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Determination of VT198-2 in rat plasma by liquid chromatography-tandem mass spectrometry and its pharmacokinetics

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Abstract: *VT198-2 is a new drug for the treatment of non-small cell lung cancer with KRAS G12 target. Ongoing clinical studies show that it has high safety and good clinical activity. Therefore, we developed a sensitive and reliable quantitative method of high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) to study the pharmacokinetic characteristics of the new drug VT198-2 in rat plasma. Terfenadine was used as the internal standard, the protein precipitation method was adopted, ZORBAX Eclipse XDB-C18 column was used, and separation and detection were realized together with triple quadrupole mass spectrometer under basic chromatographic conditions. The accuracy and precision of this method are within the specified range. In this study, it was found that VT198-2 was stable in the plasma of SD rats, with good selectivity in the linear range of 0.295-295ng/mL, and the extraction recovery rate was between 98.7% and 99.6%, without matrix effect. The $t_{1/2}$ of single intravenous and oral administration in rats was 2.02±0.01h and 2.8±1.1h, which resulted in the bioavailability of VT198-2 of 3.9%. Therefore, we believe that this method is specific, accurate and rapid, and it is suitable for the determination of VT198-2 plasma concentration in rats in preclinical research.*

Keywords: *VT198-2, KRAS, G12C, LC-MS/MS, PK*

1. Introduction

Lung cancer is the most common cause of cancer death in the world, and non-small cell lung cancer accounts for more than 85% of lung cancer cases in the world 9 [1]. KRAS protein is one of the key proteins in non-small cell lung cancer, which has GTPase activity and is a regulator of cell growth [2]. KRAS mutation is a member of RAS, the most common mutation oncogene family in cancer, which will affect intracellular signal transduction and cell growth. It is a common driving factor in non-small cell lung cancer, and its morbidity can account for 25%-30% of the global cancer cases [3].

KRAS mutations mainly exist at codons 12, 13 and 61, 117 and 146 [4,5], while G12C mutations account for the vast majority in patients with non-small cell lung cancer [6]. Because of the inherent reactivity of cysteine, it can be used to manufacture covalent small molecule inhibitors. However, before 2019, targeted therapy for non-small cell lung cancer with KRAS G12C mutation was always considered "impossible to be used as a medicine" [7], but with further research, many inhibitors against KRAS G12C mutation have achieved more and more results, and ARS-1620 is the first G12C specific inhibitor that can prove its efficacy in vivo. Sotorasib(AMG 510) and adagrasib(MRTX849) have been developed and approved by the FDA for the treatment of advanced non-small cell lung cancer patients with KRAS G12C mutation [8,9], which is a landmark breakthrough for targeted treatment of patients with KRAS mutation. In addition, two covalent inhibitors of KRAS-G12C, JNJ-74699157, and LY3499446, are undergoing clinical trials, and the results have not yet been announced [10].

However, with the deepening of research, researchers found that some patients developed resistance to KRAS G12C inhibitors, which may be due to the inherent resistance caused by lack of dependence on KRAS signal transduction, or it may be mediated by new KRAS mutation or wild RAS [11]. These

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inhibitors have been proven to have limited therapeutic activity in patients with KRAS mutant lung cancer [12], so it is particularly important to develop new drugs for the treatment of KRAS G12C mutation in non-small cell lung cancer.

VT198 is a pyridine heterocyclic compound with halogen atom connected with benzothiazole, which was developed by Suzhou Wentian Pharmaceutical Technology Co., Ltd., and has isomerism. One of the isomers is named VT198-2, which has positive significance for targeted treatment of non-small cell lung cancer with KRAS G12C mutation. Pharmacokinetics is to clarify the dynamic changes of drugs in the body by studying the absorption, distribution, metabolism, and excretion process of drugs in the body, especially the change in the law of blood drug concentration with time. It is an important part of preclinical and clinical research of drugs. In order to study the pharmacokinetic characteristics of the new drug VT198-2 in the plasma of SD rats, the method was verified according to the guidelines of the European Drug Review Committee on verifying biological analysis methods according to GLP requirements. Therefore, we initially developed and established a sensitive, simple, rapid and accurate LC-MS/MS method to determine the plasma concentration of VT198-2 in rats, and analyzed its bioavailability, which provided data support for subsequent research.

