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# Pharmacokinetics and safety of the antitumor drug everolimus in healthy Chinese subjects: a single-dose, open-label, randomized, two-sequence, two-period crossover, phase I bioequivalence study

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**Purpose:** It aims to evaluate the pharmacokinetic (PK) similarity and safety of everolimus in whole blood under fasting and postprandial conditions and its generics in Chinese healthy subjects.

**Methods:** Healthy subjects were enrolled in a single-dose, open-label, randomized, two-sequence, two-period crossover study (Clinical trial registry number: CTR20220446) under fasting and postprandial conditions. The blood concentration of everolimus was quantified by a validated and robust liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

**Results:** All 90% CIs for the geometric mean ratios of  $C_{max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-\infty}$  were within the bioequivalence range of 80.00%–125.00%. the maximum intra-individual CV% of variation were 22.76% and 18.63% under fasting and fed conditions, respectively. The gastrointestinal absorption rate was decreased by food intake: median  $T_{max}$  was 0.75 h in the fasted state, and 3.00 h after a high-fat meal. The average elimination half-life ( $t_{1/2\beta}$ , 37.19–38.43 h) and mean residence time (MRT, 23.13–25.81 h) did not appear to be affected by food intake regardless of fasting or fed state. In this study, no serious AE (SAEs) were observed throughout the trial.

**Conclusion:** The systemic availability of a single oral 5 mg dose of everolimus is significantly reduced by coadministration with fed compared with fasting conditions, the test formulation (T) and the reference formulation (R) were bioequivalent.

**Clinical Trial Registration:** [clinicaltrials.gov](https://clinicaltrials.gov), identifier CTR20220446, (<http://www.chinadrugtrials.org.cn/clinicaltrials/searchlist.dhtml>).

## KEYWORDS

bioequivalence, everolimus, healthy subject, pharmacokinetics, the antitumor drug

# 1 Introduction

Everolimus, a mammalian target of rapamycin (mTOR) inhibitor, achieves triple anti-tumor effects by blocking the PI3K-AKT-mTOR signaling pathway: inhibiting tumor cell growth, reducing tumor cell nutrient metabolism, and affecting tumor angiogenesis (Menzel et al., 2023; Park et al., 2023). Everolimus was first developed by Novartis of the Swiss company, the product name was Zortress® for transplantation in the United States and Certican in Europe, and Afinitor® for cancer.

The phase III and II clinical trials of everolimus or placebo plus best supportive care in patients with metastatic renal clear cell carcinoma who had failed tyrosine kinase inhibitor (TKI) therapy have firmly established its position in subsequent therapy among patients with this characteristic (Motzer et al., 2010; Motzer et al., 2014; Motzer et al., 2016). In 2009, it was initially approved by the Food and Drug Administration (FDA) for adult patients with advanced renal cell carcinoma who had failed previous treatment with sunitinib or sorafenib. It was then successively approved for the prevention of organ rejection after kidney transplantation, tuberous sclerosis (TS), breast cancer and neuroendocrine tumors (Yao et al., 2016; Hirabatake et al., 2022; Farmaki et al., 2023). The results of the prestigious EXIST studies have revealed that everolimus can make tuberous sclerosis complex renal angiomyolipoma (TSC-AML) shrink rapidly and permanently, and so far it is the unique drug approved worldwide for the allopathic treatment of TSC-AML and tuberous sclerosis complex subventricular giant cell astrocytoma (TSC-SEGA) (Franz et al., 2013; Bissler et al., 2013; French et al., 2016). In June 2013, Afinitor® was approved by the Chinese drug regulatory authority for the treatment of late-stage renal cell carcinoma patients who had previously failed to receive sunitinib or sorafenib treatment. However, the high cost of original products has limited accessibility for many patients. Generic products may provide a viable solution to reduce patients' financial burden and enhance the accessibility of antitumor drugs, because generic drugs usually cost less than original products.

Current publications on everolimus are more often derived from population pharmacokinetic (PPK) studies in specific populations (e.g., oncology patients) (Park et al., 2023; Tanaka et al., 2016; Combes et al., 2018; de Wit et al., 2016; Itohara et al., 2022), and have not been explored in the healthy Chinese population. The instructions for everolimus tablets show that in healthy subjects, high-fat meals reduced systemic exposure to everolimus 10 mg tablet (as measured by AUC) by 22% and the peak plasma concentration  $C_{max}$  by 54%. Light fat meals reduced AUC by 32% and  $C_{max}$  by 42%. Food, however, had no apparent effect on the post-absorption phase concentration-time profile. This data does not reflect healthy individuals in China. Everolimus is an oral mTOR inhibitor, therefore when oral drugs are administered, food-drug interactions, also known as the “food effect” affect the pharmacokinetics and efficacy of oral drug. According to the dose-dependent pharmacokinetic characteristics of everolimus in the five currently marketed generic drugs, it may be more appropriate and convenient to test it in whole blood rather than in plasma. As a result of these considerations, this study (Clinical trial registry number: CTR20220446, <http://www.chinadrugtrials.org.cn/clinicaltrials.searchlist.dhtml>) aims to evaluate the pharmacokinetic (PK) similarity and safety of everolimus in

whole blood under fasting and postprandial conditions and its generics in Chinese healthy subjects with a randomized, single-dose, open-label, two-period crossover clinical trial.

# 2 Methods

## 2.1 Subjects

This study was conducted at the Phase I clinical trial research center of Jiangxi Cancer Hospital from April to June 2022. Eligible subjects were selected from healthy Chinese male and female volunteers aged 18–45 years, with body weight  $\geq 50$  kg, and body mass index (BMI) of 19.0–28.0 kg/m<sup>2</sup>. A full medical examination was performed on all subjects including physical examinations, vital signs (oral body temperature, pulse rate, and sitting blood pressure), 12-lead ECG, and prescribed laboratory tests (routine blood, urinalysis, blood biochemical tests, coagulation function tests, Infectious diseases tests, etc.). The duration of study participation for each subject was about 33 days.

