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# A study on the pharmacokinetic bioequivalence of oral tablet formulations of riluzole among healthy volunteers utilizing HPLC-MS/MS

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## Abstract

**Introduction** This randomized, open-label, two period, two treatment, fasting bioequivalence trial was conducted to demonstrate the bioequivalence between riluzole tablets manufactured by Jiangsu Enhua Pharmaceutical Co., Ltd. and the reference preparations from Sanofi Winthrop Industry (certified by Sanofi Mature IP) in healthy individuals.

**Objective** The study aimed to compare the pharmacokinetic parameters and evaluate the bioequivalence of both preparations when taken on an empty stomach. Additionally, the safety profile of both preparations was assessed in the study population.

**Methods** Seventy-two subjects participated in the trial and received riluzole tablets once per dosing cycle while fasting. They were randomLy assigned to either a 50-mg test or reference formulation, with a 7-day washout period between cycles. Venous blood samples (4 mL) were collected 22 times from each subject, starting before dosing (0 h) and ending 48 h after. Plasma riluzole concentrations were measured using liquid chromatography tandem mass spectrometry. This clinical trial has been officially registered in the Chinese Clinical Trial Register (accessible at http://www.chinadrugtrials.org.cn/index.htmL) with the registration number CTR20230637 on March 02, 2023.

**Results** The results showed that the geometric mean ratios of key pharmacokinetic parameters—including the area under the plasma concentration-time curve from time zero to the last nonzero concentration ($AUC_{0-t}$) (102.21%; confidence interval [CI], 96.85-107.86%), AUC from time zero to infinity ($AUC_{0-\infty}$) (102.03%; CI, 96.86-107.47%), and the peak plasma concentration ($C_{max}$) (107.47%; CI, 95.03-121.54%)—all fell within the bioequivalence acceptance range of 80-125%. Importantly, no serious adverse events were reported, and no subjects withdrew due to adverse events, indicating good tolerability of both formulations among the healthy Chinese volunteers.

**Conclusion** These findings establish the bioequivalence of the 50-mg test preparation of oral riluzole tablets with the reference listed drug.

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**Keywords** Riluzole, Bioequivalence study, Pharmacokinetic study, High performance liquid chromatography, Mass spectrometry

## Introduction

Amyotrophic lateral sclerosis (ALS) is a rare multisystem neurodegenerative disease that progressively degrades motor neurons, resulting in demyelination, muscular weakness, and ultimately, respiratory failure [1]. Increasing age stands as a significant risk factor for ALS. Given the escalating elderly population worldwide, it becomes imperative to discover valuable and efficient diagnostic and treatment approaches [2]. Riluzole stands as the first disease-modifying treatment approved for ALS [3]. Although various drug classes have been approved in certain countries, riluzole remains the gold standard of therapy [3].

This benzothiazole compound, with the structural formula 2-amino-6-trifluoromethoxybenzothiazole, was approved by the U.S. Food and Drug Administration in 1995 for the treatment of ALS patients [4]. Produced by Sanofi Company, it is administered twice daily in 50 mg oral tablets (Improvutek). The mechanism of riluzole's action is not fully understood but may involve the inhibition of glutamate release, inactivation of stable voltage-dependent sodium channels, and interference with intracellular events following neurotransmitter binding to excitatory amino acid receptors [5]. Riluzole demonstrates high absorbability (approximately 91%) and an absolute bioavailability of about 60% [6]. After oral administration, it is rapidly absorbed, reaching maximum plasma concentration ($T_{max}$) within 1 to 1.5 h across studied dose ranges [7]. Exposure to riluzole has been found to be linear over a dose range of 25 to 300 mg, administered every 12 h [7].In the body, riluzole primarily binds to plasma albumin and lipoprotein, and it can penetrate the blood-brain barrier. Its metabolic process is complex, involving both phase I reactions catalyzed by cytochrome P450 (CYP) and phase II reactions [7]. Initially, the trifluoromethoxybenzene moiety on the benzothiazole ring undergoes aromatic hydroxylation to form phenolic metabolites. In the second phase, riluzole binds to the glucuronic acid of its phenolic metabolites [7]. Both riluzole and its metabolites are primarily eliminated via renal excretion, with minimal fecal excretion [7].

The riluzole tablets manufactured by Jiangsu Enhua Pharmaceutical Co., Ltd. bear the NMPA approval number (Sinopharm Zhunzi H20045977), and are currently undergoing consistency evaluation in accordance with the Opinions of the General Office of the State Council on the Consistency Evaluation of Generic Drugs and their Efficacy (Guo Ban Fa [2016] No.8). In this study, we conducted a bioequivalence evaluation of a 50 mg riluzole test preparation manufactured by Jiangsu Enhua Pharmaceutical Co., Ltd., in comparison to the reference preparation licensed by Sanofi Mature IP and produced by Sanofi Winthrop Industrie (trade name: Lirutai®, specification: 50 mg). High fat diet will absorb riluzole in gastrointestinal system, resulting in low plasma level Referring to the FDA guidelines for the bioequivalence study of riluzole tablets finalized in May 2008, if the reference preparation instruction clearly states that the drug can only be taken on an empty stomach (1 h before meals or 2 h after meals), the postprandial bioequivalence study may not be carried out [8]. Therefore, this study only evaluated the bioequivalence of riluzole tablets in healthy subjects under fasting conditions.