2. Methodology

2.1. Chemicals, reagents and animals

Compound VT198-2 (catalog number: 190156-103-1, purity 99.06%) was synthesized by Suzhou wentian pharmaceutical technology co., ltd. The internal standard compound Terfenadine (batch number: 21-04-2608, purity 99.61%) was purchased from Shanghai Pumai Biotechnology Co., Ltd. Methanol, acetonitrile, formic acid, ammonium acetate and DMSO (all HPLC grade, Thermo Fisher Scientific). SPF SD rats, SD rats, male, 6-8 weeks old, weighing 180-230 g, are operated by Shanghai Institute of Family Planning Science. All animal experiments were approved by the Animal Protection and Use Committee of Guilin Medical University.

2.2. Instruments and operating conditions

The HPLC system (SHIMADZU) consists of a liquid pump, a column temperature box, a controller, a degasser and an automatic sampler. Chromatographic method: the chromatographic column is Zorbax Eclipse XDB-C18 (2.1*50 mm, 3.5 μm, Agilent); Mobile phase: 0.1% formic acid and 5 mM ammonium acetate aqueous solution: methanol (A:B); Column temperature: 40℃; Sample volume: 5 μL; Mass spectrometry running time: 6.00 min; The elution mode is gradient elution (Table 1). Mass spectrometry conditions: API 4000 triple quadrupole tandem mass spectrometer with electrospray ESI ion source was used in positive ion mode, and the highest response ion pair was selected in MRM scanning mode for determination. Adjust the ionization source/gas parameters according to the analyte (Table 2) to create the best ionization conditions for the analyte and improve the sensitivity.

Table 1: Chromatographic elution gradient table.

Time(min)	Flow rate(mL·min^{-1})	A%	B%
0.10	0.70	70	30
4.00	0.70	30	70
4.50	0.70	30	70
5.50	0.70	70	30
6.00	0.70	Stop	Stop

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Table 2: Ion source parameters.

Parameter	Values
Ion Spray voltage	5500V
Temperature	550℃
Gas 1	55psi
Gas 2	55psi
Curtain gas	35psi
Collision gas	8 psi
Interface heater	On

2.3. Standard solution and standard curve

Weigh the VT198-2 standard, correct the quality correction coefficient, and dissolve it in DMSO to obtain the VT198-2 standard stock solution (stock solution concentration = weight × quality correction coefficient/volume). The working solution of VT198 was continuously diluted with 80% methanol water to a suitable concentration, and then about 90 μL of rat plasma was transferred to a 1.5 mL Eppendorf tube, and 10 μL of standard working solution was added, the following VT198-2 concentrations are obtained as standard curve samples: 0.295, 1.48, 2.95, 5.91, 14.8, 29.5, 59.1, 148and 295ng/mL. Quality control solutions with VT198-2 concentrations of 0.295, 0.591, 23.6, 118and 207 ng/mL were prepared in the same way. IS working solution is to weigh Terfenadine standard, and after the quality correction coefficient is corrected, pure methanol is dissolved as IS stock solution, and then the stock solution of Terfenadine is diluted to an internal standard solution with a concentration of 10 ng/mL with acetonitrile as solvent. All stock solutions are kept at -20℃ and working solutions are kept at room temperature before use. Add 100μL of standard curve sample, quality control sample and other samples into 400μL of internal standard solution (Double blank sample with 400μL of methanol), vortex for 1min, centrifuge at 4℃ for 10min at 15400 g, and inject 120 μL of supernatant into LC-MS/MS system for analysis.