## 2.2 Study drug

The *T* formulation (everolimus tablet: 5 mg, batch number: PEMA2110001) was produced by Bo Rui Pharmaceutical (Suzhou) Co., Ltd. The *R* formulation (Afinitor®: 5 mg, batch number: SYC43) was produced by Novartis Pharma Stein AG. Both *T* and *R* were given the same batch of drug throughout the trial.

## 2.3 Ethics approval and study population

This clinical study was approved by the Medical Ethics Committee of Jiangxi Cancer Hospital with written informed consent from all subjects (IRB Approval No:2022001-YW001). The study was performed following the ethical standards for studies in humans of the Declaration of Helsinki and its amendments, the International Conference on Harmonisation Guideline for Good Clinical Practice, and the Guideline for Good Clinical Principles recommended by the National Medical Products Administration (NMPA) of China. The study reported following the Consolidated Standards of Reporting Trials (CONSORT) extension for Bioequivalence Studies (Hopewell et al., 2025). The completed checklist is provided as supplementary material.

## 2.4 Study design

This whole study was a randomized, open-label, two-sequence, two-period crossover study with a 1-week washout period. Throughout the study, each subject receives *T* or *R* is determined by a random table. Two randomization tables by SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) were generated for the fasting cohort and the fed cohort through a 1:1 block randomization method. In the fasting cohort, the 28 subjects received the everolimus formulations in the sequence *T* then *R* or *R* then *T* under an overnight fast for at least 10 h; in the fed cohort, the

28 subjects received the everolimus formulations in the sequence *TR* or *RT* under the high-fat meal condition. During the trial, the unblinded pharmacist provided coded medications to the research nurse according to the study phase. The research nurse subsequently administered these medications to the participants.

The instructions for Afinitor® indicate that the  $C_{\max}$  is about 1–2 h after oral administration of 5–70 mg in patients with advanced solid tumors. The  $t_{1/2\beta}$  of everolimus is about 30 h, which can meet the requirement of sampling time of 3–5  $t_{1/2\beta}$  or more for 96 h, and the  $AUC_{0-t}$  of everolimus in fed condition can cover 80% of  $AUC_{0-\infty}$ . Venous blood samples (3 mL) were collected from an intravenous indwelling catheter before the dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, and 96 h after the 5 mg dose in the fasting cohort, and at 0 (pre-dosing), 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72, and 96 h after the dose in the fed cohort of the study. All participating subjects were under medical supervision by a physician throughout the study. After sample collection, the blood was stored directly in the  $-80^{\circ}\text{C}$  refrigerator until analyzed by LC-MS/MS.

## 2.5 Safety assessment

Safety assessments included physical examinations, vital signs, 12-lead ECGs, prescribed laboratory tests, and AE monitoring.

## 2.6 Determination of blood concentrations

In this study, the concentration of everolimus in blood was determined by a validated LC-MS/MS method. A deuterium-labeled analytical internal standard (IS, [ $^{13}\text{C}_2, ^2\text{H}_4$ ]- everolimus) was used for the quantitative determination of everolimus. To ensure the reliability of the analytical method for the determination, validation of this analytical method was performed to evaluate its selectivity, linearity, precision, accuracy, matrix effect, extraction recovery, and stability in matrix samples according to ICH M10 criteria.

A protein precipitation method was used to extract everolimus from human blood samples: An aliquot (50  $\mu\text{L}$ ) of IS (10.0 ng/mL in 50% [v/v] acetonitrile) was added to 50  $\mu\text{L}$  of blood, then 200  $\mu\text{L}$  of acetonitrile was added into each sample and mixed well (vortex 2,500 rpm  $\times$  3 min). After centrifuging the mixture at 14,000 rpm  $\times$  5 min at  $4^{\circ}\text{C}$ , the supernatant was transferred into auto-sampler vials, and 20  $\mu\text{L}$  was injected into the LC-MS/MS system for analysis. Chromatographic conditions were described as follows: Quantitation was achieved on a TRIPLE QUAD 5500 mass spectrometer (Applied Biosystems Sciex, Foster, CA, USA) in positive-ion mode with electrospray ionization (ESI) in multiple-reaction-monitoring (MRM) mode ( $m/z$  975.600/908.600 for everolimus,  $m/z$  981.600/914.600 for IS). The analyte and IS were separated on an Agela Technologies Venusil XBP C8 ( $2.1 \times 50$  mm, 5  $\mu\text{m}$ ) using a gradient elution program with a flow rate of 0.4 mL/min. The mobile phases A was methanol, and mobile phases B was 0.1% ammonium formate in water.

The lower limit of quantitation (LLOQ) of everolimus was 0.100 ng/mL with a dynamic range of 0.100–100 ng/mL. Quality control (QC) samples (Low QC, 0.300 ng/mL; Geometric Mean QC,

3.00 ng/mL; Medium QC, 30.0 ng/mL; High QC, 80.0 ng/mL; Dilution QC, 600 ng/mL) were analyzed to assess the accuracy and precision of the method. All QC samples are evenly distributed among the samples to be analyzed. The retention times of everolimus and IS were about 2.56 min, respectively. The missing pharmacokinetic concentration data were not imputed.

## 2.7 Pharmacokinetic analysis

Phoenix WinNonlin 8.2 (Pharsight Corp, Mountain View, CA, USA) and SAS software version 9.4 (SAS Institute, Cary, NC, USA) were used for statistical analysis in this study. Phoenix WinNonlin software was used to estimate and analyze the PK parameters of the non-compartmental model in the blood concentration data, and the Primary PK parameters ( $C_{\max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-\infty}$ ) and the secondary PK parameters ( $T_{\max}$ , MRT,  $t_{1/2}$ , and  $\lambda_z$ ). Sensitivity analysis was performed using Phoenix WinNonlin software.