## Methods

### Study design

A total of 72 subjects were randomized equally into 1 of the 2 treatment sequences under fasting conditions (no food from 10 h before to 4 h after dosing). A single 50-mg oral dose of riluzole tablet of test preparation with 240 mL of water, or a single 50-mg oral dose of a riluzole tablet of reference preparation with 240 mL water. The elimination half-life of riluzole ranges from 9 to 15 h. In order to ensure complete cleaning, the cleaning period in this test is 7 days. The blood sampling schedule for this test comprises 22 time points: 0 h before administration (within an hour of ingestion), and then at 10, 20, 30, 40, 50, 60, 70, 80 min, as well as 1.5, 1.75, 2, 3, 4, 6, 8, 10, 12, 14, 24, 36, and 48 h post-administration.

### Subjects

The study primarily included healthy Chinese subjects aged 18 and above, both male and female. Male subjects were required to have a minimum weight of 50 kg, while female subjects had to weigh at least 45 kg. The body mass index (BMI) of all subjects fell within the range of 19.0–26.0 kg/m$^2$, inclusive of the critical values. Subjects were required to have a comprehensive understanding of the test's purpose and potential adverse reactions, and they voluntarily signed informed consent to participate in and complete the study according to the test plan's requirements. Additionally, subjects were expected to communicate effectively with researchers, fully comprehend, and adhere to the study's demands. Key exclusion criteria involved subjects with a clinically significant history of disease or other physical abnormalities that could potentially influence drug absorption, distribution, or metabolism. Subjects with abnormal laboratory test results were also excluded from participation. During the study period, no concurrent medication was permitted,

except for medications necessary to treat adverse events. The inclusion and exclusion criteria of the subjects were put in the supplementary material 2.

### Procedures

Subjects were confined to a Phase 1 clinical trial laboratory for a minimum of 11 h prior to the first cycle of administration and up to 48 h following the second cycle. 4mL blood samples were collected from the upper limb venous blood for the quantitative detection of riluzole. Vital signs were measured 1 h before administration and at 1, 2, 4, 12, 24, 36, and 48 h after administration. The researchers continuously monitored the subjects' health during the entire trial. Adverse events were graded using the Common Terminology Criteria for Adverse Events (CTCAE) 5.0 system, ranging from level 1 to 5. The relationship between adverse events and the study medication was categorized into five levels: definitely related, probably related, possibly related, probably not related, and definitely not related.

### Analysis conditions

Determination of riluzole in human plasma utilizing high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).This analytical method used riluzole-$^{13}C$-$^{15}N_2$ as the internal standard (IS).The chromatography separation of riluzole and the IS was conducted on an ACE Excel Super C18 column (dimensions: 2.1 × 100 mm, 3 μm, ACE). The isocratic mobile phase consisted of A: a 1.0 M ammonium acetate aqueous solution mixed/ formic acid = 2/10, B: methanol (A/B = 30/70). The flow rate of the mobile phase was set at 0.35 mL/min. The column temperature was set at 40 °C. Injection volume was set at 5 μl. The retention time of riluzole is approximately 1.91 min, while the retention time of riluzole-$^{13}C$-$^{15}N_2$ is 1.90 min for a total run time of 4 min. The detection was carried out on a QTrap 5500 mass spectrometer (Applied Biosystems/ Sciex) for riluzole and riluzole-$^{13}C$-$^{15}N_2$.The ionization mode employed was positive electrospray ((+) ES), the scanning mode was multiple reaction monitoring (MRM), and the acquisition time amounted to 4 min. The MRM transitions of riluzole and the IS were m/z 235.0→166.0 and m/z 238.0→169.4. Both drug and IS were eluted in under 4.0 min. The linearity was in the range of 0.700–700 ng/mL with an lower limit of quantifcation (LLOQ) was 0.700 ng/mL. The intra-and inter-batch precision and accuracy of riluzole were less than 14.4%. The IS normalized recovery was 97.7%.The molecular structure of riluzole can be located in the supplementary material 1.

### Statistical analyses

Phoenix® WinNonlin® (version 8.2, Certara, USA) was utilized for calculating the pharmacokinetic parameters of riluzole and IS through noncompartmental analysis. The primary evaluation criteria consisted of peak concentration ($C_{max}$), the direct $AUC_{0-t}$ based on measured blood drug concentration-time data, and the area under the curve from drug administration to the lowest detectable blood drug concentration. Secondary evaluation metrics included the time to reach peak concentration ($T_{max}$) and the direct elimination rate constant ($\lambda_z$) derived from blood drug concentration-time measurements.