2.4. Method verification

According to US FDA ICH Harmonised Guideline M10 Bioanalytical Method Validation (2018) recommendations and acceptance criteria, the selectivity was studied by comparing six different batches of blank rat plasma to detect whether the internal standard and the substance to be tested interfered with each other. The linearity was evaluated by using Zero samples without analyte but with IS and nine non-zero samples with a linear range of 0.295-295 ng/ml. Plot the peak area ratio of VT198-2 and IS to the corresponding concentration, evaluate the linearity and determine LLOQ. The linearity of calibration curve is constructed by plotting the peak area ratio (y) of the analyte and the concentration (x) of the analyte with weighted ($1/x^2$) least square linear regression.

QC samples with five concentration levels (LLOQ, LQC, MQC, GMQC, and HQC) were repeatedly analyzed for six times to investigate the accuracy and precision of the detection. Six duplicate samples were analyzed on the same day to evaluate intra-batch precision and accuracy, while inter-batch precision and accuracy were evaluated by repeated analysis within three days. The precision is expressed by the coefficient of variation (CV) of QC samples, the coefficient of variation of quality control samples shall not exceed 15.0%, the coefficient of variation of the lower limit of quantification shall not exceed 20.0%, and the calculation method is standard deviation/average×100%. The calculation method of accuracy is measured value/true value×100%, the average accuracy of quality control samples and quantitative lower limit are within the range.

The recovery rate was determined by comparing the peak areas of analytes added with blank plasma at two quality control levels with the peak areas of analytes in the extracted blank matrix diluent obtained in six different batches, and the matrix effect was evaluated by analyzing the preparation of pure solution. We also prepared a diluted quality control sample DQC and a high-concentration quality control sample

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whose concentration exceeded the quantitative upper limit, then diluted the quality control sample with blank matrix to make its concentration fall within the linear range of the standard curve (repeated dilution for six times), and verified the dilution reliability by sample analysis after pretreatment according to the sample pretreatment method. The samples were stored in different environments to determine their stability, including short-term stability at room temperature for 24 hours and medical cryobox for 48 hours, long-term stability at ultra-low temperature refrigerator for 28 days, repeated freeze-thaw stability, post-treatment stability and whole blood stability.

2.5. Pharmacokinetics and bioavailability of VT198-2 in normal SD rats

Male SD rats (180-230g,n=3) of 6-8 weeks old were given a single oral dose of 10mg/kg, and blood was collected at 0.25, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, 12.0 and 24.0 hours. Each animal was given a single intravenous injection of 3 mg/kg, and blood was collected at 0, 0.25, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, 12.0 and 24.0 hours. 2~8℃, 1700g, centrifugation for 5 min for analysis. The absolute bioavailability of VT198-2 in SD rats was calculated based on the area AUC0→t under the in vivo plasma concentration-time curve, as shown in Formula (1):

$$F_{\text{absolute}}(\%)=\frac{AUC_{0\rightarrow t(\text{i.g.})}\times Dose_{i.v.}}{AUC_{0\rightarrow t(i.v.)}\times Dose_{i.g.}}\times 100\% \quad (1)$$

3. Results and discussion

3.1. Method Development

The quantitative lower limit of this method is 0.295ng/mL, and the retention time is 4.43min. This method is sensitive, simple and reproducible, which can greatly meet the requirements of drug concentration measurement in vivo. As shown in Table 3, LC-MS/MS analysis was performed in positive ion mode using nitrogen-assisted atomization. Table 3 lists the optimized mass spectrum parameters of each compound. The quantitative ion pair of VT198-2 is m/z 555.3→444.0, and the internal standard terfenadine is m/z 472.4→436.4.

Table 3: Compound parameters.