## 2.8 Bioequivalence assessment

The average bioequivalence (ABE) method was used to evaluate the bioequivalence of the *T* and *R* formulations. When the 90% confidence interval (CI) of the geometric mean ratio of  $C_{\max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-\infty}$  between the *T* and *R* fall within the range of 80%–125% in this fasting and fed study, it can be determined that the two formulations are bioequivalent. SAS software was used for statistical analysis of data, and differences were considered statistically significant at  $p < 0.05$ .

# 3 Results

## 3.1 Volunteers characteristics

Twenty-eight subjects were included in each of the fasting and fed cohort groups (fasting: male: female = 22:6; postprandial: male: female = 25:3), with mean values of age, weight, and BMI of  $27.57 \pm 8.61$  years,  $63.95 \pm 10.44$  kg, and  $22.51 \pm 2.59$  kg/m<sup>2</sup> in the former and  $26.14 \pm 7.23$  years,  $63.45 \pm 8.72$  kg, and  $22.17 \pm 2.22$  kg/m<sup>2</sup> in the latter, respectively. Table 1 shows the demographic factors and laboratory tests of the two groups. All subjects received a single 5 mg oral dose of a test everolimus tablet (BoRui Pharmaceutical (Suzhou) Co., Ltd.) and the reference product (Afinitor®, Novartis Pharma Stein AG) under fasting or fed conditions. In the fed cohort group, subject Y025 was discontinued after failing to swallow the study drug whole in period 2, All other 27 participants completed the study in full compliance with the trial protocol (Figure 1).

## 3.2 Method validation

The bioanalytical method validation results are briefly described as follows: No interfering peaks were observed near the retention times of everolimus and IS for all samples, The intra-batch and inter-batch accuracy and precision were assessed by six runs analyzed at four concentration levels of QCs. Including the LLOQ, the

TABLE 1 Demographic characteristics of the subjects.

Parameters <sup>a</sup>	Under fasting condition (N = 28)	Under fed condition (N = 28)
Age (years)	27.57 ± 8.61	26.14 ± 7.23
Sex (n %)		
Male	22 (78.57%)	25 (89.28)
Female	6 (21.43%)	3 (10.72)
Height (cm)	168.38 ± 9.75.4	169.04 ± 7.81
Weight (kg)	63.95 ± 10.44	63.45 ± 8.72
BMI (kg/m²)	22.51 ± 2.59	22.17 ± 2.22

<sup>a</sup> Values are presented as mean ± SD, except for sex, which is shown as n (%).

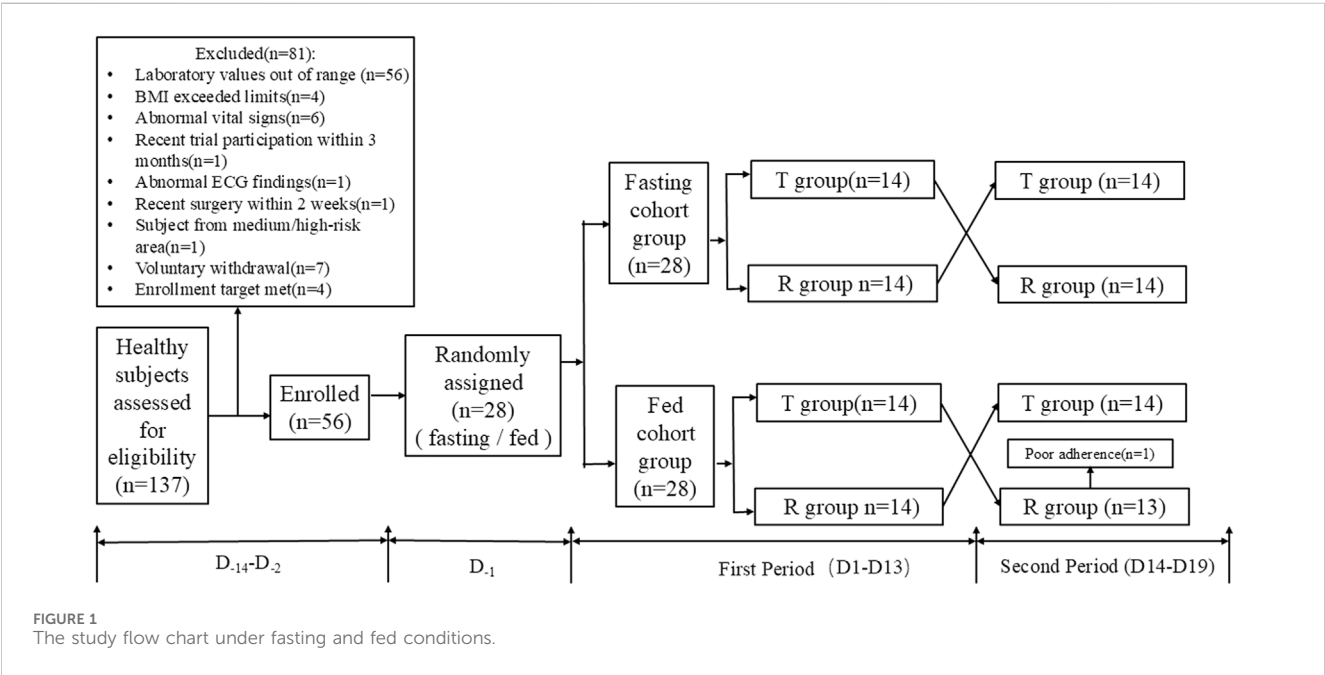


TABLE 2 Intra-batch and Inter-batch precision and accuracy of the LC-MS/MS method for determining everolimus concentration in human blood.