### Sample size calculation

We estimated the sample size based on the following six factors: average bioequivalence limit: 80.00%~ 125.00%; significance level: one-sided 5%; test power: 80%; test preparation/reference preparation ratio: 94%; intra-individual coefficient of variation (CVw): 36%. Taking into account the 20% dropout rate, it is finally planned to enroll 72 subjects for the trial.

### Random method

During the screening process, subjects were identified solely by their screening numbers, which were assigned sequentially based on the order of signing the informed consent form, starting with S001, S002, S003, and so on. The order in which each subject received either the test or reference preparation was determined using a randomization table. This table was generated randomly by our statistical units using SAS (version 9.4), following a blocked design with a 1:1 group ratio. To ensure reproducibility of the random data, the initial seed number for generating random numbers was saved. The randomization scheme was communicated by the statistical unit to the clinical study site prior to dosing. Subjects who successfully complete the screening process will be randomized on Day-1 of the trial, receiving random numbers from K001 to K072, assigned in ascending order based on their screening numbers. During the analysis phase of the study, researchers involved in sample analysis are required to maintain blindness regarding the randomization plan, ensuring unbiased analysis.Subjects who withdraw or are withdrawn from the clinical trial for any reason, whether or not they have taken the study drug, will retain their assigned random numbers. As a general rule, these subjects with their respective random numbers cannot be replaced or re-enter the trial.

## Results

### Subjects

A total of 226 subjects underwent screening, of whom 72 were enrolled, and 72 subjects were dosed. In this experiment, there were no subjects who withdrew from the trial prematurely. Throughout all parts of the study, the mean age of subjects ranged from 18 to 50 years. Women account for 9.7%, men account for 90.3%. A summary of

demographic characteristics in each study part is shown in Table 1. Supplementary meterial 3 provides subject demographic information.

### Pharmacokinetic analyses

A total of 72 subjects were enrolled in this study, and all of them successfully completed the experiment. Figure 1 illustrates the mean blood concentration-time curve for riluzole following the oral administration of 50 mg of both test preparation (T) and reference preparation (R) on an empty stomach among the 72 subjects. Table 2 presents the pharmacokinetic parameters of riluzole observed after a single oral administration of either 50 mg of the test formulation (T) or the reference formulation (R) in this trial. The bioequivalence findings for riluzole after a single oral administration of 50 mg of either test preparation (T) or reference preparation (R) under fasting conditions are summarized in Table 3.

The $C_{max}$ of riluzole, following oral administration of the test preparation on an empty stomach, was determined to be 244 ± 133 ng/mL. In comparison, the $C_{max}$ after administering the reference preparation on a similar stomach condition was 228 ± 125 ng/mL. The geometric mean ratio between the two preparations was calculated to be 107.67%. Utilizing the [1–2α] confidence interval method, the 90% confidence interval for the geometric mean ratio of $C_{max}$ between the test (T) and reference (R) preparations was found to be 95.37-121.55%, falling comfortably within the accepted range of 80.00-125.00%. This demonstrates that the pharmacokinetic parameter $C_{max}$ satisfies the equivalence criterion. Regarding $T_{max}$, the values for the test and reference preparations were 0.83(0.33, 4.00) h and 0.83(0.33, 6.00) h, respectively. A paired Wilcoxon test revealed no statistically significant difference between the two (P value = 0.7010, $P > 0.05$).In terms of $AUC_{0-t}$, the value obtained was 818 ± 372 h*ng/mL. For riluzole, the $AUC_{0-t}$ and $AUC_{0-\infty}$ values were 799 ± 376 h*ng/mL and 845 ± 385 h*ng/mL, respectively. The geometric mean ratios for $AUC_{0-t}$ and $AUC_{0-\infty}$ between the test and reference preparations were 103.11% and 102.93%, respectively. The 90% confidence intervals for these ratios, calculated using the [1–2α] method, were 97.59-108.95% and 97.59-108.56%, respectively. Both intervals fall within the acceptable range of 80.00-125.00%, indicating that the pharmacokinetic parameters $AUC_{0-t}$ and $AUC_{0-\infty}$ also meet the equivalence criterion. In this trial, supplementary material 4–5 present the summary results of the plasma concentration of riluzole following a single oral administration of either 50 mg of the test formulation (T) or the reference formulation (R) on an empty stomach.

