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Quantification of VT204 in rat plasma by LC-MS/MS and its pharmacokinetic study

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Abstract: *Although the target of KRAS G12C is no longer unbreakable, resistance to current drugs against KRAS G12C occurs. The emergence of a new drug, VT204, is expected to be a new clinical drug in this field. Preclinical pharmacokinetic (PK) studies in animal models during the formulation development phase provide preliminary evidence of the PK behaviour of a drug before clinical studies in humans and help to tailor the dosage form to the expected and necessary clinical behaviour. A high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to determine VT204 in rat plasma. A single chromatographic run was performed on a ZORBAX Eclipse Plus C18 (2.1 mm×50 mm, 3.5 μm, Agilent) and at a flow rate of 0.75 mL·min^{-1}. The intra- and inter-batch accuracies ranged from 95.3% to 103.3%, and the intra- and inter-batch precision did not exceed 15%. The $t_{1/2}$ of single intravenous and oral administration in rats was 1.09±0.05 h and 1.47±0.55 h, which resulted in the bioavailability of VT204 of 63.52%. This method is accurate and specific and provides a good solution for the pharmacokinetic study of VT204 in rats.*

Keywords: *VT204; KRAS G12C; Non-small cell lung cancer; LC-MS/MS; pharmacokinetic*

1. Introduction

The RAS genes are the most frequently mutated gene family in cancer and contain KRAS, NRAS and HRAS mutations. RAS mutations are one of the three main mutated genes screened for in cancer deaths in the U.S. [1,2]. Kirsten rat sarcoma virus proto-oncogene (KRAS) is a driver of lung, colorectal and pancreatic cancers in cancer patients [3]. KRAS is also RAS's most commonly mutated subtype, accounting for approximately 85% of oncogenic RAS mutations [4]. Among KRAS mutations, missense mutations in glycine codon 12 (G12) are the most common mutated amino acid [5]. G12 is a gain-of-function mutation that enhances the KRAS downstream signalling pathway and promotes tumour development [6]. The development of effective therapies to inhibit RAS-driven tumorigenesis has not been supported by the field for more than three decades, long before small molecule therapeutic agents became available, and RAS was considered untreatable with drugs [7]. However, the 2018 clinical approval by the U.S. Food and Drug Administration (FDA) of Sotorasib (codename AMG-510), a G12C allele-specific covalent inhibitor manufactured by Amgen, broke this impasse and represents a new milestone in the field of anticancer drugs [8]. The development of allele-specific KRAS G12C inhibitors is promising in early clinical trials, marking a new chapter in targeting oncogenic KRAS mutants in cancer [9]. Emerging clinical evidence suggests that the majority of patients are resistant to KRAS G12C inhibitors, and new therapeutic approaches should be developed to effectively target KRAS G12C and its inhibitor-associated resistance mechanisms[10]. Resistance to these new drugs may limit their efficacy in the clinic. Recently, several studies have detailed the mechanisms of resistance to KRAS G12C inhibition and pioneered the use of such combinations to overcome resistance

Only a few KRAS G12C inhibitors have entered clinical trials, highlighting the need to develop effective drug candidates for treatment of KRAS G12C-driven cancers. The development of new drugs

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can reach a breakthrough quickly and precisely by starting with already-marketed drugs. VT204, a quinazoline compound linked to a benzothiazole, is a promising drug candidate for targeting cancers associated with KRAS G12C mutant proteins, developed by Suzhou Vincentage Pharma Co., Ltd. The pharmacokinetic characterisation of drugs by the determination of blood concentrations further elucidates the mechanism of drug action and is one of the bases for pharmacodynamic and toxicological studies. In addition, the pharmacokinetic parameters obtained are the basis for generating, determining, or elucidating the magnitude of efficacy or toxicity, and also provide the basis for the efficacy or toxicity of the drug on the target organ.