Concentration Added (ng/mL)	Intra-batch (N = 6)			Intre-batch (N = 6)		
	Mean ± SD (ng/mL)	Accuracy (%DEV)	Precision (CV%)	Mean ± SD (ng/mL)	Accuracy (%DEV)	Precision (CV%)
0.100	0.102 ± 0.01	2.42	9.16	0.107 ± 0.02	6.77	14.87
0.300	0.288 ± 0.01	-3.94	4.86	0.312 ± 0.03	3.92	9.46
3.00	3.23 ± 0.09	7.50	2.81	3.18 ± 0.16	5.87	5.06
30.0	31.1 ± 1.03	3.56	3.32	30.4 ± 1.59	1.44	5.22
80.0	78.3 ± 2.91	-2.17	3.72	78.5 ± 3.87	-1.86	4.92

intra-batch accuracy deviation (%DEV) was -3.94%~7.50%, and the precision (coefficient of variation, %CV) ranged from 2.81%~9.16%. For Inter-batch accuracy and precision, the %DEV was -1.86%~6.77%, and the %CV was 4.92%~14.87% (Table 2).

The matrix effect was assessed by preparing LQC, MQC, and HQC using six different sources of normal blank matrix and three different sources of haemolysed matrix with three replicates. The average normalized matrix factor of

TABLE 3 Matrix factor and recovery of everolimus and IS.

Concentration Added (ng/mL)	Matrix factor <sup>a</sup> (N = 27 <sup>c</sup> )		Recovery <sup>b</sup> (N = 18 <sup>d</sup> )		
	Matrix factor	%CV	Analyte (%)	IS (%)	%CV
0.300	1.03	4.69	95.57	99.97	2.61
30.0	1.00	1.03	95.14		
80.0	0.99	0.71	91.78		

<sup>a</sup>Matrix factor (Non – Normalized) = Average peak area response in the presence of matrixions (Matrix Factor Blanks)/Average peak area response in the absence of matrixions (Neat Samples).  
<sup>b</sup>Recovery = Average peak area response in the presence of Pre - extraction/Average peak area response in the presence of Post - extraction.  
<sup>c</sup>Each QC, for matrix effect was assessed by six different sources of normal blank matrix with three replicates and three different sources of haemolysed matrix with three replicates.  
<sup>d</sup>Each QC, for recovery was assessed by six different sources of normal blank matrix with three replicates.

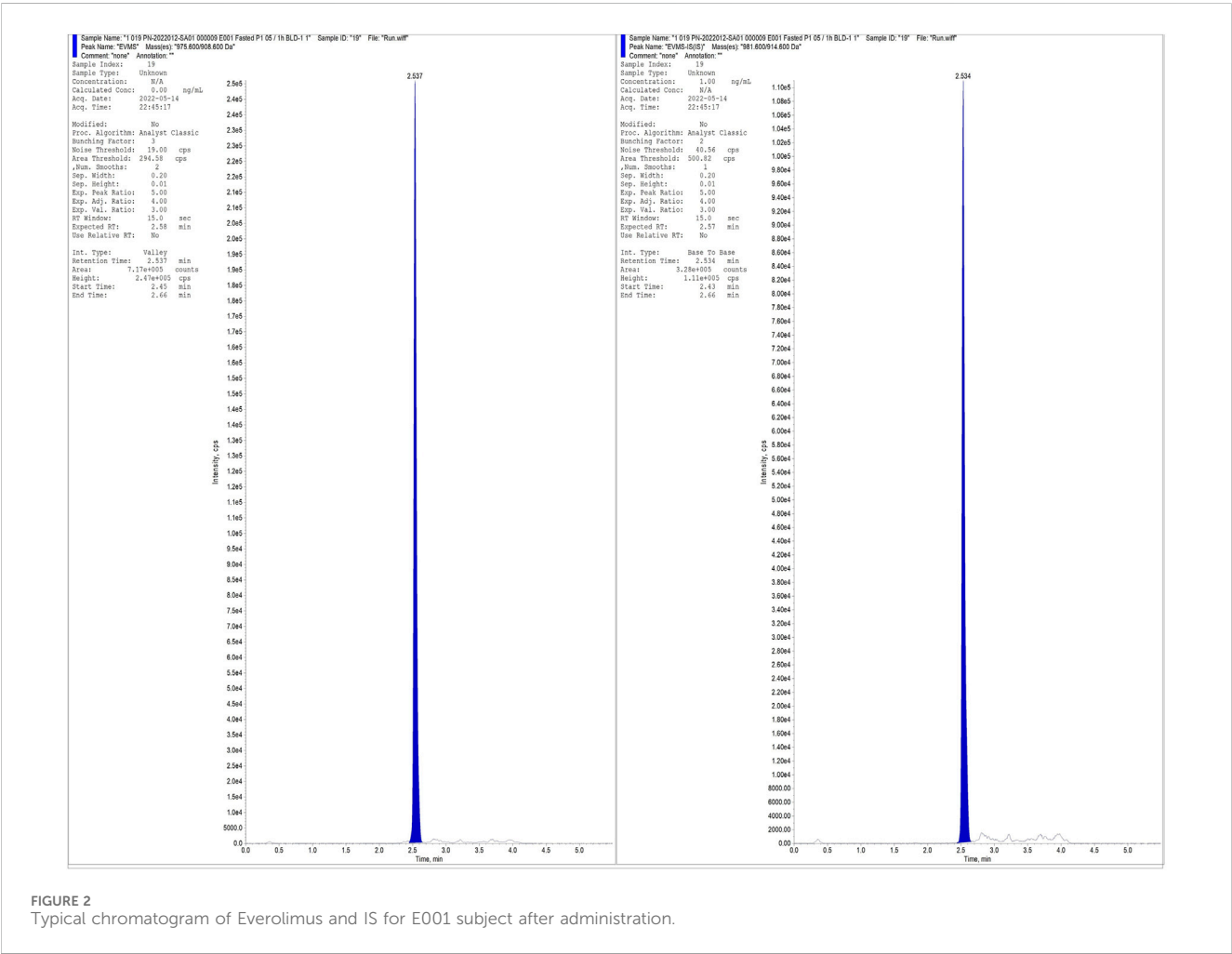


FIGURE 2 Typical chromatogram of Everolimus and IS for E001 subject after administration.