**Table 1** Basic information of subjects enrolled in the group

| Indicator | | T-R *N*(%) | R-T *N*(%) | Total *N*(%) |
|---|---|---|---|---|
| Age (one year old) | Mean ± Sd | 29 ± 8 | 28 ± 8 | 29 ± 8 |
| | M($Q_1$ ~ $Q_3$) | 29(24 ~ 31) | 27(21 ~ 32) | 27(23 ~ 32) |
| | Min ~ Max | 18 ~ 49 | 19 ~ 50 | 18 ~ 50 |
| Age group N (%) | < 18 | 0(0.0) | 0(0.0) | 0(0.0) |
| | 18–40 | 31(86.1) | 32(88.9) | 63(87.5) |
| | 41–60 | 5(13.9) | 4(11.1) | 9(12.5) |
| | > 60 | 0(0.0) | 0(0.0) | 0(0.0) |
| | Total | 36(100.0) | 36(100.0) | 72(100.0) |
| Gender N (%) | Male | 32(88.9) | 33(91.7) | 65(90.3) |
| | Famle | 4(11.1) | 3(8.3) | 7(9.7) |
| | Total | 36(100.0) | 36(100.0) | 72(100.0) |
| Ethnic N (%) | Han ethnic group | 33(91.7) | 33(91.7) | 66(91.7) |
| | Others | 3(8.3) | 3(8.3) | 6(8.3) |
| | Total | 36(100.0) | 36(100.0) | 72(100.0) |
| Height (cm) | Mean ± Sd | 168.6 ± 8.4 | 169.8 ± 8.8 | 169.2 ± 8.6 |
| | M($Q_1$ ~ $Q_3$) | 169.8(164.3 ~ 173.0) | 169.3(164.3 ~ 175.8) | 169.5(164.3 ~ 174.3) |
| | Min ~ Max | 146.5 ~ 185.0 | 150.5 ~ 189.5 | 146.5 ~ 189.5 |
| Body weight (kg) | Mean ± Sd | 63.4 ± 7.0 | 63.7 ± 6.9 | 63.5 ± 6.9 |
| | M($Q_1$ ~ $Q_3$) | 63.1(58.8 ~ 69.3) | 63.3(58.0 ~ 70.3) | 63.2(58.4 ~ 69.5) |
| | Min ~ Max | 45.9 ~ 75.6 | 53.4 ~ 80.1 | 45.9 ~ 80.1 |
| BMI($kg/m^2$) | Mean ± Sd | 22.3 ± 1.6 | 22.1 ± 1.8 | 22.2 ± 1.7 |
| | M($Q_1$ ~ $Q_3$) | 21.9(21.2 ~ 23.3) | 21.7(20.9 ~ 24.1) | 21.9(21.0 ~ 23.6) |
| | Min ~ Max | 19.6 ~ 25.7 | 19.5 ~ 25.4 | 19.5 ~ 25.7 |

Mean ± Sd, Mean ± standard deviation; M(Q1 ~ Q3), combination of median and interquartile range; Min, the minimum value; Max, the maximum value; T, test preparation; R, reference preparation; BMI, body mass index

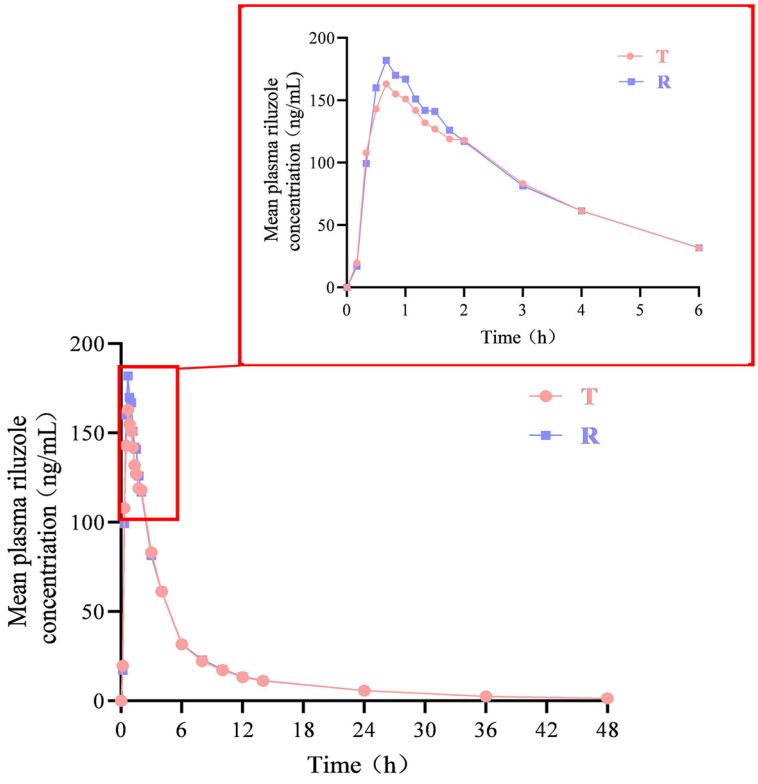


**Fig. 1** Mean plasma concentration-time curves of riluzole after administering a single 50-mg oral dose of both the test and reference formulations in fasting conditions. The upper right inset displays a time frame of 0–2 h on an enlarged scale. T, test formulation (produced by Jiangsu Nhwa Pharmaceutical Co., Ltd, China); R, reference formulation (produced by Sanofi Winthrop Industrie, France)