To study the pharmacokinetic profile of the new drug VT204 in SD rats, the method was validated according to the EC Drug Review Committee guidelines for the validation of bioanalytical methods by GLP requirements. In this chapter, a rapid, accurate, stable, wide linear range, sensitive, and simple pre-treatment LC-MS/MS assay for the determination of VT204 in the plasma of SD rats was initially developed and established to study the blood concentration of VT204 in SD rats, and bioavailability analyses were carried out to provide supporting data for subsequent studies.

2. Methodology

2.1. Chemicals, reagents, and animals

The compound VT204 (Cat. No.: 190136-197-1) was synthesised by Suzhou Vincentage Pharma Co., Ltd. The internal standard compound Terfenadine was purchased from MedChemExpres (Cat. No.: HY-B1193). Methanol, aceturate, formic acid, ammonium acetate, and dimethyl sulfoxide (all HPLC grade, Thermo Fisher Scientific).

SPF-grade SD rats, male, 6~8 weeks old, body weight 220~260g, from Changzhou Cavins Laboratory Animal Co., Ltd. All animal experiments were approved by the Animal Care and Use Committee of Guilin Medical University.

2.2. Apparatus and operation conditions

The HPLC system (Shimadzu) is composed of a degasser, infusion pump, autosampler, and column oven. Chromatographic method: the column was ZORBAX Eclipse Plus C18 (2.1 mm×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 ℃; Injection volume: 1.00 μL; Analysis time: 2.5 min; The elution mode was gradient elution (Table 1).

Mass spectrometry conditions: An API 4000 triple quadrupole tandem mass spectrometer with an electrospray ESI ion source was used in positive ion mode with a multiple reaction monitoring (MRM) scanning mode to select the highest response ion pairs for determination. The ionisation source/gas parameters (Table 2) are adjusted according to the analyte to create optimal ionisation conditions for the analyte and increase the sensitivity.

Table 1: Chromatographic elution gradient table.

Time(min)	Flow rate(mL·min^{-1})	A%	B%
0.30	0.75	60	40
0.80	0.75	10	90
1.90	0.75	10	90
2.00	0.75	60	40
2.50	0.75	Stop	Stop

Table 2: Ion source parameters.

Parameter	Values
Ion Spray voltage	5500 V
Temperature	500 ℃
Gas 1	50 psi
Gas 2	50 psi
Curtain gas	20 psi
Collision gas	8 psi
Interface heater	On

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2.3. Sample preparation

About 45 μL of rats plasma was pipetted into a 2 mL Eppendorf tube and added with 5 μL of standard solution to formulate the standard curve of VT204 with final concentrations of 1, 5, 10, 20, 50, 100, 200, 500 ng·mL^{-1}. The same method was used to formulate the QC samples of VT204 with final concentrations of 2, 80 and 400 ng·mL^{-1}. The standard and QC samples were added with 100 μL of acetonitrile and 100 μL of 2 ng·mL^{-1} acetonitrile (terfenadine), vortexed for 1 min, centrifuged for 10 min at 4 ℃ and then at 15400 g. About 150 μL of supernatant was used for analysis.

2.4. Methodological validation

The method developed was validated in line with US FDA Guidance for Industry Bioanalytical Method Validation 2001, US FDA Bioanalytical Method Validation Guidance for Industry 2018, and US FDA ICH Harmonised Guideline M10 Bioanalytical Method Validation (2018). Validation was conducted using the calibration curve and LLOQ, specificity, matrix effect, precision and accuracy, extraction recovery and stability as per the FDA recommendations and acceptance criteria.

2.5. Pharmacokinetic and bioavailability studies of VT204 in normal SD rats

Male Sprague–Dawley rats (250 ± 10 g, n = 6) received oral administration VT204 (10 mg·mL^{-1} per rat). VT204 (3 mg·mL^{-1} per rat) was injected into the tail vein. Blood was collected at 0.25, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, 12.0 and 24.0 h after each administration. Blood was centrifuged (4 °C, 3000 r·min^{-1}) for 10 min and analysed.