everolimus was 1.03, 1.00, and 0.99, respectively, and the overall %CV was 0.71~4.69%, showing that the ion inhibition or enhancement in human blood can be negligible at different matrices. By calculating the peak area ratio of LQC, MQC, and HQC, the extraction recoveries of everolimus in blood were 95.57%, 95.14%, and 91.78%, respectively, and the overall %CV was 2.97%; the extraction recovery of IS was 99.97%, and the %CV was 2.61% (Table 3).

The testing included the stability of whole blood at room temperature for 19 h, frozen-thawed cycles five times, processed samples in the autosampler (4 °C) for 212 h, and the long-term stability at –80 °C condition for 44 days, the results of matrix stability were all within the acceptable criteria.

In summary, this analytical method exhibits high sensitivity, simple sample processing steps (using protein precipitation method), no matrix effect, good recovery, and a short run time

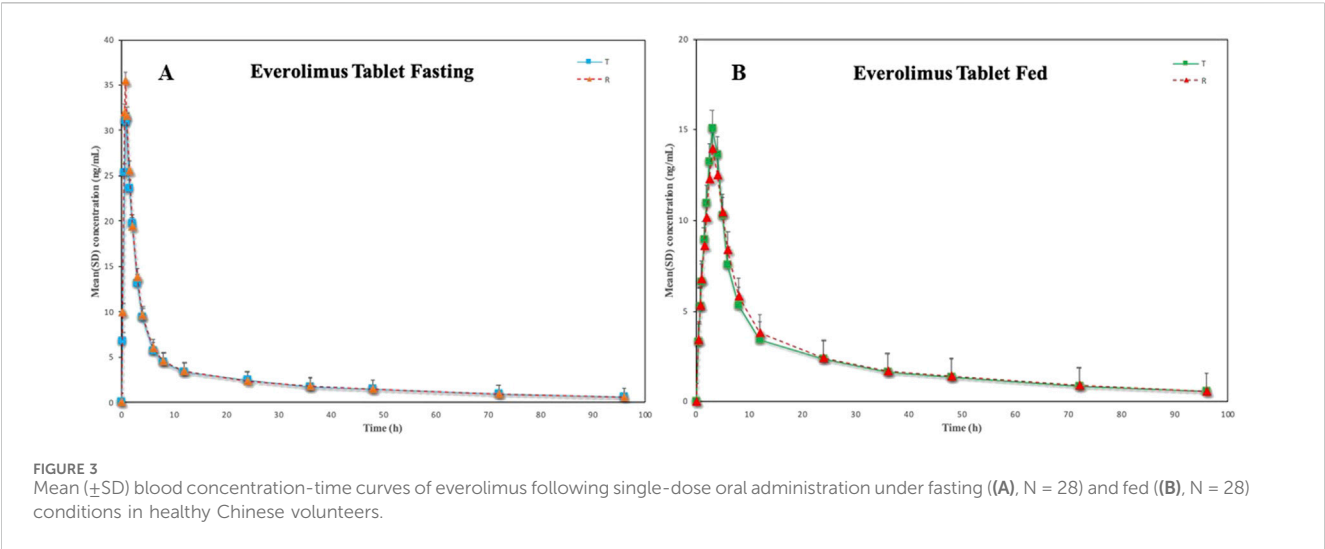


TABLE 4 Pharmacokinetic properties of T and R formulations of everolimus 5-Mg tablets after single-dose administration in healthy Chinese volunteers under fasting and fed Conditions (Mean ± SD (intra CV%)).

Parameters	Under fasting condition		Under fed condition	
	T Formulation	R Formulation	T Formulation	R Formulation
T <sub>max</sub> (h) <sup>b</sup>	0.75 (0.50, 4.00) (75.61)	0.75 (0.50, 3.00) (58.20)	2.75 (0.75, 4.00) (40.28)	3.00 (0.50, 6.00) (44.88)
C <sub>max</sub> (ng/mL)	35.38 ± 11.75 (33.21)	39.04 ± 13.38 (34.26)	18.94 ± 4.85 (25.61)	18.34 ± 5.48 (29.91)
AUC <sub>0-t</sub> (ng·h/mL)	234.62 ± 82.70 (35.25)	245.44 ± 53.81 (21.92)	212.36 ± 33.16 (15.62)	217.86 ± 43.52 (19.98)
AUC <sub>0-∞</sub> (ng·h/mL)	265.73 ± 96.28 (36.23)	278.28 ± 61.99 (22.28)	243.34 ± 39.18 (16.10)	251.30 ± 51.65 (20.55)
AUC <sub>%Extrap</sub> (%)	13.36 ± 10.64 (79.69)	11.66 ± 3.35 (28.70)	12.60 ± 2.98 (23.68)	13.15 ± 3.07 (23.32)
t <sub>1/2</sub> (h)	35.90 ± 7.13 (19.87)	37.19 ± 6.74 (18.12)	37.22 ± 4.79 (12.86)	38.43 ± 5.23 (13.60)
λ <sub>z</sub> (h <sup>-1</sup> )	0.02 ± 0.01 (25.09)	0.02 ± 0.00 (18.24)	0.02 ± 0.00 (11.14)	0.02 ± 0.00 (13.07)
MRT <sub>0-t</sub> (h)	22.88 ± 3.55 (15.52)	23.13 ± 1.83 (7.92)	25.60 ± 1.88 (7.33)	25.81 ± 1.74 (6.76)

<sup>b</sup>Tmax is represented by the median [minimum, maximum].