**Table 2** Pharmacokinetic parameters of riluzole ($N=72$)

| Pharmacokinetic parameters (units) | Mean ± SD (CV%) | |
|---|---|---|
| | Test preparation(T) | Reference preparation(*R*) |
| $T_{max}$(h) | 0.83(0.33,4.00) | 0.83(0.33,6.00) |
| $C_{max}$(ng/mL) | 244 ± 133(54.3) | 228 ± 125(54.8) |
| $AUC_{0-t}$(h*ng/mL) | 818 ± 372(45.4) | 799 ± 376(47.1) |
| $AUC_{0-\infty}$(h*ng/mL) | 845 ± 385(45.5) | 825 ± 387(46.9) |
| $AUC_{\_\%Extrap}$(%) | 3.3 ± 1.4(41.5) | 3.4 ± 1.8(53.6) |
| $\lambda_z$(1/h) | 0.0620 ± 0.00901(14.5) | 0.0622 ± 0.00928(14.9) |
| $t_{1/2}$(h) | 11.4 ± 1.84(16.1) | 11.4 ± 1.94(17.0) |

$T_{max}$ is expressed as median (minimum, maximum)h, hour; $C_{max}$, maximum plasma concentration; $AUC_{0-t}$ is area under the curve from time 0 to the time of the last measurable concentration; $AUC_{0-\infty}$ is area under the curve from time 0 to infinity; $AUC_{\_\%Extrap}$, percentage of the area under the curve extrapolated to infinity; $\lambda_z$, terminal elimination rate; $t_{1/2}$, elimination half-life; N, number of cases participating in the calculation

## Safety

After 72 subjects took the test drug T, 18 of them (25.0%) reported experiencing 31 adverse events, all categorized as adverse reactions, and all with a severity level of (1) The adverse event outcomes included 21 recoveries among 11 subjects, 2 remissions among 2 subjects, 6 lost to follow-up among 5 subjects, and 2 refusals of follow-up from 1 subject. Importantly, there were no serious adverse events or reactions, and none that led to drop-off. However, among the 18 subjects, 31 adverse events were assessed as possibly related. In the case of the reference formulation R, which was administered to 72 subjects, 23 subjects (31.9%) reported 39 adverse events. Out of these, 38 were identified as adverse reactions. In terms of severity, 23 out of 33 adverse events were level 1, while 3 out of 6 adverse events were classified as level (2) Among the reported events, 38 adverse events in 23 subjects were deemed possibly related, and 1 adverse event in 1 subject was considered possibly unrelated. The adverse event outcomes consisted of 30 recoveries among 18 subjects, 7 recoveries among 6 subjects, 1 subject lost to follow-up, and 1 subject refusing follow-up. Importantly, there were no serious adverse events or reactions, and none that resulted in drop-off. Table 4 presents categorized statistical tables of adverse events, while supplementary meterial 6 offers extensive and precise details on 70 adverse events observed in 36 subjects.

Furthermore, approximately two hours after the initial dosing cycle, one participant received non-pharmacological treatment - specifically, supine rest - owing to adverse reactions including dizziness, amaurosis, and a decrease in blood pressure. These symptoms persisted for seven minutes. Similarly, another participant experienced dizziness and a drop in blood pressure roughly six minutes

**Table 3** Bioequivalence results of main pharmacokinetic parameters of riluzole (*N* = 72)

| Pharmacokinetic parameters(Unit) | Geometric mean and ratio | | | CV (%) | 90% CI (%) |
|---|---|---|---|---|---|
| | Test preparation(T) | Reference preparation(*R*) | T/*R* (%) | | |
| $C_{max}$ (ng/mL) | 210 | 195 | 107.67 | 45.83 | 95.37-121.55 |
| $AUC_{0-t}$ (h*ng/mL) | 741 | 718 | 103.11 | 20.02 | 97.59-108.95 |
| $AUC_{0-\infty}$ (h*ng/mL) | 766 | 744 | 102.93 | 19.35 | 97.59-108.56 |

CV, Coefficient of Variation; CI, confidence interval; $C_{max}$, maximum plasma concentration; $AUC_{0-t}$ is area under the curve from time 0 to the time of the last measurable concentration; $AUC_{0-\infty}$ is area under the curve from time 0 to infinity