The absolute bioavailability of VT204 in the blood of SD rats was calculated by taking the area under the blood concentration–time curve $AUC_{0-\infty}$ in vivo after a single dose of VT204 given intravenously to SD rats at a dose of 3 mg·kg^{-1} as a reference. The absolute bioavailability of VT204 in the blood of rats given a single dose of VT204 through gavage at a dose of 10 mg·kg^{-1} was determined using Formula (1).

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

3. Results and discussion

3.1. Method development

The two main goals when optimising MS/MS conditions are adequate sensitivity and selectivity. While sample extraction, chromatographic development and initial mass spectrometry adjustments all have an impact on sensitivity and selectivity, mass spectrometry optimisation has a significant effect on the sensitivity of the method. The reliability of the method was determined by the results of the recovery experiments, which were reproducible. The requirement of 45 μL of rat plasma also ensured few wasted samples.

Tandem mass spectrometry was based on precursor ion selection (Q1), its fragmentation mainly by collision-induced dissociation (CID), and m/z measurements of the formed product ions (Q3). The optimised MS parameters for each compound are listed in Table 3. Mass-to-charge ratios of quantitatively analysed ion reactions: VT204 m/z 515.0→152.2, internal standard terfenadine m/z 472.4→436.4. The ESI positive ion secondary full scan (MS2) mass spectra of VT204 and the internal standard terfenadine are shown in Figures 1A and B.

Table 3: Compound parameters.

Analysts	Q1/Q3 (m/z)	Dwell Time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
VT204	515.0→152.2	100	130	10	37	10
Terfenadine	472.4→436.4	100	66	10	45	14

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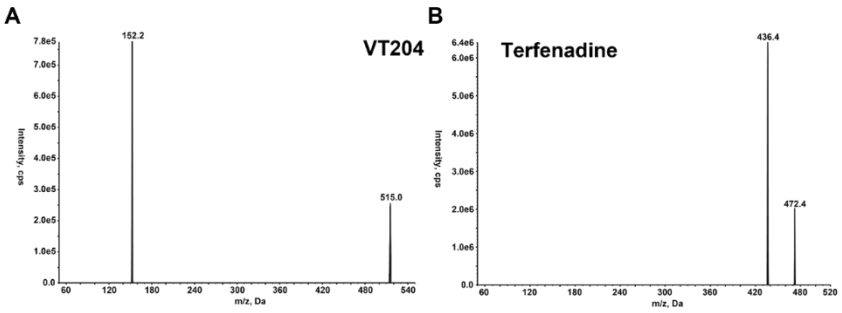

Figure 1: Ion scan of VT204 and Terfenadine. (A) MS2 scan of VT204 in positive ions mode; (B) MS2 scan of Terfenadine in positive ions mode.