(2.56 min) (Figure 2), which is particularly well-suited for routine analysis of a large number of samples.

3.3 Pharmacokinetics

Under fasting cohort, the average relative bioavailability of F<sub>0-t</sub> and F<sub>0-∞</sub> was (99.83 ± 20.11) % and (98.11 ± 18.78) %, respectively; under fed cohort, F<sub>0-t</sub> and F<sub>0-∞</sub> were (98.79 ± 12.30) % and (98.14 ± 11.80) %, respectively. The concentration-time curves of the two groups after receiving a single oral 5 mg everolimus tablet are described in Figure 3, and the main pharmacokinetic parameters are illustrated in Table 4. Under fasting cohort, the intra-subject CV % for C<sub>max</sub> was 33.21% for the test (T) formulation and 34.26% for the reference (R) formulation, while the intra-subject CV% for AUC<sub>0-t</sub> were 35.25% (T) and 21.92% (R),These results showed considerable inter-individual variability in the absorption of everolimus; In contrast, under fed cohort, the intra-subject CV% for C<sub>max</sub> of the T and R formulations were 25.61% and 29.91%, respectively, and the intra-subject CV% for AUC<sub>0-t</sub> were 15.62%

and19.98%. Food intake may moderate inter-individual pharmacokinetic differences. Based on the median and range of T<sub>max</sub>, suggesting that food intake significantly delayed drug absorption, which aligns with the expected effect of a high-fat diet on gastrointestinal motility. For R formulation, the gastrointestinal absorption rate was decreased by food intake: median Tmax was 0.75 h in the fasted state, and 3.00 h after a high-fat meal. The average elimination half-life (t<sub>1/2β</sub>, 37.19–38.43 h) and mean residence time (MRT, 23.13–25.81 h) did not appear to be affected by food intake regardless of fasting or fed state. In addition, the results of the Wilcoxon signed-rank test did not exhibit any statistical significance in the T<sub>max</sub> between the two formulations under fasting or fed condition (P > 0.05).

3.4 Bioequivalence

The average bioequivalence (ABE) method was adopted in this study. A total of 28 healthy subjects completed the fasting test, the results showed that the 90% CI of C<sub>max</sub>, AUC<sub>0-t</sub>, and AUC<sub>0-∞</sub> for



TABLE 5 Bioequivalence evaluation of *T* and *R* formulations of everolimus 5-Mg tablets in healthy volunteers under fasting condition.

Parameters	Geometric mean			90%CI (Lower ~ Upper) (%)	Power (%)	Intra-CV (%)
	<i>T</i>	<i>R</i>	( <i>T</i> / <i>R</i> )%			
<i>C</i> <sub>max</sub> (ng/mL)	30.91	29.45	95.29	85.81~105.80	86.70	22.76
AUC <sub>0-t</sub> (ng·h/mL)	203.81	198.63	97.46	91.39~103.93	99.97	13.87
AU <i>C</i> <sub>0-∞</sub> (ng·h/mL)	273.65	265.04	96.85	90.94~103.15	99.97	13.36

TABLE 6 Bioequivalence evaluation of *T* and *R* formulations of everolimus 5-Mg tablets in healthy volunteers under fed condition.

Parameters	Geometric mean			90%CI (Lower ~ Upper) (%)	Power (%)	Intra-CV (%)
	<i>T</i>	<i>R</i>	( <i>T</i> / <i>R</i> )%			
<i>C</i> <sub>max</sub> (ng/mL)	17.65	18.38	104.13	95.61~113.41	97.15	18.63
AUC <sub>0-t</sub> (ng·h/mL)	214.58	209.97	97.85	94.13~101.72	>99.99	8.38
AU <i>C</i> <sub>0-∞</sub> (ng·h/mL)	246.47	240.05	97.40	93.60~101.35	>99.99	8.43

TABLE 7 Summary of adverse events (SS) of *T* and *R* formulations of everolimus 5-Mg tablets after oral administration under fasting and fed conditions in healthy Chinese volunteers.

Projects	Fasting study		Fed study	
	<i>T</i> (N = 28)	<i>R</i> (N = 28)	<i>T</i> (N = 28)	<i>R</i> (N = 28)
At least one adverse event occurred	10 (35.71%)	5 (17.86%)	7 (25.00%)	6 (21.43%)
Related to research drugs	8 (28.57%)	5 (17.86%)	7 (25.00%)	5 (17.86%)
SAE	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Adverse events leading to study discontinuation	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

the geometric mean ratio (GMR) of the *T* and *R* formulations were 95.29% (85.81–105.80%), 97.46% (91.39–103.93%), and 96.85% (90.94–103.15%) (Table 5), the maximum intra-individual CV% (intra-CV%) of *T* and *R* formulations was 22.76% (*C*<sub>max</sub>) under fasting condition. A total of 28 healthy subjects completed the fed test, the results revealed that the 90% CI of *C*<sub>max</sub>, AUC<sub>0-t</sub>, and AUC<sub>0-∞</sub> for the GMR of the *T* and *R* were 104.13% (95.61–113.41%), 97.85% (94.13–101.72%), and 97.40% (93.60–101.35%) (Table 6), the maximum intra-CV% was 18.63% (*C*<sub>max</sub>). All parameters were within the bioequivalence range of 80.00%–125.00%.