**Table 4** Statistical table of adverse events (*N* = 72)

| Adverse events | Test preparation | | | Reference preparation | | |
|---|---|---|---|---|---|---|
| | Cases | Absolute freuquency*(%) | Relative frequency**(%) | Cases | Absolute freuquency*(%) | Relative frequency**(%) |
| Total AEs | 31 | 18(25.0) | - | 39 | 23(31.9) | - |
| Various inspections | 29 | 17(23.6) | 93.5 | 32 | 22(30.6) | 82 |
| Elevation of gamma glutamyltransferase | 2 | 2(2.8) | 6.5 | 0 | 0(0.0) | 0 |
| Elevation of alanine aminotransferase | 6 | 6(8.3) | 19.4 | 7 | 7(9.7) | 18 |
| Depression of red blood cell count | 0 | 0(0.0) | 0 | 1 | 1(1.4) | 2.6 |
| Elevation of conjugated bilirubin | 3 | 3(4.2) | 9.7 | 0 | 0(0.0) | 0 |
| Urine white blood cell positive | 1 | 1(1.4) | 3.2 | 2 | 2(2.8) | 5.1 |
| Depression of globulin | 1 | 1(1.4) | 3.2 | 0 | 0(0.0) | 0 |
| Elevation of aspartate aminotransferase | 2 | 2(2.8) | 6.5 | 1 | 1(1.4) | 2.6 |
| Extended QT interval on electrocardiogram | 2 | 2(2.8) | 6.5 | 0 | 0(0.0) | |
| ST segment depression on electrocardiogram | 0 | 0(0.0) | 0 | 1 | 1(1.4) | 2.6 |
| Abnormal ST segment on electrocardiogram | 0 | 0(0.0) | 0 | 1 | 1(1.4) | 2.6 |
| Changes in ST-T segment of electrocardiogram | 1 | 1(1.4) | 3.2 | 1 | 1(1.4) | 2.6 |
| Decreased heart rate | 1 | 1(1.4) | 3.2 | 3 | 2(2.8) | 5.1 |
| Elevation of blood bilirubin | 2 | 2(2.8) | 6.5 | 0 | 0(0.0) | 0 |
| Elevation of blood triglycerides | 1 | 1(1.4) | 3.2 | 3 | 3(4.2) | 7.7 |
| Depression of hemoglobin | 1 | 1(1.4) | 3.2 | 1 | 1(1.4) | 2.6 |
| Depression of blood potassium | 0 | 0(0.0) | 0 | 1 | 1(1.4) | 2.6 |
| Depression of blood sodium | 0 | 0(0.0) | 0 | 1 | 1(1.4) | 2.6 |
| Elevation of blood uric acid | 3 | 3(4.2) | 9.7 | 3 | 3(4.2) | 7.7 |
| Depression of blood glucose | 0 | 0(0.0) | 0 | 1 | 1(1.4) | 2.6 |
| Elevation of blood glucose | 1 | 1(1.4) | 3.2 | 0 | 0(0.0) | 0 |
| Depression of blood pressure | 2 | 2(2.8) | 6.5 | 5 | 5(6.9) | 12.8 |
| AEs of neurological disorders | 0 | 0(0.0) | 0 | 2 | 2(2.8) | 5.1 |
| Dizzy | 0 | 0(0.0) | 0 | 2 | 2(2.8) | 5.1 |
| AEs of gastrointestinal system | 0 | 0(0.0) | 0 | 1 | 1(1.4) | 2.6 |
| Nausea | 0 | 0(0.0) | 0 | 1 | 1(1.4) | 2.6 |
| AEs of heart organ | 2 | 2(2.8) | 6.5 | 3 | 2(2.8) | 5.1 |
| Nodal rhythm | 0 | 0(0.0) | 0 | 1 | 1(1.4) | 2.6 |
| Indoor conduction disorder | 1 | 1(1.4) | 3.2 | 0 | 0(0.0) | 0 |
| Sinus tachycardia | 1 | 1(1.4) | 3.2 | 2 | 2(2.8) | 5.1 |
| AEs of Eye organ | 0 | 0(0.0) | | 1 | 1(1.4) | |
| Amaurosis | 0 | 0(0.0) | | 1 | 1(1.4) | |

AE, adverse events; QT interval, the time interval between the beginning of the Q wave and the end of the T wave on the electrocardiogram; ST segment, reflects the potential changes in each part of the ventricle during the period from the end of depolarization to the beginning of repolarization; ST-T segment is the repolarization process of the ventricular muscle, reflecting the myocardial blood supply or myocardial damage

Absolute freuquency* is ratio of the number of subjects experiencing adverse events to the total number of subjects(*N* = 72); relative frequency** is ratio of the number of adverse events in subjects to the total number of cases occurring after taking the test preparation or reference preparation

after the second dosing cycle, necessitating supine rest for a duration of three minutes. Almost all AEs were mild and resolved by the end of the study.

## Discussion

The findings of this study reveal that the test preparation tablet is bioequivalent to the standard 50-mg riluzole tablet when administered in a fasted state. The bioequivalence criteria were met by the test preparation, as indicated by the geometric least squares (LS) mean ratios for $AUC_{0-t}$, $AUC_{0-\infty}$, and $C_{max}$, which were approximately 103%, 103%, and 108%, respectively. Additionally, the 90% confidence intervals (CIs) fell within the accepted bioequivalence range.In this experiment, no significant difference was observed in the occurrence of adverse events between subjects taking the test formulation and those taking the reference formulation.