3.2. Method validation

3.2.1. Specificity

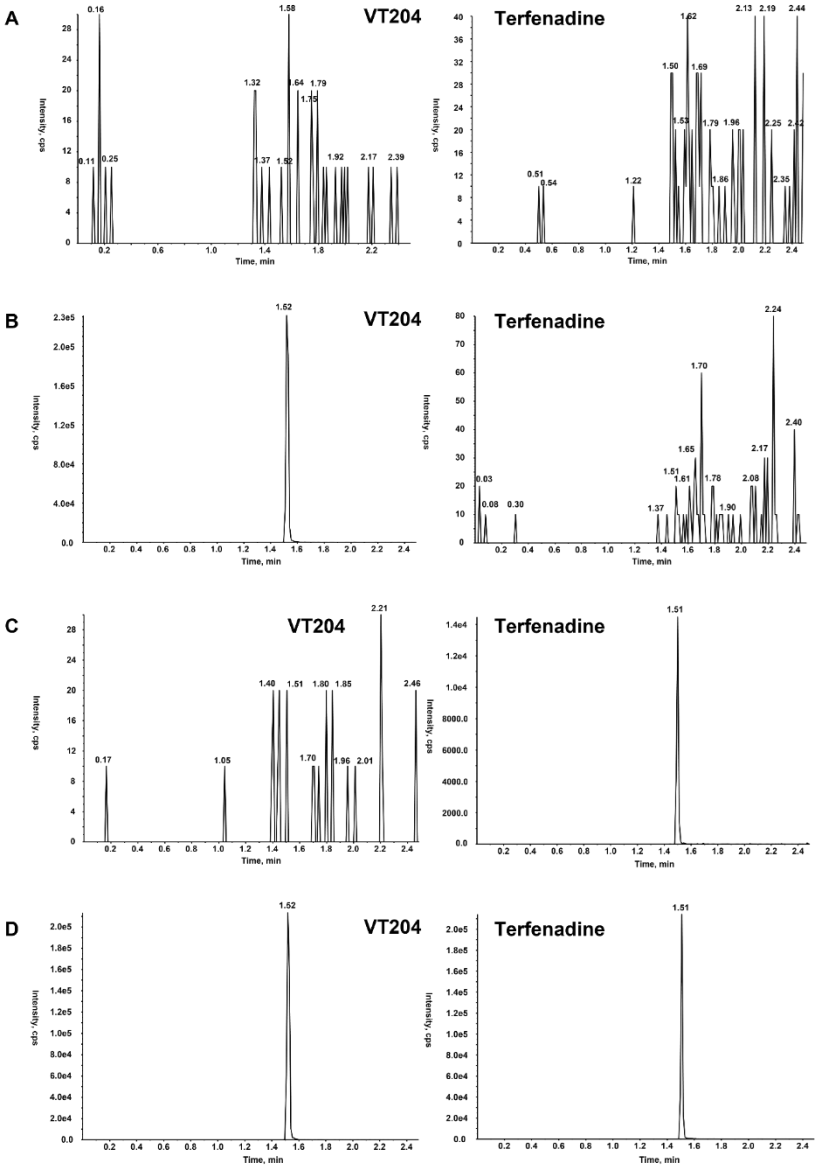

Figure 2: Specific chromatogram of rat plasma. (A) Blank plasma; (B) Blank plasma spiked with VT204 ; (C) Blank plasma spiked with Terfenadine;(D) Blank plasma spiked with VT204 and Terfenadine.

ISSN 2706-6819 Vol.5, Issue 11: 10-16, DOI: 10.25236/IJFM.2023.051102

The specificity of the LC-MS/MS method for different biological matrix samples was investigated. As shown in Figure 2, under the experimental conditions, the analytes to be measured, VT204, and the internal standard compound, terfenadine, were well separated in the biological matrix samples. The retention time of VT204 and terfenadine in rat plasma was 1.51 min. The retention time of terfenadine in rat plasma was 1.52 min, and they did not interfere with each other, and the peak shapes of the two compounds were good. The result showed no interference of endogenous substances and other substances from the biological matrix samples in the peaks of both compounds.

3.2.2. Precision and Accuracy

The precision and accuracy results for all analytes in the LLOQ and QC samples are summarised in Table 4. The intra- and inter-batch precision and accuracy of VT204 were determined by the different analytical methods in the biomatrix samples based on the requirements of the analytical methods.

The intra-and inter-batch precision of VT204 for LLOQ in plasma and the three quality control samples (low, medium and high) were less than 15.0%; the mean value of the intra-batch accuracy was 95.3%–101.9%, and that of the inter-batch accuracy was 98.2%–103.3%, indicating the suitability of the method for the determination of the requirements of biological samples.