Analysts	Q1/Q3(m/z)	Dwell Time (ms)	DP(V)	EP(V)	CE(V)	CXP(V)
VT198-2	555.3→444.0	100	130	10	45	38
Terfenadine	472.4→436.4	100	66	10	55	14

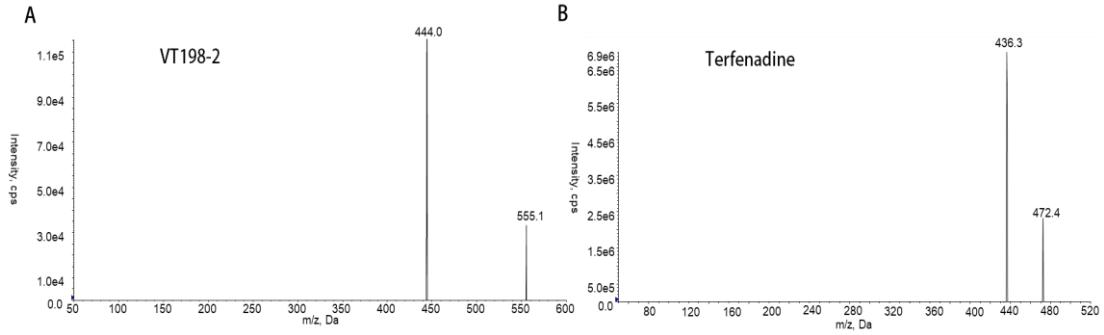

Figure 1: Ion scan of VT198-2 and Terfenadine. (A) MS2 scan of VT198-2 in positive ions mode; (B) MS2 scan of Terfenadine in positive ions mode.