Recent studies have explored the bioequivalence between various new riluzole preparations and traditional tablets, offering alternatives such as the disintegrating (Zydis) formulation of riluzole [9], Riluzole oral suspension [10], and Riluzole oral film [11] for patients experiencing dysphagia. James Wymer and colleagues conducted an open-label, randomized, single-dose, crossover study to evaluate the bioavailability and pharmacokinetics (PK) of 50 mg Riluzole oral film compared to 50 mg riluzole tablets in 32 healthy volunteers [11]. Separately, Ann Margaret Dye and her team assessed the riluzole oral suspension, which is provided with a plastic graduated oral dosing syringe to ensure precise and reproducible dosing for ALS patients [8]. A bioequivalence study comparing the novel riluzole oral suspension to Rilutek® (tablets) found that the total exposure of 50 mg riluzole tablets was equivalent to that of 5 mg/mL riluzole oral suspension [8]. Additionally, Irfan Qureshi and co-authors determined that 40 mg of sublingual BHV-0223, a Zydis-based orally disintegrating formulation of riluzole, is bioequivalent to 50 mg oral riluzole tablets [12]. The above research shows that the new dosage form may provide more convenient medication for ALS patients.

In terms of adverse reactions, the most frequently reported side effects of riluzole include transient elevations in liver enzyme concentrations (ranging from 2 to 5 times the upper limit of normal), as well as exacerbation of asthenia, nausea, vomiting, diarrhea, anorexia, dizziness, vertigo, somnolence, and mouth paresthesia [7]. Riluzole also carries the risk of liver and kidney damage, potentially resulting in neutropenia, indirect pneumonia triggered by respiratory distress, and dizziness. During the phase III clinical trial involving ALS patients treated with riluzole, the most prevalent adverse reactions reported were fatigue, nausea, and abnormalities in liver function tests [13]. No serious adverse reactions, including swallowing difficulties, occurred in this study, and none of the adverse events or reactions led to detachment. Both formulations demonstrated good safety and tolerability, with no discontinuations due to severe or serious adverse events. Most adverse events resolved by the end of the study.

In this study, the average pharmacokinetic parameter ratios for riluzole were calculated after a single oral administration of 50 mg of both test preparation T and reference preparation R to 72 subjects each. The mean ratio for riluzole was 107.67%, the average $AUC_{0-t}$ was 103.11%, and the average $AUC_{0-\infty}$ was 102.93%. These ratios were determined using a confidence interval method to calculate the peak-to-peak and average ratios between preparations T and R.The 90% confidence intervals for the peak-to-peak-to-average, $AUC_{0-t}$, and $AUC_{0-\infty}$ between test preparation T and reference preparation R were 95.37-121.55%, 97.59-108.95%, and 97.59-108.56%, respectively. These intervals align with the bioequivalence range (80.00-125.00%) specified in the technical guidelines for bioequivalence studies of generic chemical drugs, using pharmacokinetic parameters as the endpoint.

To sum up, there are still three limitations in this study.First, since riluzole is clinically used to treat nervous system diseases-amyotrophic lateral bundle sclerosis, most patients will have dysphagia when they are sick, considering the influence of tablets on medication compliance, the bioequivalence test should be designed from the perspective of improving new administration types to improve medication compliance. The second is to ensure that the bioequivalence test ensures the comprehensiveness and accuracy of pharmacokinetic results, and it is more reasonable to consider the experimental design under fasting and postprandial conditions at the same time. The third point to note is that during this trial, a total of 7 adverse events were lost to follow-up across 6 subjects. Typically, it is highly unusual to encounter a significant number of lost-to-follow-up adverse events (AEs) in bioequivalence trials. To rectify this, we plan to implement several measures: Firstly, we will ensure comprehensive disclosure to subjects during the informed consent discussion. Secondly, we will intensify our care and attention to subjects throughout the trial. Lastly, researchers must swiftly manage any adverse reactions to the study drug, guaranteeing subject safety. Additionally, we will foster stronger communication with subjects and enhance their compliance education.

## Conclusion

The findings of this study confirm that the test preparation of riluzole was bioequivalent to the reference preparation among healthy adult volunteers in a fasted condition. Additionally, the safety data indicates that

healthy Chinese participants showed positive safety and tolerability outcomes after orally ingesting both the test and reference formulations of 50 mg riluzole tablets on an empty stomach.

**Abbreviations**

| | |
|---|---|
| HPLC-MS/MS | High Performance liquid chromatography-tandem mass spectrometry |
| CI | Confidence interval |
| $AUC_{0-t}$ | Area under the curve from time 0 to t |
| $AUC_{0-\infty}$ | Area under the curve |
| $C_{max}$ | Maximum concentration |
| ALS | Amyotrophic lateral sclerosis |
| $T_{max}$ | Time to maximum concentration |
| CYP | Cytochrome P450 |
| BMI | Body mass index |
| CTCAE | Common Terminology Criteria for Adverse Events |
| IS | Internal standard |
| MRM | Multiple reaction monitoring |
| LLOQ | Lower limit of quantification |
| T | Test formulation |
| R | Reference preparation |
| P | Probability |
| Mean ± SD | Mean ± standard deviation |
| CV | Coefficient of variation |
| AE | Adverse event |
| PK | Pharmacokinetics |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40360-025-00931-1.