Table 4: The precision and accuracy of VT204 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)	Mean±SD ($ng \cdot mL^{-1}$)	Precision (%RSD)	Mean±SD ($ng \cdot mL^{-1}$)	Precision (%RSD)
1	1±0.1	8.6	101.9	1±0.1	12.9	98.9
2	2±0.2	10.9	98.6	2±0.2	10.9	98.2
80	76±6.9	9.1	95.3	82±7.1	15.0	102.5
400	403±19.5	4.8	100.8	413±20.2	5.4	103.3

3.2.3. Calibration curve and LLOQ

The VT204 standard curve consists of eight points according to the methodology established in this experiment. The lowest concentration point of the standard curve was used as the lowest lower limit of quantification (LLOQ). Figure 3 shows that the linearity of VT204 in SD rat plasma was good with a linear range of 1–500 $ng \cdot mL^{-1}$ (y=0.0646x+0.00786, r=0.9979). The lower limit of quantification (LLOQ) was 1 $ng \cdot mL^{-1}$. The results of the precision and accuracy of LLOQ are shown in Table 4. The accuracy ranged from 80.0% to 120.0%, and the precision was less than 20.0%, which satisfied the requirements for analysis of biological matrix samples.

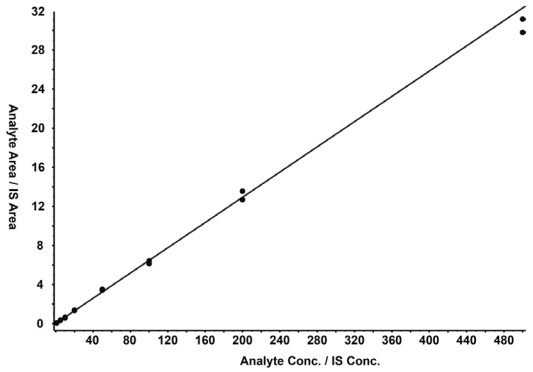

Figure 3: Representative calibration curve of Semaglutide in rat plasma by LC-MS/MS.

3.2.4. Extraction recovery and matrix effects

The results of the extraction recoveries and matrix effects are shown in Table 5. The extraction recoveries of the test substance VT204 and the internal standard terfenadine in rat plasma were in the ranges of 100.8%–108.5% and 99.6% with precision (RSD) less than 12.4% in the three quality control samples (low-, medium- and high-quality samples, respectively). The method was proved to be suitable for the extraction of VT204 from biological matrix samples with high recovery and good stability. The matrix effects were normalised to the internal standard, ranging from 96.3% to 113.9% and 126.4%, respectively, and the precision (RSD) was less than 8.0%. The matrix effects of VT204 and the internal standard terfenadine in rat plasma were stable without obvious ion-enhancing or ion-suppressing effects, which is suitable for analysis. It meets the validation requirements of the analytical method for biological

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matrix samples.

Table 5: Matrix effect and extraction recovery of VT204 and Terfenadine in plasma of rats(Mean±SD, n=6).

Compounds	Spiked Con. ($ng \cdot mL^{-1}$)	Extraction recovery (%)	RSD (%)	Matrix effect (%)	RSD (%)
VT204	2	100.8±12.5	12.4	96.3 ±7.7	8.0
	80	108.5±6.8	6.30	113.9±8.9	7.8
	400	103.1±4.5	4.30	113.8±7.0	6.2
Terfenadine	2	99.6±7.0	7.10	126.4±9.6	7.6

3.2.5. Stability

The stability of VT204 in plasma was determined under storage at room temperature for 24 hours and at -75 °C for 28 days as well as after three freeze–thaw cycles and 48 hours of storage of processed samples at autosampler temperature (4 °C). Tables 6 and 7 indicate that VT204 is stable in rat plasma under the above conditions.

Table 6: Stability of VT204 in rat plasma under different conditions (Mean±SD, n=6).

Analysts	Spiked Con. ($ng \cdot mL^{-1}$)	Short-term stability (24h, room temperature)			The processed samples (48h, 4°C)		
		Mean±SD ($ng \cdot mL^{-1}$)	Precision (%RSD)	Diff (%)	Mean±SD ($ng \cdot mL^{-1}$)	Precision (%RSD)	Diff (%)
VT204	2	2±0.3	13.6	-0.8	2.1±0.2	7.3	2.8
	400	410±28.6	7.0	2.5	409±13.3	3.2	2.3

Table 7: Stability of VT204 in rat plasma under different conditions (Mean±SD, n=6).