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3.2. Method verification

3.2.1. Selectivity

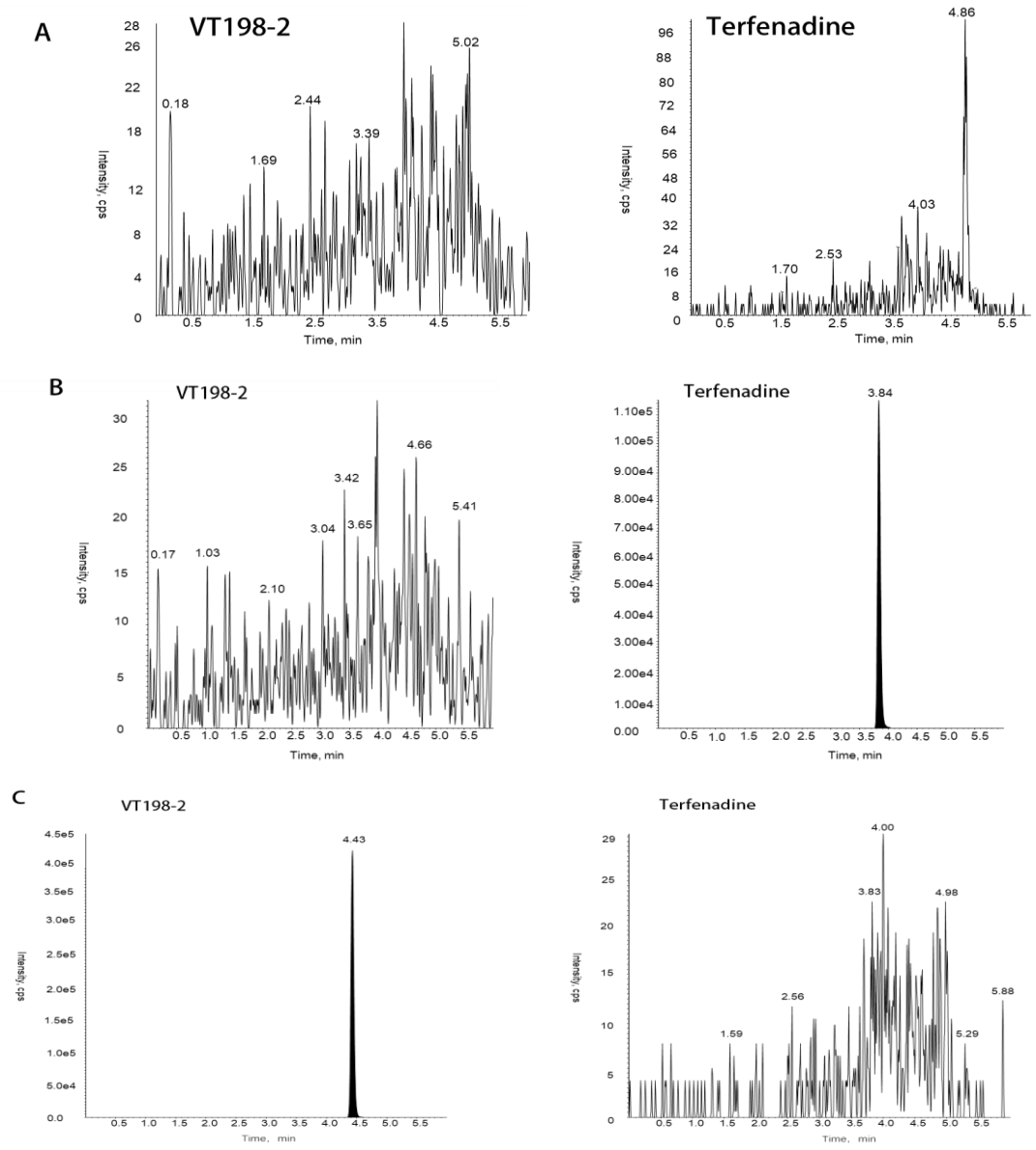

Figure 2: Specific chromatogram of rat plasma. (A) Blank plasma; (B) Blank plasma spiked with Terfenadine; (C) Blank plasma spiked with VT198-2.

As shown in Figure 2, the selectivity of LC-MS/MS method for six different batches of blank plasma was studied. In the experiment, VT198-2 and Terfenadine were well separated in the biological matrix samples with good peak shape, and there was no interference of endogenous substances or other substances during the retention time of the analyte and internal standard.

3.2.2. Accuracy and precision

After analyzing QC samples of five concentration levels (LLOQ, LQC, MQC, GMQC and HQC), it was found that the accuracy of VT198-2 content determination ranged from 85.9% to 100.0%, and the accuracy between batches was 94.3% to 100.3%. It is proved that this method has sufficient accuracy and precision in the determination of biological samples.

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Table 4: The precision and accuracy of VT198-2 in plasma of rats (Mean±SD, n=6).

Spiked Con. ($ng \cdot mL^{-1}$)	Intra-day			Inter-day		
	Mean±SD ($ng \cdot mL^{-1}$)	Precision (%RSD)	Accuracy (%RE)	Mean±SD ($ng \cdot mL^{-1}$)	Precision (%RSD)	Accuracy (%RE)
0.295	0.254±0.0	8.2	85.9	0.278	10.6	94.3
0.591	0.573±0.0	5.3	97.0	0.584	4.9	98.9
23.6	23.6±0. 6	2.4	100.0	23.7	2.3	100.3
118	116±1.3	1.1	98.3	117	2.7	98.9
207	200±7.0	3.5	96.7	201	3.2	97.2

3.2.3. Standard curve and LLOQ

In this verification, nine concentration points of the calibration curve were designed and determined four times. The linear range was 0.295-295 ng/mL, and the lowest quantitative limit (LLOQ) was the lowest concentration point of the calibration curve. Figure 3 shows that the linear range of VT198-2 in SD rat plasma is good, and the regression equation is y = 0.0131 x+0.000652 (r = 0.9986). Blank and Zero samples are included in the analysis to verify that there is no interference in the sample preparation procedure. The results of the precision and accuracy of LLOQ are shown in Table 4. The accuracy and precision meet the requirements of biological matrix sample analysis.