Supplementary Material 1

Supplementary Material 2

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**Author contributions**

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**Data availability**

No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**

The trial received ethical approval from the Ethics Committee of Zhejiang Hospital, bearing the approval number 2023 Clinical Review (02G). The study was independently conducted at the Phase I Clinical Trial Research Center of Zhejiang Hospital, spanning from April 13, 2023, to May 23, 2023. The research adhered strictly to the standards set by the Declaration of Helsinki, the Guidelines of Good Clinical Practice, and all other pertinent guiding principles. All participants involved in the trial provided informed consent.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no competing interests.

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**References**

1. Ramaish S, Sidharth M, Zuber K, Ghanshyam D Gupta, Acharan S Narula. Therapeutic potential of oleanolic acid in modulation of PI3K/Akt/mTOR/STAT-3/GSK-3β signaling pathways and neuroprotection against methylmercury-induced neurodegeneration. Neurochem Int. 2024;180(0):0. https://doi.org/10.1016/j.neuint.2024.105876.
2. Zeinab K, Iman A, Majid G. Association between cerebrospinal fluid chitotriosidase level and amyotrophic lateral sclerosis: a systematic review. Horm Mol Biol Clin Investig. 2024;46(1):0. https://doi.org/10.1515/hmbci-2024-0007.
3. Philippe C, Nathalie G, Pierre-François P, Marie-Helene S, Annie V, Philippe C. Treatment continuity of amyotrophic lateral sclerosis with available riluzole formulations: state of the art and current challenges in a 'real-world' setting. Amyotroph Lateral Scler Frontotemporal Degener. 2024;26(0):0. https://doi.org/10.1080/21678421.2024.2375330.
4. Rosario V, Filippo DM, Salvatore T, Antonio C, Umberto M, Maurizio G, Francesca P, Sara C, Enrico M, Francesca DP, Grazia Z, Giorgio P, Emilio M, Daniela P, Barbara I, Stefano C, Giuseppe F, Paolina S, Fabiola. De Marchi., Letizia, Mazzini., Cristina, Moglia., Andrea, Calvo., Adriano, Chiò. Changes to Average Survival of Patients With Amyotrophic Lateral Sclerosis (1995–2018): Results From the Piemonte and Valle d'Aosta Registry. Neurology. 2025;104(8):0. https://doi.org/10.1212/WNL.0000000000213467
5. Le Liboux A, Lefebvre. P, Le Roux Y, Truffinet. P, Aubeneau. M, Kirkesseli S, Montay G. Single- and multiple-dose pharmacokinetics of riluzole in white subjects. J Clin Pharmacol. 1998;37(9):0. https://doi.org/10.1002/j.1552-4604.1997.tb05630.x.
6. Groeneveld GJ, van Kan HJ, Toraño. JS, Veldink JH, Guchelaar HJ, Wokke JH, den Berg. Inter- and intraindividual variability of riluzole serum concentrations in patients with ALS. J Neurol Sci. 2001;191(0):0. https://doi.org/10.1016/s0022-510x(01)00613-x. van.
7. Ranjeet Prasad D, Jayachandra R, Nuggehally B, Srinivas R. Two decades-long journey from riluzole to Edaravone: revisiting the clinical pharmacokinetics of the only two amyotrophic lateral sclerosis therapeutics. Clin Pharmacokinet. 2018;57(11):0. https://doi.org/10.1007/s40262-018-0655-4.
8. Ann Margaret D, Alan S. Riluzole 5 mg/ml oral suspension: for optimized drug delivery in amyotrophic lateral sclerosis. Drug Des Devel Ther. 2017;11(0):0. https://doi.org/10.2147/DDDT.S123776.
9. Diane M, Longo., Lisl KM, Shoda., Brett A, Howell., Vladimir C, Robert M, Berman., Irfan A, Qureshi. Assessing effects of BHV-0223 40 mg Zydis Sublingual formulation and riluzole 50 mg oral tablet on liver function test parameters utilizing DILIsym. Toxicol Sci. 2020;175(2):0. https://doi.org/10.1093/toxsci/kfaa019.
10. Benjamin Rix B, Paolo B, Sara C. Riluzole oral suspension: bioavailability following percutaneous gastrostomy Tube-modeled administration versus direct oral administration. Clin Ther. 2019;41(12):0. https://doi.org/10.1016/j.clinthera.2019.09.016.
11. James W, Stephen A, Antoinette H, Alan B, Hill. Pharmacokinetics, bioavailability, and swallowing safety with riluzole oral film. Clin Pharmacol Drug Dev. 2022;12(1):0. https://doi.org/10.1002/cpdd.1168.

12. Irfan Q, et al. A Pharmacokinetic bioequivalence study comparing Sublingual riluzole (BHV-0223) and oral tablet formulation of riluzole in healthy volunteers. Clin Pharmacol Drug Dev. 2019;9(4).
13. Lacomblez L, Bensimon. G, Leigh PN, Guillet. P, Meininger V. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis/riluzole study group II. Lancet. 1996;347(9013):0. https://doi.org/10.1016/s0140-6736(96)91680-3.

## Publisher's note