Analysts	Spiked Con. ($ng \cdot mL^{-1}$)	Long-term stability (28 days, -75°C)			Freeze-thaw (three cycles)		
		Mean±SD ($ng \cdot mL^{-1}$)	Precision (%RSD)	Diff (%)	Mean±SD ($ng \cdot mL^{-1}$)	Precision (%RSD)	Diff (%)
VT204	2	2.1±0.2	10.3	6.0	2±0.2	11.6	-0.5
	400	397±17.1	4.3	-0.7	416±27.5	6.6	4.1

3.3. Pharmacokinetics of VT204 in normal Sprague Dawley rats

The mean blood concentrations and mean blood concentration–time curves of VT204 in normal SD rats after single intravenous (3 $mg \cdot kg^{-1}$) and single oral (10 $mg \cdot kg^{-1}$) administration are shown in Figure 4. VT204 was rapidly absorbed and eliminated in SD rats and in vivo after a single administration, with the peak time centred between 0.5 to 2 h, basically undetectable in vivo after 12h and eliminated in vivo after 24 h.

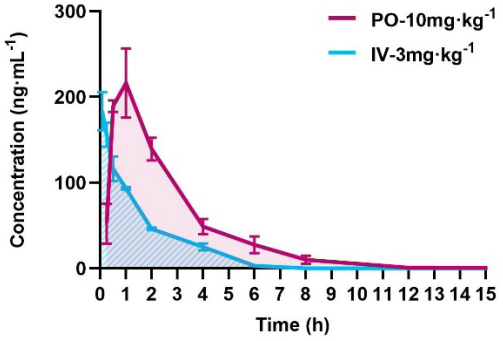

Figure 4: Mean blood concentration-time curve of VT204 in normal SD rats (Mean±SD, n=3).

Pharmacokinetic parameters of VT204 in normal SD rats were calculated using WinNonLin (non-atrial model). VT204 was rapidly eliminated from plasma after an intravenous dose of 3 $mg \cdot kg^{-1}$ with $t_{1/2}$ and CL of 1.09 ± 0.05 h; and 9932.71 ± 116.12 ($mL \cdot h^{-1} \cdot kg^{-1}$), respectively. In addition, AUC0-t and $AUC_{0-\infty}$ were 300.83±32.33 ($ng \cdot h \cdot mL^{-1}$); and 304.73±34.64 ($ng \cdot h \cdot mL^{-1}$), respectively, and the calculated F was 63.52% after converting the dose administered intravenously to be consistent with oral

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administration. After oral administration, VT204 was rapidly absorbed into the bloodstream, reaching a maximum concentration of 226.33±60.43 ($ng \cdot mL^{-1}$) C_{max} at 0.83±0.29 h after administration, AUC_{0-t} and $AUC_{0-\infty}$ were 644.45±132.11 ($ng \cdot h \cdot mL^{-1}$); 645.24±132.26 ($ng \cdot h \cdot mL^{-1}$), respectively. In addition, VT204 administered orally (10 $mg \cdot kg^{-1}$) was cleared rapidly from plasma with a $t_{1/2}$ of 1.47±0.55 h, which was close to that of the venous close, while the CL was 160004.40±3706.99 ($mL \cdot h^{-1} \cdot kg^{-1}$), which was higher than that of intravenous administration.

4. Conclusions

In this work, the researchers developed a reliable and accurate LC-MS/MS method through small-scale studies on a small number of animal and plasma samples. The method was validated against FDA-recommended permissible limits and acceptance criteria. Preclinical pharmacokinetic data were obtained for a promising KRAS G12C-targeted drug, VT204, laying the groundwork for the subsequent clinical launch of this new drug.

Author Contributions

Xuechao Yang and Shu Zhang designed and performed experiments, and analyzed the data. Xiaoqun Duan and Xiaochuan Li supervised the experiments. Xuechao Yang and Hongqian Qin wrote the manuscript draft. Yang Yang supervised the study, interpreted the data, and provided funding for this study. All authors critically reviewed the study protocol and manuscript and approved the final manuscript.

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