### 3.5 Safety

A total of 22 AEs occurred in 15 (53.57%) subjects in the fasting cohort, including 14 AEs in 10 (35.71%) subjects under the *T* and 8 AEs in 5 (17.86%) subjects under the *R*. The AEs related to the study drug was 18 AEs in 13 (46.43%) subjects (Table 7); A total of 17 AEs occurred in 13 (46.43%) subjects in the fed cohort, including 10 AEs in 7 (25.00%) subjects under the *T* and 7 AEs in 6 (21.43%) subjects under the *R*. The AEs related to the study drug was 16 AEs in 12 (42.86%) subjects (Table 7). All AEs were mild, and none led to

subject withdrawal. All AEs were followed until resolution, categorising as “improved”, “recovered”, or “unknown”.

## 4 Discussion

Due to the high cytotoxicity of anti-tumor drugs, in order to truly reflect the safety and effectiveness of drugs in patients and avoid unnecessary damage to healthy subjects, cancer patients are generally selected for human studies due to ethical requirements. However, for some non-cytotoxic drugs, such as hormones, tyrosine kinase inhibitors - due to their mild toxicity, studies based on healthy volunteers can be considered to obtain relatively accurate metabolic characteristics of drugs *in vivo* provided that subject safety is fully ensured. Healthy adult volunteers were identified for the study in accordance with the FDA’s Draft Guidance on Everolimus (U.S. Food and Drug Administration, 2018), thus healthy subjects were enrolled to complete this clinical trial. Presently, no other relevant literature has reported the exploration of the pharmacokinetic parameters of everolimus in healthy humans. The pharmacokinetic data of the original drug showed that Chinese solid tumor patients (n = 24) reached peak blood drug concentrations 2–3 h after receiving 5 mg/day and 10 mg/day, which

was consistent with the  $T_{max}$  data of healthy subjects in our experiment, indicating that clinical trials using healthy subjects can accurately reflect real-world pharmacokinetic parameters.

The concentration of the parent drug in whole blood was determined, which is a distinguishing feature of everolimus compared to the concentration monitoring of other drugs. Everolimus exhibited dose-dependent pharmacokinetic characteristics ranging from 5 to 5,000 ng/mL. Upon oral administration, approximately 75% of the gastrointestinally absorbed penetrated the erythrocytes, while the plasma drug concentration accounted for about 20% of the whole blood concentration. Despite the fact that six major metabolites, including three monohydroxylated metabolites, two hydrolysed ring-opening products, and one phosphatidylcholine conjugate were detectable in human blood, they were considered negligible, as their activity was about 1% of the original. Therefore, the concentration of the parent drug in whole blood was determined to evaluate bioequivalence.

Under both fasting and fed conditions, the 90% confidence intervals for the geometric mean ratios of the primary pharmacokinetic parameters between the test and reference formulations fell entirely within the predefined bioequivalence range. The intra-subject coefficients of variation for both formulations were within acceptable limits under both prandial states, and the mean relative bioavailability was comparable. Furthermore, statistical analysis of  $T_{max}$  showed no significant differences between the two formulations under either fasting or fed conditions ( $P > 0.05$ ). As such, the two products were bioequivalent under both fasting and fed conditions. Furthermore, the  $AUC\%_{Extrap}$  values were well below 20% (Fasting T: 13.36%, R: 11.66%; Fed T: 12.60%, R: 13.15%), confirming that the blood sampling schedule was appropriately designed to reliably estimate total drug exposure from time zero to infinity ( $AUC_{0-\infty}$ ). The elimination half-life ( $t_{1/2}$ , approximately 35–38 h) and terminal elimination rate constant ( $\lambda_z$ ) were highly consistent. This demonstrates that the elimination processes of the test and reference formulations were fundamentally equivalent, further supporting the conclusion of bioequivalence between the two products.

Sensitivity analysis was performed in this study. In the fasting cohort, because the  $AUC\%_{Extrap}$  of E019 and E020 subjects in the first period were both greater than 20%, the  $AUC_{0-\infty}$  analysis set did not include them. The sensitivity analysis of  $AUC_{0-\infty}$  in 28 subjects was carried out, Phoenix WinNonlin 8.2 software was used to analyze BES, and the results showed that the 90% CI of  $T/R$  GMR of  $AUC_{0-\infty}$  was 91.05–103.51%, and the  $P$  values of  $AUC_{0-\infty}$  were all  $<0.05$ . In the fed cohort, because the  $AUC\%_{Extrap}$  of Y007 subjects in the first and second periods were both greater than 20%, the  $AUC_{0-\infty}$  analysis set did not include them. The sensitivity analysis of  $AUC_{0-\infty}$  after inclusion showed that the 90% CI of  $T/R$  GMR of  $AUC_{0-\infty}$  was 93.65–101.11%. Both unilateral  $t$ -tests showed that  $P$  values of  $AUC_{0-\infty}$  were  $<0.05$ . The fact that both  $T$  and  $R$  formulations are bioequivalent was demonstrated in both parts of the sensitivity analysis study.

## 5 Conclusion

In the study, it is demonstrated that it is safe for healthy subjects to participate in bioequivalence studies of antitumor drugs. A simple

and high-throughput LC-MS/MS method with satisfactory accuracy, precision, and sensitivity was developed for the detection of everolimus in human blood. The validated method allows the determination of everolimus concentrations in the range of 0.100–100 ng/mL, making it is particularly applicable for routine analysis a large sample volumes.

The systemic availability of a single oral 5 mg dose of everolimus was significantly reduced when coadministration with food compared to under fasting conditions. Moreover, the trial concluded that the  $T$  formulation (5 mg tablet) was bioequivalent to the  $R$  formulation under both fasting and fed conditions.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by the Medical Ethics Committee of Jiangxi Cancer Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

XC: Conceptualization, Writing – original draft. XY: Data curation, Project administration, Writing – review and editing. YL: Data curation, Project administration, Writing – review and editing. XW: Investigation, Software, Writing – review and editing. YP: Supervision, Validation, Writing – review and editing. QW: Formal Analysis, Writing – review and editing. YS: Software, Writing – review and editing. HZ: Writing – review and editing, Conceptualization, Methodology, Project administration.

