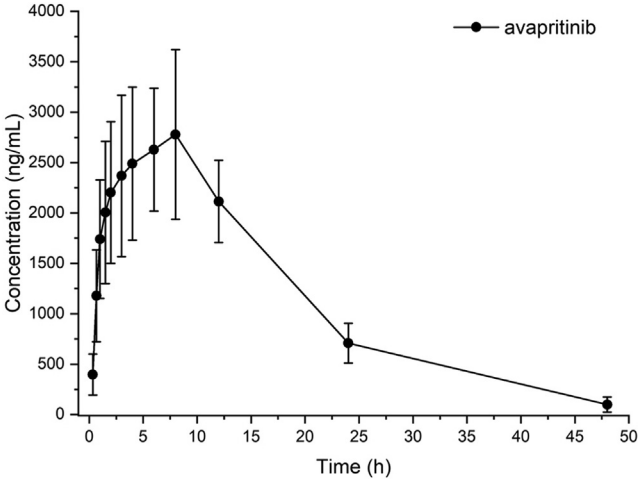

Fig. 3 Mean plasma concentration-time curves of avapritinib in rats after oral administration of avapritinib at a single dose of 30 mg/kg. (n = 6).

benefits were observed when utilizing optimized methods including rapid analysis time (only 2.0 min) and low sample preparation cost (simple and rapid precipitation of protein with acetonitrile). Post a single 30 mg/kg dosage of avapritinib orally, the optimized UPLC-MS/MS analysis technique applicability was established by rat pharmacokinetic studies. These results might provide basis for further researches of avapritinib in human.

Table 4 The main pharmacokinetic parameters of avapritinib in rat plasma after oral administration of avapritinib at a single dose of 30 mg/kg. (n = 6, Mean ± SD).

Parameters	Avapritinib
$AUC_{0\rightarrow t}$ (ng/mL•h)	54464.83 ± 9256.67
$AUC_{0\rightarrow\infty}$ (ng/mL•h)	55902.39 ± 9312.93
$MRT_{0\rightarrow t}$ (h)	12.63 ± 1.48
$MRT_{0\rightarrow\infty}$ (h)	13.69 ± 1.56
$t_{1/2}$ (h)	8.43 ± 1.68
T_{max} (h)	5.50 ± 1.76
CLz/F (L/h/kg)	0.55 ± 0.10
Vz/F (L/kg)	6.66 ± 1.52
C_{max} (ng/mL)	3161.31 ± 710.32

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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