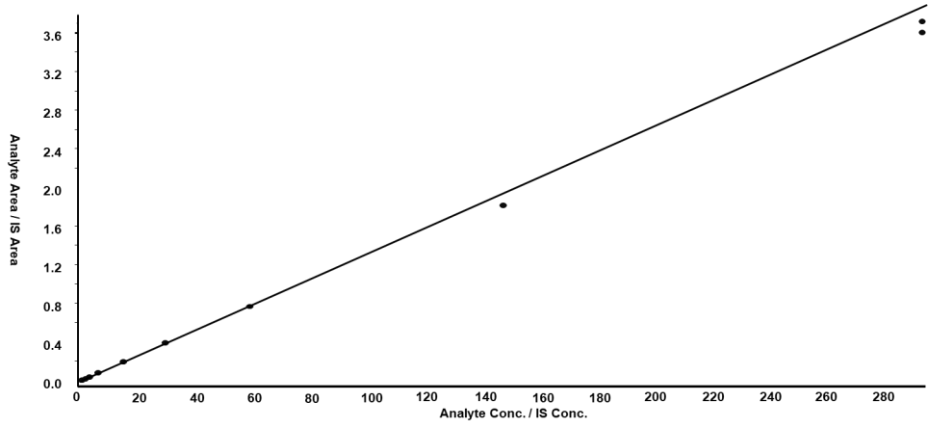

Figure 3: Representative calibration curve of VT198-2 in rat plasma by LC-MS/MS.

3.2.4. Extraction recovery and matrix effect

The results of extraction recovery and matrix effect are shown in Table 5. The recovery rates of VT198-2 and internal standard Terfenadine in rat plasma were between 98.7%-99.6% and 99.6%, and the RSD of quality control samples was less than 15%. The matrix effect ranges of the analyte and the internal standard are 94.4%-107.3% and 95.7% respectively, and the RSD is less than 15%. This proves that the method has good recovery rate, good stability and no obvious matrix effect, which meets the verification requirements of biological products analysis methods.

Table 5: Matrix effect and extraction recovery of VT198-2 and Terfenadine in plasma of rats (Mean±SD, n=6)

Compounds	Spiked Con. ($ng \cdot mL^{-1}$)	Extraction recovery (%)	RSD (%)	Matrix effect (%)	RSD (%)
VT198-2	0.591	99.6	2.9	107.3	3.9
	207	98.7	1.5	94.4	1.6
Terfenadine	10	99.6	2.9	95.7	2.9

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3.2.5. Stability

VT198-2 measured the following stability: 24-hour stability at room temperature, long-term stability of ultra-low temperature refrigerator (stored at -75℃ for 28 days), repeated freeze-thaw stability, post-extraction stability (sampled after 48 hours storage at 4℃ in automatic sampler), and whole blood stability (2 hours at room temperature). The data in the following table show that VT198-2 is stable in rat plasma under the above conditions.

Table 6: Short-term stability of VT198-2 in rat plasma under different conditions (mean SD, n = 6).

Analysts	Spiked Con. (ng·mL^{-1})	Short-term stability (24h, room temperature)			The processed samples (48h, 4℃)		
		Mean±SD (ng·mL^{-1})	Precision (%RSD)	Diff (%)	Mean±SD (ng·mL^{-1})	Precision (%RSD)	Diff (%)
VT198-2	0.591	0.631±0.0	2.8	6.9	0.624±0.0	7.5	5.6
	207	208.3±0.6	0.3	0.6	203.8±5.1	2.5	-1.6

Table 7: Long-term stability of VT198-2 in rat plasma under different conditions (mean SD, n = 6).

Analysts	Spiked Con. (ng·mL^{-1})	Long-term stability (28 days, -75℃)			Freeze-thaw (three cycles)		
		Mean±SD (ng·mL^{-1})	Precision (%RSD)	Diff (%)	Mean±SD (ng·mL^{-1})	Precision (%RSD)	Diff (%)
VT198-2	0.591	0.573±0.0	4.1	-3.1	0.571±0.0	8.2	-3.5
	207	194±5.0	2.6	-6.2	194±1.7	0.9	-6.2

In addition, samples with low and high concentration levels were prepared to investigate the stability of whole blood. The operation was as follows: The stable samples were taken out immediately after preparation and placed at room temperature for at least 2 hours, and centrifuged (2~8℃, 1700g, 5 min) to obtain plasma samples. The deviation between the measured value of the stable sample and the sample immediately after preparation should be within 15.0%, indicating that VT198-2 is stable at room temperature for 2 hours.