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## Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

## References

- Bissler, J. J., Kingswood, J. C., Radzikowska, E., Zonnenberg, B. A., Frost, M., Belousova, E., et al. (2013). Everolimus for angiomyolipoma associated with Tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet* 381, 817–824. doi:10.1016/S0140-6736(12)61767-X
- Combes, F. P., Baneyx, G., Coello, N., Zhu, P., Sallas, W., Yin, H., et al. (2018). Population pharmacokinetics-pharmacodynamics of oral everolimus in patients with seizures associated with Tuberous sclerosis complex. *J. Pharmacokinet. Pharmacodyn.* 45, 707–719. doi:10.1007/s10928-018-9600-2
- de Wit, D., Schneider, T. C., Moes, D. J., Roozen, C. F., den Hartigh, J., Gelderblom, H., et al. (2016). Everolimus pharmacokinetics and its exposure-toxicity relationship in patients with thyroid cancer. *Cancer Chemother. Pharmacol.* 78, 63–71. doi:10.1007/s00280-016-3050-6
- Farmaki, E., Nath, A., Emond, R., Karimi, K. L., Grolmusz, V. K., Cosgrove, P. A., et al. (2023). ONC201/TIC10 enhances durability of mTOR inhibitor everolimus in metastatic ER+ breast cancer. *Elife* 12, e85898. doi:10.7554/eLife.85898
- Franz, D. N., Belousova, E., Sparagana, S., Bebin, E. M., Frost, M., Kuperman, R., et al. (2013). Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with Tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 381, 125–132. doi:10.1016/S0140-6736(12)61134-9
- French, J. A., Lawson, J. A., Yapici, Z., Ikeda, H., Polster, T., Nabbut, R., et al. (2016). Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study. *Lancet* 388, 2153–2163. doi:10.1016/S0140-6736(16)31419-2
- Hirabatake, M., Mizuno, T., Kato, H., and Hashida, T. (2022). Everolimus pharmacokinetics and exposure-response relationship in Japanese patients with advanced breast cancer. *Front. Pharmacol.* 13, 984002. doi:10.3389/fphar.2022.984002
- Hopewell, S., Chan, A. W., Collins, G. S., Hrobjartsson, A., Moher, D., Schulz, K. F., et al. (2025). CONSORT 2025 statement: updated guideline for reporting randomised trials. *BMJ* 389, e081123. doi:10.1136/bmj-2024-081123
- Itohara, K., Yano, I., Nakagawa, S., Sugimoto, M., Hirai, M., Yonezawa, A., et al. (2022). Population pharmacokinetics of everolimus in adult liver transplant patients: comparison to tacrolimus disposition and extrapolation to pediatrics. *Clin. Transl. Sci.* 15, 2652–2662. doi:10.1111/cts.13389
- Menzel, K., Kuo, Y., Chen, D., Hartmann, G., Wang, Y. H., Cho, C. R., et al. (2023). Developing a mechanistic understanding of the nonlinear pharmacokinetics of letermovir and prospective drug interaction with everolimus using physiological-based pharmacokinetic modeling. *Clin. Transl. Sci.* 16, 1039–1048. doi:10.1111/cts.13509
- Motzer, R. J., Escudier, B., Oudard, S., Hutson, T. E., Porta, C., Bracarda, S., et al. (2010). Phase 3 trial of everolimus for metastatic renal cell carcinoma: final results and analysis of prognostic factors. *Cancer* 116, 4256–4265. doi:10.1002/cncr.25219
- Motzer, R. J., Barrios, C. H., Kim, T. M., Falcon, S., Cosgriff, T., Harker, W. G., et al. (2014). Phase II randomized trial comparing sequential first-line everolimus and second-line sunitinib versus first-line sunitinib and second-line everolimus in patients with metastatic renal cell carcinoma. *J. Clin. Oncol.* 32, 2765–2772. doi:10.1200/JCO.2013.54.6911
- Motzer, R. J., Alyasova, A., Ye, D., Karpenko, A., Li, H., Alekseev, B., et al. (2016). Phase II trial of second-line everolimus in patients with metastatic renal cell carcinoma (RECORD-4). *Ann. Oncol.* 27, 441–448. doi:10.1093/annonc/mdv612
- Park, J., Kim, S. H., Hahn, J., Kang, H. C., Lee, S. G., Kim, H. D., et al. (2023). Population pharmacokinetics of everolimus in patients with seizures associated with focal cortical dysplasia. *Front. Pharmacol.* 14, 1197549. doi:10.3389/fphar.2023.1197549
- Tanaka, A., Yano, I., Shinsako, K., Sato, E., Fukudo, M., Masuda, S., et al. (2016). Population pharmacokinetics of everolimus in relation to clinical outcomes in patients with advanced renal cell carcinoma. *Ther. Drug Monit.* 38, 663–669. doi:10.1097/FTD.0000000000000344
- U.S. Food and Drug Administration (2018). Draft guidance on everolimus FDA seizes 7-OH opioids to protect American consumers. Available online at: [https://www.accessdata.fda.gov/drugsatfda\\_docs/psg/Everolimus\\_draft\\_Oral%20tab\\_RLD%2022334\\_RC11-18.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/psg/Everolimus_draft_Oral%20tab_RLD%2022334_RC11-18.pdf).
- Yao, J. C., Fazio, N., Singh, S., Buzzoni, R., Carnaghi, C., Wolin, E., et al. (2016). Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. *Lancet* 387, 968–977. doi:10.1016/S0140-6736(15)00817-X

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