3.3. Pharmacokinetics of VT198 in normal SD rats

The pharmacokinetic parameters of VT198-2 in normal SD rats were calculated by WinNonLin (non-atrial model). The average plasma concentration-time curve of VT198-2 in normal SD rats after single intravenous injection and single oral administration is shown in Figure 4. After intravenous injection of 3mg/kg, VT198-2 was rapidly eliminated from plasma, with $t_{1/2}$ and CL being 2.8±1.1h; And 182.16±32.5 (mL/h/kg), $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ are 16750.90 2697.1 (ng·h/mL) respectively; And 16793.50 2726.5 (ng·h/mL), and the bioavailability was 3.9%. After oral administration, the absorption rate of VT198-2 was slow, reaching the maximum concentration of 363.7±59.7(ng/mL) at 3.33±1.2h after administration, and the $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ were 2176.33±728.3(ng·h/mL) respectively 2190.43±707.9 (ng·h/mL), the $t_{1/2}$ was 2.02±0.1h, which was close to that of intravenous administration.

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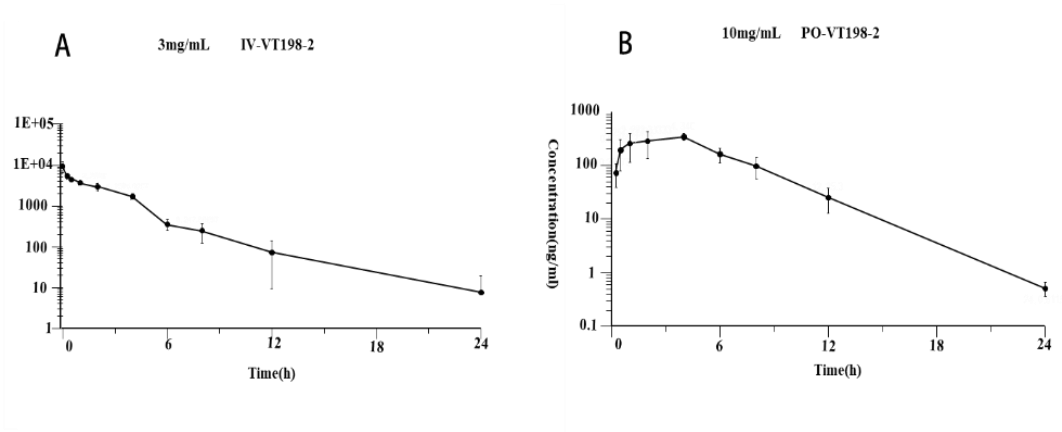

Figure 4: The average plasma concentration-time curve of VT198-2 in normal SD rats (average SD, n=3) (A) injected intravenously of 3mg/mL. (B) oral administration of 10mg/mL.

4. Conclusion

The bioavailability of VT198-2 is low, which may be related to the solubility of the drug. Later, the dissolution can be improved by changing the dosage form or adding appropriate surfactant, or by using other technologies such as solid dispersion technology and micronization technology to improve the bioavailability.

In this study, a simple, selective, sensitive and convenient liquid chromatography-tandem mass spectrometry method was developed. The linear range of VT198-2 was 0.295-295ng/mL, which was sensitive enough to determine the change of plasma concentration of VT198-2, and provided preclinical pharmacokinetic data support for VT198-2, a target drug mutated by KRAS G12C, which provided great help for the development of new drugs in the future.

Author's contribution

Lihua Qin and Xiaoqun Duan designed and implemented the experiment, and Meiqing Wang analyzed the data. Shu Zhang is responsible for supervising the experiment, and Lihua Qin and Meiqing Wang jointly wrote the first draft of the article. Xiaochuan Li supervised the study, interpreted the data and provided funds for the study. All the authors strictly reviewed the research plan and manuscript, and approved the final manuscript.

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